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Short communication

Activation of ventral tegmental $GABA_B$ receptors inhibits morphine-induced place preference in rats

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Abstract

The effect of microinjection of a $GABA_B$ receptor agonist, baclofen, into the ventral tegmental area on the rewarding effect of morphine was investigated using the conditioned place preference paradigm in rats. Morphine (1–8 mg/kg, s.c.) caused a dose-related place preference for the drug-associated place. In contrast, microinjection of baclofen (0.1–1 nmol/side) into the ventral tegmental area did not produce a significant preference for either compartment of the test box. Pretreatment with baclofen (0.1–1 nmol/side) into the ventral tegmental area dose dependently suppressed the morphine (8 mg/kg, s.c.)-induced place preference. This suppression of the morphine (8 mg/kg, s.c.)-induced place preference by baclofen (1 nmol/side) was reversed by treatment of the ventral tegmental area with the $GABA_B$ receptor antagonist, 2-hydroxysaclofen (1 nmol/side), but not with the $GABA_A$ receptor antagonist bicuculline (1 nmol/side). The present results suggest that a decrease in $GABA_B$ neurotransmission in the ventral tegmental area, which may be produced via inhibition of a tonic GABAergic input by morphine, may be involved in the expression of the rewarding effect of morphine.

Keywords: Morphine; $GABA_B$ receptor; Ventral tegmental area; Reward; Place conditioning

1. Introduction

Various lines of evidence suggest that the mesolimbic dopamine system, which originates in the ventral tegmental area and projects to the nucleus accumbens and other forebrain regions, is a major neural substrate of the rewarding effect produced by morphine. For example, rats rapidly learn self-administration of morphine into the ventral tegmental area (Bozarth and Wise, 1981), and microinjection of morphine into the ventral tegmental area produces a significant conditioned place preference which can be prevented by neurochemical destruction of dopamine neurons (Phillips et al., 1983). Morphine produces an increase in dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988), suggesting that an increase in dopamine output in the nucleus accumbens mediates the rewarding effect of morphine. In addition, an electrophysiological study demonstrated that systemic administration of morphine elicits an increase in the firing rate of dopaminergic neurons in the ventral tegmental area (Matthews and German, 1984).

The direct action of morphine in the nervous system is inhibitory, and it is therefore possible that the excitatory effect of morphine on the ventral tegmental area occurs through indirect mechanisms. Various reports have indicated that dopamine neuronal activity in the ventral tegmental area is modulated by GABAergic inhibitory input (Olpe et al., 1977; Kalivas et al., 1990; Klitenick et al., 1992). Recently, electrophysiological studies have demonstrated that morphine and the μ-opioid receptor agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) inhibit the firing frequency of non-dopamine cells in the ventral tegmental area (Matthews and German, 1984; Johnson and North, 1992), which appears to indicate that the excitatory

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effect of morphine on the ventral tegmental area occurs indirectly, possibly via inhibition of a tonic GABAergic input.

Based on previous reports, it has been hypothesized that the inhibition of tonic GABAergic input into the ventral tegmental area by morphine may be involved in the expression of the rewarding effect of morphine. However, direct evidence has not yet been provided. The aim of the present study was to investigate the role of the ventral tegmental GABAergic system on the rewarding effect of morphine using the conditioned place preference paradigm and the microinjection technique.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Japan) were individually housed at a room temperature of 24 ± 2°C and a relative humidity of 55 ± 10% with a 12 h light-dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were available ad libitum. Each experimental group contained eight rats.

2.2. Surgery

Rats weighing between 250 and 300 g were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a stereotaxic apparatus with the incisor bar 3.3 mm below the interaural line. Bilateral guide cannulae, implanted to terminate 1.4 mm dorsal to the injection site, were secured to the skull with cranial screws and dental cement. Removable obturators were inserted the full length of the cannulae to prevent obstruction by foreign substances and to limit infection. The stereotaxic coordinates used for the ventral tegmental area were 4.0 mm from the interaural line, 2.0 mm lateral to the midline, and −7.0 mm ventral to the cortical surface, according to the atlas of Paxinos and Watson (1986). The cannulae were angled 10° from the mid-sagittal plane to avoid the ventricular system of the brain. The rats were allowed to recover for 1 week before the experiments were carried out.

2.3. Microinjection procedure

Bilateral microinjection of vehicle or drugs was made into the ventral tegmental area over 30 s in a volume of 0.3 μl/side through an injection needle (31-gauge stainless steel) lowered to 1.4 mm ventral to the end of the implanted guide cannulae under light anesthesia with ether exposure. 30 s after completion of the injection, the injection needles were removed and the obturators were replaced. During this injection procedure the animals showed no evidence of pain or severe stress. At the end of the experiment, histological verification of cannulae placement was made on cresyl violet-stained sections, and rats with improper cannulae placement were excluded from the final data analysis.

2.4. Place conditioning

Place conditioning was conducted as previously described using a minor modification of an unbiased procedure (Suzuki et al., 1994). The apparatus consisted of a shuttlebox (30 × 60 × 30 cm: w × l × h) which was divided into two compartments of equal size. One compartment was white with a textured floor and the other was black with a smooth floor. For conditioning, rats were confined to one compartment after drug injections and to the other compartment after saline injections. The order of the injection (drug or vehicle) and compartment (white or black) was counterbalanced across subjects. Conditioning sessions (2 for drug: 2 for vehicle; 50 min in duration) were conducted once daily. On day 5, tests of conditioning were performed as follows: the partition separating the two compartments was raised 12 cm above the floor and a 5 × 2 cm neutral platform was inserted along the seam separating the compartments. Rats which had not been treated with either drugs or vehicle were placed on the platform of the test box. The time spent in each compartment during a 900 s session was then measured in a blinded fashion by an infrared beam sensor (KN-80, Natsume Seisakusyo, Tokyo, Japan). The position of the rat was defined by the position of its body (forelimbs and head). All sessions were conducted under conditions of dim illumination and masking white noise. Morphine (1–8 mg/kg) and baclofen (0.1–1 nmol/side) were used to make dose-response curves. Drugs and saline were injected into rats on alternate days. Rats were immediately confined to each compartment after injection. In the combination study, baclofen (0.1–1 nmol/side) or saline was injected into the ventral tegmental area 10 min before morphine (8 mg/kg, s.c.) or saline injection, and baclofen (1 nmol/side) and 2-hydroxysaclofen (0.1–1 nmol/side) or bicuculline (0.1 nmol/side) were also co-injected.

2.5. Drugs

The drugs used in the present study were morphine hydrochloride (Takeda Pharmaceutical Ind., Tokyo, Japan), baclofen (Sigma Chemical Co., St. Louis, MO, USA), 2-hydroxysaclofen (Sigma), and bicuculline methobromide (Sigma). All of the drugs were dissolved in saline.

2.6. Data analysis

Conditioning scores represent the time spent in the drug-paired place minus the time spent in the vehicle-paired place and are expressed as the means ± S.E.M. One-way

random factorial analysis of variance (ANOVA) followed by Dunnett's test was used to determine whether a particular dose produced significant conditioning.

3. Results

3.1. Saline control

Preference for the injection-associated place, which was considered a substitute for the drug, was calculated. In a control test of preference, animals which received s.c. saline during conditioning sessions exhibited no preference for either compartment of the test box (Fig. 1A). Similarly, microinjection of saline into the ventral tegmental area for 4 days did not induce a preference for either compartment of the test box (Fig. 1B).

3.2. Dose-response for place conditioning produced by morphine and baclofen

The place conditioning produced by morphine and baclofen is shown in Fig. 1. Morphine (1–8 mg/kg, s.c.) caused a dose-related ($F(4,35) = 7.748$; $P < 0.01$) preference for the drug-associated place, and significant conditioning was observed at doses of 2 mg/kg ($P < 0.05$), 4 mg/kg ($P < 0.01$) and 8 mg/kg ($P < 0.01$). In contrast,

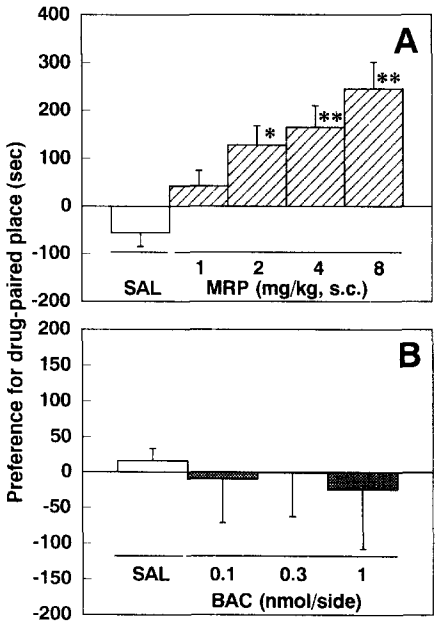

Fig. 1. Place conditioning produced by s.c. injection of morphine (MRP; 1–8 mg/kg) (A) and microinjection of baclofen (BAC; 0.1–0.3 nmol/side) (B) into the ventral tegmental area. Ordinate: mean difference (s) between times spent on the drug- and saline-paired sides of the test box. Each column represents the mean with S.E.M. from eight rats. The asterisks denote a significant preference (Dunnett's test: *, $P < 0.05$, **, $P < 0.01$).

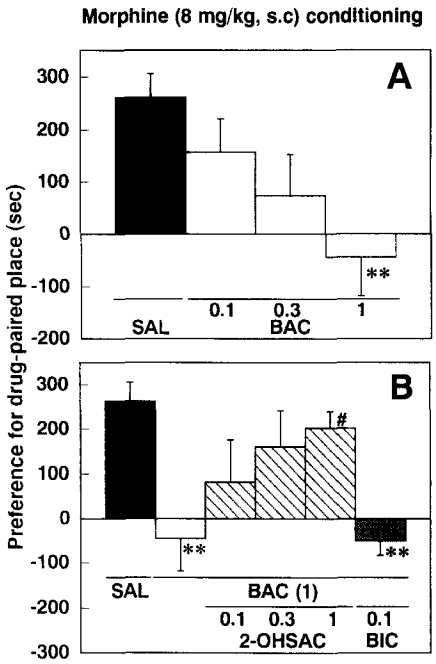

Fig. 2. Effect of microinjection of baclofen (BAC; 0.1–1 nmol/side) into the ventral tegmental area on the morphine (8 mg/kg, s.c.)-induced place preference (A) and effects of injection of 2-hydroxysaclofen (2-OHSAC; 0.1–1 nmol/side) or bicuculline (BIC; 0.1 nmol/side) into the ventral tegmental area on the suppression of the morphine (8 mg/kg, s.c.)-induced place preference by baclofen (BAC; 1 nmol/side) (B). Rats were pretreated with baclofen 10 min prior to morphine injection. 2-Hydroxysaclofen or bicuculline was co-injected with baclofen into the ventral tegmental area. Each column represent the mean with S.E.M. from eight rats. **, $P < 0.01$ vs. saline-pretreated group. #, $P < 0.05$ vs. baclofen (1 nmol/side)-pretreated group.

microinjection of baclofen (0.1–1 nmol/side) into the ventral tegmental area did not produce a significant preference for either compartment of the test box ($F(3,28) = 0.076$; $P > 0.05$).

3.3. Effect of the microinjection of baclofen into the ventral tegmental area on the morphine-induced place preference

The effect of the microinjection of baclofen into the ventral tegmental area on the morphine-induced place preference is shown in Fig. 2. Microinjection of baclofen (0.1–1 nmol/side) into the ventral tegmental area dose dependently ($F(3,28) = 3.780$; $P < 0.05$) suppressed the morphine (8 mg/kg, s.c.)-induced place preference, and a significant suppression was observed at a dose of 1 nmol/side ($P < 0.05$; Fig. 2A). This suppression of the morphine (8 mg/kg, s.c.)-induced place preference produced by baclofen (1 nmol/side) was reversed by treatment with the $GABA_B$ receptor antagonist 2-hydroxysaclofen (1 nmol/side) but not with the $GABA_A$ receptor antagonist bicuculline (0.1 nmol/side) (Fig. 2B).

4. Discussion

The present study demonstrated that morphine produced a dose-related conditioned place preference in rats. These results agree with those of a previous report (Shippenberg and Herz, 1987), which indicates that the conditioning procedure in the present study can be used to investigate the rewarding effect of morphine.

The present results indicate that microinjection of the $GABA_B$ receptor agonist baclofen into the ventral tegmental area dose dependently suppressed the morphine-induced place preference, and that this effect of baclofen was reversed by treatment with the $GABA_B$ receptor antagonist 2-hydroxysaclofen but not with the $GABA_A$ receptor antagonist bicuculline. These findings are the first direct evidence that a decrease in ventral tegmental $GABA_B$ neurotransmission, which may be produced via inhibition of tonic GABAergic input into the ventral tegmental area, is involved in the rewarding effect of morphine. It is well known that activation of the mesolimbic dopamine system by morphine is essential for the expression of the rewarding effect of morphine (Bozarth and Wise, 1981; Phillips et al., 1983; Di Chiara and Imperato, 1988; Matthews and German, 1984). However, this effect may be produced via an indirect mechanism. The stimulation of dopamine neurons in the ventral tegmental area by iontophoretic morphine is not reversed by naloxone, while the inhibition of non-dopamine cells is naloxone-reversible (Matthews and German, 1984). Similarly, it was recently reported that μ-opioid receptor agonists inhibited only non-dopamine cells in tissue slices from the rat ventral tegmental area (Johnson and North, 1992). Moreover, the lack of a direct effect of morphine on dopamine cells was corroborated in a study with autoradiographic analysis in which destruction of dopamine neurons with 6-hydroxydopamine did not alter ^{125}I-DAMGO binding in the ventral tegmental area (Dilts and Kalivas, 1989). Therefore, this electrophysiological and autoradiographic evidence indicates that morphine acts on non-dopamine neurons to stimulate dopamine cells in the ventral tegmental area.

Many of the non-dopaminergic neurons in the ventral tegmental area are thought to be GABAergic neurons. It has been previously shown that GABA, the GABAergic enzyme glutamic acid decarboxylase and its mRNA exist in the ventral tegmental area (Nagai et al., 1983; Fonnum et al., 1978; Zhang et al., 1991), and that GABAergic neurons innervate dopamine cells within the ventral tegmental area (Nagai et al., 1983). In addition, the presence of $GABA_B$ receptors has been demonstrated within the ventral tegmental area (Bowery et al., 1987), and it is also clear that $GABA_B$ receptors are located on dopamine neurons, based on a study which recorded the intracellular activity of dopamine neurons in slices of tissue from the ventral tegmental area (Johnson and North, 1992). Therefore, activation of ventral tegmental $GABA_B$ receptors may directly inhibit dopamine cells within the ventral tegmental area. Indeed, baclofen decreases impulse generation in dopamine cells (Olpe et al., 1977) and extracellular dopamine content in the ventral tegmental area (Klitenick et al., 1992). Along with these previous reports, the present data indicate that the activation of $GABA_B$ receptors, which are located on dopamine cells within the ventral tegmental area, by baclofen inhibits the capacity of morphine to activate dopamine neurotransmission. Baclofen thereby prevents morphine-induced place preference. Previous reports demonstrated that microinjection of baclofen into the ventral tegmental area reversed the DAMGO-induced elevation of postmortem dopamine metabolites in the nucleus accumbens (Kalivas et al., 1990), and the morphine-induced increase in the extracellular dopamine concentration within the ventral tegmental area (Klitenick et al., 1992). These neurochemical findings may support our present hypothesis.

Finally, we present our hypothesis for the mechanism for the expression of the rewarding effect of morphine based on the present and previous electrophysiological, neurochemical and behavioral data: dopamine cells in the ventral tegmental area contain $GABA_B$ receptors, while GABAergic afferents to the ventral tegmental area contain opioid receptors. Morphine produces a decrease in $GABA_B$-mediated neurotransmission in the ventral tegmental area via hyperpolarization of GABA afferents. This would lead to excitation of mesolimbic dopamine neurons and an increase in dopamine output in the nucleus accumbens by disinhibition, which may contribute to the expression of the rewarding effect of morphine.

In conclusion, the present study demonstrated that microinjection of the $GABA_B$ receptor agonist baclofen into the ventral tegmental area dose dependently suppressed morphine-induced place preference. These results are the first evidence that a decrease in $GABA_B$-mediated neurotransmission within the ventral tegmental area is an essential process in the expression of the rewarding effects of morphine.

References

Bowery, N.G., A.L. Hudson and G.W. Price, 1987, $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system, Neuroscience 20, 365.

Bozarth, M.A. and R.A. Wise, 1981, Intracranial self-administration of morphine into the ventral tegmental area in rats, Life Sci. 28, 551.

Di Chiara, G. and A. Imperato, 1988, Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats, J. Pharmacol. Exp. Ther. 244, 1067.

Dilts, R.P. and P.W. Kalivas, 1989, Autoradiographic localization of μ-opioid and neurotensin receptors within the mesolimbic dopamine system, Brain Res. 488, 311.

Fonnum, F., Z. Gottesfeld and I. Grofova, 1978, Distribution of glutamate decarboxylase, choline acetyltransferase and aromatic acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for striatopallidal, striatopeduncular and striatonigra GABAergic fibers, Brain Res. 143, 125.

Johnson, S.W. and R.A. North, 1992, Opioids excite dopamine neurons by hyperpolarization of local interneurons, J. Neurosci. 12, 483.

Kalivas, P.W., P. Duffy and H. Eberhardt, 1990, Modulation of A10 dopamine neurons by γ-aminobutyric acid agonists, J. Pharmacol. Exp. Ther. 253, 858.

Klitenick, M.A., P. DeWitte and P.W. Kalivas, 1992, Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: An in vivo microdialysis study, J. Neurosci. 12, 2623.

Matthews, R.T. and D.C. German, 1984, Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine, Neuroscience 11, 617.

Nagai, T., P.L. McGeer and E.G. McGeer, 1983, Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain, J. Comp. Neurol. 218, 220.

Olpe, H.R., W.P. Koella, P. Wolf and H.L. Haas, 1977, The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area, Brain Res. 134, 577.

Paxinos, G. and C. Watson, 1986, The rat brain in stereotaxic coordinates, 2nd edn. (Academic Press, Tokyo).

Phillips, A.G., F.G. LePaine and H.C. Fibiger, 1983, Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat, Life Sci. 33, 2505.

Shippenberg, T.S. and A. Herz, 1987, Place conditioning reveals the involvement of D_1-dopamine receptors in the motivational properties of μ- and κ-opioid agonist, Brain Res. 436, 169.

Suzuki, T., T. Mori, M. Tsuji, M. Misawa and H. Nagase, 1994, The role of delta-opioid receptor subtypes in cocaine- and methamphetamine-induced place preferences, Life Sci. 55, PL339.

Zhang, J.H., M. Sato and M. Tohyama, 1991, Region-specific expression of the mRNAs encoding β subunits (β_1, β_2, and β_3) of $GABA_A$ receptor in the rat brain, J. Comp. Neurol. 303, 637.