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Evidence that 5-hydroxytryptamine in the forebrain is involved in naloxone-precipitated jumping in morphine-dependent rats

L. Cervo, S. Romandini & R. Samanin

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62, 20157 Milan, Italy

1 A withdrawal syndrome was precipitated by naloxone in morphine-dependent rats injected with 5,7-dihydroxytryptamine (5,7-DHT) in the ventromedial tegmentum (VMT) at the level of the nucleus interpeduncularis.

2 5,7-DHT, which markedly depleted 5-hydroxytryptamine (5-HT) in the forebrain but not in the brainstem, significantly reduced jumping in abstinent rats with no significant effect on other withdrawal signs.

3 The effect of morphine 10 mg kg^{-1} on responses on the hot plate was unchanged in 5,7-DHT-treated rats.

4 The findings suggest that 5-HT in the forebrain is selectively involved in the jumping of morphine-abstinent rats.

Introduction

There is evidence that forebrain regions such as central amygdala, globus pallidus and medial thalamus are involved in jumping of morphine-abstinent rats (Wei, Loh & Way, 1972; Calvino, Lagowska & Ben-Ari, 1979; Tremblay & Charton, 1981). Recent studies using 5-hydroxytryptamine (5-HT) antagonists have suggested that brain 5-HT is involved in the development of naloxone-precipitated jumping in morphine-dependent rats (Samanin, Cervo & Rochat, 1980; Cervo, Rochat, Romandini & Samanin, 1981) and intraventricular injection of 5-HT neurotoxins was found to reduce jumping in morphine-abstinent rats (Herz & Bläsig, 1978).

In the present study the role of forebrain 5-HT in the naloxone-precipitated syndrome of morphine-dependent rats was investigated by administering 5,7-dihydroxytryptamine (5,7-DHT), a neurotoxin for 5-HT-containing neurones (Baumgarten, Björklund, Lachenmayer & Nobin, 1973), in the ventromedial tegmentum (VMT) at the level of the nucleus interpeduncularis where most 5-HT fibres innervating the forebrain are found (Palkovits, Saavedra, Jacobowitz, Kizer, Zaborszky & Brownstein, 1977; Azmitia, 1978). In order to see whether 5,7-DHT injection changed the acute sensitivity to morphine, the effect of a single dose of morphine in the hot plate test was studied as well.

Methods

Male CD-COBS rats (Charles River, Italy) were used, weighing about 220 g at the beginning of the experiments. The animals were maintained on standard laboratory chow and tap water and housed 3 per cage at constant room temperature (21 ± 1°C) and relative humidity (60%) with a 12 h light/12 h dark cycle (dark period starting at 19 h 30 min).

5,7-Dihydroxytryptamine injections

Fifteen minutes before surgery, rats were injected with nomifensine (30 mg kg^{-1}, i.p.), a blocker of catecholamine uptake (Samanin, Bernasconi & Garattini, 1975), to prevent damage to catecholamine-containing nerve endings. Then, under ether anaesthesia, the animals were immobilized in a Stoelting stereotaxic instrument. 5,7-DHT (5,7-dihydroxytryptamine creatinine sulphate, Serva) was dissolved in a solution of ascorbic acid (1 mg ml^{-1}) and injected at a constant infusion rate

(1 $\mu l\,min^{-1}$). The animals received 5,7-DHT (calculated as free base) 6 μg in 3 μl bilaterally into the ventromedial tegmentum: A = 2.0, L = ±0.5, D = 2.2, according to König & Klippel (1963). Controls received the same amount of the vehicle but not 5,7-DHT.

Induction of dependence and withdrawal testing

On day 9 after surgery the rats received two intraperitoneal injections (at 10 h 00 min and 18 h 00 min) of morphine HCl (Farmitalia-Carlo Erba, Italy) 10 $mg\,kg^{-1}$ (calculated as free base); the daily dose of morphine was doubled every other day to reach a total of 160 $mg\,kg^{-1}$ on the 7th day and this regimen was continued for 3 more days. At 10 h 00 min on the 11 th day the animals received the last injection of morphine and 4 h later an abstinence syndrome was precipitated by intraperitoneal injection of 1 mg/kg naloxone (Endo Lab., N.Y.). Some animals were subjected to the same schedule of treatment but received saline instead of morphine.

Withdrawal signs within 30 min were recorded, according to a procedure described previously (Cervo *et al.*, 1981). The data are expressed as the proportion of positive animals and differences were analysed statistically by the χ^2 test.

Hot plate

Nine days after 5,7-DHT injection, before, and 30, 60, 120 and 180 min after a subcutaneous injection of morphine HCl 10 $mg\,kg^{-1}$ rats were placed on a hot plate with the temperature thermostatically held at 55°C. A Plexiglas cylinder (26 cm high, 20 cm diameter), open at the top, confined the rat to a defined area of the hotplate. The time between contact with the plate and licking of paws (or occasionally jumping to the edge of the cylinder) was recorded to the nearest 0.1 s. If an animal failed to reach this criterion it was removed from the plate after 30 s and given a score of 30.

Assay of 5-hydroxytryptamine

Some 5,7-DHT-treated animals that had received no morphine were randomly chosen from the experimental groups and killed by decapitation. Their brains were rapidly removed and dissected into the fore-brain and lower brainstem (mesencephalon + pons + medulla oblongata) for biochemical assay. 5-HT was measured by high performance liquid chromatography using the method described by Ponzio & Jonsson (1979). In some animals catecholamine levels were also measured in the forebrain by the method of Keller, Oke, Mefford & Adams, (1976).

Results

In agreement with previous findings (Samanin *et al.*, 1980; Cervo *et al.*, 1980) vehicle-treated animals, that had received morphine for 11 days, showed mainly jumping, ptosis and diarrhoea during naloxone-precipitated withdrawal. Other signs such as teeth chattering, wet-dog shakes and ataxic posture were observed less frequently and were not considered in the analysis of the data. None of the vehicle or 5,7-DHT-treated animals that had received no morphine showed any withdrawal signs when injected with naloxone. The rats that had received 5,7-DHT in the VMT showed significantly less jumping than controls during naloxone-precipitated withdrawal. Ptosis and diarrhoea were not significantly affected (Table 1). Inspection of motor coordination (righting response and cage climbing) in 5,7-DHT-treated animals revealed no motor dysfunction that could have interfered with jumping.

As shown in Table 2, no significant differences were found between vehicle and 5,7-DHT-treated rats in the effect of morphine 10 $mg\,kg^{-1}$ on the nociceptive response. In 5,7-DHT-treated rats, 5-HT levels in the forebrain but not in the brainstem were markedly reduced compared to vehicle-treated animals. Values in ng g^{-1} ± s.e. were: in forebrain vehicle 206 ± 9 and 5,7-DHT 61 ± 3, $P < 0.01$ (Student's t test); in the brainstem, vehicle 536 ± 5 and 5,7-DHT 471 ± 44, not significantly different. Noradrenaline levels were not significantly affected, but a small but significant decrease in dopamine was found in the forebrain of animals that had received

Table 1 Abstinence signs precipitated by naloxone in morphine-dependent rats that had received 5,7-dihydroxytryptamine (5,7-DHT) in the ventromedial tegmentum (VMT)

Treatment	*Proportion of positive animals*		
	Jumping	*Diarrhoea*	*Ptosis*
Vehicle	16/18	13/18	15/18
5,7-DHT	6/18*	16/18	12/18

*$P < 0.01$ compared with vehicle (χ^2 test).

Table 2 Effect of morphine on nociceptive responses on the hot plate in animals that had received 5,7-dihydroxytryptamine (5,7-DHT) in the ventromedial tegmentum (VTA)

Treatment	*Nociceptive response at different times (min) after injection*				
	0	30	60	120	180
Vehicle + saline	4.7 ± 0.7	5.9 ± 1.0	4.9 ± 1.0	4.6 ± 0.8	5.8 ± 1.0
Vehicle + morphine	5.2 ± 0.7	23.3 ± 2.3*	19.7 ± 2.2*	11.3 ± 2.8	5.6 ± 0.8
5,7-DHT + saline	4.7 ± 0.6	5.7 ± 0.8	5.6 ± 0.2	5.8 ± 0.6	5.2 ± 0.7
5,7-DHT + morphine[a]	5.3 ± 0.6	17.2 ± 2.7*°	23.3 ± 3°	10.4 ± 3.3	6.1 ± 1.0

Each value is the mean ± s.e. mean of 8 animals.
The animals were injected subcutaneously with morphine 10 mg kg^{-1}.
*$P < 0.01$ compared with vehicle + saline } Tukey's test
°$P < 0.01$ compared with 5,7-DHT + saline }
[a] = no significant interaction between 5,7-DHT and morphine (following 2 × 2 analysis of variance)

5,7-DHT in the VMT (values in ng g^{-1} ± s.e. were: controls 1431 ± 78 and 5,7-DHT 1196 ± 47, $P < 0.05$, Student's t test).

Discussion

Injection of 5,7-DHT in the VMT markedly reduced jumping in morphine-abstinent rats with no significant effects on other signs. It is unlikely that the slight reduction of forebrain dopamine found in 5,7-DHT-treated rats contributed to the effect, as Bläsig Herz & Gramsch (1975) found no change in jumping of morphine-abstinent rats in which a similar decrease of brain dopamine was achieved by injecting 6-hydroxydopamine intraventricularly 12 days before withdrawal precipitation. Moreover, haloperidol, a potent blocker of dopamine receptors (Janssen & Van Bever, 1977) was recently found to have no effect on jumping of morphine-abstinent rats when administered concurrently with morphine during the development of dependence or immediately before naloxone (Cervo *et al.*, 1981). The effect of 5,7-DHT could not be attributed to any change in motor coordination. It is also unlikely that a general decrease in morphine sensitivity was involved since the antinociceptive effect of a single dose of morphine in the hot plate test was unchanged in 5,7-DHT-treated animals.

It has been shown previously that intraventricular injection of 5,6-dihydroxytryptamine reduces jumping with no effect on other withdrawal signs (Herz & Bläsig, 1978). The present study confirms and extends these findings, showing that forebrain 5-HT may be particularly involved. Although in experiments with 5,7-DHT no distinction can be made between development and expression of jumping in morphine dependent rats, cyproheptadine and methergoline, two 5-HT antagonists (Clineschmidt & Lotti, 1974; Fuxe, Agnati & Everitt, 1975) were recently found to reduce jumping when administered concurrently with morphine during development of dependence (Cervo *et al.*, 1981; Samanin *et al.*, 1980) but had no effect when given immediately before naloxone–precipitated withdrawal (unpublished results). Therefore, reduced development rather than reduced expression of jumping may occur in animals with impairment of 5-HT transmission in the forebrain.

As regards the lack of effect of 5,7-DHT on the antinociceptive activity of morphine on the hot plate, there is evidence that 5-HT in the brain and spinal cord facilitates the effect of morphine on various nociceptive responses (Vogt, 1974; Samanin, Miranda & Mennini, 1978) and intraventricular injections of 5,6-DHT were reported to reduce the effect of morphine on the tail flick response (Genovese, Zonta & Mantegazza, 1973). The present findings suggest that 5-HT in regions other than the forebrain is involved in the antinociceptive effect of morphine, at least as regards nociceptive responses in the hot plate test.

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