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Short Communication

Blockade of Central 5-Hydroxytryptamine Receptors by Methiothepin

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Summary. Methiothepin (1-[10,11-dihydro-8-(methylthio)dibenzo[b,f] thiepin-10-yl]-4-methylpiperazine maleate), a representative of a new class of neuroleptics, is shown to be an antagonist of 5-hydroxytryptamine (5-HT) at receptors in the CNS. The neuropharmacological evidence was obtained by observing its effects on ponto-geniculo-occipital (PGO) spikes induced in curarized cats by either the benzoquinolizine Ro 4-1284 or p-chlorophenylalanine. Methiothepin a) increased the number of drug-induced spikes, b) antagonized the inhibitory action of 5-HTP and LSD on the spikes, and c) induced PGO-spikes by itself in untreated animals. The turnover of 5-HT in the rat brain was accelerated by methiothepin as indicated by virtually unaltered 5-HT levels, an increase of 5-hydroxyindole acetic acid, an enhancement of the 5-HT increase induced by a MAO inhibitor and by an increase of tryptophan levels in the brain.

Key words: Brain Serotonin — PGO-Spikes — Methiothepin — Serotonin Antagonist.

A number of drugs is thought to antagonize effects of the central transmitters noradrenaline (NA) or dopamine (DA) or both at their receptor sites within the central nervous system (CNS). There is some evidence that NA receptors in the CNS might be similar to α-adrenergic receptors in the periphery: α-adrenolytics not only block NA effects in various smooth muscles and in neuronal structures in the periphery, such as autonomic ganglia, but increasing evidence indicates that they do so also in the CNS if able to pass the blood-brain barrier. The same probably holds for DA receptors in the CNS and in renal arteries.

For 5-hydroxytryptamine (5-HT) receptors the situation is more complicated (Vogt, 1968): potent antagonists of 5-HT at smooth muscle receptors (such as LSD and methysergide) do not alter the effects of 5-HT in peripheral neuronal structures (Haefely, in preparation). Reports on agents claimed to act as antagonists of central effects of 5-HT are very conflicting, due to a great part to the lack of a generally accepted way for assessing a central 5-HT-antagonistic action. In view of the suspected implications of 5-HT in many important functions of the brain, a central

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5-HT antagonist would be of great experimental and possibly of therapeutic interest. A neurophysiological observation (Delorme *et al.*, 1965, 1966) recently adapted for neuropharmacological investigations of central monoamines (Jalfre *et al.*, 1970) led us to presume a central 5-HT antagonistic activity of methiothepin (1-[10,11-dihydro-8-(methylthio)dibenzo-[b,f] thiepin-10-yl]-4-methylpiperazine maleate) (Pelz *et al.*, 1968), a member of the new class of dibenzothiepins with neuroleptic activity. Turnover studies of brain 5-HT corroborated this assumption.

Methods and Materials

In unanaesthetized curarized cats (surgery was carried out under ether anaesthesia and all pressure points were infiltrated with a local anaesthetic) maintained at constant body temperature the electrical activity in the lateral geniculate bodies (LGB) was recorded with stainless steel macroelectrodes. The animals were either pretreated with p-chlorphenylalanine (PCPA) 300 mg/kg i.p. 3 and 2 days before or injected i.p. with Ro 4-1284 (Pletscher *et al.*, 1959), 20 mg/kg, at the beginning of the experiment. With this dose schedule the 5-HT content in the lower brain stem was reduced to 35% (Keller, 1972) and 55% (Jalfre *et al.*, 1971), respectively, at the moment when methiothepin and other drugs were studied. Reduction of cerebral 5-HT by inhibitors of storage (Ro 4-1284) or synthesis (PCPA) of amines induces the occurrence of monophasic potential changes in the LGB resembling those observed immediately before and during the rapid eye movement (REM)-phases of sleep and which are called ponto-geniculo-occipital (PGO) spikes. The results reported below are based on at least 3 animals per dose and substance or drug combination.

The turnover of 5-HT was measured in female rats from a closed randomized colony (Füllinsdorf strain of Wistar descent) by measuring the increase of 5-HT after inhibition of monoamine oxidase by pargyline and the accumulation of 5-hydroxyindoleacetic acid (5-HIAA). Methiothepin was injected intraperitoneally. At different times after drug application 5-HT (Bogdanski *et al.*, 1956), 5-HIAA (Giacalone and Valzelli, 1966) and tryptophan (Denckla and Dewey, 1967) were measured in the whole brain. In order to prevent hypothermia the animals were kept in an environmental temperature of 32° C.

The drugs used were: L-5-hydroxytryptophan (5-HTP), lysergic acid diethylamide tartrate (LSD), Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-benzo[a]quinolizine hydrochloride), chlorpromazine hydrochloride, chlorprothixene hydrochloride, cinanserine (base), clothiapine (base), cyproheptadine hydrochloride, haloperidol (ampoules of Haloperidol®, Janssen), methiothepin maleate, methysergide dimaleate, prochlorperazine dimaleate, p-chlorophenylalanine, pargyline hydrochloride.

Results and Discussion

PGO-spikes originate in the pontine reticular formation; their number depends (inter alia) on the amount of tonic inhibition exerted by 5-HT neurons upon reticular "generator cells". Drugs and procedures which increase 5-HT at these critical synapses will decrease or abolish PGO-spikes induced by partial depletion of 5-HT by Ro 4-1284 or PCPA,

Table 1. *Effect of some neuroleptics on Ro 4-1284-induced PGO-spikes*

Compound	Cumulative doses (mg/kg i.v.)				
	0.1	0.3	1	3	10
Chlorpromazine	129 ± 19	127 ± 17	128 ± 22	140 ± 23	173 ± 56
Clothiapine	91 ± 13	110 ± 3	121 ± 3	120 ± 11	90 ± 18
Haloperidol	123 ± 3	138 ± 5	147 ± 20	149 ± 35	32 ± 21
Methiothepin	110 ± 9	136 ± 16	217 ± 38*	289 ± 42*	283 ± 61*
Prochlorperazine	90 ± 3	81 ± 11	93 ± 11	104 ± 16	72 ± 23
Chlorprothixene	102 ± 3	117 ± 12	125 ± 9	121 ± 16	71 ± 15

Values are the means (± S.E.) of the number of PGO-spikes recorded over 30 min, expressed in per cent of animals which received saline instead of a neuroleptic. 3 animals per drug were used.

* $p < 0.05$ when compared with control.

whereas a further reduction of the release of 5-HT or occupation of 5-HT receptors should lead to an additional increase of their number.

5-Hydroxytryptophan (5-HTP), a 5-HT precursor, and 5-HT-like agents, e.g. LSD, given i.v. rapidly abolish the Ro 4-1284- and PCPA-induced PGO-spikes already at low doses (Jalfre *et al.*, 1970). Five representative neuroleptics tested (chlorpromazine, clothiapine, haloperidol, prochlorperazine, chlorprothixene) did not significantly increase the number of Ro 4-1284-induced PGO-spikes, whereas methiothepin produced a marked, dose-dependent increase (Table 1). Some agents claimed to be central 5-HT antagonists in the spinal cord, namely cyproheptadine, cinanserin and methysergide (Banna and Anderson, 1968) did not increase PGO-spikes; methysergide was even able to abolish the spikes, suggesting a 5-HT-like activity under our experimental conditions.

That the effect of methiothepin was the result of blockade of 5-HT receptors is indicated by the finding that the inhibition of PGO-spikes by 5-HTP was antagonized by the drug: methiothepin (10 mg/kg i.v.) shifted the dose-response curve of 5-HTP (i.v.) to the right by a factor of approximately 5. The compound (3 mg/kg i.v.) also prevented the inhibitory effect of LSD (0.1 mg/kg i.v.) on PCPA-induced PGO-spikes. As one would expect of a central 5-HT-receptor blocking agent, methiothepin (10 mg/kg i.v.) induced PGO-spikes in cats which had not received Ro 4-1284 or PCPA.

NA and DA receptor blocking agents increase the turnover of these catecholamines probably by neuronal feedback mechanisms (Nybäck and Sedvall, 1970). Methiothepin increased brain levels of 5-HIAA, while decreasing 5-HT levels by only 9%. The increase in 5-HIAA was dose-dependent, a maximal effect was obtained with 20 mg/kg (Fig. 1). The

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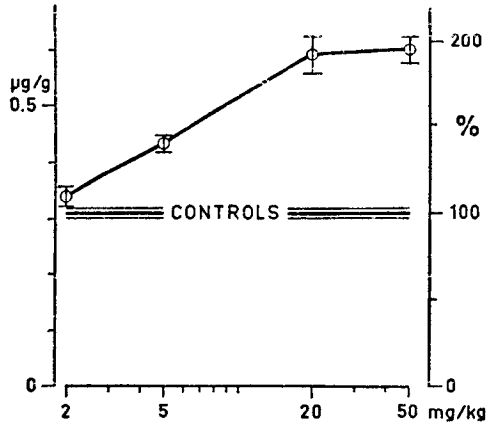

Fig. 1. Levels of 5-HIAA in rat brain 2 h after i.p. injections of methiothepin. Each point represents the mean ± S.E. of at least 5 pairs of pooled brains

increase of 5-HT produced within 1 h by the MAO inhibitor pargyline (75 mg/kg i.p.) was further augmented from 171% ± 5 ($n = 16$) to 207% ± 7 ($n = 15$) by methiothepin (50 mg/kg i.p. 30 min before pargyline). Furthermore, the amount of tryptophan in the brain was significantly increased by methiothepin (results not shown). An increased turnover of 5-HT is often accompanied by increased levels of brain tryptophan (Tagliamonte *et al.*, 1971).

Brain 5-HT has been connected with many functions of the CNS. Its supposed involvement in sleep mechanisms is based on ample experimental evidence. A likely function of the 5-HT contained in neurons of the raphé nuclei, at least in the cat, is to prevent PGO-spikes, possibly by a direct inhibitory action on generator cells for PGO-spikes. The regularity with which PGO-spikes are obtained in the cat after reduction of brain 5-HT either by reserpine-like agent, or by PCPA, and the ease with which 5-HTP (Jalfre *et al.*, 1970) and inhibitors of 5-HT uptake (Jalfre *et al.*, 1971) can abolish the drug-induced PGO-spikes make them a useful method for studying central 5-HT agonists and antagonists. Methiothepin produced all the effects anticipated for a central 5-HT antagonist: a) increase of the number of PGO-spikes induced by Ro 4-1284 by blocking the effect of the reduced amounts of 5-HT still present and released at nerve endings, b) induction of PGO-spikes by itself without previous treatment with 5-HT depletors, c) prevention of the inhibitory effect of 5-HTP and LSD on Ro 4-1284- and PCPA-induced PGO-spikes, respectively.

Biochemical investigations support the proposed 5-HT receptor-blocking effect of methiothepin by showing that the turnover of central 5-HT as measured by two different methods was increased. A study of the action of methiothepin on the depressant effects of microelectrophoretically administered 5-HT on single cells in the LGB (Tebēcis, 1972) is in full agreement with our results. Interestingly enough, methiothepin has 5-HT antagonistic actions in smooth muscle preparations, but not on autonomic ganglia (unpublished results).

Methiothepin is not a selective antagonist of central 5-HT receptors, since it blocks also central DA receptors as indicated by an increase of DA turnover (unpublished results); nevertheless, its marked effect on 5-HT receptors might be helpful in elucidating the rôle of central 5-HT neurons.

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