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SHORT COMMUNICATIONS

Reduction of morphine's effect on striatal dopamine metabolism in rats treated with a low dose of apomorphine or agents increasing serotonin transmission

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Morphine administered systemically increases striatal levels of homovanillic acid (HVA) [1–4] and blocks amphetamine-induced stereotypy [5], an effect commonly attributed to increased release of dopamine (DA) from nerve terminals in the striatum [6, 7]. *P*-Chlorophenylalanine (PCPA), an inhibitor of serotonin (5-HT) synthesis [8], was recently found to prevent the inhibitory effect of morphine on stereotypy caused by *d*-amphetamine [5]. Moreover, stereotyped movements were observed in rats treated with a 5-HT antagonist before a single dose of morphine [9, 10]. 5-HT-sensitive sites inhibitory of DA release from nerve terminals in the striatum have been described [11–13] and there is evidence that a single morphine injection activates 5-HT mechanisms in the brain [14–18]. Morphine injection in the substantia nigra pars compacta, site of origin of striatal DA neurons [19], causes stereotyped movements and increases DA metabolism in the striatum [20] suggesting that opiate sites exciting DA neurons exist in the brain.

It has been recently suggested that systemical administration of morphine, resulting in increased 5-HT transmission, masks the drug's ability to activate dopamine function in the brain [5].

If this is true, one would expect an increase of 5-HT transmission to reduce the effect of morphine on DA metabolism in the brain. This hypothesis has been tested by studying the effect of morphine on striatal HVA levels of animals treated with d-fenfluramine, a releaser of 5-HT from nerve terminals [21], and *m*-chlorophenylpiperazine (CCP), a potent 5-HT agonist [22]. To confirm that activation of inhibitory sites on DA neurons may reduce morphine's effects on DA metabolism, animals treated with a low dose (50 μg/kg) apomorphine were used as well. This dose of apomorphine is believed to selectively activate presynaptic DA receptors [23] and, to our knowledge, has never been studied in combination with morphine on striatal DA metabolism.

Materials and methods

Male CD–COBS (Charles River, Italy) rats, weighing 175–200 g, were used. They were kept at constant room temperature (21 ± 1°) and relative humidity (60%) with a 12 hr light : 12 hr dark cycle (dark period commencing at 7 p.m.). The animals were injected subcutaneously with 10 mg/kg morphine hydrochloride (Farmitalia-Carlo Erba, Milan, Italy) and killed by decapitation 1 hr later for biochemical assay. The following drugs were given in combination with morphine at doses reported to act significantly on particular monoamine mechanisms (references for each compound are given in brackets): 2.5 mg/kg *d*-fenfluramine hydrochloride (Servier Laboratory, Neuilly-sur-Seine, France) [21]), 2.5 mg/kg CPP hydrochoride (Aldrich Europe) [24] and 0.050 mg/kg apomorphine hydrochloride (Sandoz, Milan, Italy) [23].

d-Fenfluramine and CPP were dissolved in saline and administered intraperitoneally 30 min before morphine. Apomorphine, dissolved in saline, was injected subcutaneously 30 min after morphine. Striatum was dissected as described by Glowinski and Iversen [25] and immediately frozen on dry ice. HVA was measured by high performance liquid chromatography with electrochemical detection according to the method of Wightman *et al.* [26] with minor modifications [27].

The data were statistically analyzed by ANOVA 2 × 2 [28]. *F* test for significant interaction were followed by Tukey's test to compare the experimental groups with controls.

Results

As shown in Table 1, neither *d*-fenfluramine and CPP nor apomorphine significantly modified HVA levels in the striatum. All the drugs markedly reduced the increase in HVA levels caused by morphine (*F* interaction $P < 0.05$). In order to check whether the drugs had decreased the effect of morphine by reducing its entry into the brain, in some animals, taken randomly from the various groups, brain levels of morphine were measured according to a slightly modified version of the method described by Todd *et al.* [29]. No decrease in morphine levels (measured 1 hr after morphine injection) was found in the brain of treated animals compared to controls. Morphine levels in apomorphine treated rats were, in fact, higher than in controls ($P < 0.001$ Dunnett's test). Morphine levels in ng/g ± S.E. (mean values of 6 determinations) were: controls 281 ± 9; *d*-fenfluramine 331 ± 4; CPP 330 ± 17; apomorphine 467 ± 21.

Table 1. Effect of *d*-fenfluramine (d-F), m-chlorophenylpiperazine (CPP) and apomorphine (APO) on the rise in striatal homovanillic acid (HVA) levels induced by 10 mg/kg morphine s.c. in rats

Treatment (mg/kg)	HVA (ng/g ± S.E.)
Saline + saline	655 ± 43
d-F (2.5) + saline	685 ± 31
Saline + morphine (10)	846 ± 31*
d-F (2.5) + morphine (10)	659 ± 54***
Saline + saline	615 ± 31
CPP (2.5) + saline	680 ± 50
Saline + morphine (10)	1017 ± 26*
CPP (2.5) + morphine (10)	725 ± 80***
Saline + saline	622 ± 42
Saline + APO (0.05)	540 ± 54
Morphine (10) + saline	988 ± 73**
Morphine (10) + APO (0.05)	436 ± 29***

Each value is the mean of 6 determinations.
d-F and CPP were administered intraperitoneally 30 minutes before morphine.
Apomorphine was injected subcutaneously 30 min after morphine and the animals were killed 1 hr after morphine.
* $P < 0.05$ compared with saline + saline.
** $P < 0.01$ compared with saline + saline.
*** $P < 0.05$ (*F* interaction).

Discussion

The fact that doses of apomorphine reported to activate inhibitory presynaptic receptors in DA-containing neurons [23] completely blocked the increase in striatal HVA caused by 10 mg/kg morphine is compatible with the hypothesis that this dose of morphine mainly acts by exciting DA-containing neurons in the nigrostriatal system. Should inhibitory opiate sites on DA terminals [30] be involved, one would in fact expect a cooperative effect of apomorphine and morphine on DA metabolism in the striatum. Morphine may increase DA transmission by acting on mechanisms controlling the activity of DA cells in the substantia nigra [20].

Morphine's effect on HVA was partially antagonized in animals which had received *d*-fenfluramine and CPP, two drugs reported to selectively increase 5-HT transmission at the doses used in the present study [21, 22, 24]. It is unlikely that the effects of *d*-fenfluramine and CPP were due to a direct action on dopamine metabolism since at the doses used in the present study neither drug modified the levels of striatal HVA. Moreover, these drugs have little or no effect on the uptake and release of DA in synaptosomal prepatations or binding of ^{3}H-ligands for DA receptors in striatal membranes [31, 22, 24].

Crunelli *et al.* [32] reported that 10 mg/kg *d*-fenfluramine increased the concentrations of HVA in the rat striatum. The effect, however, was prevented by procedures aimed at blocking *d*-fenfluramine's effect on 5-HT-containing neurons [32], suggesting that even a relatively high dose of *d*-fenfluramine influences DA activity through an action on 5-HT mechanisms. The changes in dopamine metabolism found with doses of CPP higher than that used in the present study [33] may also depend on its ability to activate 5-HT mechanisms. This is suggested by the fact that cyproheptadine, a serotonin antagonist, was found to prevent the depression of dopamine synthesis caused by 10 mg/kg CPP in rats [34]. It is likely therefore that activation of 5-HT transmission is the mechanism by which 2.5 mg/kg *d*-fenfluramine and CPP reduce the morphine-induced increase in striatal HVA. This dose of *d*-fenfluramine or CPP may respectively increase 5-HT availability or mimic its action on 5-HT-sensitive sites which inhibit DA transmission in the nigro-striatal system [11, 12, 35]. Higher doses of these compounds may change DA metabolism by 5-HT mechanisms not necessarily identical to those involved in the effects found in the present study. In apparent contrast with the present results, Demarest and Moore [36] reported that the increase in striatal DA turnover caused by morphine was attenuated by intracerebroventricular injections of 5,7-dihydroxytryptamine, a 5-HT depletor, or intraperitoneal injections of metergoline, a 5-HT antagonist. We have been unable to significantly modify the effect of morphine on striatal HVA by using parachlorophenylalanine, an inhibitor of 5-HT synthesis, or two 5-HT antagonists, metergoline and mianserine [37]. Besides the fact that Demarest and Moore themselves considered that disruption of 5-HT transmission had no dramatic effect on morphine-induced increase in striatal DA turnover and, at least in the case of metergoline, this was probably due to an increased variability in experimental animals, we have no clear explanations for the apparent differences between Demarest and Moore's and our results. Nevertheless, it cannot be excluded that DA synthesis as measured by DA decline with alphamethyltyrosine and DA metabolism are under different control by 5-HT mechanisms in the brain. That activation of 5-HT mechanisms in the brain contributes to reducing morphine-induced excitation of DA-containing neurons in the striatum is suggested by the fact that stereotypy rather than catalepsy, signs commonly associated with activation and inhibition of DA functions respectively [1, 6, 7, 38] has been found in animals which had received PCPA or a 5-HT antagonist before morphine injection [9, 10]. Moreover, it has been shown that PCPA completely prevents the inhibittory effect of morphine on amphetamine-induced stereotypy [5].

These data, together with the present study, support the hypothesis that a single moderate dose (about 10 mg/kg) of morphine activates dopaminergic mechanisms in the brain and the functional output of this effect is masked by a concomitant activation of 5-HT mechanisms, which act by inhibiting DA-containing neurons in the brain [11–13]. That a single dose of morphine activates 5-HT mechanisms in the brain is suggested by various findings [14–18].

In summary the effect of 10 mg/kg morphine s.c. on striatal levels of homovanillic acid (HVA) was studied in rats which had received an intraperitoneal injection of *d*-fenfluramine (2.5 mg/kg), *m*-chlorophenylpiperazine (2.5 mg/kg) or apomorphine (0.05 mg/kg). None of these drugs significantly modified the striatal levels of HVA. All, however, markedly reduced the rise in HVA caused by morphine. Brain levels of morphine were not reduced in any experimental group.

The results suggest that an increase in serotonin transmission, like activation of presynaptic dopamine receptors, inhibits morphine-induced enhancement of dopamine metabolism in the striatum of rats.

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Effect of GABA and photoaffinity labelling on the affinity of drugs for benzodiazepine receptors in membranes of the cerebral cortex of five-day-old rats

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Recently, a number of new compounds, usually with a structure unrelated to that of benzodiazepines, like the β-carbolines [1], Ro 15-1788 [2] or CGS 8216 [3], have been discovered which seem to exert their pharmacological effects by an interaction with benzodiazepine receptors which differs from that of classical benzodiazepines [1]. Although these novel drugs do not exhibit a pharmacologically relevant selectivity for subtypes of benzodiazepine receptors, it appears possible that their mode of interaction with these subtypes of receptors differs with respect to their (partial) agonistic or (partial) inverse agonistic effects.

Currently two *in vitro* methods can be used to indicate whether a ligand for benzodiazepine receptors has agonist respectively inverse agonist properties, namely the investigation of affinity changes induced by GABA [4, 5] or by photoaffinity labelling [6] of one of the presumed four benzodiazepine binding sites of the GABA/benzodiazepine receptor complex [7].

We have used these two methods in order to investigate whether ligands for benzodiazepine receptors differ in their mode of interaction with subtypes of benzodiazepine receptors. For this, we used membranes derived from the cerebral cortex of five-day-old rats. In these membranes, like in those from hippocampus of animals of the same age, the type I benzodiazepine receptor, which contains the P_{51} photolabelled peptide, constitutes only approximately one third of total binding capacity [8–10] (W. Sieghart, personal communication). The remaining two other thirds of the binding sites consist of receptors which contain the P_{53}, P_{55} and P_{59} photolabelled peptides [9]. We therefore investigated membranes from the cerebral cortex of five-day-old rats as a tissue enriched in other benzodiazepine receptors than type I and compared the results with those from the cerebellum of adult rats [6]. It has previously been shown that the benzodiazepine receptor for the latter tissue contains >90% type I receptors respectively the P_{51} photolabelled peptide [10].

Methods and materials

Membranes from cerebral cortex of five-day-old Wistar rats were prepared at 0–4° in 50 mM Tris–citrate buffer, pH 7.1 [6]. The membranes were washed 5 times and stored frozen. Photoaffinity labelling with washed cortex membranes was performed essentially as already described [6]. Membranes were preincubated at 0° with 20 nM flunitrazepam 20 min and then irradiated for 60 min with long wave length (366 nm) u.v. light. Membranes were then washed twice in order to remove non-incorporated ligand. For binding assays [^{3}H]-flunitrazepam (72.4 Ci/mmole), [^{3}H]-βCCE (83.7 Ci/mmole) and [^{3}H]-Ro 15-1788 (87.5 Ci/mmole) were used. Control and photoaffinity labelled membranes, equivalent to 10 mg wet weight of tissue, were incubated at 0° in 1 ml 50 mM Tris–citrate buffer pH 7.1, which contained 200 mM NaCl, the drugs