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European Journal of Pharmacology 302 (1996) 21–26

ejp

Chronic morphine increases hippocampal acetylcholine release: possible relevance in drug dependence

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Received 22 November 1995; revised 8 January 1996; accepted 12 January 1996

Abstract

Previous studies have shown that cocaine and amphetamine stimulate acetylcholine release in the hippocampus via an action of endogenously released dopamine on dopamine D_1 and D_2 receptors. The present study was aimed at clarifying if the property of stimulating hippocampal acetylcholine release was shared by morphine. The acute administration of morphine (10 mg/kg i.p.) failed to modify acetylcholine release in the hippocampus. However, after repeated administration (10 mg/kg i.p. twice daily) morphine acquired the ability to stimulate hippocampal acetylcholine release. Thus, at days 5 and 7 of chronic morphine treatment, a challenge dose of morphine (10 mg/kg i.p.) increased acetylcholine release by 50 and 100%, respectively. Concomitantly with the development of the stimulant property on acetylcholine release, morphine also acquired that of producing behavioural stimulation and lost that of producing sedation and catalepsy. The morphine-induced increase in acetylcholine output was suppressed by the blockade of dopamine D_1 receptors with SCH 23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) (0.1 mg/kg s.c.), which also suppressed the morphine-induced motor stimulation. Moreover, repeated morphine administration markedly potentiated the stimulant effect of the dopamine D_1/D_2 receptor agonist apomorphine (R(-)-10,11-dihydroxyaporphine) (0.1 or 0.5 mg/kg s.c.) both on hippocampal acetylcholine release and on behaviour. These results may suggest that the enhancement of hippocampal acetylcholine release as well as the development of behavioural sensitisation after chronic morphine could be related to the development of dopamine receptor supersensitivity. Moreover, increased acetylcholine transmission in the hippocampus may play a role in the 'memory' of the rewarding effects of drugs of abuse.

Keywords: Acetylcholine release, hippocampus; Morphine administration, acute and chronic; Dopamine receptor, sensitization

1. Introduction

It has recently been shown that two drugs of abuse, cocaine (Imperato et al., 1993b) and amphetamine (Imperato et al., 1993b; Nilsson et al., 1992), markedly enhance acetylcholine release in the hippocampus via endogenously released dopamine acting upon both dopamine D_1 and D_2 receptors (Imperato et al., 1993a).

Accordingly, neuroanatomical observations indicate that dopaminergic neurons originating in the ventro-tegmental area project to the lateral septal area (Lindvall, 1975; Simon et al., 1979). Moreover, neurochemical and pharmacological evidence suggests that dopaminergic neurons do not control the septo-hippocampal cholinergic neurons directly, but via a γ-amino-*n*-butyric acid (GABA) interneuron (Cheney and Panula, 1986; Robinson et al., 1979). Thus, dopamine-induced activation of cholinergic neurons would result from the suppression of the GABA inhibitory control (Blaker et al., 1986; Imperato et al., 1993c, 1994; Wood et al., 1979).

The consideration that acetylcholine release in the hippocampus plays a central role in cognitive functions (Brito et al., 1983), and that learning and memory are critically involved in the initiation and maintenance of drug-seeking behaviour (Stewart and Badiani, 1993) led us to attempt to clarify if the property of stimulating acetylcholine release in the hippocampus was shared by another drug of abuse, namely morphine.

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Elsevier Science B.V.
PII S0014-2999(96)00047-7

Brain microdialysis studies have shown that morphine stimulates dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988) at doses that induce conditioned place preference and self-administration (Mackey and Van der Kooy, 1985; Mucha and Herz, 1985; Rossi and Reid, 1976). Conversely, the rewarding responses were suppressed, at least partially, by blockade of dopamine receptors (Bozarth and Wise, 1981; Ettenberg et al., 1982; Gerber and Wise, 1989; Shippenberg and Herz, 1987; Spyraki et al., 1983).

Moreover, since repeated administration of morphine produces sensitisation to both the motor stimulant effects of the drug (Babbini and Davis, 1972; Eidelberg and Schwartz, 1970; Kalivas and Duffy, 1987; Kumar et al., 1971; Vasko and Domino, 1978) and its reinforcing properties (Gaiardi et al., 1991; Lett, 1989), we compared the effect of acute and chronic morphine on acetylcholine release in the hippocampus. The interaction of the dopamine D_1 receptor SCH 23390 with morphine responses was also tested.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (225–250 g; Charles River) were housed in groups of 3/cage for at least 10 days before use. Food and water were freely available and animals were maintained under an artificial 12-h/12-h light/dark cycle (lights were on from 07:00 to 19:00 h). Experiments were carried out between 08:00 and 17:00 h.

2.2. Microdialysis implantation and experimental procedure

Rats were anaesthetised with chloral hydrate (0.4 g/kg i.p.) and implanted with a dialysis tube (AN 69-HF, wet tube o.d. 320 μm; Hospal-Dasco, Bologna, Italy) at the level of the dorsal hippocampus (A −3.0 from bregma; V −3.5 from the skull) according to the Paxinos and Watson atlas (Paxinos and Watson, 1986). The localisation of the dialysis probe was verified at the end of experiments using a microtome cryostat. Surgery was carried out by using the transversal microdialysis technique recently revised in order to reduce tissue damage and the glial reaction, thus, allowing the monitoring of neurotransmitter output for several weeks after tube implantation (Imperato et al., 1992).

Experiments started 48 h after surgery.

Ringer solution containing (mM) KCl 3, NaCl 125, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $NaHCO_3$ 23, a potassium phosphate buffer 1.5, pH 7.3 was pumped through the dialysis probe at a constant rate of 2 l/min. For the determination of acetylcholine, neostigmine bromide (3 (dimethylamino)carbonyloxy-*N,N,N,*-trimethylbenzaminium bromide) 10^{-7} M was added to the Ringer solution. Samples were collected every 20 min, corresponding to a vol. of 40 μl, and were injected in a high-performance liquid chromatograph (HPLC) with electrochemical detection according to the techniques described by Damsma and Westerink (Damsma and Westerink, 1991), for the evaluation of acetylcholine. The detection limit for acetylcholine was 0.05 pmol/injection.

The basal extracellular concentration of acetylcholine from the hippocampus, at 48 h after surgery, was 2.7 ± 0.19 pmol/40 μl ($n = 14$).

On each day of experiment, rats were perfused for ~ 80–120 min before receiving the morphine challenge.

It should be pointed out that the effects of morphine on acetylcholine release in morphine-treated animals were tested once acetylcholine basal values were stable (3 or 4 samples not differing more than 10% from each other). No drift in acetylcholine baseline was observed during perfusion, suggesting that no local morphine withdrawal effects are induced by microdialysis perfusion.

Gentle injection of vehicle did not significantly change acetylcholine release.

In order to evaluate if the morphine-induced stimulation of acetylcholine output was due to a different responsiveness of the cholinergic system in the different days after the operation, we performed additional experiments. Naive rats were implanted, with the dialysis tube, 2, 6 and 11 days before they were given an acute challenge of morphine (10 mg/kg i.p.). No change in basal acetylcholine release in naive rats, at any day after surgery, was observed.

2.3. Behaviour

Behaviour was studied in the same animals in which the microdialysis experiments were performed. Rats were individually placed in plexiglass cages (40 cm × 40 cm) in the morning of the experiment and they were allowed to adapt to the cages during the time necessary to collect the basal release values (~ 120 min). The behaviour was videotaped and is expressed as the percentage of time spent (mean ± S.E.M.) by rats in performing several behavioural items, including locomotion, grooming, confined sniffing and gnawing, over a 2-h observation period.

It is worth noting that rats which received morphine every 12 h displayed none of the symptoms of withdrawal (like diarrhea, wet-dog shakes, teeth-chattering, jumping, etc.).

2.4. Drugs

Morphine sulphate, SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HCl (Schering Research Laboratories, Kenilworth, NJ) and apomorphine (*R*(−)-10,11-dihydroxyaporphine) HCl (Sigma, Italy) were dissolved in distilled

water. Morphine sulphate was administered i.p. in a vol. of 0.3 ml/100 g (every 12 h, ~09:30 and 21:30 h). SCH 23390 and apomorphine HCl were injected s.c. in a vol. of 0.1 ml/100 g.

2.5. Statistical analysis

Between-group comparisons were performed using a two-way ANOVA for repeated measures, the factors being treatment (2 levels = vehicle compared with morphine, as in Fig. 1, or vehicle compared with SCH 23390 as in Fig. 2) and time points (5 levels = 5 days as in Fig. 1, or 10 levels = 10 time points as in Fig. 2).

Data presented in Fig. 3 were analysed by two-way ANOVA, the factors being pretreatment (2 levels = vehicle compared with morphine as in Fig. 3) and treatment (3 levels = vehicle compared with apomorphine 0.1 and 0.5 mg/kg as in Fig. 3).

Posthoc comparisons were performed by Student's *t*-test for paired and unpaired data.

3. Results

Fig. 1 shows the effect of chronic morphine treatment (10 mg/kg i.p. twice daily, for 10 days) on hippocampal acetylcholine release.

The values reported are expressed as percentage changes in acetylcholine release over 2 h after morphine vs. vehicle treatment.

As shown in the Fig. 1, while the first morphine injection failed to modify acetylcholine release, the subsequent treatments became progressively more effective so that morphine increased acetylcholine release by 50% on day 5, and by 100% on day 7. This maximum stimulatory effect of morphine was maintained up to day 10 of treatment.

Changes in acetylcholine release were associated with changes in the behavioural response to morphine. Whereas catalepsy and immobility were present on the first days of the treatment combined with a low-intensity stereotypy, such as grooming behaviour, they gradually decreased, to disappear on day 5. In contrast locomotor activity and more intense stereotypies, like confined sniffing and gnawing behaviour, gradually increased to a maximum on days 5 and 7, respectively.

The possibility that the acquired ability of morphine to facilitate acetylcholine release might be due to dopamine was investigated.

Fig. 2 shows that on day 10 of the chronic morphine treatment the enhancement of acetylcholine release was antagonised by the blockade of dopamine D_1 receptors with the dopamine D_1 receptor antagonist SCH 23390 (0.1 mg/kg s.c.), given 60 min after morphine. Blockade of the morphine-induced acetylcholine release was also observed when SCH 23390 (0.1 mg/kg s.c.) was given just after morphine treatment (results not shown).

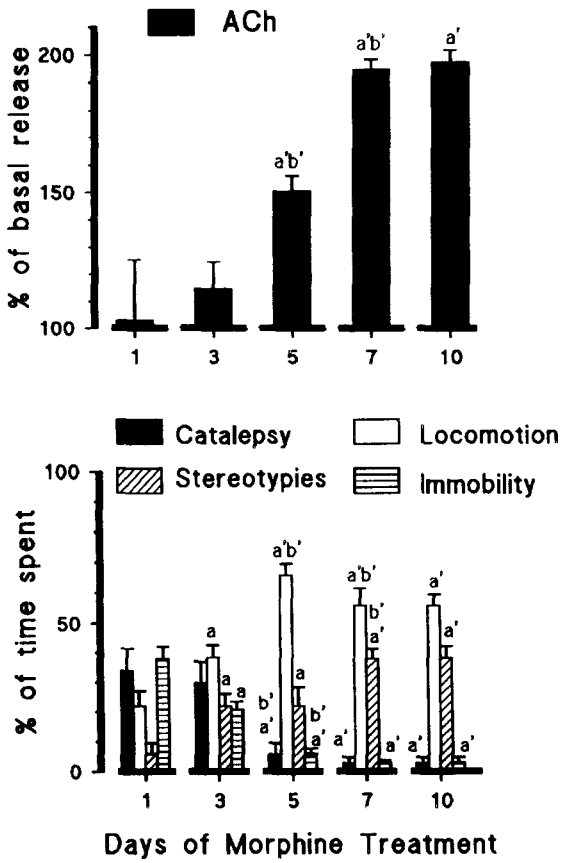

Fig. 1. Effect of morphine (10 mg/kg i.p.) on acetylcholine release from the hippocampus during 10 days of treatment (10 mg/kg i.p. every 12 h). In the lower panel are shown the behavioural effects of morphine (10 mg/kg i.p.) during the treatment. In the upper panel, values are expressed as percentage changes from basal values (mean ± S.E.M.) over 2 h after morphine injection. In the lower panel, data are expressed as a percentage of time spent (mean ± S.E.M.) by rats in performing several behavioural items including locomotion, grooming, confined sniffing and gnawing, over 2 h of observation. For the effect of chronic treatment with morphine on acetylcholine release, ANOVA revealed a significant main effect of treatment ($F(1,39) = 216.029$; $P < 0.001$); a significant main effect of repeated measures ($F(4,39) = 30.717$; $P < 0.001$); and a significant interaction between factors ($F(4,39) = 29.445$; $P < 0.001$). $n = 4$ for each group. ^{a} $P < 0.05$, $^{a'}$ $P < 0.01$ vs. 1st day. ^{b} $P > 0.05$, $^{b'}$ $P < 0.01$ vs. previous day.

SCH 23390 (0.1 mg/kg s.c.) also antagonised the behavioural stimulation induced by morphine (10 mg/kg i.p.) (results not shown).

In order to clarify whether the acquired ability of morphine to release acetylcholine might depend on the sensitisation of dopamine receptors, we investigated if chronic morphine treatment potentiated the acetylcholine-releasing effect of apomorphine.

Previous studies have shown that apomorphine releases acetylcholine in the hippocampus by stimulating both dopamine D_1 and D_2 receptors (Imperato et al., 1993a).

Fig. 3 shows that apomorphine was more effective in stimulating acetylcholine release in the rats chronically treated with morphine than in saline-treated controls. The enhancement of acetylcholine release after apomorphine

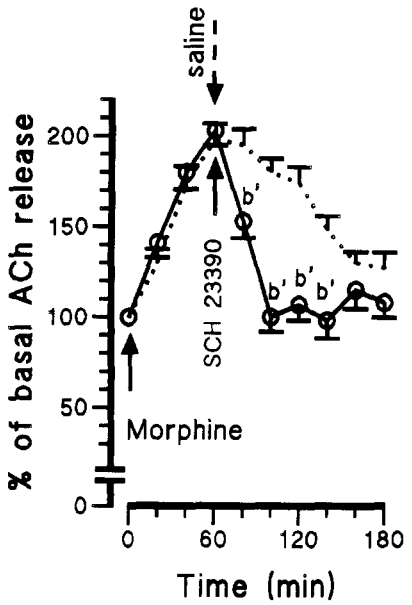

Fig. 2. SCH 23390 0.1 mg/kg s.c. (administered 60 min after morphine) blocks the enhancement of acetylcholine (ACh) release induced by morphine (10 mg/kg i.p.) on day 10 of chronic treatment. Data are expressed as the mean ± S.E.M. percentage variation from basal values. For the interaction morphine–SCH 23390 compared with morphine–saline, ANOVA revealed a significant main effect of treatment ($F(1,79) = 150.204$; $P < 0.001$); a significant main effect of repeated measures ($F(9,79) = 82.729$; $P < 0.001$); and a significant interaction between factors ($F(9,79) = 22.727$; $P < 0.001$). $n = 4$ for each group. $^{b'}$ $P < 0.01$ vs. saline-injected rats.

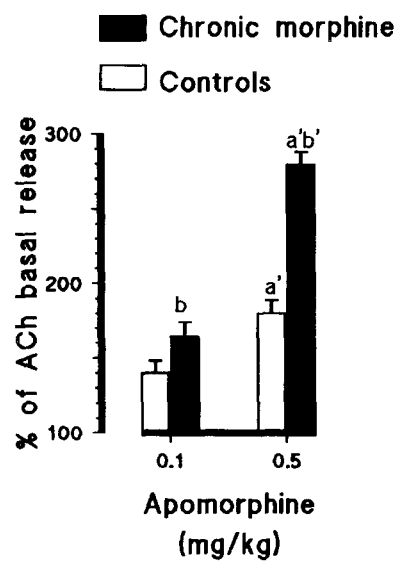

Fig. 3. Effect of apomorphine (0.1 and 0.5 mg/kg i.p.) on hippocampal acetylcholine (ACh) release in control rats (empty columns) and in chronic morphine-treated rats (full columns). Values are expressed as mean ± S.E.M. percentage variation from basal values. ANOVA revealed a significant main effect of pretreatment ($F(1,29) = 1000$; $P < 0.001$); a significant main effect of treatment ($F(2,29) = 1000$; $P < 0.001$); and a significant interaction between factors ($F(2,29) = 300.955$; $P < 0.001$). $n = 5$ for each dose. $^{a'}$ $P < 0.01$ vs. lower dose. ^{b} $P < 0.05$, $^{b'}$ $P < 0.01$ vs. controls.

was associated with a parallel increase in the motor stimulant response to the drug (results not shown).

4. Discussion

Our results show that the acute administration of morphine neither increases acetylcholine release in the hippocampus nor stimulates motor activity. However, after chronic treatment, morphine acquired the ability both to increase acetylcholine release and to produce behavioural stimulation, while it lost its sedative and cataleptogenic effects.

Morphine-induced acetylcholine output, as well as motor stimulation, seem to be due to the activation of dopamine receptors in the limbic system.

Indeed, different studies have shown that morphine stimulates the firing of dopamine neurons in the ventrotegmental area (Matthews and German, 1984) and increases dopamine release in limbic areas (Di Chiara and Imperato, 1988). Moreover, we have shown that stimulation of dopamine D_1 or D_2 receptors enhances acetylcholine release in the hippocampus and, vice versa, blockade of dopamine D_1 receptors prevents the stimulant effect of dopamine D_1 or D_2 receptor agonists on acetylcholine release (Imperato et al., 1993a, 1993b).

Accordingly, the present study has shown that the ability of morphine to increase both acetylcholine release and motor activity is suppressed by the dopamine D_1 receptor antagonist SCH 23390.

Since acute morphine increases dopamine release, the question arises as to why acute morphine is ineffective in releasing acetylcholine. One possible explanation is that dopamine-mediated stimulation of acetylcholine release is counterbalanced by a direct inhibitory effect of morphine on cholinergic neurons in the septum, via somatodendritic μ-opioid receptors, which have been shown to be localised on cholinergic neurons in the septum (Costa et al., 1983; Mansour et al., 1988; Moroni et al., 1977; Wood et al., 1979). It is possible that, after chronic treatment, μ-opioid receptors responsible for the inhibitory effects may become tolerant to morphine, whereas no tolerance develops to the stimulatory effect of morphine on the activity of dopaminergic neurons in the ventral tegmental area (Diana et al., 1995).

Alternatively, the acquired ability of morphine to release acetylcholine might be sustained by an increase in the dopamine-releasing effect of morphine (Kalivas and Duffy, 1987, Kalivas and Duffy, 1990) or in the postsynaptic events.

Regarding the first possibility, studies are in progress in our laboratory in order to address this issue.

The possibility that the acquired ability of morphine to induce acetylcholine release might depend on enhanced dopamine transmission downstream of postsynaptic dopamine receptors is supported by a number of studies

indicating that chronic morphine modifies intracellular messengers coupled to dopamine receptors, such as G-proteins, adenylate cyclase and C-AMP-dependent protein kinase in limbic areas (Guitart and Nestler, 1989; Terwilliger et al., 1991). In agreement with this possibility, we found that chronic morphine potentiated the response to the dopamine D_1/D_2 receptor agonist apomorphine, both on acetylcholine release and on motor activity.

The fact that similar changes are also produced by repeated cocaine or amphetamine administration, and that they are confined to the limbic areas involved in the reinforcing effect of drugs of abuse, supports the idea that they share a common substrate of drug dependence.

The increase in acetylcholine release after chronic morphine may represent a biochemical marker of enhanced dopaminergic transmission in the septal nucleus.

Irrespective of the mechanism involved, the finding that morphine acquires the ability to increase acetylcholine in the hippocampus, a property shared by amphetamine and cocaine, suggests that this phenomenon may play an important role in the memory of the rewarding property of these drugs of abuse and, therefore, in the maintenance of drug dependence.

Acknowledgements

We wish to thank SANDOZ Prodotti Farmaceutici S.p.A. Italy for having generously supported this research with a grant 'Project in Neuroscience' dedicated to Aleksander R. Lurija.

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