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The Effects of Quipazine on 5-HT Metabolism in the Rat Brain

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Summary. Since quipazine is a potent 5-HT agonist in peripheral organs, its possible stimulatory effects on serotoninergic receptors in the rat brain were investigated. Quipazine administration (10 mg/kg, i.v.) induced a significant decrease in the synthesis and turnover rates of serotonin in the brain stem as well as in the forebrain. It is not likely that these changes were mediated by a negative feed-back mechanism triggered by a *direct* action of quipazine on central 5-HT postsynaptic receptors. Indeed, in contrast to LSD and 5-methoxy-N,N-dimethyltryptamine, this compound failed to activate the 5-HT sensitive adenylate cyclase in colliculi homogenates of newborn rats. However, quipazine exerted direct effects on serotoninergic terminals. It inhibited competitively the reuptake process in synaptosomes (Ki $= 1.38 \times 10^{-7}$ M) and stimulated the K^+ evoked release of newly synthesized ^{3}H-5-HT in slices of the brain stem. Injected in vivo in a dose which affected 5-HT uptake and release, quipazine did not modify MAO activity. However, this activity was non-competitively inhibited by high concentration of the drug in vitro (Ki $= 3.0 \times 10^{-5}$ M). These actions are very likely *indirectly* responsible for the stimulation of central 5-HT receptors.

Key words: Quipazine – Serotonin uptake – MAO activity – 5-HT sensitive adenylate cyclase.

INTRODUCTION

Earlier pharmacological studies have revealed that quipazine can mimic the effects of serotonin (5-HT) on peripheral serotoninergic receptors. The drug was shown to be very potent in provoking contractions of uterine smooth muscle (Hong and Pardo, 1966). It was also active in other preparations such as the rabbit aorta, the guinea pig ileum and the guinea pig trachea (Hong et al., 1969). Furthermore, it induced bronchoconstriction in the guinea pig. These effects were blocked by potent antagonists of peripheral 5-HT receptors such as methysergide and morphine (Hong and Pardo, 1966; Hong et al., 1969). Interesting central effects were also observed initially in the cat. The i.v. injection of quipazine induced behavioural alterations characterized primarily by shamrage reactions, lack of responsiveness to external stimuli and catatonic stereotyped postures as well as EEG synchronization (Hong et al., 1969; Rodriguez et al., 1973). These effects, similar to those detected after the injection of large doses of 5-hydroxytryptophan (5-HTP) were antagonized by cinanserin or cyproheptadine (Hong et al., 1969; Rodríguez et al., 1973). Similar to 5-HTP, quipazine was found to antagonize the tremorine induced tremor (Rodríguez, 1972) and to induce a head-twitch (Malick et al., 1975) in mice. The latter effect was blocked by antiserotoninergic drugs but potentiated by a monoamine oxidase inhibitor. It was thus suggested that quipazine acted not only by a direct activation of 5-HT receptors but also indirectly by stimulating the release of 5-HT (Malick et al., 1975). An indirect action of the drug was substantiated by two other studies. In rats, the stereotyped responses induced by quipazine were selectively abolished by lesions of the medial and/or dorsal raphe nucleus, leading to the degeneration of serotoninergic systems innervating telencephalic structures (Costall and Naylor, 1975). Furthermore, in contrast to 5-HT, the iontophoretic application of quipazine seemed to have little effect on the activity of raphe neurons; however, it prolonged the action of 5-HT and also that of noradrenaline in some neurons of the rat brain stem (Briggs, 1975). Quipazine may act as well on other processes of 5-HT metabolism. Thus, it reduced the rate of decline of 5-hydroxyindole

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acetic acid (5-HIAA) levels induced by pargyline in the whole brain of rats suggesting a reduction of the transmitter turnover (Grabowska et al., 1974). Other reports have mentioned an inhibitory effect of quipazine on the uptake process of 5-HT in brain slices (Medon et al., 1973; Jacoby et al., 1975) and in synaptosomes of the rat forebrain (Burkard, 1975). These various results led us to examine more extensively the effects of quipazine on the synthesis, the release and the inactivation of 5-HT in the rat brain. Its potential action on central 5-HT receptors was analyzed by measuring its effect on the 5-HT sensitive adenylate cyclase of colliculi homogenates of newborn rats (Von Hungen et al., 1975). In most experiments, the effects of quipazine were compared to those of chlorimipramine, a well-known inhibitor of 5-HT uptake (Carlsson et al., 1969) and those of LSD, a potent agonist of 5-HT receptors (Andén et al., 1968).

MATERIALS AND METHODS

The following drugs were used: quipazine [2-(1-piperazinyl)quinoline maleate, Miles], chlorimipramine (Ciba-Geigy), pargyline (Abbott), Ro4-4602 (Hoffman La Roche), methiothepin (Hoffman-La Roche), N,N-dimethyl-5-methoxytryptamine (Regis), d-LSD (d-lysergic acid diethylamide, Sandoz) and 4-methyl-α-ethylmetatyramine (H 75/12 AB Hässle).

L-^{3}H-tryptophan (generally labelled, 4.5 Ci/mmole) and ^{3}H-5-HT (generally labelled, 17.3 Ci/mmole) from Radiochemical Centre (Amersham) were purified by ion exchange chromatography before each experiment (Hamon et al., 1974). α-^{32}P-ATP (10–20 Ci/mmole) was purchased from New England Nuclear.

In vivo Studies

Male Charles River rats, weighing 250–350 g, were kept for at least 10 days in a controlled environment (24 °C; relative humidity: 60%; alternative cycles of 12 h light and 12 h darkness) before the experiments. Each drug was injected intraperitoneally in a saline solution (1 ml per rat). At times indicated in the "Results" section, treated and control (receiving saline only) rats were sacrificed by decapitation. On occasion, blood was collected from trunk vessels and allowed to clot in the cold for 1 h. The brain was rapidly removed and divided in two by a midcollicular frontal cut. From the posterior region, the brain stem was reserved after having discarded the cerebellum. A sagittal cut was made in the anterior region and one half of the forebrain was also reserved for the study. These two regions were separately weighed and homogenized in an ethanol–water (74:16 v/v) solution. After storage at −30 °C for 12 h, homogenates were centrifuged and the clear supernatant was used as the starting material for the determination of tryptophan, 5-HTP, tyrosine, 5-HT and 5-HIAA as already described in detail (Hamon et al., 1973; Bourgoin et al., 1974, 1975). Briefly, aromatic amino acids were selectively retained on a Dowex AG 50 WX4 column; 5-HT was isolated on an Amberlite CG 50 column and the acidic metabolite was adsorbed on a Sephadex G10 column. Each compound in the corresponding column eluate was determined by a specific spectrofluorimetric method. When blood was collected, the serum was submitted to ultrafiltration through an Amicon CF 50 membrane cone in order to isolate the unbound fraction of tryptophan (Bourgoin et al., 1974). The levels of free tryptophan and those of total tryptophan and tyrosine were estimated in serum according to the methods described for tissues with slight modifications (Bourgoin et al., 1974).

In vitro Studies

A. Conversion of ^{3}H-Tryptophan into ^{3}H-5-HT and ^{3}H-5-HIAA in Brain Stem Slices

Rats treated with quipazine or saline solution were killed by decapitation. The brain stem was rapidly removed in the cold and sliced with a McIlwain tissue chopper. The thickness of slices was 300 µm. Tissues (100 ± 7 mg) were then suspended in 5 ml of a physiological medium: in mM: glucose, 5; NaCl, 136; KCl, 5.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $CaCl_2$, 2.2; $NaHCO_3$, 16.2 containing in some cases pargyline (10^{-4} M), quipazine ($10^{-6}-2\times10^{-5}$ M), chlorimipramine ($10^{-6}-10^{-5}$ M) and/or LSD (10^{-5} M). When the K^+ concentration was raised to 30 mM, Na^+ concentration was decreased by 24.4 mM in order to maintain the isotonicity of the incubating medium. Each incubation flask was kept at 37 °C under a constant stream of O_2 : CO_2 (95:5%) for 5 min. ^{3}H-Tryptophan (16–22.5 µCi in 50 µl) was added at the starting point of the incubation period, which lasted a total of 30 min. Then, tissues and medium were separated by filtration under vacuum through Whatman paper no. 3, and submitted to biochemical analyses. Newly synthesized ^{3}H-5-HT still contained in tissues or spontaneously released in the incubating medium was selectively extracted by ion exchange chromatography on an Amberlite CG 50 column. ^{3}H-Tryptophan, passing through the column, was retained on a Dowex AG 50 WX4 column. Finally, ^{3}H-5-HIAA in the effluent was adsorbed on Sephadex G10. These procedures have been described in detail elsewhere (Hamon et al., 1973). They allow one to estimate the synthesis rate of 5-HT from tryptophan in brain stem slices. As previously discussed, the conversion index, i.e. the ratio of ^{3}H-5-hydroxyindoles formed over the specific activity of ^{3}H-tryptophan in tissues, is quantitatively related to the actual rate of synthesis of 5-HT under these experimental conditions (Héry et al., 1972; Hamon et al., 1973).

B. Uptake and Release of Exogenous ^{3}H-5-HT

a) Uptake. In order to measure the initial accumulation of ^{3}H-5-HT in brain stem slices, tissues were prepared as described above. They were incubated for 15 min with ^{3}H-5-HT (7 µCi–10^{-7} M) at 37 °C under a constant stream of O_2 : CO_2 (95/5%) in the presence of pargyline (10^{-4} M) and various concentrations of quipazine or chlorimipramine ($10^{-7}-10^{-4}$ M). They were isolated by filtration and then washed on the filter with 5 ml of cold physiological medium. Their ^{3}H-5-HT content was determined by using the procedure described above. For uptake experiments on synaptosomes, a crude synaptosomal P_2 fraction from the brain stem region was obtained according to Gray and Whittaker (1962). An aliquot (0.5 ml) of the synaptosomal suspension in a physiological medium identical to that used for slices was added to 4.5 ml of the physiological medium containing pargyline (10^{-4} M), ^{3}H-5-HT (3.9 µCi–4.5×10^{-8} M) and various amounts of cold 5-HT to reach a final concentration of the indoleamine ranging from 4.5×10^{-8} to 5.5×10^{-7} M. Each sample was then incubated for 4 min at 37 °C under a constant stream of O_2 : CO_2 (95/5%). Under these conditions, the uptake of ^{3}H-5-HT was linear both as a function of time (0–4 min) and protein concentration in the incubating medium (0.1–0.6 mg/ml). Blanks were made by incubating synaptosomes

at 0° C. The uptake was stopped by pouring 1 ml of the incubating suspension in 4 ml of ice-cold medium and centrifuging the whole mixture in a refrigerated centrifuge for 3 min at 5000 × g. The synaptosomal pellet was washed with 1 ml of ice-cold medium and finally sonicated in 1 ml of water. Proteins and total radioactivity were then determined. Authentic ^{3}H-5-HT represented more than 95% of the total radioactivity accumulated in tissues.

b) Release. Brain stem slices were first incubated for 15 min in 5 ml of physiological medium containing pargyline (10^{-4} M) and ^{3}H-5-HT (7 μCi – 10^{-7} M). They were then isolated by filtration, washed in 5 ml of cold physiological medium and immediately reincubated in 5 ml of the same medium containing pargyline (10^{-4} M) and quipazine (10^{-7} – 10^{-4} M) or chlorimipramine (10^{-7} – 10^{-4} M) but not ^{3}H-5-HT. This second incubation period lasted for 15 min. At the end, the ^{3}H-5-HT contents in tissues and incubating medium were determined by ion-exchange chromatography (see above).

C. MAO Activity

This enzymatic activity was measured using ^{3}H-5-HT as the substrate according to a procedure adapted from Robinson et al. (1968). The brain stem of rats was homogenized in 10 vol. of 0.1 M phosphate buffer pH 7.4 with a Polytron PT 10 OD apparatus. The assay mixture contained: 0.2 ml of the homogenate, 0.2 ml of the phosphate buffer, 0 – 10^{-4} M quipazine, ^{3}H-5-HT (62.5 nCi) and 5-HT (2.3×10^{-5} M – 1.8×10^{-4} M) in a final volume of 0.5 ml. The mixture was incubated for 20 min at 37° C in open tubes. Under these conditions MAO activity was linear with respect to both time (0 – 20 min) and protein concentration (1 – 5 mg/ml in the assay mixture). The amount of ^{3}H-5-HT catabolised was determined by the difference between that originally added to the medium and that recovered at the end of the incubation. Blanks were made by incubating samples with boiled homogenates.

D. Adenylate Cyclase Activity

Adenylate cyclase activity was measured by the conversion of α-^{32}P-ATP into ^{32}P-cyclic AMP according to the method described previously (Bockaert et al., 1976). The enzyme source was a whole homogenate of colliculi of newborn rats (less than 24 h old) made in 20 vol. (v/w) of an isotonic buffer containing 2 mM Tris maleate pH 7.2, 2 mM ethyleneglycol-bis-(β-aminoethylether)N,N'-tetra acetic acid (EGTA-Sigma) and 300 mM sucrose. The assay mixture (40 μl) was incubated for 3 min at 30° C and newly formed ^{32}P-cyclic AMP was selectively extracted according to Salomon et al. (1974). Proteins were determined by the procedure of Lowry et al. (1951) with bovine serum albumin as the standard. Statistical calculations were done according to Snedecor and Cochran (1967). When P values were higher than 0.05, differences were not considered to be significant. To calculate the kinetic parameters of 5-HT uptake and MAO activity, the best slopes of the experimental curves were determined by linear regression analysis.

RESULTS

A. Effects of Quipazine on 5-HT, 5-HIAA and Tryptophan Levels in the Brain Stem and the Forebrain

Animals were hyperactive immediately after the administration of quipazine (10 mg/kg, i.p.). This effect lasted for about 30 to 45 min. Biochemical changes were also observed: 5-HT levels increased significantly only in the brain stem 60 min after the drug administration (Fig. 1). At this time, 5-HIAA levels were significantly reduced both in the brain stem and the forebrain. The decline in 5-HIAA levels was even more pronounced 2 h after the injection (Fig. 1). The concentration of tryptophan was significantly altered only in the brain stem 30 min after the injection of quipazine (controls: 4.68 ± 0.27 μg/g; quipazine: 5.97 ± 0.37 μg/g, means ± S.E.M. of 8 determinations; $P < 0.05$). Neither the concentration of whole tryptophan nor that of the free fraction of the amino acid in serum were changed during the 120 min following the drug administration.

B. Effects of Quipazine on the Synthesis of 5-HT in the Brain Stem and the Forebrain

The estimation of the rate of 5-HT synthesis was made according to the procedure of Carlsson et al. (1972) which consists of measuring the initial rate of 5-HTP accumulation in tissues after the blockade of 5-HTP decarboxylase activity with Ro 4-4602 (800 mg/kg, i.p.). When injected 45 min before the decarboxylase inhibitor, quipazine (10 mg/kg, i.p.) reduced the rate of accumulation of 5-HTP both in the brain stem (−24%) and in the forebrain (−28%) (Table 1). The decrease in 5-HT levels seen normally 30 min after the injection of Ro 4-4602 could no longer be detected after the quipazine treatment (Table 1).

The effect of quipazine on the synthesis of 5-HT was also examined in brain stem slices by measuring the rate of formation of ^{3}H-5-HT and ^{3}H-5-HIAA from ^{3}H-tryptophan. The drug (10 mg/kg, i.p.) was injected in vivo 45 min before sacrificing the animals. As indicated by the conversion index (C.I.) of 5-HT synthesized from tryptophan (^{3}H-5-HT + ^{3}H-5-HIAA/specific activity of tryptophan), quipazine treatment significantly reduced the transmitter synthesis (Table 2). In contrast, the direct addition of quipazine (10^{-6}, 10^{-5} M) to the incubating medium of control brain stem slices failed to alter the rate of ^{3}H-5-HT formation.

C. Effects of Quipazine on the Uptake and Release of 5-HT

Various approaches were used to study the effects of quipazine on the uptake and release of 5-HT. In most experiments, the effects of quipazine were compared to those obtained with chlorimipramine, a well-known potent blocker of 5-HT uptake.

a) Effects of Quipazine and Chlorimipramine on the Efflux of Newly Synthesized ^{3}H-5-HT from Brain

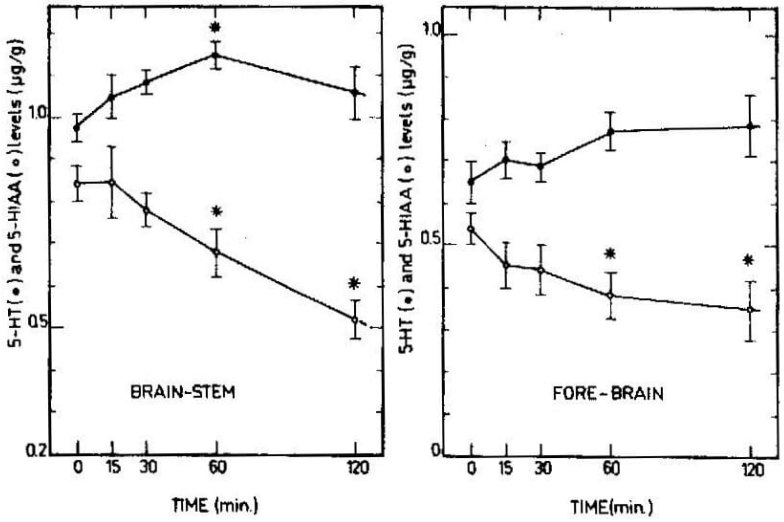

Fig. 1
Effects of quipazine treatment on 5-HT and 5-HIAA levels in the brain stem and forebrain of the rat. Rats were injected with saline (zero time) or quipazine (10 mg/kg, i.p.) and were sacrificed at varying times up to 120 min. For both brain regions, each value is the mean ± S.E.M. of 7–8 determinations. * $P < 0.05$ when compared to corresponding values of saline treated rats

Table 1. Effects of quipazine treatment on the synthesis and utilization rates of 5-HT in the rat brain. Quipazine (10 mg/kg) or saline was injected intraperitoneally to groups of 16 rats. Half of each group was then treated with Ro 4-4602 (800 mg/kg, i.p.) or saline (i.p.) 45 min later. All rats were killed 30 min after the second injection. Each value is the mean ± S.E.M. of 5-HTP or 5-HT levels (in µg per g or fresh tissue) estimated in groups of 8 rats. nd: not detectable. * $P < 0.05$ when compared to saline treated rats. ** $P < 0.05$ when compared to rats treated with Ro 4-4602 alone

Treatment	5-HTP (µg/g)		5-HT (µg/g)	
	brain stem	forebrain	brain stem	forebrain
Saline	nd	nd	0.868 ± 0.018	0.494 ± 0.014
Ro 4-4602	0.226 ± 0.013	0.152 ± 0.015	0.719 ± 0.035* (−17.2%)	0.395 ± 0.017* (−20.0%)
Quipazine	nd	nd	1.029 ± 0.027	0.569 ± 0.017
Quipazine + Ro 4-4602	0.172 ± 0.015**	0.110 ± 0.012**	0.935 ± 0.037 (−9.1%)	0.539 ± 0.037 (−5.3%)

Table 2. Effect of the in vivo quipazine treatment on the synthesis of 5-HT in brain stem slices. Quipazine (10 mg/kg) or saline was injected intraperitoneally to groups of 8 rats 45 min before sacrifice. Brain stem slices were then incubated for 30 min in the presence of ^{3}H-tryptophan (3.2 µCi/ml; 0.7 µM). ^{3}H-tryptophan (^{3}H-TRP) and the specific activity of the amino-acid (^{3}H-TRP S.A.) were estimated in slices at the end of the incubation period. The conversion index (C.I.) of tryptophan into 5-HT is the ratio of the total amount of ^{3}H-5-HT and ^{3}H-5-HIAA accumulated in tissues and their incubation medium over the specific activity of ^{3}H-tryptophan in tissues [^{3}H-5-HT + ^{3}H-5-HIAA (µCi/g)/^{3}H-TRP S.A. (µCi/nmole)]. Each value is the mean ± S.E.M. * $P < 0.05$ when compared to determinations made with tissues of saline treated rats

Determination	Saline	Quipazine	% Change
^{3}H-TRP (µCi/g)	7.05 ± 0.32	6.51 ± 0.31	− 7.6
^{3}H-TRP S.A. (µCi/nmole)	0.220 ± 0.016	0.204 ± 0.009	− 7.3
^{3}H-5-HT (µCi/g)	0.396 ± 0.013	0.326 ± 0.027*	− 17.7
^{3}H-5-HIAA (µCi/g)	0.194 ± 0.020	0.109 ± 0.012*	− 43.8
C.I.	2.68 ± 0.18	2.13 ± 0.16*	− 20.5

Stem Slices. The levels of ^{3}H-5-HT were significantly increased in the medium of brain stem slices incubated for 30 min with ^{3}H-tryptophan in the presence of quipazine (10^{-6} M) or chlorimipramine (10^{-6} M) (Table 3). In vivo treatment with quipazine (10 mg/kg, i.p., 20 min before sacrifice) resulted in a similar but less pronounced effect (Table 3). When the incubation was performed with both quipazine and chlorimipramine, the change in ^{3}H-5-HT efflux was only slightly more pronounced than that occurring in the presence of each compound alone (Table 3). The changes in ^{3}H-5-HT levels in the incubating medium induced by quipazine could be related to effects on the reuptake and/or the release of the newly synthesized transmitter. Further experiments were thus carried out to precise the mechanisms involved.

b) Protecting Effects of Quipazine and Chlorimipramine on the Depletion of 5-HT Induced by H75/12. H75/12, a compound which depletes 5-HT stores in the brain (Carlsson et al., 1969) has been used by some authors to determine the effects of drugs on the uptake of the

transmitter. Indeed, its depleting effect is markedly reduced by potent inhibitors of the uptake process in serotoninergic nerve terminals (Carlsson et al., 1969). Marked behavioural changes (tremor, hyperreactivity) occurred immediately after H75/12 administration (100 mg/kg, i.p.). They were partially prevented by the prior injection of quipazine (10 mg/kg, i.p.) or chlorimipramine (10 mg/kg, i.p.). Two hours after H75/12 treatment, the levels of 5-HT were reduced by more than 50% both in the brain stem and the forebrain (Fig. 2). In rats pretreated with quipazine or chlorimipramine 30 min before H75/12 injection, the 5-HT decline was significantly less pronounced than that observed in animals injected with the depleting agent alone (Fig. 2).

Table 3. Effects of quipazine and chlorimipramine on the spontaneous efflux of ^{3}H-5-HT newly synthesized from ^{3}H-tryptophan in brain stem slices. Brain stem slices were incubated for 30 min in the presence of ^{3}H-tryptophan (4.5 μCi/ml; 1 μM) and ^{3}H-5-HT was estimated in tissues and their incubating media at the end of the incubation period. Quipazine was either injected 20 min before sacrifice or added, as well as chlorimipramine, directly to the incubation medium of control slices 5 min before the radioactive amino acid. Each value is the mean ± S.E.M. of 7 determinations. * $P < 0.05$ when compared to control values

Groups	^{3}H-5-HT (nCi/g)	
	tissue	medium
Control	561.4 ± 61.7	54.5 ± 5.6
Quipazine (10 mg/kg)	563.4 ± 51.6	82.4 ± 6.8*
Quipazine (1 μM)	593.9 ± 52.5	100.6 ± 3.4*
Chlorimipramine (1 μM)	565.6 ± 48.6	108.4 ± 5.6*
Quipazine (1 μM) + Chlorimipramine (1 μM)	537.8 ± 25.4	122.4 ± 6.5*

c) Effects of Quipazine and Chlorimipramine on the Initial Accumulation and the Release of Exogenous ^{3}H-5-HT from Brain Stem Slices. Brain stem slices were first incubated for 15 min with exogenous ^{3}H-5-HT in the presence of various concentrations of quipazine or chlorimipramine. Under these conditions, quipazine added in small concentrations appeared to be a weaker inhibitor of the initial accumulation of ^{3}H-5-HT than chlorimipramine (Fig. 3). However, this difference was no longer obvious at higher concentrations (10 μM; 0.1 mM) of these drugs (Fig. 3). The efflux of ^{3}H-5-HT previously taken up in brain stem slices was increased by the addition of quipazine or chlorimipramine to the incubating medium. In this respect, chlorimipramine was still slightly more efficient than quipazine (Fig. 3). However, for both compounds, the dose response curve of their releasing effect was shifted to the right when compared to that of their inhibitory action on the initial accumulation of ^{3}H-5-HT (Fig. 3). The respective concentrations of quipazine or chlorimipramine which induced a 50% inhibition of ^{3}H-5-HT accumulation were about 1.5 order of magnitude lower than that required to deplete by 50% ^{3}H-5-HT previously taken up in tissues (Fig. 3). Much the same as chlorimipramine, quipazine appeared to have a greater effect on the uptake than on the release process under these experimental conditions.

d) Effects of Quipazine and Chlorimipramine on the Uptake of ^{3}H-5-HT in Synaptosomes of the Brain Stem. The in vitro addition of quipazine (5×10^{-7} M) to a crude synaptosomal suspension (P_2) prepared from the brain stem resulted in a marked reduction of ^{3}H-5-HT uptake. As revealed by double reciprocal

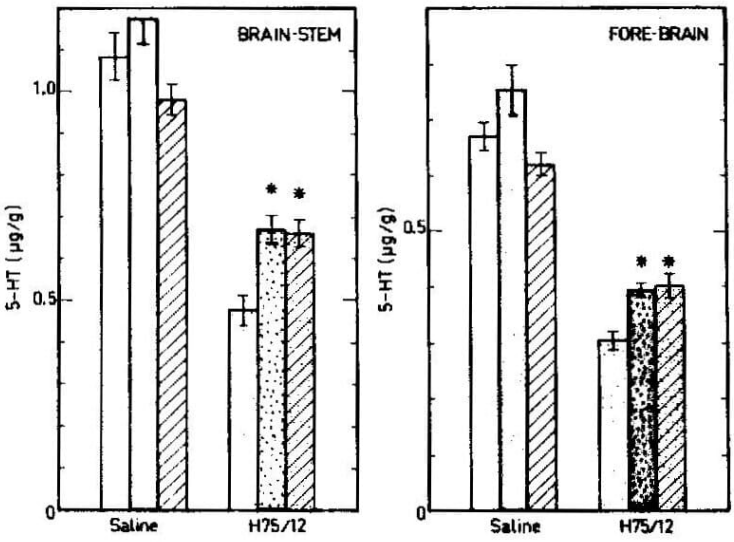

Fig. 2
Effects of quipazine or chlorimipramine on the 5-HT depletion induced by H75/12 treatment. Quipazine (10 mg/kg) or chlorimipramine (10 mg/kg) was injected intraperitoneally to groups of 12–14 rats. Thirty minutes after this first injection, half of the animals in each group were treated with H75/12 (100 mg/kg, i.p.) and the other half with saline. Other rats were treated with saline or H75/12 alone. All animals were killed 2 h after the last injection (saline or H75/12) for 5-HT estimation. Each bar is the mean ± S.E.M. of 6–8 determinations. * $P < 0.05$ when compared to corresponding values of rats treated with H75/12 alone. Empty bars: first injection of saline; solid bars: first injection of chlorimipramine; hatched bars: first injection of quipazine

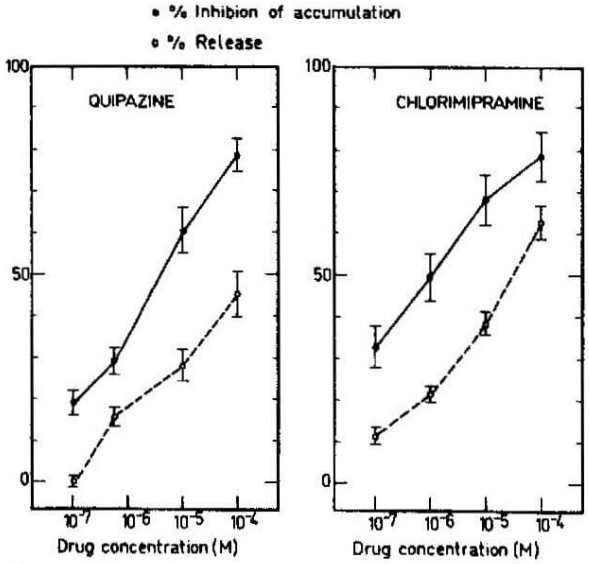

Fig. 3. Effects of chlorimipramine or quipazine on accumulation and release of ^{3}H-5-HT in brain stem slices of the rat. In accumulation experiments, slices were incubated for 15 min at 37° C in the presence of ^{3}H-5-HT (1.4 µCi/ml; 10^{-7} M) and quipazine or chlorimipramine. The data were calculated as % inhibition of ^{3}H-5-HT accumulation according to the formula: % inhibition = $100 \times \frac{^3\text{H-5-HT in control tissues} - ^3\text{H-5-HT in tissues incubated with the drug}}{^3\text{H-5-HT in control tissues}}$

Each point is the mean of 5 determinations. At the end of the incubation period, 8.97 ± 0.33 µCi of ^{3}H-5-HT per g of fresh tissue were accumulated in control slices. In release experiments, ^{3}H-5-HT was allowed to accumulate in brain stem slices for 15 min. Then the slices were filtered, washed and resuspended in fresh medium (5 ml). Quipazine or chlorimipramine was then added and incubation continued for 15 min. Slices were then filtered, washed and assayed for their ^{3}H-5-HT content. The data were calculated as % release according to the formula: % release = $100 \times \frac{^3\text{H-5-HT in control slices} - ^3\text{H-5-HT in slices incubated with the drug}}{^3\text{H-5-HT in control slices}}$

Each point is the mean of 5 determinations. In control conditions, tissues contained 6.48 ± 0.35 µCi of ^{3}H-5-HT per g at the end of the second incubation

plots, quipazine competitively inhibited the uptake of ^{3}H-5-HT (Fig. 4A). When estimated by the Dixon graphical analysis (Fig. 4B) the Ki of quipazine was 1.38×10^{-7} M, a value about 6 times higher than that of chlorimipramine (2.2×10^{-8} M).

D. Effect of Quipazine on MAO Activity

As described in § A, quipazine injected in vivo reduced the levels of 5-HIAA in brain tissues. This led us to examine its possible effect on MAO activity. The oxidative deamination of exogenous ^{3}H-5-HT by brain stem homogenates was significantly inhibited by quipazine. The drug acted as a non-competitive inhibitor with a Ki equal to 3×10^{-5} M (Fig. 5). However, no significant inhibition of the enzyme activity could be detected when the MAO assay was performed on homogenates prepared from rats treated with quipazine (10 mg/kg i.p.) 20 or 60 min before their sacrifice.

E. Effect of Quipazine on 5-HT Receptors

Methiothepin, a potent blocker of 5-HT receptors, has been shown to stimulate the turnover of 5-HT in the brain (Monachon et al., 1972). When injected 2 h before sacrificing the animals, the drug markedly increased the levels of 5-HIAA and those of tryptophan in the whole brain. The administration of quipazine (10 mg/kg, i.p.) or that of LSD (2 mg/kg, i.p.) 20 min before methiothepin treatment largely prevented the effect of the 5-HT antagonist on 5-HIAA levels (Table 4). In contrast to LSD, quipazine also prevented the rise in tryptophan levels induced by methiothepin.

The effects of quipazine and LSD on the K^+ evoked release of newly synthesized ^{3}H-5-HT were compared to further examine the effect of quipazine on 5-HT receptors. Indeed, it is likely that LSD (10^{-5} M) reduced the K^+ induced release of ^{3}H-5-HT from brain slices by stimulating autoreceptors (Hamon et al., 1974). Brain stem slices were incubated for 30 min with ^{3}H-tryptophan in the presence of K^+ (30 mM) and chlorimipramine (10^{-5} M). In presence of the 5-HT uptake inhibitor, the depolarizing cation markedly increased the release of newly synthesized ^{3}H-5-HT (Fig. 6). As expected, this effect was significantly reduced by LSD (10^{-5} M). In contrast, quipazine (10^{-5} M) stimulated the releasing action of K^+ in presence of chlorimipramine (Fig. 6). In the absence of chlorimipramine, quipazine at a con-

Table 4. Effects of Quipazine or LSD treatment on Tryptophan, 5-HT and 5-HIAA levels in the whole brain of rats injected with methiothepin. Quipazine (10 mg/kg) or LSD (2 mg/kg) was injected intraperitoneally 20 min before methiothepin treatment (20 mg/kg, i.p.). All rats were killed 2 h after the last injection (methiothepin or saline for control animals). Each value is the mean ± S.E.M. of 8 determinations. * $P < 0.05$ when compared to saline treated rats. ** $P < 0.05$ when compared to rats injected with methiothepin alone

Treatment	TRP (µg/g)	5-HT (µg/g)	5-HIAA (µg/g)
Saline	5.28 ± 0.21	0.709 ± 0.022	0.496 ± 0.023
Methiothepin	8.13 ± 0.77*	0.636 ± 0.027	0.724 ± 0.042*
Methiothepin + Quipazine	6.29 ± 0.57	0.834 ± 0.067	0.596 ± 0.041**
Methiothepin + LSD	9.54 ± 0.72*	0.750 ± 0.031	0.476 ± 0.022**

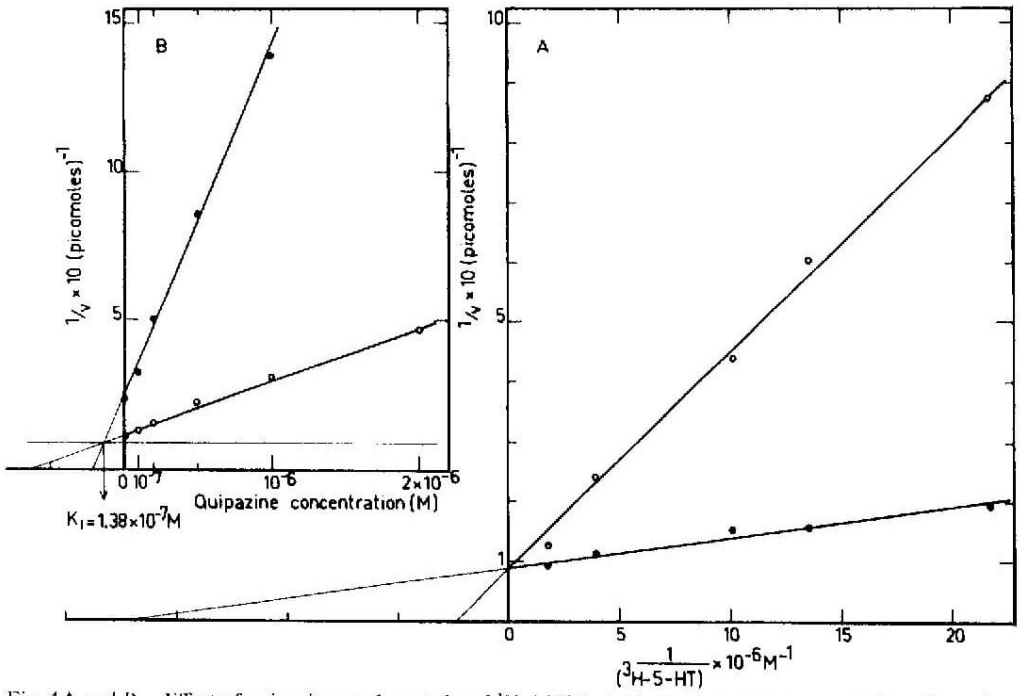

Fig. 4A and B. Effect of quipazine on the uptake of ^{3}H-5-HT in brain stem synaptosomes. (A) Double reciprocal plot of ^{3}H-5-HT uptake versus ^{3}H-5-HT concentration. ^{3}H-5-HT uptake was estimated with various concentrations of ^{3}H-5-HT (0.045 µM; 0.075 µM; 0.1 µM; 0.25 µM; 0.55 µM) in the absence (●) or in the presence (○) of quipazine (0.5 µM). The velocity V is expressed in picomoles of ^{3}H-5-HT taken up in 4 min by brain stem synaptosomes contained in 0.1 ml of the initial sucrose homogenate (see "Methods" – 0.1 ml of the synaptosomal suspension contained 0.320 mg of proteins). Each point is the mean of triplicate determinations. (B) Dixon plot of the inhibition of ^{3}H-5-HT uptake by quipazine. The uptake was estimated at two concentrations of ^{3}H-5-HT: 0.04 µM (●) and 0.25 µM (○) in the presence of various concentrations of quipazine. Each point is the mean of triplicate determinations

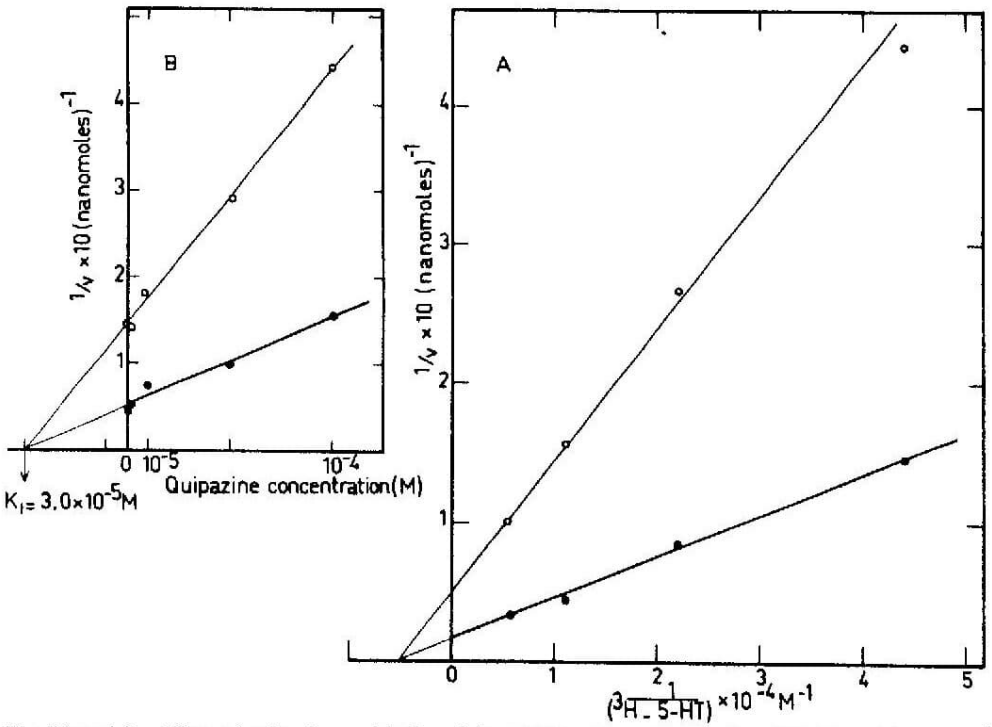

Fig. 5A and B. Effect of quipazine on MAO activity. (A) Double reciprocal plot of MAO activity versus ^{3}H-5-HT concentration. MAO activity was estimated with various concentrations of ^{3}H-5-HT (23 µM; 45 µM; 90 µM; 0.180 mM) in the absence (●) or in the presence (○) of quipazine (0.1 mM). The velocity V is expressed in nanomoles of ^{3}H-5-HT catabolised in 20 min in the presence of 0.2 ml of brain stem homogenate containing 2.41 mg of proteins. Each point is the mean of triplicate determinations. (B) Dixon plot of the inhibition of MAO activity by quipazine. MAO activity was estimated with two different concentrations of ^{3}H-5-HT: 23 µM (○) and 90 µM (●) in the presence of various concentrations of quipazine. Each point is the mean of triplicate determinations

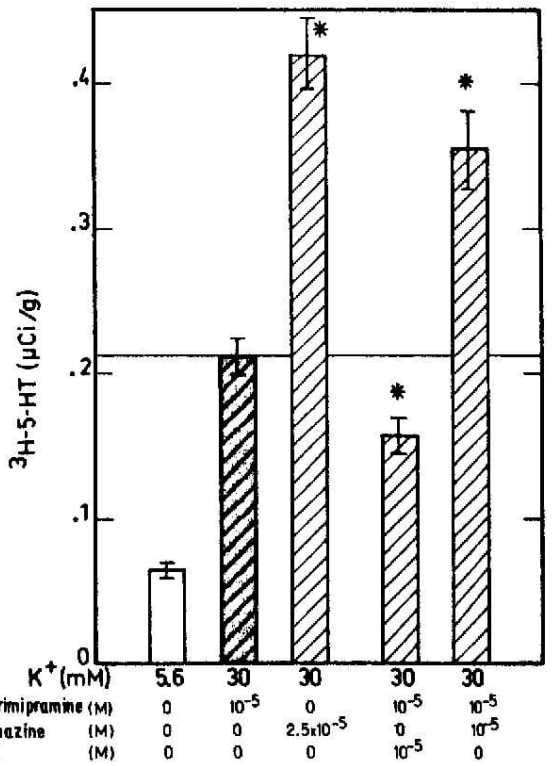

Fig. 6. Effects of chlorimipramine, quipazine and LSD on the K^+ induced release of newly synthesized ^{3}H-5-HT from brain stem slices. Brain stem slices were incubated for 30 min in a physiological medium containing pargyline (10^{-4} M), ^{3}H-tryptophan (3.25 µCi/ml, 0.72 µM) and 5.6 or 30 mM K^+. In the latter case, iso-osmolarity was maintained by decreasing Na^+ concentration in the incubating medium by 24.4 mM. The drugs were added to the incubating medium 5 min before the radioactive substrate amino acid. Each bar represents the mean ± S.E.M. of ^{3}H-5-HT (expressed per mg of tissue slices) found at the end of the incubation period in the medium of 8 samples in each case. Increasing the K^+ concentration in the incubating medium in the presence of chlorimipramine resulted in a decreased accumulation of ^{3}H-5-HT in brain stem slices: $K^+ = 5.6$ mM: 568.7 ± 13.7 nCi/g; $K^+ = 30$ mM + 10^{-5} M chlorimipramine: 404.5 ± 34.0 nCi/g (means ± S.E.M. of 8 determinations; $P < 0.05$). In all situations in which 30 mM K^+ were added, the concentration of ^{3}H-5-HT in tissues were not significantly different from that found in slices incubated with 30 mM K^+ and chlorimipramine. * $P < 0.05$ when compared to ^{3}H-5-HT released in presence of 30 mM K^+ and 10 µM chlorimipramine

centration (2×10^{-5} M) which inhibited the uptake of exogenous ^{3}H-5-HT in slices (Fig. 3) to the same extent as chlorimipramine (10^{-5} M) also markedly increased the release of ^{3}H-5-HT induced by K^+. This latter effect was much more pronounced than that observed with chlorimipramine alone (Fig. 6).

The above results suggested that, in contrast to LSD, quipazine was not stimulating 5-HT autoreceptors.

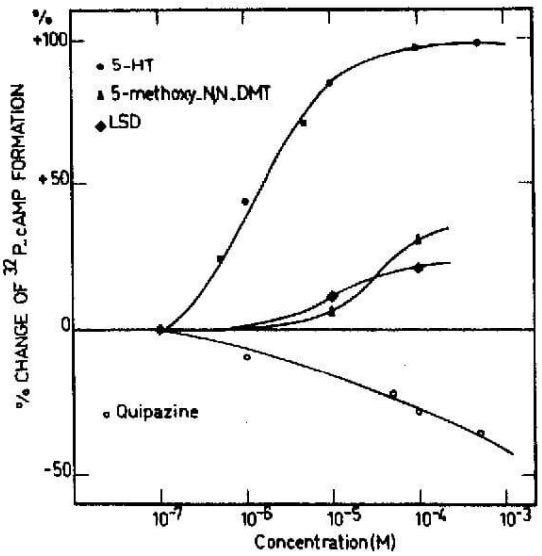

Fig. 7. Effects of 5-HT, 5-HT agonists and quipazine on the adenylate cyclase of colliculi homogenates of newborn rats. Each point is the mean of triplicate determinations of ^{32}P-cyclic AMP formed in assay conditions described in "methods". The basal activity (0% change) corresponds to 4.57 picomoles of ^{32}P-cyclic AMP formed per min and per mg of protein

Recently, Von Hungen et al. (1975) demonstrated that the stimulation of 5-HT receptors in colliculi homogenates of newborn rats was associated with the activation of a specific adenylate cyclase. In agreement with these authors, we observed that 5-HT, N,N-dimethyl-5-methoxytryptamine and LSD stimulated this enzymatic activity in colliculi homogenates of new born rats (Fig. 7).

In contrast, quipazine decreased the basal activity of the enzyme. Furthermore, the percent increase in adenylate cyclase activity induced by 5-HT (+ 90% at 10^{-4} M) was not altered when quipazine (up to 5×10^{-4} M) was included in the assay mixture.

DISCUSSION

Quipazine exerts various actions on the metabolism of 5-HT in the central nervous system. Its main effect was to inhibit reuptake and stimulate release, actions that should increase the extraneuronal levels of the transmitter. Although less potent than chlorimipramine, in small concentrations quipazine markedly inhibited the uptake of exogenous ^{3}H-5-HT in synaptosomes ($Ki = 1.38\times10^{-7}$ M) as well as in slices of the brain stem. These results are in agreement with previous findings obtained on synaptosomes (Burkard, 1975) and brain slices (Medon et al., 1973; Jacoby et al., 1975). In addition, as with other 5-HT uptake

inhibitors, quipazine was able to prevent the depleting action of H75/12 on 5-HT stores. On the basis of the experimental model originally designed by Heikkila et al. (1975) to differentiate the effects of drugs on the uptake and release of monoamines, quipazine appeared much more potent in inhibiting the uptake process than in releasing exogenous ^{3}H-5-HT previously taken up in slices. However, the action of the drug could be more complex. Indeed, quipazine (2×10^{-5} M) markedly increased the K^+ induced release of newly synthesized ^{3}H-5-HT from ^{3}H-tryptophan in brain stem slices. This effect was more pronounced than that of chlorimipramine (10^{-5} M) although both compounds in these concentrations similarly inhibited 5-HT uptake in slices. Furthermore, in the presence of chlorimipramine, quipazine was still able to stimulate the effect of high K^+ concentration on ^{3}H-5-HT release. These results could suggest that quipazine directly stimulates the release of newly synthesized 5-HT in depolarized tissues.

Quipazine ($10^{-6} - 10^{-5}$ M) was without effect on HT synthesis when added directly to the incubating medium of brain slices. However, there is little doubt that the drug injected in vivo decreases the rate of 5-HT synthesis. This was observed both by measuring the rate of the limiting step of 5-HT synthesis in vivo (thanks to the use of a 5-HTP decarboxylase inhibitor) and by estimating the rate of conversion of tryptophan into 5-HT in slices. The decreased rate of 5-HT synthesis was probably associated with a reduction in the rate of turnover as suggested by the slower decline of 5-HT levels seen after the inhibition of synthesis by Ro 4-4602. The reduction of 5-HT synthesis can be compared to that observed after LSD (Hamon et al., 1974) or chlorimipramine (Schubert et al., 1970) administration. It can thus be speculated that the effect of quipazine on 5-HT synthesis and turnover might be related to a decreased activity of 5-HT neurons as already shown for LSD (Aghajanian, 1972) and chlorimipramine (Sheard et al., 1972). Thus, the reduction of 5-HT synthesis and turnover induced by quipazine may result from a negative feed back process triggered by a direct or indirect activation of 5-HT receptors.

Dopaminergic postsynaptic receptors are associated with an adenylate cyclase (Kebabian et al., 1972). This may also be the case for 5-HT postsynaptic receptors. Indeed, a 5-HT sensitive adenylate cyclase was found in colliculi homogenates of newborn rats. The stimulating effect of 5-HT was shared by other serotoninergic agonists such as LSD and 5-methoxy-N,N-dimethyltryptamine and could be blocked by specific antagonists (Von Hungen et al., 1975). In contrast to these two later agonists, quipazine was unable to stimulate the 5-HT sensitive adenylate cyclase in this preparation. It is therefore doubtful that quipazine exerts a direct stimulatory action on central 5-HT receptors as it does on peripheral receptors (Hong et al., 1969). This hypothesis is in agreement with that of other authors whose conclusions were based on behavioural (Costall and Naylor, 1975) and electrophysiological (Briggs, 1975) studies. We were also unable to detect any stimulating effect of quipazine on serotoninergic autoreceptors. Indeed, in contrast to LSD, the K^+ induced release of ^{3}H-5-HT was not reduced by quipazine.

Quipazine in high concentrations (above 10^{-5} M) inhibited MAO activity using 5-HT as substrate. Such an inhibition has been shown in vivo when a high dose of the drug is injected (50 mg/kg, i.p., Green et al., 1976). However, in a smaller dose (10 mg/kg) which affected 5-HT uptake and release, the MAO activity measured in crude homogenates was not altered. Taking in account the possible accumulation and compartmentation of quipazine in intact tissues, one cannot exclude that local concentrations of the drug could be high enough to inhibit MAO activity in vivo even after the injection of low doses of quipazine.

In conclusion, by enhancing the synaptic levels of 5-HT, quipazine should be of great help in further studies of the functional properties of central serotominergic neuronal systems.

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