ELSEVIER

European Journal of Pharmacology 286 (1995) 311–314

ejp

Short communication

Intermittent morphine treatment causes a protracted increase in cholinergic striatal neurotransmission measured ex vivo

Anton N.M. Schoffelmeer *, Patrizia Nestby, Guno H.K. Tjon, George Wardeh, Taco J. De Vries, Louk J.M.J. Vanderschuren, Arie H. Mulder

Graduate School Neurosciences Amsterdam, Research Institute Neurosciences Vrije Universiteit, Department of Pharmacology, Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam, Netherlands

Received 12 June 1995; revised 31 August 1995; accepted 19 September 1995

Abstract

Considering the long-lasting neuroadaptations that occur in the brain after exposure to drugs of abuse, we found that the facilitatory effect of an EC_{50} concentration (0.1 μM) of the acetylcholinesterase inhibitor physostigmine, unlike that of the muscarinic receptor agonist oxotremorine, on K^+-induced [^{3}H]dopamine release from rat striatal slices was enhanced about 2-fold 1 month after cessation of intermittent morphine treatment. Similarly, the inhibitory effect of physostigmine on K^+-induced [^{14}C]acetylcholine release from the slices was enhanced subsequent to morphine treatment, whereas that of oxotremorine appeared to be unchanged. Therefore, intermittent morphine administration may cause a very long-lasting increase of muscarinic receptor activation by released endogenous acetylcholine in rat striatum, which may play a pivotal role in the enduring character of stimulus hyperresponsiveness after exposure to drugs of abuse.

Keywords: Morphine; Acetylcholine; Muscarinic receptor; Striatum

1. Introduction

Intermittent exposure of rats to drugs of abuse or emotional stress is well known to induce a long-lasting hyperresponsiveness towards these stimuli (behavioral sensitization), which may play a crucial role in the acquisition of an addiction-prone state (for reviews, see Wise and Bozarth, 1987; Kalivas and Stewart, 1991; Robinson and Berridge, 1993). With regard to the neuroadaptive changes underlying the long-term expression of drug-induced behavioral sensitization attention has as yet primarily been focussed on dopaminergic neurotransmission in the dorsal and ventral striatum. Thus, intermittent treatment of rats with psychostimulants or morphine has been shown to cause an enduring increase in the reactivity of central dopaminergic neurons towards depolarization and addictive drugs (Kalivas and Duffy, 1993; Tjon et al., 1994; Paulson and Robinson, 1995) as well as a long-lasting supersensitivity of postsynaptic dopamine D_1 receptors in the striatal complex (Tjon et al., 1994 and references quoted therein). Recently, we demonstrated that intermittent morphine or cocaine treatment also causes an enduring increase of[^{14}C]acetylcholine release from cholinergic interneurons in slices of the dorsal and ventral striatum (Tjon et al., 1995). Therefore, assuming a similar effect of drugs of abuse on the release of *endogenous* acetylcholine, an adaptive increase in cholinergic neurotransmission could also be involved in the expression of sensitization. Since such an enduring enhanced release may be compensated by reduced neurotransmitter synthesis, enhanced breakdown by acetylcholinesterase or desensitization of muscarinic receptors, the present study examined as to whether or not intermittent morphine administration indeed causes a protracted increase of muscarinic receptor activation by released endogenous acetylcholine in superfused rat striatal slices.

* Corresponding author. Research Institute Neurosciences Vrije Universiteit, Department of Pharmacology, Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam, Netherlands. Tel.: 020-4448104, fax: 020-4448100.

SSDI 0014-2999(95)00588-9

2. Materials and methods

2.1. Morphine 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 once daily for 3 days before drug treatment. Intermittent morphine administration consisted of one daily s.c. injection of 10 mg/kg morphine hydrochloride for 14 days at 13.00 h. The control group received s.c. saline injections.

2.2. Determination of neurotransmitter release

One month after the last injection, rats were decapitated and striatal 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., 1986). 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 (pH 7.4) and subsequently incubated for 15 min in this medium, containing 0.1 μM [^{3}H]dopamine and 1 μM [^{14}C]choline under an atmosphere of 95% O_2-5% CO_2 at 37°C. After labelling, the slices were washed and transferred to each of 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°. The superfusate was collected as 10-min samples after 40 min of superfusion ($t = 40$ min). Calcium-dependent neurotransmitter release was induced during superfusion by exposing the slices to medium in which the KCl concentration was raised to 10 mM (NaCl was reduced in order to maintain isomolarity) for 10 min at $t = 50$ min. The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 N 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 K^+-induced release of neurotransmitter was calculated by subtracting the spontanous efflux of radioactivity from the total overflow of radioactivity during stimulation and the following 10 min. A linear decline from the 10-min interval before to that 20–30 min after the onset of stimulation was assumed for calculation of the spontanous efflux of radioactivity. The evoked release was expressed as percent of the content of radioactivity of the slices at the start of the stimulation period.

2.3. Determination of adenylate cyclase activity

Striatal slices were washed twice with the Krebs-Ringer bicarbonate medium described above, containing 1 mM 3-isobutyl-1-methylxanthine in order to prevent breakdown of cyclic AMP by phosphodiesterase. The slices were incubated with [^{3}H]adenine (4 μCi/ml) for 1 h and subsequently transferred to each of 24 chambers of a superfusion apparatus (4 mg tissue per chamber; 0.2 ml volume) and superfused (0.1 ml/min) with medium containing 3-isobutyl-1-methylxanthine and gassed with 95% O_2-5% CO_2 at 37°C. At $t = 30$ min the slices were exposed to drugs for 30 min. At $t = 60$ min the slices were extracted with 5% ice-cold trichloroacetic acid containing 1 mM of both unlabelled ATP and cyclic AMP during 15 min. The superfusate of this 15-min extraction period was further used for chromatography.

[^{3}H]Cyclic AMP was separated from other tritiated nucleotides, predominantly [^{3}H]ATP, by chromatography on Dowex and alumina columns (Salomon et al., 1974). The amounts of [^{3}H]cyclic AMP and [^{3}H]ATP were determined by liquid-scintillation counting. The activity of adenylate cyclase was expressed as the percentage of total tissue [^{3}H]ATP that was converted to [^{3}H]cyclic AMP.

2.4. Statistics

Statistical significance of differences between the raw data was determined by one-way analysis of variance followed by a post-hoc Student-Newman-Keuls test ($P < 0.05$). Subsequently, drug effects were expressed as percentage of respective control values.

2.5. Radiochemicals and drugs

[^{3}H]Adenine (25 Ci/mmol), [^{14}C]choline (15 mCi/mmol) and [^{3}H]dopamine (47 Ci/mmol) were purchased from the Radiochemical Center (Amersham, UK). Morphine hydrochloride was obtained from OPG (Utrecht, Netherlands) and 3-isobutyl-1-methylxanthine, physostigmine, oxotremorine and forskolin from Sigma (St. Louis, MO, USA).

3. Results

One month after intermittent treatment, the 10 mM K^+-induced release of [^{3}H]dopamine and [^{14}C]acetylcholine from superfused striatal slices of saline-treated rats amounted to about 2.7 and 9.5% of total tissue radioactivity, respectively. The evoked release of these

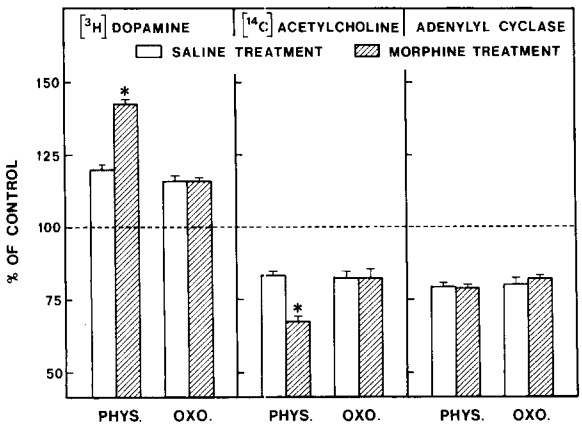

Fig. 1. Effect of intermittent morphine administration on the muscarinic receptor-mediated modulation of K^+-induced [^{3}H]dopamine and [^{14}C]acetylcholine release and forskolin-stimulated adenylyl cyclase activity by physostigmine and oxotremorine in rat striatal slices. One month after cessation of intermittent saline or morphine treatment, the effect of 0.1 μM physostigmine (phys.) and oxotremorine (oxo.) on 10 mM K^+-induced release of the radiolabelled neurotransmitters as well as on the percent conversion of [^{3}H]ATP in [^{3}H]cyclic AMP induced by 3 μM forskolin was determined in superfused striatal slices. Evoked [^{3}H]dopamine and [^{14}C]acetylcholine release in slices from saline-treated rats amounted to $2.75 \pm 0.11\%$ and $9.54 \pm 0.24\%$ of total tissue radioactivity, respectively, whereas in slices of morphine-treated rats the release of these neurotransmitters was enhanced to $3.45 \pm 0.08\%$ and $11.82 \pm 0.23\%$ ($P < 0.01$). The conversion of [^{3}H]ATP in [^{3}H]cyclic AMP (in the presence of forskolin) in slices of saline-treated rats amounted to $1.24 \pm 0.12\%$ and was not significantly different in slices of morphine-treated rats. Data, expressed as percentage of control values, represent means ± S.E.M. of 16 observations obtained in 4 separate experiments. * Significantly different than found in slices of saline-treated rats ($P < 0.05$).

neurotransmitters was significantly, i.e. 20%, higher in slices of morphine-treated rats. The spontanous release of [^{3}H]dopamine and [^{14}C]acetylcholine (amounting to about 3.5 and 2% of total tissue radioactivity, respectively), was not at all affected by prior morphine treatment. The muscarinic receptor antagonist atropine (0.1 μM) did not affect K^+-induced neurotransmitter release in slices of saline or morphine-treated rats, consistent with rapid inactivation of released acetylcholine by acetylcholinesterase (data not shown). The conversion of [^{3}H]ATP in [^{3}H]cyclic AMP in the presence of 3 μM forskolin amounted to about 1.2% in superfused *non-depolarized* striatal slices of rats treated intermittently with either saline or morphine.

At their EC_{50} concentration of 0.1 μM (see Schoffelmeer et al., 1986, 1988), the facilitatory effect of the acetylcholinesterase inhibitor physostigmine, unlike that of the muscarinic receptor agonist oxotremorine, on K^+-induced [^{3}H]dopamine release was enhanced 2-fold 1 month after cessation of morphine administration (Fig. 1). A similar differential adaptive effect was observed studying the inhibitory effect of these drugs on K^+-induced [^{14}C]acetylcholine release. In addition, Fig. 1 shows that inhibition of acetylcholinesterase by physostigmine (and direct activation of muscarinic receptors with oxotremorine) caused a similar inhibitory effect on adenylate cyclase activity in *non-depolarized* striatal slices of saline- or morphine-treated rats.

4. Discussion

Through muscarinic receptor activation, cholinergic interneurons regulate the activity of afferent and efferent neurons of the dorsal and ventral striatum (Di Chiara et al., 1994). For instance, muscarinic receptor agonists and acetylcholinesterase inhibitors enhance dopamine release at the level of dopaminergic nerve terminals within the striatal complex (Lehmann and Langer, 1982; Raiteri et al., 1984; Schoffelmeer et al., 1986). Moreover, at the level of efferent (γ-aminobutyric/opioid peptide/substance P) neurons activation of muscarinic receptors inhibits the functioning of postsynaptic dopamine D_1 receptors (Schoffelmeer et al., 1988). In view of the crucial role of the striatal complex in the acute and long-term behavioral effects of drugs of abuse, we recently investigated the possibility that drugs of abuse may persistently alter the functioning of these important interneurons. Interestingly, intermittent morphine or cocaine administration appeared to enhance depolarization-induced [^{14}C]acetylcholine release from slices of the dorsal as well as from the ventral striatum until at least 3 weeks after cessation of drug treatment (Tjon et al., 1995). With regard to the release of *endogenous* acetylcholine and the activation of functional muscarinic receptors in the striatum following morphine treatment, our present data indeed show an enduring increase in the activation of muscarinic autoreceptors by released endogenous acetylcholine. Thus, unlike the inhibitory effect of the muscarinic receptor agonist oxotremorine, that of endogenous acetylcholine (following partial inhibition of acetylcholinesterase by physostigmine) on K^+-induced [^{14}C]acetylcholine release was enhanced 1 month after cessation of intermittent morphine administration. Therefore, our data show that intermittent morphine treatment persistently enhances the extracellular concentration of endogenous acetylcholine, leading to enhanced autoreceptor activation upon inhibition of acetylcholinesterase without changes in the apparent affinity of the muscarinic receptors. Moreover, this study shows that the facilitatory effect of physostigmine on K^+-induced [^{3}H]dopamine release, caused by released endogenous acetylcholine at muscarinic (allo)receptors postsynaptic to cholinergic interneurons, is also enhanced 1 month after morphine administration.

Regarding the magnitude of this long-lasting neuroadaptive effect, one might wonder whether the observed 2-fold increase in the effect of an EC_{50} concen-

tration of physostigmine on [^{14}C]acetylcholine and [^{3}H]dopamine release can be explained by an increase in endogenous acetylcholine release of perhaps no more than the 20% observed when studying the release of radiolabelled acetylcholine. In fact, it might be argued that the enhanced effect of physostigmine can also be partly attributed to the occurrence of muscarinic receptor supersensitivity and/or adaptive changes in the binding characteristics of acetylcholinesterase. However, since the modulatory effect of oxotremorine (being no substrate for acetylcholinesterase) on K^+-induced neurotransmitter release was not affected by morphine administration, supersensitivity of muscarinic receptors does not seem to occur. Previous experiments have shown that extracellular acetylcholine in *non-depolarized* striatal slices causes muscarinic receptor-mediated inhibition of adenylyl cyclase activity, provided that acetylcholinesterase activity is inhibited by e.g. physostigmine (Schoffelmeer et al., 1988). Since morphine treatment did not change spontaneous [^{14}C]acetylcholine efflux, this inhibitory effect of physostigmine can be used as a sensitive parameter of acetylcholinesterase activity. In this respect, our experiments showed that the inhibitory effect of physostigmine (and oxotremorine) on forskolin-stimulated adenylyl cyclase activity in *non-depolarized* striatal slices was not affected following intermittent morphine treatment, indicating that previous opiate administration did not change the functioning of acetylcholinesterase. In view of these data, it is possible that the adaptive increase in depolarization (K^+)-induced [^{14}C]acetylcholine release is much lower than that of endogenous (unlabelled) acetylcholine at the level of the muscarinic receptors. We would like to emphasize, that the unchanged inhibitory effect of physostigmine on adenylyl cyclase activity in non-depolarized striatal slices from morphine-treated rats should not be taken as evidence against enhanced activation of these postsynaptic muscarinic receptors by depolarization-induced release of endogenous acetylcholine. Unfortunately, investigation of this issue is hampered by the fact that muscarinic receptor-mediated inhibition of adenylyl cyclase activity can no longer be detected when the slices are exposed to a high K^+ concentration (unpublished observations), probably due to the simultanous release of multiple transmitters acting on adenylyl cyclase-coupled receptors.

Taken together, the present data show that intermittent morphine administration causes a very long-lasting increase in pre- and postsynaptic muscarinic receptor activation in rat striatal slices due to an enduring increase in the reactivity of cholinergic interneurons. In view of the strategic role of these neurons in the integration of neurotransmission in the dorsal and ventral striatum, we tentatively suggest that a protracted increase in cholinergic neurotransmission may play a facilitatory role in the hyperresponsiveness of rats to drugs of abuse long after previous contact with these drugs.

References

Di Chiara, G., M. Morelli and S. Consolo, 1994, Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions, Trends Neurosci. 17, 228.

Kalivas, P.W. and P. Duffy, 1993, Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals, J. Neurosci. 13, 266.

Kalivas, P.W. and J. Stewart, 1991, Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity, Brain Res. Rev. 16, 223.

Lehmann, J. and S.Z. Langer, 1982, Muscarinic receptors on dopamine terminals in the cat caudate nucleus: neuromodulation of [^{3}H]dopamine release in vitro by endogeneous acetylcholine, Brain Res. 248, 61.

Paulson, P.A. and T.E. Robinson, 1995, Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats, Synapse 19, 56.

Raiteri, M., R. Leardi and M. Marchi, 1984, Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain, J. Pharmacol. Exp. Ther. 228, 209.

Robinson, T.E. and K.C. Berridge, 1993, The neural basis of drug craving: an incentive-sensitization theory of addiction, Brain Res. Rev. 11, 157.

Salomon, Y., C. Londos and M. Rodbell, 1974, A highly sensitive adenylate cyclase assay, Anal. Biochem 58, 541.

Schoffelmeer, A.N.M., B.J. Van Vliet, G. Wardeh and A.H. Mulder, 1986, Muscarinic receptor-mediated modulation of [^{3}H]dopamine and [^{14}C]acetylcholine release from rat neostriatal slices: selective antagonism by gallamine but not pirenzepine, Eur. J. Pharmacol. 128, 291.

Schoffelmeer, A.N.M., F. Hogenboom and A.H. Mulder, 1988, M-1 and M-2 muscarinic receptor-mediated inhibition of dopamine-sensitive adenylate cyclase in rat neostriatum: a permissive role for D-2 dopamine receptors, J. Pharmacol. Exp. Ther. 245, 658.

Tjon, G.H.K., T.J. De Vries, E. Ronken, F. Hogenboom, G. Wardeh, A.H. Mulder and A.N.M. Schoffelmeer, 1994, Repeated and chronic morphine administration causes differential long-lasting changes in dopaminergic neurotransmission in rat striatum without changing its delta- and kappa-opioid receptor regulation, Eur. J. Pharmacol. 252, 205.

Tjon, G.H.K., T.J. De Vries, P. Nestby, G. Wardeh, A.H. Mulder and A.N.M. Schoffelmeer, 1995, Intermittent and chronic morphine treatment induce long-lasting changes in δ-opioid receptor-regulated acetylcholine release in rat striatum and nucleus accumbens, Eur. J. Pharmacol. 283, 169.

Wise, R.A. and M.A. Bozarth, 1987, A psychomotor stimulant theory of addiction, Psychol. Rev. 94, 469.