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Muscimol injections in the nucleus raphé dorsalis block the antinociceptive effect of morphine in rats: apparent lack of 5-hydroxytryptamine involvement in muscimol's effect

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1 The effect of morphine (3 mg kg^{-1} subcutaneously) on tail flick in the tail immersion test was studied in rats which had received a muscimol (100 ng) injection either in the nucleus raphé dorsalis (DR) or medianus (MR).

2 The levels of 5-hydroxyindoleacetic acid (5-HIAA) were measured in the hippocampus and striatum of muscimol-injected animals.

3 Muscimol injections in the DR reduced 5-HIAA concentrations in the striatum but not in hippocampus, whereas in animals which had received muscimol in the MR a selective decrease in hippocampal 5-HIAA levels was found.

4 Muscimol injections in the DR blocked the effect of morphine while no effect was seen in animals which had received muscimol in the MR.

5 An injection of 5,7-dihydroxytryptamine (6 μg in 3 μl) in the DR did not change the effect of morphine or muscimol.

6 These findings indicate that muscimol-sensitive neurones in the DR, which are probably not 5-hydroxytryptaminergic, are involved in the effect of morphine on tail flick in tail immersion. The muscimol-sensitive neurones involved in this effect of morphine do not seem to exist in the MR.

Introduction

Injections of muscimol, a γ-aminobutyric acid (GABA) receptor agonist (Krogsgaard-Larsen, Johnston, Curtis, Game & McCulloch, 1975), in the raphé nuclei dorsalis (DR) and medianus (MR) have been recently reported to reduce 5-hydroxytryptamine (5-HT) metabolism in the striatum and hippocampus respectively of rats (Przewlocka, Stala & Scheel-Krüger, 1979; Forchetti & Meek, 1981). These findings suggest that muscimol injection in the raphé nuclei inhibits the activity of 5-HT neurones originating in these brain areas (Przewlocka *et al.*, 1979; Forchetti & Meek, 1981). Muscimol injections may therefore constitute a useful tool for investigating the role of 5-HT neurones in the raphé nuclei. Studies with electrical lesions and stimulation of either DR or MR in rats have suggested an involvement of the raphé nuclei in the antinociceptive effect of morphine (Samanin, Gumulka & Valzelli, 1970; Sasa, Munekiyo, Osumi & Takaori 1977; Chance, Krynock & Rosecrans, 1978), but it is not yet completely clear whether 5-HT neurones are implicated (Bläsig, Reinhold & Herz, 1973; Buxbaum, Yarbrough & Carter, 1973). Much more attention has been recently paid to the possible role of 5-HT neurones originating in the nucleus raphé magnus in morphine analgesia in rats (Proudfit & Anderson, 1975; Mohrland & Gebhart, 1980). In the present study the roles of DR and MR in morphine analgesia were re-investigated, using muscimol injections into these nuclei. Since muscimol in the DR (but not in the MR) blocked the effect of morphine, 5,7-dihydroxytryptamine, a neurotoxin for 5-HT neurones (Baumgarten, Björklund, Lachenmayer & Nobin, 1973) was also administered in the DR to examine the extent to which destruction of 5-HT neurones in this area mimicked or modified the effect of muscimol.

Methods

Male CD-COBS rats (Charles River, Italy), 200–225 g, were anaesthetized with ethyl ether and mounted on a Stoelting Stereotaxic Instrument. Muscimol 100 ng (Fluka) was dissolved in 0.5 μl saline and injected through an Agla microsyringe (needle 0.3 mm o.d.) at a rate 0.5 μl min^{-1} in the DR and MR. Injections were made at a 20° angle to avoid the saggital sinus. The following coordinates were used for DR and MR respectively A = 0.3, L = 0, D = −0.6; A = 0.4, L = 0, D = −2.6 (König & Klippel, 1963). Controls received an equal volume of the vehicle. Fifteen minutes after muscimol the animals were given a subcutaneous injection of 3 mg kg^{-1} morphine hydrochloride (Farmitalia Carlo Erba, Milan Italy) or saline and tested for their reactions to noxious stimuli.

Tail immersion in hot water

The method described by Janssen, Niemegeers & Dony (1963) was used. The animals were placed in a specially constructed rat holder with the tail hanging out freely. The tail was dipped in a beaker containing water kept at 55°C and the time elapsed until its sudden withdrawal was measured to the nearest 0.1 s. If an animal failed to respond within 30 s, a score of 30 was recorded. Animal responses were measured 30, 60, 90 and 120 min after morphine injection.

At the end of the experiments, the rats were killed by decapitation, the brains immediately frozen in minced dry ice and 40 μm sections cut in a cryostat. In these conditions the trace of the needle was clearly seen and served to locate exactly the site of injection. Independently of their results with morphine, data from animals in which the tip was not exactly placed in the desired area were not included.

Some animals were randomly chosen from groups injected only with muscimol or vehicle in the DR or MR and killed by decapitation for assay of 5-hydroxyindoleacetic acid, (5-HIAA). The striatum and hippocampus were removed according to the technique described by Glowinski & Iversen (1966) and stored at − 20°C until assay by high-performance liquid chromatography as described by Wightman, Plotsky, Strope, Delcore & Adams (1977).

5,7-Dihydroxytryptamine injection

Six μg of 5,7-DHT creatinine sulphate (Serva, Heidelberg, Germany), calculated as free base, were dissolved in 3 μl distilled water containing 0.1% ascorbic acid and infused at a rate of 1 μl min^{-1} into the DR of ethyl ether anaesthetized rats. Stereotaxic coordinates for DR were the same as for injections with muscimol. To protect catecholamine-containing neurones from the action of 5,7-DHT, 30 min before 5,7-DHT the rats received intraperitoneally 15 mg kg^{-1} nomifensine hydrochloride (Hoechst, Frankfurt, Germany), an inhibitor of catecholamine uptake into nerve terminals (Samanin, Bernasconi & Garattini, 1975). Controls received the same dose of nomifensine and the same amount of vehicle for 5,7-DHT. The rats were tested 11 days after 5,7-DHT injection. In some 5,7-DHT treated rats, not

Table 1 Effect of morphine on tail flick of rats which had received muscimol (100 ng) in the nucleus raphé dorsalis (DR) or medianus (MR)

Treatment	*Latency (s) to tail flick at different times after injection:*			
	30	60	90	120 min
DR injections				
Vehicle + saline	2.6 ± 0.6	3.8 ± 0.3	5.1 ± 1	4.4 ± 0.8
Vehicle + morphine	19.7 ± 5.8*	26.2 ± 2.4*	26.2 ± 2.4*	17.7 ± 3.2*
Muscimol + saline	1.4 ± 0.1	2.3 ± 0.5	3.5 ± 0.8	4.0 ± 0.7
Muscimol + morphine	2.5 ± 0.2°	6.4 ± 2.4°[a]	7.9 ± 0.9°[a]	9.2 ± 1.8°
MR injections				
Vehicle + saline	2.7 ± 0.9	3.4 ± 1.4	2.8 ± 0.2	2.5 ± 0.3
Vehicle + morphine	13.6 ± 4.6*	24.5 ± 3.4*	24.5 ± 3.4*	22.2 ± 5.0*
Muscimol + saline	1.5 ± 0.2	2.0 ± 0.3	2.9 ± 0.2	2.8 ± 0.3
Muscimol + morphine	12.6 ± 5.6	23.7 ± 4.4	18.9 ± 5.4	17.6 ± 5.6

Each value is the mean ± s.e. mean of 8 animals.
Morphine 3 mg kg^{-1} was administered subcutaneously to rats 15 min after muscimol (100 ng).
[a] $P < 0.01$ (F interaction between muscimol and morphine).
* $P < 0.01$ compared with vehicle + saline
° $P < 0.01$ compared with vehicle + morphine
Tukey's test.

injected with muscimol or morphine, the striatum was removed and 5-HT assayed as described by Ponzio & Jonsson (1979).

Data were analysed by ANOVA split-plot design. F tests for significant treatment effects were followed by Tukey's test for unconfounded means to compare the experimental groups.

Results

Muscimol injections in the DR significantly reduced 5-HIAA levels in the striatum of rats with no effect on hippocampal 5-HIAA. Data expressed as ng g^{-1} ± s.e.mean were: striatum, vehicle 514 ± 11, muscimol 396 ± 29, $P < 0.01$ Student's t test: hippocampus, vehicle 295 ± 20, muscimol 284 ± 12. Muscimol injections in the MR significantly lowered 5-HT concentrations only in the hippocampus. Values in ng g^{-1} ± s.e.mean were: hippocampus, vehicle 362 ± 25, muscimol 263 ± 9, $P < 0.05$ Student's t test; striatum; vehicle 491 ± 77, muscimol 442 ± 23.

As shown in Table 1 and 2 muscimol injections in raphé nuclei, particularly in the DR, reduced the latency to tail-flick, although the effect was not statistically significant ($P > 0.05$, Tukey's test). The inhibitory effect of 3 mg kg^{-1} morphine on tail-flick response was significantly reduced by muscimol injections in the DR ($P < 0.01$, F interaction) whereas muscimol injections in the MR did not significantly modify the effect of morphine (Table 1).

Table 2 shows the results of experiments with 5,7-DHT injections in the DR. The inhibitory effect of morphine on the nociceptive response in the tail immersion test was not significantly modified in animals which had received 5,7-DHT in the DR 11 days before. The ability of muscimol injection in the DR to prevent the effect of morphine was also not significantly affected by 5,7-DHT treatment.

Striatal levels of 5-HT in 5,7-DHT treated rats were significantly lower than in vehicle-treated animals (values in ng g^{-1} ± s.e.mean, vehicle 270 ± 11; 5,7-DHT 103 ± 23, $P < 0.01$ Student's t test).

Discussion

Muscimol injections in the DR caused a decrease in 5-HT metabolism in the striatum but not in the hippocampus whereas a selective effect on hippocampal 5-HIAA was found in animals which had received muscimol in the MR. These data confirm that muscimol injections may serve to study the separate roles of 5-HT neurones in the DR and MR. The effect of morphine on tail immersion was completely prevented by muscimol injections in the DR but was not affected in animals which had received muscimol in the MR. These findings suggest that muscimol-sensitive neurones located in the DR, but not in the MR, are involved in the effect of morphine in the tail immersion test.

Although GABA-ergic sites in the DR, which presumably mediate muscimol's effect, have been found to inhibit 5-HT cells in this area (Gallager & Aghajanian 1976; Gallager, 1978), it is unlikely that these cells are involved in the action of muscimol, as

Table 2 Effect of 5,7-dihydroxytryptamine (5,7-DHT) injections in the nucleus raphé dorsalis (DR) on morphine and muscimol effect on tail flick of rats

Treatment	*Latency (s) for tail flick at different times after injection:*			
	30	60	90	120 min
Vehicle in the DR				
Vehicle + saline	3.2 ± 0.3	3.1 ± 0.7	3.2 ± 0.4	3.9 ± 0.6
Vehicle + morphine	24.4 ± 3.5*	23.8 ± 3.8*	19.2 ± 3.1*	10.5 ± 4.9
Muscimol + saline	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.2	1.8 ± 0.3
Muscimol + morphine	2.3 ± 0.3°	4.9 ± 0.7°	5.3 ± 2.1°	4.8 ± 0.7
5,7-DHT in the DR[b]				
Vehicle + saline	3.7 ± 0.7	4.2 ± 0.7	3.8 ± 0.5	3.3 ± 1.5
Vehicle + morphine	23.5 ± 4.8*	27.2 ± 2.8*	20.1 ± 4.1*	17.4 ± 5.6*
Muscimol + saline	3.0 ± 0.6	2.6 ± 0.2	3.1 ± 0.2	2.3 ± 0.2
Muscimol + morphine	4.6 ± 0.4°	5.7 ± 1.3°	9.1 ± 5.2°	9.2 ± 1.9

Each value is the mean ± s.e. mean of 8 animals.
Morphine 3 mg kg^{-1} was administered subcutaneously 15 min after muscimol (100 ng); 5,7-DHT (6 μg) was injected in the DR 11 days before testing.
[b] No significant interaction between vehicle and 5,7-DHT-treated rats
*$P < 0.01$ compared with vehicle + saline-treated animals Tukey's test.
°$P0 < 01$ compared vehicle + morphine groups.

borne out by the fact that 5,7-DHT-induced destruction of 5-HT neurones in this area neither mimicked nor changed the effect of muscimol. It has been reported that 5-HT neurotoxins in the DR do not change the effect of morphine on tail flick in rats (Deakin & Dostrovsky 1978). However, it cannot be excluded that in both our and in Deakin's & Dostrovsky's experiments some 5-HT cells in the DR had escaped the neurotoxic action. The neurones left, together with development of hypersensitivity in postsynaptic 5-HT receptor (Nelson, Herbert, Bourgoin, Glowinski & Hamon, 1978), may have compensated for the loss of 5-HT neurones in 5,7-DHT-treated animals. Glutamate-sensitive neurones which project to the nucleus raphé magnus have recently been described in the vicinity of DR (Behbehani & Fields, 1979). Since activation of these neurones causes analgesia in the rat, they may be involved in the effect of muscimol injections in the DR on morphine analgesia. Muscimol injections in the cerebral ventricles or in the periaqueductal grey (PAG) matter 2 mm anterior to the site of injection used in the present study, have been found to block morphine's effect in the tail-flick test (Zambotti, Zonta, Parenti, Tommasi, Vicentini, Conci & Mantegazza, 1982). It is unlikely that muscimol injected in the DR had diffused to the area used by Zambotti *et al.* (1982) or the aqueduct of Sylvius since an equal volume of methylene blue solution did not reach these structures. Moreover, in Zambotti *et al.*'s study muscimol in the PAG caused slight sedation while in the present experiments, as previously reported by Przewlocka *et al.* (1979), behavioural activation was noted in muscimol-injected rats. It seems therefore that muscimol-sensitive sites in different brain regions influence the effect of morphine on tail-flick response in rats. As regards the lack of effect of muscimol injection in the MR, it has previously been shown that electrolytic lesions in this area reduce the effect of morphine on various nociceptive responses (Samanin *et al.*, 1970; Garau, Mulas & Pepeu, 1975; Chance *et al.*, 1978). These findings, however, did not provide clear-cut evidence that 5-HT neurones in MR were involved, although in one study 5-hydroxytryptophan, a precursor of 5-HT, was able to restore morphine activity in MR-lesioned animals (Samanin & Bernasconi, 1972). The present findings apparently argue against an involvement of MR 5-HT neurones in the effect of morphine on tail flick in rats, although it cannot be excluded that muscimol-insensitive 5-HT neurones in this area play a role. Experiments are in progress in which various nociceptive responses are studied in animals given 5,7-DHT and muscimol injections in the MR.

In conclusion, the present study has shown that muscimol-sensitive neurones in the DR, probably not containing 5-HT as transmitter, are involved in the effect of morphine on tail flick in 'tail immersion' procedure. Although muscimol-sensitive sites involved in morphine's effect on tail flick may exist in other brain areas, the nucleus raphé medianus, an area previously involved in the effect of morphine in various nociceptive responses (Samanin *et al.* 1970), seems to be devoid of such neurones.

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