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Paradoxical Decrease of Brain 5-HT Turnover by Metergoline, a Central 5-HT Receptor Blocker

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Summary. Since metergoline (1-methyl-8-beta-carbobenzyloxy-aminomethyl-10-alpha-ergoline) is a potent 5-HT antagonist in peripheral organs, its possible blocking effects on 5-HT receptors in the rat brain were investigated. In vitro, metergoline inhibited both the specific high affinity binding of ^{3}H-5-HT onto synaptosomal membranes (IC 50 = 18 nM) and the stimulating effect of 10 μM 5-HT on the adenylate cyclase activity in colliculi homogenates from newborn rats (IC 50 = 12 μM). In vivo, the administration of metergoline (10 mg/kg i.p., 60 min before death) resulted in a significant decrease in the ^{3}H-5-HT binding capacity of synaptosomal membranes from the forebrain of adult rats. Taken together, these data clearly indicated that metergoline is a potent blocker of some serotoninergic receptors in the rat brain. Surprisingly, the changes in 5-HT turnover occurring in the brainstem and in the forebrain 1 h after metergoline (2–10 mg/kg) treatment were similar to those normally induced by a central 5-HT agonist: both the rate of 5-HT utilisation and that of 5-HT synthesis were significantly decreased. These changes were in contrast to the acceleration of 5-HT turnover induced by the administration of another potent central 5-HT antagonist, methiothepin. These results are discussed in relation to the possible existence of several types of serotoninergic receptors in the rat brain. It is possible that the positive feedback regulation of 5-HT turnover is triggered by the blockade of serotoninergic receptors sensitive to methiothepin, but not to metergoline.

Key words: Receptors – Serotonin – 5-HT-Sensitive adenylate cyclase – 5-HT High affinity binding – 5-HT Turnover.

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Introduction

Among the drugs blocking the effects of serotonin (5-HT) on peripheral organs, methiothepin is the first compound which has been shown to act as a direct 5-HT antagonist in the central nervous system. Indeed, on the basis of electrophysiological (Tebećis, 1972; Ruch-Monachon et al., 1976; Dray and Straughan, 1976), biochemical (Monachon et al., 1972; Jacoby et al., 1975; Bourgoin et al., 1977a; Enjalbert et al., 1978b) and behavioural (Andén, 1974) experiments, most authors conclude that methiothepin is a potent blocker of central 5-HT receptors. However, further studies revealed that this drug also blocks the dopaminergic and the noradrenergic receptors in the brain (Lloyd and Bartholini, 1974). Enjalbert et al. (1978b) even observed that methiothepin is much more active as a dopamine (DA) antagonist than as a 5-HT antagonist in the rat brain. Therefore, methiothepin cannot be used to specifically block 5-HT receptors in the brain. In contrast, recent behavioural (Fuxe et al., 1975) and electrophysiological (Sastry and Phillis, 1977) studies suggested that metergoline, a well-known 5-HT antagonist in the periphery (Beretta et al., 1965), exerts specific anti-5-HT effects in the rat brain.

In the present paper, the effects of metergoline and methiothepin on the 5-HT-sensitive adenylate cyclase in homogenates of colliculi from newborn rats (Von Hungen et al., 1975; Enjalbert et al., 1978a) and on the specific high affinity binding of ^{3}H-5-HT onto synaptosomal membranes (Fillion et al., 1976; Bennett and Snyder, 1976) were first determined to define and compare the possible direct action of these two drugs on receptor sites for 5-HT in the rat brain. Since previous studies (Monachon et al., 1972; Bourgoin et al., 1977a) revealed that methiothepin accelerates the turnover of 5-HT in the brain, the effects of metergoline treatment on the rates of 5-HT synthesis and utilisation were also investigated.

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Although the present biochemical data confirm that metergoline is a direct 5-HT antagonist in the rat brain, they also suggest that some of the receptor sites which are blocked by methiothepin are not affected in a similar way by metergoline.

Materials and Methods

The following drugs were used: L- and D-LSD (isomers of lysergic acid diethylamide, Sandoz), methiothepin (Hoffmann-La Roche), benserazid (Ro 4-4602, Hoffmann-La Roche), metergoline (1-methyl-8-beta-carbobenzyloxy-aminomethyl-10-alpha-ergoline, Liserdol, Farmitalia), and pargyline (Abbott). L-5(n)-^{3}H-tryptophan (27.5–29 Ci/mmol Radiochemical Centre, Amersham) and G-^{3}H-5-HT (generally labelled, 10 Ci/mmol Radiochemical Centre, Amersham) were purified by ion-exchange chromatography just before the experiments (in vitro synthesis and release with brain slices, ^{3}H-5-HT binding studies) as described earlier (Hamon et al., 1974). α-^{32}P-ATP (10 Ci/mmol) and ^{3}H-cyclic AMP (25 Ci/mmol) were purchased from New England Nuclear.

In Vivo Experiments

Adult male Sprague-Dawley (Charles River strain) rats weighing 250–300 g were kept for at least 10 days in a controlled environment (24° C, 60% relative humidity, alternate cycles of 12 h light and 12 h darkness, food and water freely available) before any pharmacological treatment. Drugs were administered i.p. either suspended (methiothepin) or solubilized (D-LSD, benserazid) in saline or in 5% ascorbic acid (metergoline). The doses (refering to the bases) and the time of treatments are described in the Results section. Control animals received the same volume of solvent with time schedules similar to those used in treated rats. The body temperature was measured at various times after the i.p. injection of solvents or drugs using a mercury thermometer inserted 4 cm inside the rectum.

In all cases, animals were killed by decapitation and their brains were rapidly removed in the cold (4° C). The brain was generally divided into 2 parts by a coronal midcollicular section. The brainstem (minus the cerebellum) and the left forebrain (sagittal section) were saved for biochemical determinations. Endogenous 5-HT, tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) were selectively extracted by ion-exchange and adsorption chromatography (see Hamon et al., 1973) and estimated separately by specific spectrofluorimetric methods (Curzon and Green, 1970; Denckla and Dewey, 1967). A Dowex resin (AG50 WX4) was used for the isolation of 5-hydroxytryptophan (see Hamon et al., 1976) which was finally measured as described by Tachiki and Aprison (1975).

In Vitro Experiments

^{3}H-5-HT Synthesis and Release Using Brain Stem Slices. Immediately after dissection in the cold (4° C), the brain stem was cut into 0.3 mm thick slices using a McIlwain tissue chopper. Tissues were suspended in 5 ml of a Krebs-Henseleit medium (Hamon et al., 1973) equilibrated at pH 7.35 (at 37° C) with a constant atmospheric mixture of O_2 : CO_2 (95 : 5). Samples were preincubated for 5 min at 37° C in a shaking water bath. ^{3}H-tryptophan (19.6–27.5 µCi in 50 µl) was then added and the incubation proceeded for 30 min thereafter. At the end of the experiment, tissues and medium were rapidly separated by filtering samples through Whatman paper no. 3 under slight vacuum. The medium was collected in ice-cold glass tubes containing 50 µl of 5% ascorbic acid and 0.1 ml of 2% EDTA. Tissues were immediately homogenized in 6 ml of an ethanol : water solution (74 : 16, v/v) containing 0.05% ascorbic acid and EDTA. Labelled and cold tryptophan, ^{3}H-5-HT and ^{3}H-5-HIAA were than selectively extracted from tissues and medium by ion-exchange and adsorption chromatography as previously described (Hamon et al., 1973).

When incubations were performed in the presence of drugs, they were added at the beginning of the preincubation period. In order to keep the osmolarity of the incubating medium constant, the concentration of NaCl was reduced from 136 to 108 mM when that of KCl was raised from 5.6 to 33.6 mM.

^{3}H-5-HT Binding to Crude Synaptosomal Membranes. The preparation of crude synaptosomal membranes was achieved at 4–6° C except when indicated. Freshly dissected forebrains were homogenized in 10 vol. (v/w) of 0.32 M sucrose using a Potter-Elvejehm apparatus fitted with a Teflon pestle. Homogenates were centrifuged at 1000 × g for 10 min. The supernatant was then centrifuged at 10000 × g for 30 min to yield the crude synaptosomal fraction, P_2. The P_2 pellet was then suspended and gently stirred in 10 vol of ice-cold water for 30 min. Membranes from this lysed synaptosomal fraction were collected by centrifugation at 35000 × g for 20 min at 4° C. The sedimented material was suspended in 30 vol of 0.05 M Tris-HCl buffer (pH 7.4) and incubated for 10 min at 37° C in a shaking water bath. During this step, endogenous 5-HT still contaminating the lysed P_2 fraction was enzymatically destroyed. Omitting this step resulted in a 50% decrease in the specific ^{3}H-5-HT binding capacity of synaptosomal membranes (unpublished observations). The incubated membranes were collected by centrifugation (35000 × g, 20 min), washed in 40 vol of 0.05 M Tris-HCl (pH 7.4) and finally spun down at 35000 × g for 20 min. The sedimented material, suspended in 60 vol of 0.05 M Tris-HCl buffer (pH 7.4) containing 0.1% ascorbic acid, 10 µM pargyline and 4 mM $CaCl_2$ (Bennett and Snyder, 1976) was the final preparation of synaptosomal membranes used in the binding studies. For this purpose, 2 ml aliquots of this preparation were incubated with 2.2–3.5 nM of ^{3}H-5-HT in the absence or the presence of drugs, for 7 min at 37° C in a shaking water bath. In agreement with Bennett and Snyder (1976), we observed that the amount of ^{3}H-5-HT bound to membranes was maximum at the end of such an incubation period. The binding assay was stopped by filtering the samples through GF/B Whatman filters. The incubation tubes and filter wells were rapidly rinsed with two 5 ml aliquots of ice cold Tris buffer. Each filter well was then rinsed with an additional 5 ml of this buffer. Finally, filters were dried and immediately immersed in 10 ml of Scintix® (Isotec) scintillating fluid in counting vials. A vigorous shaking of the vials resulted in an homogenous gel-like mixture immediately suitable for radioactivity counting. Blanks were made by incubating samples with an excess (10 µM) of cold 5-HT. The specific binding of ^{3}H-5-HT was defined as the difference between the radioactivity bound to the filter in the absence of cold 5-HT minus that bound in blank samples (containing 10 µM 5-HT). Control and blank samples were run in quadriplicate for each concentration of ^{3}H-5-HT or added drug.

5-HT-Sensitive Adenylate Cyclase. The enzyme source was an isotonic homogenate of colliculi from 24 h-old rats (see Hamon et al., 1976; Enjalbert et al., 1978a, b). The assay mixture (40 µl) contained 25 mM Tris-maleate (pH 7.2), 0.5 mM ATP, 1 mM $MgSO_4$, 0.5 mM EGTA [ethyleneglycol-bis-(β-aminoethyl ether) N,N′-tetraacetic acid, Sigma], 0.2 mg/ml creatine kinase, 20 mM creatine phosphate, 10 mM theophylline, 2 µCi (α-^{32}P)-ATP and 1 nCi ^{3}H-cyclic AMP. The reaction was initiated by adding 10 µl of homogenate and was allowed to proceed for 5 min at 37° C. It was stopped by the addition of 100 µl of a solution containing 5 mM ATP, 5 mM cyclic AMP, 50 mM Tris-HCl (pH 7.4) and 1% sodium lauryl sulfate. The ^{32}P-cyclic AMP formed and the ^{3}H-cyclic AMP added as a recovery marker were finally isolated according to Salomon et al. (1974). The conversion of α-^{32}P-ATP into ^{32}P-cyclic AMP in the presence of a supramaximal concentration (10 µM) of 5-HT was used as an index of the activity of 5-HT-sensitive adenylate cyclase. With such a

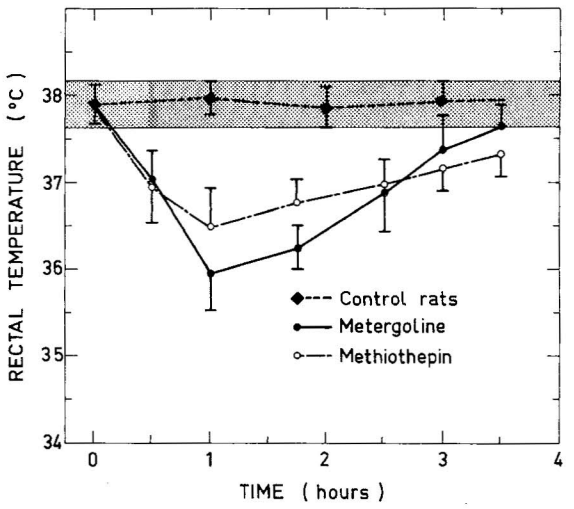

Fig. 1. Effects of metergoline or methiothepin treatment on the rectal temperature of rats. Rats were kept in groups of 6 per cage at an ambient temperature of 25° C. Rectal temperature was measured at various times after the i.p. injection of saline or 5% ascorbic acid (control rats), metergoline (10 mg/kg) or methiothepin (20 mg/kg). Each point is the mean ± S.E.M. of 6 determinations

concentration of 5-HT, the enzymic activity was doubled when compared to the basal adenylate cyclase activity (Enjalbert et al., 1978a). For each concentration of any tested drug, 5-HT-sensitive adenylate cyclase assays were run in triplicate.

Proteins were estimated with bovine serum albumin as the standard (Lowry et al., 1951). Statistical calculations were done as described by Snedecor and Cochran (1967). When a p value was higher than 0.05 (Student's t test), the difference was considered to be non-significant.

Results

A. Effects of Metergoline or Methiothepin Treatment on Behaviour and Body Temperature in the Adult Rat

Whereas a long lasting (for at least 24 h) catalepsy rapidly developed in rats after the i.p. injection of methiothepin (20 mg/kg), only minor and short lasting behavioural alterations were noted in rats treated with metergoline (2–10 mg/kg i.p.). Difficulties in locomotor behaviour were observed during the first hour after the administration of metergoline at a dose as low as 0.2 mg/kg; the most obvious sign was that the hind limbs remained flaccid and abducted. When methiothepin (20 mg/kg i.p.) or metergoline (10 mg/kg i.p.) was injected 10 min before LSD (1 mg/kg i.p.), the "typical LSD behavioural reaction" (Trulson et al., 1976) was largely prevented. In particular, animals were markedly less hyper-responsive to noise and puff stimuli. In rats receiving the combined administration of metergoline or methiothepin and LSD, the fur remained dry in contrast to that of rats treated with LSD alone.

When control animals were kept in groups of 6 per cage at an ambient temperature of 25° C, their rectal temperature remained constant for several hours and was not affected by saline or 5% ascorbic acid injections (Fig. 1). The i.p. injection of metergoline (10 mg/kg) or methiothepin (20 mg/kg) resulted in a pronounced decrease in the body temperature of rats (Fig. 1). The maximum decrease (1.5–2° C, $P < 0.05$) was observed 1 h after the injection of each drug, and the rectal temperature had returned to normal 210 min after drug administration. When metergoline (10 mg/kg) and methiothepin (20 mg/kg, 10 min after) were injected into the same animal, the resulting hypothermia was no more pronounced than that occurring after the administration of metergoline alone (data not shown).

B. Effects of Methiothepin and Metergoline on 5-HT Receptors in the Rat Brain

a) 5-HT-Sensitive Adenylate Cyclase

When metergoline or methiothepin was added to the assay mixture of adenylate cyclase, neither drug altered the basal activity provided that its concentration was lower than 50 μM. Higher concentrations of these compounds were found to induce significant increases in the basal activity of this enzyme (+40% with 0.1 mM metergoline, +230% with 0.1 mM methiothepin) but these effects were unrelated to the presence or the absence of 5-HT-sensitive adenylate cyclase in the assay mixture (see Enjalbert et al., 1978b). In particular, using dissected tissues from new born rats, the stimulating effect of methiothepin on adenylate cyclase activity was observed in homogenates of cerebellum (which contained a very low 5-HT-sensitive adenylate cyclase activity, Enjalbert et al., 1978a) but not in homogenates of striatum or cerebral cortex (although these two areas contained the 5-HT-sensitive enzyme, Enjalbert et al., 1978a).

As shown in Figure 2A, methiothepin and metergoline, in concentrations which did not alter the basal adenylate cyclase activity (< 50 μM), reduced significantly the stimulatory effect of 5-HT (10 μM) in colliculi homogenates. In this preparation, methiothepin (IC 50 = 2.9 μM) was more potent than metergoline (IC 50 = 12 μM) in inhibiting 5-HT-sensitive adenylate cyclase (Fig. 2A).

b) ^{3}H-5-HT Binding onto Synaptosomal Membranes

In agreement with Fillion et al. (1976), ^{3}H-5-HT was found to bind with a high affinity (Kd = 1.5 nM) onto specific sites of synaptosomal membranes from the forebrain of adult rats. In control samples, the radioac-

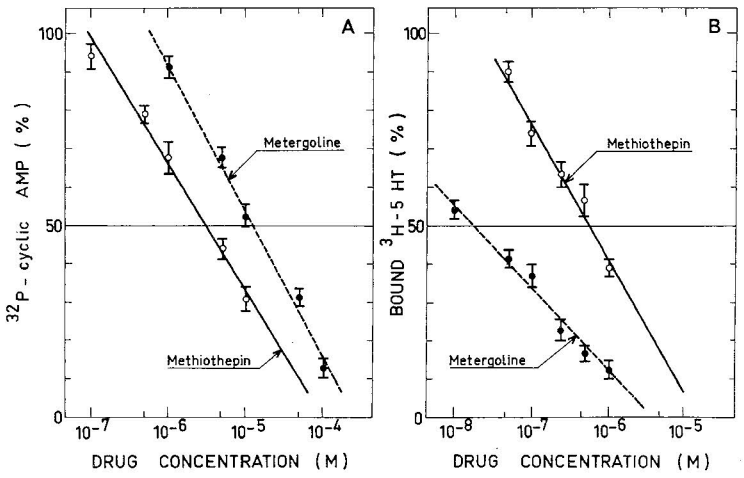

Fig. 2
Effects of metergoline and methiothepin on the 5-HT-sensitive adenylate cyclase (A) and on the specific high affinity binding of ^{3}H-5-HT onto synaptosomal membranes (B). (A) 5-HT-sensitive adenylate cyclase activity was measured in colliculi homogenates from new born rats in the presence of 10 μM 5-HT and various concentrations of metergoline or methiothepin. In the absence of drug, 5-HT sensitive adenylate cyclase catalysed the formation of 10.05 pmoles ^{32}P-cyclic AMP per min and per mg prot. (100 %). Each point is the mean ± S.E.M. of triplicate determinations. (B) The specific high affinity binding of ^{3}H-5-HT onto synaptosomal membranes from the forebrain of adult rats was measured in the presence of 2.2 nM of the labeled amine and various concentrations of metergoline or methiothepin. In the absence of drug, 1 mg prot. of synaptosomal membranes specifically bound 38.9 femtomoles ^{3}H-5-HT (100 %). Each point is the mean ± S.E.M. of quadriplicate determinations

Table 1. *Effects of in vivo administration of metergoline (10 mg/kg i.p.) or methiothepin (20 mg/kg i.p.) on the ^{3}H-5-HT binding capacity of synaptosomal membranes from the rat forebrain.* Animals were killed 1 h or 20 h after the i.p. injection of solvent (saline or 5 % ascorbic acid, control rats), metergoline or methiothepin. Synaptosomal membranes from the forebrain were prepared as described in Methods and assayed for ^{3}H-5-HT binding using 3.5 nM of the labelled amine. Each value is the mean ± S.E.M. of 4 determinations (corresponding to 4 rats in each group); * $P < 0.05$ when compared to respective control values

	Bound ^{3}H-5-HT (femtomol/mg prot.)	
	1 h	20 h
Control	57.7 ± 3.7	63.7 ± 2.0
Metergoline	30.9 ± 1.9*	67.3 ± 4.8
Methiothepin	31.6 ± 1.4*	49.8 ± 1.6*

tivity corresponding to ^{3}H-5-HT specifically bound (according to that defined in "Methods") was about twice that occurring in the presence of 10 μM 5-HT (blank samples). In forebrain preparations, the maximum capacity of the high affinity binding sites was equal to 90.5 ± 12.6 femtomoles of ^{3}H-5-HT bound per mg protein (mean ± S.E.M. of 4 independent determinations). As shown in Figure 2B, both methiothepin and metergoline induced a concentration-dependent reduction in the amount of ^{3}H-5-HT specifically bound to membranes. However, in contrast to that observed with 5-HT-sensitive adenylate cyclase assays, metergoline was more potent than methiothepin in inhibiting ^{3}H-5-HT binding: the concentration of drug inducing a 50 % decrease in ^{3}H-5-HT binding was equal to 18 nM for metergoline, a value about 30 times lower than that required with methiothepin (IC 50 = 0.56 μM, Fig. 2B). Neither metergoline nor methiothepin inhibited the binding of ^{3}H-5-HT which persisted in the presence of 10 μM 5-HT (non-specific binding).

When forebrain membranes were prepared from rats pretreated with 10 mg/kg of metergoline or 20 mg/kg of methiothepin 1 h before sacrifice, their ability to bind ^{3}H-5-HT was significantly lower than that of a similar preparation from control animals (Table 1). When metergoline was administered 20 h before death, this effect was no longer detected. In contrast, a significant reduction in ^{3}H-5-HT binding capacity was still observed in forebrain membranes prepared from rats treated with methiothepin (20 mg/kg) 20 h before sacrifice, but this effect was less pronounced than that occurring one hour after the drug's injection (Table 1).

C. In Vivo Effects of Metergoline on 5-HT Metabolism in the Brain

a) Effects of Metergoline Treatment on the Endogenous Levels of Tryptophan, 5-HT, and 5-HIAA in the Brainstem and Forebrain of Rats

Whereas methiothepin treatment was found to increase tryptophan levels in the brain (Monachon et al., 1972; Jacoby et al., 1975; Bourgoin et al., 1977a), no significant change in tryptophan levels was detected 30 min, 1 h, 2 h or 3 h after the i.p. administration of 5 mg/kg of metergoline. The injection of various doses of metergoline (0.2, 0.5, 2 or 10 mg/kg i.p.), 1 h before death, also failed to alter the concentration of tryptophan in the rat brain. In contrast, metergoline induced significant changes in the levels of 5-hydroxyindoles. Since the amplitude of these alterations was maximum 1 h after the i.p. injection of metergoline (data not shown), the effects of various doses of this

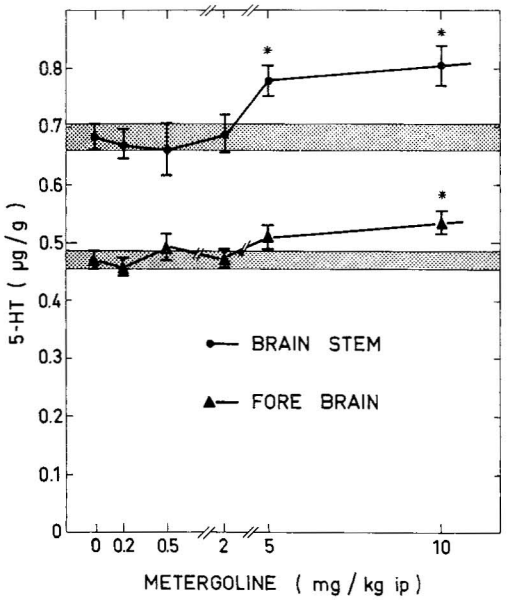

Fig. 3. Effects of metergoline treatment of 5-HT levels in the brain stem and the forebrain of rats. Animals were killed 1 h after the i.p. administration of various doses of metergoline. Control rats (dose equal to 0) received an i.p. injection of 5% ascorbic acid (1 ml) 1 h before death. Each point is the mean ± S.E.M. of values determined in groups of 8 rats. * $P < 0.05$ when compared to respective control values

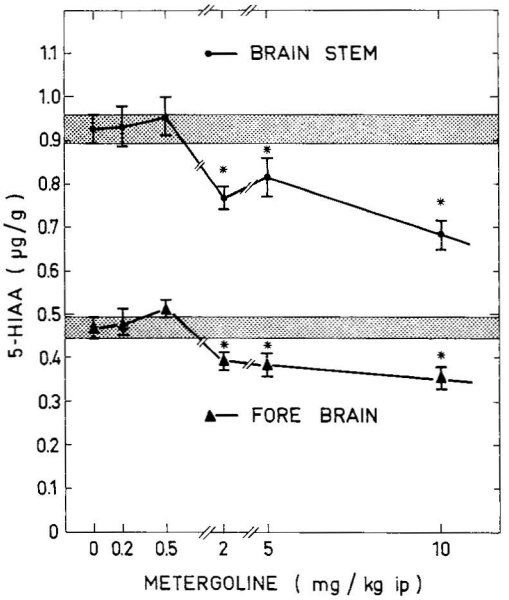

Fig. 4. Effects of metergoline treatment on 5-HIAA levels in the brain stem and the forebrain of rats. The protocole was that described in the legend to Figure 3. Each point is the mean ± S.E.M. of 8 independent determinations. * $P < 0.05$ when compared to respective control valucs

drug were analyzed at this time. As shown in Figure 3, a significant increase in 5-HT levels was noted in the brain stem as well as in the forebrain with a dose of metergoline equal to 10 mg/kg i.p. Conversely, the levels of 5-HIAA were significantly reduced following the administration of 2 – 10 mg/kg of this drug (Fig. 4). The changes in 5-hydroxyindole levels induced by metergoline treatment were exactly the reverse to those observed after the administration of methiothepin (Bourgoin et al., 1977a).

b) Effects of Metergoline Treatment on the Changes in 5-HIAA/5-HT Ratio Induced in the Brain Stem and in the Forebrain by the Administration of Methiothepin or D-LSD

As a result of both a slight decrease in 5-HT levels (10 – 13%) and a large increase in 5-HIAA levels (31 – 52%) in the two brain areas, the ratio of 5-HIAA to 5-HT levels in tissues of rats treated with methiothepin (20 mg/kg i.p., 70 min before death) was significantly higher than that found in control animals (Fig. 5). Conversely, this ratio was significantly decreased in tissues of rats treated with D-LSD (1 mg/kg i.p., 70 min before sacrifice, Fig. 5). As shown in Figure 5, the administration of metergoline (10 mg/kg i.p., 60 min before death) also resulted in a significant reduction in the 5-HIAA/5-HT ratio in the brain stem and in the forebrain; indeed, the values calculated for metergoline-treated rats were not significantly different from those found in D-LSD-treated animals. When metergoline (10 mg/kg) was administered 10 min after methiothepin, the changes in the 5-HIAA/5-HT ratio normally induced by the latter drug were completely prevented (Fig. 5). In contrast, the effects of D-LSD treatment on the 5-HIAA/5-HT ratio in the brain stem and in the forebrain were not significantly altered by the administration of metergoline 10 min after the i.p. injection of the hallucinogenic drug (Fig. 5). Thus, metergoline differed from methiothepin since we previously observed that the latter drug was able to block, at least partially, the decrease in the 5-HIAA/5-HT ratio evoked by D-LSD treatment (Bourgoin et al., 1977a).

c) Effects of Metergoline Treatment on the In Vivo Synthesis of 5-HT in the Rat Brain

The rate of 5-HT synthesis was estimated as proposed by Carlsson et al. (1972) by measuring the accumulation of 5-HTP in tissues after the blockade of 5-HTP decarboxylase by benserazid (800 mg/kg i.p.). In contrast to methiothepin (Bourgoin et al., 1977a), metergoline caused a reduction in the rate of 5-HTP accumu-

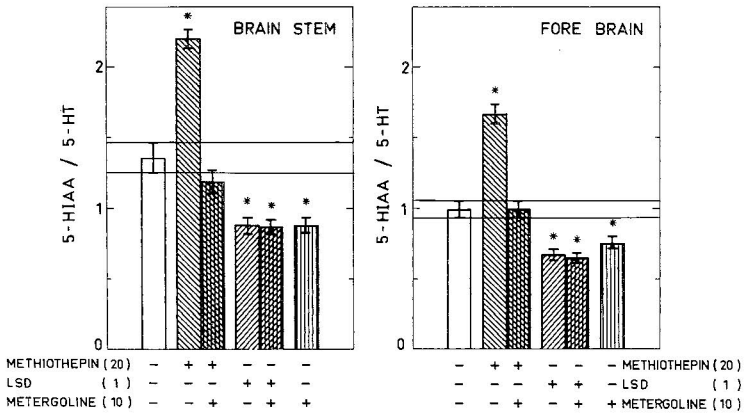

Fig. 5
Effects of metergoline and/or LSD or methiothepin treatments on the 5-HIAA/5-HT ratio in the brain stem and the forebrain of rats. Methiothepin (20 mg/kg) or D-LSD (1 mg/kg) was injected i.p. 70 min before death. Metergoline (10 mg/kg i.p.) was administered 60 min before death. Treatments were performed as indicated under the figure. Each bar represents the mean ± S.E.M. of the ratio of 5-HIAA to 5-HT levels (in μg/g) calculated in 8 rats. * $P < 0.05$ when compared to respective control values (open bars)

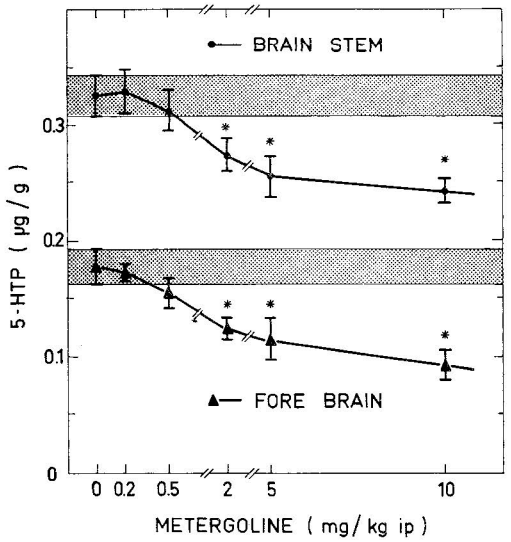

Fig. 6. Effects of metergoline treatment on the rate of 5-HTP accumulation in the brain stem and the forebrain of rats after the blockade of 5-HTP decarboxylase by benserazid. Various doses of metergoline were administered to groups of 16 rats. One hour later, 8 animals in each group were treated with benserazid (800 mg/kg i.p.) while saline was injected to others. All rats were killed 30 min after this second injection. The net accumulation of 5-HTP induced by the blockade of 5-HTP decarboxylase by benserazid was then measured in the brain stem and the forebrain. Each point is the mean ± S.E.M. of 5-HTP (in μg/g) accumulated in tissues for 30 min. * $P < 0.05$ when compared to respective values in rats treated with 5% ascorbic acid (0 on the abscissa) instead of metergoline

lation in the brain stem as well as in the forebrain (Fig. 6). A significant effect was noted with a dose of metergoline as low as 2 mg/kg i.p. For a given dose, and particularly with the highest dose used (10 mg/kg i.p.), the reduction was generally more pronounced in the forebrain (−48%) than in the brain stem (−25%, Fig. 6).

Table 2. *Effects of metergoline (10 μM) on the synthesis of ^{3}H-5-hydroxyindoles from ^{3}H-tryptophan in brain stem slices.* Brain stem slices were incubated for 30 min at 37° C in the presence of 19.6 μCi of ^{3}H-tryptophan per 5 ml of medium (corresponding to a concentration of 0.14 μM). ^{3}H-TRP is the amount of ^{3}H-tryptophan (in μCi) accumulated per g of tissue at the end of the incubation period. ^{3}H-5-HT and ^{3}H-5-HIAA correspond to the total quantities of newly synthesized ^{3}H-5-hydroxyindoles found in tissues + medium at the end of the experiment. CI, the conversion index, is the ratio of the total amount of newly synthesized ^{3}H-5-hydroxyindoles (in μCi) over the specific activity of ^{3}H-tryptophan (in μCi/nmol) in tissues. Each value is the mean ± S.E.M. of 7 determinations

	Control	Metergoline
^{3}H-TRP (μCi/g)	12.9 ± 1.0	12.5 ± 0.5
^{3}H-5-HT (μCi/g)	0.66 ± 0.10	0.65 ± 0.05
^{3}H-5-HIAA (μCi/g)	0.25 ± 0.02	0.27 ± 0.01
CI (nmol./g)	2.1 ± 0.2	2.4 ± 0.1

D. In Vitro Effects of Metergoline on 5-HT Metabolism in Brain Stem Slices

a) Effects of Metergoline on the Synthesis of ^{3}H-5-Hydroxyindoles from ^{3}H-Tryptophan

Metergoline (5 − 10 μM) altered neither the uptake of ^{3}H-tryptophan in tissues nor the synthesis of ^{3}H-5-HT and ^{3}H-5-HIAA in brain stem slices incubated for 30 min at 37° C in normal Krebs-Henseleit medium (Table 2). In contrast, methiothepin (10 − 20 μM) has been found to double the synthesis of ^{3}H-5-HIAA from ^{3}H-tryptophan under similar conditions (Bourgoin et al., 1977a).

b) Effects of Metergoline on the K^+-Induced Release of Newly Synthesized ^{3}H-5-HT

Brain stem slices were incubated for 30 min with ^{3}H-tryptophan under various experimental conditions. At

Table 3. *Effects of LSD and/or metergoline on the K^+-induced release of newly synthesized ^3H-5-HT from brain stem slices.* Brain stem slices were incubated for 30 min in the presence of 27.5 μCi of ^3H-tryptophan (corresponding to a concentration of 0.19 μM), 5.6 mM or 33.6 mM K^+, and L-LSD, D-LSD or/and metergoline as indicated in the table. Newly synthesized ^3H-5-HT found in tissues and in medium was then determined as described in Methods. Each value is the mean ± S.E.M. of 7 determinations of the ratio of ^3H-5-HT accumulated in the medium to that found in tissues. Δ (%) corresponds to the relative increase in this M/T ratio induced by various conditions when compared to that occurring in the presence of 33.6 mM K^+ alone (100 corresponding to 164.0 − 63.8 = 100.2). * $P < 0.05$ when compared to the value obtained with samples incubated with 33.6 mM K^+ in the absence of drug

Conditions	$[(^3H\text{-}5\text{-}HT)_M/(^3H\text{-}5\text{-}HT)_T \times 10^3$	Δ (%)
5.6 mM K^+	63.8 ± 7.3	
33.6 mM K^+	164.0 ± 8.1	100
33.6 mM K^+ + 10 μM L-LSD	163.0 ± 14.8	99
33.6 mM K^+ + 10 μM D-LSD	119.2 ± 9.4*	55
33.6 mM K^+ + 10 μM D-LSD + 0.2 μM metergoline	123.4 ± 8.0*	59
33.6 mM K^+ + 10 μM D-LSD + 10 μM metergoline	146.2 ± 10.6	82
33.6 mM K^+ + 10 μM metergoline	177.1 ± 13.8	113

the end of this period, the ratio of ^3H-5-HT found in the medium to that accumulated in tissues was considered to be an index of the release of newly synthesized ^3H-5-HT. As shown in Table 3, raising the concentration of K^+ from 5.6 to 33.6 mM in the incubating medium resulted in a marked increase (+157%) in this ratio. The K^+-induced change in ^3H-5-HT release was unaffected by the addition of 10 μM L-LSD or 10 μM metergoline to the incubating medium. In contrast, D-LSD (10 μM) partially inhibited the K^+-evoked release of newly synthesized ^3H-5-HT (Table 3). As already observed with methiothepin (Bourgoin, 1976), metergoline (10 μM but not 0.2 μM) was able to prevent the inhibitory effect of D-LSD (Table 3).

Discussion

Two direct biochemical methods are available to define the mechanism of action of a given drug on 5-HT receptors in the brain. They consist of measuring the specific high affinity binding of ^3H-5-HT onto synaptosomal membranes (Fillion et al., 1976; Bennett and Snyder, 1976) and the 5-HT-sensitive adenylate cyclase activity (Enjalbert et al., 1978 b) in the presence of this drug. Since these two markers of 5-HT receptors are not affected by the degeneration of serotoninergic neurons (Bennett and Snyder, 1976; Bourgoin et al., 1977b), they can be considered as specific for receptors located on the postsynaptic site. Using these two methods, we observed-first, that metergoline inhibited the high affinity binding of ^3H-5-HT onto synaptosomal membranes indicating a direct interference with the 5-HT receptor sites and-second, that this drug reducing the stimulatory effect of 5-HT on the adenylate cyclase activity in homogenates of colliculi from newborn rats acted as a central 5-HT antagonist. This conclusion is in complete agreement with previous behavioural (Clineschmidt and Lotti, 1974; Fuxe et al., 1975) and some electrophysiological (Sastry and Phillis, 1977) findings. In concentrations higher than 50 μM, metergoline (as well as methiothepin) stimulated the basal adenylate cyclase activity in homogenates of various brain areas from newborn rats (Enjalbert et al., 1978b). However, in contrast to the stimulatory effect of D-LSD (Von Hungen et al., 1975; Enjalbert et al., 1978b), that induced by metergoline did not result from the drug interaction with 5-HT receptors (Enjalbert et al., 1978b). Accordingly, metergoline must be considered as a pure 5-HT antagonist in the CNS, at least in rats.

The comparison of the effects induced by metergoline and methiothepin revealed striking differences. Whereas methiothepin was more potent than metergoline in inhibiting 5-HT sensitive adenylate cyclase, the reverse was true for the inhibition of ^3H-5-HT binding onto specific sites. As previously discussed (Enjalbert et al., 1978b), this suggests that the receptor coupled with an adenylate cyclase is not identical to the one characterized by the high affinity binding of ^3H-5-HT. This might also explain the 650-fold difference in potency of metergoline in inhibiting the ^3H-5-HT-high affinity binding (IC 50 = 18 nM) and the 5-HT-sensitive adenylate cyclase (IC 50 = 12 μM).

The in vitro release of newly synthesized or previously taken up ^3H-5-HT induced by depolarization with K^+ or electrical field stimulation seems to be under the control of presynaptic 5-HT receptors (Farnebo and Hamberger, 1974; Hamon et al., 1974; Bourgoin et al., 1977a). In agreement with this hypothesis, we observed that D-LSD, but not the inactive isomer, L-LSD, was able to reduce the K^+-induced release of newly synthesized ^3H-5-HT from brain stem slices. In contrast to methiothepin (Farnebo and Hamberger, 1974; Bourgoin et al., 1977a), metergoline did not increase the transmitter release elicited by depolarization. However, it was able to prevent the negative effect induced by the stimulation of presynaptic 5-HT receptors by D-LSD. These data suggest that metergoline may also have some antagonistic effect on presynaptic 5-HT receptors.

Previous in vivo studies have shown that the blockade of central 5-HT receptors by methiothepin accelerates the turnover of 5-HT in the rat brain (Monachon et al., 1972; Jacoby et al., 1975; Bourgoin

et al., 1977a). Surprisingly, the administration of metergoline induced an opposite effect, i.e., a decrease in the rate of 5-HT turnover. Therefore, this effect of metergoline was similar to that observed with 5-HT agonists, like D-LSD (Andén et al., 1968; Hamon et al., 1974), hallucinogenic phenylethylamines (Andén et al., 1974) and quipazine (Hamon et al., 1976). In addition, as already observed with D-LSD (Bourgoin et al., 1977a), metergoline administration was able to completely prevent the increase in 5-HT utilization resulting from methiothepin treatment. These paradoxical "5-HT agonist" properties of metergoline recall those already reported for some other putative 5-HT antagonists. Kehr (1977) mentioned that the administration of methysergide and lisuride, two well-known blockers of serotoninergic receptors in the periphery, also induced a significant decrease in the turnover rate of 5-HT in the rat brain. These data indirectly suggest that some peripheral 5-HT antagonists might in fact stimulate some of the 5-HT receptors in the brain. Indeed, Haigler and Aghajanian (1974) reported that these peripheral antagonists (including metergoline) were able partly to mimic the effects of 5-HT when applied directly to neurons located in the lateral geniculate nucleus, the optic tectum and the amygdala. Similar findings were reported by Segal (1976) and Sharma (1977) who analyzed the effects of microiontophoretic application of 5-HT and peripheral antagonists on hippocampal and cortical neurons. However, as discussed by these authors (Haigler and Aghajanian, 1974; Segal, 1976; Sharma, 1977), the depressant effect of most 5-HT antagonists on neuronal firing may be related to their local anaesthetic action. Another difficulty in analyzing the effects of a given drug when applied locally to neurons is that the resulting change in nerve impulse flow largely depends on the baseline firing rate of these cells; according to Szabadi et al. (1977), an excitatory drug is converted into an inhibitory compound when the neuronal firing increases. This could explain why some authors (Sastry and Phillis, 1977) concluded that metergoline exerts anti-5-HT effects (i.e. acceleration of neuronal firing) on cortical neurons, whereas others (Haigler and Aghajanian, 1974) mentioned that it depresses nerve impulse flow, as 5-HT does, in neurons located in other brain areas. In contrast to these inconclusive data, those obtained on the basis of behavioural (Clineschmidt and Lotti, 1974; Fuxe et al., 1975) and ^{3}H-5-HT binding and 5-HT-sensitive adenylate cyclase assays (Enjalbert et al., 1978b; this paper) experiments strongly support the hypothesis that metergoline is a potent 5-HT antagonist in the rat brain. Therefore, the failure of metergoline administration to trigger an acceleration of 5-HT synthesis (like that occurring after methiothepin treatment, Bourgoin et al., 1977a) suggests that this drug does not act on the 5-HT receptors involved in the positive feedback control of 5-HT synthesis seen after methiothepin treatment.

In conclusion, as already demonstrated for the CNS of invertebrates (Gerschenfeld and Paupardin-Tritsch, 1974) and the periphery of mammals (Gaddum and Picarelli, 1957), more than one type of 5-HT receptor may exist in the rat brain. Biochemical evidence already supports the presence of presynaptic (Farnebo and Hamberger, 1974; Hamon et al., 1974) and postsynaptic (Bennett and Snyder, 1976; Bourgoin et al., 1977b) 5-HT receptors. The present data further extend the hypothesis of a multiplicity of 5-HT receptors. They suggest that (at least) two types of postsynaptic 5-HT receptors are present in the rat brain.

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References

Andén, N. E.: Effect of acute axotomy (spinal cord transection) on the turnover of 5-hydroxytryptamine. In: Serotonin-New Vistas. Adv. Biochem. Psychopharmacol. **10**, 35–43 (1974)

Andén, N. E., Corrodi, H., Fuxe, K., Hökfelt, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Br. J. Pharmacol. **34**, 1–7 (1968)

Andén, N. E., Corrodi, H., Fuxe, K., Meek, J. L.: Hallucinogenic phenylethylamines: interactions with serotonin turnover and receptors. Eur. J. Pharmacol. **25**, 176–184 (1974)

Bennett, J. P., Jr., Snyder, S. H.: Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic serotonin receptors. Mol. Pharmacol. **12**, 373–389 (1976)

Beretta, G., Glässer, A. H., Nobili, M. B., Silvestri, R.: Antagonism of 5-hydroxytryptamine-induced bronchospasm in guinea-pigs by 8-B-carbobenzyloxyaminomethyl-1-dimethyl-10-ergoline. J. Pharm. Pharmacol. **17**, 423–428 (1965)

Bourgoin, S.: Evolution du métabolisme central de la sérotonine chez le rat au cours du dévelopement. Thèse de Doctorat es Sciences, pp. 1–303. Université de Paris VII, 1976

Bourgoin, S., Artaud, F., Enjalbert, A., Héry, F., Glowinski, J., Hamon, M.: Acute changes in central 5-HT metabolism induced by the blockade or stimulation of serotoninergic receptors during ontogenesis in the rat. J. Pharmacol. Exp. Ther. **202**, 519–531 (1977a)

Bourgoin, S., Enjalbert, A., Adrien, J., Héry, F., Hamon, M.: Midbrain raphe lesion in the new-born rat: II. Biochemical alterations in serotoninergic innervation. Brain Res. **127**, 111–126 (1977b)

Carlsson, A., Davis, J. N., Kehr, W., Lindqvist, M., Atack, C. V.: Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in vivo in brain, using an inhibitor of the aromatic amino acid decarboxylase. Naunyn-Schmiedeberg's Arch. Pharmacol. **275**, 153–168 (1972)

Clineschmidt, B. V., Lotti, V. J.: Indoleamine antagonists: relative potencies as inhibitors of tryptamine and 5-hydroxytryptophan-evoked responses. Br. J. Pharmacol. **50**, 311–313 (1974)

Curzon, G., Green, A. R.: Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in small regions of rat brain. Br. J. Pharmacol. **39**, 653–655 (1970)

Denckla, W. D., Dewey, H. K.: The determination of tryptophan in plasma, liver and urine. J. Lab. Clin. Med. **69**, 160–169 (1967)

Dray, A., Straughan, D. W.: Synaptic mechanisms in the substantia nigra. J. Pharm. Pharmacol. **28**, 400–405 (1976)

Enjalbert, A., Bourgoin, S., Hamon, M., Adrien, J., Bockaert, J.: Postsynaptic serotonin-sensitive adenylate cyclase in the central nervous system. I. Development and distribution of serotonin- and dopamine-sensitive adenylate cyclases in rat and guinea pig brain. Mol. Pharmacol. **14**, 1–10 (1978a)

Enjalbert, A., Hamon, M., Bourgoin, S., Bockaert, J.: Postsynaptic serotonin-sensitive adenylate cyclase in the central nervous system. II. Comparison with dopamine- and isoproterenol-sensitive adenylate cyclases in rat brain. Mol. Pharmacol. **14**, 11–23 (1978b)

Farnebo, L. O., Hamberger, B.: Regulation of (^{3}H)-5-hydroxytryptamine release from rat brain slices. J. Pharm. Pharmacol. **26**, 642–643 (1974)

Fillion, G., Fillion, M. P., Spirakis, C., Bahers, J. M., Jacob, J.: 5-hydroxytryptamine binding to synaptic membranes from rat brain. Life Sci. **18**, 65–74 (1976)

Fuxe, K., Agnati, L., Everitt, B.: Effects of metergoline on central monoamine neurones. Evidence for a selective blockade of central 5-HT receptors. Neurosci. Lett. **1**, 283–290 (1975)

Gaddum, J. H., Picarelli, Z. P.: Two types of tryptamine receptors. Br. J. Pharmacol. **12**, 323–328 (1957)

Gerschenfeld, H. M., Paupardin-Tritsch, D.: Ionic mechanisms and receptor properties underlying the responses of moluscan neurons to 5-hydroxytryptamine. J. Physiol. (Lond.) **243**, 427–456 (1974)

Haigler, H. J., Aghajanian, G. K.: Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotoninergic input. J. Neural Trans. **35**, 257–273 (1974)

Hamon, M., Bourgoin, S., Glowinski, J.: Feed-back regulation of 5-HT synthesis in rat striatal slices. J. Neurochem. **20**, 1727–1745 (1973)

Hamon, M., Bourgoin, S., Jagger, J., Glowinski, J.: Effects of LSD on synthesis and release of 5-HT in rat brain slices. Brain Res. **69**, 265–280 (1974)

Hamon, M., Bourgoin, S., Enjalbert, A., Bockaert, J., Héry, F., Ternaux, J. P., Glowinski, J.: The effects of quipazine on 5-HT metabolism in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **294**, 99–108 (1976)

Jacoby, J. H., Shabshelowitz, H., Fernstrom, J. D., Wurtman, R. J.: The mechanisms by which methiothepin, a putative serotonin receptor antagonist, increases brain 5-hydroxyindole levels. J. Pharmacol. Exp. Ther. **195**, 257–264 (1975)

Kehr, W.: Effect of lisuride and other ergot derivatives on monoaminergic mechanisms in rat brain. Eur. J. Pharmacol. **41**, 261–273 (1977)

Lloyd, K. G., Bartholini, G.: The effect of methiothepin on cerebral monoamine neurons. In: Serotonin New vistas. Adv. Biochem. Psychopharmacol. **10**, 305–309 (1974)

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265–275 (1951)

Monachon, M. A., Burkard, W. P., Jalfre, M., Haefely, W.: Blockade of central 5-hydroxytryptamine receptors by methiothepin. Naunyn-Schmiedeberg's Arch. Pharmacol. **247**, 192–197 (1972)

Ruch-Monachon, M. A., Jalfre, M., Haefely, W.: Drugs and PGO waves in the lateral geniculate body of the curarized cat. II. PGO wave activity and brain 5-hydroxytryptamine. Arch. Int. Pharmacodyn. Ther. **219**, 269–286 (1976)

Salomon, Y., Londos, C., Rodbell, M.: A highly sensitive adenylate cyclase assay. Anal. Biochem. **58**, 541–548 (1974)

Sastry, B. S. R., Phillis, J. W.: Metergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. Can. J. Physiol. Pharmacol. **55**, 130–133 (1977)

Segal, M.: 5-HT antagonists in rat hippocampus. Brain Res. **103**, 161–166 (1976)

Sharma, J. N.: Microiontophoretic application of some monoamines and their antagonists to cortical neurones of the rat. Neuropharmacology **16**, 83–88 (1977)

Snedecor, G. W., Cochran, W. G.: Statistical methods. Ames: Iowa State College Press 1967

Szabadi, E., Bradshaw, C. M., Bevan, P.: Excitatory and depressant neuronal responses to noradrenaline, 5-hydroxytryptamine and mescaline: the role of the baseline firing rate. Brain Res. **126**, 580–583 (1977)

Tachiki, K. H., Aprison, M. H.: Fluorometric assay for 5-hydroxytryptophan with sensitivity in the picomole range. Anal. Chem. **47**, 7–11 (1975)

Tebećis, A.: Antagonism of 5-hydroxytryptamine by methiothepin shown in microelectrophoretic studies of neurones in the lateral geniculate nucleus. Nature New Biol. **238**, 63–64 (1972)

Trulson, M. E., Ross, C. A., Jacobs, B. L.: Behavioral evidence for the stimulation of CNS serotonin receptors by high doses of LSD. Psychopharmacol. Commun. **2**, 149–164 (1976)

Von Hungen, K., Roberts, S., Hill, D. F.: Serotonin-sensitive adenylate cyclase activity in immature rat brain. Brain Res. **84**, 257–267 (1975)

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