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CONTENTS

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I Kivastik, T., Vuorikallas, K., Piepponen, T. P., Zharkovsky, A., Ahtee, L. Morphine- and cocaine-induced conditioned place preference: effects of quinpirole and preclamol. Pharmacology Biochemistry and Behavior, 54: 371–375, 1996.

II Kivastik, T., Rutkauskaite, J., Zharkovsky, A.
Nitric oxide synthesis inhibition attenuates morphine-induced place preference. Pharmacology Biochemistry and Behavior, 53: 1013–1015, 1996.

III Zharkovsky, A., Moisio, J., Kivastik, T., Ahtee L.
Role of dopamine receptors in the dual effect of naloxone on quinpirole-induced yawning in morphine pretreated rats. Naunyn — Schmiedeberg's Archive of Pharmacology, 347: 478–482, 1993.

IV Piepponen, T. P., Katajamäki, J., Kivastik, T., Zharkovsky, A., Ahtee, L. Behavioural and neurochemical sensitization of morphine-withdrawn rats to quinpirole. Pharmacology Biochemistry and Behavior, 54: 787–792, 1996.

V Piepponen, T. P., Kivastik, T., Katajamäki, J., Zharkovsky, A., Ahtee, L. Involvement of opioid μI-receptors in morphine-induced conditioned place preference in rats. Submitted to Pharmacology Biochemistry and Behavior. Accepted 24 November 1996

ABBREVIATIONS

DPDPE	[D-Pen^2,D-Pen^5]enkephalin
DSLET	[D-Ser^2,Leu^5]enkephalin-Thr^6
GABA	gamma-aminobutyric acid
ICV	intracerebroventricular(ly)
IP	intraperitoneal(ly)
IV	intravenous(ly)
L-NOARG	N$^{\omega}$-nitro-L-arginine
NMDA	N-methyl-D aspartate
6-OHDA	6-hydroxydopamine
7-OH-DPAT	7-hydroxy-(2-N,N-dipropylamino)-tetraline
SC	subcutaneous(ly)
VTA	ventral tegmental area

1. INTRODUCTION

> "For thousands of years we have pursued altered mental states and other-wordly insights, whether through prayer and meditation, through art or sexual ecstacy, or through psychoactive substances" (R.Campbell-Johnston, The Times, August 14, 1996).

Drug addiction is defined as a behavioral syndrome consisting of compulsive pattern of drug use, characterized by overwhelming involvement with the use of a drug, the securing of its supply, and a high tendency to relapse after withdrawal [abstinence] (Jaffe, 1975). In other words, in the case of addiction the use of a drug appears to dominate over behaviors that once had a higher value, the ones essential for the organism's survival and well-being included. This intrinsic feature of addiction is well characterized by the term *motivational toxicity* (Bozarth and Wise, 1985).

In the contemporary world drug addiction has become a major health problem. Despite the vast amount of scientific information available, the ability of drugs to create addiction is still poorly understood, and research in this area is characterized by controversial findings. Furthermore, no effective treatment is available as yet.

2. REVIEW OF LITERATURE

2.1. REINFORCEMENT THEORY

The most popular approach has been the behavioristic one, that is, to analyze the addiction process within the framework of drug reinforcement theory (which is an extension of operant psychology). Accordingly, drug addiction is viewed as a behavior controlled by its consequences, which means that "... the behavior of drug taking is governed by the direct and immediate consequences of that behavior — drug administration and the ensuing pharmacological actions of the drug" (Bozarth, 1987). The term *reinforcement* is used in order to describe the relationship between the behavior and its consequences. By nature reinforcement can be either positive or negative. In the case of positive reinforcement the presentation of a stimulus (i.e., the drug) increases the frequency (or probability) of the behavior that presentation of the stimulus is contingent upon; whereas negative reinforcement refers to a situation in which the frequency of the behavior is increased by the *removal* of the stimulus (e.g., withdrawal syndrome). It is important to note that reinforcement merely describes the changes in the probability of behavior and does not offer any physiological or psychological explanations to this.

Reward is another term that is widely used in the present context. It is, unfortunately, somewhat ill-defined and therefore used in the literature in many different meanings. Many researchers refer to reward interchangeably with reinforcement, and the present study will do the same.

2.2. PRINCIPAL MODELS OF DRUG ADDICTION

There are two general models of drug addiction that have evolved from reinforcement theory. According to the negative reinforcement model, addictive behavior (drug seeking and drug taking) is sustained because of the state that is alleviated by drugs. In this model the central role is given to the aversive consequences of drug withdrawal. The second model concentrates on the positive reinforcing properties of a drug, asserting that addictive behavior is sustained not because of the condition that the drugs remove or lessen, but because of the state the drugs produce.

Traditional negative reinforcement model, focusing on withdrawal and tolerance, though having predominated previously, has been challenged both experimentally and clinically (for discussion see Wise and Bozarth, 1987; Robinson and Berridge, 1993). For example, as noted by Wise and Bozarth (1987), "physical dependence is neither a necessary nor sufficient condition for drug addiction". This is not to deny any role at all for the withdrawal distress in addictive behavior, but it is evidently not the most important factor. In fact, addiction is nowadays regarded as an integrated process, containing both positively and negatively reinforcing components; however, a substantial body of evidence suggests an intrinsic role for positive reinforcing properties of drugs (Wise and Bozarth, 1987; Bozarth, 1994). These have been attributed to the drug-induced pleasurable effects (e.g., Wise, 1987), yet the question is far from clearness. Thus, it is currently generally accepted that drugs may serve as positive reinforcers, but whether the underlying reason is hedonic by nature or, for instance, a relatively autonomic drug craving as proposed by Robinson and Berridge (1993), remains a matter for discussion.

2.3. ROLE OF SENSITIZATION

Some behavioral effects (e.g., locomotor activation) of opioids and psychomotor stimulants are increased upon repeated intermittent administration. Such a phenomenon, called behavioral sensitization, is characteristic of many other addictive drugs as well (for review see Robinson and Berridge, 1993). Moreover, the neural substrates that mediate behavioral sensitization, at least to some extent coincide with those mediating the reinforcing properties (see below). It is unclear, however, whether behavioral sensitization reflects sensitization to the reinforcing properties of drugs. For instance, in the presence of sensitization to psychomotor effects of opioids, their positive reinforcing properties have been shown to be unchanged (Martin *et al.*, 1988) or to exhibit tolerance (Shippenberg *et al.*, 1988) or sensitization (Lett, 1989). Thus, the role of sensitization is fairly unclear hitherto, though some researchers suggest it be a major determinant of drug addiction (Lett, 1989; Robinson and Berridge, 1993)

2.4. ANIMAL MODELS OF DRUG ADDICTION

Most drugs that are addictive in humans can serve as reinforcers in other mammals. This fact refers to the involvement of brain mechanisms common to all mammals; on the other hand, it justifies the most widely implemented ap-

proach, that is, to study drug addiction using animal models that involve positive reinforcement processes. The following short description is restricted to the three major experimental paradigms, place conditioning (or, more exactly, conditioned place preference if one is concerned with positive reinforcement), drug self-administration, and intracranial self-stimulation. There are, of course, other methods in use, as for instance, drug discrimination. This method is unspecific to positive reinforcement, for besides the reinforcing properties, a myriad of other effects (as in the case of opioids are analgesia, changes in the autonomic nervous system, e.t.c.) could be responsible for stimulus cues. However, there appears to be a rather good concordance between the drug discrimination studies and human addiction liability (Bozarth, 1987).

Conditioned place preference: In this paradigm the classical (Pavlovian) conditioning is involved. The test apparatus is typically a box consisting of two distinct compartments, which usually differ in visual, tactile and/or olfactory cues, and may be connected by a "neutral" section. The drug administration is repeatedly paired with one of the compartments, whereas the other one is paired with the injections of drug vehicle; later the drug-free animals are given free choice between both compartments. The drug is presumed to be positively reinforcing if the animals increase the time spent in the compartment that was associated with the drug. Thus, in the case of conditioned place preference the drug acts as an unconditioned stimulus and the drug-related environment as a conditioned one; i.e., the environment acquires reinforcing properties through conditioning.

Conditioned place preference is relatively quick and simple, and thus probably the most valuable method to preliminarily screen the drugs for addiction liability, as well as in the development of new compounds in the treatment of addiction. Further, the method appears to be specific for positive reinforcing effects of drugs. Because the animals are tested in an undrugged state, the drug's direct influence on sensory and motor processes may be ruled out. Other considerable alternatives are in some instances, the antiaversive properties of drugs (see below), and the effect of novelty, the significance of which, however, has been challenged by several studies (Mucha *et al.*, 1982; Mucha and Iversen, 1984).

There are two principal ways to run place conditioning, the so called unbiased and biased (or balanced and unbalanced) paradigms. In the case of the latter paradigm the animals exhibit an initial preference for one of the compartments, whereas in the former one it is absent. The biased type of procedure has been a matter for discussion because antiaversive rather than positively reinforcing properties of a given drug may be regarded as determinative (van der Kooy, 1987). Yet, the problem with the unbiased procedure is that it often appears to involve additional biases rather than removing the bias, making thus the situation even more complicated (Bozarth, 1987). An optimal way has been proposed to counterbalance the drug treatment between the nonpreferred and

preferred sides (Bozarth, 1987). With opioids, however, the experiments comparing the biased and counterbalanced procedures have provided consistent results (Blander *et al.*, 1984; Mucha and Iversen, 1984).

Drug self-administration: There are several self-administration procedures, which comprehend intravenous, intracranial, oral, and some others. Of these, the intravenous self-administration has been the most widely used procedure.

Intravenous self-administration can be viewed as an operant behavior, where the term *operant* indicates that the responses have defined reinforcing consequences and are instrumental in the attainment of a goal (Stolerman, 1992). In this procedure the drug is available for the experimental animals via implanted catheters, and the response, usually the leverpress, is followed by a drug injection. The drug is thought to serve as a positive reinforcer if the drug responding is higher than the response on a control lever (which does not result in drug administration); if response rate is higher when compared to the animals receiving injections of drug vehicle; or if the response rate is higher than in yoked controls, i.e., the animals receiving drug injection simultaneously with reinforced animals but independently of their own lever-pressing behavior (Yokel, 1987).

Intravenous self-administration is the most direct method to study drug reinforcement and has a high degree of validity. Though with some exceptions, drugs that are abused by humans serve as positive reinforcers in experimental animals in this paradigm (Yokel, 1987). On the other hand, the paradigm has some disadvantages in that it is sensitive to non-specific drug effects such as the influence on motor activity. Other problems are of a technical nature, for the intravenous preparation is relatively difficult to maintain, and the method is rather time-consuming (weeks or sometimes months of testing may be required for response patterns to stabilize).

Intracranial self-stimulation: This method is an indirect one. It involves training animals to work for electrical brain stimulation and determining the effects of drugs on this behavior. The drugs that serve as positive reinforcers appear to enhance or facilitate intracranial self-stimulation. This effect can be expressed either by the increased rate of lever-pressing in the case of fixed intensity brain stimulation, or as a lowering of current thresholds for brain stimulation. Such an enhancement of intracranial self-stimulation behavior has been demonstrated for practically all known drugs of abuse (Bozarth *et al.*, 1980). The problem with the method is that its neurochemical basis is relatively poorly understood. Furthermore, it may prove to be sensitive to the factors other than positive reinforcement. Among these are a nonspecific, stimulation produced energization and other manipulations that influence performance capacity, relief of anxiety or pain (Liebmann, 1989). There is, however, a reliable correlation between the positive reinforcing properties of drugs and their facilitative effect on intracranial self-stimulation behavior; likewise, the overall concordance with human addiction liability data is very good (Bozarth, 1987).

2.5. POSITIVE REINFORCING PROPERTIES OF MORPHINE: NEUROCHEMICAL BASIS

It is nowadays generally accepted that the drugs may serve as positive reinforcers because of their interaction with the endogenous reinforcement mechanisms in the brain (or, reward pathways as they are widely called; e.g., Wise, 1987; Koob 1992). An important fact is that the same pathways appear to govern the organism's normal behavior such as eating, drinking, mating behavior, predatory attack, and nest building (Glickman and Schiff, 1967; Stellar *et al.*, 1979) — the acts essential for individual and species survival. The following short review is concerned with the two major substrates, the endogenous opioid systems and the mesotelencephalic dopamine, which make up the endogenous reward pathways for many addictive drugs, opioids and psychomotor stimulants included (Koob, 1992). The role of a glutamate-related neurotransmitter, free radical gas nitric oxide, is also discussed. Certainly, other neurotransmitter systems are significantly involved, as, for instance, serotonin. This, however, remains beyond the scope of the present study.

The part of our work that concerns dopamine, involves the experiments with cocaine. Though the initial idea was to use cocaine as a reference psychomotor stimulant, it proved to be of great interest by itself. The dopaminergic mechanisms of cocaine reinforcement have been well demonstrated using the intravenous self-administration paradigm but not so clearly as far as the place conditioning is concerned. Therefore, also this problem will be briefly discussed.

2.5.1. Role of opioid receptors

The endogenous opioid systems, which are distributed throughout the central nervous system, constitute three distinct functional systems designated according to their precursor molecules: β-endorphin from proopiomelanocortin, enkephalins from proenkephalin, and dynorphins from prodynorphin (for review see Khachaturian *et al.*, 1993). These peptides exert their physiological actions by interacting with various classes of opioid receptors (see below), which are present both on pre- and postsynaptic membranes of opioidergic and their target neurons. There is a wide variety of physiological functions that appear to be influenced by opioidergic neurotransmission. Among these are modulation of nociceptive response to painful stimuli and stressors, reward, homeostatic and adaptive functions as eating, drinking, and thermoregulation (Koob, 1992; Khachaturian *et al.*, 1993).

Three main types of opioid receptors have been characterized pharmacologically: μ, δ, and κ (Lord *et al.*, 1977; Martin *et al.*, 1976), which according to the recent cloning studies belong to the family of seven transmembrane G-

protein-coupled receptors (Mansour *et al.*, 1995). Of these, the μ receptor is regarded as the primary site mediating the effects of opioids as morphine, heroin, and methadone. Thus, these drugs exert their actions, including positive reinforcement, mainly (but not exclusively) via activation of the μ receptor (Di Chiara and North, 1992).

Binding studies have identified two subtypes of μ receptor, one with a high affinity to both morphine and enkephalins (μ1), and another with a lower affinity that binds morphine far more potently than enkephalins (μ2; Wolozin and Pasternak, 1981). The physiological roles of these subtypes have mainly been characterized with opioid antagonist naloxonazine that is selective for μ1 receptor (Pasternak and Wood, 1986). Naloxonazine antagonizes a variety of morphine effects including analgesia, without affecting the other ones such as respiratory depression (Pasternak and Wood, 1986). Further evidence suggests that the opioid analgesia in supraspinal level be mediated by μ1 receptor, whereas the spinal analgesia and the respiratory depression are mediated by μ2 receptor (Ling *et al.*, 1985; Paul *et al.*, 1989).

There is data available showing that the μ1 receptor has no determining role in many signs of morphine withdrawal (Ling *et al.*, 1984), which means that this subtype is apparently not involved in the negative reinforcing properties of opioids. It thus appears that the physical dependence and respiratory depression can be separated from opioid analgesia. An intriguing question is, which of the μ receptor subtypes is involved in positive reinforcement, or in other words, whether one can dissociate the μ-opioid's positive reinforcing properties from analgesia as well. Needless to say, the thing is of great practical value. Thus far the data have been rather equivocal: in a study by Suzuki *et al.* (1993) naloxonazine did not antagonize morphine-induced place preference in mice, yet a rather selective μ1 agonist etonitazene (Moolten *et al.*, 1993) can serve as a positive reinforcer in rats (Caroll and Meisch, 1979; Sala *et al.*, 1992).

Since the brain dopamine has been strongly implicated in opioid reinforcement (see below), an important question concerns the commitment of μ receptor subtypes in this matter. The current knowledge is fairly inconsistent, for naloxonazine did not affect morphine-induced increase in striatal and limbic dopamine metabolism (Wood and Pasternak, 1983; Piepponen and Ahtee, 1995), yet it did partially antagonize the enhanced dopamine metabolism produced by μ receptor agonist [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAGO; Latimer *et al.*, 1987).

As far as morphine is concerned, albeit relatively selective for μ receptor, it has also some affinity to the δ receptor (Corbett *et al.*, 1993). Selective δ receptor agonists are able to serve as positive reinforcers (Shippenberg *et al.*, 1987; Suzuki *et al.*, 1991), and furthermore, there is a significant predominance of δ receptors over the μ type in the nucleus accumbens (Goodman *et al.*, 1983; Mansour *et al.*, 1988). Hence, in the case of morphine also the δ receptor may

be a likely candidate for mediating the reinforcement process. Nevertheless, the issue is not clear and opposing results are available. For instance, the intracerebral administration of δ receptor antagonist ICI 174,864 did not affect the place preference induced by morphine given ICV (Shippenberg *et al.*, 1987).

In contrast to μ and δ, the administration of selective agonists at κ receptors exhibits negative reinforcing effects, for in the place conditioning paradigm the animals actively avoid the stimuli that were previously associated with these drugs (Mucha and Herz, 1985). Concerning morphine, peripheral κ receptors have been proposed to mediate its aversive effect that occurred after conditioning with a small dose (0.05 mg/kg) administered intraperitoneally (Bechara and van der Kooy, 1985; 1987). Also, the aversive properties of selective κ agonists were suggested to be brought about by peripheral receptors (Bechara and van der Kooy, 1987). Later studies, however, have demonstrated that the activation of κ receptors in the central nervous system is sufficient to induce aversive effects (Bals-Kubik *et al.*, 1989; 1993).

2.5.2. Role of brain dopamine

Dopaminergic substrate of opioid reinforcement: The mesotelencephalic dopamine has been proposed to serve as a common neural substrate mediating positive reinforcing properties of many addictive drugs (Wise and Bozarth, 1987). Furthermore, the same substrate is critically involved in the phenomenon of behavioral sensitization. Current evidence refers more precisely to the importance of the so-called mesolimbic dopamine system (Di Chiara and Imperato, 1988; Kaliwas *et al.*, 1993). This system is formed by the cell bodies of A10 dopaminergic neurons in the ventral tegmental area (VTA) and their projections to the ventral striatum (nucleus accumbens). A common feature for many addictive drugs, including opioids and psychomotor stimulants, is their ability to enhance the mesolimbic dopaminergic transmission (Di Chiara and Imperato, 1988); on the other hand, drugs with aversive properties (e.g., agonists at κ opioid receptors) have an opposite effect.

Behaviorally relevant doses of opioids enhance both the firing of the dopaminergic neurons in the VTA (Gysling and Wang, 1983; Matthews and German, 1984) and the release of dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988). This effect is indirect by nature. Thus, the activity of A10 dopaminergic neurons is modulated by inhibitory GABA-ergic interneurons, which express μ opioid receptors. Opioids hyperpolarize the GABA-ergic interneurons via activation of μ receptors, and thus reduce the inhibitory control over A10 dopaminergic neurons (Gysling and Wang, 1983).

The activity of the mesolimbic dopaminergic system is likewise regulated by a negative feedback mechanism that involves dopamine receptors located on

the dopaminergic cell itself, i.e., autoreceptors. Dopamine and exogenous dopaminergic agonists inhibit the firing of most midbrain dopaminergic neurons by stimulating autoreceptors (Carlsson *et al.*, 1975). Dopamine autoreceptors exhibit pharmacological characteristics of D2 or D3 receptors (White and Wang, 1984; Sokoloff *et al.*, 1990; see below for dopamine receptor classification).

According to the mode of coupling to adenylate cyclase, dopamine receptors were originally divided into two main groups, designated as D1 and D2 receptors (Kebabian and Calne, 1979). The current cloning studies have extended this initial classification into D1-like and D2-like receptor families, the former comprising D1 and D5 receptors, and the latter D2, D3, and D4 receptors (Seeman and Van Tol, 1994). Several studies have demonstrated that dopamine receptor antagonists and lesions of dopaminergic neurons interfere with the opioid reinforcement (Bozarth and Wise, 1981; Spyraki *et al.*, 1983; Smith, *et al.*, 1985). More recent studies refer to the critical involvement of D1 receptors (Shippenberg and Herz, 1988; Shippenberg *et al.*; 1993; but see Gerrits *et al.*, 1994), and there is also some evidence about the role of dopamine autoreceptors in opioid reinforcement (De Fonseca *et al.*, 1995). One can find data, however, to indicate that the reinforcing actions of opioids may also involve dopamine-independent mechanisms. In a study by Ettenberg *et al.* (1982), dopaminergic antagonist α-flupentixol did not reduce the self-administration of heroin, but in doses that caused motor impairment; and similar effect (or rather, the lack of it) on the initiation of heroin self-administration has been observed with selective D1 receptor antagonist SCH23390 (Gerrits *et al.*, 1994). Furthermore, the study by Pettit *et al.* (1984) demonstrated that selective lesions of the dopaminergic terminals in the nucleus accumbens significantly attenuated the self-administration of cocaine but not that of heroin.

As far as the aversive properties of κ opioids are concerned, again an involvement of dopaminergic mechanisms has been suggested. κ Receptors are present both in the VTA and its dopaminergic terminal fields in ventral striatum (nucleus accumbens), and the aforementioned structures are innervated by lateral hypothalamic neurons containing κ-agonistic opioid peptides, dynorphins (Mansour *et al.*; 1988; 1995). Furthermore, the administration of κ agonists, U50,488 and bremazocine, has been shown to cause a decrease in synaptic dopamine concentrations both in the caudate and nucleus accumbens (Di Chiara and Imperato, 1988).

Dopaminergic substrate of cocaine reinforcement: Positive reinforcing properties of cocaine are dopamine-dependent as it has been demonstrated by several self-administration studies (e.g., Roberts *et al.*, 1977; Caine and Koob, 1995). Interestingly enough, both the neuroleptic drugs and the 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens have failed to influence conditioned place preference induced by intraperitoneal cocaine (Morency *et al.*,

1986, Spyraki *et al.*, 1982). The effect of either intracerebroventricularly or intravenously administered cocaine was still blocked by pimozide (Morency *et al.*, 1986) and haloperidol (Spyraki *et al.*, 1987), respectively. In view of these data it has been questioned whether the effect of intraperitoneal cocaine truly reflects its central reinforcing properties, and alternative explanations have been proposed including local anaesthesic action of cocaine (Spyraki *et al.*, 1982). Drug discrimination studies (Colpaert *et al.*, 1979), however, do not support the local anaesthesia hypothesis. Furthermore, according to the recent place conditioning study by Cervo and Samanin (1995), the effect of intraperitoneally administered cocaine was impaired by D1 receptor antagonist SCH23390, and another study (Hemby *et al.*, 1994) clearly demonstrates the predominant involvement of central components in place preference induced by intraperitoneal cocaine.

Behavioral sensitization to opioids and the brain dopamine: The sensitizing effects of opioids are closely related to the reinforcing ones as the same neural substrate appears to be involved in both. However, as discussed above, the relationship between behavioral sensitization and positive reinforcement is still somewhat problematic, and so is the role of dopamine in opioid reinforcement. Several lines of evidence suggest that the dopaminergic mechanisms underlie the behavioral sensitization to both opioids and psychomotor stimulants. With repeated administration, the aforementioned drugs become increasingly effective in activating the mesotelencephalic dopamine system (for review see Robinson and Becker, 1986; Kaliwas and Stewart, 1991). Evidence suggests the involvement of independent neural substrates in the induction and expression of behavioral sensitization. Thus, the sensitization is induced by the administration of amphetamine and μ-opioids to the region of dopamine cell bodies in the VTA but not to the nucleus accumbens, whereas the terminal fields (nucleus accumbens) are critical in its expression (Vezina *et al.*, 1987; Cador *et al.*, 1995). The exact nature of the phenomenon is not fully understood. Beside the most consistent finding, i.e., the augmented mesoaccumbens dopamine release in response to psychomotor stimulant and μ-opioid challenge (Kaliwas and Stewart, 1991; Kaliwas *et al.*, 1993), also changes in sensitivity of postsynaptic dopamine receptors have been detected. Concerning opioids, an enhancement of behavioral effects of dopamine receptor agonists have been observed both after acute morphine administration (Vedernikov, 1970; Martin and Takemori, 1985; 1987) and during withdrawal from chronic treatment (e.g., Carlson and Almasi, 1979). The results are somewhat inconsistent, however. While most authors (e.g., Carlson and Almasi, 1979; De la Baume *et al.*, 1979; Carlson and Seeger, 1982) found an enhancement of apomorphine-induced stereotypy, climbing, and locomotor activity during morphine withdrawal, some have failed to detect any changes (Kuschinsky, 1975). Furthermore, the issue is unclear with respect to the type of the dopamine receptor, because all of these studies

used apomorphine, which is an unselective dopamine receptor agonist (Seeman and Van Tol, 1994).

2.5.3. Role of nitric oxide

The free radical gas nitric oxide (NO) is an unconventional messenger molecule. It is generated from the amino acid L-arginine by a family of enzymes, called NO synthases (Moncada *et al.*, 1991). The function of NO in the central nervous system is widely related to excitatory amino acids, as in neurons it is formed in response to glutamate acting upon the NMDA receptor (Garthwaite *et al.*, 1988). This process is catalyzed by the constitutive, neuronal isoform of NO synthase (Förstermann and Kleinert, 1995), and can be effectively suppressed by the compounds that inhibit the enzyme. Yet, most such drugs are L-arginine analogs that unselectively block also the other known NO synthases, the (constitutive) endothelial, and inducible isoforms, and have therefore remarkable vascular effects (Moncada *et al.*, 1991). Such circumstances make the interpretation of the results sometimes fairly complicated. There is, however, a novel group of inhibitors, 7-nitro indazole and related indazoles, which lack the capacity to elevate blood pressure. These compounds suppress the neuronal and inducible NO synthases, but *in vivo* apparently do not influence the activity of the endothelial isoform (Moore *et al.*, 1993 a b; Wolff and Gribin, 1994).

The release of NO is involved in many glutamate actions in the central nervous system, including cellular events that may underlie the processes of learning and memory (Schuman and Madison, 1991). Likewise, NO has been shown to regulate the release of neurotransmitters, dopamine included (Zhu and Luo, 1992). According to the results of recent studies NO may be implicated in the actions of opioids — so it has been demonstrated that the inhibitors of NO synthase could prevent morphine tolerance (Kolesnikov *et al.*, 1992; Pasternak *et al.*, 1995) and attenuate the development and expression of the abstinence syndrome (Kimes *et al.*, 1993; Cappendijk *et al.*, 1995). NO has been likewise shown to modulate morphine induced changes in locomotion and food intake in mice (Calignano *et al.*, 1993), but hitherto there were no reports available regarding NO with relation to the positive reinforcing properties of opioids.

3. AIMS OF THE STUDY

The present study was addressed to clarify the following questions:

— *Role of opioid receptors in positive reinforcing properties of morphine.* Stress was laid upon the subtypes of the μ receptor and the δ receptor. Therefore we investigated the influence of the μ1 receptor antagonist naloxonazine and the δ receptor antagonist naltrindole on place preference induced by morphine. By way of comparison, the effect of unselective opioid antagonist naltrexone was studied. To evaluate the availability and selectivity of naloxonazine, we also measured its effects on morphine-induced antinociception, hyperthermia, and catatonia (a state of immobilization that is regarded as a mixture of muscle rigidity and akinesia). In rats catatonia is elicited by large doses of opioids (Ahtee and Kääriäinen, 1973) and has been shown to be mediated by the μ receptor, particularly by the μ1 subtype (Ling *et al.*, 1986). As for morphine-induced hyperthermia, it is readily antagonized by unselective opioid antagonist naloxone (Clark and Clark, 1980), but no data have been available concerning selective μ opioid antagonists (see above about analgesia).

— *Role of brain dopamine in positive reinforcing properties of morphine with an emphasis on dopamine autoreceptors.* The idea was that drugs that activate dopamine autoreceptors, and hence decrease dopaminergic transmission, could interfere with morphine reinforcement. To test this hypothesis, we studied the influence of the two dopamine autoreceptor activating drugs, quinpirole and preclamol, on morphine- and (by way of comparison) cocaine-induced place preferences. Biochemical and behavioral investigations indicate that the selective D2/D3 receptor agonist quinpirole (LY171555) in small doses could act selectively at dopamine autoreceptors (White and Wang, 1986; Widzovsky and Gori-Slechta, 1993). Preclamol ([-]3PPP) is a partial dopamine autoreceptor agonist that also exhibits antagonistic properties at postsynaptic dopamine receptors (for review see Clark *et al.*, 1985 a b).

— *Changes in sensitivity of dopamine D2/D3 receptors brought about by acute or repeated intermittent treatment with morphine.* Therefore we investigated the influence of acute morphine pretreatment and withdrawal from chronic morphine on quinpirole-induced yawning behavior, hypolocomotion, and stereotyped behavior in rats.

The yawning behavior is, however, a somewhat problematic model, because the location of the D2-like receptors that mediate yawning, is under discussion. While some authors have proposed that it may be mediated via presynaptic, i.e., autoreceptors (Protais *et al.*, 1983), the others strongly suggest the postsynaptic location (Serra *et al.*, 1986; Ståhle, 1992).

— *Role of NO in positive reinforcing properities of morphine.* With this purpose the influence of NO synthase inhibitor L-NOARG on morphine-induced conditioned place preference was studied. In order to clarify the consequences of mnemonic and motivational processes (see below), the effect of L-NOARG on κ opioid agonist U50,488 induced place aversion was also investigated.

4. MATERIALS AND METHODS

4.1. ANIMALS

Male Wistar rats weighing 200–405 g were used. The rats were housed in groups of 4–6 with food and water available ad libitum, under 12 hours light/dark cycle (lights on at 6–7 a.m.). The experiments were carried out during the light phase of the cycle.

4.2. DRUGS

The doses of morphine, naltrexone, and naloxonazine indicate the amount of the free base. Other doses refer to the salt. Morphine HCl (Ph. Eur. 2nd ed.) or morphine sulfate (ampoules containing 20 mg/ml of morphine sulfate; Antigen Pharmaceuticals LTD, Roscrea, Ireland), naltrexone HCl, and naloxone HCl (both Sigma Chemical Co., St. Louis, MO, USA), quinpirole HCl (LY17155; gift of Eli Lilly & Co, Indianapolis, Ind., USA), preclamol HCl ([-]3PPP; RBI, Natick, MO, USA, and gift of Suomen Astra OY), SCH23390 maleate [R-(+)-8-chloro-2,3,4,-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate; gift of Schering Corp., Bloomfield, N.J., USA], and U50,488 (trans-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate; RBI, Natick, MO, USA) were administered SC into the neck region. Cocaine HCl (Ph. Eur. 2nd ed.) was injected IP. All the above named compounds were dissolved in 0.9% NaCl solution and given in a volume of 1–2 ml/kg. Naltrindole (RBI Natick, MO, USA) was dissolved in 22.5% w/v solution of 2-hydroxypropyl-β-cyclodextrin and administered IP in a volume of 2 ml/kg. Nalxonazine (RBI Natick, MO, USA) and N^{ω}-nitro-L-arginine (L-NOARG; Sigma Chemical Co., St. Louis, MO, USA) were administered IP in a volume as above as 2.5% Tween® 80 solution.

4.3. CONDITIONED PLACE PREFERENCE

The apparatus consisted of two square-base compartments (h 40 × 30 × 30 cm), one with white, and the other with gray walls and floor. Compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. The apparatus was placed into a dimly lit room.

Before starting the experiment the rats were acclimated to experimenter contact for three days by handling and weighing in the experiment room. The experiment consisted of three phases.

Preconditioning. During three days (days 1, 2, and 3) rats were given free access to both compartments of the apparatus for 15 minutes each day. On day 3, the time spent by rats in each compartment was recorded (the position of the rat was defined by the position of its front paws) and these values served as a baseline. According to the baseline values the animals were divided into treatment groups with a similar initial preference. Since most of the rats preferred the gray compartment (i.e., they spent over 50% of time on that side), the ones preferring the white compartment were excluded from the experiment.

Conditioning was conducted during four days (days 4, 5, 6, and 7) and included two sessions each day. The rats were conditioned in the initially nonpreferred chamber immediately after the administration of morphine or cocaine, and in the preferred one after the administration of saline (in control animals both compartments were paired with the injection of saline). An interval of four hours separated the two sessions. The order of drug (i.e., morphine or cocaine) and saline presentation, paired with the given environment, was balanced across treatment groups. Conditioning times of 45–60 and 45 min were used for morphine and cocaine, respectively.

The doses of morphine (3 mg/kg SC) and cocaine (5 mg/kg IP) were selected according to the previous studies (Bardo *et al.*, 1995). Although there is no reliable dose-dependence with the doses of morphine above 1 mg/kg, the effect of this dose appears to be fairly unstable (Shippenberg and Herz, 1988; Bardo *et al.*, 1995). For this reason, morphine was administered at 3 mg/kg.

Opioid antagonists naltrexone (2.5 mg/kg SC), naloxonazine (15 mg/kg IP), and naltrindole (2 mg/kg IP) were administered 20 min, 12 hours, and 15 min prior to morphine administration, respectively. Quinpirole (0.05 mg/kg SC) was administered 5 and 10 min before morphine and cocaine, respectively. Preclamol (2 or 8 mg/kg) was given 15 minutes before morphine or cocaine administration, and L-NOARG (5 or 20 mg/kg IP) 15 minutes prior to morphine administration. In separate groups of rats the place conditioning effects of all the aforementioned pretreatment drugs were assessed.

Postconditioning. The postconditioning test was carried out on day 8 (24 hours after the last drug or vehicle administration). No injections were given prior to test. The rats had free choice in the apparatus for 15 minutes and the

time spent in each compartment was recorded by an observer unaware of the previous drug treatment.

4.4. CONDITIONED PLACE AVERSION

Conditioned place aversion experiments were carried out similarly to the place preference ones, with the exception that after the administration of U50,488 (1 mg/kg SC) the rats were conditioned for 45 min in the initially preferred compartment. L-NOARG (20 mg/kg IP) was given 15 minutes prior to U50,488 treatment.

4.5. ANALGESIA AND RECTAL TEMPERATURE

The pain sensitivity in rats was measured with the hot plate (Woolfe and Mac-Donald, 1944). The animals were gently placed on a 55°C copper plate and as a latency period, the time to the onset of paw-licking movements was measured. Cutt-off time of 30 seconds was used. Naloxonazine (15 mg/kg IP) was given 12 hours prior to morphine (3 mg/kg SC). Latencies were measured 30 and 60 minutes after the administration of morphine. The antinociceptive effect was calculated as a percentage of maximum possible effect (% MPE):

$$\%\,MPE = \frac{LTT - LTC}{CT - LTC} \times 100,$$

where LTT = latency time of treated animals, LTC = latency before treatment, and CT = cut-off time.

Rectal temperature was measured immediately prior to the hot-plate test by an electrical thermometer (Ellab, Copenhagen, Denmark) using a 4 cm long rectal probe. The animals were unrestrained during the measurements.

4.6. CATATONIA

Catatonia was measured every 30 min during 150 min after the administration of morphine (15 mg/kg SC). Four tests were used: 1) both front limbs of the rat were gently placed onto a 3 cm high horizontal bar; 2) both front limbs of the rat were placed onto a 9 cm high horizontal bar; 3) the front and hind limbs were placed onto parallel horizontal bars with a 6 cm distance between the bars; 4) the rat was placed on a metal grid positioned at an angle of 45°. Each

test was scored from 0 to 2: the score 1 was given if the animal remained immobile for 10 seconds; the score 2 was given in case the animal remained immobile for 20 seconds or more. The four tests were repeated five times during 2.5h experiment; the scores were summed and taken as a measure of catatonia (maximum sum was 40). Naloxonazine (15 mg/kg IP) and naltrindole (2 mg/kg IP) were given 24 hours and 15 min after morphine administration, respectively.

4.7. CHRONIC MORPHINE TREATMENT

Morphine was administered SC twice a day at 8.00 and 18.00 according to the schedule as follows: day 1: 10 and 10 mg/kg; day 2: 15 and 10 mg/kg; day 3: 15 and 15 mg/kg; day 4: 20 and 15 mg/kg; day 5: 20 and 20 mg/kg; day 6: 25 and 20 mg/kg; day 7: 25 and 25 mg/kg. On day 8 the animals were given morphine (30 mg/kg) in the morning only and placed back to their home cages for 24 hours (this group is later referred to as morphine withdrawn animals. A separate group of animals was given saline repeatedly.

4.8. LOCOMOTOR ACTIVITY

Locomotor activity was measured in a microcomputer-controlled photocell activity monitor, which contained 5 Plexiglas activity boxes of 31 × 21 × 20 cm (one rat per box). Locomotor activity was monitored either every 30 min during 180 min after the acute administration of morphine (3 mg/kg SC), or, in the case of chronic morphine treatment, 24 hours after the last morphine administration every 5 min over a 20 min period after quinpirole (0.025 mg/kg SC) administration. The rats had no previous experience with the activity box.

4.9. YAWNING BEHAVIOR

The rats were placed into individual Plexiglas boxes (31 × 21 × 20 cm) immediately after the administration of quinpirole (0.01–1 mg/kg SC) and the number of yawning episodes was recorded during 30 min. In the case of chronic morphine treatment yawning was assessed 24 hours after the last morphine administration.

4.10. STEREOTYPY

The intensity of stereotypy was rated according to a four-point severity scale by two independent observers for 60 min after quinpirole (1 mg/kg SC) administration. The scoring system was as follows: 0 — no stereotypy; 1 — periodic sniffing with some locomotion; 2 — continuos sniffing; 3 — periodic biting, gnawing, or licking; 4 — continuos (1 min) biting, gnawing, or licking, no locomotion (Costall *et al.* 1977).

4.11. STATISTICS

The data of place conditioning experiments were subjected to two-factor analysis of covariance (ANCOVA), where the time spent in the drug-paired compartment during the postconditioning test served as a dependent variable, drug treatments as categorical variables, and the baseline as covariate. Where necessary, the post-hoc comparisons were conducted by using either the Tukey-compromise test or the contrast analysis with Bonferroni levels (i.e., the critical level 0.05 was divided by the number of the comparisons made).

Data from the hot-plate test were analyzed either with the Wilcoxon test (effects of acute drug) or the Mann-Whitney U-test (effects of pretreatment). Catatonia scores were compared with the Mann-Whitney U-test; rectal temperatures were compared with the paired t-test.

Locomotor activity data were analyzed with the unpaired t-test, or with repeated measures ANOVA, followed by the contrast analysis where necessary. The data on yawning behavior were subjected to ANOVA or the Kruskall-Wallis test; the post-hoc comparisons were conducted with either the Newman-Keuls test or the Mann-Whitney U-test, respectively.

5. RESULTS

5.1. OPIOID RECEPTORS

5.1.1. Effect of opioid receptor antagonists on morphine-induced place preference

Results are shown in Fig. 1 (panels A, B, and C). In all experiments morphine induced significant place preference [morphine factor, $F(1,47) = 5.18$, $p = 0.028$; $F(1,49) = 12.56$, $p = 0.0009$; and $F(1,28) = 23.66$, $p < 0.001$; for the experiments with unselective opioid antagonist naltrexone, μ1 receptor antagonist naloxonazine, and δ receptor antagonist naltrindole, respectively]. Likewise, significant morphine x naltrexone and morphine x naloxonazine interactions [$F(1,47) = 10.65$, $p = 0.002$; and $F(1,49)= 6.88$, $p = 0.002$; respectively] were established, and further multiple comparison revealed that the effect of morphine was significantly antagonized by naltrexone and naloxonazine. Naltrexone and naloxonazine by themselves did not induce any significant place conditioning. Naltrindole had neither significant place conditioning effect by itself, nor did it influence the effect of morphine.

Taken together, morphine-induced place preference was significantly attenuated by unselective opioid antagonist naltrexone and μ1 receptor antagonist naloxonazine, whereas δ receptor antagonist naltrindole failed to show any reliable effect.

5.1.2. Effect of opioid receptor antagonists on morphine-induced catatonic state, antinociceptoin, and hyperthermia

Morphine (15 mg/kg, SC) produced a marked catatonic effect, which lasted for about 120 min. This catatonia was significantly antagonized by naloxonazine ($U = 13.5$, $p = 0.017$ as compared vehicle pretreatment, Mann-Whitney U-test) but not by naltrindole.

Naloxonazine clearly antagonized morphine-induced antinociception (Table 1). Morphine induced significant increase in rectal temperature at 30 and 60 min after its administration. Naloxonazine had no reliable influence on this effect (Table 1).

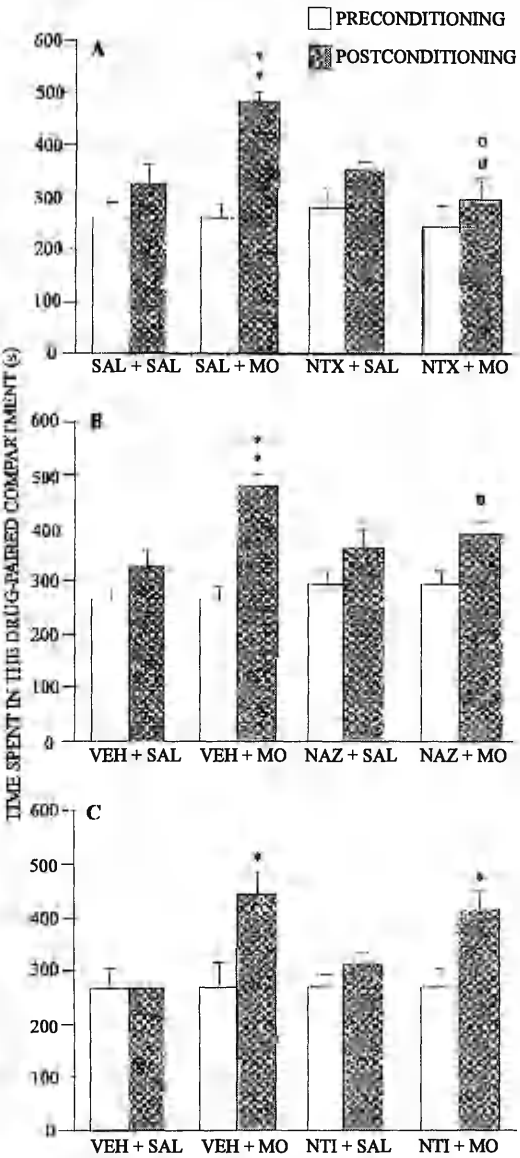

Figure 1. Effect of unselective opioid antagonists naltrexone (2.5 mg/kg SC, panel A), μ1 receptor antagonist naloxonazine (15 mg/kg IP, panel B), and δ receptor antagonist naltrindole (2 mg/kg IP) on place preference induced by morphine (3 mg/kg SC). The columns depict the mean (± SE) time, the rats (n = 7–18) spent in the initially nonpreferred (i.e., drug-paired) compartment during pre- and postconditioning tests. Abbreviations: SAL — saline, VEH — vehicle, MO — morphine, NTX — naltrexone, NAZ — naloxonazine, NTI — naltrindole. * $p < 0.05$, ** $p < 0.01$ vs. control (SAL + SAL or VEH + SAL) group. ° $p < 0.05$; °° $p < 0.01$ vs. morphine group (Tukey-compromise test).

Table 1

The effect of naloxonazine pretreatment (15mg/kg, IP, 12 h) on morphine-induced (3 mg/kg, SC) antinociception and hyperthermia. Antinociception was measured by estimating the latency (s) and mean percentage of maximum possible effect (% MPE). The rectal temperatures (Trect, °C) were measured in the same animals immediately before placing them on the hot plate. The median values ± 95% confidence limits (latency and %MPE) or the mean values ± SE (Trect, °C) of 7–8 animals are given.

	Time after morphine administration		
Pretreatment	0 min	30 min	60 min
		Latency	
Vehicle	8.4±2.9	16.2±3.2 *	9.4±1.4
Naloxonazine	12.8±3.3	12.3±3.0	10.0±2.9
		% MPE	
Vehicle		31.6±15.1	0.5±14.4
Naloxonazine		−12.2±26.1#	−2.6±23.2
		Trect °C	
Vehicle	38.0±0.1	38.6±0.2 *	38.7±0.2 *
Naloxonazine	38.3±0.1	38.9±0.1 *	38.9±0.2 *

* $p < 0.05$ vs. corresponding value at 0 min, Wilcoxon test (latency and % MPE) or paired t-test (Trect, °C). # $p < 0.05$ vs. vehicle pretreatment; Mann-Whitney U-test.

5.2. DOPAMINE RECEPTORS

5.2.1. Effect of quinpirole and preclamol on place preference induced by morphine and cocaine

Dopamine D2/D3 receptor agonist quinpirole (0.05 mg/kg) or dopamine autoreceptor agonist preclamol (2 or 8 mg/kg) by themselves had no significant place conditioning effect (Fig. 2 panel A).

Morphine induced significant place preference [$F(1,67) = 14.56$, $p < 0.01$]; neither quinpirole nor preclamol (8 mg/kg) had any significant influence on this effect (Fig. 2 panel C).

Likewise, cocaine brought about significant place preference [$F(1,69) = 18.9$, $p < 0.01$]. This effect was significantly impaired by 8 mg/kg of preclamol but was unaffected by quinpirole and preclamol at the dose 2 mg/kg (Fig. 2 panel B). In fact, ANCOVA revealed a nonsignificant quinpirole X cocaine interaction [$F(1,69) = 0.44$, $p = 0.5$], while the preclamol X cocaine interaction was significant [$F(2,70) = 5.1$, $p = 0.009$]. Two planned post-hoc compari-

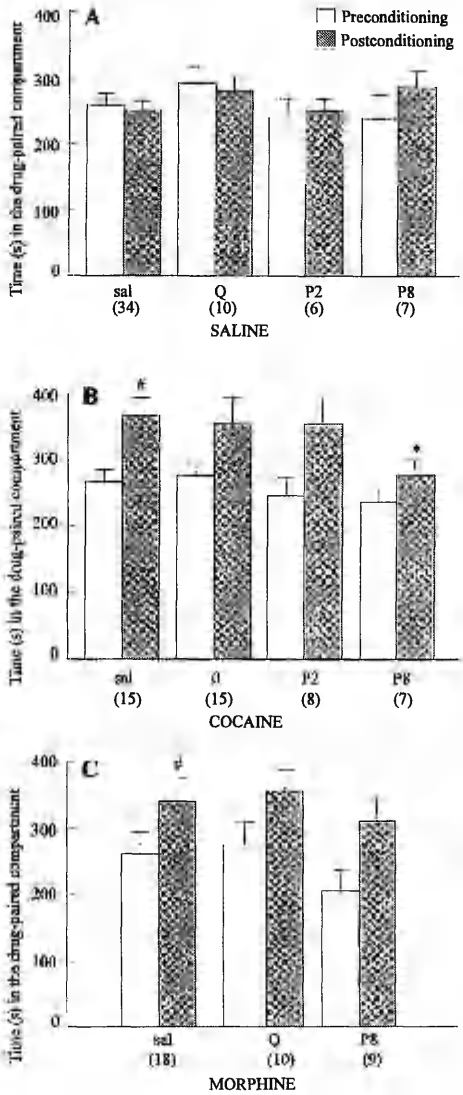

Figure 2. Effect of quinpirole and preclamol on conditioned place preference induced by morphine or cocaine in rats. Panel A: the effect of quinpirole and preclamol in saline treated control rats. Panel B: the effect of drugs on place preference induced by cocaine (5 mg/kg IP). Panel C: the effect of drugs on place preference induced by morphine (3 mg/kg SC). The columns depict the mean (± SE) time, spent in the initially nonpreferred (i.e., drug-paired) compartment during pre- and postconditioning tests. Abbreviations under columns indicate the pretreatment during conditioning: sal — saline, Q — quinpirole (0.05 mg/kg SC), P2 — preclamol (2 mg/kg SC), P8 — preclamol (8 mg/kg SC). Number of animals in brackets. # $p < 0.01$ compared with control (saline + saline) group; * $p < 0.05$ compared with saline-pretreated cocaine group (contrast analysis with Bonferroni adjustment).

sons (contrast analysis) revealed no significant difference between the treatment groups preclamol 2 mg/kg + cocaine and saline + cocaine, whereas the difference between the groups preclamol 8 mg/kg + cocaine and saline + cocaine was significant [$F(1,70) = 6.45$, $p = 0.013$, which is below the corresponding critical p value 0.025 for two comparisons].

5.2.2. Effect of acute morphine administration on quinpirole-induced yawning in rats

Quinpirole, over a wide dose-range (0.01–0.1 mg/kg SC), induced yawning behavior with maximum effects occurring at doses 0.05 and 0.1 mg/kg SC. Further increase in the dose of quinpirole brought about a decrease in yawning and the appearance of low intensity stereotyped behavior.

The effect of morphine (3 mg/kg SC) on quinpirole induced yawning behavior was dependent on the pretreatment interval. Morphine, given 15 min prior to quinpirole (0.1 mg/kg SC), nearly totally inhibited yawning, and a significant reduction was also present when morphine was administered 60 min prior to quinpirole. In the case of pretreatment intervals of 90 and 120 min, there was no significant differences in yawning between the morphine-pretreated and control groups.

5.2.3. Effect of naloxone on quinpirole-induced yawning in morphine-pretreated rats

Naloxone, given 1 mg/kg 10 min before quinpirole (0.1 mg/kg SC), had no significant influence on yawning in control rats, yet it restored the yawning behavior that was inhibited by 15 min morphine pretreatment. In contrast, naloxone further reduced the yawning behavior in rats that received morphine as a 90 min pretreatment. When administered to rats that received morphine 150 min before quinpirole, naloxone had no significant effect.

5.2.4. Effect of SCH23390 on quinpirole-induced yawning in morphine pretreated rats

D1 receptor antagonist SCH23390 given at 0.01 mg/kg SC did not affect the quinpirole-induced yawning by itself. Nor did it reliably influence yawning behavior in the rats that were administered morphine 15 min prior to quinpirole. However, in the case of 90 min morphine pretreatment, SCH23390 clearly enhanced the quinpirole-induced yawning.

5.2.5. Effect of morphine withdrawal on quinpirole-induced yawning behavior in rats

Results are shown in Fig. 3. The occurrence of yawning episodes followed an inverted U-shaped curve with the maximum effect at 0.01–0.1 mg/kg. Morphine withdrawal significantly enhanced yawning induced by 0.01 and 0.1 mg/kg of quinpirole. Quinpirole, given at 1 mg/kg, caused significantly less yawning in morphine-withdrawn rats than in controls.

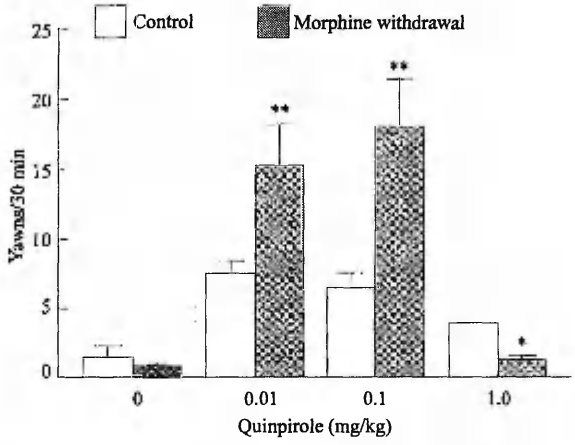

Figure 3. Quinpirole-induced yawning in morphine-withdrawn rats. The columns depict the mean (± SE), n = 8–10 animals. *p < 0.05; **p < 0.01 as compared to corresponding control group (Mann-Whitney U-test).

5.2.6. Effect of acute morphine treatment and morphine withdrawal on locomotor activity in rats. Influence of morphine withdrawal on quinpirole-induced hypolocomotion

Acute administration of morphine (3.0 mg/kg SC) exhibited a clear biphasic effect on locomotor activity. An initial phase of immobility that persisted up to 30 min, was turned into a significant increase in activity. The phase of locomotor activation started about 60 min after morphine administration, lasted for about 60 min, and returned to the control level within 150–180 min after morphine administration.

Both quinpirole (0.025 mg/kg SC) and withdrawal from chronic morphine treatment significantly suppressed locomotor activity [$F(1,28) = 10.5$, $p < 0.01$; and $F(1,28) = 10.0$, $p < 0.01$, respectively]. In fact, ANOVA revealed also significant effects for quinpirole X morphine withdrawal interaction [$F(1,28) = 6.8$, $p < 0.05$], indicating that morphine withdrawal further potentiated the inhibitory effect of quinpirole. However, this could not be revealed by post-hoc

comparisons. Within subject analysis revealed that it was only the effect of quinpirole that was significantly dependent on time [$F(3,84) = 7.5$, $p < 0.05$].

5.2.7. Effect of morphine withdrawal on quinpirole-induced stereotyped behavior in rats

In the control rats quinpirole, given at 1mg/kg induced stereotypy of low intensity as indicated by the sniffing episodes. No licking or gnawing could be observed in these animals. Withdrawal from repeated morphine treatment resulted in an enhancement of quinpirole-induced stereotypy. In these rats the stereotyped response was expressed not only by intense sniffing but also by the appearance of licking and occasional gnawing.

5.3. NITRIC OXIDE

5.3.1. Effect of NO synthase inhibitor L-NOARG on morphine-induced place preference

Results are shown in Fig. 4 panel A. ANCOVA revealed a significant effect for the morphine factor [$F(1,44) = 13.5$, $p = 0.001$] indicating that morphine brought about reliable place preference. In addition, a significant effect [$F(2,44) = 3.2$, $p = 0.049$] was established for L-NOARG and a nearly significant one for the L-NOARG x morphine interaction [$F(2,44) = 2.9$, $p = 0.066$]. In order to further clarify the nature of these effects, four post-hoc comparisons were conducted: the treatment groups L-NOARG 5 mg/kg + saline and L-NOARG 20 mg/kg + saline were tested against the group vehicle + saline, whereas the groups L-NOARG 5 mg/kg + morphine and L-NOARG 20 mg/kg + morphine against the group vehicle + morphine. In most cases no significant differences were found [$F(1,44) = 0.02–0.5$; $p = 0.48–0.88$]. However, for the comparison L-NOARG 20 mg/kg + morphine vs. vehicle + morphine the contrast analysis revealed $F(1,44) = 11.6$, $p = 0.001$. This can be considered significant because it is well below the corresponding critical p value 0.0125 for four comparisons. Thus, L-NOARG when given at 20 mg/kg reliably attenuated the effect of morphine.

5.3.2. Effect of NO synthase inhibitor L-NOARG on U50,488-induced place aversion

Results are shown in Fig. 4 panel B. κ Receptor agonist U50,488 (1 mg/kg SC) induced significant place aversion [$F(1,32) = 14.13$, $p = 0.001$]. ANCOVA likewise revealed no significance for the factor L-NOARG and L-NOARG x U50,488 interaction. Hence, L-NOARG neither induced any place conditioning by itself, nor did it influence the effect of U50,488.

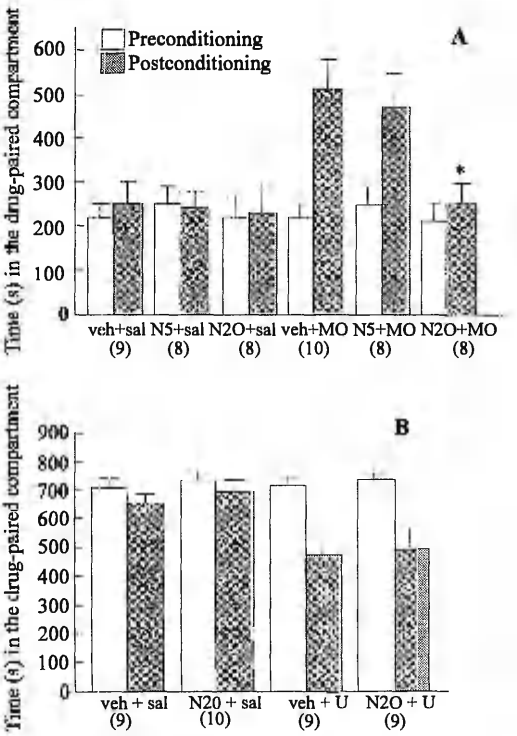

Figure 4. Effect of NO synthase inhibitor L-NOARG on place preference induced by morphine (3 mg/kg SC, panel A), and place aversion, induced by κ opioid receptor agonist U50,488 (1 mg/kg SC, panel B). The columns depict the mean (± SE) time, spent in the drug-paired compartment (initially nonpreferred or preferred, for panels A and B, respectively) during pre- and postconditioning tests. Number of animals in brackets. Abbreviations: sal — saline, veh — vehicle, Mo — morphine, N5 — L-NOARG 5 mg/kg IP, N20 — L-NOARG 20 mg/kg IP, U — U50,488. * — $p < 0.05$ compared with the group veh + Mo (contrast analysis with Bonferroni adjustment).

6. DISCUSSION

6.1. ROLE Of OPIOID RECEPTOR SUBTYPES IN POSITIVE REINFORCING PROPERTIES OF MORPHINE

In our experiments morphine, given 3 mg/kg SC, induced reliable place preference in rats, which well agrees with the numerous previous findings. Thus, morphine has been shown to produce such an effect over its entire usable dose range, from a small dose of 0.08 mg/kg (IV) to the doses sublethal for opioid naive rats (10–15 mg/kg, IV; for discussion see van der Kooy, 1987; Bardo *et al.*, 1995). On the other hand, a small dose (0.05 mg/kg) of morphine, but only when administered intraperitoneally, has been shown to elicit place aversion (Bechara and van der Kooy, 1985; 1987).

Morphine-induced place preference was significantly antagonized by the unselective opioid antagonist naltrexone, and the selective μ1 opioid antagonist naloxonazine. Hence, our results indicate a critical role of the μ1 receptor in positive reinforcing properties of morphine. The involvement of the μ2 receptor remains unclear, since no selective antagonist is available as yet.

Unlike the rats, in mice the blockade of μ1 receptors by naloxonazine did not affect morphine-induced place preference (Suzuki *et al.*, 1993). This could be explained by the fact that mice differ from rats in many respects, including the distribution and proportion of opioid receptors in various areas of the brain (Goodman and Pasternak, 1985; Goodman *et al.*, 1988; Waksman *et al.*, 1986; Mansour *et al.*, 1988). Rats and mice also differ in their behavioral responses to morphine, for large doses of morphine induce catatonia in rats (μ1-effect) but locomotor activation in mice (Kuschinsky and Hornykiewicz, 1974; Saito 1989).

Naloxonazine is an azine derivative of naloxone, and its reversible action closely resembles that of naloxone in binding studies (i.e., it binds all types of opioid receptors; Hahn *et al.*, 1985). Only irreversible binding of naloxonazine has been shown to be μ1-selective, and under *in vivo* conditions the best μ1-selectivity is reached when the drug is given about 24 hours before agonist (Ling *et al.*, 1986). In our place conditioning experiments, naloxonazine was administered 12 h before morphine (this was done for methodological reasons in order to prevent overlapping with conditioning sessions). One may argue that at this time point (i.e., 12 h after morphine administration) naloxonazine may have had affinity to other opioid receptors than the μ1 subtype. However, the

fact that naloxonazine was unable to antagonize morphine-induced hyperthermia, an effect that is readily antagonized by the unselective antagonist naloxone (for an extensive review see Clark and Clark 1980), indicates that naloxonazine acted selectively at the μ1 site.

Our conclusion is likewise supported by earlier studies. The μ1 receptor subtype has been shown to be involved in natural rewards as feeding (Mann *et al.*, 1988 a b; Simone *et al.*, 1985), drinking (Mann *et al.*, 1988 a; Simone *et al.*, 1985), and maternal behavior (Mann *et al.*, 1990). Moreover, rats readily self-administer orally etonitazene (Caroll *et al.*, 1979), an opioid agonist that is rather selective for the μI receptor (Moolten *et al.*, 1993). Etonitazene also induces place preference (Sala *et al.*, 1992). Therefore, it appears likely that although the μ1 selective analgesics would lack some undesirable side effects as the respiratory depression and inhibition of gastrointestinal transit, they can serve as positive reinforcers and cause addiction.

Though morphine is regarded as a μ-opioid, it has some affinity to the δ receptors as well (Corbett *et al*, 1993). δ Receptors predominate over the μ type in the nucleus accumbens (Goodman *et al.*, 1983; Mansour *et al.*, 1988), and have been proposed to mediate reinforcing effects produced by intra-accumbal morphine (Shippenberg *et al.*, 1993). Significant commitment of δ receptors has been likewise shown in cocaine reinforcement (Menkens *et al.*, 1992). Therefore, the δ receptors could well mediate a part of reinforcing effects of systemically administered morphine. Our results, however, do not support this idea, because naltrindole was without any effect on morphine-induced place preference. Nor did the intracerebral administration of δ receptor antagonist ICI 174,864, modify the place preference induced by morphine administered ICV (Shippenberg *et al.*, 1987). Furthermore, naltrindole affected the self-administration of heroin only in doses 10 and 15 mg/kg (Negus *et al.*, 1993), which were 10–1000 times larger than the ones needed to antagonize the antinociception induced by the selective δ receptor agonists DPDPE and DSLET (Crook *et al.* 1992). Considering that naltrindole blocks both putative subtypes (i.e., δ1 and δ2) of δ receptors (Pasternak, 1993; Traynor and Elliott, 1993), the involvement of δ receptors in morphine reinforcement seems unlikely.

6.2. ROLE OF BRAIN DOPAMINE IN MORPHINE AND COCAINE REINFORCEMENT

In the present study both the dopamine D2/D3 receptor agonist quinpirole, and the partial dopamine autoreceptor agonist preclamol failed to reliably affect morphine-induced place preference. The effect of cocaine was not influenced

by quinpirole, whereas it was significantly attenuated by relatively high dose of preclamol.

A self-administration study by Caine and Koob (1993) demonstrated that the dopamine D2/D3 receptor agonists quinpirole and 7-hydroxy-(2-N,N-dipropylamino)-tetraline (7-OH-DPAT), when co-administered with cocaine (at doses that were not self-administered by themselves), reduced cocaine intake by increasing the intervals between injections without disrupting self-administration. The same effect occurs when the dose of cocaine is increased (Yokel, 1987). The authors suggested that "... D3 selective dopamine agonists may interact presynaptically to enhance cocaine's reinforcing properties". In another study the administration of 7-OH-DPAT inhibited both the expression and the acquisition of morphine-induced place preference (De Fonseca *et al.*, 1995). The results of our study do not agree with either of these works, for the effect of preclamol was antagonistic to cocaine, and neither preclamol nor quinpirole significantly influenced the effect of morphine. Moreover, according to our results it appears that the activation of dopamine autoreceptors was not sufficient to antagonize positive reinforcing properties of cocaine. Thus, cocaine-induced place conditioning was inhibited by the higher dose of preclamol, whereas the smaller dose of preclamol that has been shown to activate the autoreceptors (Arnt, 1983), and quinpirole were ineffective. Quinpirole, acting upon dopamine autoreceptors, reduces the release of dopamine both *in vivo* (See *et al.*, 1991) and in vitro (Bull and Sheenan, 1991), yet, it does not block it entirely. It has been shown, furthermore, that only extensive lesions (> 90%) with 6-OHDA could effectively reduce psychomotor stimulant reward (Roberts *et al.*, 1977, Roberts *et al.*, 1979). Martin-Iverson *et al.* (1985) proceeding from their work with indirect dopaminergic agonists, methylphenidate and nomifensine, proposed that "... even a slight increase in activation of DA (dopamine) receptors could be sufficient to produce a rewarding effect". Such an explanation also seems to be appropriate in our case, in order to interpret the lack of quinpirole's effect. Preclamol, besides being a partial dopamine autoreceptor agonist, has antagonistic properties upon postsynaptic dopamine receptors (Clark *et al.*, 1985a b). Therefore, the effect of the larger dose of preclamol could be due to either its postsynaptic or the combination of its pre- and postsynaptic actions.

Concerning cocaine, one of the reasons for the inconsistency in our vs. Caine and Koob's (1993) results could be the difference in paradigms used: intravenous self-administration vs. conditioned place preference. As far as intravenous self-administration is concerned the animals are tested under the direct influence of drugs (that is not the case in place conditioning). Thus, besides the changes in reinforcing effects, the changes in motor behavior may serve as an underlying cause: a decrease or increase in the response rate may result from an inhibition or stimulation of motor behavior, respectively (Yokel, R. A. 1987). Since both 7-OH-DPAT and quinpirole in "autoreceptor selective" doses

reduce locomotor activity (Daly, *et al.*, 1993; Jackson, *et al.*, 1989), their direct influence on test performance may serve as a confounding factor. According to a later study, however, the pretreatment with 7-OH-DPAT does appear to specifically enhance the reinforcing properties of cocaine (Caine and Koob, 1995). Yet, in this event the same research would rather refer to the involvement of postsynaptic D2/D3 receptors than dopamine autoreceptors.

As to morphine, its place conditioning effect was unaltered both by quinpirole and preclamol in our study, whereas it was attenuated by 7-OH-DPAT (De Fonseca *et al.* 1995). This attenuation could be brought about by 7-OH-DPAT inhibiting the mesolimbic dopamine, provided that this system is critical in opioid reinforcement. As the authors note, however, there are alternative explanations available, including the disruption of attentional functions necessary for place conditioning to develop. In fact, the acquisition of morphine-induced place preference was attenuated by relatively high doses (0.25 and 5 mg/kg) of 7-OH-DPAT that could elicit disorganized behavior (De Fonseca *et al.*, 1995).

The central role of the dopaminergic substrate in positive reinforcing properties of opioids is referred to by a substantial body of evidence (Bozarth and Wise, 1981; Smith *et al.*, 1985; Spyraki *et al.*, 1982). A place conditioning study by Shippenberg *et al.* (1993) suggests the significance of the D1 receptor in the nucleus accumbens (but see below, Gerrits *et al.*, 1994). It has been also demonstrated that selective D1 receptor antagonist SCH23390, over a large dose range increases the responding for heroin, which was interpreted as a decrease in heroin reinforcement (Nakajima and Wise, 1987). On the other hand, several studies do not agree with such a dopaminergic hypothesis (e.g., Ettenberg *et al.*, 1982; Mackey and van der Kooy, 1985; Pettit *et al.*, 1984). Dopamine receptor antagonist, alpha-flupentixol, while eliminating intravenous self-administration of cocaine, did not reduce self-administration of heroin unless given in doses that impaired locomotor activity (Ettenberg *et al.*, 1982). Neither did small doses of alpha-flupentixol cause a compensatory increase in responding for heroin (Ettenberg *et al.*, 1982). Likewise, SCH23390, when given systemically, affected the initiation of heroin self-administration only in doses that inhibited motor behavior as well, and lacked the effect in the case of local administration in the nucleus accumbens (Gerrits *et al.*, 1994). In our study preclamol at the dose that impaired the cocaine place conditioning, had no significant effect on place preference induced by morphine. Our data thus agree with the earlier reports indicating the dopamine-independent components in positive reinforcing effects of opioids.

6.3. DOPAMINE D2/D3 RECEPTORS AND MORPHINE-INDUCED BEHAVIORAL SENSITIZATION

In accordance with previous studies (Babbini and Davis, 1972; Smee and Overstreet, 1976; Genç *et al.*, 1983), acute administration of morphine (3 mg/kg) produced a biphasic effect on locomotor activity. An initial phase of inhibition, lasting for about 30 min, was followed by stimulated locomotor activity over about a 60 min period. The phase of morphine-induced hypoactivity was accompanied by a decrease in quinpirole-induced yawning. A similar effect of morphine on apomorphine-induced yawning has been described previously by Berendsen and Gower (1986). The fact that naloxone reversed this inhibitory action refers to the involvement of opioid receptors.

During the phase of locomotor stimulation the yawning behavior in morphine-treated rats was similar to the quinpirole controls. In view of the fact that D1 receptor antagonist strongly enhanced yawning in morphine-treated rats (but not in controls) suggests an increase in the sensitivity of D2/D3 receptors. Thus, morphine-induced locomotor activation is dependent on the activity of D1 receptors, for it is specifically inhibited by D1 receptor antagonist SCH23390 (Longoni *et al.*, 1987). On the other hand, it has been shown that the activation of D1 receptors by the selective agonist SKF38393 suppresses apomorphine-induced yawning, and this effect is can be reversed by SCH23390 (Zharkovsky and Cereska, 1989). Hence, the blockade of D1 receptor during the morphine-induced locomotor activation removed the inhibitory influence of the activated D1 receptors, and therefore the effect of quinpirole-activated supersensitive D2/D3 receptors could manifest itself.

Likewise, withdrawal from repeated morphine administration appeared to enhance the sensitivity of D2/D3 receptors. In morphine withdrawn rats an enhancement of yawning behavior could be observed after small doses (0.01 and 0.1 mg/kg) of quinpirole, whereas in the case of a large dose (1 mg/kg) the yawning response was reduced in comparison with controls. This could be explained by the occurrence of the stereotyped behavior in morphine withdrawn rats, for as shown by Protais *et al.* (1983), the stereotypies and yawning behavior are mutually exclusive. The increased score of the stereotyped behavior in morphine withdrawn rats was due to the appearance of the high-degree stereotypy, licking and gnawing, which were not seen in control rats. This was rather unexpected because previous studies have revealed the requirement of concomitant activation of D1 receptors for high degree stereotypy to occur (Dall'Ollio *et al.*, 1988; Vasse *et al.*, 1988).

Both withdrawal from repeated morphine and the administration of quinpirole clearly reduced locomotor activity. It appears, however, that in morphine-withdrawn rats the locomotor activity was already nearly maximally inhibited, and therefore any further reduction by quinpirole could hardly be detected. The

locomotor activity test thus proved to be fairly insensitive in our experimental conditions.

Taken together, both acute morphine administration and withdrawal from repeated morphine treatment induced the supersensitivity of D2-like receptors that mediate yawning behavior. The significance of the latter with respect to morphine as a positive reinforcer remains, however, an unresolved question. It is especially so if one has in mind the results of the present study, which do not refer to any significant involvement of these receptors in morphine reinforcement (see above).

Table 2

Manipulations influencing opioidergic, dopaminergic, and NMDA / NO circuits in the central nervous system — their impact on positive reinforcing properties of μ-opioids (morphine or heroin). The table does not include some studies that contradict the present research (these have been discussed in the text)

Manipulation	Effect on μ-opioid reinforcement	References
	Opioid receptors	
Blockade of μ1 receptors	attenuation	Present study
Blockade of δ receptors	no effect	Present study Shippenberg *et al.*, 1987
	Dopamine	
Blockade of D1 receptors	inconclusive data	Shippenberg and Herz, 1988; Shippenberg *et al.*, 1993 Gerrits et al, 1994
Blockade of (postsynaptic) D2 receptors	inconclusive data	Leone and Di Chiara, 1987 Shippenberg and Herz, 1988; Shippenberg *et al.*, 1993
Activation of dopamine autoreceptors	no effect	Present study
Lesions of the nucleus accumbens	no effect	Pettit *et al.*, 1984
	NMDA / NO cascade	
Blockade of NMDA receptors	attenuation	Tzschentke and Schmidt, 1995
NO synthesis inhibition	attenuation	Present study

6.4. ROLE OF NITRIC OXIDE IN POSITIVE REINFORCING PROPERTIES OF MORPHINE

The effect of morphine was significantly attenuated by NO synthase inhibitor L-NOARG given at 20 mg/kg intraperitoneally. L-NOARG itself had no reliable place conditioning effect.

Apparently the L-NOARG's influence on morphine place conditioning was not due to some of its aversive properties because it failed to elicit any place aversion, regardless whether paired with the preferred or nonpreferred side. Nevertheless, the nature of L-NOARG's effect is a rather perplexing question, because several processes may be underlying. The acquisition of place preference involves thus both mnemonic and motivational components, which can be manipulated separately (White and Carr, 1985). Since NO is involved in long-term potentiation (Schuman and Madison, 1991), L-NOARG may have impaired the acquisition of place preference due to its impact on mnemonic processes rather than interference with motivational properties of morphine. The role of NO in different forms of learning and memory, however, is somewhat problematic. In the study by Bohme *et al.* (1993) L-NOARG in the dose 25 mg/kg IP (i.e., similar to the one effective in the present study) given over four days, was ineffective both in impairing radial maze learning in rats and blocking LTP in ex vivo prepared hippocampal slices. Yet the same dose almost totally inhibits the brain NO synthase activity (Salter and Duffy, 1995). This suggests that the observed inhibition of morphine-induced place preference cannot be explained solely by the impairment of mnemonic processes, and hence, our finding may have been based on the changes of motivational origin. Moreover, in our study L-NOARG (20 mg/kg IP) failed to affect the U50,488-induced place aversion, which it could have done provided that the target for L-NOARG was the mnemonic component of place conditioning. This fact also offers some rationale to propose the motivational changes in the case of morphine (however, we cannot exclude the possibility that the mechanisms mediating the mnemonic processes in place conditioning with morphine and U50,488, do not coincide). On the other hand, it shows that NO is not involved in the aversive properties of κ opioids.

Evidence suggests that NO may participate in the modulation of dopaminergic neurotransmission, for NO release-inducing agents sodium nitroprusside and L-arginine have been shown to enhance the release of dopamine in striatal slices (Zhu and Luo, 1992). However, as far as the reinforcement process is concerned, the available data are rather equivocal. The inhibition of NO synthesis attenuated cocaine-induced dopaminergic behaviors in mice, place preference included (Kim and Park, 1995). On the other hand, in rats it failed to affect both dopamine-dependent lateral hypothalamic brain stimulation reward, and the ability of cocaine to lower reward thresholds for electrical brain stimu-

lation (Bozarth *et al.*, 1994). As discussed above, the role of dopamine in opioid reinforcement is, in fact, a fairly obscure question. Hence, supposing that there is no commitment of NO in the dopamine related reinforcement process in rats, it is possible that NO is involved in the dopamine-independent mechanisms of opioid reinforcement.

In the brain NO is akin to glutamate (Garthwaite *et al.*, 1988), and with considerations as above it is tempting to speculate about the glutamate's commitment in positive reinforcing effects of morphine. Thus, NMDA receptor antagonists have been shown to inhibit morphine-induced place preference (Tzschentke and Schmidt, 1995), which the authors proposed to result from the disruption of associative learning, or the interference with dopaminergic processes in the brain. Such an explanation is strongly sustained by the involvement of glutamate in cellular plasticity processes (Collingridge and Singer, 1990). Moreover, the activity of the mesotelencephalic dopaminergic transmission is regulated via afferent glutamatergic projections from the prefrontal cortex to the VTA (Gariano and Groves, 1988), and from hippocampus, amygdala, and prefrontal cortex to the nucleus accumbens (Mogenson and Yang, 1991; Yoshikava *et al.*, 1991). However, according to our results and those by Bozarth *et al.* (1994) we speculate that glutamate likewise participates in such components of morphine reinforcement that are motivational in nature and relatively independent on associative learning or mesotelencephalic dopamine. A characteristic feature of the glutamatergic circuits in the central nervous system gives support to this proposal, for as stated by Kaliwas (1995) "... considering the widespread distribution of EAA (exitatory amino acids)-ergic projections, it is likely that any relatively global modification of neurotransmission in the brain, such as that produced by systemic administration of a drug of abuse, will ultimately impact on EAA transmission".

There are, however, at least two alternative explanations for L-NOARG's effect, which cannot be excluded on the basis of the present study. First, L-NOARG may have altered the pharmacokinetics of morphine due to its vascular effects. Though behavioral results suggest that the pharmacokinetic area-under-the-curve of cocaine was unaffected by the inhibition of NO synthase (Bozarth *et al.*, 1994), it does not imply morphine. Second, L-NOARG may have interfered with the effect of morphine due to the inhibition of locomotor activity (Starr and Starr, 1995). However, at least the acute effect of the drug on motor behavior may be ruled out as the postconditioning test was carried out 24 hours after the last L-NOARG administration. Moreover, the decreased locomotor activity has been shown to enhance the expression of morphine-induced place preference (Neisewander *et al.*, 1990).

7. CONCLUSIONS

1) The activation of μ1 opioid receptors is critical in the positive reinforcing properties of morphine, whereas the δ opioid receptors could be insignificant in this respect. The results also confirm the importance of μ1 opioid receptors in the mediation of morphine-induced antinociception and catatonia.

2) As far as the place preference paradigm is concerned, the activation of dopamine autoreceptors appears insufficient to reliably modify the positive reinforcing effects of both morphine and cocaine. Furthermore, there is a divergence in the endogenous pathways that mediate positive reinforcing properties of μ-opioids (morphine) and psychomotor stimulants (cocaine). Reinforcing effects of morphine involve some dopamine-independent mechanisms, whereas concerning cocaine the role of brain dopamine is critical.

3) Both acute morphine administration and withdrawal from repeated morphine treatment may induce the supersensitivity of dopamine D2/D3 receptors. However, in the case of acute morphine administration the expression of this supersensitivity in behavioral level is inhibited by concomitant activation of dopamine D1 receptors. The significance of the supersensitive D2/D3 receptors with respect to morphine as a positive reinforcer remains, however, an unresolved question.

4) NO is involved in the positive reinforcing properties of morphine. This involvement is apparently related to the motivational rather than mnemonic components of the reinforcement process. Besides, there is no commitment of NO in the aversive properties of κ opioids.

5) There are reward pathways in the brain, the activity of which is not suppressed by the inhibition of the mesotelecephalic dopaminergic system. The activation of these reward pathways by μ-opioids appears sufficient to produce positive reinforcing effects. These mechanisms are related to endogenous opioid systems and involve μ1 receptors. We propose also the participation of glutamate / NO cascade.

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RAVIMISÕLTUVUSE MEHHANISMID: TÄHELEPANU KESKMES ON MORFIINI POSITIIVSED SARRUSOMADUSED

Kokkuvõte

Töö eesmärgiks oli selgitada:

— μ-opioidiretseptorite alatüüpide ning δ-opioidiretseptorite osa morfiini positiivsetes sarrusomadustes (*positive reinforcing properties*). Käitumismudelina kasutasime tingitud paigaeelistust rottidel. Uurisime selektiivse μ1-opioidiretseptori antagonisti naloksonasiini ning δ-retseptori antagonisti naltrindooli mõju morfiiniga indutseeritud paigaeelistusele. Võrdlusena uurisime ka mitteselektiivse opioidiretseptorite antagonisti naltreksooni toimet. Naloksonasiini selektiivsuse hindamiseks uurisime selle mõju morfiini analgeetilisele, hüpertermilisele ning katatoonilisele toimele.

— Kesknärvisüsteemi dopamiinergilise süsteemi osa morfiini positiivsetes sarrusomadustes rõhuasetusega dopamiini autoretseptoritel. Dopamiinergilise substraadi kriitilisuse korral võiksid dopamiini autoretseptoreid aktiveerivad (ja seetõttu dopamiinergilist neurotransmissiooni pärssivad) ained pärssida ka morfiini positiivseid sarrusefekte. Selle hüpoteesi kontrollimiseks uurisime rottidel dopamiini D2/D3-retseptorite agonisti kvinpirooli ning dopamiini autoretseptorite agonisti preklamooli mõju morfiiniga indutseeritud paigaeelistusele. Võrdluseks uurisime ka nimetatud ainete mõju kokaiiniga indutseeritud paigaeelistusele.

— Dopamiini D2/D3-retseptorite tundlikkuse muutusi morfiini akuutse ning kroonilise manustamise korral. Selleks uurisime rottidel morfiini akuutse ning kroonilise manustamise mõju kvinpirooliga indutseeritud käitumistele (haigutamiskäitumine, lokomotoorse aktiivsuse muutused, stereotüüpia).

— Lämmastikoksiidi (NO) osa morfiini positiivsetes sarrusomadustes. Uurisime NO süntetaasi inhibiitori L-NOARG-i mõju morfiiniga indutseeritud paigaeelistusele. Mälu ning motivatsiooniga seotud protsesside osatähtsuse hindamiseks uurisime ka L-NOARG-i mõju κ-opioidiretseptori agonisti U50,488-ga indutseeritud paigavältimisele.

Morfiin (3 mg/kg SC) indutseeris statistiliselt olulise paigaeelistuse, mis on hästi kooskõlas varasemate töödega (van der Kooy, 1987). Seda toimet pärssisid oluliselt mitteselektiivne opioidiretseptorite antagonist naltreksoon (2,5 mg/kg SC) ja selektiivne μ1-opioidiretseptori antagonist naloksonasiin

(15 mg/kg IP). Naloksonasiin pärssis ka morfiini analgeetilist ning katatoonilist toimet, kuid ei mõjustanud oluliselt morfiini hüpertermilist efekti. Nende tulemuste põhjal väidame, et antud annuse ning manustamisviisi korral (s.t. 15 mg/kg IP) toimis naloksonasiin valikuliselt μI opioidretseptori suhtes. Selektiivne δ-opioidiretseptori antagonist naltrindool (2 mg/kg IP) morfiiniga indutseeritud paigaeelistusele olulist mõju ei avaldanud.

Kvinpiroolil (0,05 mg/kg SC) ning preklamoolil (8 mg/kg SC) ei olnud olulist mõju morfiini paigaeelistusele. Preklamool, manustatuna suhteliselt suures doosis (8 mg/kg) pärssis kokaiiniga (5 mg/kg IP) indutseeritud paigaeelistust, kuid seda oletatavasti osaliselt või täielikult postsünaptiliste dopamiiniretseptorite blokeerimise tõttu.

Morfiini akuutne (3 mg/kg SC) ning krooniline (20–50 mg/kg/24 h 7 päeva jooksul) manustamine põhjustasid dopamiini D2/D3-retseptorite tundlikkuse suurenemise kvinpirooli suhtes. Sellise nähtuse peamiseks avalduseks oli kvinpirooliga (0,01–0,1 mg/kg SC) indutseeritud haigutamiskäitumise suurem intensiivsus morfiinigrupi loomadel. Morfiini akuutse manustamise korral tekkis selline toime 90 min pärast morfiini injektsiooni ning selle avaldumiseks oli vajalik dopamiini D1-retseptorite blokaad.

NO sünteesi inhibeerimine L-NOARG-ga (20 mg/kg IP) pärssis rottidel oluliselt morfiiniga indutseeritud paigaeelistust. L-NOARG ise olulist paigatingimuslikku toimet ei põhjustanud. Kuna L-NOARG ei mõjutanud oluliselt ka κ-opioidiretseptori agonisti U-50,488-ga indutseeritud paigavältimist, siis oletame, et NO sünteesi inhibeerimine põhjustas muutusi morfiini motivatsiooniga seotud sarruskomponentides.

Järeldused

1. μ1-opioidiretseptori aktivatsioonil on keskne roll morfiini positiivsetes sarrusomadustes. Seevastu δ-opioidiretseptori aktivatsioon olulist tähendust morfiini korral ilmselt ei oma. Samuti kinnitavad käesoleva töö tulemused μ1-opioidiretseptori olulist osa morfiini analgeetilises ning katatoonilises toimes.

2. Dopamiini autoretseptorite aktivatsioon ei ole küllaldane faktor, et mõjutada oluliselt nii morfiini kui ka kokaiini positiivseid sarrusomadusi. μ-opioidide (morfiini) ning psühhomotoorsete stimulaatorite (kokaiini) positiivseid sarrusomadusi vahendavates endogeensetes närviteedes on olulised erinevused. Morfiini sarrusomadused sisaldavad dopamiinist sõltumatuid komponente, seevastu kokaiini korral on dopamiinergilistel mehhanismidel keskne roll.

3. Nii akuutne kui ka krooniline morfiini manustamine võib põhjustada dopamiini D2/D3-retseptorite suurenenud tundlikkust. Akuutse morfiini manustamise korral on sellise toime avaldumine käitumuslikul tasemel aga varjutatud samaaegse D1-retseptorite aktivatsiooni poolt. Mis aga puutub morfiini posi-

tiivsetesse sarrusomadustesse, siis dopamiini D2/D3-retseptorite suurenenud tundlikkuse olulisus jääb selles suhtes ebaselgeks.

4. NO osaleb morfiini positiivseid sarrusomadusi vahendavates närviteedes. See roll on seotud pigem sarrusefekti motivatsioonilise kui mäluga seotud komponendiga. κ-opioidide aversiivsetes toimetes NO ilmselt ei osale.

5. Ajus eksisteerivad positiivse sarrustuse mehhanismid, mille aktiivsus säilib ka pärast mesotelentsefaalse dopamiinergilise süsteemi pärssimist. Need mehhanismid on seotud endogeensete opioidsüsteemidega ning oluline roll on neis μ1-opioidiretseptoritel. Me oletame, et nendes närviteedes osalevad ka NO ja glutamaat.

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Morphine- and Cocaine-Induced Conditioned Place Preference: Effects of Quinpirole and Preclamol

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KIVASTIK, T., K. VUORIKALLAS, T. P. PIEPPONEN, A. ZHARKOVSKY AND L. AHTEE. *Morphine- and cocaine-induced conditioned place preference: Effects of quinpirole and preclamol.* PHARMACOL BIOCHEM BEHAV **54**(2) 371–375, 1996. – The role of dopamine in opioid reward is unresolved. Furthermore, the issue is somewhat unclear regarding cocaine and the place preference paradigm. In the present study we investigated whether the drugs activating dopamine autoreceptors affect cocaine- and morphine-induced place preference in rats. Neither the dopamine D_2/D_3 receptor agonist, quinpirole (0.05 mg/kg, SC), nor the partial dopamine autoreceptor agonist, preclamol (2 or 8 mg/kg, SC), induced place conditioning by itself. Quinpirole had no significant influence on the place preference induced either by morphine (3 mg/kg, SC) or cocaine (5 mg/kg, IP). Preclamol, when given at the dose of 8 mg/kg SC, significantly attenuated the effect of cocaine but failed to modify the effect of morphine. Our results suggest that the rewarding properties of morphine involve DA-independent mechanisms whereas in the cocaine-induced reward the role of brain DA is critical. Furthermore, as regards place conditioning, we propose that the activation of DA autoreceptors is not sufficient to reliably modify the rewarding effect of cocaine.

Reward Cocaine Morphine DA autoreceptors Quinpirole Preclamol Rats Place preference

MOTIVATIONAL effects of addictive drugs have been attributed to the interaction of exogenous substances with endogenous reward pathways. A great deal of evidence suggests that the mesolimbic dopamine (DA) system could serve as a common neural substrate mediating the appetitive properties of different classes of drugs. Thus, a common feature for many addictive drugs, including opioids and psychomotor stimulants, is their ability to enhance the mesolimbic DA transmission (11).

Behaviourally relevant doses of opioids enhance both the firing of the dopaminergic neurons in the ventral tegmental area (22) and the release of DA in the nucleus accumbens (11). Several studies have demonstrated that DA receptor antagonists and lesions of dopaminergic neurons interfere with the opioid reward (2,30,32). One can find data, however, to indicate that the reinforcing actions of opioids may also involve DA-independent mechanisms. Thus, DA antagonists do not reduce the self-administration of heroin unless given in doses causing motor impairment (12). Furthermore, it has been demonstrated that selective lesions of the DA terminals in the nucleus accumbens significantly attenuate the self-administration of cocaine but not that of heroin (25).

The rewarding properties of cocaine appear to depend on the integrity of the mesolimbic DA system as measured by IV drug self-administration (26). However, the issue is somewhat unclear regarding the place conditioning paradigm because both the neuroleptic drugs and the 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens have failed to influence conditioned place preference (CPP) induced by IP cocaine (23,33). Still, the effect of either ICV- or IV-administered cocaine was blocked by pimozide (23) and haloperidol (34), respectively. In view of these data, it has been questioned

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whether the effect of IP cocaine truly reflects its central rewarding properties, and alternative explanations have been proposed, including local anaesthetic action of cocaine (33). Drug discrimination studies (9), though, do not support the local anaesthesia hypothesis. Furthermore, according to a recent place conditioning study (17), clozapine impairs the effect of IP-administered cocaine, and another study (14) clearly demonstrates the predominant involvement of central components in the CPP induced by IP cocaine.

The activity of the mesolimbic DA system is regulated by a negative feedback mechanism that involves DA receptors located on the DA cell itself (i.e., autoreceptors). Thus, DA and exogenous DA agonists inhibit the firing of most midbrain dopaminergic neurons by stimulating DA autoreceptors (6). DA autoreceptors exhibit pharmacological characteristics of DA D_2-like receptors (35). It has been demonstrated that D_3 receptors, known to be D_2-like, also act as autoreceptors (31). According to some recent studies, DA autoreceptors could be implicated in the rewarding and discriminative stimulus properties of cocaine (4,5).

Biochemical and behavioural investigations indicate that the selective DA D_2/D_3 receptor agonist quinpirole (LY171555) in small doses could act selectively at DA autoreceptors (36,38). Preclamol ([−]3PPP) is a partial DA autoreceptor agonist that also exhibits antagonistic properties at postsynaptic DA receptors [for extensive review see (7,8)].

The present study was devised to further clarify the role of brain DA in cocaine and morphine reward, with an emphasis on DA autoreceptors. Our idea was that drugs activating DA autoreceptors, and hence decreasing DAergic transmission, could interfere with morphine and cocaine reward. To test this hypothesis, a series of experiments was carried out, where we investigated whether quinpirole or preclamol affect cocaine- and morphine-induced place preference.

METHOD

Animals

Male Wistar rats weighing 250–400 g were used. The rats were housed in groups of four to five with food and water available ad lib, under 12 L : 12 D cycle (lights on at 0600 h). The experiments were carried out during the light phase of the cycle.

Drugs

The doses of drugs, except morphine, refer to the salt. The dose of morphine refers to the amount of the free base. All compounds were dissolved in 0.9% NaCl solution and injected in a volume 2 ml/kg. Morphine HCl (Ph. Eur. 2nd ed.) was administered SC into the neck region. Cocaine HCl (Ph. Eur. 2nd ed.) was injected IP. Quinpirole HCl (LY171555; gift of Eli Lilly & Co, Indianapolis, IN) and preclamol HCl ([−]3PPP; RBI, Natick, MO, and gift of Suomen Astra OY) were administered SC into the neck region. The doses of preclamol (2 or 8 mg/kg, SC) used were based on biochemical and behavioural studies where they were found to inhibit DA synthesis in autoreceptor models (7) and cocaine discrimination [(5); for further information about doses and pretreatment intervals see the Experimental Procedure section].

Place Preference Apparatus

An apparatus similar to that described previously (18) was used. It consisted of two square-base compartments (h 40 × 30 × 30 cm), one with white and the other with gray walls and floor. Compartments were separated by a guillotine door and were covered with a transparent Plexiglas ceiling. The apparatus was placed into a dimly lit room with masking noise provided by ventilation fan.

Experimental Procedure

Before starting the experiment the rats were acclimated to experimenter contact for 3 days by handling and weighing in the experiment room.

Each experiment consisted of three phases.

1. Preconditioning: For 3 days (days 1, 2, and 3) rats were given free access to both compartments of the apparatus for 15 min (900 s) each day. On day 3, the time spent by rats in each compartment was recorded and these values served as a baseline.
2. Conditioning was conducted for 4 days (days 4, 5, 6, and 7) and included two sessions each day. The rats were conditioned in the initially nonpreferred chamber after administration of morphine (3 mg/kg, SC) or cocaine (5 mg/kg, IP), and in the preferred one after administration of saline. An interval of 4 h separated the two sessions. The order of drug (i.e., morphine or cocaine) and saline presentation, paired with the given environment, was balanced across treatment groups. Conditioning times of 60 and 45 min were used for morphine and cocaine, respectively. Quinpirole (0.05 mg/kg, SC) was administered 5 and 10 min before morphine and cocaine, respectively. Preclamol (2 or 8 mg/kg) was given 15 min before morphine or cocaine administration. For assessing the conditioning induced by quinpirole and preclamol, separate groups of rats were administered saline immediately, and quinpirole 5 min or preclamol 15 min before placing the rat in the nonpreferred chamber.
3. Postconditioning: On day 8 no injections were given. The rats had free choice in the apparatus for 15 min and the time spent in each chamber was recorded.

Statistics

The data from each drug combination were subjected to two-factor analysis of covariance (ANCOVA) where the time spent in the drug-paired compartment during postconditioning test served as dependent variable, pretreatment (quinpirole or preclamol) and treatment (morphine or cocaine) as categorical variables, and the baseline as covariate. Where necessary, post hoc comparisons were conducted by using the contrast analysis with Bonferroni levels (i.e., the critical level 0.05 was divided by the number of the comparisons made).

RESULTS

Figure 1A shows that neither quinpirole (0.05 mg/kg) nor preclamol (2 or 8 mg/kg) induced a significant place conditioning effect.

Cocaine induced significant CPP, $F(1, 69) = 18.9$, $p < 0.01$. This effect was significantly impaired by 8 mg/kg of preclamol but was unaffected by quinpirole and preclamol at the dose 2 mg/kg (Fig. 1B). In fact, the ANCOVA revealed a nonsignificant quinpirole × cocaine interaction, $F(1, 69) = 0.44$, $p = 0.5$, whereas the preclamol × cocaine interaction was significant, $F(2, 70) = 5.1$, $p = 0.009$. Post hoc comparisons showed that there was no significant difference between the treatment groups preclamol 2 mg/kg + cocaine and saline + cocaine, whereas the difference between the groups precla-

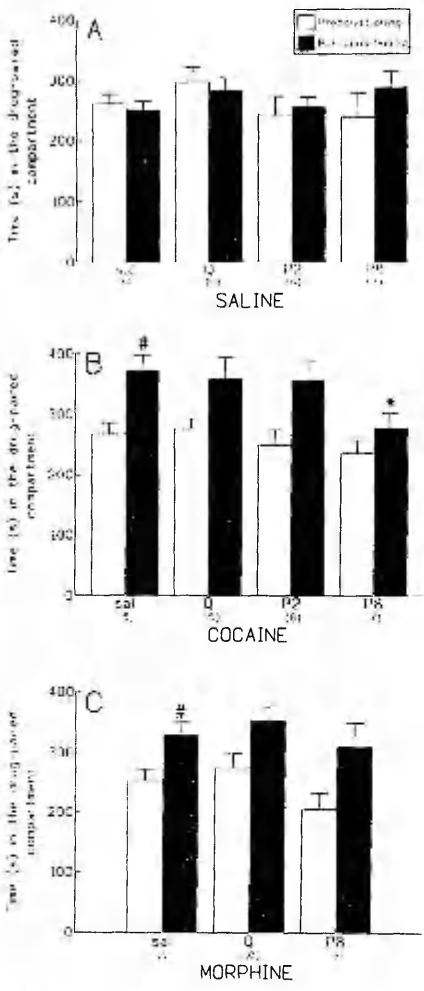

FIG. 1. Effect of quinpirole and preclamol on conditioned place preference (CPP) induced by morphine or cocaine in rats. (A) The effect of quinpirole and preclamol in saline-treated control rats. (B) The effect of drugs on CPP induced by cocaine (5 mg/kg, IP). (C) The effect of drugs on CPP induced by morphine (3 mg/kg, SC). The columns depict the mean ± SE time spent in the initially nonpreferred (i.e., drug-paired) compartment during preconditioning (open columns) and postconditioning (filled columns) tests. Abbreviations under columns indicate the pretreatment during conditioning: sal—saline, Q—quinpirole (0.05 mg/kg, SC), P2—preclamol (2 mg/kg, SC), P8—preclamol (8 mg/kg, SC). Number of animals in parentheses. #$p < 0.01$ compared with control (saline + saline) group; *$p < 0.05$ compared with saline-pretreated cocaine group (contrast analysis with Bonferroni adjustment).

mol 8 mg/kg + cocaine and saline + cocaine was significant, $F(1, 70) = 6.45$, $p = 0.013$.

Morphine also induced significant CPP, $F(1, 67) = 14.56$, $p < 0.01$, but neither quinpirole nor preclamol (8 mg/kg) had significant influence on this effect (Fig. 1C).

DISCUSSION

In our study conditioned place preference induced by cocaine was significantly attenuated by the partial DA autoreceptor agonist, preclamol (8 mg/kg), but not by the DA D_2/D_3 receptor agonist, quinpirole. Neither preclamol nor quinpirole significantly influenced the CPP induced by morphine. Quinpirole and preclamol by themselves had no place conditioning effect. The finding that quinpirole lacked the effect on place conditioning at a dose of 0.05 mg/kg agrees with the earlier results, indicating that quinpirole can induce CPP within the limited dose range (0.1–1.0 mg/kg), whereas smaller and larger doses are ineffective (15,37).

Our data agree with the recent results demonstrating that preclamol dose-dependently (0.625–10 mg/kg) reduces the discriminative stimulus properties of cocaine (5). However, it appears that the activation of DA autoreceptors is not sufficient to antagonize cocaine reward, because quinpirole and the smaller dose of preclamol that was shown to activate the autoreceptors (1) were ineffective. Quinpirole, acting upon DA autoreceptors, reduces the release of DA both in vivo (28) and in vitro (3), yet it does not block it entirely. It has been shown, furthermore, that only extensive lesions (>90%) with 6-OHDA could effectively reduce psychomotor stimulant reward (26,27). Martin-Iverson et al. (21), proceeding from their work with indirect DA agonists, methylphenidate and nomifensine, proposed that " . . . even a slight increase in activation of DA receptors could be sufficient to produce a rewarding effect." Such an explanation also seems to be appropriate in our case, to interpret the lack of quinpirole's effect. It is also unclear to what extent quinpirole at such a small dose (0.05 mg/kg) activates the postsynaptic DA receptors. However, the discriminative stimulus properties of 0.05 mg/kg SC of quinpirole were shown to be mediated via DA autoreceptors (38). Preclamol, besides being a partial DA autoreceptor agonist, has antagonistic properties on postsynaptic DA receptors (7,8). Therefore, the effect of the larger dose of preclamol could be due to either its postsynaptic action or to the combination of its pre- and postsynaptic actions.

A recent self-administration study (4) demonstrated that the D_3 receptor agonists quinpirole and 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetraline (7-OH-DPAT), when coadministered with cocaine (at doses that were not self-administered by themselves), reduced cocaine intake by increasing the intervals between injections without disrupting self-administration. The same effect occurs when the dose of cocaine is increased (39). Because 7-OH-DPAT did not alter self-administration of a direct DA agonist, apomorphine, the authors suggested that " . . . D_3 selective dopamine agonists may interact presynaptically to enhance cocaine's reinforcing properties." The results of our study, however, do not agree with this proposal, for quinpirole neither potentiated nor inhibited the effect of cocaine. One of the reasons for this discrepancy could be the difference in paradigms used: IV self-administration vs. CPP. As far as IV self-administration is concerned, the animals are tested under direct influence of drugs (that is not the case in CPP). Thus, besides the changes in reinforcing effects, the changes in motor behaviour may be underlying: a decrease or increase in response rate may result from an inhibition or

stimulation of motor behaviour, respectively (39). Because both 7-OH-DPAT and quinpirole in "autoreceptor-selective" doses reduce locomotor activity (10,16), their direct influence on test performance may serve as a confounding factor. However, the fact that 7-OH-DPAT did not alter self-administration of apomorphine (4) could possibly rule it out. An alternative explanation for the inconsistency in ours vs. Caine and Koob's results is that CPP and IV self-administration, due to some fundamental differences, reflect different aspects of cocaine reward (e.g., in the case of the former paradigm the acquisition phase is routinely studied whereas the latter one considers usually the maintenance). This view is further sustained by the discrepancy in results concerning clozapine's effect on cocaine self-administration and CPP (17,19).

A substantial body of evidence refers to the central role of the dopaminergic substrate in opioid reward (2,30,32). Moreover, a recent place conditioning study (29) suggests the significance of DA D_1 receptor in the nucleus accumbens [but see below and (13)]. It was also demonstrated that selective DA D_1 receptor antagonist, SCH23390, over a large dose range increases the responding for heroin, which was interpreted as a decrease in heroin reward (24). On the other hand, several studies do not agree with such a DA hypothesis (12,20,25). Thus, DA receptor antagonist, α-flupentixol, although eliminating IV self-administration of cocaine, did not reduce self-administration of heroin, unless given in doses impairing locomotor activity (12). Neither did small doses of α-flupentixol cause a compensatory increase in responding for heroin (12). Likewise, SCH23390 affects the initiation of heroin self-administration only in doses that inhibit motor behaviour as well (13). In our study preclamol, at the dose that impaired the effect of cocaine, did not affect significantly place preference induced by morphine. Hence, our data agree with the earlier reports indicating the existence of different endogenous substrates in opioid and psychomotor stimulant reward.

In conclusion, the results of the present study confirm the involvement of the central DAergic substrate in CPP induced by IP cocaine. Furthermore, our data suggest that the endogenous pathways mediating rewarding effects of morphine and cocaine differ. Rewarding properties of morphine appear to also involve DA-independent mechanisms, whereas in the case of cocaine the role of brain DA is critical. However, the role of DA autoreceptors remains fairly unclear. Thus, cocaine-induced CPP was impaired by preclamol at a dose that may have antagonistic properties at postsynaptic DA receptors, whereas a small dose of preclamol as well as quinpirole in an "autoreceptor-selective" dose lacked the effect. We propose that, as far as the place preference paradigm is concerned, the activation of DA autoreceptors is not sufficient to reliably modify the rewarding effect of cocaine.

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Nitric Oxide Synthesis Inhibition Attenuates Morphine-Induced Place Preference

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KIVASTIK, T., J. RUTKAUSKAITE AND A. ZHARKOVSKY. *Nitric oxide synthesis inhibition attenuates morphine-induced place preference.* PHARMACOL BIOCHEM BEHAV 53(4) 1013–1015, 1996. – Nitric oxide (NO) has been implicated in the actions of opioids. The aim of the present study was to investigate the role of NO in the mechanisms mediating the rewarding effects of morphine. Therefore, the influence of NO synthase inhibitor L-*N*-nitroarginine (L-NOARG) on morphine-induced place preference in rats was studied. L-NOARG, when given at 20 mg/kg, IP, significantly inhibited the effect of morphine. L-NOARG by itself, when administered at 5 or 20 mg/kg, IP, appeared to have no reliable effect on place conditioning. The results suggest a possible role of NO in the opioid reward process.

Nitric oxide Morphine Place preference Rats

THE FREE RADICAL gas nitric oxide (NO) is a highly unconventional messenger molecule. Its function in the CNS is widely related to excitatory amino acids. Thus, NO is formed in response to glutamate acting upon NMDA receptor, and its release is involved in many glutamate actions in the CNS including cellular events that may underlie the processes of learning and memory (12). According to the results of recent studies, NO may be implicated in the actions of opioids; it has been demonstrated that inhibitors of NO synthase could prevent morphine tolerance (6) and attenuate the development and expression of the abstinence syndrome (5). The issue is largely unclear, however, as far as the opioid reward process and NO are concerned. Thus, the present study was addressed to investigate whether NO is involved in the mechanisms that mediate the rewarding effects of morphine. We studied the influence of NO synthase inhibitor L-*N*-nitroarginine (L-NOARG) on morphine-induced conditioned place preference (CPP) in rats.

METHODS

Animals

Male Wistar rats weighing 245–405 g were used. The rats were housed in groups of four to five with food and water available ad lib, under a 12 L : 12 D cycle (lights on at 0700 h). The experiments were carried out during the light phase of the cycle.

Drugs

Morphine sulfate (ampoules containing 20 mg/ml of morphine sulfate; Antigen Pharmaceuticals, Roscrea, Ireland) was dissolved in 0.9% NaCl solution and injected in a volume of 1 ml/kg, SC, into the neck region. The dose of morphine refers to the amount of the free base. L-NOARG (Sigma Chemical Co., St. Louis, MO) was administered IP in a volume of 2 ml/kg as 2.5% Tween 80 solution.

Place Preference Apparatus

The apparatus consisted of two square-base compartments (H 40 × 30 × 30 cm), one with white and the other with gray walls and floor. Compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. The apparatus was placed into a dimly lit room.

Experimental Procedure

Before starting the experiment, the rats were acclimated to experimenter contact for 3 days by handling and weighing in the experiment room. The experiment consisted of three phases.

Preconditioning. During 3 days (days 1, 2, and 3), rats were given free access to both compartments of the apparatus for 15 min each day. On day 3, the time spent by rats in each compartment was recorded (the position of the rat was defined

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by the position of its front paws) and these values served as a baseline. According to the baseline values, the animals were divided into treatment groups with similar initial preference. Because most of the rats (51 from 56 animals) preferred the gray compartment (i.e., they spent over 50% of time on that side), the ones preferring the white compartment were excluded from the experiment.

Conditioning. Conditioning was conducted during 4 days (days 4, 5, 6, and 7) and included two sessions each day. The rats were conditioned for 45 min in the initially nonpreferred compartment immediately after administration of morphine, and in the preferred one after administration of saline. An interval of 4 h separated the two sessions. The order of morphine and saline presentation, paired with the given environment, was balanced across treatment groups. L-NOARG (or its vehicle) was given 15 min before morphine (or saline) administration. The following treatment groups were included: a) control [i.e., the animals receiving L-NOARG vehicle pretreatment, and saline immediately before the conditioning session (veh + sal)]; b) L-NOARG 5 mg/kg plus saline (N5 + sal); c) L-NOARG 20 mg/kg plus saline (N20 + sal); d) vehicle + morphine 3 mg/kg (veh + Mo); e) L-NOARG 5 mg/kg plus morphine 3 mg/kg (N5 + Mo); and f) L-NOARG 20 mg/kg + morphine 3 mg/kg (N20 + Mo). The dose of morphine (3 mg/kg, SC) was selected according to earlier studies (11) in which it was shown to produce reliable CPP.

Postconditioning. The postconditioning test was carried out on day 8 (24 h after the last drug administration). No injections were given before test. The rats had free choice in the apparatus for 15 min, and the time spent in each compartment was recorded by an observer unaware of the previous drug treatment.

Statistics

The data were subjected to two-factor analysis of covariance (ANCOVA) according to a 3 × 2 factorial design, in which the time spent in the drug-paired compartment during the postconditioning test served as the dependent variable, L-NOARG and morphine as categoric variables, and the baseline as covariate. Posthoc comparisons were conducted by using the contrast analysis with Bonferroni levels (i.e., the critical level 0.05 was divided by the number of the comparisons made).

RESULTS

Figure 1 shows the results. ANCOVA revealed a significant effect for the morphine factor [$F(1, 44) = 13.5$, $p = 0.001$] indicating that morphine brought about reliable CPP. In addition, a significant effect [$F(2, 44) = 3.2$, $p = 0.049$] was established for L-NOARG and a nearly significant one for the L-NOARG × Morphine interaction [$F(2, 44) = 2.9$, $p = 0.066$]. To further clarify the nature of these effects, four posthoc comparisons were conducted: the groups N5 + sal and N20 + sal were tested against the group veh + sal, whereas the groups N5 + Mo and N20 + Mo against the group veh + Mo. In most cases, no significant differences were found [$F(1, 44) = 0.02$–0.5, $p = 0.48$–0.88]. However, for the comparison of N20 + Mo vs. sal + Mo, the contrast analysis revealed $F(1, 44) = 11.6$, $p = 0.001$. This can be considered significant, as it is well below the corresponding critical p value of 0.0125 for four comparisons. Thus, L-NOARG when given at 20 mg/kg reliably attenuated the effect of morphine.

DISCUSSION

In accordance with previous studies, morphine given 3 mg/kg, SC, induced reliable CPP (11,14). This effect of morphine was significantly attenuated by NO synthase inhibitor L-NOARG given at 20 mg/kg, IP. L-NOARG itself appeared to have no reliable affect on place conditioning.

Because in our apparatus most of the rats prefer one particular (i.e., the gray) compartment, we ran the biased type of place conditioning (i.e., drug treatment was paired with the less preferred side). Such a type of procedure has been a matter for discussion, as antiaversive rather than rewarding prop-

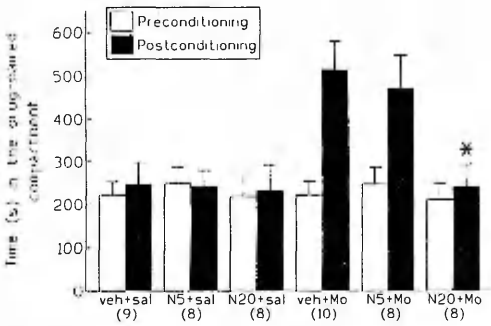

FIG. 1. Effect of NO synthase inhibitor L-NOARG on place preference induced by morphine (3 mg/kg, SC). The columns depict the mean ± ± SEM time spent in the initially nonpreferred (i.e., drug-paired) compartment during pre- and postconditioning tests (open and closed columns, respectively). The number of animals is given in brackets. sal, Saline; veh, vehicle; Mo, morphine; N5, L-NOARG 5 mg/kg, IP; N20, L-NOARG 20 mg/kg, IP. *$p < 0.05$ compared with the group veh + Mo (contrast analysis with Bonferroni adjustment).

erties of a given drug may be regarded as determinative (14). An optimal way has been proposed to counterbalance the drug treatment between the nonpreferred and preferred sides (3). With morphine, however, the experiments comparing the biased and counterbalanced procedures have provided consistent results (1,7).

The nature of L-NOARG's influence on morphine-induced CPP is a rather perplexing question, because several processes may underlie this effect. The acquisition of CPP thus involves both mnemonic and motivational components, which can be manipulated separately (15). Since NO is involved in long-term potentiation (12), L-NOARG may have impaired the acquisition of CPP as a result of its impact on mnemonic processes rather than interference with motivational properties of morphine. The role of NO in different forms of learning and memory, however, is still somewhat problematic. In the study by Bohme et al. (2), L-NOARG in the dose 25 mg/kg (i.e., similar to the one effective in the present study) given IP over 4 days was ineffective both in impairing radial-maze learning in rats and blocking LTP in ex vivo prepared hippocampal slices. The same dose almost totally inhibits brain NO synthase activity (10). This suggests that the observed inhibition of morphine-induced CPP cannot be explained solely by the impairment of mnemonic processes, and hence, our finding may have been based on the changes in the motivational properties of morphine.

Brain dopamine has been proposed to be a common neural substrate mediating the rewarding properties of different classes of abused drugs including opioids (16). NO release-inducing agents sodium nitroprusside and L-arginine have been shown to enhance the release of dopamine in striatal slices (17). However, as far as the reward process is concerned, the inhibition of NO synthesis failed to affect dopamine-dependent lateral hypothalamic brain stimulation reward (4). The role of dopamine in opioid reinforcement is, in fact, a fairly obscure question, and several studies refer to the existence of dopamine-independent components [e.g., (9)]. Hence, supposing that there is no commitment of NO in the dopamine-related reward process, it is possible that NO is involved in dopamine-independent mechanisms of opioid reward.

There are, however, alternative explanations for our finding, which cannot be excluded on the basis of the present study. First, L-NOARG may have altered the pharmacokinetics of morphine as a result of its vascular effects. Behavioural results suggest that the pharmacokinetic area under the curve of cocaine was unaffected by the inhibition of NO synthase (4); yet, it does not imply morphine. Second, L-NOARG may have interfered with the effect of morphine as a result of the inhibition of locomotor activity (13). However, at least the acute effect of the drug on motor behaviour may be ruled out, as the postconditioning test was carried out 24 h after the last L-NOARG administration. Moreover, the decreased locomotor activity has been shown to enhance the expression of morphine-induced CPP (8). Third, L-NOARG could have interfered with morphine-induced CPP as a result of some of its (possibly peripheral) aversive properties. In the present study, this effect could have been dampened by a possible floor effect, because the biased type of CPP was used. However, in our recent experiments, L-NOARG, when paired with the initially preferred compartment, failed to have any reliable place conditioning effect (unpublished results).

In conclusion, the main finding of the present study is that NO synthesis inhibition antagonizes the rewarding effects of morphine as revealed by the CPP paradigm. Our results refer to the potential involvement of NO in the opioid reward process.

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III

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Role of dopamine receptors in the dual effect of naloxone on quinpirole-induced yawning in morphine pretreated rats

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Summary. The present study was undertaken to determine the state of sensitivity of dopamine D2/D3 receptors involved in the mediation of yawning behaviour at various times following acute morphine administration to rats. Morphine (3.0 mg/kg, s.c.) induced a biphasic effect on locomotor activity: an initial inhibitory phase lasting for about 30 min was after about an hour followed by a phase of locomotor activation lasting for about 60 min. Dopamine D2/D3 receptor agonist quinpirole (0.01–0.1 mg/kg, s.c.) induced yawning behaviour in rats. Morphine given at 15 or 60 min before (inhibitory phase) inhibited the yawning response to quinpirole (0.1 mg/kg) but not when given at 90 or 120 min before (stimulatory phase). Naloxone (1.0 mg/kg) given 10 min before quinpirole restored yawning inhibited by morphine pretreatment during the inhibitory phase (15–60 min after morphine). However, during the morphine-induced stimulatory phase naloxone strongly inhibited the yawning response to quinpirole. D1 receptor antagonist SCH 23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate] at 0.01 mg/kg did not affect quinpirole-induced yawning or its inhibition by morphine. However, in rats which received morphine 90 min prior to testing yawning, SCH 23390 enhanced quinpirole-induced yawning behaviour as compared with morphine- or saline-pretreated animals. The data obtained in the present study indicate that morphine pretreatment initially induces a lack of responsiveness of the D2/D3 receptors mediating yawning behaviour and subsequently increases their sensitivity. However, the behavioural expression of hypersensitivity of these receptors seems to be attenuated by the concomitant activation of D1 receptors after morphine pretreatment, and thus the enhanced response to quinpirole is first seen after blockade of D1 receptors.

Key words: Morphine – Naloxone – Quinpirole – SCH 23390 – Yawning – D2/D3 receptor hypersensitivity

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Introduction

Brain dopaminergic systems have been widely implicated in the behavioural effects of opioids (Shippenberg and Herz 1987; for review see also Feigenbaum and Yanai 1984; Ahtee and Attila 1987). It has been suggested that acute and chronic opioid administration might change the sensitivity of dopamine receptors (Carlson and Almasi 1979; Tye et al. 1979; De la Baume et al. 1979; Martin and Takemori 1985, 1987). Further, acute morphine administration increases the dose of haloperidol required to inhibit apomorphine-induced climbing behaviour in mice (Martin and Takemori 1987), prolongs the apomorphine-induced stereotypies (Vedernikov 1970) and increases apomorphine-induced locomotor activity in rats (Martin and Takemori 1985) suggesting an increased sensitivity of postsynaptic dopamine receptors.

A number of behavioural studies have suggested that intense stereotypy and climbing behaviour seen after administration of dopamine receptor agonists are dependent on the concurrent activation of both D1 and D2 types of dopamine receptors (Clark and White 1987; Vasse et al. 1988; Dall'Olio et al. 1988). Therefore it is difficult to establish on the basis of the studies where stereotypy or climbing behaviour were taken as the measures of dopamine receptor sensitivity which receptor subtype is responsible for the observed behavioural supersensitivity after acute morphine administration. The selective dopamine D2 receptor agonist, quinpirole, in low doses acts selectively at presynaptic D2 receptors and, like many other dopamine D2 receptor agonists, induces yawning behaviour, but in contrast to mixed D1/D2 receptor agonist apomorphine, does not induce intense stereotyped behaviour (Tsuruta et al. 1981; Stoof and Kebabian 1984). Such a feature makes quinpirole a valuable tool for studying the sensitivity of dopamine D2 receptors mediating yawning behaviour. Although it was initially suggested (Mogilnicka and Klimeck 1977) and supported by later studies (Dourish and Hutson 1985; Stoessl et al. 1987) that yawning response is mediated via D2 autoreceptors, the data obtained recently (Serra et al.

1986; for review see also Ståhle 1992) suggest in favour of postsynaptic localization of the dopamine receptors mediating yawning. Furthermore, recently it was shown that quinpirole has about 100 times higher affinity at D3 receptors than at D2 receptors (Sokoloff et al. 1990). Therefore it is not excluded that some of the behavioural (yawning?) and biochemical effects of quinpirole might be attributed to its action at D3 receptors. The biphasic effect of morphine on locomotor activity of rats which is well documented (Babbini and Davis 1972; Smee and Overstreet 1976; Genç et al. 1983) might be due to changes in dopamine receptor sensitivity. In the present study we examined changes in the sensitivity of D2/D3 receptors by scoring quinpirole-induced yawning behaviour after acute morphine administration during both phases, the initial phase of locomotor inhibition and the later stimulatory phase.

We and others previously showed that activation of D1 receptor inhibits yawning behaviour (Zharkovsky and Cereska 1989; Yamada et al. 1990b). Furthermore, D1 receptor might be involved in the morphine-induced hypermotility (Longoni et al. 1987). Therefore, we examined the role of D1 receptor in the expression of quinpirole-induced yawning behaviour in morphine preteated rats. We used a selective D1 receptor antagonist SCH 23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate]. In radioligand binding studies and adenylate cyclase assays SCH 23390 has been shown to interact selectively with D1 receptors with much lower affinity at α_2-adrenoceptor, 5-HT_2, D2 and D3 receptors (Hyttel 1983; Iorio et al. 1983; Sokoloff et al. 1990).

Materials and methods

Animals. Male Wistar rats weighing 200–300 g housed in groups of 6, with free access to standard rat diet and tap water, in a room with 12 h light-dark cycle (lights on at 6.00 h). All behavioural experiments were performed between 9.00 and 13.00 h.

Behavioural studies. Locomotor activity was measured in a microcomputer controlled photocell activity monitor containing 5 plexiglass activity boxes of 31×21×20 cm (one rat per box). Locomotor activity was monitored every 30 min during 180 min after morphine administration. The rats had no previous experience with the box and were placed in it immediately after morphine injection.

To assess yawning the rats were placed individually in the similar boxes immediately after quinpirole injection. The number of yawning episodes was recorded during 30 min after quinpirole administration. The intensity of stereotypy was rated on fourpoint severity scale 20 min after quinpirole administration by two independent observers. The following scoring system was adopted. 0 = No stereotypy; 1 = periodic sniffing with some locomotion; 3 = periodic biting, gnawing or licking; 4 = continuous (1 min) biting, gnawing or licking, no locomotion (Costall et al. 1977).

Drugs. Morphine hydrochloride (Ph. Eur. 2nd ed.) was dissolved in 0.9% NaCl solution (saline) and injected s.c. in the back of the neck in the volume of 2 ml/kg body weight. The doses of morphine refer to the base. Quinpirole-HCl (gift of Eli Lilly & Co, Indianapolis, Ind., USA), D1 receptor antagonist SCH 23390 maleate (gift of Schering Corp., Bloomfield, N.J., USA) and naloxone hydrochloride (Sigma, St. Louis, Mo., USA) were dissolved in saline and administered s.c. in the volume of 1 ml/kg body weight. SCH 23390 was administered 15 min and naloxone 10 min prior to quinpirole administration.

Statistics. Locomotor activity data were analysed by Student's *t*-test. Data from yawning were analysed by the analysis of variance. If it was significant, individual group comparisons were made by using Newman-Keuls test.

Results

Effect of morphine on locomotor activity and quinpirole-induced yawning

In order to determine the pattern and duration of morphine-induced changes of locomotor activity, in the pilot experiment animals were administered morphine (3 mg/kg) and their locomotion was measured during 180 min thereafter. Morphine induced a clear biphasic effect on locomotor activity of rats (Fig. 1). An initial phase of immobility that persisted up to 30 min was turned in about an hour into a significant increase in activity lasting for about 60 min and then locomotor activity returned to control level at about 180 min after morphine administration.

Quinpirole over a wide range of doses (0.01–0.1 mg/kg) induced in rats yawning behaviour with maximum effects occurring at doses 0.05–0.1 mg/kg (Fig. 2). Further increasing the dose of quinpirole led to diminution of the yawning response and appearance of low intensity stereotyped behaviour.

In order to explore the effect of morphine on quinpirole-induced yawning, we administered 0.1 mg/kg of quinpirole at 15, 60, 90 and 120 min following morphine and counted yawnings for 30 min after quinpirole administration. The intensity of quinpirole-induced yawning behaviour was dependent on the time interval between morphine and quinpirole administration. Figure 3 shows that the quinpirole-induced yawnings were nearly totally inhibited when quinpirole was administered at 15 min after morphine and then returned to control level gradually with lengthening the interval between morphine and quinpirole administration. When quinpirole was administered at 90 and 120 min after morphine the number of yawns did not differ from control.

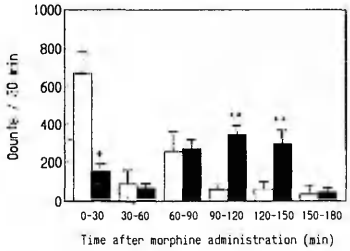

Fig. 1. Morphine-induced changes in the locomotor activity of rats. Locomotor activity was scored for every 30 min during 180 min following morphine (3.0 mg/kg, s.c.). Control rats (*open columns*) were given saline s.c. The values given are means±SEM, $n = 10$. *$P<0.05$; **$P<0.01$ as compared with corresponding control (Student's *t*-test)

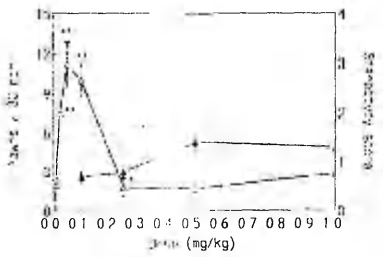

Fig. 2. Dose-response relationship of quinpirole-induced yawnings and stereotypies in rats. The values are means ± SEM. Each group consists of 6–10 animals. $^{*}P<0.05$; $^{**}P<0.01$ as compared with control (1.2 ± 0.4 yawns/30 min; Newman-Keuls test). No stereotypies could be observed in control animals. △ Yawning, ▲ stereotypy

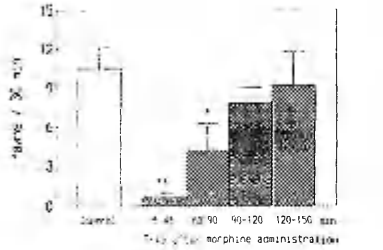

Fig. 3. Effect of morphine pretreatment on quinpirole-induced yawning behaviour in rats. Quinpirole (0.1 mg/kg, s.c.) was administered at various times (15–120 min) following morphine (3.0 mg/kg, s.c.). Control rats were given saline s.c. followed by quinpirole. Values are means ± SEM. Each group consists of 8–12 animals. $^{*}P<0.05$; $^{**}P<0.01$ compared with saline-treated control group (Newman-Keuls test)

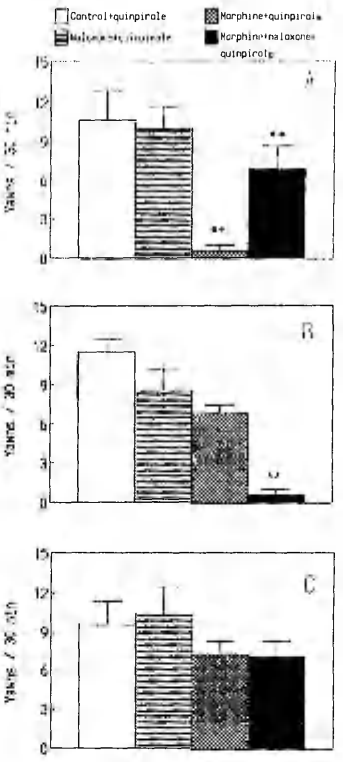

Fig. 4A–C. Effect of naloxone on yawning behaviour in rats treated with morphine (3.0 mg/kg, s.c.). Quinpirole (0.1 mg/kg, s.c.) was administered at 15 min (**A**), 90 min (**B**) or 150 min (**C**) after morphine. Naloxone (1.0 mg/kg, s.c.) was administered 10 min before quinpirole. The values are means ± SEM. Each group consists of 6–8 animals. $^{**}P<0.01$ compared with control + quinpirole group; $^{**}P<0.01$ compared with morphine + quinpirole pretreated group (Newman-Keuls test)

Effect of naloxone on quinpirole-induced yawnings in morphine pretreated rats

Naloxone in the dose of 1 mg/kg 10 – in before quinpirole did not alter the yawning response of the control rats, but restored yawnings inhibited by morphine administered at 15 min before quinpirole (Fig. 4A). By contrast, in rats given quinpirole 90 min after morphine naloxone totally inhibited yawning response (Fig. 4B). When this experiment was repeated in rats 150 min after morphine pretreatment neither morphine nor naloxone altered the quinpirole-induced yawning response (Fig. 4c).

Effect of SCH 23390 on quinpirole-induced yawnings in morphine pretreated rats

SCH 23390 given alone in the dose of 0.01 mg/kg did not affect quinpirole-induced yawning. Neither did SCH 23390 given simultaneously with morphine affect morphine-induced inhibition of yawning response tested at 15–45 min after morphine (Fig. 5A). However, when SCH 23390 was administered to rats which had received morphine 90 min prior to testing, it clearly enhanced quinpirole-induced yawning (ANOVA: $F(1.25) = 4.9$, $P<0.05$; Fig. 5B).

Discussion

In accordance with previous studies (Babbini and Davis 1972; Smee and Overstreet 1976; Genç et al. 1983) morphine in our experiments produced a biphasic effect on the locomotion of rats. An initial phase of inhibition lasting for about 30 min was followed after an hour by a period of locomotor stimulation lasting for about 60 min. In accordance with previous studies quinpirole induced yawning behaviour with maximum effect at 0.05–0.1 mg/kg. The phase of morphine-induced locomotor inhibition was accompanied by a decrease in quinpirole-induced yawning behaviour. A similar inhibition of apomorphine-induced yawnings by morphine was

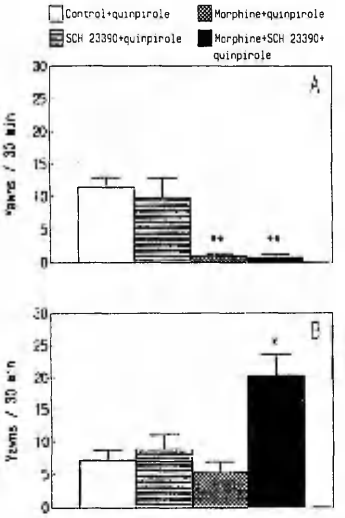

Fig. 5. Effect SCH 23390 on yawning behaviour in rats treated with morphine (3 mg/kg, s.c.) at 15 min (A) or 90 min (B) before quinpirole (0.1 mg/kg, s.c.). SCH 23390 (0.01 mg/kg) was administered s.c. 15 min before quinpirole. The values are means ± SEM. Each group consists of 8 animals. * $P < 0.05$; ** $P < 0.01$ compared with control + quinpirole group (Newman-Keuls test)

found previously (Berendsen and Gower 1986). This inhibitory effect of morphine was reversed by naloxone indicating that opioid receptors are involved in the inhibitory action of morphine on yawning behaviour. When quinpirole was administered during the phase of locomotor stimulation (90 min after morphine) quinpirole-induced yawning behaviour was only slightly weakened. Naloxone administered during this phase instead of antagonizing the action of morphine, further inhibited quinpirole-induced yawning behaviour. When quinpirole was given at 150 min after morphine naloxone did not alter yawning behaviour showing that opioid mechanisms were restored at this time. These data indicate that naloxone, depending on the duration of morphine pretreatment produces opposite effects on D2/D3 receptor-mediated yawning behaviour: restores yawnings during the first (inhibitory) phase and inhibits them during the second (stimulatory) phase.

The phase of locomotor stimulation might represent an adaptive phase which develops in response to the initial phase of morphine-induced locomotor inhibition. One possible explanation for the dual effect of naloxone is that it might enhance this adaptive response by inducing an additional activation of neuronal mechanisms responsible for the inhibition of yawnings. Such a possibility was thought to be unlikely to explain biphasic effect of morphine on locomotor activity (Havemann and Kuschinsky 1982). According to these authors (see also Möller and Kuschinsky 1986) the different sets of opioid receptors with different location in respect to dopaminergic system are involved in the mediation of akinesia, muscular rigidity and locomotor activation. Other neurotransmitter systems as well as different brain structures also might be involved in the naloxone-induced inhibition of quinpirole-induced yawning reported here. For example, during the phase of locomotor stimulation naloxone might activate noradrenergic system (Ayhan and Randrup 1973; Yamada et al. 1990a) or inhibit oxytocin release (Melis et al. 1989) and thus reduce yawning.

Also, the observed naloxone-induced inhibition of quinpirole yawnings during the phase of locomotor activation after acute morphine might be due to an activation of postsynaptic dopamine D1 receptors. Recent studies have shown that morphine-induced behavioural activation is strongly inhibited by SCH 23390 (Longoni et al. 1987) suggesting that activation of D1 receptor might be involved in locomotor effects of morphine. Our previous study showed that administration of D1 receptor agonist SKF 38393 inhibited apomorphine-induced yawning, and D1 receptor antagonist SCH 23390 antagonised this effect of SKF 38393 restoring yawning behaviour (Zharkovsky and Cereska 1989). In the present study SCH 23390 in a dose which did not affect yawning in control animals, strongly enhanced it in morphine pretreated rats during the phase of locomotor stimulation. This finding contrasts the data found by Serra et al. (1987) that SCH 23390 inhibited quinpirole-induced yawning. However, the doses of SCH 23390 used by these authors were somewhat higher than those used in the present study. The enhancement of quinpirole-induced yawning by SCH 23390 in morphine pretreated rats suggests that the intensity of quinpirole-induced yawning behaviour in morphine pretreated rats is dependent on the activity of D1 receptor. The activation of D1 receptor seems to occur in the later phase after morphine administration. Such an activation might mask an activation of D2/D3 receptors mediating yawning behaviour. The activation of D1 receptors might develop as a compensatory response to their inhibition during the initial hypoactivity phase following morphine pretreatment and might be further enhanced by naloxone administration.

In conclusion, our data show that acute morphine administration might induce dopamine D2/D3 receptor hypersensitivity during morphine-induced phase of locomotor activation. However, the expression of D2/D3 receptor mediated behaviour is attenuated by the concomitant activation of postsynaptic D1 receptors.

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IV

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Behavioural and Neurochemical Sensitization of Morphine-Withdrawn Rats to Quinpirole

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PIEPPONEN, T. P., J. KATAJAMÄKI, T. KIVASTIK, A. ZHARKOVSKY AND L. AHTEE. *Behavioural and neurochemical sensitization of morphine-withdrawn rats to quinpirole.* PHARMACOL BIOCHEM BEHAV 54(4) 787–792, 1996.—The sensitivity of dopamine D_2-like receptors in morphine-withdrawn rats was studied using the selective agonist quinpirole. Morphine was administered twice daily increasing the daily dose from 20 to 50 mg/kg during 7 days. Twenty-four hours after the last morphine administration the rats were given quinpirole (0.01–1 mg/kg), and their behaviour was assessed. Withdrawal from repeated morphine treatment enhanced yawning behaviour and penile erections induced by small doses (0.01–0.1 mg/kg) as well as the intensity of stereotypy induced by a large dose (1.0 mg/kg) of quinpirole. In the morphine-withdrawn rats the dose of 1 mg/kg of quinpirole caused less yawning than in the control rats, whereas the number of erections induced by this dose was enhanced as compared with the control animals. In the control rats the striatal and limbic concentrations of dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA), were not clearly affected by the smallest dose of quinpirole. However, the small dose of quinpirole (0.01 mg/kg) significantly reduced the levels of DOPAC and HVA in the striatum and limbic forebrain of the rats withdrawn from morphine either for 24 or 48 h. These findings indicate that withdrawal from repeated morphine treatment enhances the sensitivity of dopamine D_2-like receptors.

Quinpirole Morphine withdrawal Yawning Penile erections Stereotypy Locomotor activity Cerebral dopamine metabolism

BRAIN dopaminergic systems have been widely implicated in the behavioural effects of opioids (4,14,25). Morphine withdrawal results in a reduction of dopamine (DA) metabolism and release (1,3–5,7), which one would expect to lead to supersensitive dopamine receptors. The sensitization of DA receptors might be reflected by an enhancement of the behavioural effects of DA receptor agonists. However, behavioural studies in morphine-withdrawn animals, where DA receptor agonist apomorphine was used, provided controversial results. While most authors (8,9,13,27,35) found an enhancement of apomorphine-induced stereotypy, climbing, and locomotor activity during morphine withdrawal, some studies (21) failed to detect any changes in apomorphine-induced stereotypy.

The studies of the behavioural supersensitivity to DA receptor agonists in morphine-withdrawn animals are complicated by the existence of the multiple DA receptors within the central nervous system. DA receptors have been classified into two major classes, D_1-like (D_1, D_5) and D_2-like (D_{2A}, D_{2B}, D_3, D_4) receptors (28,30,33,34,36). Postsynaptic DA receptors are represented by D_1- and D_2-like receptors and they are involved in the mediation of behavioural effects of high doses of apomorphine (12,23,26,37). Presynaptic receptors (autoreceptors) are presumably D_2-like and their activation seems to result in the inhibition of DA release and metabolism (2,6,10, 26). There is some evidence suggesting that inhibition of locomotor activity and induction of yawning are also mediated via presynaptic D_2-like receptors (15,16,31,32,39). According to other authors (24,29), yawning behaviour is related to the activation of a distinct subtype of D_2-like receptors located postsynaptically. Functionally, post- and presynaptic DA receptors play an opposite role in the regulation of dopaminergic neurotransmission. Activation of postsynaptic receptors enhances, whereas activation of presynaptic receptors inhibits the activity of the dopaminergic system. Classical DA receptor agonist

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apomorphine is regarded as a nonselective DA receptor agonist, because in high doses it acts at both D_1 and D_2 receptors, and simultaneous activation of both D_1 and D_2 receptors, located postsynaptically, is required for induction of high grade stereotypy (12,37). In contrast, another DA receptor agonist, quinpirole, possesses rather selective action at D_2-like receptors (28,34). Similarly to small doses of apomorphine, quinpirole inhibits locomotor activity, induces yawning behaviour, and inhibits release and metabolism of DA, but in contrast to apomorphine, it does not induce high grade stereotypy even in high doses (30,34). Therefore, we used quinpirole to study whether the behavioural supersensitivity in morphine-withdrawn rats is associated with supersensitivity of DA D_2-like receptors, and investigated the behavioural and neurochemical effects of quinpirole in rats withdrawn from repeated morphine administration.

METHOD

Male Wistar rats, weighing 200–300 g at the start of the experiments, were housed in groups of four to six, with free access to standard rat diet and tap water, in a room with 12 L:12 D cycle (lights on at 0600 h). Five separate experiments were performed with different groups of animals. In each experiment, animals were randomly assigned to two treatment groups, saline (control) and morphine. Morphine hydrochloride (Ph. Eur. 2nd ed.) was dissolved in 0.9% NaCl solution (saline) so that the salinity of the solution was isotonic with physiological saline. Saline and morphine were given subcutaneously in the back of the neck in the volume of 2 ml/kg body weight. The daily dose of morphine was divided in two parts and administered at 0800 and 1800 h according to the following schedule: day 1: 10 and 10 mg/kg; day 2: 15 and 10 mg/kg; day 3: 15 and 15 mg/kg; day 4: 20 and 15 mg/kg; day 5: 20 and 20 mg/kg; day 6: 25 and 20 mg/kg and day 7: 25 and 25 mg/kg. On day 8, the animals were given only the morning dose (30 mg/kg) of morphine and placed back into their home cages for 24 or 48 h, and were referred to as morphine-withdrawn animals. The doses of morphine refer to the base. Control animals were given repeatedly saline.

Experiment 1

Twenty-four hours after the last saline or morphine administration control (n = 22) and morphine-withdrawn (n = 24) rats were randomly assigned to three groups each, and received quinpirole (quinpirole-HCl, gift of Eli Lilly & Co, Indianapolis, IN) either 0.01, 0.1, or 1.0 mg/kg. Immediately after that animals were placed into Plexiglas observation boxes of 31 × 21 × 20 cm (one rat per box). The number of yawning episodes and number of erections were counted during 30 min. Quinpirole was dissolved in saline and administered subcutaneously in the volume of 1 ml/kg body weight. Quinpirole doses refer to the salt. The rats had no previous experience with the box.

Experiment 2

Twenty-four hours after the last saline or morphine administration control (n = 10) and morphine-withdrawn (n = 12) rats were assigned to two groups each and were given either saline or 0.025 mg/kg quinpirole. Immediately after saline or quinpirole administrations the locomotor activity of the animals was measured by a microcomputer controlled photocell activity monitor containing five activity boxes of 31 × 21 × 20 cm (one rat per box). Locomotor activity was monitored every 5 min over a 20-min period after quinpirole administration. The rats had no previous experience with the box.

Experiment 3

Twenty-four hours after the last saline or morphine administration control (n = 8) and morphine-withdrawn (n = 8) rats were administered quinpirole (1 mg/kg). Immediately after that animals were placed into observation boxes for the assessment of stereotypy. The intensity of stereotypy was rated on a four-point severity scale over a 60-min period after quinpirole administration by two independent observers. The following scoring system was adopted: 0 = no stereotypy; 1 = periodic sniffing with some locomotion; 2 = continuous sniffing; 3 = periodic biting, gnawing or licking; 4 = continuous (1 min) biting, gnawing or licking, no locomotion (11). According to this scoring system scores 1–2 reflect low degree stereotypy and scores 3–4 reflect high degree stereotypy. The rats had no previous experience with the box.

Experiment 4

Twenty-four hours after the last saline or morphine administration control (n = 22) and morphine-withdrawn rats (n = 26) were assigned to three groups of 5–11 animals each and were administered either saline or 0.01 or 0.1 mg/kg of quinpirole. Animals were decapitated 30 min after quinpirole. The striatum and the limbic forebrain were dissected as described in detail previously (5). Shortly, beginning at the dorsal surface of the rat brain a transverse vertical cut was made 2 mm from the pole, just exposing the rostral ends of caudate nuclei. From the resulting small piece the area ventral to the rhinal fissure was removed and saved as a part of the limbic forebrain. On the ventral side of the brain the borders of the olfactory tubercle were cut to a depth of 2 mm, and the brain was turned to expose the dorsal surface. Cortical hemispheres were spread apart and separated by cutting the corpus callosum, and the hippocampi were peeled away from adjacent cortical tissue and discarded. The area bordered by the anterior commissure and stria terminalis was cut; these cuts continued those made earlier around the olfactory tubercle, and the resulting block of tissue was removed as the main part of the limbic forebrain. The medial borders of the striata were cut free, and the striata removed without the underlying cortex. The brain was then turned to expose the ventral surface and the amygdalae, situated on either side of the diencephalon, were pinched off and added to the limbic forebrain sample. The weights of striata and limbic forebrains were 71.2 ± 8.6 and 172.0 ± 20.9 mg, respectively (n = 48, mean ± SD). Each sample was frozen immediately on dry ice and stored at −80°C. The concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were estimated by HPLC with electrochemical detection as described by Haikala (18).

Experiment 5

Forty-eight hours after the last saline or morphine administration control (n = 15) and morphine-withdrawn rats (n = 15) were assigned to two groups of seven to eight animals each and were administered either saline or 0.01 mg/kg of quinpirole. Animals were decapitated 30 min after quinpirole. The striatum and limbic forebrain were dissected as described previously (19) with the difference that amygdaloid nuclei from the third rostral slice were discarded. Shortly described, the brain was placed on brain mold (RBM-4000C, ASI Instru-

ments. USA) and sectioned coronally at 2.7, −0.3 and −4.6 mm from bregma (22). The striata were punched from the second rostral slice (2.7 to −0.3) with needle (inner diameter 3 mm), and the limbic forebrain (containing the nucleus accumbens and the olfactory tubercle) was dissected from the tissue ventral to the striata. The weights of striata and limbic forebrains were 23.2 ± 2.2 and 53.6 ± 7.3 mg, respectively (n = 30, mean ± SD). The smaller tissue weights in Experiment 5 than in Experiment 4 are due to the fact that only 3 mm-thick section of the striata was dissected out in Experiment 5 as compared to the whole striata in Experiment 4, and the amygdaloid nuclei were discarded from the limbic forebrain sample. Further, the samples in Experiment 5 obviously contain less surrounding cortical tissues. The concentrations of DA and its metabolites were estimated as described above. In spite of the different weights of the limbic areas in the two experiments, the amounts of limbic DOPAC and HVA per sample were similar in both experiments.

Statistics

The yawning and penile erections data were analyzed by the Kruskall–Wallis analysis of variance. If it was significant, individual group comparisons were made using Mann–Whitney U-test at each dose used. The stereotypy data were analyzed by the Mann–Whitney U-test at each time point. Locomotor activity data were analyzed by multifactor repeated-measures ANOVA [morphine, quinpirole, and time (repeated factor) as independent variables]. Concentrations of DA metabolites were analyzed by the two-way ANOVA followed by the Newman–Keuls test. As the test for interaction in the two-way ANOVA at the ordinary significance levels is known to be of weak power (17), the significance level of 0.15 was considered to be appropriate to indicate morphine withdrawal × quinpirole interaction (38).

RESULTS

Experiment 1. Yawning and Penile Erections

The spontaneous occurrence of yawnings and penile erections in the morphine-withdrawn rats did not significantly differ from that in the control rats. The number of yawns in the control rats was 1.6 ± 0.7 and in the morphine-withdrawn rats 0.9 ± 0.3 (Fig. 1). The number of spontaneous penile erections was 0.3 ± 0.2 in the control, and 0.4 ± 0.2 in the morphine-withdrawn rats (Fig. 2). Quinpirole induced a behavioural syndrome, which consisted of recurrent yawning and intermittent penile erections accompanied by episodes of grooming. The occurrence of yawning episodes and penile erections followed inverted U-shaped curve with the maximum effect at 0.01–0.1 mg/kg of quinpirole, and with a smaller effect at the dose 1.0 mg/kg. Morphine-withdrawal enhanced significantly the yawning induced by 0.01 and 0.1 mg/kg of quinpirole (Fig. 1). Higher dose (1 mg/kg) of quinpirole caused significantly ($p < 0.05$) less yawning in the morphine-withdrawn rats than in the controls (Fig. 1). In contrast, in the morphine-withdrawn animals the number of erections was enhanced after all doses (0.01, 0.1, and 1 mg/kg) of quinpirole (Fig. 2).

Experiment 2. Inhibition of Locomotor Activity

The locomotor responses to quinpirole in the control and in the morphine-withdrawn animals are shown in Fig. 3. Multifactor ANOVA with repeated measures revealed an overall significant inhibitory effect of both quinpirole, $F(1, 28)$ = 10.5, $p < 0.01$, and morphine withdrawal, $F(1, 28) = 10.0$, $p < 0.01$, as well as a significant interaction, $F(1, 28) = 6.8$, $p < 0.05$. Within group analysis showed that only the effect of quinpirole was significantly dependent on time, $F(3, 84) = 7.5$, $p < 0.05$.

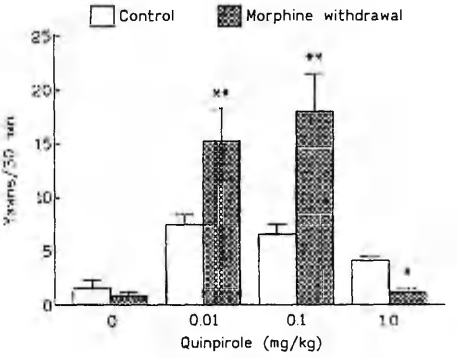

FIG. 1. Quinpirole-induced yawning behaviour in morphine-withdrawn rats. Values are means ± SE, n = 8–10 animals. *$p < 0.05$; **$p < 0.01$ as compared to corresponding control (Mann–Whitney U-test).

Experiment 3. Stereotypy

In the control rats the high dose of quinpirole (1 mg/kg) induced only low intensity stereotypy as indicated by the sniffing episodes. No licking or gnawing could be observed after administration of quinpirole alone. Withdrawal from repeated morphine treatment resulted in an enhancement of the quinpirole-induced stereotypy. In the morphine-with-

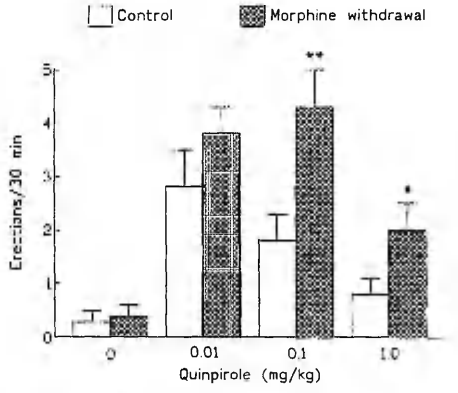

FIG. 2. Quinpirole-induced penile erections in morphine-withdrawn rats. Values are means ± SE, n = 8–10 animals. *$p < 0.05$; **$p < 0.01$ as compared to corresponding control (Mann–Whitney U-test).

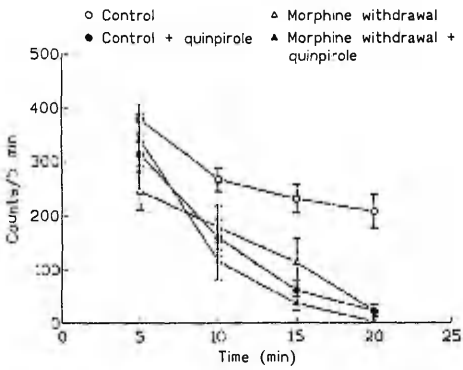

FIG. 3. Effect of quinpirole (0.025 mg/kg SC) on the locomotor activity in morphine-withdrawn rats. Values are means ± SE, $n = 8$ animals.

drawn rats, the stereotyped response was expressed not only by the intense sniffing but also by the appearance of licking and occasional gnawing, which contributed to the stereotypy score (Fig. 4).

Experiments 4 and 5. Dopamine Metabolism

Striatal and limbic DA metabolism was studied in rats withdrawn either for 24 or 48 h from repeated morphine treatment. None of the treatments altered the DA concentrations. The larger concentrations of the limbic DOPAC and HVA in the Experiment 5 than in the Experiment 4 (Table 1) are due to the different dissections of the brains in the two experiments (see the Method section).

Withdrawal from repeated morphine tended to decrease the concentrations of DA metabolites (DOPAC: 1–8%; HVA: 10–17%), but only limbic HVA in Experiment 5 was significantly decreased. In the control animals quinpirole, at the dose of 0.01 mg/kg, did not significantly reduce the levels

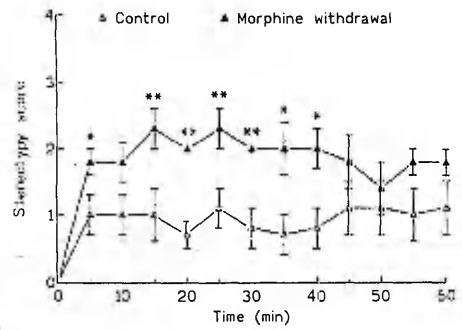

FIG. 4. Quinpirole (1.0 mg/kg SC)-induced stereotypy in morphine-withdrawn rats. Values are means ± SE, $n = 8$ animals. $*p < 0.05$; $**p < 0.01$ (Mann–Whitney U-test).

DOPAC (5–11%) and HVA (3–6%) except striatal DOPAC in Experiment 4 (by 27%; Table 1). The larger dose of quinpirole (0.1 mg/kg) significantly reduced DOPAC (26–32%) and HVA (33–35%) in the control animals. The larger dose of quinpirole reduced the DA metabolites in the morphine-withdrawn rats to about same degree as in the control rats (DOPAC: 32–46%; HVA 38–40%). When the smaller dose of quinpirole (0.01 mg/kg) was given to rats withdrawn from morphine for 24 or 48 h the concentrations of striatal (DOPAC: 20–29%; HVA: 18–23%) and limbic (DOPAC: 15–26%; HVA: 16–26%) DA metabolites were reduced significantly as compared with those of morphine-withdrawn rats not given quinpirole (Table 1). Furthermore, in the rats withdrawn from morphine for 48 h and given quinpirole (0.01 mg/kg) the concentrations of limbic and striatal DOPAC and HVA were significantly smaller than in the control rats given the same dose of quinpirole. However, two-way ANOVA did not reveal significant morphine withdrawal × quinpirole interactions using the ordinary significance level of 0.05. Because both the morphine-withdrawal and quinpirole affected DA metabolism in the same direction, the test for interaction in the two-way ANOVA at the ordinary significance levels is known to be of weak power (17). Still, there was a strong tendency of interaction in the levels of limbic HVA at 24 h withdrawal [0.01 mg/kg quinpirole; $F(1, 32) = 2.3$, $p = 0.14$] and of striatal and limbic DOPAC at 48 h withdrawal, $F(1, 26) = 3.1$, $p = 0.09$; $F(1, 26) = 2.4$, $p = 0.14$, respectively], indicating that quinpirole's effect was enhanced by withdrawal from morphine.

DISCUSSION

The present study shows that withdrawal from repeated morphine treatment enhances yawning behaviour and penile erections induced by small doses of quinpirole in rats. Thus, withdrawal from repeated morphine administration apparently results in hypersensitivity of DA D_2-like receptors involved in the mediation of yawning and penile erections. There is some controversy concerning the precise location of DA receptors mediating yawning response. While some authors (16,23,39) think that yawning is mediated via presynaptic receptors (autoreceptors), others suggest a postsynaptic location for the receptors mediating yawning (24,29).

An enhancement of yawning in the morphine-withdrawn rats could be observed only after small doses of quinpirole. A large dose (1.0 mg/kg) of quinpirole reduced the yawning response in the morphine-withdrawn animals as compared with the controls. This observation might be explained by the finding of Protais et al. (23), who demonstrated that the appearance of stereotypy results in the reduction of yawning because these two behaviours are mutually exclusive in rats. Thus, the enhancement of stereotyped response observed in the morphine-withdrawn rats after a large dose of quinpirole might result in the reduction of yawning. In contrast, penile erections do not seem to be dependent on the intensity of stereotypy because even 1 mg/kg of quinpirole induced more penile erections in the morphine-withdrawn animals than in the controls.

The increased score of the stereotyped responses in the morphine-withdrawn rats was due to the appearance of the elements of high degree stereotypy, licking and gnawing, which were not seen in the control rats. Previous studies have demonstrated that high degree stereotypy (e.g., continuous licking, biting, and gnawing) is induced by coadministration of D_1 receptor agonist SKF 38393 with quinpirole (12,37). This suggests that activation of D_1 receptor plays a permissive role

TABLE 1

EFFECT OF QUINPIROLE ON THE STRIATAL AND LIMBIC CONCENTRATIONS OF DOPAC AND HVA OF RATS WITHDRAWN FROM REPEATED SALINE OR MORPHINE TREATMENT

	DOPAC (ng/g)		HVA (ng/g)	
Quinpirole mg/kg	Control	Morphine Withdrawal	Control	Morphine Withdrawal
Experiment 4 (24 h)				
Striatum				
0	1015 ± 50	944 ± 39	808 ± 62	696 ± 48
0.01	744 ± 53*	672 ± 36*	784 ± 51	570 ± 29*
0.1	796 ± 49*	642 ± 54*	523 ± 90*	433 ± 37*
Limbic forebrain				
0	268 ± 12	247 ± 7	233 ± 15	195 ± 13
0.01	254 ± 7	210 ± 11*	226 ± 11	143 ± 7*‡
0.1	183 ± 21†	133 ± 11†§	156 ± 45*	117 ± 15*
Experiment 5 (48 h)				
Striatum				
0	1269 ± 69	1253 ± 60	849 ± 50	768 ± 70
0.01	1211 ± 56	1003 ± 31†‡	800 ± 50	592 ± 32*‡
Limbic forebrain				
0	949 ± 35	877 ± 39	530 ± 31	438 ± 31‡
0.01	841 ± 43	651 ± 35†§	497 ± 26	369 ± 17§

Quinpirole (0.01 or 0.1 mg/kg SC) was administered at 24 h (Experiment 4) or 48 h (Experiment 5) after the last saline or morphine administration, and the rats were decapitated 30 min later. Given are means ± SE, n = 5–13.

*$p < 0.05$, †$p < 0.01$ as compared with corresponding nonquinpirole group (repeated saline or repeated morphine).

‡$p < 0.05$, §$p < 0.01$ as compared with corresponding control (repeated saline + acute saline or quinpirole) group (Newman-Keuls test).

in the expression of the intense stereotypy induced by D_2 receptor agonists. The appearance of high degree stereotypy in the morphine-withdrawn rats was rather unexpected and suggested an additional activation of D_1 receptor.

The withdrawal from repeated morphine clearly reduced the locomotor activity. Although quinpirole significantly inhibited locomotor activity in the control rats, it failed to further reduce the locomotor activity in the morphine-withdrawn rats. This, however, might be explained by the fact that the locomotor activity in these rats was nearly maximally reduced due to the morphine withdrawal. Thus, the locomotor activity cannot be considered as a good indicator of the changes in DA receptor sensitivity under our experimental conditions.

Further, our results suggest that there is an enhancement of the effect of quinpirole on the cerebral DA metabolism in the morphine withdrawn rats. This enhancement was most clearly seen in the rats withdrawn from morphine for 48 h. Thus, these findings show that sufficiently long withdrawal from morphine augments the effects of quinpirole, and suggest that the sensitivity of DA receptors involved in the inhibitory action of quinpirole on the DA release and metabolism increases during morphine withdrawal. The findings that the D_2-like receptors involved in the regulation of DA metabolism seem to be sensitized later after morphine withdrawal than the receptors involved in the behavioural actions, agree with the time courses of behavioural and neurochemical sensitization after withdrawal from psychostimulants (20).

In conclusion, our data demonstrate that withdrawal from repeated morphine treatment induces behavioural supersensitivity to the D_2-like agonist quinpirole as reflected by an increase in the number of yawnings and penile erections. Further, our data suggest that the DA receptors controlling the release and metabolism of the striatal and limbic DA are sensitized as well in the morphine-withdrawn rats.

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INVOLVEMENT OF OPIOID μ1-RECEPTORS IN MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

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Abstract

PIEPPONEN, T. P., T. KIVASTIK, J. KATAJAMÄKI, A. ZHARKOVSKY AND L. AHTEE. *Involvement of opioid μ1-receptors in morphine-induced place preference in rats.* PHARMACOL BIOCHEM BEHAV. The main purpose of this study was to evaluate the role of μ1-opioid receptors in morphine reward. Therefore, we studied the ability of μ1-selective antagonist, naloxonazine (15 mg/kg, IP), to antagonize the conditioned place preference (CPP) induced by morphine (3 mg/kg, SC). In addition, effects of naloxonazine on morphine-induced catalepsy (15 mg/kg), analgesia (3 mg/kg) and hyperthermia (3 mg/kg) were studied. For comparison, the effects of a non-selective opioid receptor antagonist, naltrexone (2.5 mg/kg, SC), and a selective δ-opioid receptor antagonist, naltrindole (2 mg/kg, IP), on CPP induced by morphine were investigated. Morphine-induced CPP was clearly antagonized by pretreatment with naloxonazine and naltrexone (12 h and 20 min prior to morphine, respectively) but not by naltrindole (15 min before morphine). Naloxonazine also antagonized morphine-induced catalepsy and analgesia but not morphine-induced hyperthermia. Naltrindole did not modify morphine-induced catalepsy. These results suggest an active role for μ1-opioid receptors in morphine reward, whereas morphine-induced hyperthermia does not appear to be mediated by μ1-opioid receptors. Furthermore, δ-opioid receptors seem to be without significance in morphine-induced reward.

Naltrexone, Naloxonazine, Naltrindole, Conditioned place preference, Catalepsy, Analgesia, Hyperthermia, Opioid receptors

THE μ-opioid receptors are regarded as the primary site of action for the rewarding effects of opioids (6). Binding studies have identified two subtypes of μ-opioid receptors, one, μ1, with a high affinity for both morphine and enkephalins, and one, μ2, with a lower affinity that, however, binds morphine far more potently than it binds the enkephalins (42). The pharmacological roles of these subtypes have mainly been characterized with the μ1-selective antagonist, naloxonazine (29). Naloxonazine antagonizes a variety of morphine's actions including analgesia without affecting a number of other ones such as respiratory depression and increased striatal dopamine turnover (29). It is not known how these subtypes are involved in the rewarding effects of μ-receptor activating drugs in rats. Therefore, we have studied whether μ1-receptors are involved in the morphine reward by investigating the effect of naloxonazine on morphine-induced conditioned place preference. To evaluate the effectiveness and selectivity of the dose of naloxonazine used we also measured its effects on morphine-induced antinociception and hyperthermia. In smaller doses, systemically given morphine elicits antinociception in rats predominantly supraspinally through μ1-opioid receptors, whereas in larger doses μ2-opioid receptors become predominant (35). Morphine-induced hyperthermia is readily antagonized by naloxone (3), which blocks all subtypes of opioid receptors. The possible role of μ1- and μ2-opioid receptors in hyperthermia has not been investigated prior to this study.

For comparison, we investigated the effects of the non-selective opioid antagonist, naltrexone, and the selective δ-opioid antagonist, naltrindole, on morphine-induced place preference. Besides the μ-receptor, morphine has some affinity to δ-opioid receptors (4), which have been shown to mediate rewarding effects as well (12, 33, 36). There is also evidence that in mice blockade of δ-opioid receptors prevents rewarding effects of morphine (39). Therefore, to validate the effectiveness and selectivity of the dose used we also wanted to clarify whether δ-opioid receptors are involved in the acute rewarding effects of morphine in rats.

In rats, large doses of opioids elicit catalepsy (1), a state of immobilization that is regarded as a mixture of muscle rigidity and akinesia. Opioid-induced catalepsy has been shown to be mediated by μ-opioid receptors, especially by μ1-receptors (16, 27), whereas the activation of δ-receptors has been shown to mediate only stimulatory effects, e.g. locomotor activation and stereotypies (18, 24). Therefore, to validate the effectiveness and selectivity of the doses used we also studied the effects of naloxonazine and naltrindole on morphine-induced catalepsy.

METHOD

Animals

Male Wistar rats weighing 250–400 g were used. The rats were housed in groups of four to six under a 12 L : 12 D cycle (lights on at 0600) with food and water ad lib. The experiments were carried out during the light phase of the cycle.

Catalepsy

Catalepsy was measured every 30 min for 150 min after the administration of morphine (15 mg/kg, SC). Four tests were used: (1) both front limbs of the rat were gently placed onto a 3 cm high horizontal bar; (2) onto a 9 cm high bar; (3) the front and hind limbs were placed onto parallel horizontal bars with a 6 cm distance between bars; (4) the rat was placed on a metal grid positioned at an angle of 45°. Each test was scored from 0 to 2; a score of 1 was given if the animal remained immobile for 10 s; a score of 2 was given if the animal remained immobile for 20 s or more. The four tests were repeated five times during the 2.5 h experiments; the scores were summed and taken as a measure of the catalepsy (maximum sum was 40). Naloxonazine (15 mg/kg, IP) and naltrindole (2 mg/kg, IP) were given 24 h and 15 min before morphine, respectively.

Analgesia testing and rectal temperature measurement

The pain sensitivity of rats was tested with hot-plate (43). The animals were gently placed on a 55 °C copper plate and the time to onset of paw-licking movements was taken as the latency period. The cut-off time was 30 s. Naloxonazine (15 mg/kg, IP) was given 12 h prior to morphine (3 mg/kg, SC). Latencies were measured 30 and 60 min after administration of morphine. Antinociceptive effect was calculated as a percentage of maximum possible effect (% MPE):

$$\%\,MPE = \frac{LTT - LTC}{CT - LNC} \times 100,$$

where LTT = latency time of treated animals, LTC = latency before treatment and CT = cut-off time.

Rectal temperature was measured immediately prior to hot-plate test by an electrical thermometer (Ellab, Copenhagen, Denmark) using a 4 cm long rectal probe. The animals were unrestrained during measurements.

Conditioned Place Preference (CPP)

CPP was studied in an apparatus consisting of two square-base compartments (h 40 x 30 x 30 cm), one with white and the other with dark gray walls and floor. The compartments were separated by a guillotine door and covered with a transparent Plexiglass ceiling. The apparatus was placed into a dimly lit room with a masking noise provided by a ventilation fan.

Experimental Procedure

Before starting the experiments, the rats were acclimatised to experimenter contact for 3 days by handling and weighing them. The procedure was similar to that described previously (13).

Each experiment consisted of three phases.

1. Preconditioning: For 3 days (days 1, 2, and 3) rats were given free access to both compartments of the apparatus for 15 min (900 s) each day. On day 3, the time spent by the rats in each compartment was recorded and these values served as a baseline.

2. Conditioning was conducted for 4 days (days 4, 5, 6, and 7) and included 2 sessions each day. Rats were given SC morphine (3 mg/kg) or saline (controls) immediately before placing in the nonpreferred compartment for 60 min. After an interval of 4 h all the rats were given saline SC and placed in the preferred compartment for 60 min. The order of morphine and saline presentation paired with the given environment was balanced across treatment groups. Naltrexone (2.5 mg/kg, SC), naloxonazine (15 mg/kg, IP) and naltrindole (2 mg/kg, IP) were given 20 min, 12 h and 15 min prior to morphine, respectively.

3. Post-conditioning: On day 8, the rats had free choice in the apparatus for 15 min (no drugs were administered), and the time spent in each compartment was recorded.

Drugs

Naloxonazine (RBI, Natick, MO, USA) was suspended in 2.5 % Tween® 80 solution. Naltrindole HCl (RBI, Natick, MO, USA) was dissolved in a 22.5 % w/v solution of 2-hydroxypropyl-ß-cyclodextrin. Morphine HCL (Ph. Eur. 2nd ed.) and naltrexone HCl (Sigma, MO, USA) were dissolved in saline. Drugs were administered at a dose of 2 ml/kg, the doses except for naltrindole are given as a base.

Statistics

The data obtained in CPP experiments were subjected to 2-way analysis of covariance (ANCOVA), the baseline serving as a covariate. For multiple comparisons, the Tukey-compromise post hoc test was used. Data from hot-plate tests were analyzed either with the Wilcoxon signed-rank test (effects of acute drug) or the Mann-Whitney U-test (effects of pretreatment). Catalepsy scores were compared with Mann-Whitney U-test. The rectal temperatures were compared with a paired t-test (two-tail).

RESULTS

Catalepsy, Antinociception and Rectal Temperature

Morphine (15 mg/kg, SC) produced a marked cataleptic effect, which lasted for about 120 min. This catalepsy was significantly antagonized by naloxonazine ($U = 13.5$, $p = 0.017$ as compared to vehicle pretreatment, Mann-Whitney U-test) but not by naltrindole (Fig. 1).

Naloxonazine clearly antagonized morphine-induced antinociception (Table 1). Naloxonazine itself tended to increase the baseline latency ($U = 16$, $p = 0.0924$, Mann-Whitney U-test).

Morphine induced a significant hyperthermic effect at 30 and 60 min after its administration. Naloxonazine did not alter this effect of morphine (Table 1).

Place preference conditioning

In all the experiments, rats treated with morphine showed significant preference [$F(1, 47) = 5.18$, $p = 0.0276$; $F(1, 49) = 12.56$, $p = 0.0009$; $F(1, 28) = 23.66$, $p < 0.0001$, the experiments with naltrexone, naloxonazine and naltrindole, respectively] for the drug associated compartment (Fig. 2). This preference was clearly antagonized by both the non-selective opioid antagonist, naltrexone, and the μ1-receptor selective antagonist, naloxonazine, [$F(1, 47) = 8.32$, $P=0.0059$; $F(1, 49) = 3.49$, $P = 0.0678$, the interactions with morphine: $F(1, 47) = 10.649$, $p = 0.002$; $F(1, 49) = 6.88$, $p = 0.012$, respectively]. The δ-selective antagonist, naltrindole, did not interact with the effect of morphine, [$F(1, 28) = 0.09$, $p = 0.76$, the interaction with morphine $F(1, 28) = 1.57$, $p = 0.22$]. None of the antagonists alone significantly affected the CPP.

DISCUSSION

The conditioned place preference (CPP) paradigm has proved to be a valuable tool in the investigation of rewarding (or aversive) properties of drugs (for reviews see 10,11). In this method the subjects learn to associate the primary rewarding stimulus with the environmental stimulus, or in other words, the environmental secondary stimulus (place) acquires rewarding properties through the conditioning. In our experiments the morphine-induced place preference was significantly antagonized by the non-selective opioid antagonist, naltrexone, as well as by the μ1-opioid receptor selective antagonist, naloxonazine. Thus, our results indicate that μ1-opioid receptors are critically involved in the rewarding properties of morphine. This is not surprising because μ1-opioid receptors have been shown to be involved in natural rewards like feeding (19, 20, 34), drinking (19, 34), and maternal behaviour (21). Naloxonazine has also been shown to partially antagonize the increase of locomotor activity induced by the selective μ-opioid agonist, DAGO (15). Further,

rats readily orally self-administer etonitazene (2), a potent opioid, that has recently been shown to be rather selective agonist for μ1-opioid receptor (25). Etonitazene also induces CPP (31). Taken together, it seems likely that although μ1-selective opioid analgesics may lack some undesirable effects like respiratory depression and inhibition of gastrointestinal transit, they would not be without rewarding effects.

Naloxonazine is an azine derivative of naloxone, and in binding studies the behaviour of reversibly bound naloxonazine closely resembles that of naloxone, i.e., it binds to all types of opioid receptors (9). Only irreversible binding of naloxonazine has been shown to be μ1-selective, and under *in vivo* -conditions the best μ1-selectivity with this drug is reached when it is given about 24 hours before agonist (17). In our experiments naloxonazine was for methodological reasons (to prevent overlapping with conditioning sessions) given 12 h before (except in the catalepsy experiment where it was given 24 h before) morphine. It may be argued that at this time (12 h after administration) naloxonazine may have affinity to other opioid receptors besides the μ1-opioid receptors. However, the fact that naloxonazine was not able to antagonize morphine-induced hyperthermia, an effect that is readily antagonized by the non-selective antagonist naloxone (for an extensive review see 3), strongly indicates its clear selectivity for the putative μ1-site using this way of administration. Furthermore, our finding suggests that morphine-induced hyperthermia is not mediated by μ1-receptors.

In contrast to our findings in rats, in mice the blockade of μ1-receptors by naloxonazine did not affect morphine-induced CPP (37). This may be because mice differ from rats in many respects like in the distribution and proportion of opioid receptors in various areas of brain (7, 8, 22, 41). Rats and mice also differ in their behavioural response to morphine, large doses of morphine induce catalepsy in rats (μ1-effect) but locomotor activation in mice (14, 30). Differences in opioid receptor mediated functions also occur between different strains of rats, as etonitazene, a possible μ1-opioid receptor selective agonist, serves as a reinforcer in one rat line but not in another one (38).

Recent reports have emphasized the role of δ-receptor in the rewarding properties of cocaine (23). Also, δ-receptors have been proposed to mediate rewarding effects produced by intra-accumbal morphine (32). Furthermore, δ-opioid antagonists were shown to abolish the morphine-induced place preference in mice (39). Although the affinity of morphine to δ-receptors is relatively low as compared to μ-receptors (4), δ-receptors could mediate part of rewarding effects of morphine in rats as well. Our results, however, do not support this idea, because naltrindole was without effect on morphine-induced CPP. Neither did intracerebral administration of δ-antagonist, ICI 174,864, modify the CPP induced by ICV morphine (33). Furthermore, naltrindole affected heroin self-administration only at doses (10 and 15 mg/kg, 26), which were 10-1000 fold larger than the ones needed to antagonize the antinociception induced by the selective δ-agonists, DPDPE or DSLET (5). As naltrindole blocks both putative subtypes (δ1 and δ2) of δ-receptors (28, 40), it seems unlikely that δ-opioid receptors are involved in the rewarding effects of morphine in rats.

In conclusion, our results indicate a distinct role for μ1-opioid receptors in the rewarding effects of morphine in rats, and δ-opioid receptors appear to be without significance in this respect as well as in the mediation of morphine-induced catalepsy. Furthermore, our results confirm that μ1-opioid receptors play an active role in the mediation of morphine-induced antinociception and catalepsy. In contrast, μ1-opioid receptors do not seem to be involved in the morphine-induced hyperthermia.

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TABLE 1

The effect of naloxonazine pretreatment (15mg/kg, IP, 12 h) on morphine-induced (3 mg/kg, SC) antinociception and hyperthermia. Antinociception was measured by estimating the latency (s), and mean percentages of maximum possible effect (% MPE) were calculated. The rectal temperatures (Trect, °C) were measured from the same animals immediately before placing them on the hot plate. The median values ± 95% confidence limits (latency and %MPE) or the mean values ± SE (Trect°C) of 7–8 animals are given.

	Time after morphine administration		
Pretreatment	0 min	30 min	60 min
		Latency	
Vehicle	8.4±2.9	16.2±3.2 *	9.4±1.4
Naloxonazine	12.8±3.3	12.3±3.0	10.0±2.9
		% MPE	
Vehicle		31.6±15.1	0.5±14.4
Naloxonazine		–12.2±26.1 †	–2.6±23.2
		Trect °C	
Vehicle	38.0±0.1	38.6±0.2 *	38.7±0.2 **
Naloxonazine	38.3±0.1	38.9±0.1 **	38.9±0.2 *

* $p < 0.05$; ** $p < 0.01$ vs. corresponding value at 0 min, Wilcoxon signed-rank test (latency and % MPE) or Student's paired two tail *t*-test (Trect).

† $p < 0.05$ vs vehicle pretreatment; Mann-Whitney U-test.

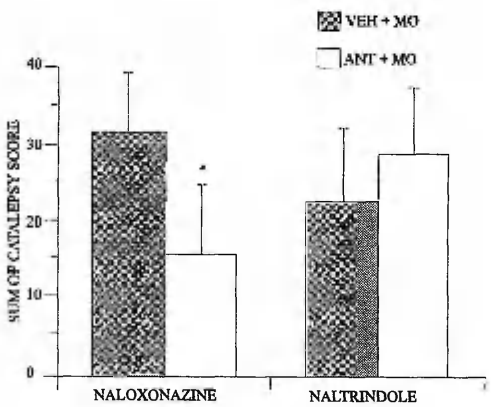

Fig. 1. Effects of opioid antagonists naloxonazine (15 mg/kg, IP, 12 h before morphine) and naltrindole (2 mg/kg, IP, 15 min before morphine) on catalepsy induced by morphine (15 mg/kg, SC). The control rats received vehicle 12 h or 15 min before morphine, respectively. The columns show the summed catalepsy scores of five measurements at 30 min intervals after morphine administration. The median values ± 95% confidence limits are given, n=6–9. Abbreviations: VEH = vehicle, MO = morphine, ANT = antagonist. * p<0.05 (Mann-Whitney U-test).

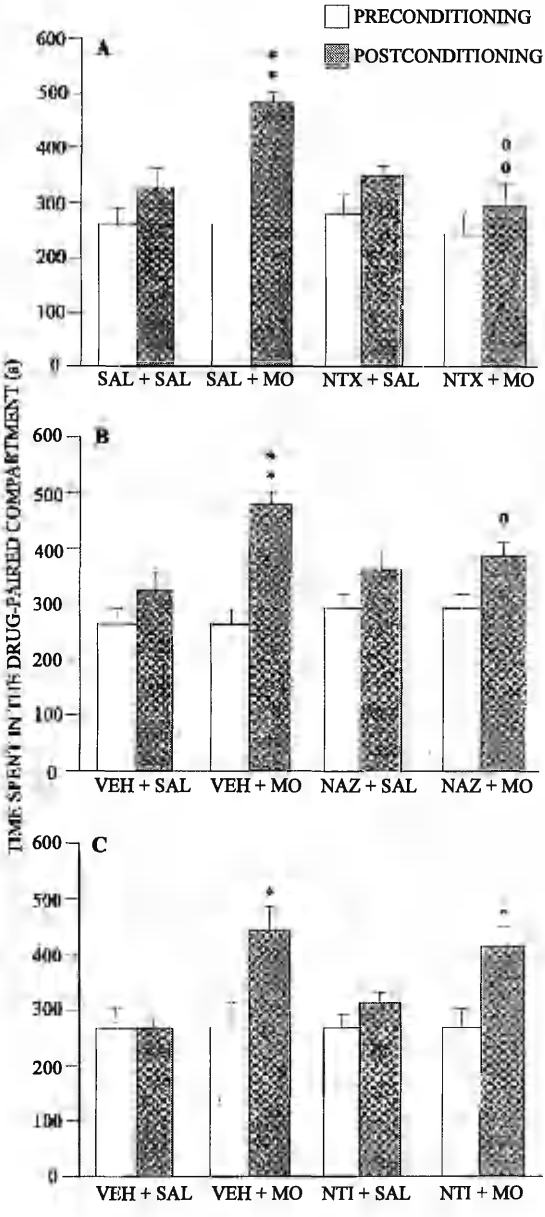

Fig. 2. Effects of opioid antagonists naltrexone (NTX, 2.5 mg/kg SC, panel A), naloxonazine (NAZ, 15 mg/kg, IP, panel B) and naltrindole (NTI, 2 mg/kg, IP, panel C) on conditioned place preference induced by morphine (3 mg/kg, SC). The antagonists were administered 20 min,12 h, and 15 min before morphine, respectively. The control rats received saline (SAL) or vehicle (VEH). The columns show the times (means ± SE) the rats (n= 7–18) spent in the initially non-preferred (white) compartment during preconditioning (shaded columns) and postconditioning (filled columns).
* p< 0.05, ** p<0.01 vs control group. o p<0.05, oo p<0.01 vs morphine group (Tukey-compromise test).

CURRICULUM VITAE

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Educational highlights

1984–1991 Medical faculty of the University of Tartu, MD upon graduation.
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During 1992–1994 I have worked for 9 months in the Division of Pharmacology and Toxicology, Department of Pharmacy, University of Helsinki, as a visiting scientist.

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Senine teadustegevus

Ravimisõltuvuse mehhanismid, eriti opioidide ning psühhomotoorsete stimulaatorite positiivsed sarrusomadused.

Publikatsioonid

22 teaduslikku publikatsiooni.

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