EFFECTS OF APOMORPHINE ON MORPHINE ANALGESIA DURING THE STATE OF DOPAMINERGIC SUPERSENSITIVITY AFTER CHRONIC TREATMENT WITH HALOPERIDOL

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Morphine induced-reduction in response to a repetitive electrical stimulation of the tail was measured in acute rats and rats chronically treated with haloperidol (1 mg/kg/d, for 7 d) following pretreatment with apomorphine. In acute experiments, a significant enhancement of the anti-struggling action of morphine was produced by haloperidol (1 mg/kg, *i.p.*). Low doses of apomorphine (30–480 μ g/kg, *i.p.*) had no influence on the suppressing action of morphine on the struggling response induced by the tail stimulation. Following chronic treatment with haloperidol, the inhibitory action of morphine on the tail stimulation-induced struggling response was dose-dependently inhibited by very low doses of apomorphine (60–480 μ g/kg, *i.p.*). A significant increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels was observed after administration of haloperidol or morphine in acute rats, whereas no change in DOPAC levels was found after administration of morphine or apomorphine in chronically haloperidol-treated rats. In these animals, basal striatal DOPAC levels were significantly decreased compared with those of vehicle-treated animals controls.

The present results suggest that in rats treated chronically with haloperidol, the suppressive action of a low dose of apomorphine on morphine analgesia is due to an increased sensitivity of postsynaptic dopaminergic receptors to apomorphine.

Keywords — Morphine analgesia; dopamine system; supersensitivity; haloperidol; rat

INTRODUCTION

An accumulating body of evidence suggests that the nigro-striatal dopamine (DA) system responds to a variety of noxious stimuli, *e.g.* tail pinch, sciatic nerve stimulation or radiant heat.^{1–3)} Moreover, biochemical evidence indicates that peripheral stimulation influences the release of DA in both the caudate nucleus and the substantia nigra.⁴⁾ Jurna and Heinz⁵⁾ reported that an intracaudate microinjection of opiates caused a marked anti-nociceptive action and this effect was abolished by intraperitoneal injection of naloxone or apomorphine. Impairment of dopaminergic impulse flow following a microinjection of 6-hydroxydopamine into the substantia nigra or systemic administration of reserpine or haloperidol depressed the tail-flick re-

sponse.^{6,7)} The caudate nucleus of the rat is particularly rich in opiate receptors.^{8–10)} This evidence strongly suggests that the nigro-striatal dopaminergic system and the caudate nucleus may play an important role in morphine analgesia. If this is the case, then morphine analgesia should be influenced by apomorphine in animals treated chronically with haloperidol because of an increase in the sensitivity of postsynaptic dopaminergic receptors to apomorphine. The present study tested this hypothesis in an attempt to elucidate the role of the nigro-striatal dopaminergic system in the anti-nociceptive action of morphine. In addition, striatal 3,4-dihydroxyphenylacetic acid (DOPAC) levels were measured after administration of morphine, haloperidol and apomorphine in acute rats and

rats chronically treated with haloperidol to study alterations in dopamine metabolism associated with changes in morphine analgesia.

METHODS

All experiments were carried out on male rats of the Wistar-strain weighing approximately 150 g. For the analgesic assay, the struggling response to electrical stimulation of the tail was used as the indicator of pain. This method is relatively easy to manipulate and the analgesic assay can be carried out under light anesthesia. The response is uniform and reproducible without evidence of tachyphylaxis. The detailed procedure for recording the struggling response following a repetitive electrical stimulation of the tail has been reported elsewhere.^{11,12} Specific details are as follows: the animals were anesthetized with α -chloralose (50 mg/kg, *s.c.*) and urethane (500 mg/kg, *s.c.*). The effects of analgesics were determined as an inhibitory action on the struggling response induced by electrical stimulation of the tail. When the tail was repetitively stimulated, the rat squeaked and struggled violently. The movement of the head significantly increased in parallel with struggling. The movement of the head associated with the struggling was recorded by means of a force displacement transducer (Nihon Kohden, SB1T). To express the struggling response in a quantitative manner, the area under this response graph was determined. Repetitive stimulation was given at 15 min intervals following drug administration until the extent of struggling response returned to the pre-medication level. The repetitive stimulation of the tail was performed for 5 s each time. For stimulation, rectangular wave pulses were applied through a pair of stainless steel electrodes placed on the root of the tail at a frequency of 50 Hz. The intensity and duration of the pulse was 5–15 V and 2.5 ms, respectively.

Drug Administrations — All drugs (salt) were administered intraperitoneally through a cannula. In the acute study, animals were divided into three groups: 1) the saline-morphine group, in which each animal received a single dose of

saline (1 ml/kg) followed by various doses of morphine (0.94–5.6 mg/kg); 2) the haloperidol-morphine group, in which each animal received a single dose of haloperidol (1 mg/kg) followed by various doses of morphine; and 3) the apomorphine-morphine group, in which each animal received various doses of apomorphine (30–480 μ g/kg), followed by a single dose of morphine (2.2 mg/kg).

For the chronic study, animals were administered either 0.3% carboxymethyl cellulose (CMC) (vehicle) or haloperidol (1 mg/kg) daily for 7 d. After the last administration, the animals were allowed a 3 d-washout period, and these rats received a test dose of morphine (2.2 mg/kg). This dose is the ED_{50} in acute animals. After this control observation for morphine's anti-struggling action was made, the effects of various doses of apomorphine (30–480 μ g/kg) on the inhibitory action of morphine on the struggling response induced by a electrical stimulation of the tail were investigated.

Biochemical Assays — DA was absorbed onto aluminum oxide from a neutralized perchloric acid extract of striatal homogenates, eluted with diluted hydrochloric acid, oxidized with potassium ferricyanide and measured fluorimetrically.¹³ DOPAC levels were determined by means of high performance liquid chromatography (HPLC) with electrochemical detection according to the method of Sasa and Blank.¹⁴ The striatum (about 100 mg) was quickly removed from rats treated with various drugs and homogenized in 3 ml of butanol containing 0.05 M ethylenediaminetetraacetic acid (EDTA), 0.025 N HCl and 6 μ g/ml 3,4-dihydroxyphenyl propionic acid using a ground glass homogenizer. The homogenate was saturated with 1 g NaCl and shaken for 60 min. After centrifugation at 3000 rpm for 5 min, 2 ml of the butanol layer were then transferred to another vial containing *n*-heptane and 0.1 N HCl and shaken for 10 min. After centrifugation at 3000 rpm for 5 min, 0.2 M Tris-HCl (pH 8.5) containing 0.05% ascorbic acid was added to the organic layer and vibrated for 1 min. Following centrifugation

gation at 3000 rpm for 5 min, 10 μ l of aqueous layer were injected into the HPLC.

Statistical Evaluation — Paired Student *t*-test and Aspin-Welch method was used for statistical evaluation of the data. The 0.05 level of probability was accepted as significant.

RESULTS

Acute Study

It has previously been reported by Kamata *et al.*^{11,12)} that subcutaneous administration of 50 mg/kg of α -chloralose and 500 mg/kg of urethane produced no appreciable alteration in the struggling response to electrical stimulation of the tail. Furthermore, the slow wave patterns in the electroencephalogram following administration of the above doses of α -chloralose and urethane switched to desynchronization following tail stimulation at 5 V for 10 s, indicating that the pain response persisted even during the state of anesthesia. This finding was confirmed in the present study (data not shown). Under these conditions, 3.75 mg/kg of morphine significantly inhibited the struggling response by approximately 90% and this action was antagonized by naloxone (1 mg/kg, *i.p.*) as shown in Fig. 1.

As shown in Table I, neither haloperidol (1

mg/kg) nor apomorphine (120 μ g/kg) alone produced significant changes in the struggling response induced by the tail stimulation. However, when haloperidol was administered 15 min before morphine administration, the inhibitory action of morphine on the struggling response was significantly increased. Figure 2 represents the effect of haloperidol on the dose-response effects of morphine on the struggling response.

FIG. 1. *Anti-struggling Effect of Morphine in Acute Experiments*

The columns presents the mean values of the anti-struggling response of morphine to electrical stimulation of the tail. $\square$, effects of morphine (3.75 mg/kg) on the struggling response; $\blacksquare$, effect of naloxone (1 mg/kg) on the anti-struggling response of morphine. a) $p < 0.001$.

TABLE I. *Change in Struggling Response Induced by Tail Stimulation Following Treatment of Rats with Various Drugs in Acute and Chronic Experiments*

Treatment		n	Area of struggling ($\text{mm}^2 \pm \text{S.E.}$)		p
			Control	After treatment	
Acute	Apomorphine (120 μ g/kg) (APO)	5	233.2 $\pm$ 16.1	234.1 $\pm$ 26.1	N.S.
	Haloperidol (2 mg/kg) (HAL)	5	210.0 $\pm$ 11.5	221.3 $\pm$ 29.5	N.S.
	Morphine (2.2 mg/kg) (MOR)	6	215.6 $\pm$ 7.6	111.2 $\pm$ 14.9	<0.001
	APO (120 μ g/kg) + MOR (2.2 mg/kg)	5	202.1 $\pm$ 9.2	102.8 $\pm$ 16.5	<0.001
	HAL (2 mg/kg) + MOR (2.2 mg/kg)	5	220.8 $\pm$ 6.6	20.0 $\pm$ 4.7	<0.001
Chronic	APO (120 μ g/kg)	5	207.0 $\pm$ 8.1	203.0 $\pm$ 12.1	N.S.
	MOR (2.2 mg/kg)	7	204.0 $\pm$ 4.6	93.7 $\pm$ 19.1	<0.001
	APO (120 μ g/kg) + MOR (2.2 mg/kg)	5	204.1 $\pm$ 7.1	196.4 $\pm$ 24.3	N.S.

Quantitative determinations of the struggling response were made by measuring the area of struggling circumscribed by the base line. Haloperidol and apomorphine were administered 15 and 5 min, respectively, before morphine administration. Testing for reaction times was performed 20 min after the administration of apomorphine, 30 min after haloperidol and 15 min after morphine. n = number of animals. Aspin-Welch method was used for statistical evaluation of the data.

Haloperidol (1 mg/kg) shifted the dose-response curves for morphine to the left and decreased the ED_{50} of morphine by about 2.5-fold. On the other hand, apomorphine had no influence on the morphine-induced suppression of the struggling response.

Chronic Study

Even after chronic administration of haloperidol, there was no change in the struggling response in morphine-naïve rats. The effect of morphine on the struggling response was generally similar to that in acute rats (Table I). As shown in Table I, apomorphine alone did not produce significant changes in the struggling response induced by the tail stimulation following chronic treatment with haloperidol. When apomorphine was administered to the chronic rat 5 min before morphine administration, however, the inhibitory action of morphine on tail stimulation-induced struggling was completely abolished as shown in Table I. In order to determine the extent of the effect of apomorphine on the inhibitory action of morphine on the tail stimulation-induced struggling response, a dose-

FIG. 2. Dose Response Curves for the Anti-struggling Action of Morphine in the Presence or Absence of Haloperidol

Effect of morphine on the tail stimulation-induced struggling response in the presence (—●—) or absence (—○—) of haloperidol (1 mg/kg). Haloperidol was administered 15 min before morphine administration.

response curve was generated following chronic treatment with haloperidol or vehicle (Fig. 3). As can be seen in Fig. 3, apomorphine at various doses (60–480 μ g/kg) produced a significant decrease in morphine's effects on tail stimulation-induced struggling in chronically haloperidol-treated rat but not in vehicle-treated rats.

Biochemical Study

The mean striatal levels of DA or DOPAC in anesthetic treated animals (control; $n=5$) were 8.08 ± 0.68 and 0.76 ± 0.05 μ g/g (means $\pm$ S.E.), respectively. Preliminary studies indicated that subcutaneous administration of α -chloralose and urethane up to 50 and 500 mg/kg in doses, respectively, did not produce any alterations in DA or DOPAC levels. In accordance with this finding, Massotti and Longo¹⁵ have reported that intraperitoneal administration of α -chloralose, at doses up to 100 mg/kg, did not alter DA content and metabolism in the rat brain. In the present study, therefore, drug ef-

FIG. 3. Effects of Various Doses of Apomorphine (30–480 μ g/kg) on the Suppressive Action of Morphine (2.2 mg/kg) on the Struggling Response Induced by Electrical Stimulation the Tail

Three days after cessation of chronic haloperidol (■) or vehicle (□) treatment, control response for morphine's anti-struggling action was checked. Each symbol, (closed circle, chronic haloperidol-treated rats; open circle, chronic vehicle-treated rats), represents the mean $\pm$ S.E. of 8 determinations. Significantly different from control: a) $p < 0.05$; b) $p < 0.001$.

fects were tested under conditions similar to those in the analgesic test.

DA levels in the striatum were not altered by haloperidol (1 mg/kg), morphine (2.2 mg/kg),

FIG. 4. Effects of Various Drugs on Striatal DOPAC Levels in Acute and Chronic Experiments

All rats received α -chloralose (50 mg/kg, s.c.) and urethane (500 mg/kg, s.c.) 2 h before sacrifice. Haloperidol (Hal, 1 mg/kg), apomorphine (Apo, 120 μ g/kg) and morphine (Mor, 2.2 mg/kg) were administered 30, 20 and 15 min, respectively, before sacrifice. A, acute experiments; B, chronic experiments (▨, chronic vehicle; ■, chronic haloperidol). In chronic experiments, rats were killed 3 d after cessation of chronic treatment with haloperidol. The vertical lines indicate the S.E. of the mean of 5 determinations. The control DOPAC levels in acute and chronic experiments were 0.76 ± 0.05 and 1.06 ± 0.02 μ g/g wet wt. (mean $\pm$ S.E.), respectively. Significantly different from control: a) $p < 0.05$; b) $p < 0.001$. a: Mor vs. Apo ($p < 0.05$); b: Mor vs. Mor + Hal ($p < 0.001$).

apomorphine (120 μ g/kg), haloperidol + morphine or apomorphine + morphine in both acute and chronic haloperidol-treated rats.

In acute rats, haloperidol (1 mg/kg) and haloperidol + morphine (2.2 mg/kg) induced a great increase in striatal DOPAC levels and morphine alone produced a slight but significant increase. Apomorphine (120 μ g/kg) produced no change in striatal DOPAC levels (Fig. 4A). An increasing effect of morphine on striatal DOPAC levels was antagonized by pretreatment with apomorphine. Three days after cessation of chronic treatment with haloperidol (1 mg/kg/d, *i.p.* for 7 d), basal striatal DOPAC levels were significantly decreased compared with those of chronic vehicle-treated animals. In contrast to acute rats, morphine (2.2 mg/kg) failed to modify DOPAC levels in the chronic haloperidol-treated rats. Also, apomorphine (120 μ g/kg) and apomorphine + morphine exerted no effect on striatal DOPAC levels in rats treated chronically with haloperidol (Fig. 4B).

DISCUSSION

In the acute study, haloperidol showed a marked potentiation of morphine analgesia. The haloperidol-induced potentiation is possibly due to a removal of the inhibitory effect of the dopaminergic system on morphine analgesia *via* blockade of dopamine receptors in the striatum by haloperidol. This is likely because the same dose of haloperidol dramatically increased DOPAC level, in the striatum, presumably reflecting a feedback increase of the activity of dopaminergic neurons resulting from dopamine receptor blockade.¹⁶⁻¹⁹⁾

Morphine itself produced a slight but significant increase in DOPAC levels. This result is in agreement with several lines of evidence. Fukui and Takagi,²⁰⁾ Algeri *et al.*²¹⁾ and Van Loon and Kim²²⁾ have reported that acute administration of morphine or morphine-like peptides increase striatal DA turnover. Iwatsubo and Clouet²³⁾ and Matthews and German²⁴⁾ reported that intravenous administration of morphine increased the rate of spontaneous firing of DA neurones in

the substantia nigra zona compacta. Apomorphine alone did not produce significant alterations but inhibited the morphine-induced increase in DOPAC concentrations. Nevertheless, apomorphine did not show any influence on morphine analgesia even at high doses (up to 480 $\mu\text{g/kg}$). Taken together, these results suggest that physiological stimulation of DA receptors attains the maximal inhibitory effect on morphine analgesia in the rat in the normal situation and that further stimulation of the DA receptor by apomorphine does not modify morphine analgesia. This inhibition can be removed when the DA receptor is blocked by haloperidol, thereby resulting in a potentiation of morphine analgesia.

Supporting evidence comes from the chronic study in which it was found that DA receptor stimulation by apomorphine decreased the analgesic effect of morphine when the rat was in a state of dopaminergic supersensitivity following chronic treatment with haloperidol. The dose and length of haloperidol treatment employed in the present experiment were well established to produce dopaminergic supersensitivity in the striatum.²⁵⁻²⁸ In this supersensitive state, as can be seen from DOPAC levels, the activity of dopaminergic neurones was dramatically lowered. This is probably the result of a compensatory decrease in the activity of dopaminergic neurons due to increased sensitivity of DA receptors or a gradual development of depolarization block of DA cells after chronic haloperidol.²⁹⁻³⁴ If the activity of dopaminergic neurons was completely suppressed, morphine analgesia should be enhanced by the treatment. In animals treated chronically with haloperidol, however, morphine exerted an analgesic action similar to that in chronic vehicle-treated rats. It is conceivable, therefore, that some physiological activity of dopaminergic neurones after long-term treatment with haloperidol is still contributing to the inhibition of morphine analgesia because of an increased sensitivity of the DA receptor. Further stimulation of DA receptors by apomorphine produced additional inhibition of mor-

phine analgesia, presumably due to the supersensitivity state of the striatal dopaminergic system.

In conclusion, on the basis of the results from the present experiments, the suppressive action of apomorphine on morphine analgesia in chronic haloperidol-treated rats, which could not be detected in acute experiments, may be due to an increased sensitivity of the postsynaptic DA receptor to apomorphine. This provides additional evidence to support the concept that the nigro-striatal dopaminergic system is playing a role as an inhibitory modulator of the analgesic action of morphine in rats.

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