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15

STUDIES ON PERIPHERAL MARKERS OF CENTRAL SEROTONERGIC ACTIVITY AND BEHAVIOUR

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IV **Kiive, E**., Fischer, K., Harro, M., & Harro, J. Platelet monoamine oxidase activity in association with adolescent inattentive and hyperactive behaviour: data from a prospective longitudinal study. (Submitted to *European Neuropsychopharmacology*)

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADHD	Attention deficit hyperactivity disorder
CRH	Corticotropin releasing hormone
DA	Dopamine
DSM	Diagnostic and Statistical Manual of Mental Disorders
GH	Growth hormone
GHRH	Growth hormone releasing hormone
HDL	High density lipoprotein
HPA	Hypothalamic-pituitary-adrenal axis
5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
MAO	Monoamine oxidase
MÅDRS-S	Montgomery-Åsberg Depression Rating Scale self-assessment version
PEA	Phenylethylamine

1. INTRODUCTION

1.1. Serotonin in the central nervous system

In search for a better understanding of the neurobiological basis of certain personality traits, behavioural deviations and their possible markers, attention has been focused on the monoamine transmitter systems. Particularly the serotonin (5-HT) system has been of interest in biological models of human personality and altered 5-HT function has also been implicated in the pathogenesis of several psychiatric conditions. The majority of 5-HT neurons in the mammalian brain are located along the midline of the brainstem and their axons innervate almost every area in the brain (Jacobs and Azmitia, 1992). 5-HT is synthesized from the amino acid tryptophan. The primary catabolic pathway for 5-HT is oxidative deamination by the enzyme monoamine oxidase (MAO), which converts 5-HT to 5-hydroxyindoleacetaldehyde, which is, in turn, oxidized by an NAD+ dependent aldehyde dehydrogenase to form 5-hydroxyindoleacetic acid (5-HIAA). The 5-HT system has been suggested to be involved in very many functions of the brain and participates in the control of pituitary secretion, particularly in the regulation of prolactin, adrenocorti-cotropic hormone (ACTH), and growth hormone (GH) release.

1.2. Hyperactive and inattentive behaviour in children and young adults in association with central serotonergic activity

In addition to the well-known role of dopaminergic (DA) neurotransmitter system in human hyperactive and impulsive behaviour, the 5-HT-ergic system is also implicated because of the modulation of emotion and cognition by the 5-HT midbrain raphe system and the complex interaction between 5-HT-ergic and DA-ergic neurotransmitter systems. There is evidence that disturbances in central 5-HT function have a role in hyperactive and inattentive behaviour in adults (Rogers et al., 1999). Central 5-HT function, measured by the prolactin response to fenfluramine challenge, has been found to correlate negatively with impulsivity in normal controls (Manuck et al., 1998). Reduced synaptic availability of 5-HT has been implicated in poor impulse regulation also in young children (Oades et al., 2002). Furthermore, differences in the 5-HT turnover in children with attention deficit hyperactivity disorder (ADHD) have been described with the tendency towards lower levels of blood 5-HT in the children with more severe disorder (Spivak et al., 1999). Concerning genetic regulation of 5-HT neurotransmission, previous studies of clinical ADHD

suggest that functional polymorphisms in the 5-HT transporter gene may be associated with the disorder (Manor et al., 2001). Also, 5-HT_{1B} receptor gene has been associated with ADHD (Quist et al., 2003) and recently, a more active MAO-A 941G allele in children with ADHD was found (Domschke et al., 2005).

Studies investigating the 5-HT function in childhood behavioural problems via peripheral and central measures have, nevertheless, yielded inconsistent results, which may be partly related to the stability of measures of 5-HT function. Developmental factors should be considered when studying neurobiological functions in children.

1.3. Depression and serotonergic activity

The 5-HT system has been shown to be involved in modulating neural circuits that regulate emotion and mood in humans. There is considerable evidence available in the literature supporting the idea that brain 5-HT system plays an important role in the etiology and pathogenesis of affective disorders, particularly major depression.

A deficiency in brain 5-HT activity has been associated with both depression (Maes and Meltzer, 1995) and suicide (Mann, 1998). Studies of the patients with major depression have reported decreased plasma tryptophan (Coppen and Wood, 1978), reduced cerebrospinal fluid 5-HIAA levels (Åsberg et al., 1984), and decreased platelet 5-HT uptake (Healy and Leonard, 1987) compared to healthy controls. Also, neuroendocrine studies have suggested that various groups of depressed patients may exhibit abnormal 5-HT-mediated responses. Blunted neuroendocrine responses have been demonstrated in challenge studies of different 5-HT receptors suggesting decreased brain 5-HT responsiveness (Siever et al., 1984; Cowen and Charig, 1987; Mann et al., 1995; Sher et al., 2003) in depressive patients compared to healthy controls. Clinical studies have provided further evidence that 5-HT is implicated in the causation and treatment of depression, as both 5-HT precursors and selective inhibitors of 5-HT uptake are effective in the treatment of depression. Antidepressant drugs may act, in part, by enhancing 5-HT-ergic activity.

1.4. Peripheral markers of serotonin-related behaviour

5-HT measures used in neurochemistry and behaviour studies include neurochemical assays, receptor-density studies, functional neuroimaging studies, and candidate-gene research. Peripheral markers of the 5-HT system in human platelets have been widely used in psychiatric research: with regard to 5-HT uptake and 5-HT_2 receptors, the platelet has been considered to be a valid model for the central 5-HT neuron (Pletscher, 1987; Da Prada et al., 1988). In

particular, platelet activity of MAO is used as a possible indicator of central 5-HT function because of its easy accessibility in platelets.

1.4.1 Monoamine oxidase

In 1928, Mary Hare isolated a new enzyme, which catalyzed the oxidative deamination of tyramine (Hare, 1928). Later Blaschko and colleagues showed that the same enzyme also oxidized catecholamines (Blaschko et al., 1937), and to reflect the more general activity, Zeller proposed the name monoamine oxidase (MAO) (Zeller, 1938). Monoamine oxidase (MAO; E.C. 1.4.3.4) is an integral protein of outer mitochondrial membranes (Schnaitman et al., 1967), and it occurs as two subtypes, MAO-A and MAO-B, which have different inhibitor and substrate specificities (Johnston, 1968). MAO-A and MAO-B are encoded by separate genes that are closely linked on the X chromosome, and share 70% similarity in amino acid sequence (Bach et al., 1988). MAO-A and MAO-B are important in catalyzing the oxidative deamination of many exogenous and endogenous monoamines and also trace amines such as phenylethylamine (PEA). MAO-A is selectively inhibited by e.g., clorgyline and has 5-HT and noradrenaline as preferred substrates. MAO-B is selectively inhibited by L-deprenyl (selegiline) with DA and a number of exogenous monoamines as preferred substrates (Oreland, 1993). Most human tissues, including the brain, express both MAO-A and MAO-B, but human platelets and lymphocytes express only MAO-B (Donnelly and Murphy, 1977). The existence of MAO in platelets was first reported in 1964 by Finnish researchers (Paasonen et al., 1964), the platelet MAO-B has the same amino acid sequence as MAO-B in the brain (Chen et al., 1993). There are multiple biochemical and pharmacological similarities existing between blood platelets and 5-HT-containing neurons of the central nervous system. Since human platelets contain almost exclusively MAO-B, they can be used as a source for the characterization of this enzyme (Da Prada et al., 1988). The catalytic activity of platelet MAO is under strong genetic control: studies have shown heritability factor of about 0.75 for both males and females (Oxenstierna et al., 1986; Pedersen et al., 1993). There was some uncertainty whether platelet MAO-B activity reflected the activity of brain MAO-B. Although brain and platelet MAO-B are identical, there are studies that had found no significant correlation between MAO activity in the brain and in platelets (Winblad et al., 1979; Young et al., 1986). However, an in-vivo study using PET technique measured the reduction of cerebral MAO-B and found it to be significantly correlated with the reduction in platelet MAO-B (Bench et al., 1991). Platelet MAO activity is characterized by a considerable variability between individuals; compared to inter-individual variation, the intra-individual variation of platelet MAO activity has reported to be low, with a minor increase in activity after the age of about 40 years (Murphy et al., 1976; Bridge et al., 1985). This age related increase of

platelet MAO is more prominent in females (Veral et al., 1997). Women have approximately 10–20% higher platelet MAO activity when compared to men (Murphy et al., 1976). Females have also repeatedly been reported to show higher variation than males, presumably because of changes due to menstrual cycles (Belmaker et al., 1974). There are several factors, which can alter the platelet MAO activity such as physical exercise (Gawel et al., 1977; Owen et al., 1977) and abstinence reaction after alcohol abuse (Wiberg, 1979; Alexopoulos et al., 1981; Major et al., 1981). Vitamin B12 deficiency drastically increases the enzyme activity (Glover et al., 1980), and, consequently, the supplemention of vitamin B12 reduces the activity to normal levels in these cases (Regland et al., 1991). Reduced platelet MAO activity has also been found during pregnancy (Wahlund et al., 1986).

1.4.1.1. Platelet monoamine oxidase activity, personality traits, and psychiatric disorders

In 1976, Buchsbaum and colleagues presented the so-called "vulnerability hypothesis" of platelet MAO (Buchsbaum et al., 1976). The implication was that low activities of platelet MAO are associated with personality traits, which increase the vulnerability to several psychiatric disorders and maladaptive behaviours, such as drug abuse and social maladjustment. These personality traits include impulsiveness, sensation seeking, monotony avoidance and, to some degree, aggressiveness. These personality traits as well as low platelet MAO activity have been found in disorders such as alcoholism (Wiberg et al., 1977; Mukasa et al., 1990; Anthenelli et al., 1995; von Knorring and Oreland, 1996; Coccini et al., 2002; Demir et al., 2002), psychopathy (Lidberg et al., 1985; Alm et al., 1996), suicidal behaviour (Oreland et al., 1981; Tripodianakis et al., 2000; Stalenheim, 2001), and eating disorders (Verkes et al., 1996; Carrasco et al., 2000; Diaz-Marsa et al., 2000). Platelet MAO activity has also been shown to correlate with different personality traits of healthy controls in several studies. Studies on MAO and personality have found that people with low MAO activity are more likely to score high in risk-taking, sensation seeking or novelty seeking in personality questionnaires (Ward et al., 1987; Shekim et al., 1989; Reist et al., 1990; Smith, 1994; Howard et al., 1996).

Von Knorring and colleagues studied 18-years old boys selected from the general population, and they found low MAO subjects to be more sensation seeking and to have higher scores in impulsiveness and monotony avoidance: they also had higher use of tobacco and alcohol, and had more frequently drug abuse. When low MAO subjects were subdivided according to their intellectual level, low MAO subjects with low intellectual level were found to have more use and abuse of alcohol and drugs, i.e., less accepted forms of sensation seeking. The authors suggested this subgroup to be the real "high risk" group that according to the high-risk paradigm could be expected to show more

alcohol abuse (von Knorring et al., 1987). King and colleagues (1990) found significant differences between 53 drug abusers and 20 controls on sociability, impulsiveness, and neuroticism as assessed by the Eysenck Personality Inventory. The same authors subsequently suggested a neurochemical trait model of risk for drug abuse (King et al 1992). According to their model, differences in personality traits that predispose to drug usage have their basis in certain neuromodulatory systems. Drug consumption is a response to temperamental factors, and is motivated by self-medication for these traits. Aggressiveness may also be related to vulnerability for drug abuse (Stattin and Magnusson, 1989) as well as impulsiveness, hyperactivity, and poor self-regulation (Gittelman et al., 1985; Block and Keyes, 1988; Cloninger et al., 1988; Tarter and Edwards, 1988). It has been shown that type A personalities tend to have low platelet MAO activity (Smith, 1994). The findings agree with previous reports in showing relationships between MAO activity and certain personality traits and support the notion that low activity of MAO may contribute to sympathetic hyperreactivity in type A individuals. Type A personality is characterized by impatience with slowness, a heightened pace of living, ambitiousness, dissatisfaction with life, excessive drive, and easily aroused hostility (Jenkins et al., 1967). There is also an association between platelet MAO activity and certain neuropsychological measures (af Klinteberg et al., 1990). Response time in a computerized test was found to be significantly correlated to platelet MAO and in a perceptual maze test, there was a significant relationship between low platelet MAO activity and short check times (time from last decision until pressing the 'finish' button) after completing the task. Furthermore, there was a significant correlation with failed inhibitions, a measure of how many mistakes the individuals make when shown a visual sign indicating 'press the button' and, at the same time, auditory signal canceling this order. These results show that subjects with low platelet MAO activity prefer speed over accuracy. Similar performance patterns have also been demonstrated to correlate with such personality traits as extraversion and non-conformity (Newman et al., 1985). In principally similar tasks, deficient performance has been strongly associated with 5-HT-ergic depletion in animal studies (Soubrie, 1986).

There have been less studies concerning personality and MAO activity in children, and these studies have mostly been carried out in small samples of subjects with psychiatric or behavioural disorders (Rogeness et al., 1982; Shekim et al., 1986; Bowden et al., 1988; Plizska et al., 1988; Stoff et al., 1989). Plizska and colleagues (1988) found no difference in platelet MAO activities in children with behavioural disorders but a positive correlation with their degree of anxiety. Others have found a positive correlation of platelet MAO activity with impulsiveness in prepubertal boys with externalizing symptoms of disruptive behaviour (Stoff et al., 1989). Rogeness and colleagues (1982) found no difference in platelet MAO activity in children with conduct disorder, while some other studies have found lower activity in children with attention deficit disorder and hyperactivity (Shekim et al., 1982; Bowden et al.,

1988). These inconsistencies may also be related to the stability of measures of neurobiological function as they may undergo developmental changes.

The above-cited studies were carried out in psychiatric patients. Regarding healthy children, a study of af Klinteberg and Oreland (1995) found that aggressiveness and hyperactivity (the sum of scores of motor restlessness and concentration difficulties) in 13 years old male adolescents (n=84), according to teachers' ratings, were negatively associated with platelet MAO activity, which was measured when the subjects had reached adulthood. Both hyperactivity and aggressiveness may be observed in various degrees in normal schoolchildren and be conceptualized as traits or dimensions (Schachar and Taylor, 1986). However, motor restlessness and concentration difficulties are common components of ADHD. According to DSM, ADHD occurs in about 3–5% of school-age children, with lower prevalence in girls than in boys. The concept of ADHD has evolved over the last 35 years. DSM-II (American Psychiatric Association, 1968) defined a disorder called "hyperkinetic reaction of childhood or adolescence". DSM-III (American Psychiatric Association, 1980) described the disorder as a developmentally inappropriate degree of inattention, impulsiveness, and hyperactivity and, similarly, DSM-IV (American Psychiatric Association, 1994) includes three subtypes of ADHD based on elevated symptoms of inattention and hyperactivity-impulsivity. The predominantly inattentive type describes individuals with significant portion of inattentive symptoms in the absence of significant hyperactivity-impulsivity symptoms. The predominantly hyperactive-impulsive type includes children and adolescents with symptoms of hyperactivity-impulsivity but not inattention. The combined type describes individuals with simultaneous symptoms of inattention and hyperactivity-impulsivity. Symptoms in children with ADHD include hyperkinesis and distractibility, variability of task performance, disorganization, an inability to plan or follow through on a plan, an inability to shift, set and reprogram activities when needed, and deficient rule-governing behaviour. Unfortunately, no consistent biological marker or identifiable genetic abnormality to define the ADHD subgroups has yet been found. Diagnosis must therefore depend on evaluation of the behaviours said to characterize the syndrome-inattention, impulsiveness, and overactivity. However, the dimensions of these behaviours may be blurred not only in the eye of the beholder but by the lack of a clear dividing line between normal and abnormal, the characteristics of questionnaires, and the ubiquity of the symptoms in other disorders.

Aggressive behaviour of patients with ADHD was first time described in the literature in 1977 (Cantwell, 1977). Childhood aggression has been shown to predict young adult drug use and deviant behaviour (Brook et al., 1996). Drug use and delinquency during early and late adolescence served as the mediator between childhood aggression and young adult drug use. Moreover, adolescent drug use is associated with later delinquency. In a ten-year prospective follow-up study of Hechtman and colleagues (1984), young adults who had been diagnosed as hyperactive in childhood were found to have had greater involve-

ment with alcohol and drug use and with courts and police than did matched controls during the five years preceding evaluation. Several studies have proposed platelet MAO activity as a reliable biochemical marker for alcoholism and perhaps addiction to other substances of abuse (Pandey et al., 1988; Devor et al., 1993; Faraj et al., 1994). There are several lines of research that lend support to the potential significance of platelet MAO as an indicator of vulnerability to drug abuse. In a series of papers, von Knorring and colleagues (1984, 1985, 1987) reported results of an investigation of 18-years old adolescents. The smokers were more likely to abuse glue, alcohol, cannabis and amphetamine. As a group, the smokers not only had significantly lower platelet MAO activity, but also there was more drug abuse (as well as alcohol and tobacco use) among subjects with low platelet MAO activity compared with subjects with higher MAO activity. Subjects with mixed substance abuse had significantly lower platelet MAO activity, while subjects with only alcohol abuse did not have low platelet MAO activity.

1.4.1.2. Monoamine oxidase activity and smoking

Several human studies have shown that cigarette smokers have lower platelet MAO activity than non-smokers (Coursey et al., 1979; Norman et al., 1982), and that those who had stopped smoking had platelet MAO activities similar those of the non-smokers (Oreland et al., 1981). The authors suggested that the low MAO activity found in smokers is due to either a long term effect of one or more of the constituents of cigarettes upon the synthesis or breakdown of platelet MAO, or that individuals with low platelet MAO activity are more prone to smoking. The finding of low platelet MAO in smokers has been confirmed several times in studies both on males and females (Norman et al., 1987; Oreland et al., 1999). Whitfield and colleagues (2000) demonstrated that smoking reduced platelet MAO activity in a significant and dose related manner with no evidence of lower MAO in ex-smokers or in nonsmoking subjects. Likewise, the activities of MAO-A and MAO-B are decreased in animals exposed to cigarette smoke in vivo (Carr and Rowell, 1990) and in vitro (Yu and Boulton, 1987), and heavy smokers have reduced levels of MAO in peripheral tissues (Berlin et al., 1995). By using positron emission tomography, it was demonstrated that cigarette smoking reduced brain levels of MAO-A (Fowler et al., 1996a) and MAO-B, (Fowler et al., 1996b). The degree of MAO-B inhibition in smokers relative to non-smokers is quite variable between subjects, ranging between 17 and 67% (Fowler et al., 1996b). Despite the evidence that smoking inhibits MAO activity, the mechanism of MAO inhibition by cigarette smoke is not known. Although nicotine is the main pharmacologically active compound in tobacco, its effects on MAO catalytic activity are unclear. At physiological concentrations, nicotine does not affect cerebral MAO-A and MAO-B activity (Carr and Basham, 1991; Fowler et al.,

1998) or MAO-B activity in platelets (Oreland et al., 1981). Two other components in cigarette smoke, hydrazine and phenylpyridine, do not inhibit MAO in vivo (Carr and Basham, 1991). However, it was reported that 2-naphthylamine decreases mouse brain MAO-A and MAO-B activity in vitro (Hauptmann and Shih, 2001). 2-naphthylamine is a carcinogen found in high concentrations in cigarette smoke. Studies of these compounds may provide insight into some aspects of the pharmacological and toxicological properties of tobacco smoke. The inhibitory effect of 2-naphthylamine on both MAO-A and MAO-B catalytic activity supports the hypothesis that smoking decreases MAO activity in vivo, instead that people with lower MAO activity are more prone to become a smoker. Berlin and colleagues (2000) have found that platelet MAO-B activity in current smokers is inversely associated with plasma cotinine level, an index of smoked tobacco use, but not with the number of cigarettes smoked. Further studies are needed to investigate whether the measurement of platelet MAO-B activity can be used as a long-term index of tobacco use and smoke exposure. However, it has become apparent that MAO activity is directly inhibited by some component of cigarette smoke, and smokers have low platelet MAO activity. Since the prevalence of smoking is higher in many of the conditions in which low MAO has been implicated (Vanable et al., 2003), the MAO susceptibility associations may be partly, or entirely, false. However, several studies have reported that personality traits of smokers are similar to the personality traits of individuals with low platelet MAO activity (von Knorring and Oreland, 1985; Terracciano and Costa, 2004). It has been found that when the smoking factor is under control, there are clear associations between low platelet MAO activity and eating disorders (Carrasco et al., 2000; Diaz-Marsa et al., 2000), and some aspects of impulsivity (Eensoo et al., 2004). Furthermore, platelet MAO activity was found to be related to the behaviour of newborn babies, those with lower MAO displaying more screaming and restlessness (Sostek et al., 1981). In addition, low platelet MAO has been associated with social incompetence in monkeys (Fahlke et al., 2002), and those associations cannot be attributed to smoking. These findings strongly suggest that platelet MAO activity is linked to behaviour. However, the effect of smoking has to be considered as a serious confounding factor in investigations on platelet MAO as a correlate of behavioural traits.

1.4.2. Cholesterol, serotonergic activity, and behaviour

Low brain 5-HT-ergic activity is causally related to impulsive and aggressive behaviour (Westergaard et al., 2003). Behaviours associated with low 5-HT neurotransmission have also been observed in persons with low cholesterol. Many studies have reported a significant relationship between low cholesterol levels, impulsivity and violent acts (New et al., 1999; Buydens-Branchey et al., 2000; Golomb et al., 2000). It has been suggested that low cholesterol and

decreased 5-HT neurotransmission may be linked to each other (Engelberg, 1992; Steegmans et al., 1996; Papakostas et al., 2003, 2004) and to impulsive and aggressive behaviour (Kaplan et al., 1994). Cholesterol plays an integral role in the structure and function of the cell membrane and may also affect neurotransmission in the central nervous system. Low membrane cholesterol has been proposed to decrease the number of 5-HT receptors and therefore lowered plasma cholesterol concentration may contribute to a decrease in brain 5-HT (Heron et al., 1980; Engelberg, 1992). Excess cholesterol may adversely affect the function of membrane-bound 5-HT structures. However, it has been proposed that also elevated cholesterol may lead to lower 5-HT function either directly by binding to membrane-bound receptors or transporter or indirectly by altering the fluidity of the neuronal membrane and thereby conformation of these structures (Papakostas et al., 2004).

1.4.3. Hormonal responsivity to maximal exercise

Altered regulation of the 5-HT-ergic system has been suggested to play a role in response to exercise stress. 5-HT system plays an important role in modulation of the hypothalamic-pituitary-adrenal (HPA) axis, via both hormonal and direct neural pathways (Lowry, 2002). The HPA axis mediates perceived stress along a chain that involves the hypothalamus, the anterior pituitary gland, and the adrenal cortex. Individual differences in the hormonal response are thought to reflect dimensional variability in central 5-HT-ergic activity. Several studies have shown that acute physical exercise activates the HPA axis resulting in rapid increases in plasma cortisol (Sowers et al., 1977; Van der Pompe et al., 1999; Gispen-de Wied et al., 2000), growth hormone (GH), and prolactin (Luger et al., 1988; Di Luigi et al., 2003) levels.

1.4.3.1. Growth hormone

Human GH or somatotropin is the major growth–promoting hormone secreted by the pituitary gland. GH secretion underlies the regulation of growth hormone releasing hormone (GHRH) and somatostatin: GHRH stimulates release of GH (Mayo et al., 1995), whereas somatostatin inhibits its release (Brazeau et al., 1973; Vale et al., 1975). In response to GHRH, somatotropes in the anterior pituitary release GH into the systemic circulation. Spontaneous GH secretion occurs in pulsatile fashion with most being secreted during the early hours of night sleep in males, but in a more variable pattern and with higher output during the day in females (Van Cauter et al., 1998). Functions that can be influenced by GH and GHRH include sleep, mood, neuroprotection, and cognitive functions (Sartorio et al., 1996). In depressive patients, reduced nocturnal and 24-h GH secretion has been found (Voderholzer et al., 1993;

Fiasche et al., 1995), other authors have found increased 24-h GH secretion (Mendlewicz et al., 1985). Several studies have found that depressed patients have reduced GH response in pharmacological challenge tests (Voderholzer et al., 1993; Mokrani et al., 2000). Healthy young men with high depression and anxiety scores show a reduced or no increase in GH secretion after physical exercise (Harro et al., 1999). A decreased GH secretion in response to dynamic testing has been found also in children with depression (Ryan et al., 1994; Dahl et al., 2000).

1.4.3.2. Cortisol

The HPA axis activation is a response of the organism to psychological and physical stress, resulting in elevated levels of glucocorticoids, mainly cortisol in humans. Corticotropin releasing hormone (CRH) stimulates the secretion of ACTH from corticotrophes in the anterior pituitary, which, in turn, stimulates cortisol secretion from the cells of the adrenal cortex (Akil et al., 1999). One of the most replicated findings in biological psychiatry is the activation of the HPA axis in the patients with major depressive disorder. Frequently reported findings include elevated cortisol and CRH (Dinan, 1994), non-suppression in the dexamethasone suppression test (Holsboer and Barden, 1996), a blunted ACTH response to CRH (Rupprecht et al., 1989), and hippocampal volume reduction possibly due to cortisol overproduction (Bremner et al., 2000) among the subjects with affective disorders.

1.4.3.3. Prolactin

Prolactin is regulated by the hypothalamus, it lacks a major releasing hormone but is controlled by prolactin inhibiting factor (Frohman et al., 1999). Prolactin exhibits a pulsatile secretion pattern (Van Cauter et al., 1981), and prolactin concentrations are higher in females than in males as a result of estrogens. DA serves as the major prolactin inhibiting factor, it is secreted into portal blood by hypothalamic neurons, binds to receptors on lactotrophs, and inhibits both the synthesis and secretion of prolactin. Prolactin has an essential role in the maintenance of immune system functions, and is also a stress-related hormone because its release increases in response to psychological (Delitala et al., 1987; Biondi and Picardi, 1999) and physiological stress (de Vries et al., 2000). Prolactin is regulated, in part, throughout the 5-HT pathways. It has been suggested that 5-HT has a direct action on pituitary prolactin secretion (Van de Kar et al., 1989; Balsa et al., 1998). Drugs that act as 5-HT-ergic precursors, releasing agents and agonists elevate plasma prolactin levels (Van de Kar et al., 1989; Yatham and Steiner, 1993), while 5-HT antagonists decrease plasma prolactin levels (Goodall et al., 1993).

2. AIMS OF THE STUDY

The aims of the present dissertation are listed as follows:

1. To investigate the association of platelet MAO activity with simultaneously rated aggressive and hyperactive behaviour, alcohol and illicit drug use in a population derived sample of healthy children and adolescents in cross-sectional and longitudinal studies with considering the confounding effect of smoking on platelet MAO activity.
2. To investigate the changes in platelet MAO activity and in plasma cholesterol levels and their possible associations with changes in aggressive and hyperactive behaviour, smoking, alcohol and illicit drug use in a longitudinal study on healthy adolescents.
3. To study whether MAO activity in platelets is stable across the time and the extent to which it varies with development in normal healthy adolescents.
4. To study the 5-HT-ergic component of major depression through the hormonal responses to the maximal physical exercise.

3. METHODS

3.1. Participants

The data were collected in 1998/1999 and three years later, in 2001/2002. The original sample (**Article I** and **II**) represented the proportion of urban and rural, Estonian and Russian boys and girls of the certain age living in Tartu county. According to the proportion of all 9-and 15-years old children (data obtained from local statistics), the planned number of children in each subgroup was calculated. The main unit of sampling was a school. Headmasters of 54 schools out of the 56 with 9- and 15-years old children in Tartu county agreed to participate in the study. A random sample of 25 schools was selected using cluster sampling (urban and rural schools with younger and older children from Estonian and Russian language schools) and probability proportional to school size. Of each school sampled, all 9- and 15-years old children were asked to participate in the study. Parents and children gave their written consent. Of all subjects invited to participate (n=1486), 76% of children (n=1129) and their parents agreed. The agreement rate was highest in urban Estonian girls and the lowest in younger Russian children. The follow-up studies (**Articles IV** and **V**) were performed with the group of older children (15-years old in 1998/1999) three years later when they were 18 years old. In **Article IV** data of 365 18 years old adolescents (149 boys, 216 girls) were used, the analysis included all adolescents who had platelet MAO measured at age 15. In **Article V**, the analysis included 320 adolescents (132 boys, 188 girls) who had platelet MAO activity measured at age 15 and 18.

In **Article III**, participants were 24 patients, recruited at the in- and outpatient services of the Clinic of Psychiatry, Tartu University Clinics, and 22 healthy volunteers. Subjects were all males, age 24–68; to be included in the study the patients had to fulfill the DSM-IV diagnostic criteria (American Psychiatric Association, 1994) for current major depressive disorder. Diagnoses were confirmed with use of the Mini-International Neuropsychiatric Interview, version 5.0.0 (Sheehan et al., 1998). The exclusion criteria were significant psychiatric comorbidity, including schizophrenia or other psychotic disorder, organic mental disorder, mental retardation, bipolar disorder, anxiety disorders if primary and/or predominant, alcohol abuse or dependence in the last 12 months, and unstable or significant comorbid somatic or neurological disease. Most of the patients were using antidepressant medication. The control subjects were also interviewed by a psychiatrist to exclude depression or any other psychiatric disorder. All participants provided written informed consent, and the study protocol was approved by Ethics Review Committee on Human Research of the University of Tartu.

3.2. Psychological measures

In **Articles I**, **II**, **IV,** and **V,** ratings on Aggressiveness, Motor Restlessness and Concentration Difficulties were obtained from the teachers who had known the child for at least 3 years. Behaviour was rated on a seven point scale as described by af Klinteberg (1988) with verbal descriptions of extreme manifestations as follows:

Aggressiveness. (1) They work in harmony with the teacher and have positive contacts with classmates. Their relations to others easily become warm and affectionate. (7) They are aggressive against teachers and classmates. They may, for example, be impertinent and imprudent, actively obstructive or inciting to rebellion. They like disturbing and quarrelling with classmates.

Motor Restlessness. (1) They have no difficulty at all in satisfying even great demands on silence and quietness. (7) They find it very difficult to sit still during lessons. They fidget uneasily in their seats or wish to move about in the classroom, even during lessons. They may also be talkative and noisy.

Concentration Difficulties. (1) They have marked ability to concentrate on a task and persevere with it. They never allow themselves to be distracted, and do not give up as long as the task suits their level of intelligence. (7) They cannot concentrate on their work but are occupied with irrelevant things, or sit daydreaming. For a few moments they may work but are soon lost in other thoughts again. They usually give up quickly, even when the work is suited to their level of intelligence.

The teachers were instructed to use the boys and girls in their own class as reference groups. Hyperactivity score was calculated after af Klinteberg and Oreland (1995) by summing the scores of Motor Restlessness and Concentration Difficulties.

In **Article IV**, the additional teacher-report version of the Swanson, Nolan and Pelham (SNAP) Questionnaire (Swanson, 1992) was also used to assess ADHD symptoms among the adolescents. Each of the 18 items of the SNAP-IV (Swanson, 1995) provides a word-to-word description of a symptom of DSM-IV ADHD (American Psychiatric Association, 1994), and asks the rater to indicate whether the child exhibits the symptom “not at all, “just a little”, “pretty much” or “very much”. The scores of SNAP-IV divide into inattention and hyperactivity/impulsivity subscores.

At age 18, adolescents filled in the Montgomery-Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979) self-assessment version (MÅDRS-S). The data about mood at age 18 were used in **Article V**. Also, in **Article III,** MÅDRS-S was used to assess subjective symptoms of depression of the patients and healthy volunteers.

3.3. Data about smoking, alcohol and illicit drug use

Children reported whether they currently smoke, drink alcohol, have used illicit drugs or not in an anonymous questionnaire in a laboratory out of school with no teachers or parents present. Questions about smoking habits were asked as follows: "Do you smoke?" and "How often do you smoke?" Children who reported that they smoke either regularly or occasionally were considered as smokers. Children answered the questions- "Have you ever tasted alcohol?" with possible answers *no/yes/I do not know,* and alcohol consumption frequency ("How frequently do you consume beer, wine, strong spirits and cider?" with possible answers *never/less than once per month/at least once per month/once per week/every day*. The frequency of consumption of each of the four types of alcoholic beverages was asked separately. On the basis of the most frequently used type of alcohol the total alcohol consumption score was formed, ranging from 1 to 5 (*1- never uses any kind of alcohol, 2- drinks alcohol less than once per month, 3- drinks alcohol at least once per month, 4- drinks alcohol once per week, 5- drinks alcohol every day*).

At age 15, adolescents also reported their experiences with illicit drugs. ("Have you ever tried illicit drugs?", with possible answers *no/once/several times/current user*). The illicit drug use score was formed, ranging 1 to 4 (*1- never tried any illicit drug, 2- has tried an illicit drug once, 3- has tried illicit drugs several times, 4- is a current user*). At age 18 adolescents reported if they had tried illicit drugs and answered to more specific questions about how often do they use marijuana, cocaine, steroids, amphetamines, heroin, barbiturates, inhalants, or prescriptional drugs without doctor´s orders. The frequency of the most often used drug ranging from 1 (*never tried*) to 8 (*I use it every day*) was used to form the total illicit drug use score. The data about drug use at age 18 were used in **Article V.**

3.4. Blood sampling

In **Articles I**, **II**, **IV,** and **V,** blood samples were collected at 08:30 hr, after 12 hour fast. Blood (4.5 ml) was obtained by antecubital venipuncture into Vacutainer® tubes containing 0.054 ml ethylenediaminetraacetic acid (EDTA) for prevention of clotting. The samples were immediately centrifugated for 10 min, with 800 rpm, at room temperature obtaining platelet rich plasma. The platelet concentration was estimated electronically with Sysmex SE-9000 in the certified clinical laboratory of Tartu University Clinics. One ml of platelet-rich plasma was stored at -80˚C until the measurement of MAO activity.

In **Article III**, the experiment started between 09:00 and 09:15 hr. An indwelling venous catheter was inserted into the antecubital vein and the first blood sample was drawn into Vacutainer® tubes containing EDTA 10 minutes

before the exercise started. Venous blood was sampled again immediately after exercise, and during the recovery phase three additional times with 30 min intervals. Blood draws were standardized for each subject. Blood samples were coded and plasma was separated by centrifugation. Plasma samples were stored at –80˚C until analyzed.

3.5. Measurement of platelet monoamine oxidase activity

In **Articles I**, **II**, **IV**, and **V**, activities of platelet MAO were analyzed by a radiometric assay with ^{14}C-labeled 2-phenylethylamine (ß-PEA) ("Amersham") as substrate as described by Hallman and colleagues (1987). After melting the platelet-rich plasma on ice in the laboratory, platelets were sonificated with Bandelin Sonoplus Ultrasonic Homogenizer HD2070 at 4x10 seconds with intervals for 5 sec at 4˚C. Then, 50 µl of 0.1 mM (5 nmol) [^{14}C] ß-PEA (0.5 µCi/ml) was mixed with 50 µl of sonificated plasma, following 4 min incubation in 37˚C water bath. After that, 30 µl of 1.0M HCl was added to stop the reaction and all tubes were put into bath for another 10 minutes. Thereafter, the radioactive aldehyde product formed was extracted under vigorous shaking for 30 seconds, into 750 µl toluene:ethylacetate (1:1). The samples were then centrifugated at room temperature for 5 minutes at 1000 rpm. 500 µl of the organic phase was pipetted into vials with 8 ml of scintillation liquid (Optiphase HiSafe, Wallace). For standard samples 50 µl of 0.1 mM [^{14}C] ß-PEA was added to 8 ml of scintillation solution. All samples were analyzed in duplicate and blindly, and corrected using a reference sample. Radioactivity was measured in a ß-counter (Wallac 1409). MAO activity was calculated as [the amount of the substrate (nmol) x ß-count of the sample (cpm) x 1.5] / [ß- count of the standard (cpm) x incubation time (min) x the count of platelets in 50 µl of platelet-rich plasma (10^{10} of platelets)], and is expressed as nmol of substrate oxidised per 10^{10} platelets per min (nmol x min^{-1} x 10^{10}platelets $^{-1}$).

3.6. Physical exercise testing

In **Article III**, the subjects underwent a bicycle cardiopulmonary exercise testing using stepwise increasing workload by 25W per two minutes (Ergometry System, Siemens). Expired gases, ventilation and heart rate analyses were computed simultaneously and displayed with "Oxycon Record" (Erich Jaeger). Ventilatory anaerobic threshold (AnT) was determined following the Wassermann et al. (1999) basic criteria. Peak oxygen consumption and working capacity was registered.

3.7. Biochemical measures

GH, cortisol and prolactin were measured by chemiluminescence immunoassay using commercially available kits (Immulite) and conventional methods (Babson et al., 1991) by a technician blind to sample coding. Detection limit for GH, cortisol and prolactin was 0.05 ng/ml.

Fasting basal cholesterol (total and HDL) was measured at the Laboratories Department of the Tartu University Clinics by conventional techniques.

3.8. Statistical methods

Statistical analysis in **Articles I, II** and in **Articles III, V** was carried out with StatView 4.5 and with 5.0 packages for Macintosh, respectively. In **Article IV**, software package R 2.0.0 for Windows (http://www.r-project.org) was used.

In **Article I** differences between younger and older boys and girls and the low and high MAO groups in relation to behavioural ratings were tested by means of analysis of variance (ANOVA) and Mann-Whitney *U*-test. Spearman rank correlation was used to indicate the relationship between continuous variables.

In **Article II**, the prevalence of substance use and differences between sex and age groups were calculated with chi-square test with post hoc calculations. Differences between sex and age groups in alcohol consumption score, platelet MAO activity, behavioural ratings, and between substance use groups were calculated using ANOVA with Fisher's PSLD test. Relationships between the variables were studied with Spearman rank correlation. Multiple regression analysis was performed with different independent variables to find significant predictors for alcohol and illicit drug consumption.

In **Article III**, for hormone responses to maximal physical exercise, comparisons between the control group and patients' group were tested by ANOVA repeated measures and additional analyses were performed using ANCOVA with maximal heart rate and peak oxygen uptake as covariates. Within group comparisons were made by paired t-test and Mann-Whitney *U*-test. Spearman rank correlation was used to indicate the relationship between continuous variables.

In **Article IV** differences in platelet MAO activity and behavioural ratings at age 15 and 18 were studied by paired t-test. Pearson correlation was used to indicate the relationship between continuous variables. Comparisons between groups were made by linear regression, adjusting for gender.

In **Article V** differences in changes of the behavioural ratings between decreased, unchanged and increased MAO activity groups were tested by ANOVA with post hoc Fisher´s PLSD test. ANOVA was also used to study the differences in total and HDL cholesterol levels between MAO activity groups.

Differences in platelet MAO activity and behavioural ratings at age 15 and 18 were studied by paired t-test. Pearson correlation was used to test the potential associations between behavioural measures, plasma cholesterol, MAO activity, and mood. Regression analysis was used to model the relationship between behavioural measures and physiological variables.

4. RESULTS AND DISCUSSION

4.1. Effect of gender, age, and smoking on platelet monoamine oxidase activity (Articles I, II, IV, and V)

Gender related differences in platelet MAO activity with females exhibiting higher enzyme activity have repeatedly been found in adults (Bagdy and Rihmer, 1986; Snell et al., 2002; Coccini et al., 2005). Some studies have reported similar differences in children (Murphy et al., 1976, Young et al., 1980), while others have not found any difference between boys and girls (Shekim et al., 1989). In our study it was confirmed in a large population based randomly selected sample of children and adolescents that boys have, on average, significantly lower MAO activity when compared to girls. Boys had significantly lower MAO activity than girls in both age groups, at age 9- and 15-years (**Articles I** and **II**). In the follow-up studies performed with the same 15-years old subjects three years later at age 18, boys continuously had lower enzyme activity when compared to girls (**Article IV, Table 1**).

Positive correlation between age and MAO activity has been found by several authors (Robinson, 1975; Berlin et al., 2000). We also found that 9-years old children had lower MAO activity when compared with 15-years old adolescents (**Article I**). However, this difference was significant only in girls if the data of smokers and non-smokers was analyzed together. Difference in platelet MAO activity between younger and older boys became significant when the smokers were excluded from the analysis. The data suggested that there is a slight increase in platelet MAO between ages 9 to 15. A small but significant increase in platelet MAO activity level was also observed from age 15 to 18 (**Article IV**). This could be, in part, attributed to the developmental changes, as there is a possibility that MAO activity levels have not yet reached their adult levels. The correlation between two MAO measurements was not very high (0.55–0.56) (**Articles IV** and **V**). There is a possibility that MAO levels had not reached the stability at age 15, which may cause this moderate correlation between the two platelet MAO activity measurements. The longitudinal data of our studies show that in most of the tested individuals, platelet MAO activity is a relatively stable measure, however, there was a significant number of subjects with large intra-individual variability of MAO activity over three years (**Article V, Figure 1**). It is not known whether this reflects the changes in central 5-HT-ergic activity but this possibility cannot be excluded. Smoking has been found to reduce platelet MAO activity in adults in a significant and dose related manner but the effect appears to be significant in subjects smoking more than 10 cigarettes a day (Whitfield et al., 2000; Eensoo et al., 2004). In the large sample of adolescents of the present study, this effect was present both in boys and girls. It is also possible that in adolescents MAO activity is more sensitive

to less extensive smoking than in adults. Platelet MAO levels were negatively associated with cigarette use: 15-years old smokers had a significantly lower MAO activity than adolescents who had not tried smoking or tried, but not smoking currently (**Article I**) and this difference was also present at age 18 (**Article IV**). The difference remained significant after adjusting for gender. A significant negative correlation was found between the changes in platelet MAO activity and frequency of smoking over the period of three years, reflecting the reduction in platelet MAO activity by more frequent smoking (**Article V**).

4.2. Effect of gender, age, and smoking on behavioural characteristics (Articles I, II, and IV)

The well-known gender difference with boys displaying more overactivity and attention problems was observed: as expected, boys were given higher scores in Aggressiveness, Motor Restlessness and Concentration Difficulties scales than girls at age 9- and 15-years (**Article I, Table 1**; **Article II, Table 2**). The gender differences remained also at age 18 (**Article IV, Table 1**). Some authors have defined aggression so as to include both direct (most common in boys) and indirect (most common in girls) forms of aggression (Pulkkinen and Pitkänen, 1993). Boys might be given a higher score because they cause more disruptions in class, which gives us a reason to believe that teachers notice more direct than indirect aggression. Also, boys were rated to have significantly more hyper-active/impulsive and inattentive symptoms than girls at age 18 by teachers using the SNAP-IV scale (**Article IV, Table 1**). 15-years old adolescents were given higher scores in Aggressiveness but not Motor Restlessness and Concentration Difficulties than 9-years old children (**Article I, Table 1**). In the continuous longitudinal study, the scores of Aggressiveness and Hyperactivity (the sum of Motor Restlessness and Concentration Difficulties) were significantly lower when measured at age 18 compared to age 15. However, the decrease in the score of Concentration Difficulties was statistically not significant (**Article IV**). These results are in concordance with earlier studies, which have shown that symptoms of hyperactivity and impulsivity tend to decline more with age than symptoms of inattention (Biederman et al., 2000). Among 15- and 18-years old adolescents, smokers were rated more aggressive and to have more concent-ration difficulties than nonsmokers of the respective age, after adjusting for gender (**Article IV, Figure 1**). Adolescents with inattention problems may be more likely to experiment with tobacco smoking and to become regular users, this may be attributed, in part, to nicotine's beneficial effects on cognitive function (Tercyak et al., 2002; Potter and Newhouse, 2004). Smokers were given higher scores in Motor Restlessness scale at age 15 but not at age 18 (**Article IV**). According to the regression analysis (**Article IV**) male sex and smoking at age 15 were associated with aggressive and hyperactive behaviour at

the same age. When rated at age 18 by teachers using the SNAP-IV scale, smokers were rated to have more inattentive but not hyperactive/impulsive symptoms and had also higher overall score on SNAP-IV scale than adolescents who were not smoking currently (**Article IV, Table 2**). There is also a possibility that adolescents who score higher in these behavioural traits are more likely to become smokers, tend to start smoking earlier in life, or smoke more frequently.

4.3. Platelet monoamine oxidase activity, smoking, aggressive and hyperactive behaviour (Articles I, IV and V)

Significant negative correlations between 15-years old adolescents' platelet MAO activity and the scores of Aggressiveness, Motor Restlessness, Concentration Difficulties and Hyperactivity were found (**Article I, Table 2**). Adolescents, but not 9-years old children with lower platelet MAO activity scored significantly higher in all scales except Aggressiveness (**Article I, Figure 1**). When adolescent boys and girls with high or low platelet MAO activity were studied separately, Motor Restlessness was found to be significantly higher in both boys and girls with low platelet MAO activity. Hyperactivity score was also higher in the low platelet MAO group, but only in boys. However, if the smokers were excluded from these analyses, all differences appeared non-significant (**Article I, Table 2**). As the exclusion of smokers from the sample rendered the association between platelet MAO activity and aggressive-hyperactive behaviour non-significant, it could be argued that the previously described association between these measures has been an artefact due to the smoking effect. However, exclusion of those subjects who smoke may also mask the true association between behaviour and platelet MAO, because there are differences in personality traits between smokers and nonsmokers (Patton et al., 1993; Canals et al., 1997; Vollrath and Torgersen, 2002). Platelet MAO activity measured at age 15 was not independently associated with aggressive and hyperactive behaviour. As the smoking habit is closely related to these behavioural traits, is more common in boys, and, as very recently demonstrated, is also non-linearly related to platelet MAO activity (Harro et al., 2004), it is problematic to differentiate cross-sectionally the impact of smoking or MAO activity on these behavioural characteristics. Longitudinal analysis revealed that low platelet MAO activity and smoking at age 15 are independently associated with high scores of Motor Restlessness and Inattention at age 18 (**Article IV, Table 3**). Low platelet MAO activity measured at age 18 and male sex were associated with high score on Motor Restlessness scale, however, adding smoking status to the analysis rendered the association non-significant. This can be, similarly to the cross-sectional analysis

of data obtained at age 15, related to the complex associations between behaviour, smoking effect, and the enzyme activity. There is a possibility that adolescents who score higher in Motor Restlessness and Inattention scales, started to smoke earlier in life, however, we do not know whether this is due to low platelet MAO activity. Since cigarette use is more prevalent in older adolescents, and more adolescents start to smoke with an increasing age, MAO activity measured at age 18 may not reflect well the predisposition to behavioural disturbances.

Changes in platelet MAO activity over the period of three years were not significantly related to the reduction in aggressiveness and motor restlessness. An increase in platelet MAO activity was associated with a decrease of concentration difficulties. However, when boys and girls were analyzed separately, the significant negative correlation between changes in concentration difficulties and platelet MAO activity was found only in the sample of girls but not in boys. Subjects with decreased or unchanged platelet MAO activity did not have such a reduction (**Article V**).

4.4. Association of aggressive and hyperactive behaviour and platelet monoamine oxidase activity with alcohol and illicit drug use (Articles II and V)

Several studies have suggested that children having high levels of hyperactive, impulsive, and inattentive behaviour are at greater risk for substance experimentation, use, and abuse (Lambert and Hartsough, 1998; Kuperman et al., 2001). We found a significant positive correlation between Aggressiveness, Hyperactivity and alcohol drinking in 15-years old adolescents, however, the correlation was weak (**Article II, Table 3**). These associations were not seen in 9-years old children but it should also be noted that the number of alcohol users among 9-years old children was small. Also, a weak but significant correlation was found between illicit drug use and hyperactivity among adolescents (**Article II, Table 3**). Adolescents, who reported frequent alcohol use, showed the highest scores in Aggressiveness and Hyperactivity and those who had tried illicit drugs were given higher scores in Hyperactivity. However, the number of adolescents, who had tried drugs, was also too small to make any conclusions. The regression analysis confirmed that in adolescents, higher aggressiveness and higher hyperactivity, together with older age, are significant predictors for the alcohol consumption (**Article II, Table 4**). Adding platelet MAO activity to the regression models did not change the significance of other parameters in the models. We did not find any differences in platelet MAO activity between consumers and non-consumers of alcohol or illicit drugs in 9- or 15-years old children. Also, the change in platelet MAO activity was not associated with change in frequency of alcohol and drug use among the subjects from age 15 to

18 (**Article V**). Thus, it seems, that the link between platelet MAO activity and alcohol abuse repeatedly observed in adults (von Knorring and Oreland, 1996) may rather be based on association of MAO activity with vulnerability to abuse or antisocial behaviour than with early experimentation with drugs. It is also possible that environmental factors are more important in determining experimentation with substances than biological pre-dispositions. However, platelet MAO activity is associated with one ore more specific behavioural characteristics, which may interact with other variables (biological or environmental) to produce alcohol and drug use.

4.5. Platelet monoamine oxidase activity, plasma total and HDL cholesterol levels, and behavioural characteristics (Article V)

The change in platelet MAO activity was associated with changes in total and HDL cholesterol levels with higher cholesterol increase in subjects with decreased platelet MAO activity (**Article V, Figure 2 a, b**). Excluding the regular smokers from the analysis did not change the outcome. Analyzing boys and girls separately, the associations turned non-significant, probably due to reduced statistical power, as the trends in both sexes were similar. Lower platelet MAO activity and cholesterol levels have both been associated with lower central 5-HT-ergic activity, but there is also evidence of possible negative relationship between plasma cholesterol levels and central 5-HT function (Papakostas et al., 2004). These results provide additional support to the notion that cholesterol levels and 5-HT-ergic activity may be related in a non-linear manner. It has been suggested that reduced cholesterol may alter membrane fluidity, viscosity and function, including the function of 5-HT receptors and 5-HT transporter (Engelberg, 1992; Salter, 1992), and therefore would result in a reduced 5-HT-ergic function. Diebold and colleagues (1998) proposed that a decrease in plasma total cholesterol would induce a relative increase in brain cell membrane fluidity, with increased presynaptic 5-HT reuptake and decreased postsynaptic 5-HT function. However, recent findings indicate that also high cholesterol levels may lead to lower 5-HT receptor sensitivity or 5-HT transporter activity (Papakostas et al., 2004).

In a multiple regression model, increases in platelet MAO activity and HDL cholesterol levels were independently associated with the decrease in concentration difficulties when smoking and gender were taken into account. Regression models for changes in the scores of Aggressiveness, Motor Restlessness and Hyperactivity were statistically non-significant. The possible effect of mood at age 18 on the associations between concentration difficulties, platelet MAO activity and cholesterol levels was examined by using the MÅDRS-S scores. Cholesterol levels, platelet MAO activity and concentration difficulties at this

age and changes in three years in these measures were not associated with the MÅDRS-S score. Negative correlation between changes in total cholesterol level and motor restlessness was found in boys. In girls, negative correlation between changes in HDL cholesterol, aggressiveness, motor restlessness, and hyperactivity was found. (**Article V**). Some authors have also found that the lipid fraction associated with neuroendocrine indices of reduced 5-HT function was low HDL cholesterol, not total cholesterol (Buydens-Branchey et al., 2000). Whether it is total cholesterol, or one of its subfractions that plays the important role in psychological and behavioural problems is a question that remains to be answered. However, since plasma total and HDL cholesterol levels had significantly increased whereas aggressive and hyperactive behaviour had decreased from age 15 to 18 among the subjects, these correlations may also reflect developmentally occurring biochemical and behavioural changes during adolescence and not indicate a causal relationship between behaviour and cholesterol levels. Plasma cholesterol level may be a peripheral biochemical result of diet and stress involving behaviour. Also, the change in cholesterol level as well as behavioural changes, could be related to both age and maturation and likely reflect the influence of sex hormones on plasma lipoprotein metabolism (Berenson et al., 1981). We failed to find any association between cholesterol levels and more complex behaviours such as alcohol and illicit drug use.

4.6. Growth hormone, cortisol, and prolactin responses to exercise in depressed patients and healthy controls (Article III)

In this study, no differences in baseline GH, prolactin or cortisol levels between patients and healthy volunteers were found. This result is in concordance with the results of many earlier studies, which have found that baseline GH levels are similar in depressed patients compared to controls (Fiasche et al., 1995). Also, several authors have reported no differences in baseline prolactin levels between depressed patients and controls (Riedel et al., 2002; Sagud et al., 2002). No group differences in baseline cortisol levels were expected, since previous research indicates that HPA system is relatively active in the morning hours and cortisol levels in normal adults were likely to be maximal at the time of the current experiment (Kanaley et al., 2001).

Acute physical exercise has been shown to stimulate the HPA axis (Luger et al., 1988; Leal-Cerro et al., 2003). Plasma GH was significantly elevated after physical exercise in controls as well as in patients. Following the increase in plasma GH secretion associated with aerobic exercise, GH release decreased to baseline levels (**Article III, Figure 1**). The result was somewhat different that we had expected: in a previous study (Harro et al., 1999) a lower response of

GH secretion to physical challenge in volunteers with psychometrically measured depressiveness was found. Therefore we had a reason to think that depressed patients would have a significantly lower GH response to exercise than healthy volunteers. However, there were no differences between healthy volunteers and depressives' mean GH secretion increase in response to physical exercise. This discrepancy may be, in part, explained by the antidepressant treatment of depressed patients, which may influence neuroendocrine functioning via modulation of several neurotransmitter systems. Also it should be noted that the GH increase was much smaller than in the study of Harro et al (1999), maybe partly because of older age of the participants in the present study. It has been found that GH response to exercise is lower in older men (Zaccaria et al., 1999) and the magnitude of GH release is reduced by four- to seven-fold in older individuals compared to younger ones (Wideman et al., 2002).

The activation of the HPA axis resulting in rapid increases in plasma cortisol (**Article III, Figure 2**) in response to acute exercise was also expected. However, there were no differences between the two groups' mean cortisol secretion increase in response to physical exercise. There are also other studies, which have found similar cortisol output after physical challenge in depressed patients and healthy controls (Gispen-de Wied et al., 2000).

In contrast to GH and cortisol, prolactin levels were elevated by exercise only in the patients' group (**Article III, Figure 3**). Since multiple neural pathways, which influence prolactin secretion converge on the hypothalamus from other parts of the brain, the effect of exercise on the secretion of prolactin may also reflect the action of different neural inputs on the activity of the hypothalamic-pituitary axis. Prolactin release in response to stress is mediated via 5-HT release in the hypothalamic paraventricular nucleus (Minamitani et al., 1987), and one can speculate that altered regulation of the 5-HT-ergic system may have a role in response to exercise stress. A study of Porter and colleagues, investigating 5-HT_{1A} receptor function in depression, has reported an enhanced prolactin response to L-tryptophan in depressed patients compared with a matched control group (Porter et al., 2003). Similarly to our results, there was also no difference between patients and control subjects in cortisol levels in this study. As the 5-HT_{1A} receptor function and prolactin release at the pituitary level may be reduced by cortisol, the enhanced prolactin response to L-tryptophan in depressed patients may be explained by the lack of hypercortisolaemia. However, it should be noted that the patients in our study were on various antidepressants and in different stages of treatment response. The higher prolactin response in the patients does not appear to be due to antidepressant treatment, however, there was no apparent distinction between subgroups of patients treated with different antidepressants or being drug-free. Given the small number of drug-free controls, the role of a pharmacodynamic effect is not excluded, but the similarity of data of patients treated with different drugs suggests that the effect would not be on a single molecular target.

There were no differences in baseline plasma hormone levels or in GH and cortisol response to physical exercise between low or high scorers on MÅDRS-S scale. Prolactin levels were increased during the exercise only in the group of high MÅDRS-S scorers. Thus, group comparison of the high and low scorers on MÅDRS-S revealed similar outcome as the comparison of the patients and controls.

5. CONCLUSIVE REMARKS

Platelet MAO activity was not independently associated with aggressive and hyperactive behaviour assessed at the same time in 9-, 15- and 18-years old children and adolescents. However, a longitudinal analysis revealed that low platelet MAO activity and smoking at age 15 are independently associated with hyperactive behaviour at age 18. This result supports the notion that platelet MAO activity is associated, independently of smoking, with specific aspects of hyperactivity and attention deficit in adolescents.

Platelet MAO activity was not associated with alcohol and drug use among the subjects. Thus, it seems, that the frequently reported link between platelet MAO activity and drug abuse may rather be based on association of MAO activity with vulnerability to abuse than with early experimentation with drugs.

The longitudinal data of our studies show that in most of the tested individuals, platelet MAO activity is a relatively stable measure, however, there was a significant number of subjects with large intra-individual variability of MAO activity over three years. It is not known whether this reflects the changes in central 5-HT-ergic activity, but this possibility cannot be excluded. The change in platelet MAO activity was associated with changes in plasma cholesterol levels with higher cholesterol increase in subjects with decreased platelet MAO activity. These results provide additional support to the notion that cholesterol levels and 5-HT-ergic activity may be related in a non-linear manner.

No differences in growth hormone or cortisol responses to physical exercise were found between healthy controls and depressives, however, prolactin levels were elevated only in the depressed patients group during the exercise. This result suggests that acute exercise may increase serotonin availability in depressed patients, which is reflected by increased plasma prolactin concentration.

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8. SUMMARY IN ESTONIAN

Kesknärvisüsteemi serotonergilise aktiivsuse ja käitumise spetsiifiliste korrelaatide uuringud

Kindlate käitumiseelistuste, iseloomuomaduste ja psüühiliste häirete neurobioloogiliste aluste otsinguil on palju tähelepanu pälvinud kesknärvisüsteemi serotonergiline (5-HT) närviülekanne. Iseärasusi 5-HT süsteemi aktiivsuses on muuhulgas täheldatud ka hüperaktiivse ja agressiivse käitumisega lastel ja noorukitel ning meeleoluhäiretega isikutel. Lisaks on leitud, et madala 5-HT-ergilise aktiivsusega inimestele omaseid käitumiseelistusi võib täheldada ka liigmadala või liigkõrge kolesteroolitasemega inimeste hulgas. Kuna kolesteroolil on oluline roll rakumembraanide struktuuri ja funktsiooni säilitamisel, siis võib liigselt madal või kõrge kolesteroolitase mõjustada ka 5-HT-ergilist närviülekannet. 5-HT-ergilise närviülekande aktiivsuse perifeerse korrelaadina on kasutatud vereliistakute monoamiinide oksüdaasi (MAO) aktiivsust, mida peetakse täiskasvanud inimeste hulgas stabiilseks näitajaks.

5-HT süsteem moduleerib hüpotaalamuse-ajuripatsi-neerupealiste telje tegevust läbi hormonaalsete ja otseste neuraalsete teede, mistõttu on 5-HT-ergilisel närviülekandel roll ka füüsilise stressreaktsiooni reguleerimises. Uuringud on näidanud, et füüsiline pingutus aktiveerib hüpotaalamuse-ajuripatsi-neerupealiste telje, mille tulemusena suureneb vereplasmas kortisooli, kasvuhormooni ja prolaktiini tase. Eelnevate uuringute põhjal on alust arvata, et depressiivsetel isikutel on selles süsteemis märkimisväärsed muutused.

Käesoleva töö eesmärgid on sõnastatud järgmiselt:

- uurida läbilõike- ja longituuduuringus vereliistakute MAO aktiivsuse seost agressiivse ja hüperaktiivse käitumisega ning alkoholi ja illegaalsete uimastite tarbimisega ulatuslikus tervete laste ja noorukite valimis arvestades suitsetamise võimalikku mõju MAO aktiivsusele (**Artiklid I**, **II**, **IV** ja **V**)
- uurida longituuduuringus muutusi vereliistakute MAO aktiivsuses ja vereplasma kolesteroolisisalduses ning nende muutuste võimalikku seost muutustega agressiivses ja hüperaktiivses käitumises, suitsetamises ning alkoholi ja illegaalsete uimastite tarbimises tervete noorukite valimis (**Artikkel V**)
- uurida, kas vereliistakute MAO aktiivsus on ajas püsiv marker ning kui suur on MAO aktiivsuse arenguline varieeruvus tervete noorukite valimis (**Artiklid IV** ja **V**)
- uurida depressiooni 5-HT-ergilist komponenti läbi hormonaalsete vastuste maksimaalsele füüsilisele pingutusele (**Artikkel III**)

Läbilõikeuuring 9- ja 15- aastaste laste hulgas leidis olulise negatiivse seose 15-aastaste noorukite agressiivsuse, motoorse rahutuse, keskendumisraskuste,

hüperaktiivsuse ja vereliistakute MAO aktiivsuse vahel. Madala MAO aktiivsusega noorukid said teistest madalama skoori kõikidel alaskaaladel, va. agressiivsuse alaskaala. Seos kadus, kui suitsetajad analüüsist välja jäeti. Noorema vanusegrupi puhul olulist seost käitumise ja vereliistakute MAO aktiivsuse vahel ei ilmnenud. 15- aastaste suitsetajate MAO aktiivsus oli oluliselt madalam mittesuitsetajate MAO aktiivsusest ning suitsetajad said võrreldes mittesuitsetajatega kõrgemaid skoore agressiivsuse ja hüperaktiivsuse skaalal (**Artikkel I**). Kuna sigaretisuitsul on leitud olevat oluline MAO aktiivsust pärssiv toime ning suitsetajatel esineb nimetatud käitumisviise sagedamini kui mittesuitsetajail, siis on läbilõikeuuringus raske hinnata MAO aktiivsuse mõju käitumisele. Jätkuuuring kolme aasta pärast samas valimis näitas, et vereliistakute MAO aktiivsus 15-aastaselt, sõltumata soost ja suitsetamisest, on seotud hüperaktiivse käitumise ja keskendumisraskustega 18-aastaselt (**Artikkel IV**). Meessugu ja 18-aastaselt mõõdetud madal vereliistakute MAO aktiivsus olid seotud samal ajal hinnatud kõrge motoorse rahutusega, kuid suitsetamise näitaja kaasamine analüüsi muutis seose ebaoluliseks (**Artikkel IV**).

Nõrk seos ilmnes agressiivse ja hüperaktiivse käitumise ning alkoholitarbimise vahel ning hüperaktiivse käitumise ja illegaalsete uimastite tarbimise vahel 15-aastastel noorukitel. Uuringus ei õnnestunud näidata vereliistakute MAO aktiivsuse seost alkoholi ja illegaalsete uimastite tarbimisega 9- ja 15-aastastel lastel (**Artikkel II**). Sarnaselt ei seostunud vereliistakute MAO aktiivsuse muutus muutusega alkoholi ja illegaalsete uimastite tarbimise sageduses kolm aastat hiljem läbi viidud jätku-uuringus (**Artikkel V**). Vereliistakute MAO aktiivsus võib seega olla pigem haavatavuse markeriks uimastite kuritarvitamise suhtes, kui et ennustada uimastitega eksperimenteerimist varases eas.

Longituuduuringus leiti mõõdukas korrelatsioon (0.55–0.56) kahe vereliistakute MAO aktiivsuse mõõtmise vahel kolmeaastase vahega (**Artikkel IV** ja **V**). Selline mitte väga tugev korrelatsioon võib tuleneda sellest, et MAO aktiivsus ei olnud 15-aastaste noorte seas veel jõudnud täiskasvanu tasemele. Siiski – enamusel noortest oli MAO aktiivsus kolme aasta jooksul üsna stabiilne, ent leidus märkimisväärne hulk neid, kelle vereliistakute MAO aktiivsus varieerus enam, kui seda lubaks mõõtmisviga (**Artikkel V**). Pole teada, kas see peegeldab muutusi kesknärvisüsteemi 5-HT-ergilises aktiivsuses, kuid seda võimalust ei saa välistada. Muutused vereliistakute MAO aktiivsuses olid seotud muutustega kolesterooli tasemetes – kolesteroolitase oli enam tõusnud nende noorte hulgas, kelle MAO aktiivsus oli kolme aasta jooksul oluliselt vähenenud (**Artikkel V**). See tulemus viitab võimalusele, et kolesterooli tase ja 5-HT süsteemi aktiivsus on omavahel mittelineaarselt seotud. Vereliistakute MAO aktiivsuse ja kolesterooli tasemete tõus oli seotud keskendumisraskuste vähenemisega, kuid mitte muutusega alkoholi või illegaalsete uimastite tarbimises (**Artikkel V**).

Uurides terveid vabatahtlikke ja depressiooniga patsiente ei leitud kasvuhormooni, kortisooli ega prolaktiini baastasemetes erinevusi gruppide vahel.

Maksimaalne füüsiline pingutus aktiveeris hüpotaalamuse-ajuripatsi-neerupealiste telje, suurendades vereplasma kasvuhormooni ja kortisooli sisaldust nii patsientidel kui kontrollisikutel. Prolaktiini tase tõusis vaid patsientide grupis, mis viitab suuremale 5-HT vabastamisele patsientide hulgas (**Artikkel III**). Seda efekti ei saa omistada antidepressantravile, sest uurides eri tüüpi ravimeid või ravimeid üldse mitte saanud patsientide gruppe ei ilmnenud nende vahel erinevusi prolaktiini vabanemises vastusena maksimaalsele füüsilisele pingutusele.

9. PUBLICATIONS

Kiive, E., Eensoo, D., Harro, M., & Harro, J. (2002). Platelet monoamine oxidase activity in association with childhood aggressive and hyperactive behaviour: the effect of smoking? *Personality and Individual Differences 33*, 355–363.

Merenäkk, L., Harro, M., **Kiive, E**., Laidra, K., Eensoo, D., Allik, J., Oreland, L., & Harro, J. (2003). Association between substance use, personality traits, and platelet MAO activity in preadolescents and adolescents. *Addictive Behaviors 28*, 1507–1514.

Kiive, E., Maaroos, J., Shlik, J., Tõru, I., & Harro, J. (2004). Growth hormone, cortisol and prolactin responses to physical exercise: the higher prolactin response in depressed patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry 28*, 1007–1013.

Kiive, E., Fischer, K., Harro, M., & Harro, J. Platelet monoamine oxidase activity in association with adolescent inattentive and hyperactive behaviour: data from a prospective longitudinal study. (Submitted to *European Neuropsychopharmacology*)

Kiive, E., Merenäkk, L., Harro, M., & Harro, J. (in press) Changes in platelet monoamine oxidase activity, cholesterol levels and hyperactive behaviour in adolescents over the period of three years. *Neuroscience Letters*

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PLATELET MONOAMINE OXIDASE ACTIVITY IN ASSOCIATION WITH ADOLESCENT INATTENTIVE AND HYPERACTIVE BEHAVIOUR: DATA FROM A PROSPECTIVE LONGITUDINAL STUDY

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ABSTRACT

Platelet monoamine oxidase (MAO) activity is a peripheral marker of central serotonergic activity, and has been associated with psychiatric vulnerability. This investigation in adolescents studied the relationship between aggressive and hyperactive behaviour and platelet MAO activity. The measures were taken at two points of time with an interval of three years. The psychological data were obtained from teachers by using the Hyperactivity Scale (af Klinteberg, 1988) and, at age 18, the teacher-report version of the SNAP-IV. Female subjects had significantly higher MAO activity levels than males, whereas regular smokers had significantly lower MAO activity levels than nonsmokers. Low levels of platelet MAO and smoking at age 15 were independently associated with Motor Restlessness and Inattention symptoms at age 18. These longitudinal data suggest that specific aspects of hyperactivity and attention deficit are associated with platelet MAO activity.

Keywords: Platelet monoamine oxidase; Hyperactivity; Inattention; Smoking; Adolescents

1. INTRODUCTION

Experimental and clinical findings suggest that platelet monoamine oxidase (MAO) isoenzyme B activity is a marker for the capacity of the serotonergic activity in the CNS (Oreland and Hallman, 1995). Studies associating platelet MAO activity with human behaviour have a long history since Cookson and colleagues (1975) reported a relationship between low levels of MAO activity and the schizophreniform psychosis. Soon afterwards, low platelet MAO activity was associated with overall heightened vulnerability for several psychiatric disorders (Buchsbaum et al., 1976); according to this hypothesis, people with low platelet MAO activity have certain personality traits, which are predisposing to psychiatric disorders. Low platelet MAO activity has been shown to correlate with personality traits such as monotony avoidance, sensation seeking and impulsiveness (von Knorring et al., 1987; af Klinteberg et al., 1990; Stalenheim et al., 1997) and extraversion (Gattaz and Beckmann, 1981; af Klinteberg et al., 1987). Low platelet MAO levels are also found in forms of psychopathology characterized by impulsive tendencies to seek immediate rewards without regard for consequences (Stalenheim, 2004). Children with attention-deficit/hyperactivity disorder (Shekim et al., 1986; Cederblad et al., 1992) have been reported to have significantly lower platelet MAO activity than controls. All these associations have recently been questioned on the basis of a direct inhibitory effect of cigarette smoke on MAO activity. Several studies have reported that both male and female cigarette smokers have lower platelet MAO activity than nonsmokers (Coursey et al., 1979; Berlin and Anthenelli, 2001); also, low brain MAO A and B in smokers compared to former smokers and nonsmokers have been demonstrated by using positron emission tomography (PET) (Fowler et al., 1996a,b). Studies reporting normal platelet MAO levels in former smokers (Oreland et al., 1981; Norman et al., 1987; Rose et al., 2001; Gilbert et al., 2003) provide additional evidence that low MAO activity may be a pharmacological effect of the smoke rather than a biological characteristic of smokers. A study of Whitfield et al. (2000) carried out on a large sample of subjects has found that current smoking reduced platelet MAO activity in a significant and dose-related manner, with no evidence of lower MAO in ex-smokers or in nonsmoking subjects. This study found no association between alcoholism and platelet MAO activity when smoking was taken into account. However, we have recently demonstrated that platelet MAO activity is lower in non-smoking subjects with alcohol-related socially deviant behaviour, and thus association of low platelet MAO and problem drinking is not an artifact of smoking (Eensoo et al., 2004). In non-human primates, low platelet MAO activity is also related to higher intake of alcohol and social incompetence (Fahlke et al., 2002).

While there have been numerous studies of association of platelet MAO, personality and psychopathology in adults, children have remained much less studied. The study of af Klinteberg and Oreland (1995) in healthy children

found that more aggressive and hyperactive adolescents had lower platelet MAO activity as measured in adulthood, but the possible direct inhibitory effect of smoking on platelet MAO activity was not considered. In our previous study aimed at reproducing these findings in a larger sample and full cross-sectional design (Kiive et al., 2002), we found significant associations between platelet MAO activity and hyperactive behaviour in 15 years but not 9 years old children. However, excluding smokers from the analysis, the associations turned out non-significant. This could be taken as evidence that association between hyperactivity and platelet MAO activity is an artifact; nevertheless, it is also possible that subjects with lower platelet MAO are also more likely to become smokers, what would distort any cross-sectional correction for smoking. Therefore it was concluded that the causal relationship between behavioural measures and platelet MAO activity could only be revealed with longitudinal studies, considering smoking as a confounding factor. Indeed, in the first longitudinal study on platelet MAO activity and smoking we have found that not only does smoking reduce platelet MAO activity but low (and also high) platelet MAO activity leads to higher probability of regular smoking three years later (Harro et al., 2004). This suggests that smoking is associated with low platelet MAO activity not only because of the direct inhibitory effect of tobacco constituents on the enzyme, but also because subjects with both low and high platelet MAO activity are more likely to become smokers.

The purpose of the present study was to investigate the relationship between platelet MAO activity and aggressiveness, hyperactivity and inattention controlled for the confounding factor of cigarette smoking using a prospective longitudinal design.

2. METHODS

2.1. Subjects

The details of original sample formation (Harro et al., 2001) and the follow-up study (Harro et al., 2004) have been described previously. In the present analysis data of 365 adolescents (149 boys, 216 girls) from the Tartu city and county, Estonia, were used. The data were collected in 1998/1999 when subjects were in average 15.4 (SD=0.5) years old and three years later, in 2001/2002 when the subjects were 18.2 (SD=0.5) years old. The present analysis included all adolescents who had platelet MAO activity measured at age 15. Parents and children gave their written informed consent before participating in the study, which was conducted according to the protocol approved by Ethics Review Committee on Human Research of the University of Tartu.

2.2. Psychological measures

Ratings on aggressiveness, motor restlessness and concentration difficulties both at age 15 and 18 were obtained from the class teachers, using the seven point Hyperactivity Scale described by af Klinteberg (1988). Hyperactivity score was calculated as in af Klinteberg and Oreland (1995) by summing the scores of two of the three subscales, Motor Restlessness and Concentration Difficulties subscales. Data of Aggressiveness, Motor Restlessness and Concentration Difficulties obtained at age 15 and 18 were available about 358 and 242 adolescents, respectively.

At age 18, the teacher-report version of the SNAP-IV (Swanson, 1992) was also used to assess Attention Deficit-Hyperactivity Disorder (ADHD) symptoms among the adolescents. Each of the 18 items of the SNAP-IV provides a word-to-word description of a symptom of DSM-IV ADHD (APA, 1994), and asks the rater to indicate whether the child exhibits the symptom "not at all, "just a little", "pretty much" or "very much". The scores of SNAP-IV divide into inattention and hyperactivity/impulsivity subscores which are used in further analysis. Data of SNAP-IV were available for 232 adolescents.

2.3. Measurement of platelet MAO activity

MAO activity in platelets was measured twice with three years interval, first when the subjects were 15 years old and the second time when the subjects were 18 years old. Blood samples were collected at 08.30, before breakfast after 12 hours fast. Blood (4.5 ml) was obtained by antecubital venipuncture into Vacutainer® tubes containing 0.054 ml K_3 EDTA as an anticoagulant. The preparation of platelet rich plasma and platelet MAO activity measurement procedure has been described (Harro et al., 2001). The substrate used was ^{14}C-labelled β–phenyl-ethylamine ("Amersham", U.K.). All experiments were performed in duplicate and the enzyme activity is expressed as nanomoles of substrate oxidized per 10^{10} platelets per minute. Data about platelet MAO activity measured at age 18 were available for 319 adolescents.

2.4. Smoking

Adolescents reported their smoking habits in an anonymous questionnaire in a laboratory out of school with no teachers and parents present. Four levels of smoking were defined: (1) never tried smoking, (2) have tried, but not smoking currently, (3) irregular smoking (up to twice a week) and (4) regular smoking (smoking every day or almost every day). For data analysis, subjects who reported that they smoke either irregularly or regularly were considered as smokers.

Data about smoking at age 15 were available for 363 adolescents and at age 18 for 334 adolescents.

2.5. Data analysis

Software package R 2.0.0 for Windows (http://www.r-project.org) was used for statistical analysis. Differences in platelet MAO activity and behavioural ratings at age 15 and 18 were studied by paired t-test. Pearson correlation was used to indicate the relationship between continuous variables. Comparisons between groups were made by linear regression, adjusting for gender. Variables that had a skewed distribution were logarithmically transformed before using them in the regression analysis. In the statistical analysis, the conventional 5% level was used to assess the significance.

3. RESULTS

3.1. Platelet MAO activity, age, gender, and smoking

Mean platelet MAO activity (±SD) measured at age 15 and 18 was 9.87±3.16 and 10.39±3.20 nmol X 10^{10} platelets^{-1} X min^{-1}, respectively. The correlation between two MAO measurements with three years interval was 0.55. Mean platelet MAO activities (±SD) in boys and girls measured at age 15 and 18 are presented in Table 1. As expected, boys had a significantly lower platelet MAO activity compared to girls measured both at age 15 and 18. Overall, there has been a significant increase in platelet MAO activity level from age 15 to 18 ($t= -2.82$, $df= 318$, $p< 0.005$).

Platelet MAO levels were negatively associated with cigarette use: 15 years old smokers had a significantly lower MAO activity than adolescents who had not tried smoking or tried, but not smoking currently (10.13±3.03 and 9.07±3.43, $t= 2.79$, $df= 361$, $p< 0.01$) and this difference was also present at age 18 (10.71±3.15 and 9.44±3.19, $t= 3.12$, $df=315$, $p< 0.005$). The difference remained significant after adjusting for gender: $p< 0.01$ and $p< 0.05$ at age 15 and 18, respectively. The smoking statuses of the subjects are presented in Table 1.

3.2. Behavioural characteristics, age, gender, and smoking

Scores of Aggressiveness and Hyperactivity (the sum of Motor Restlessness and Concentration Difficulties), measured by using the Hyperactivity scale of af Klinteberg were significantly lower, when measured at age 18 compared to age

15. There was no difference in Concentration Difficulties measured at age 15 and 18. Boys were given higher scores in Aggressiveness, Motor Restlessness, Concentration Difficulties and Hyperactivity than girls both at age 15 and 18 (Table 1).

At age 15 smokers had significantly higher scores in Aggressiveness, Motor Restlessness, Concentration Difficulties and Hyperactivity than adolescents who had not tried smoking or had tried, but were not smoking currently ($t= –4.29$, $df= 354$; $t= –5.28$, $df= 354$; $t= –6.12$, $df= 354$; $t= –6.19$, $df= 354$, respectively, $p< 0.0001$ for all parameters, after adjusting for gender) (Figure 1). 18 years old smokers had significantly higher scores in Aggressiveness, Concentration Difficulties and Hyperactivity but not Motor Restlessness than adolescents who had not tried smoking or had tried, but were not smoking currently ($t= –2.41$, $df= 215$, $p< 0.05$; $t= –3.30$, $df= 215$, $p< 0.001$; $t= –2.85$, $df= 215$, $p< 0.005$; $t= –1.66$, $df= 215$, $p=0.08$), respectively (Figure 1).

SNAP-IV scale was used to rate hyperactive/impulsive and inattentive symptoms only when the subjects were 18 years old. The correlation between the SNAP-IV total score and the Hyperactivity score (the sum of Motor Restlessness and Concentration Difficulties, measured by using the Hyperactivity Scale of af Klinteberg) measured at age 18 was 0.77, $p< 0.0001$.

Boys were rated to have significantly more hyperactive/impulsive symptoms and inattentive symptoms than girls (Table 1). Smokers were rated to have more inattentive but not hyperactive/impulsive symptoms. Smokers had also higher overall score on SNAP-IV scale than adolescents who had not tried smoking or had tried, but were not smoking currently (Table 2).

3.3. Regression analysis of platelet MAO activity and behavioural characteristics

For regression analysis, subjects were divided into three groups based on platelet MAO activity at age 15 and 18, forming groups with low (<25th percentile), average (25–75th percentile) and high (>75th percentile) enzyme activity, expressed as nmol X 10^{10} platelets^{-1} X minute^{-1}. Other explanatory variables examined were sex and smoking status of the participants.

According to the multiple regression analysis, male sex and smoking at age 15 were associated with aggressive and hyperactive behaviour at the same age, however, platelet MAO activity measured at age 15 did not have any significant independent effect on adolescent behaviour (data not shown).

Linear regression model revealed the association between platelet MAO activity measured at age 15 and Motor Restlessness (but not Aggressiveness, Concentration Difficulties or Hyperactivity) measured at age 18. The regression model showed that 11% of variance in Motor Restlessness score at age 18 was

explained by gender, smoking status and platelet MAO activity levels of the subject at age 15 (Table 3).

The mean score of Motor Restlessness (Mean± SD), measured at age 18 in boys and girls with low, average, and high MAO activity at age 15 were 3.1±1.8; 2.6±1.7; 2.1±1.5 and 2.2±1.5, 1.8±1.2, 1.7±1.3, respectively. According to the linear regression model, male sex, low MAO activity and smoking at age 15 are associated with approximately 1.3-fold increase in Motor Restlessness score at age 18.

Regression model also revealed the association between platelet MAO activity measured at age 15 and Inattention at age 18 (Table 3). The mean score of Inattention, measured at 18 in boys and girls with low, average, and high MAO activity at age 15 were 8.4±5.7, 7.2±5.4, 6.8±5.6 and 8.1±6.4, 5.4±4.5, 6.1±5.1, respectively.

SNAP total score at age 18 was influenced by gender, MAO activity and smoking status of the participants at age 15 (Table 3), however, MAO activity in this model is borderline significant. The mean score of SNAP-IV, measured at 18 in boys and girls with low, average, and high MAO activity at age 15 were 12.6±8.7, 11.2±8.9, 9.6±10.1 and 10.2±8.3, 7.3±6.6, 7.0± 6.0, respectively. According to the linear regression model, both male sex and low MAO activity at age 15 are associated with 1.3-fold increase in SNAP total score at age 18, trying smoking and regular smoking at age 15 are associated with 1.6 and 2.0-fold increases in SNAP total score, respectively.

Platelet MAO activity measured at age 18 and smoking status at age 18 had poor predictive value for most behavioural ratings, as the corresponding regression coefficients were statistically non-significant. When smoking status was not considered in regression analysis, low MAO activity at age 18 and male sex were associated with high score on Motor Restlessness scale, however, adding MAO activity at age 15 to explanatory variables, the association between MAO activity measured at age 18 and Motor Restlessness disappeared leaving MAO activity at 15 as a significant predictor.

4. DISCUSSION

It has been claimed that the variation in platelet MAO activity of the same individual over time is rather small (Murphy et al., 1976; Bagdy and Rihmer, 1986), but in these studies time between two platelet MAO activity measurements has ranged from 8 to 10 weeks. Our data, however, show a significant but not very high correlation between two MAO activity measurements over an interval of three years. As most earlier research of platelet MAO activity has concentrated on adults, little is known of children and adolescents. There is a possibility that MAO activity levels had not reached the stability at age 15, which may cause the moderate correlation between the two MAO measurements.

With regards to gender, boys had a lower MAO activity than girls both at age 15 and 18. Gender-related differences in MAO activity are in agreement with previous studies (Murphy et al., 1976; Bagdy and Rihmer, 1986). It was also found that smokers had a significantly lower platelet MAO activity compared to nonsmokers which was also an expected result since smoking has been found to reduce MAO activity in a significant and dose-related manner (see Oreland et al., 2002 for review).

Scores of Aggressiveness and Motor Restlessness had declined with age, however, the score of Concentration Difficulties had not. These results are in concordance with earlier studies, which have shown that symptoms of hyperactivity and impulsivity tend to decline at a higher rate than symptoms of inattention (Biederman et al., 2000). Hyperactivity may be prominent during early school age, but is less prevalent in older subjects (Ross and Ross, 1982). However, a significant amount of subjects experience concentration difficulties during adulthood. It has been estimated that clinically referred inattention and hyperactivity (ADHD) persists in 60% to 70% of young adults having had the disorder in childhood (Barkley et al., 2002). The well-known gender difference with boys displaying more overactivity and attention problems was also observed: both at age 15 and 18, boys were rated to exhibit more aggressive and hyperactive behaviour measured by using the scale described by af Klinteberg (1988) than girls at the same age. Recent research has indicated that male and female adults with ADHD have a similar number of symptoms and a similar distribution of symptom clusters of inattention and hyperactivity/impulsivity, however, inattentive symptoms are more common in female subjects for both pediatric and adult samples (Biederman et al., 2004). It should be noted that neither hyperactivity nor attention problems in these settings is a valid measure of ADHD. Hyperactivity here refers to a disposition to behave in a restless, inattentive, and impulsive fashion.

Fifteen years old regular and irregular smokers were rated to have more concentration difficulties and to behave in a more restless way than nonsmokers of respective age. Also, 18 years old smokers exhibited more symptoms of Inattention, measured by SNAP-IV scale, than nonsmokers. Adolescents with inattention problems may be more likely to experiment with tobacco smoking and to become regular users, this is thought to be attributable, in part, to nicotine's beneficial effects on cognitive function (Tercyak et al., 2002; Potter and Newhouse, 2004). According to the multiple regression analysis male sex and smoking at age 15 were associated with aggressive and hyperactive behaviour at the same age. Platelet MAO activity measured at age 15 was not independently associated with behaviour. As smoking habit is closely related to those behavioural traits, is more common in boys, and is also non-linearly related to platelet MAO activity (Harro et al., 2004), it is problematic to differentiate cross-sectionally the impact of smoking or MAO activity on these behavioural characteristics.

Longitudinal analysis revealed that low platelet MAO activity and smoking at age 15 are independently associated with high scores of Motor Restlessness and Inattention at age 18. The score of Motor Restlessness had declined with age among all subjects but had declined less among adolescents with low platelet MAO activity. The results of this study thus indicate that smoking and low platelet MAO activity are independently associated with inattentive and overactive behaviour. At age 18, platelet MAO activity was not revealed to be associated with behavioural characteristics when obtained at age 18. This can be, similarly to the cross-sectional analysis of data obtained at age 15, related to the complex associations between behaviour, smoking effect and the enzyme activity. There is a possibility that adolescents who score higher in Motor Restlessness and Inattention scales started to smoke earlier in life, however, we do not know whether this is due to low platelet MAO activity or whether MAO activity is lowered by the cigarette smoke. Since cigarette use seems to be more normative for older adolescents, and more adolescents start to smoke with an increasing age, MAO activity measured at age 18 may not reflect the predisposition for behavioural disturbances.

In summary, this longitudinal analysis supports the notion that platelet MAO activity is associated, independently of smoking, with specific aspects of hyperactivity and attention deficit in adolescents.

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TABLES

Table 1. Baseline characteristics of the participants.

	Time 1		Time 2	
	Boys (n=149)	Girls (n=216)	Boys (n=131)	Girls (n=188)
Age: mean (SD)	15.4 (0.5)	15.4 (0.5)	18.2 (0.6)	18.2 (0.5)
Smoking: n (%)				
Never tried	32 (22%)	82 (38%)	26 (20%)	46 (23%)
Tried but not smoking	74 (50%)	83 (39%)	33 (25%)	62 (30%
Smoking occasionally	14 (9%)	28 (13%)	23 (17%)	34 (17%)
Smoking regularly	29 (19%)	21 (10%)	50 (38%)	60 (30%)
Behaviour characteristics: mean (SD)	n=147	n=211	n=102	n= 140
Aggressiveness	3.17 (1.51)***	2.35 (1.13)	2.44 (1.31)**	2.07 (1.15)
Motor Restlessness	3.20 (1.70)***	2.19 (1.37)	2.57 (1.70)*	1.86 (1.26)
Concentration Difficulties	3.33 (1.60)***	2.65 (1.41)	3.01 (1.64)**	2.54 (1.45)
Hyperactivity	6.52 (3.03)***	4.84 (2.52)	5.58 (2.93)*	4.39 (2.32)
			n=98	n=134
Hyperactivity/Impulsivity	–	–	3.67 (4.78)**	1.81 (3.08)
Inattention	–	–	7.53 (5.37)*	5.89 (5.15)
SNAP-IV total score	–	–	11.20 (8.83)**	7.70 (6.79)
MAO activity: mean (SD)	9.34 (3.00)*	10.23 (3.22)	9.55 (3.30)***	10.97 (3.00)

***p< 0.0001; **p< 0.005; * p< 0.05 different from girls of respective age group.

Table 2. Mean (±SD) scores of Hyperactivity/Impulsivity, Inattention and SNAP-IV total in 18 years old smokers and nonsmokers.

		Hyperactivity/ Impulsivity	Inattention	SNAP-IV total
18 years old boys	Smokers (n=45)	4.50±5.86	8.70±6.03*	13.21±9.99*
	Nonsmokers (n=41)	2.78±3.66	5.85±4.56	8.63±7.57
18 years old girls	Smokers (n=63)	1.87±2.92	7.18±5.56*	9.05±6.89*
	Nonsmokers (n=60)	1.92±3.46	4.88±4.45	6.80±6.74

* p< 0.05 different from nonsmokers of respective gender group

Table 3. Linear regression models for Motor Restlessness (measured at age 18 by using the Hyperactivity Scale), Inattention and SNAP-IV total score, measured at age 18 by using SNAP-IV scale.

	log (Motor Restlessness)		Inattention		log (SNAP-IV total)	
Included independent variables	Regression coefficient (SE)	p	Regression coefficient (SE)	p	Regression coefficient (SE)	p
Low platelet MAO activity at age 15	0.18 (0.07)	0.04	1.52 (0.79)	0.05	0.22 (0.13)	0.10
Nonsmokers	0	–	0	–	0	–
Tried smoking at age 15	0.24 (0.09)	0.007	2.06 (0.78)	0.008	0.36 (0.13)	0.008
Smoking at age 15	0.29 (0.10)	0.005	4.27 (0.90)	<0.0001	0.65 (0.15)	<0.0001
Male sex	0.24 (0.08)	0.002	–	ns	0.30 (0.12)	0.01
R^2	0.11		0.12		0.15	

The scores of Motor Restlessness and SNAP-IV total are log-transformed for normality. Regression coefficient for low platelet MAO activity is calculated regarding average and high platelet MAO activity as reference, coefficients for smoking status are calculated with nonsmokers as reference group. Low (< 7.5), average (7.5–11.6) and high (>11.6 nmol X 10^{10} platelets^{-1} X minute^{-1}) platelet MAO activity was defined according to the percentile distribution.

FIGURES

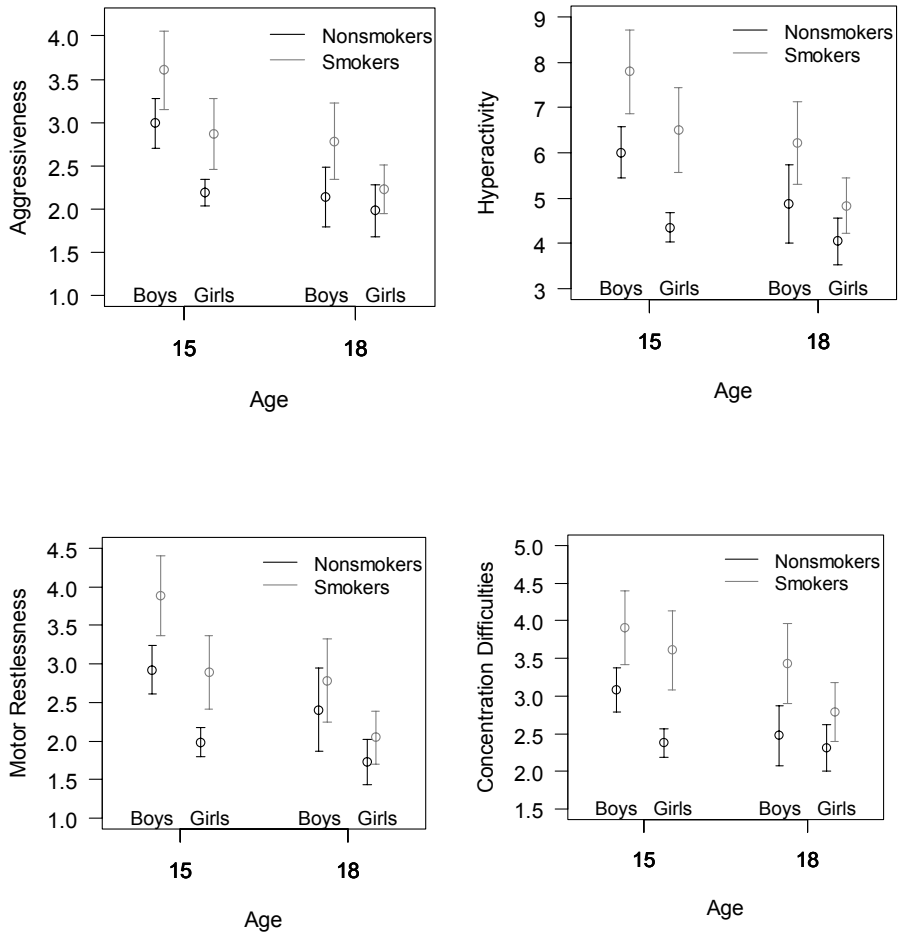

Figure 1. Mean (error bars: 95% confidence interval) scores of Aggressiveness, Motor Restlessness, Concentration Difficulties, and Hyperactivity in 15- and 18-years old smokers and nonsmokers. See Results for statistical evaluation of the data.

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Articles

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Platelet monoamine oxidase activity in association with childhood aggressive and hyperactive behaviour: the effect of smoking?

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Abstract

Monoamine oxidase (MAO) activity in platelets is known to be a marker of certain personality traits and behavioural preferences in adults, but less is known of the association of platelet MAO and behaviour in children. Platelet MAO activity was measured in 1129 randomly sampled 9- and 15-year old children by a radioenzymatic method with β-phenylethylamine as the substrate. Ratings of children's Aggressiveness, Motor Restlessness and Concentration Difficulties were obtained from teachers. Smokers had significantly lower MAO activity and they were also given significantly higher scores in Aggressiveness, Motor Restlessness and Concentration Difficulties than non-smokers. In 15-year but not 9-year-old children, significant associations were found between platelet MAO activity and behavioural ratings. However, these associations disappeared when smokers were excluded from the analysis. It is concluded that the causal relationship between behavioural measures and platelet MAO can only be revealed with longitudinal studies, considering smoking as a confounding factor.

Keywords: Monoamine oxidase (MAO); Aggressiveness; Motor restlessness; Concentration difficulties; Children; Smoking

1. Introduction

In the search for a better understanding of the neurobiological basis for behavioural deviations, and their possible markers, attention has been focused on the monoamine transmitter systems. The activity of the enzyme monoamine oxidase (MAO) in platelets has been of particular interest.

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Platelet MAO is believed to reflect central serotonergic activity (Oreland & Shaskan, 1983; Oreland, Ekblom, Garpenstrand, & Hallman, 1998), as it is positively correlated with cerebrospinal fluid levels of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin (Oreland et al., 1981). Low serotonin turnover, in turn, is likely to be associated with disinhibitory tendencies (Soubrie, 1986).

In adults, platelet MAO activity is characterized by a considerable variability between individuals, whereas the intraindividual variation of activity over time is low. Many studies have shown a high degree of heritability for platelet MAO. For example, Pedersen, Oreland, Reynolds, and McClearn (1993) reported a heritability factor of 76% for this enzyme activity. The mean activity has repeatedly been reported to be 10–27% higher in females than in males (Bridge, Soldo, Phelps, Wise, Francak, & Wyatt, 1985; Murphy, Wright, Buschbaum, Nichols, Costa, & Wyatt, 1976). In children, however, the gender differences are not so clear (Shekim, Davis, Bylund, Brunngraber, Fikes, & Lanham, 1982; Stoff, Friedman, Pollock, Vitiello, Kendall, & Bridger, 1989).

According to the vulnerability hypothesis, as it was first formulated by Buchsbaum, Coursey, and Murphy (1976), low platelet MAO activity is associated with personality traits which are predisposing to psychiatric vulnerability. This hypothesis was based on the observation that the subjects with low platelet MAO activity had more psychiatric disorders and had relatives who were more psychiatrically disturbed than the relatives of the high platelet MAO subjects. Low MAO activity in platelets has been shown to correlate with such personality characteristics as high sensation seeking, impulsiveness, and monotony avoidance (Schalling, Åsberg, Edman, & Oreland, 1987).

There have been fewer studies concerning psychiatric disorders, personality and MAO activity in children, and those studies have mostly been carried out in small samples of subjects (Bowden, Deutsch, & Swanson, 1988; Plizska, Rogeness, & Medrano, 1988; Rogeness, Hernandez, Macedo, & Mitchell, 1982; Stoff et al., 1989). Pliszka et al. (1988) found no difference in platelet MAO activities in children with behavioural disorders but a positive correlation with their degree of anxiety. Others have found a positive correlation of MAO activity with impulsiveness in prepubertal boys with externalizing symptoms of disruptive behaviour (Stoff et al., 1989). Rogeness et al. (1982) found no difference in platelet MAO activity in children with conduct disorder while some other studies have found lower activity in children with attention deficit disorder and hyperactivity (Bowden et al., 1988; Shekim et al., 1986).

The earlier cited studies were carried out on psychiatric patients. Regarding healthy children, a study of af Klinteberg and Oreland (1995) found that aggressiveness and hyperactivity (the sum of scores of motor restlessness and concentration difficulties) in 13-year old male adolescents ($n = 84$), according to teachers' ratings, was negatively associated with platelet MAO activity which was measured when the subjects had reached adulthood.

It has been found that there are some components in tobacco smoke that irreversibly inhibit platelet MAO activity (Norman, Chamberlain, & French, 1987; Yu & Boulton, 1987). Thus, the results of previous studies which did not consider the inhibitory effect of smoking on MAO activity appear to need re-evaluation. The aim of the present investigation was to study the association of platelet MAO activity with simultaneously rated aggressive and hyperactive behaviour in a population derived large sample of healthy boys and girls, with the possibility of considering the confounding effect of smoking.

2. Method

2.1. Subjects

The data were collected as a part of the European Youth Heart Study from September until June 1998–1999. A random sample of 25 schools in the Tartu region, Estonia was selected using cluster sampling (urban and rural schools with 9- and 15-year-old children) and probability proportional to school size. The participation rate was 76%. Parents and children gave their written consent before participating in the study. Details of the sample formation are described elsewhere (Harro et al., 2001).

Platelet MAO activity was measured in 1129 subjects, 520 boys and 609 girls; 584 of them were 15 years old (257 boys and 327 girls), 545 were 9 years old (264 boys and 281 girls). Psychological data were obtained from teachers of 508 15-year old children, (229 boys, 279 girls), and 493 9-year old children, (229 boys, 264 girls). Data about smoking was available for 526 15-year old children, (236 boys, 290 girls), and 562 9-year old children (267 boys, 295 girls).

2.2. Measurement of platelet MAO activity

Blood samples were obtained before breakfast. MAO activity was analyzed in platelet-rich plasma according to the procedure described by Hallman, Oreland, Edman, and Schalling (1987) and Harro et al. (2001). The substrate used was ^{14}C-labelled β-phenyl-ethylamine (''Amersham''). All experiments were performed in duplicate and the enzyme activity is expressed as nanomoles of substrate oxidized per 10^{10} platelets per minute.

2.3. Ratings of aggressiveness, motor restlessness and concentration difficulties

Ratings on Aggressiveness, Motor Restlessness and Concentration Difficulties were obtained from the teachers who had known the child for at least 3 years. Aggressiveness, Motor Restlessness, and Concentration Difficulties were rated on a seven-point scale as described by af Klinteberg (1988). The original study using this instrument in its Swedish version in a longitudinal design conservatively estimated the test-retest reliability for the rating variables at 0.80 (Magnusson, Duner, & Zetterblom, 1975; quoted by af Klinteberg, 1988). The teachers were

Table 1
Teacher's scorings of children's Aggressive and Hyperactive behaviour

	Aggressiveness	Motor Restlessness	Concentration Difficulties	Hyperactivity	Total score
15-year-old boys ($n=229$)	3.27 ± 1.56^{a}	3.28 ± 1.73^{a}	3.47 ± 1.58^{a}	6.74 ± 3.05^{a}	10.01 ± 4.31^{a}
15-year-old girls ($n=279$)	2.43 ± 1.23	2.26 ± 1.49	2.73 ± 1.49	4.99 ± 2.71	7.42 ± 3.64
9-year-old boys ($n=229$)	2.92 ± 1.61^{ab}	3.36 ± 1.82^{a}	3.43 ± 1.85^{a}	6.80 ± 3.39^{a}	9.71 ± 4.71^{a}
9-year-old girls ($n=264$)	2.22 ± 1.26^{b}	2.26 ± 1.48	2.56 ± 1.51	4.81 ± 2.76	7.04 ± 3.77

All values are means±S.D. Hyperactivity refers to the sum of scores of Motor Restlessness and Concentration Difficulties.

[a] $P<0.0001$ vs. girls of respective age group.

[b] $P<0.005$ vs. older children of the same gender.

instructed to use the boys and girls in their own class as reference groups. Hyperactivity score was calculated as in af Klinteberg and Oreland (1995) by summing the scores of Motor Restlessness and Concentration Difficulties. As all three behavioural ratings were highly correlated in our sample, we also calculated the sum of all three variables (Total score).

2.4. Smoking

Children reported their smoking habits in an anonymous questionnaire in a laboratory out of school with no teachers or parents present. Children who reported that they smoke either regularly or occasionally were considered as smokers.

2.5. Statistical methods

Differences between younger and older boys and girls and the low and high MAO groups in relation to the scores of Aggressiveness, Motor Restlessness and Concentration Difficulties were tested by means of analysis of variance (ANOVA) and Mann–Whitney test using the StatView 4.5 package for Macintosh. ANOVA was also used to study the differences in MAO between boys and girls and younger and older children. Spearman rank correlations were used to indicate the associations between continuous variables.

3. Results

As expected, boys had a significantly lower mean platelet MAO activity compared to girls in both age groups in this sample (Harro et al., 2001). There was also a significant difference in MAO activity between age groups, the younger children having lower platelet MAO activity on average. The proportion of smokers among 9-year-old children was 4% in boys and 1% in girls, and 31% and 16%, respectively, in 15-year-old children. While no difference was found in platelet MAO activity between smokers and non-smokers in younger children, 15-year-old smokers of both sexes had significantly lower MAO activity than non-smokers.

Older children were given higher scores in Aggressiveness than children in the younger age group, $F(1, 1008) = 8.05$, $P = 0.005$. No difference was found in scores of Motor Restlessness, Concentration Difficulties, Hyperactivity, or Total score between the two age groups (Table 1). In both age groups, boys were given higher scores in Aggressiveness, Motor Restlessness and Concentration Difficulties scales than girls.

Among 15-year-old children, cigarette smokers were rated more aggressive, more motor restless, and to have more concentration difficulties than non-smokers of the respective age group [$F(1, 513) = 51.49$, 50.51, 65.41, respectively, $P < 0.0001$]. This difference was not observed in younger children.

Significant negative correlations were found between children's platelet MAO activity and the scores of Aggressiveness, Motor Restlessness, Concentration Difficulties, Hyperactivity, and the Total score, in the 15-year-old group (Table 2), but these correlations were not significant when the smokers were excluded from the analysis. If 15-year-old boys and girls (smokers included) were studied separately, a significant correlation was found only between platelet MAO activity

and Motor Restlessness in both sexes. No significant correlations between the enzyme activity and behavioural variables were found in the younger age group (data not shown).

Next, all children were divided into two groups on the basis of their individual platelet MAO activity, forming groups with higher and lower activity. The cutting points between the groups were at the median value of the activity in every certain age and gender group, and were 9.74,

Table 2
Spearman correlations between platelet MAO activity, and Aggressiveness, Motor Restlessness and Concentration Difficulties in 15-year-old adolescents

	Smokers and non-smokers (r)	P-value	Non-smokers (r)	P-value
Aggressiveness	−0.09	0.046	−0.001	NS
Motor Restlessness	−0.19	0.0001	−0.10	NS
Concentration Difficulties	−0.13	0.0047	−0.04	NS
Hyperactivity	−0.16	0.0002	−0.07	NS
Total score	−0.15	0.0011	−0.05	NS

All values are mean ± S.D. Hyperactivity refers to the sum of scores of Motor Restlessness and Concentration Difficulties. NS, not significant.

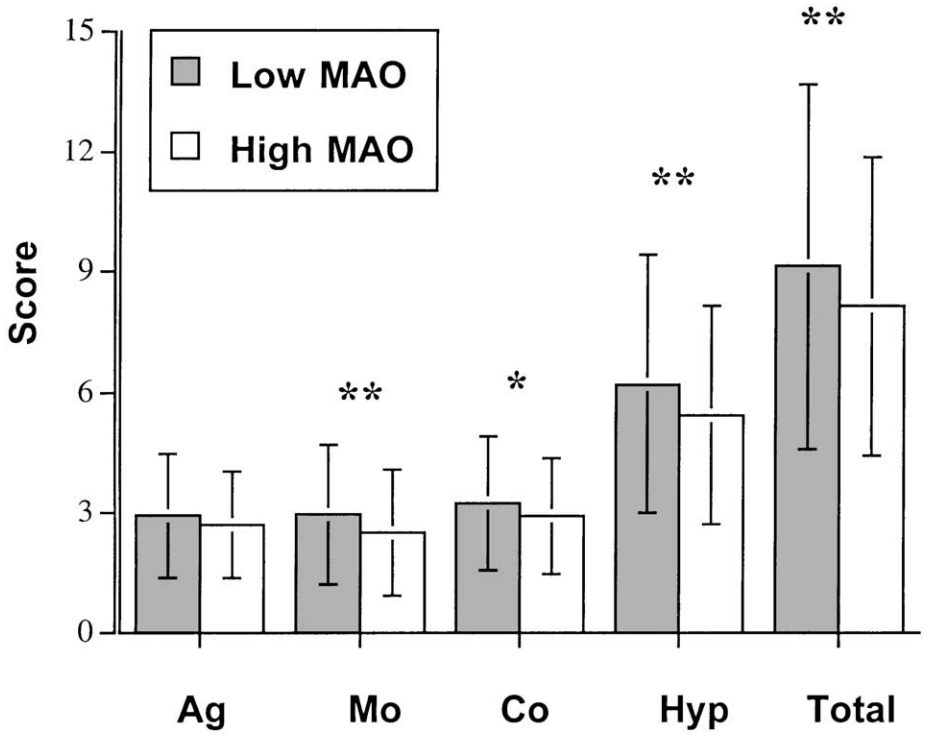

Fig. 1. Teachers' scorings of 15-year-old schoolchildren's Aggressiveness (Ag), Motor Restlessness (Mo) and Concentration Difficulties (Co) associated with children's lower and higher platelet MAO activity. * $P<0.05$; ** $P<0.005$. Children were divided into two groups on the basis of median MAO activity of particular sex and age groups. Hyperactivity refers to the sum of scores of Motor Restlessness and Concentration Difficulties.

8.66, 9.10 and 8.10 nmol oxidized/min/10^{10} platelets in older girls, older boys, younger girls and younger boys, respectively. Adolescents, but not 9-year-old children with lower platelet MAO activity scored significantly higher in all scales except Aggressiveness [$F(1, 507) = 9.61$, $P = 0.002$, 4.60, $P = 0.03$, 8.19, $P = 0.004$, 7.05, $P = 0.004$ for Motor Restlessness, Concentration Difficulties, Hyperactivity, and Total score, respectively] (Fig. 1). When adolescent boys and girls with high or low platelet MAO activity were studied separately, Motor Restlessness was found to be significantly higher in both boys and girls with low platelet MAO activity. Hyperactivity and Total score were also lower in the low platelet MAO group, but only in boys. However, if the smokers were excluded from these analyses, all differences appeared nonsignificant (data not shown).

4. Discussion

In the present study, there was a negative association between platelet MAO activity and behavioural traits of Motor Restlessness and Concentration Difficulties in adolescents. The results obtained thus seem to support earlier findings (af Klinteberg and Oreland, 1995) about the negative relationship between platelet MAO activity (determined in adulthood) and teachers' ratings of the subjects' Hyperactivity (Motor Restlessness and Concentration Difficulties) but not of ratings of Aggressiveness (assessed at the age of 13 years). In the present study, no association was found between platelet MAO and behavioural ratings in younger children. It could be speculated that as hyperactivity is a matter of maturation (Taylor, 1986) it could be defined differently by teachers for younger children and adolescents. However, no statistically significant association between behavioural measures and platelet MAO activity was found in the present study when the smokers were excluded from the analysis.

Smoking has been found to reduce platelet MAO activity in adults in a significant and dose related manner (Whitfield et al., 2000). In the large sample of adolescents of the present study, this effect was also present. As the exclusion of smokers from the sample rendered the association between platelet MAO activity and hyperactive behaviour nonsignificant, it could be argued that the previously described association between these measures (af Klinteberg and Oreland, 1995) has been an artifact due to the smoking effect. However, exclusion of those subjects who smoke may also mask the true association between behaviour and platelet MAO, because there are differences in personality between smokers and nonsmokers (Canals, Blade, & Domenech, 1997; Patton, Barnes, & Murray, 1993). In the present study, children who smoked occasionally or regularly were given higher scores in Aggressiveness, Motor Restlessness and Concentration Difficulties. There is thus also a possibility that adolescents who score higher in these behavioural traits are more likely to become smokers, tend to start smoking earlier in life, or smoke more frequently. On the other hand, it can not be excluded that the more aggressive and hyperactive males in the previous study (af Klinteberg and Oreland, 1995) had started to smoke after the assessment of behaviour and before the measurement of MAO activity. Therefore, until a longitudinal study starting before future smokers have acquired the smoking habit is conducted, no final conclusion can be made regarding the association between hyperactive behaviour and platelet MAO activity. As smoking is wide spread among adolescents, and more children start to smoke with increasing age, it is important, however, to obtain behavioural ratings and blood samplings simultaneously.

There are only a few studies that have controlled the influence of cigarette smoking on the association between personality traits and platelet MAO activity, and the inhibitory effect of cigarette smoke on the enzyme has caused some doubt on the validity of previous findings on platelet MAO, behaviour, and personality (Anthenelli et al., 1998; Whitfield et al., 2000). However, recently it has been found that when the smoking factor is under control, there are clear associations between low platelet MAO activity and eating disorders (Carrasco, Diaz-Marsa, Hollander, Cesar, & Saiz-Ruiz, 2000; Diaz-Marsa, Carrasco, Hollander, Cesar, & Saiz-Ruiz, 2000). Furthermore, platelet MAO activity was found to be related to the behaviour of newborn babies, those with lower MAO displaying more screaming and restlessness (Sostek, Sostek, Murphy, Martin, & Smith Born, 1981). In addition, low platelet MAO has been associated with specific behavioural patterns in monkeys (Åsberg et al., 1999; Redmond, Murphy, & Baulu, 1979; Redmond, Murphy, Baulu, Ziegler, & Lake, 1975). These findings strongly suggest that platelet MAO activity is linked to behaviour. However, the effect of smoking has to be considered as a serious confounding factor and may have biased many of the previous investigations on platelet MAO as a correlate of behavioural traits.

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Short Communication

Association between substance use, personality traits, and platelet MAO activity in preadolescents and adolescents

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Abstract

This study examined the relationship between alcohol/illicit drug use, the Five-Factor Model (FFM) personality traits, aggressiveness (Agg), and hyperactivity (Hyp), and platelet monoamine oxidase (MAO) activity in a population-derived representative sample of preadolescents and adolescents ($n = 1172$). Alcohol and illicit drug use was self-reported. The FFM personality inventories were filled in by mothers of the participants, and Agg and Hyp were rated by their class teachers. Higher scores in extraversion (E), Agg, and Hyp and lower scores in conscientiousness (C) together with older age were significant predictors of more frequent alcohol use in adolescents. No significant association was found between alcohol illicit drug use, and platelet MAO activity.

Keywords: Alcohol; Five-factor personality traits; Aggressiveness; Hyperactivity; Monoamine oxidase; Children

1. Introduction

Although a number of social, psychological, and environmental factors have been implicated in the aetiology of alcoholism, there is a growing body of evidence that

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personality and temperament are important determinants of vulnerability to becoming an alcoholic. Besides alcoholism, more frequent substance use in adolescents has been found to be related to a number of personality traits: these subjects are more aggressive, hyperactive, sensation seeking, impulsive, and have higher concentration difficulties (for review, see Chassin & DeLucia, 1996). During the last decades, the Five-Factor Model (FFM) of personality, which includes the dimensions of Neuroticism (N), Extraversion (E), Openness to Experience (O), Agreeableness (A), and Conscientiousness (C) (McCrae & John, 1992) has become the norm against which different personality trait taxonomies are tested. It has been demonstrated that the same five dimensions can be measured in children and adolescents, demonstrating a remarkable stability through the course of development (Caspi & Roberts, 1999).

Platelet monoamine oxidase (MAO) activity is one of the most consistently reported biological correlates of personality and psychopathology. Low platelet MAO activity has repeatedly been found in alcoholics and drug abusers. Platelet MAO activity correlates with the same personality traits (sensation seeking, impulsiveness, monotony avoidance, anxiety, and extraversion) found to be related to heavy alcohol consumption or alcoholism (for review, see Oreland, 1993). Very little is known about the possible association of platelet MAO activity, personality, and behavior in children. af Klinteberg and Oreland (1995) have described a link between lower platelet MAO and higher hyperactivity (Hyp) and aggressiveness (Agg) in adolescents, but no attempt was made to measure substance use in that sample. Recently, it has been demonstrated that some compounds in cigarette smoke directly inhibit platelet MAO activity (Oreland et al., 1999). Thus, if the association between MAO and other variables is studied, it is necessary to consider the influence of smoking (Harro et al., 2001; Whitfield et al., 2000).

The aim of our investigation was to examine the relationship between alcohol/illicit drug use, FFM personality traits, Agg, Hyp, and platelet MAO activity in a population-derived representative sample of preadolescents and adolescents.

2. Methods

2.1. Subjects

Twenty-five schools were sampled using random numbers and probability proportional to the number of students in the school. From each school sampled, all third and ninth graders were invited to participate in the study. Children and their parents gave their informed consent. The participation rate was 76%. The main cause for not participating was the fear of venous blood sampling. The total number of subjects studied was 1172, including 581 children with mean age of 9.6 ± 0.5 years (ranging from 8 to 11 years) and 591 adolescents with mean age of 15.4 ± 0.6 years (ranging from 14 to 17 years). Permission for the study was obtained from the Committee of Ethics of the University of Tartu, Estonia.

2.2. Measures

Alcohol, illicit drug usage, and smoking habits were reported by the children. Personality traits according to FFM were obtained from mothers, while Agg and Hyp of students were rated by their class teachers.

Children reported if they had tasted alcohol and how often do they consume cider, beer, wine, and strong spirits. The frequency of the most often consumed type of alcohol ranging from 1 (*never*) to 5 (*everyday*) was used to form the total alcohol consumption score. The adolescents were divided into three groups: (1) nonconsumers (including those who had not tasted alcohol and those who had tasted but reported that they never use it), (2) moderate

Table 1
Alcohol consumption, experience with illicit drugs, smoking, and platelet MAO activity in participants

	Preadolescents				Adolescents			
	Boys ($n=278$)		Girls ($n=303$)		Boys ($n=260$)		Girls ($n=331$)	
	%	*n*	%	*n*	%	*n*	%	*n*
Alcohol								
Have not tried	55	153	63	191	5	14	6	19
Have tried	45	125	37	112	95	246	94	312
Nonconsumers	85	235	90	271	19	50	24	80
Moderate consumers	13	38	10	31	59	152	64	213
Frequent consumers	2	5	0	1	22	58	12	38**
Total alcohol consumption score[a]	1.2 ± 0.6	278	1.1 ± 0.4	303**	2.5 ± 1.1	260	2.2 ± 1.0	331****
Illicit drugs								
Have not tried	–[b]		–		94	240	95	308
Have tried	–		–		6	15	5	17
Illicit drug consumption score[a]	–[b]		–		1.08 ± 0.4	255	1.06 ± 0.3	325
Smoking								
Nonsmokers	92	255	96	292*	64	165	75	247****
Experimenters	7	21	4	11*	11	29	13	43
Smokers	1	2	0	0	25	66	12	40****
Platelet MAO activity (nmol $\times 10^{10}$ platelets$^{-1} \times$ min^{-1})[a]	8.48 ± 2.90	264	9.15 ± 2.38	279****	8.92 ± 2.81	257	10.14 ± 3.31	325**

[a] Mean ± S.D. is presented.

[b] Preadolescents was not asked about their experiences with illicit drugs.

* $P<.05$, statistically significant difference between boys and girls of the same age group according to χ^2 test and ANOVA.

** $P<.01$, statistically significant difference between boys and girls of the same age group according to χ^2 test and ANOVA.

**** $P<.0001$, statistically significant difference between boys and girls of the same age group according to χ^2 test and ANOVA.

consumers (who used alcohol less often than once per week), and (3) frequent (once per week or more often) consumers. In the younger age group, only two groups were formed: (1) nonconsumers and (2) alcohol consumers. Adolescents (not younger children) reported also how many times they had tried illicit drugs and if they were current users forming a score from 1 (*never tried*) to 4 (*I am a current user*). All participants were divided into three groups according their smoking habits: (1) nonsmokers, (2) experimenters (who smoked less often than once a week), and (3) smokers (smoking at least once per week).

Following an example of the "Common Language" California Child Q-Set (John, Caspi, Robins, Moffitt, & Stouthamer-Loeber, 1994), a short 40-item questionnaire was constructed to measure personality traits according to the FFM. Each of the dimensions (N, E, O, A, and C) was measured using eight items in a five-point Likert format. Cronbach α's for the five factors were .73, .74, .52, .67, and .77, respectively. The constructed personality measure was previously validated against NEO-PI-R with which domains the convergent correlations ranged from .52 (A) to .71 (E).

Class teachers were asked to rate the participants' Agg, motor restlessness, and concentration difficulties in a seven-point scale previously used by af Klinteberg and Oreland (1995). The sum of the scores for motor restlessness and concentration difficulties was used as an indicator of Hyp.

Venous blood samples were taken after 12-h fasting into tubes with EDTA and platelet MAO activity was assessed according to the method previously described by us (Harro et al., 2001).

3. Results

In both age groups, boys had a significantly higher total alcohol consumption score, higher prevalence of smoking, and lower platelet MAO activity if compared with girls (Table 1).

Table 2
Mean ± S.D. scores of the FFM personality traits, Agg, and Hyp by age group and sex

	Age		Sex	
	Younger	Older	Boys	Girls
N	23.7 ± 5.0	22.1 ± 5.1****	22.8 ± 5.2	23.0 ± 5.0
E	29.3 ± 5.6	26.6 ± 5.3****	28.4 ± 5.6	27.7 ± 5.6
O	28.2 ± 4.0	26.8 ± 4.3****	27.5 ± 4.1	27.5 ± 4.3
A	28.0 ± 4.6	28.7 ± 4.8*	28.1 ± 4.7	28.6 ± 4.7
C	26.7 ± 5.4	28.2 ± 5.9****	26.9 ± 5.6	27.9 ± 5.8##
Agg	2.55 ± 1.48	2.80 ± 1.45***	3.10 ± 1.60	2.33 ± 1.25####
Hyp	5.74 ± 3.22	5.77 ± 3.00	6.77 ± 3.22	4.91 ± 2.74####

* $P < .05$, significantly different from younger group.
*** $P < .001$, significantly different from younger group.
**** $P < .0001$, significantly different from younger group.
$P < .01$ significantly different from boys. No significant age and sex interaction was found.
$P < .0001$ significantly different from boys. No significant age and sex interaction was found.

Table 3
Spearman bivariate correlation matrix of all variables in preadolescents and adolescents

	AL	IL	MAO	N	E	O	A	C	Agg	Hyp	Smo	Sex	Age
AL	–	–	−.05	.08	.03	.01	−.09	−.06	.06	.07	−.03	−.08	.07
IL	.20#	–	–	–	–	–	–	–	–	–	–	–	–
MAO	−.06	.02	–	.01	.01	−.04	−.01	.08	−.07	−.03	−.05	.15**	.07
N	−.02	.02	−.01	–	−.18#	−.19#	−.37#	−.40#	.11*	.11*	.05	−.02	−.01
E	.15**	.06	−.03	−.14**	–	.46#	.05	−.03	−.02	.04	.07	−.06	−.04
O	.11*	.09*	.06	−.13**	.45#	–	.10*	.14#	−.04	−.02	−.01	−.03	.01
A	−.01	−.01	.06	−.44#	.001	.09*	–	.36#	−.20#	−.17***	−.04	.08	.01
C	−.08	−.08	.13**	−.49#	.02	.21#	.41#	–	−.27#	−.34#	−.04	.08	.06
Agg	.16#	.08	−.09*	.14**	−.01	−.09	−.10*	−.25#	–	.71#	.06	−.22#	.05
Hyp	.17#	.10*	−.16***	.18***	.06	−.08	−.09	−.28#	.68#	–	.10*	−.30#	.05
Smo	.41#	.20#	−.22#	.08	.12**	.01	−.002	−.27#	.31#	.35#	–	−.11*	.02
Sex	−.12**	−.02	.19#	.09	−.004	.07	.02	.11*	−.28#	−.31#	−.18#	–	−.13**
Age	.19#	.09*	−.09*	−.03	−.06	.05	.07	.03	.09*	.07	.11*	−.08	–

Results for preadolescents are presented above diagonal and adolescents below diagonal. AL: total alcohol consumption score, IL: illicit drug consumption, Smo: smoking, Sex: 1 = male and 2 = female, Age in years.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

# $P < .0001$.

Sex- and age-related differences of personality traits are presented in Table 2. A weak positive correlation was found between alcohol consumption and E, O, Agg, and Hyp in adolescents but not in preadolescents (Table 3). The correlation between illicit drug consumption and O and Hyp was also significant but very weak (Table 3). Platelet MAO activity correlated significantly and positively with C and negatively with Agg and Hyp only in adolescents. Nevertheless, if partial correlation was performed with smoking as a covariate, the correlation remained significant only between the score of Hyp and platelet MAO activity ($r = -.11$, $P < .05$).

If the participants were divided into groups according to their substance use, younger children, who reported to be consumers of alcohol, were given significantly higher scores in N and lower scores in A if compared with nonconsumers ($P < .05$, data not shown). Those 6% of adolescents who had not tried alcohol in their lives were given significantly lower scores in E ($P < .05$, data not shown) if compared with others. In adolescents, a dose-dependent relationship was found between three alcohol consumption groups, Agg and Hyp with those reporting to consume alcohol frequently showing the highest scores in Agg and Hyp ($P < .001$). Adolescents who had tried illicit drugs were given significantly higher scores in O and Hyp ($P < .05$, data not shown).

In adolescents, older age, higher E, and lower C (Table 4) were significant predictors for the total alcohol consumption score using multiple regression analysis. In two separate regression analysis, higher Agg and higher Hyp, both together with older age, were found to be significant predictors for the alcohol consumption. In preadolescents, only male sex was found to be a significant predictor for alcohol consumption (data not shown). If experience with illicit drugs was analysed as a dependent variable, only older age and lower C were

Table 4
Regression analyses for alcohol consumption score and experiences with illicit drugs with age, sex, and (a) the FFM of personality traits, (b) Agg, or (c) Hyp as independent variables in adolescents

Dependent variable	Adjusted r^2	F	df	Significant predictors	β	t
(a) Alcohol score	.07	6.46****	7/478	E	0.02	2.34*
				C	−0.02	−2.12*
				Age	0.37	4.69****
(b) Alcohol score	.06	11.94****	3/514	Agg	0.11	3.42***
				Age	0.27	3.60**
(c) Alcohol score	.06	11.59****	3/513	Hyp	0.05	3.25**
				Age	0.28	3.72***
(a) Experiences with drugs	.02	2.00^{ns}	7/470	C	−0.01	−2.00*
				Age	0.05	2.31*
(b) Experiences with drugs	.02	2.54^{ns}	3/504	Age	0.05	2.16*
(c) Experiences with drugs	.01	3.00*	3/503	Age	0.06	2.17*

ns: nonsignificant.
* $P < .05$, statistically significant.
** $P < .01$, statistically significant.
*** $P < .001$, statistically significant.
**** $P < .0001$, statistically significant.

significant predictors for illicit drug use in models including age, sex, FFM, and/or Hyp as independents (Table 4). Adding platelet MAO activity to any of these models did not change the significance or other parameters of the models (data not shown).

3.1. Limitations of the study

This study was cross-sectional and we cannot attribute causation to any associations found between substance use and personality traits. A longitudinal study (now going on) in the same children may give the answer to the question of causality between personality traits and alcohol use in adolescents.

4. Discussion

The results of previous research and our study clearly suggest that although there exists an association between personality traits and substance use, personality variables independently contribute only modestly to the prediction of such behaviors as alcohol and illicit drug use and smoking. Nevertheless, it is important to note that the association between personality traits and substance use can be found already in preadolescents.

We did not find any difference in platelet MAO between consumers and nonconsumers of alcohol or illicit drugs. It is possible that either systematic or random environmental factors are more important in determining early experimentation with substances than biological predispositions. It is conceivable that platelet MAO is rather a marker of vulnerability to abuse/addiction than of experimentation with drugs.

Because of the complex relationship between smoking behavior and personality on one hand and the direct effect of cigarette smoke on MAO activity (Oreland et al., 1999) on the other, the true relationship between platelet MAO, smoking, and personality traits associated with smoking can only be revealed in a prospective longitudinal study.

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Growth hormone, cortisol and prolactin responses to physical exercise: higher prolactin response in depressed patients

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Abstract

This study was designed to compare growth hormone, cortisol and prolactin responses to physical exercise in depressed patients and healthy comparison subjects. Patients fulfilled the DSM-IV diagnostic criteria for current major depressive disorder; subjective depressive symptoms were rated with Montgomery-Åsberg Depression Rating Scale (MÅDRS) immediately before the experiment. Growth hormone, cortisol and prolactin were measured before and immediately after physiologically stressful bicycle cardiopulmonary exercise test. After exercise, there were three additional hormone measurements, with 30-min intervals. No significant difference was found in baseline growth hormone, cortisol or prolactin levels between patients and the control group. Plasma growth hormone and cortisol levels increased significantly during physical exercise in both patients and controls and returned to baseline in 90 min. There was no significant difference in growth hormone or cortisol responses to physical exercise between the two groups. However, prolactin levels increased only in the depressed patients group during the exercise. We hypothesize that acute exercise may have a stronger effect on serotonin (5-HT) release in depressed patients, which is reflected in increased plasma prolactin concentration.

Keywords: Cortisol; Depression; Growth hormone; MÅDRS; Physical exercise; Prolactin

1. Introduction

Numerous endocrine abnormalities have been described in depressive illness. Patients show increased plasma cortisol levels and blunted growth hormone and prolactin release in pharmacological challenge tests. Abnormality of the hypothalamic-pituitary-adrenal (HPA) axis has been one of the most consistently demonstrated biological alterations in depressive disorder. A significant proportion of patients with major depression hypersecrete cortisol (Dinan, 1994) and cortisol levels often remain elevated throughout the day with an absence of normal diurnal variation (Sachar et al., 1973). A number of authors have proposed that increased cortisol secretion down-regulates serotonin (5-HT) neurotransmission which leads to clinical depression in vulnerable individuals (Deakin and Graeff, 1991; Dinan, 1994). Depressives have a dysfunction in the 5-HT_{1A} receptor activity, which could be due to a hypersecretion of cortisol (Pitchot et al., 2001).

The regulation of the release of prolactin also involves the monoamine neurotransmitter systems that have been implicated in the pathophysiology of depression. Fenfluramine-induced release of 5-HT in the hypothalamus causes the release of prolactin and the prolactin response is used as an index of central 5-HT function (Siever et al., 1984; Mann, 1999). The prolactin response to a challenge dose of fenfluramine is attenuated in patients with affective or personality disorders (Coccaro et al., 1989). The interindi-

Abbreviations: GHRH, Growth hormone releasing hormone; HPA, hypothalamic-pituitary-adrenal; MÅDRS, Montgomery-Åsberg Depression Rating Scale; 5-HT, serotonin.

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vidual variability in serotonergic responsivity, adjusted for baseline prolactin concentration, age, sex, and drug concentration during the challenge, has been shown to be moderately reproducible (Flory et al., 2002). Individual differences in the hormonal response are thus thought to reflect dimensional variability in central serotonergic activity.

Depressed patients have a blunted growth hormone response to clonidine (Siever et al., 1982; Mokrani et al., 2000; Dahl et al., 2000). Clonidine stimulates growth hormone release, which appears to be mediated by postsynaptic α_2-adrenergic hypothalamic receptors via growth hormone releasing hormone (GHRH) release (Devesa et al., 1991). The blunted growth hormone response to clonidine in depression is believed to be secondary to a decreased sensitivity of postsynaptic α_2-adrenergic receptors. Adults with growth hormone deficiency have been reported to suffer from increased levels of depression and apathy compared with healthy controls (Zenker et al., 2002).

We have previously found that the growth hormone response to physiological stimuli is reduced in healthy young men with higher psychometrically measured depressiveness (Harro et al., 1999). However, it is unclear if patients with depression have a similar blunted response of growth hormone to physical stressors. Neither is it known whether cortisol and prolactin levels increase in response to physical stress in depressed patients.

Major depression and the stress response share similar phenomena, mediators and circuitries. The hormonal response to stress is directly related to the intensity of the stimulus, but also greatly depends on the individual's perception of potentially stressful situations. Responses to stressful events are generally regarded as reactions of the organism to accommodate to or compensate for stress. This reaction is often described as an activation of the sympathoadrenal system and HPA axis. Several studies have shown that acute physical exercise activates the HPA axis resulting in rapid increases in plasma cortisol (Sowers et al., 1977; Van der Pompe et al., 1999; Gispen-de Wied et al., 2000). Activation of the release of growth hormone and prolactin into blood also occurs during various types of stress. Exercise stimulates the HPA axis and releases growth hormone and prolactin in an intensity-dependent fashion (Luger et al., 1988).

The purpose of this study thus was to examine growth hormone, cortisol and prolactin responses to an aerobic fitness measurement test in depressed patients and normal volunteers.

2. Methods

2.1. Participants

Study participants were 24 patients and 22 healthy volunteers. This study was approved by University of Tartu Ethic Committee for Studies on Humans and all subjects gave written informed consent after complete description of the study. Subjects were all males, age 24–68. The mean (±S.D.) age, body weight and height for patients were 43.5±1.8 years, 81.2±2.2 kg and 179±1 cm, respectively, and for healthy volunteers 42.8±3.0 years, 84.7±2.7 kg, 180±1 cm, respectively. The control subjects and the depressed patients did not differ significantly in age, height, weight, or body mass index. One control subject was excluded from the experiment because of using antidepressant medication.

The patients were recruited at the in- and outpatient services of the Clinic of Psychiatry, Tartu University Clinics. Twenty inpatients and four outpatients participated in the study. To be included in the study, the patients had to fulfill the DSM-IV diagnostic criteria (American Psychiatric Association, 1994) for current major depressive disorder. Diagnoses were confirmed with use of the Mini-International Neuropsychiatric Interview, version 5.0.0 (Sheehan et al., 1998). Most of the patients were using antidepressant medication (clomipramine, nortriptyline, fluoxetine, paroxetine, citalopram, mirtazapine, moclobemide). Two outpatients reported no antidepressant drug use. Six patients were treated with tricyclic antidepressants (clomipramine and nortriptyline), eight patients with SSRI-s (fluoxetine, paroxetine, citalopram), three patients with mirtazapine and one patient was treated with moclobemide. Four patients reported antidepressant use but the type of drug was not specified. The exclusion criteria were significant psychiatric comorbidity, including schizophrenia or other psychotic disorder, organic mental disorder, mental retardation, bipolar disorder, anxiety disorders if primary and/or predominant, alcohol abuse or dependence in the last 12 months, and unstable or significant comorbid somatic or neurological disease.

Immediately before the experiment, the Montgomery-Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979) self-assessment version (MÅDRS-S) was used to assess subjective symptoms of depression. The control subjects were also interviewed by a psychiatrist to exclude depression or other psychiatric disorder.

2.2. Physical exercise testing

The experiment started between 0900 and 0915 h. Two to four subjects were tested each day. They had been instructed to fast since the evening of the previous day and not to smoke in the morning before the experiment. First they filled in the MÅDRS-S in a quiet room. An indwelling venous catheter was then inserted into the antecubital vein and the first blood sample was drawn into Vacutainer® tubes containing EDTA 10 min before the exercise started. The subjects underwent a bicycle cardiopulmonary exercise testing using stepwise increasing workload by 25 W per 2 min (Ergometry System, Siemens). Expired gases, ventilation and heart rate analyses were computed simultaneously and displayed with "Oxycon Record" (Erich Jaeger). Ventilatory anaerobic threshold (AnT) was determined

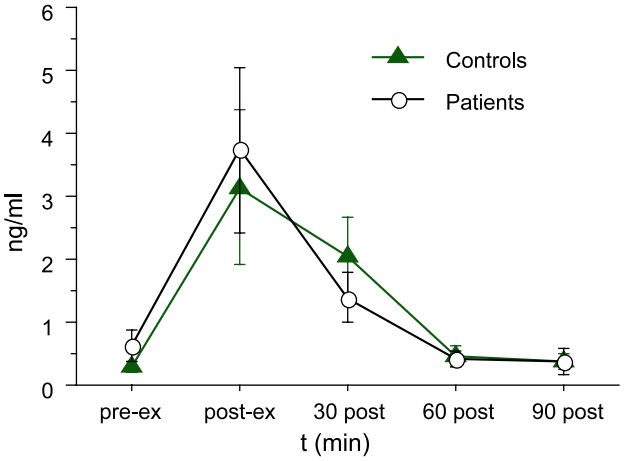

Fig. 1. Growth hormone response to physical exercise in depressed patients and controls. Pre-ex refers to mean basal value of plasma growth hormone assessed before exercise, post-ex refers to mean plasma growth hormone value assessed immediately after exercise; 30 post, 60 post and 90 post refer to mean plasma growth hormone values assessed after exercise with 30-min intervals.

following the Wassermann et al. (1999) basic criteria. Peak oxygen consumption and working capacity was registered. All subjects were continuously verbally encouraged during the test. Venous blood was sampled immediately after exercise, and during the recovery phase three additional times with 30-min intervals. Blood draws were standardized for each subject. Blood samples were coded and plasma was separated by centrifugation. Plasma samples were stored at −80 °C until analyzed.

2.3. Hormone measurement

Growth hormone, cortisol and prolactin were measured by chemiluminescence immunoassay using commercially available kits (Immulite) and conventional methods (Babson et al., 1991) by a technician blind to sample coding. Detection limit for growth hormone, cortisol and prolactin was 0.05 ng/ml.

2.4. Statistical analysis

Comparisons between the control group and patients group were tested by means of analysis of variance (ANOVA) repeated measures, using the StatView 5.0 package for Macintosh. For hormone responses, additional analyses were performed using ANCOVA with maximal heart rate (HR) and peak oxygen uptake ($VO_{2\ peak}$) as covariates. Within-group comparisons were made by paired *t*-test and two-tailed Mann–Whitney *U*-test. Parametric and nonparametric statistics revealed similar results. Spearman correlation was used to indicate the relationship between continuous variables. Differences of hormone responses to physical exercise between the control group and patients groups using different types of antidepressant medication were also tested by ANOVA.

3. Results

3.1. Pre-exercise growth hormone, cortisol and prolactin levels

The mean (±S.E.M.) plasma levels of hormones in control group and in patients' group, measured before the exercise session, were 0.29±0.08 and 0.61±0.25 ng/ml for growth hormone, 7.30±1.10 and 6.30±0.60 ng/ml for prolactin and 12.32±0.83 and 13.10±0.76 ng/ml for cortisol, respectively. No significant differences between the two groups' baseline hormone levels were found.

3.2. Growth hormone, cortisol and prolactin secretion in response to physical exercise

The increase in hormone secretion in response to physical exercise was calculated as hormone levels measured 5 min after exercise minus baseline hormone levels. Growth hormone (Fig. 1) and cortisol (Fig. 2) levels were significantly elevated after physical exercise in controls as well as in patients: $t=-2.78$, $df=21$, $P<0.01$ and $t=-2.42$, $df=23$, $P<0.05$ for growth hormone and $t=-2.91$, $df=21$, $P<0.01$ and $t=-2.89$, $df=23$, $P<0.01$ for cortisol, respectively. The mean increase in response to exercise in growth hormone levels was 2.85±1.18 and 3.14±1.13 ng/ml in controls and patients, respectively. In cortisol levels, the mean increase was 2.94±1.02 and 1.78±0.61 ng/ml in controls and patients, respectively.

Repeated measures analysis revealed the interaction between group and time of prolactin ($F(4,176)=2.4$, $P<0.05$), but not growth hormone or cortisol, sampling. Prolactin levels were elevated only in the group of patients: $t=-3.04$, $df=23$, $P<0.01$ between pre- and post-testing levels. The mean increase to physical exercise in prolactin

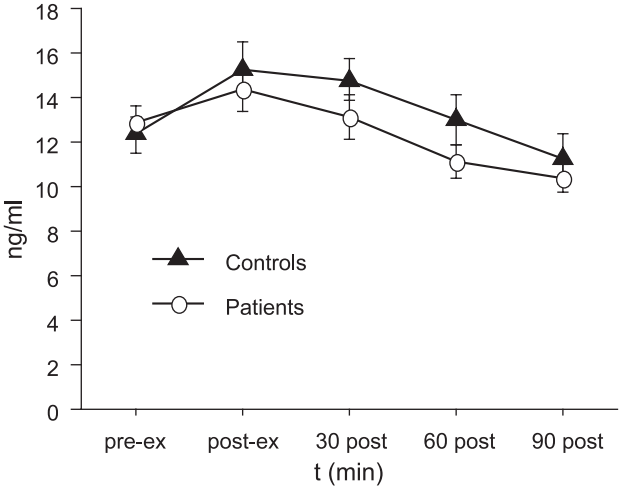

Fig. 2. Cortisol response to physical exercise in depressed patients and controls. Pre-ex refers to mean basal value of plasma cortisol assessed before exercise, post-ex refers to mean plasma cortisol value assessed immediately after exercise; 30 post, 60 post and 90 post refer to mean plasma cortisol values assessed after exercise with 30-min intervals.

was 1.44 ng/ml (Fig. 3). There were no significant differences in growth hormone, cortisol or prolactin responses to physical exercise between subgroups of patients treated with different antidepressants and drug-free patients, but the subgroups were too small to allow a conclusive statistical analysis. Nevertheless, all these subgroups of patients had on average higher elevation of prolactin levels than controls, and this elevation was of similar magnitude except for the small (n=3) subgroup of patients on mirtazapine who had the highest prolactin response.

3.3. Exercise testing characteristics and hormone secretion

There was no significant difference in working rate between the controls and the patients (714.6±186.7 and 693.7±145.4 kg/min, respectively).

The mean $VO_{2\ peak}$ was 34.9±2.3 and 29.1±1.3 ml/kg/min in controls and patients, respectively. Thus, mean $VO_{2\ peak}$ was significantly higher in controls than in patients (F(1,45)=7.6; P<0.05). However, $VO_{2\ peak}$ as a covariate did not eliminate the significant difference in prolactin response between the groups.

$VO_{2\ peak}$ was significantly positively correlated with changes in growth hormone, but not cortisol or prolactin levels during exercise in both controls and patients: r=0.54, P<0.05 and r=0.48, P<0.05, respectively. No differences between the two groups' ventilation or maximal heart rate were found.

3.4. Age and hormone secretion

In the group of patients, but not in controls, age was significantly negatively correlated with growth hormone response to physical exercise, r=−0.46, P<0.05. No such a correlation was found between age, cortisol and prolactin levels.

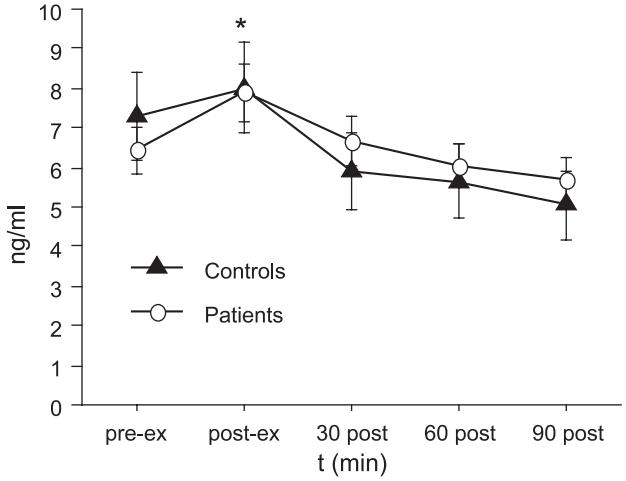

Fig. 3. Prolactin response to physical exercise in depressed patients and controls. Pre-ex refers to mean basal value of plasma prolactin assessed before exercise, post-ex refers to mean plasma prolactin value assessed immediately after exercise; 30 post, 60 post and 90 post refer to mean plasma prolactin values assessed after exercise with 30-min intervals. Prolactin levels were elevated only in the group of patients: P<0.01 between pre- and post-testing levels.

3.5. MÅDRS-S score and hormone secretion

The depressed patients scored significantly higher in MÅDRS-S than controls: (F(1,44)=39.2, P<0.0001). The mean scores (±S.D.) of MÅDRS-S were 5.5±4.0 in controls and 18.3±8.5 in patients.

All subjects were divided into low or high scorers in MÅDRS-S on the basis of median value of the sample. Low scorers were those who have MÅDRS score 11 or less (n=19). Two persons from the control group had MÅDRS-S scores 12 or more and three persons from patients' group had MÅDRS-S scores below 11. There were no differences in baseline plasma hormone levels or in growth hormone and cortisol response to physical exercise between low or high scorers. Prolactin levels were increased during the exercise only in the group of high MÅDRS-S scorers: t=−2.66, df=20, P<0.01. Thus, group comparison of the high and low scorers on MÅDRS-S revealed similar outcome as the comparison of the patients and controls.

4. Discussion

4.1. Pre-exercise growth hormone, cortisol and prolactin levels

We found no differences in baseline growth hormone, prolactin or cortisol levels between patients and healthy volunteers. This result is not unexpected: many studies have found that baseline growth hormone levels are similar in depressed patients compared to controls (Fiasche et al., 1995) and several authors have also reported no differences in baseline prolactin levels between depressed patients and controls (Riedel et al., 2002; Sagud et al., 2002). No group differences in baseline cortisol levels were expected, since previous research indicates that cortisol levels in normal adults are higher in the morning and were likely to be maximal at the time of the current experiment (Kanaley et al., 2001). A positive correlation between the scores on the MÅDRS and cortisol levels has been described in women (Sagud et al., 2002), but the present study did not find evidence for such a correlation in men.

4.2. Growth hormone, cortisol and prolactin secretion in response to physical exercise

Acute physical exercise is a well-known stimulus to growth hormone secretion (Hartman et al., 1993; Giustina and Veldhuis, 1998). Plasma growth hormone was significantly elevated after physical exercise in controls as well as in patients. Following the increase in plasma growth hormone secretion associated with aerobic exercise, growth

hormone release decreased to baseline levels. In our previous study (Harro et al., 1999), lower response of growth hormone secretion to physical challenge in volunteers with psychometrically measured depressiveness was found. Therefore, we hypothesized that depressed patients would have a significantly lower growth hormone response to exercise than healthy volunteers. However, in the present study, there were no differences between healthy volunteers and depressives' mean growth hormone secretion increase in response to physical exercise. This discrepancy may be explained by the antidepressant treatment of depressed patients, which may influence neuroendocrine functioning via modulation of several neurotransmitter systems. Also it should be noted that the growth hormone increase was small in the present study.

The results of the present study that acute bicycle exercise activates the HPA axis resulting in rapid increases in plasma cortisol were also expected. However, there were no differences between the two groups' mean cortisol secretion increase in response to physical exercise.

In contrast, prolactin levels were elevated by exercise only in the patients' group. Since multiple neural pathways which influence prolactin secretion converge on the hypothalamus from other parts of the brain, the effect of exercise on the secretion of prolactin may also reflect the action of different neural inputs on the activity of the hypothalamic-pituitary axis. Thus, we do not know at present which neurochemical mechanisms mediate the difference in prolactin response to physical exercise in depressed patients, but would like to discuss the possible role of serotonin. Serotonergic neurons originating from the dorsal raphe nucleus and terminating in the hypothalamus stimulate the secretion of prolactin (Van de Kar et al., 1996). It is also possible that prolactin release in response to stress is mediated via 5-HT release in the hypothalamic paraventricular nucleus (Minamitani et al., 1987). Thus, altered regulation of the serotonergic system may have a role in response to exercise stress.

In nondepressed humans, administration of L-tryptophan, a serotonin precursor, produces an increase in plasma prolactin concentrations. Prolactin responses to L-tryptophan are reported to be blunted in depressed patients (Cowen and Charing, 1987; Price et al., 1991). However, a significant minority of studies have not found blunted prolactin response (Park et al., 1996). Interestingly, a recent study investigating 5-HT_{1A} receptor function in depression has reported an enhanced prolactin response to L-tryptophan in depressed patients compared with a matched control group (Porter et al., 2003). The study was performed with unmedicated subjects and there was also no difference between patients and control subjects in cortisol levels in this study. As the 5-HT_{1A} receptor function and prolactin release at the pituitary level may be reduced by cortisol, the enhanced prolactin response to L-tryptophan in depressed patients may also be explained by the lack of hypercortisolaemia. Another possible explanation of enhanced prolactin response is being secondary to a greater reduction in dopaminergic inhibition in the depressed group as it is known that dopaminergic transmission is increased by corticosteroids (Wolkowitz et al., 1985). The results of our present study are similar to the results of the study of Porter et al. (2003). Similarly to the study of Porter et al. (2003), no differences in two groups' baseline cortisol levels were found. However, the patients in our study were on various antidepressants and in different stages of treatment response. Some studies have found enhancement of fenfluramine induced prolactin release after antidepressant treatment including clomipramine and fluoxetine (O'Keane et al., 1992; Shapira et al., 1992), others have not found such an effect with fluoxetine (Kavoussi et al., 1999). A study using L-5-hydroxytryptophan also demonstrated an increase in prolactin and cortisol response in fluoxetine-treated patients compared to tricyclic or unmedicated subjects (Meltzer et al., 1997). Comparison of untreated subjects with antidepressant-treated patients naturally is confounded by the pharmacological actions, also in the present study. However, in the present study, the higher prolactin response in patients does not appear to be due to antidepressant treatment, because there was no apparent distinction between subgroups of patients treated with different antidepressants or being drug-free. Given the small number of drug-free controls, the role of a pharmacodynamic effect is not excluded, but the similarity of data of patients treated with different drugs suggests that the effect would not be on a single molecular target.

4.3. Exercise testing characteristics, age and hormone secretion

Several investigators have suggested that intensity, work output, duration of acute exercise and used muscle mass may all alter growth hormone response to exercise (Weltman et al., 1992). Mean peak oxygen consumption was significantly higher in controls than in patients. $VO_{2\ peak}$ was significantly positively correlated with growth hormone release during exercise in both controls and patients. However, the increase of growth hormone release during the exercise was similar to the control group. This indirectly suggests that the exercise loads were comparable for the two groups in respect to the capabilities of the subjects.

It has been shown by several researchers that aging is associated with a diminution of growth hormone secretion (Holt et al., 2001). Also, in the present study, participants were significantly older which may affect the results: it is found that growth hormone response to exhaustive exercise is much lower in older men than in younger men (Zaccaria et al., 1999) and the magnitude of growth hormone release is reduced by four- to seven-fold in older individuals compared with younger individuals (Wideman et al., 2002). In our previous study (Harro et al., 1999), increase of growth hormone secretion in response to maximal exercise was higher than in the present study.

4.4. MÅDRS-S score and hormone secretion

As expected, patients had significantly higher scores on MÅDRS-S scale than healthy controls. When divided into groups by median scores, three persons from the patients' group moved to the low score group, and two persons from the control group moved to the high score group. The reasons for this may be the onset of the effect of antidepressant treatment or individual differences of patients and perhaps the presence of the subjective depressive symptoms among some of the volunteers, which did not meet diagnostic criteria of depressive disorder. However, this adjustment of the groups had no effect on the hormone responses to maximal exercise, compared to the original patients vs. controls design.

5. Conclusions

To conclude, in the present study, no differences in growth hormone and cortisol responses to physical exercise were found between healthy volunteers and depressives. However, prolactin levels were elevated only in the depressed patients group during the exercise. This result suggests that acute exercise may increase 5-HT availability in depressed patients, which is reflected by increased plasma prolactin concentration.

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Changes in platelet monoamine oxidase activity, cholesterol levels and hyperactive behaviour in adolescents over a period of three years

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Abstract

Platelet monoamine oxidase (MAO) activity is a peripheral marker of central serotonergic activity, and has been associated with aggressive, impulsive and hyperactive behaviour, alcohol and drug abuse. Central serotonergic activity has also been associated with plasma cholesterol levels. In the present longitudinal investigation in adolescents ($n = 320$) changes in platelet MAO activity and in plasma cholesterol levels over three years were measured, and their possible association with changes in aggressive and hyperactive behaviour, smoking, alcohol and drug use was studied. The measures were taken at age 15 and 18 years. Psychological data were obtained from teachers by using the Hyperactivity Scale [B. af Klinteberg, Studies on Sex-related Psychological and Biological Indicators of Psychosocial Vulnerability: A Developmental Perspective, University of Stockholm, Department of Psychology, 1988]. The results of the study show that in most of the tested individuals, platelet MAO activity is a relatively stable measure, however, there was a significant number of subjects with a noticeable change in MAO activity. In subjects with decreased platelet MAO activity, total and HDL cholesterol levels were significantly increased. Also, changes in HDL cholesterol and in platelet MAO activity were inversely associated with changes in the score of Concentration Difficulties. The changes in platelet MAO activity and cholesterol level were not associated with alcohol and drug use among the subjects. This longitudinal analysis provides preliminary evidence that changes in platelet MAO activity and cholesterol, which may reflect changes in central serotonergic activity are associated with attention deficit in adolescents.

Keywords: Monoamine oxidase; Cholesterol; Concentration difficulties; Alcohol; Illicit drugs; Smoking

Experimental and clinical findings suggest that platelet monoamine oxidase (MAO) isoenzyme B activity is a genetic marker for the capacity of the serotonergic (5-HT) activity in the CNS [10,23]. The positive association between platelet MAO activity and 5-HT turnover is believed to occur via common gene promoter sequences and co-regulation of the platelet MAO gene and some unidentified gene-coding proteins important in 5-HT-ergic neurotransmission [23]. Platelet MAO activity is characterized by a considerable variability between individuals, compared to inter-individual variation, which has been reported to be low [4,20]. Subjects with low-platelet MAO activity are more likely to score high in risk-taking, sensation seeking or novelty seeking in personality questionnaires [28,30,31,35]. Low-platelet MAO activity is also associated with alcohol and drug abuse [22]. As platelet MAO activity has been shown to be strongly genetically controlled [11,26], the association of this marker of central 5-HT-ergic activity is thought to reflect the heritable aspects of the link between 5-HT function and behaviour. There are several factors, which may affect platelet MAO activity including strenuous physical exercise [12], abstinence reaction after alcohol abuse [3], Vitamin B12 deficiency [27], and pregnancy [34]. Also, platelet MAO activity has been shown to be inhibited by cigarette smoke in dose-related manner, and individuals who had stopped smoking, have MAO levels similar to non-smoking subjects [37]. These short-term factors are not thought to influence the association of person-

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ality and behaviour with the CNS mechanisms reflected by the enzyme activity in "normal" subjects, as platelet MAO activity is usually considered not a causal factor in behavioural regulation but a mirror of as yet unidentified mechanisms controlling central 5-HT-ergic activity.

Nevertheless, it still remains to be studied whether long-term changes in personality and behaviours, which are associated with central 5-HT-ergic mechanisms are reflected in changes in platelet MAO activity. Time periods between MAO activity measurements in the few studies examining inter-individual stability of platelet MAO have been relatively short, typically from 8 to 10 weeks, and the number of subjects has been small [4,20]. The largest study which re-examined platelet MAO activity in 72 subjects with relatively high- or low-enzyme activity over a period of 9–12 months found a "regression toward the mean" and concluded that environmental factors modulate the genetically determined platelet MAO activity [11]. Whether such changes have behavioural correlates is not known.

Low-brain 5-HT activity is causally related to impulsivity and violent behaviour [36]. Behaviours associated with low-5-HT neurotransmission have also been observed in persons with low cholesterol. Many studies have reported a significant relation between low-cholesterol levels, impulsivity and violent acts [7,13,21]. A relationship between 5-HT and cholesterol has been postulated and discussed by several authors: it has been suggested that low cholesterol and decreased 5-HT neurotransmission may be linked to each other [9,24,25,32] and to impulsive and aggressive behaviour [17]. Low-membrane cholesterol has been suggested to decrease the number of 5-HT receptors and therefore lowered plasma cholesterol concentration may contribute to a decrease in brain 5-HT [9,16]. Excess cholesterol may adversely affect the function of membrane-bound 5-HT structures. However, it has recently been proposed that elevated cholesterol may also reduce 5-HT function either directly by binding to membrane-bound receptors or transporters or indirectly by altering the fluidity of the neuronal membrane and thereby their conformation [24].

The aim of this investigation was to examine the changes in two peripheral markers of 5-HT-ergic activity, platelet MAO activity and plasma cholesterol levels, and their possible associations with changes in such 5-HT-related behavioural measures as aggressive and hyperactive behaviour, smoking, alcohol and illicit drug use, in a longitudinal study of a representative sample of adolescents.

The details of original sample formation [15] and the follow-up study [14] have been described previously. The data were collected from a regionally representative sample of healthy adolescents in 1998/1999 ($n = 584$) when subjects were on average 15.4 (S.D. = 0.5) years old, and again three years later, in 2001/2002 when the subjects were 18.1 (S.D. = 0.6) years old. In the present analysis data of 320 adolescents (132 boys, 188 girls) from the Tartu city and county, Estonia, were used. The present analysis included all adolescents who had platelet MAO activity measured both at age 15 and 18. Platelet MAO activity was not measured in subjects who were inaccessible at the follow-up or did not agree with venipuncture. Parents and children gave their written informed consent before participating in the study, which was conducted according to the protocol approved by Ethics Review Committee on Human Research of the University of Tartu.

Ratings on aggressiveness, motor restlessness and concentration difficulties both at age 15 and 18 were obtained from the class teachers, using the seven-point Hyperactivity Scale described by af Klinteberg [1]. Hyperactivity score was calculated as in af Klinteberg and Oreland [2] by summing the scores of two of the three subscales, Motor Restlessness and Concentration Difficulties subscales. Data of Aggressiveness, Motor Restlessness and Concentration Difficulties obtained at age 15 and 18 were available about 314 and 207 adolescents, respectively. At age 18, subject filled in the self-report format of the Montgomery–Åsberg Depression Rating Scale (MÅDRS) [19].

Adolescents reported alcohol, illicit drug use and their smoking habits in a questionnaire in a laboratory out of school with no teachers and parents present both at age 15 and 18. Four levels of smoking were defined: (1) never tried smoking, (2) have tried, but not smoking currently, (3) irregular smoking (up to twice a week) and (4) regular smoking (smoking every day or almost every day). For additional data analysis, subjects who reported that they smoke either irregularly or regularly were considered as smokers. Data about smoking at age 15 were available for 318 adolescents and at age 18 for 309 adolescents.

Adolescents reported if they had tasted alcohol, and how often do they consume cider, beer, wine, and strong spirits. The frequency of the most often consumed type of alcohol ranging from 1 (never) to 5 (every day) was used to form the total alcohol consumption score. Data about alcohol usage at age 15 were available for 319 adolescents and at age 18 for 302 adolescents.

At age 15 adolescents reported also how many times they had tried illicit drugs and if they were current users, forming a score from 1 (never tried) to 4 (current user). At age 18 adolescents reported if they had tried illicit drugs and how often do they use marijuana, cocaine, steroids, amphetamines, heroin, barbiturates, inhalants or prescriptional drugs without doctor's orders. The frequency of the most often used drug ranging from 1 (never tried) to 8 (I use it every day) was used to form the total illicit drug use score. Data about illicit drug usage at age 15 were available for 312 adolescents and at age 18 for 303 adolescents.

MAO activity in platelets was also measured twice with the three years interval as previously described [15]. The substrate used was ^{14}C-labelled β-phenyl-ethylamine ("Amersham", UK). All experiments were performed in duplicate and the enzyme activity is expressed as nanomoles of substrate oxidized per 10^{10} platelets per minute. The intra- and inter-assay variation coefficients for platelet MAO activity were 6.9 and 4.1%, respectively. Fasting basal

cholesterol (total and HDL) was measured by conventional techniques.

Differences in changes of the scores of Aggressiveness, Motor Restlessness, Concentration Difficulties and Hyperactivity between decreased, unchanged and increased MAO activity groups were tested by means of analysis of variance (ANOVA) with post hoc Fisher's PLSD test. ANOVA was also used to study the differences in total and HDL cholesterol levels between MAO activity groups. Differences in platelet MAO activity and behavioural ratings at age 15 and 18 were studied by paired and unpaired *t*-tests. Pearson correlation was used to test the potential associations between behavioural measures, plasma cholesterol and MAO activity. Regression analysis was used to model the relationship between behavioural measures and physiological variables.

Mean platelet MAO activity (±S.D.) measured at age 15 and 18 in boys and girls was 9.38 ± 3.03 nmol 10^{10} platelets^{-1} min^{-1} and 10.28 ± 3.15 nmol 10^{10} platelets^{-1} min^{-1}, and 9.54 ± 3.29 nmol 10^{10} platelets^{-1} min^{-1} and 10.97 ± 3.00 nmol 10^{10} platelets^{-1} min^{-1}, respectively. Significant negative correlations were found between platelet MAO activity and the scores of Motor Restlessness and Concentration Difficulties measured at age 15 ($r = -0.17$, $p < 0.05$ and $r = -0.20$, $p < 0.005$, respectively), but not at age 18. The correlation between two MAO measurements with three years interval was 0.56. The percentual change in platelet MAO activity over three years was calculated as "(MAO activity measured at age 18 minus MAO activity measured at age 15) × 100/MAO activity measured at age 15" (Fig. 1). It can be observed that whereas the majority of subjects had similar platelet MAO values at age 15 and 18 years, there were significant numbers of subjects who had MAO activity values either increased or decreased considerably more than inter-assay variation would allow. An increase or a decrease in enzyme activity was defined as belonging of the subject to the first or the fourth quartile of the sample. Because males are known to have, on average, significantly lower platelet MAO activity than females, the division of the subjects into quartiles was carried out in both sexes separately. The decreased MAO group includes boys who had the enzyme activity decreased at least by 18% and girls with at least a 12% decrease. The increased MAO group includes boys with at least 23.5% and girls with at least 31% increase in platelet MAO activity. Significant negative correlation was found between the change in platelet MAO activity and in the change in the score of Concentration Difficulties ($r = -0.16$, $p < 0.05$) but not Aggressiveness, Motor Restlessness or Hyperactivity. When boys and girls were analyzed separately, the significant negative correlation between changes in Concentration Difficulties and platelet MAO activity was significant only in girls ($r = -0.19$, $p < 0.05$). Analyzing the change in the score of Concentration Difficulties separately in groups based on change of platelet MAO activity indicated that concentration difficulties were significantly decreased only in the subjects with increased platelet MAO activity ($t = 2.36$, d.f. = 61, $p < 0.05$) but not in the subjects with decreased or unchanged MAO activity. No differences were found in change of alcohol and illicit drug use between the groups of decreased, unchanged and increased platelet MAO activity. There was a significant negative correlation between changes in platelet MAO activity and smoking behaviour over three years ($r = -0.13$, $p < 0.05$) reflecting the reduction in platelet MAO activity by more frequent smoking, but this association was weak probably because of smoking intensity was in most cases insufficient to reduce MAO activity.

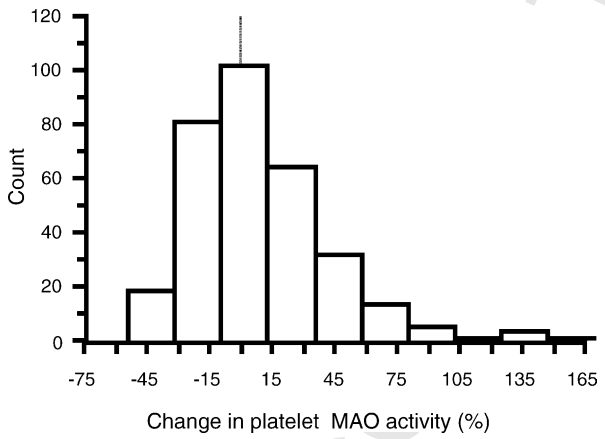

Fig. 1. Frequency distribution of percentual change in platelet MAO activity over three years ($n = 320$).

Mean total and HDL cholesterol levels (±S.D.) measured at age 15 in boys and girls were 3.92 ± 0.67 mmol/l and 4.34 ± 0.76 mmol/l, and 1.33 ± 0.26 mmol/l and 1.42 ± 0.28 mmol/l, respectively. At age 18, mean total and HDL cholesterol levels (±S.D.) measured in boys and girls were 4.11 ± 0.72 mmol/l and 4.59 ± 0.80 mmol/l, and 1.32 ± 0.28 mmol/l and 1.53 ± 0.32 mmol/l, respectively. Boys had significantly lower cholesterol (total and HDL) levels than girls both at age 15 and 18. Significant negative correlation was found between total and HDL cholesterol levels and the score of Motor Restlessness measured at age 18 ($r = 0.14$, $p < 0.05$) but not at age 15. A significant correlation was found between total and HDL cholesterol levels measured at age 15 and 18, $r = 0.65$ and 0.66, $p < 0.0001$, respectively, the mean increase was 0.23 mmol/l (S.D. = 0.66) and 0.059 mmol/l (S.D. = 0.025) in total and HDL cholesterol, respectively. Changes in total and HDL cholesterol levels were calculated as cholesterol level measured at age 18 minus cholesterol level measured at age 15. There was no significant difference in total cholesterol change between boys and girls. However, a significant difference was found in the change of HDL cholesterol levels between boys and girls $t = -4.34$, d.f. = 312, $p < 0.0001$. Mean HDL cholesterol levels were less increased in boys than in girls. A significant negative correlation between changes in total cholesterol levels and Motor Restlessness ($r = -0.24$, $p < 0.05$) was found in boys. In girls, significant negative correlations were found between the changes in HDL cholesterol and ratings of Aggressiveness ($r = -0.24$, $p < 0.01$), Motor Restlessness

($r = -0.19$, $p < 0.05$) and Hyperactivity ($r = -0.19$, $p < 0.05$). In the overall sample, borderline significant correlations were found between changes in total and HDL cholesterol levels and ratings of Motor Restlessness (−0.13 and −0.14, $p < 0.07$, respectively). The scores of Aggressiveness, Motor Restlessness, and Hyperactivity had significantly declined with age in the sample, but decrease in the score of Concentration Difficulties was statistically not significant. The score of Aggressiveness had declined less among girls ($p < 0.05$), however, boys were rated more aggressive than girls both at age 15 and 18. No sex-related differences were found in changes of Motor Restlessness, Concentration Difficulties or Hyperactivity scores.

Cross-sectionally, there was no correlation between platelet MAO activity and cholesterol levels. ANOVA revealed a significant difference in total cholesterol changes between groups based on change of platelet MAO activity $F(2, 311) = 3.14$, $p < 0.05$. Fisherĭs PLSD test indicated that total cholesterol levels were significantly less increased in the groups with unchanged and increased MAO activity compared to the group of individuals with decreased MAO activity (Fig. 2a). Also, significant differences were found in change of HDL cholesterol levels between MAO groups $F(2, 311) = 3.30$, $p < 0.05$. Again, HDL cholesterol levels were significantly more increased in the group with decreased MAO activity (Fig. 2b). Exclusion of regular smokers from the statistical analysis ($n = 9$, 13, and 9) in the groups with decreased, unchanged, and increased MAO activity, respectively, did not influence the outcome. When boys and girls were analyzed separately, there were very similar trends in both sexes, but there were no group differences due to reduced statistical power. Regression analyses were performed for behavioural changes as dependent variables with gender and changes in HDL and total cholesterol levels, MAO activity, smoking behaviour included as independent variables. According to the multiple regression analysis, decreases in HDL cholesterol level (regression coefficient (S.E.) = -1.27 ± 0.60, $p < 0.05$) and in platelet MAO activity (regression coefficient (S.E.) = -0.009 ± 0.004, $p < 0.01$) were associated with an increased score of Concentration Difficulties. Regression models for changes in the scores of Aggressiveness, Motor Restlessness and Hyperactivity were statistically non-significant. Regression models for changes in alcohol and illicit drug use with gender and changes in HDL and total cholesterol levels, MAO activity, frequency of smoking, included as independent variables were non-significant.

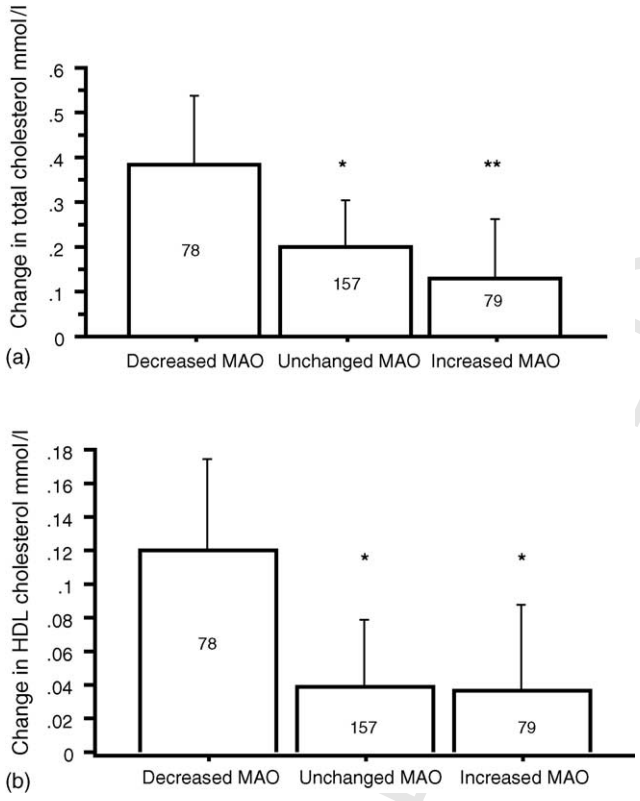

Fig. 2. Mean (error bars: 95% confidence interval) changes of total (a) and HDL cholesterol (b) levels in groups of adolescents with decreased, unchanged and increased platelet MAO activity over the period of three years. $^{*}p < 0.05$ and $^{**}p < 0.01$ different from the decreased platelet MAO group. Numbers printed on columns represent the number of participants in the particular MAO group.

The possible effect of mood at age 18 on the associations between concentration difficulties, platelet MAO activity and cholesterol levels was examined by using the MÅDRS scores. Cholesterol levels, platelet MAO activity and concentration difficulties at this age and changes in three years in these measures did not correlate with the MÅDRS score, and adding MÅDRS score as a covariate to the ANOVA analysis when studying the associations between the changes did not reveal any significant effect of mood.

The results of our study show that in most of the tested individuals, platelet MAO activity is a relatively stable measure. However, there were a significant number of subjects with large intra-individual variability of MAO activity over three years. Whether this reflects changes in their central 5-HT-ergic activity is not known but cannot be excluded. The change in platelet MAO activity was not associated with a change in the frequency of alcohol and drug use among the subjects. We have previously shown that platelet MAO activity has no independent association with alcohol and drug use in children and adolescents [18], and thus the link between platelet MAO activity and alcohol abuse repeatedly observed in adults [33] may rather be based on association of MAO activity with vulnerability to abuse or antisocial behaviour than with early experimentation with drugs. Cigarette smoking reduces platelet MAO activity directly [6] but this effect appears to be significant in subjects smoking more than 10 cigarettes per day [8,37]. That the effect of smoking in the present study was limited was also confirmed by the significant but very weak relationship found between changes in frequency of smoking and platelet MAO activity. Changes in platelet MAO were not significantly related to the reduction in Aggressiveness and Motor Restlessness. An increase in platelet MAO activity was associated with a decrease of con-

centration difficulties in the sample of girls but not in boys. Subjects with decreased or unchanged platelet MAO activity did not have such a reduction.

Plasma total and HDL cholesterol levels had significantly increased from age 15 to 18 among the subjects. These changes could be related to both age and maturation and likely reflect the influence of sex hormones on plasma lipoprotein metabolism [5]. Negative correlation between changes in total cholesterol level and motor restlessness was found in boys. In girls, negative correlation between changes in HDL cholesterol, aggressiveness, motor restlessness, and hyperactivity was found. These correlations, however, may also reflect developmentally occurring biochemical and behavioural changes during adolescence and not indicate a causal relationship. We failed to find any association between cholesterol levels and more complex behaviours such as alcohol and illicit drug use.

Even though there was no correlation between cholesterol levels and platelet MAO activity at a given age, which suggests that these two peripheral indicators of 5-HT-ergic neurotransmission are probably independent, a change in platelet MAO activity was associated with changes in total and HDL cholesterol levels with higher cholesterol increase in subjects with decreased platelet MAO activity. Lower platelet MAO activity and cholesterol levels have both been associated with lower central 5-HT-ergic activity. Nevertheless, there is also evidence of possible negative relationship between plasma cholesterol levels and central 5-HT function [24]. The present results provide additional support to the notion that cholesterol levels and 5-HT-ergic activity may be related in a non-linear manner. Mechanisms underlying the association between cholesterol and 5-HT activity have not yet been elucidated, however, it has been suggested that reduced cholesterol may alter membrane fluidity, viscosity and function, including the function of 5-HT receptors and 5-HT transporter [9,29]. Also, high cholesterol levels may lead to lower 5-HT receptor sensitivity or 5-HT transporter activity [24]. With regard to platelet MAO activity as a peripheral marker of central 5-HT-ergic activity, researchers have found that common transcriptional factors are regulating both the expression of platelet MAO and components of the central monoaminergic systems. Furthermore, it has been proposed that such transcription factors may not directly regulate platelet MAO expression, but rather mitochondrial density, or outer mitochondrial membrane surface [22]. It could thus be speculated that changes in cholesterol levels modify function of 5-HT neurons, which elicits secondary changes in recruitment of transcription factors influencing simultaneously both regulatory genes of 5-HT neurons and the gene coding for MAO-B.

The limitations of this study include missing data which reduce the sample size, and the possibility of Type I error. Because about 35% of the original sample did not participate in the second study wave mainly because they had changed the school, the sample of the longitudinal analysis was of reduced size and representativeness. This should, however, only reduce the variations within the group. Given the number of analyses performed, the results may also be because of a Type I error and need independent replication. However, it should be noted that changes in both total and HDL cholesterol were similarly related to changes in platelet MAO activity. Behavioural measures in this study were obtained from teachers, which may influence the outcome but is probably a more reliable assessment of concentration difficulties and hyperactivity that the rating by the adolescents themselves. Studies on adults have found moderate positive correlations between self- and informant ratings on concentration difficulties and hyperactivity, but informants have revealed more significant inattentive symptom severity [37].

In conclusion, this longitudinal analysis provides preliminary evidence that changes in platelet MAO activity and cholesterol level are independently associated with attention deficit in adolescents.

Uncited reference

[38].

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