5-Hydroxytryptamine-like properties of *m*-chlorophenylpiperazine: comparison with quipazine

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Studies on the central 5-hydroxytryptaminergic system are handicapped by the lack of selective centrally active agonists of the 5-HT receptor. LSD and quipazine were used as such, but the action of LSD is complex (Aghajanian 1972), while quipazine produces behavioural effects suggesting that it also stimulates the dopamine receptor (Grabowska et al 1974).

m-Chlorophenylpiperazine (CPP), a metabolite of an antidepressant drug, trazodone (Melzacka et al 1979), was reported to inhibit the membrane uptake mechanism for 5-HT without being a 5-HT releasing agent (Garattini et al 1976). A subsequent study by Maj et al (1979), employing the spinal rat flexor preparation for detection of possible action of drugs on 5-HT neurons, revealed that CPP, unlike fenfluramine, exerted a stimulatory action on postsynaptic 5-HT receptors. These authors also reported that CPP induced a behavioural syndrome characteristic for central 5-HT-ergic stimulation. Like quipazine, CPP produced hyperthermia in rabbits, which could be blocked by pretreatment with cyproheptadine (Maj et al 1980).

We have investigated to see if CPP could be regarded as a specific stimulant of the central 5-HT-ergic system, and particularly if it affected central dopaminergic functions. To this end, we have compared the action of CPP with that of quipazine in respect of their influence on the in vivo activities of tyrosine and tryptophan hydroxylases (Carlsson et al 1972) in male Wistar rats, 160–220 g, kept under standard laboratory conditions with free access to pelleted food and water. The animals received 100 mg kg^{-1} of 3-hydroxybenzylhydrazine hydrochloride (synthesized by Dr S. Misztal), an inhibitor of cerebral decarboxylase of aromatic amino acids (Carlsson et al 1972), 30 min before decapitation. The whole brain concentrations of dopa and 5-hydroxytryptophan (5-HTP) were assayed spectrofluorometrically after separation of Dowex 50 X4 column (Atack & Magnusson 1978). Quipazine hydrochloride (courtesy Miles Laboratories), cyproheptadine hydrochloride (Periactin, Merck, Sharp & Dohme, isolated from tablets) and CPP hydrochloride (synthesized by Dr S. Misztal) were given at the times specified below. All drugs were dissolved in 0·9% NaCl (saline) and given intraperitoneally in a volume of 4 ml kg^{-1}. After administration of CPP or quipazine the animal behaviour was observed until death.

CPP, 10 mg kg^{-1}, produced sedation, head twitches and limb abduction, persisting for over 6 h. This behaviour sharply contrasted with that induced by quipazine, which was dominated by locomotor stimulation and sterotyped sniffing, although head twitches and abduction of hind limbs were also observed; the behavioural changes were similar to those reported by Grabowska et al (1974). Head twitches and abduction of the limbs produced by either drug were prevented by cyproheptadine, 0·5 mg kg^{-1}, given 15 min earlier.

CPP rapidly depressed the accumulation of 5-HTP, without affecting the accumulation of dopa for at least 90 min. After that time the dopa accumulation also declined, to approximately 35% of the control value. The concentrations of both amino acids returned to control values within 12 h (Fig. 1). Quipazine produced rapid depression of accumulation of both amino acids,

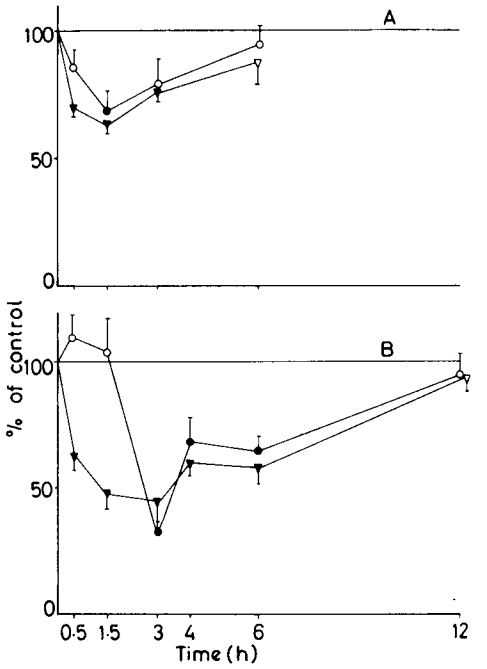

FIG. 1. The effect of quipazine (A) and CPP (B) on accumulation of dopa (circles) and 5-HTP (triangles) in the rat brain. Each rat received 100 mg kg^{-1} (i.p.) 3-hydroxybenzylhydrazine 30 min before death. Each point represents the mean (±s.e.m.) of 5–8 results. Full symbols denote results significantly different from control values ($P<0·05$). Abscissa: time (h) after injection of a 5-HT antagonist. Ordinate: amino acid concentration as percentage of control values. Control value of dopa was 165 ± 7 (n = 48), and of 5-HTP: 173 ± 4 (n = 47) ng g^{-1} of tissue.

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the fall in dopa concentration being relatively much more marked than that in 5-HTP concentration (Table 1).

Cyproheptadine, 0·5 mg kg^{-1}, when given separately, elevated the accumulation of 5-HTP, but did not counteract the depressing action of CPP and quipazine, in spite of abolishing the behavioural '5-HT-ergic syndrome'. Cyproheptadine completely antagonized the depression of dopa accumulation induced by CPP, but only partially that observed after quipazine (Table 1).

These results together with those of Maj et al (1979, 1980) indicate that CPP is an agent stimulating the central 5-HT-ergic system without directly affecting the dopamine receptor. CPP produces the '5-HT-ergic syndrome' without any sign of general stimulation or stereotyped behaviour, and this syndrome is completely blocked by cyproheptadine. CPP also inhibits the accumulation of 5-HTP in the brain after inhibition of cerebral decarboxylase: this indicates an inhibition of the central 5-HT synthesis (Carlsson et al 1972), and is compatible with the assumption that the drug stimulates the 5-HT receptor.

Unlike quipazine, which rapidly inhibited the accumulation of dopa, CPP did not affect it immediately after administration but only at the time of the full inhibitory effect of CPP on 5-HTP accumulation. This course of action of CPP suggests that changes in the activity of dopamine neurons were secondary to the alterations in the 5-HT-ergic system. This suggestion is corroborated by the fact that cyproheptadine completely abolished this effect.

In contrast to CPP, the action of quipazine on dopa accumulation seems to be more complex: not only is it rapid, but also only partially prevented by cyproheptadine. This suggests that the depression of dopa accumulation by quipazine is caused by two different mechanisms: a direct one, related to the stimulatory action of quipazine on the central dopamine receptor, as postulated by Grabowska et al (1974), and a secondary one, triggered by the stimulatory action of the drug on the 5-HT receptor.

Our demonstration of a considerable delay in the action of CPP on the activity of tyrosine hydroxylase in vivo corresponds well with the observation of Fuller & Snoddy (1977) that CPP produces an initial rise followed by a fall of the cerebral concentration of 3,4-dihydroxyphenylacetic acid in the rat.

As CPP does not stimulate the dopamine receptor, the decrease in dopa accumulation in the brain produced by the drug should be interpreted as a depression of dopaminergic transmission. It seems, therefore, that a prolonged stimulation of the 5-HT receptor leads to a decreased activity in the dopaminergic system. This situation is opposite to the effect of stimulating the dopamine receptor, which leads to a relatively prompt activation of the 5-HT-ergic system (Grabowska 1975). One possible explanation of the effect of 5-HT-ergic stimulation is that it produces a prolonged hypomotility,

Table 1. The effect of cyproheptadine and 5-HT-mimetics on the accumulation of dopa and 5-HTP in the brain of rats pretreated with m-hydroxybenzylhydrazine. All rats received 100 mg kg^{-1} of *m*-hydroxybenzylhydrazine 30 min before decapitation. Cyproheptadine was given 60 min before death, quipazine—50 min, and CPP—3 h before death. The data are means ± s.e.m.; amino acid concentrations are expressed in ng g^{-1} of frozen tissue.

Treatment, dose (mg kg^{-1})	5-HPT	Dopa
Saline	173 ± 6 (22)	181 ± 9 (20)
Cyproheptadine, 0·5	195 ± 11 (8)	229 ± 6 (7)
Quipazine, 5	136 ± 10 (5)	74 ± 7 (4)
Quipazine, 5 + Cyproheptadine, 0·5	144 ± 6 (6)[b]	134 ± 6 (5)[c]
CPP, 10	80 ± 11 (8)	57 ± 5 (5)
CPP, 10 + Cyproheptadine, 2 × 0·5[a]	102 ± 9 (8)[b]	150 ± 11 (8)[c]

3 × 2 Analysis of variance has shown a highly significant effect of 5-HT-mimetics on accumulation of 5-HTP ($F = 54{\cdot}16$, df 2/34) and dopa ($F = 22{\cdot}48$, df 2/30). Cyproheptadine exerted a highly significant effect on dopa accumulation ($F = 84{\cdot}61$, df 1/30), but much less significant on 5-HTP accumulation ($F = 4{\cdot}42$, df 1/34). The interaction between cyproheptadine and 5-HT-mimetics in the action on dopa accumulation was highly significant ($F = 77{\cdot}95$, df 2/30), while no interaction in respect to the effect on 5-HTP accumulation was observed ($F = 0{\cdot}32$, df 2/34).

[a] Additional dose of cyproheptadine was given 30 min before CPP. [b] No significant difference from the group receiving a 5-HT-mimetic alone ($P > 0{\cdot}05$, Newman-Keul's test. [c] Significantly different from the group receiving the 5-HT-mimetic alone ($P < 0{\cdot}01$, Newman-Keul's test).

which may lead to a decreased demand for and utilization of dopamine.

The result escaping explanation is the dissociation in the action of cyproheptadine on the behavioural effects of CPP and quipazine and their effect on dopa accumulation on the one hand, and cyproheptadine's ineffectiveness on the drug-induced depression of 5-HTP accumulation on the other. It is well known, however, that 5-HT antagonists are not capable of reversing other effects of 5-HT-ergic stimulation, e.g. hypothermia in the rat (Pawłowski 1976). This suggests that there exist various types of 5-HT receptors, of which only some are inhibited by cyproheptadine and other presently known antagonists. However, these cyproheptadine-sensitive receptors are present in the neural circuits responsible for the behavioural '5-HT-ergic syndrome' and interaction with the dopamine system.

It is conceivable that 5-HT-mimetics stimulate both pre- and post-synaptic 5-HT receptors. Their post-synaptic action results in behavioural effects and indirect biochemical changes, while the pre-synaptic stimulation leads to the depression of the 5-HT synthesis rate. It is tempting to assume that cypro-

heptadine-like antagonists inhibit only the post-synaptic receptor, thus counteracting the effects of post-synaptic stimulation, but are incapable of inhibition of the pre-synaptic receptor, thus producing negligible changes in the 5-HT synthesis, and not counteracting the inhibitory action of 5-HT-mimetics on 5-HT synthesis rate.

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Effect of cyclic AMP and cholera toxin on the migration of rat polymorphonuclear leucocytes in boyden chambers

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The accumulation of polymorphonuclear leucocytes (PMN) at inflammatory loci is generally considered to be the result of the chemotactic attraction of these cells to substances (cytotaxins) produced and/or released at these sites. Since these cells play a major role in inflammation through phagocytosis and release of their lysosomal enzymes, modification of their accumulation via the pharmacological manipulation of chemotaxis is a possible method by which inflammatory processes could be controlled. Therefore PMN migration has been studied. The techniques used in vitro to do so include both the direct microscopic observation of cell migration on glass slides and the measurement of the degree of migration of cells into filters. We have found, as have Allan & Wilkinson (1978), that the parallel use of both types of technique is advantageous in that they furnish some different information. Several workers have studied the effect of cyclic (c) AMP on chemotaxis (see review by Hill 1978) and both an inhibition of migration (Rivkin et al 1975) and a lack of effect (Borel 1973) have been reported. We have recently studied the effect of cAMP and cholera toxin (which raises intracellular cAMP concentrations in rat) (Roch-Arveiller et al 1979) on the chemotaxis of rat PMN towards a laser-lysed erythrocyte as observed under the phase-contrast microscope. Both substances produced a marked inhibition of the chemotactic response (Bradshaw et al 1978). The results now presented are those of a similar study but in which a filter technique instead of a microscopic technique was used for the assessment of cell migration.

The method was a modification of the Boyden chamber technique (Boyden 1962) with chambers similar to those described by Keller et al (1975). Casein (5 mg ml^{-1}) was placed in the lower compartment as chemoattractant. Casein solution was prepared by dissolving it at 10 mg ml^{-1} in dil. NaOH (pH 11·5) and re-adjusting the pH to 7·2 with Na H_2PO_4, to this was added an equal volume of a solution of 9 ml distilled water, 1 ml Hanks solution and 0·1 ml $NaHCO_3$ (7·5% w/v). The filters (Millipore) of pore diameter 3·0 μm, were made of a mixture of cellulose esters. PMN were obtained from the pleural cavity of rats 4 h after the intrapleural injection of 1 ml isologous serum. The cells were washed three times with Hanks solution and their concentration was then adjusted to 5×10^6 cells ml^{-1} before incubation for 15 min at 37 °C in Hanks solution, cAMP, or cholera toxin. 100 μl of the cell suspension was then taken and added to the upper compartment of the chemotaxis chamber. The chambers were then incubated in air for 90 min at 37 °C. After incubation, ethanol was added to fix the cells onto the filter after which they were stained with haematoxylin. Cell migration in five high-power fields for triplicate filters was assessed under the light microscope using the leading front technique (Zigmond & Hirsch 1973).

Dibutyryl cAMP (db cAMP) at various doses was tested for an effect on cell migration by the method described above but was found to have no apparent

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