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Intermittent and chronic morphine treatment induces long-lasting changes in δ-opioid receptor-regulated acetylcholine release in rat striatum and nucleus accumbens

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Abstract

Intermittent treatment of rats with morphine (10 mg/kg s.c., once daily) caused an increase (of about 30%) of the electrically evoked release of [^{14}C]acetylcholine from cholinergic interneurons of superfused striatal slices 1–21 days after morphine withdrawal. Similarly, chronic treatment with escalating doses of morphine (5–50 mg/kg s.c., 3 times daily), causing physical dependence (unlike intermittent treatment), resulted in an enduring enhanced response of these neurons towards depolarization. Following chronic morphine treatment this adaptive increase of acetylcholine release was associated with a slight but long-lasting decrease of the (δ-opioid receptor-mediated) maximal inhibitory effect of [Met^5]enkephalin, whereas upon intermittent drug treatment δ-opioid receptor desensitization was observed 1 day after opiate withdrawal only. Also in slices of the nucleus accumbens both intermittent as well as chronic morphine administration caused a long-lasting increase of the electrically evoked [^{14}C]acetylcholine release. Therefore, we hypothesize that an enhanced (re)activity of striatal and accumbal cholinergic neurons, which are regulated by dopaminergic neurons of the ventral mesencephalon, may represent a long-lasting neuroadaptive effect of morphine (and possibly other drugs of abuse) playing a crucial role in behavioral sensitization associated with enhanced vulnerability to drugs of abuse.

Keywords: Acetylcholine release; Striatum; Nucleus accumbens; Morphine; Opioid receptor; Sensitization

1. Introduction

Several studies have shown that dopaminergic neurotransmission in rat striatum and nucleus accumbens may play a prominent role in the acute and long-lasting effects of morphine and other drugs of abuse (for reviews see Wise and Bozarth, 1987; Di Chiara and North, 1992; Robinson and Berridge, 1993). Presynaptically, drugs of abuse including morphine increase dopamine release acutely in rat striatum and nucleus accumbens (Di Chiara and Imperato, 1988). In addition, withdrawal from chronic administration of such addictive drugs has been shown to induce an attenuation of dopamine release in these brain regions (Acquas and Di Chiara, 1992; Rossetti et al., 1992; King et al., 1993).

Interestingly, the neurobiochemical and behavioral effects of morphine and psychostimulants such as cocaine and amphetamine seem to depend on the temporal pattern of drug exposure (Robinson and Becker, 1986; Wise and Bozarth, 1987). Unfortunately, the terminology used in the literature to denote treatment schedules is marked by inconsistency (Robinson and Becker, 1986). However, in this respect, two types of drug administration may be discerned: (1) the 'intermittent' type using relatively low dosage with long intervals and (2) the 'chronic' type (leading to tolerance and physical dependence) involving either high and/or escalating dosage regimens with short intervals or continuous administration using subcutaneous pellets or osmotic minipumps. Intermittent morphine and psychostimulant administration was shown to result in

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an enhanced drug- and depolarization-induced dopamine release in vitro (King et al., 1993; Tjon et al., 1994) and in vivo (Robinson and Becker, 1986; Kalivas and Duffy, 1993; Spanagel and Shippenberg, 1993). This is apparent several days after drug withdrawal and seems to be associated with the progressive drug effects on psychomotor activity (behavioral sensitization), which have been observed to last long after the last drug administration (Kalivas and Duffy, 1993; Bartoletti et al., 1983). Although similar effects have also been observed *long* after cessation of chronic morphine treatment (Acquas and Di Chiara, 1992; Spanagel and Shippenberg, 1993), the *initial* phase of withdrawal from this morphine and cocaine treatment regimen is characterized by tolerance/dependence and a state of depression and dysphoria associated with a decreased basal dopamine output in vivo (Acquas and Di Chiara, 1992; Weiss et al., 1992; Rossetti et al., 1993) and dopamine release in vitro (King et al., 1993; Tjon et al., 1994).

With regard to the time course of morphine-induced neuronal adaptation at the level of dopamine nerve terminals, we recently reported that long-term alterations in dopamine release depend on the temporal pattern of morphine administration (Tjon et al., 1994). Thus, 3 weeks after withdrawal from intermittent morphine treatment, the electrically evoked [^{3}H]dopamine release from superfused rat striatal slices appeared to be increased. In marked contrast, the release of this neurotransmitter was reduced persistently subsequent to chronic morphine administration. However, regarding long-lasting neurochemical adaptations after morphine withdrawal, little attention has as yet been paid to changes in neurotransmitter release from neurons that are (tonically) modulated by released dopamine in rat striatum and nucleus accumbens. For instance, the activity of cholinergic interneurons in these brain regions is well known to be tonically inhibited by released dopamine through activation of dopamine D_2 receptors (Stoof et al., 1992). In this respect, morphine-induced changes in cholinergic neurons in rat striatum and nucleus accumbens may serve as a model for postsynaptic neural plasticity involved in the long-term effects of drugs of abuse. In addition, several lines of evidence indicate that the central cholinergic system may be involved in opiate-induced effects (Frederickson and Pinsky, 1975; Holland et al., 1993). Thus in naive rats, morphine acutely attenuates striatal acetylcholine release in vivo (Taguchi et al., 1993) and in morphine-dependent rats (Jhamandas and Sutak, 1974) basal acetylcholine release has been shown to be lower than in saline-treated controls. Moreover, Rada et al. (1991) showed that naloxone-precipitated withdrawal is associated with a rebound acetylcholine increase in rat nucleus accumbens in vivo.

In this study, we investigated whether or not morphine withdrawal is associated with long-term adaptive alterations in cholinergic interneurons of rat striatum and nucleus accumbens depending on the temporal pattern of morphine treatment. Therefore, we examined the electrically evoked [^{14}C]acetylcholine release from slices of rat striatum and nucleus accumbens 1–21 days after two different modes of morphine administration (intermittent vs. chronic). Moreover, we also studied the inhibitory effect of [Met^5]enkephalin on acetylcholine release, in view of the fact that (1) blockade of δ-opioid receptor has been shown to attenuate certain behavioral effects by various drugs of abuse (Abdelhamid et al., 1991; Heidbreder et al., 1993, Jones et al., 1993), (2) morphine treatment may be associated with changes in enkephalin biosynthesis (Uhl et al., 1988; Pierce et al., 1992; Basheer and Tempel, 1993), and (3) enkephalin potently inhibits acetylcholine release in rat striatum and nucleus accumbens through activation of a homogeneous population of δ-opioid receptors (Mulder et al., 1984; Schoffelmeer et al., 1988; Heijna et al., 1990).

2. Material and methods

2.1. Drug treatment

Male Wistar rats (180–220 g body weight), purchased from Harlan (Zeist, Netherlands), were housed in groups of 3 per cage in a temperature-controlled room with a 12 h light/dark cycle (lights on at 07.00 h) and were given food and water ad libitum. The animals were handled at least 3 days before the drug treatment. Intermittent morphine administration consisted of one daily s.c. injection of 10 mg/kg morphine hydrochloride, for 14 days at 14.00 h. Morphine dependence in rats was induced by chronic treatment, using an escalating dosage regimen consisting of 3 daily s.c. injections (at 10.00, 14.00, and 18.00 h) of morphine hydrochloride: 5 mg/kg (day 1), 10 mg/kg (day 2), 20 mg/kg (day 3), 30 mg/kg (day 4), 40 mg/kg (day 5) and 50 mg/kg (day 6). Respective control groups received s.c. saline injections. Previous experiments had shown that, in contrast to intermittent drug treatment, chronic morphine administration induced severe naloxone (1 mg/kg)-precipitated physical withdrawal effects. These were no longer observed 3 weeks after morphine withdrawal.

2.2. Determination of neurotransmitter release

The rats were decapitated and the striatum or nucleus accumbens was rapidly dissected from the brain. Slices ($0.3 \times 0.3 \times 2$ mm) were prepared using a McIlwain tissue chopper, then incubated and superfused essentially as described previously (Schoffelmeer et al., 1988). In short, slices were washed twice with 5 ml

Krebs-Ringer bicarbonate medium containing 121 mM NaCl, 1.87 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 1.22 mM $CaCl_2$, 25 mM $NaHCO_3$, 10 mM D-(+)-glucose and subsequently incubated for 15 min in this medium containing 0.1 μM [^{14}C]choline in an atmosphere of 95% O_2/5% CO_2 at 37°C. After labelling, the slices were washed and transferred to each of the 24 chambers of a superfusion apparatus (about 4 mg tissue per chamber; 0.2 ml volume) and superfused (0.25 ml/min) with medium gassed with 95% O_2/5% CO_2 at 37°C. In each experiment neurotransmitter release from brain slices of morphine and the respective saline-treated rats was studied simultaneously in 24 chambers of one superfusion apparatus. The superfusate was collected as 10-min samples after 40 min of superfusion ($t = 40$ min). Ca^{2+}-dependent neurotransmitter release was induced during superfusion by exposing the slices to electrical biphasic blockpulses (1 Hz, 30 mA, 4 ms pulses) for 10 min at $t = 50$ min (electrical field stimulation). Drugs were added to the medium 20 min prior to stimulation. The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 M HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting. The efflux of radioactivity during each collection period was expressed as a percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. The electrically evoked release of neurotransmitter was calculated by subtracting the spontaneous, Ca^{2+}-independent efflux of radioactivity from the total overflow of radioactivity during stimulation and for the next 10 min. A linear decline from the 10-min interval before to that 20–30 min after the start of stimulation was assumed for calculation of the spontaneous efflux of radioactivity. The release evoked was expressed as percentage of the content of radioactivity of the slices at the start of the stimulation period.

2.3. Radiochemicals and drugs

[^{14}C]Choline (15 mCi/mmol) was purchased from the Radiochemical Centre (Amersham), morphine hydrochloride from Onderlinge Pharmaceutische Groothandel (Utrecht, Netherlands) and [Met^5]enkephalin from Peninsula Laboratories.

2.4. Statistics

One-way analysis of variance of release data was conducted for a set of replicate experiments within one time point. If replicate experiments were not significantly different the release data were considered to represent one population and the observations were pooled for two-tailed Student t-test analysis to determine statistical significance of differences between saline and morphine groups.

3. Results

3.1. Depolarization-induced Ca^{2+}-dependent [^{14}C]acetylcholine release

It has been well established that the electrically evoked overflow of radioactivity from rat brain slices incubated with a radiolabelled neurotransmitter or its precursor (such as [^{14}C]choline), represents depolarization-induced exocytotic neurotransmitter release (e.g. see Orrego, 1979; Lehmann and Langer, 1983; Starke et al., 1989). Thus, under conditions of relatively low frequency stimulation, such as used in our present study, the evoked release has been shown to be completely blocked in the absence of calcium, abolished by calcium channel blockers, tetrodotoxin and strongly enhanced following blockade of potassium channels. We have studied the time course of adaptive changes in electrically evoked Ca^{2+}-dependent [^{14}C]acetylcholine release induced by two modes of morphine exposure, i.e. intermittent and chronic treatment. To control for the variability of the evoked [^{14}C]acetylcholine release between experiments, both saline-and morphine-treated tissue was always studied simultaneously within a single experiment (i.e. in parallel chambers of one superfusion apparatus), measuring both

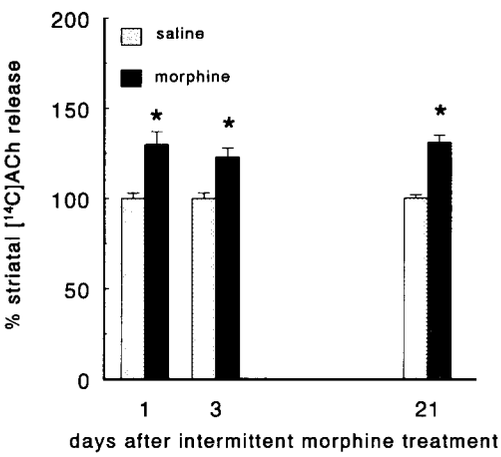

Fig. 1. Intermittent morphine treatment increased electrically evoked [^{14}C]acetylcholine release from rat striatal slices. Slices of rat striatum were incubated with [^{14}C]choline and subsequently superfused with Krebs-Ringer medium. [^{14}C]Acetylcholine release was induced electrically and control release (percentage of total radioactivity) in excess of spontaneous release from slices of saline-pretreated rats amounted to 4.91±0.29% (day 1), 5.20±0.23% (day 3) and 6.42±0.31% (day 21) respectively. The results are expressed as percentage of respective saline groups (saline = 100%). Values represent means ±S.E.M. from 9–18 observations. Triplicate observations were made in each experiment. * $P < 0.05$.

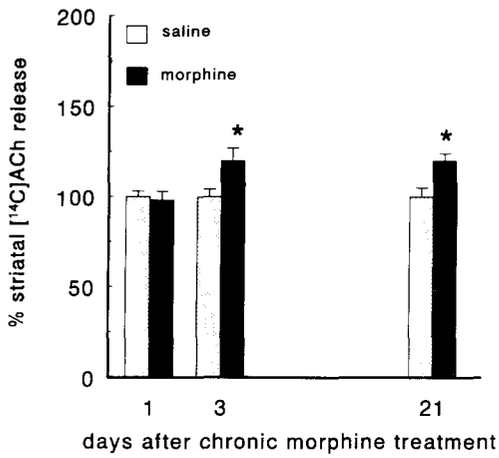

Fig. 2. Striatal [^{14}C]acetylcholine release is increased after chronic morphine administration. After incubation with [^{14}C]choline, rat striatal slices were superfused with medium and [^{14}C]acetylcholine release was stimulated electrically. Control release (percentage of total tissue radioactivity) in excess of spontaneous release from slices of saline-pretreated rats was 5.61 ± 0.42% (day 1), 5.97 ± 0.27% (day 3) and 4.30 ± 0.23% (day 21) respectively. The results are expressed as percentage of respective saline groups (saline = 100%). Values represent means ± S.E.M. from 6–18 observations. Triplicate observations were made in each experiment. * $P < 0.05$.

neurotransmitter release and its inhibition by 10^{-8}–10^{-6} M [Met^5]enkephalin. Neither the spontaneous release of [^{14}C]acetylcholine (amounting to about 2% of total tissue radioactivity) nor the uptake of [^{14}C]choline (about 7500 dpm/mg wet tissue) was significantly changed by either morphine treatment. Subsequent to intermittent once-daily morphine administration, evoked [^{14}C]acetylcholine release from striatal slices was significantly increased by 30 ± 7% on day 1 of withdrawal, by 23 ± 5% on day 3 of withdrawal and by 31 ± 4% on day 21 of withdrawal (Fig. 1). Such a long-lasting increase of striatal [^{14}C]acetylcholine release was also found after chronic morphine administration: 20 ± 7% on day 3 and 20 ± 4% on day 21 of withdrawal, with the exception that [^{14}C]acetylcholine release was unchanged on day 1 of withdrawal (−2 ± 5%) (Fig. 2). In order to investigate whether similar long-term adaptations occur in the nucleus accumbens, we also performed experiments 3 weeks after cessation of either drug exposure using slices of this limbic part of the striatal complex. These experiments indeed showed increases in [^{14}C]acetylcholine release of 51 ± 4% and 36 ± 4% following both intermittent and chronic morphine treatment, respectively (Fig. 3.).

3.2. δ-Opioid receptor regulation of [^{14}C]acetylcholine release

Previous studies have shown that in rat striatal slices, the inhibitory effect of opioids on [^{14}C]acetylcholine release is exclusively mediated by activation of a homogenous population of δ-opioid receptors (Schoffelmeer et al., 1988, 1993; Mulder and Schoffelmeer, 1993). In order to study morphine treatment-induced changes in δ-opioid receptor efficacy, δ-opioid receptor-mediated inhibition of electrically evoked [^{14}C]-acetylcholine release was determined using of 10^{-8}–10^{-6} M [Met^5]enkephalin as (endogenous) agonist. Earlier work has shown that [Met^5]enkephalin inhibits striatal [^{14}C]acetylcholine release with an EC_{50} of about 1×10^{-7} M, acting as a full high-affinity agonist at functional δ-opioid receptors under the present in vitro conditions (Mulder et al., 1989). Our results indicate that morphine-induced changes in the efficacy of the δ-opioid receptor were rather modest and only

Table 1
The effect of (A) intermittent and (B) chronic morphine treatment on the δ-opioid receptor-mediated inhibition of electrically evoked [^{14}C]acetylcholine release by [Met^5]enkephalin in rat striatal slices

[Met^5]Enkephalin concentration	[^{14}C]Acetylcholine release (as % of respective control)			
	1 day of withdrawal		3 weeks of withdrawal	
	Saline	Morphine	Saline	Morphine
(A) Intermittent morphine treatment				
–	100 ± 3	100 ± 6	100 ± 3	100 ± 3
10^{-8} M	88 ± 4	90 ± 3	93 ± 2	91 ± 4
10^{-7} M	80 ± 5	82 ± 5	83 ± 4	74 ± 3
10^{-6} M	55 ± 2	64 ± 2 [a]	60 ± 2	58 ± 3
(B) Chronic morphine treatment				
–	100 ± 3	100 ± 5	100 ± 2	100 ± 2
10^{-8} M	94 ± 4	93 ± 4	90 ± 5	104 ± 4
10^{-7} M	80 ± 3	82 ± 7	77 ± 6	88 ± 6
10^{-6} M	52 ± 2	67 ± 5 [a]	39 ± 4	58 ± 4 [a]

Striatal slices of rats treated with saline or morphine were labelled with [^{14}C]-choline and perfused with medium. [Met^5]Enkephalin was added 20 min prior to electrical stimulation. Values represent means ± S.E.M. from 6–18 observations. Control releases (percentage of total tissue radioactivity) are given in the legends to Figs. 1 and 2. In each experiment triplicate observations were made. [a] $P < 0.05$.

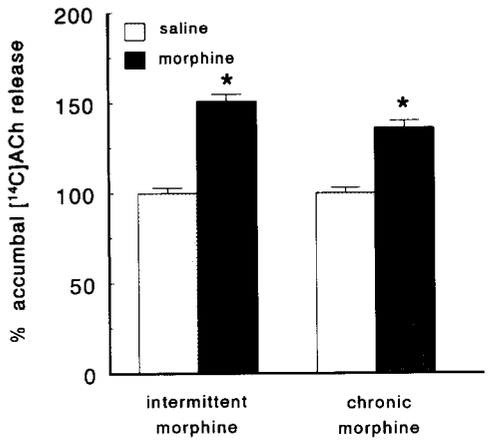

Fig. 3. Intermittent and chronic morphine induce an enduring increase of [^{14}C]acetylcholine release from slices of rat nucleus accumbens. Rat accumbal slices, labelled with [^{14}C]choline, were superfused with medium and [^{14}C]acetylcholine release was evoked electrically. Control release (percentage of total tissue radioactivity) in excess of spontaneous release from slices of saline-pretreated rats was $1.82 \pm 0.10\%$ and $2.47 \pm 0.14\%$ after intermittent and chronic administration, respectively. The results are expressed as percentage of respective saline groups (saline = 100%). Values represent means $\pm$ S.E.M. from 12–18 observations. Triplicate observations were made in each experiment. * $P < 0.05$.

reached statistical significance when the receptor was maximally activated, i.e. with 10^{-6} M [Met^5]enkephalin. On day 1 of withdrawal, intermittent and chronic morphine treatment resulted in a slight but significant reduction of the maximal inhibitory effect of [Met^5]enkephalin by about 20 and 30%, respectively (Table 1). However, the reduction induced by intermittent morphine treatment did not persist, in contrast to the long-lasting decrease observed after chronic morphine treatment, which was still apparent on day 21. In support of these data are the results of additional experiments performed on day 3 of withdrawal indicating reductions of the maximal effect of 15 and 30% after intermittent and chronic morphine treatment, respectively. These results, however, did not reach statistical significance (saline vs. morphine: $58 \pm 3\%$ vs. $64 \pm 3\%$ ($P = 0.052$) for intermittent morphine; $56 \pm 3\%$ vs. $69 \pm 5\%$ ($P = 0.08$) for chronic morphine). Since investigating the dose-dependent inhibitory effect of [Met^5]enkephalin on [^{14}C]acetylcholine release would require a very large number of rats in case of a small rat brain region such as the nucleus accumbens, δ-opioid receptor efficacy was investigated in striatal slices only.

4. Discussion

In the present study intermittent morphine treatment is defined as the administration of the same dose of 10 mg/kg of the drug once daily (leading to only transiently elevated brain levels), whereas the daily administration of 3 (escalating) doses of morphine is denoted as chronic treatment. Thus, intermittently treated rats received one daily s.c. injection of a relatively low dose of morphine (10 mg/kg) during 14 days, whereas chronic morphine administration consisted of 3 daily s.c. injections with increasing doses (5–50 mg/kg) of the drug for a period of 6 days. Although the latter drug regimen is not identical to drug delivery via morphine pellets or osmotic minipumps, it has long been known to induce similar behavioral effects, i.e. severe but reversible physical dependence and tolerance followed by a long-lasting behavioral sensitization (Kaymakcalan and Woods, 1956; Gunne, 1963; Acquas and Di Chiara, 1992; Spanagel and Shippenberg, 1993). On the other hand, behavioral sensitization caused by the once-daily intermittent administration of morphine (10 mg/kg) is not preceded by a period of physical dependence in our hands (Tjon et al., 1994). Recently, we observed these two different administration patterns to cause differential enduring changes in the electrically evoked [^{3}H]dopamine release from rat striatal slices. Although both treatments resulted in an attenuated electrically evoked [^{3}H]dopamine release on day 1 of withdrawal, the increase of exocytotic dopamine release found after intermittent morphine withdrawal seemed to slowly develop in time. Thus, after intermittent morphine administration, dopamine release was decreased on day 1, unchanged on day 3, but increased on day 21 after cessation of morphine treatment (Tjon et al., 1994). In contrast, subsequent to the chronic morphine exposure dopamine release remained reduced throughout the 3-week withdrawal period. Interestingly, the present study shows a strikingly different temporal pattern regarding changes in the in vitro [^{14}C]acetylcholine release. Thus, intermittent as well as chronic morphine administration caused an enduring augmentation of electrically evoked [^{14}C]-acetylcholine release. Moreover, this postsynaptic adaptive change was present in both rat striatum and nucleus accumbens and lasted until at least 3 weeks after cessation of treatment.

Acetylcholine release in rat striatum and nucleus accumbens is tonically inhibited by dopamine through activation of dopamine D_2 receptors in vivo and in vitro (Stoof et al., 1992). Consequently, it might be argued that the observed augmentation of [^{14}C]acetylcholine release is caused by an attenuation of dopamine release within the brain slices as seen after chronic morphine exposure (Acquas and Di Chiara, 1992; Tjon et al., 1994). However, pharmacological blockade of the dopamine D_2 receptor with (−)-sulpiride only slightly (by about 10%) enhances the electrically evoked [^{14}C]acetylcholine release in vitro (Stoof et al., 1992). Furthermore, the enhanced acetylcholine release was

observed in vitro following both modes of morphine treatment, which differentially affect dopamine release. Hence, although an indirect mechanism of action can as yet not be excluded, we hypothesize that the enhanced response of cholinergic interneurons towards depolarization results from adaptive changes within these neurons.

Interestingly, previous in vitro studies in our laboratory have shown that acetylcholine release in the striatum is not inhibited by μ-opioid receptors, unlike accumbal acetylcholine release (Mulder et al., 1984; Schoffelmeer et al., 1988, 1993; Heijna et al., 1990; for review, see Mulder and Schoffelmeer, 1993). Consequently, the long-lasting adaptive increase of [^{14}C]-acetylcholine release in slices of both brain regions upon morphine withdrawal may not result from activation by the μ-agonist morphine of μ-opioid receptors within the membranes of cholinergic interneurons. Therefore, it is possible that the initial decrease of dopamine release after both intermittent and chronic treatment (Tjon et al., 1994) causes an enduring postsynaptic adaptation in the excitability of cholinergic neurons, independent of the subsequent presynaptic changes in dopamine release. Obviously, morphine-induced alterations in other neurotransmitter systems may (also) contribute to the observed cholinergic adaptation (Uhl et al., 1988; Gifford and Johnson, 1992; Lindefors et al., 1992; Rada et al., 1993a,b).

Cholinergic interneurons in rat striatum receive input from enkephalin immunoreactive terminals (Martone et al., 1992) and the release of enkephalin is thought to cause a profound inhibition of the activity of cholinergic interneurons through selective activation of δ-opioid receptors (Mulder et al., 1984; Schoffelmeer et al., 1988; Heijna et al., 1990). Moreover, at least two lines of evidence indicate that the central enkephalin system is involved in the behavioral effects of drugs of abuse. First, drugs of abuse including morphine have been shown to alter striatal opioid peptide gene expression (Uhl et al., 1988; Hurd et al., 1992). Second, several reports have indicated that naltrindole, a δ-opioid receptor antagonist, is able to attenuate certain behavioral effects of opiates and psychostimulants (Abdelhamid et al., 1991; Menkens et al., 1992; Heidbreder et al., 1993; Jones et al., 1993). In this regard it is of interest to know whether δ-opioid receptor efficacy changes after morphine treatment. Interestingly, the present study shows a modest desensitization of the δ-opioid receptor-mediated inhibition of [^{14}C]acetylcholine release by [Met^5]enkephalin one day after both morphine regimens. This desensitization appeared to be reversible in case of the intermittent treatment and was no longer apparent 3 days after opiate withdrawal. In contrast, a long-lasting desensitization of functional δ-opioid receptors was observed after chronic morphine exposure lasting for at least 21 days. In this respect, it is important to note that withdrawal from chronic morphine treatment has also been reported to induce a decrease in rat striatal preproenkephalin gene expression and enkephalin immunoreactivity (Shani et al., 1979; Uhl et al., 1988; Pierce et al., 1992; but see Mocchetti and Costa, 1987). Together with δ-opioid receptor desensitization this may cause an even further increase of acetylcholine release in vivo. It should be noted here that at high concentrations morphine may also act as a agonist on δ-opioid in addition to μ-opioid receptors. Hence, the observed long-term desensitization upon chronic morphine treatment might result from activation of δ-opioid instead of μ-opioid receptors. The occurrence of desensitization of enkephalin-selective δ-opioid receptors does not seem to be a general phenomenon. It may be restricted to e.g. presynaptic δ-opioid receptors in view of the absence of desensitization of δ-opioid receptors mediating inhibition of postsynaptic dopamine-sensitive adenylate cyclase in striatal slices of rats previously treated with morphine (Tjon et al., 1994).

Taken together, this study is the first to show long-lasting changes in acetylcholine release and its δ-opioid receptor-mediated inhibition following cessation of intermittent and chronic morphine treatment. Moreover, unlike the increase of acetylcholine release 1–21 days upon morphine withdrawal, the persistent (modest) desensitization of inhibitory δ-opioid receptors appeared to depend on the temporal pattern of morphine administration and did not occur following intermittent treatment. Interestingly, various in vivo studies indicate a possible important role of acetylcholine in the behavioral effects of drugs of abuse. Thus, Katz and Valentino (1984) reported that cholinergic agents, such as physostigmine, mimic many initial signs of opiate withdrawal in rhesus monkeys. Furthermore, Rada et al. (1991) showed that in morphine-dependent rats, clonidine attenuated the naloxone-precipitated behavioral withdrawal symptoms as well as the rebound acetylcholine release increase in the nucleus accumbens. Finally, it was reported that i.c.v. injection of muscarinergic antagonists diminished the initial physical withdrawal signs observed after morphine withdrawal (Holland et al., 1993).

Indeed cholinergic interneurons have been suggested to play a pivotal role in the regulation of the activity of afferent and efferent neurons of the striatum and nucleus accumbens (Di Chiara et al., 1994). In this regard our present neurochemical data suggest that the enduring increase of the cholinergic neurotransmission may also play a role in the long-lasting behavioral effects of morphine such as psychomotor sensitization and enhanced vulnerability towards the acquisition of drug dependence (for review see Robinson and Berridge, 1993). For instance, activation of cholinergic nicotine and muscarine receptors on dopaminergic

neurons causes depolarization (Lacey et al., 1990; Lichtensteiger et al., 1982) and exocytotic dopamine release (Rapier et al., 1990; Ronken et al., 1994; Schoffelmeer et al., 1986). Therefore, we speculate that the morphine withdrawal-induced increase in acetylcholine release might result in a partial depolarization of the dopaminergic neurons arising from the ventral mesencephalon, which could lead to an enduring hyperexcitability of these neurons and enhanced dopamine release per action potential. Thus, enhanced activation of both nicotinic and muscarinic receptors could be involved in the facilitated effect of a challenge with drugs of abuse on dopamine neurons associated with behavioral sensitization as observed with in vivo microdialysis (Acquas and Di Chiara, 1992; Spanagel and Shippenberg, 1993; Kalivas and Duffy, 1993). In this respect it should be noted that we have recently observed a similar long-lasting increase of acetylcholine release in rat striatal and accumbal slices following intermittent cocaine administration, suggesting that the present observation with morphine can be extended to differentially acting drugs of abuse (De Vries et al., in preparation).

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