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EFFECT OF TRIPERIDOL ON PROCESSES INVOLVING ACETYLCHOLINE IN RAT BRAIN *IN VITRO*

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Abstract—The effect of triperidol on the synthesis of free acetylcholine in fresh tissue, on the activity of the choline acetylase system in extracts of acetone powders, and on the activity of acetylcholinesterase in homogenates was studied in various regions of rat brain. Triperidol at a concentration 0·2 mM decreases the free acetylcholine synthesis in brain cortex slices by about 80 per cent. This decrease, although considerably lower, is statistically significant even at a concentration of the drug of 0·002 mM. Triperidol is without effect on the activity of the choline acetylase system in cerebral cortex at concentrations varying from 0·001 mM to 0·2 mM. Triperidol is without effect on the activity of acetylcholine esterase in all regions studied at concentrations ranging from 0·001 mM to 0·2 mM.

IN A PREVIOUS paper we have shown that triperidol, 1-[3-(4-fluorobenzoyl)propyl]-4-(3-trifluoromethylphenyl)-piperidin-4-ol, a strong neuroleptic agent, decreases the oxidative processes of glucose and pyruvate metabolism in rat brain cortex slices.[1]

It is well known that acetylcholine is synthetized when brain cortex slices are allowed to respire in a medium containing eserine. The rate of synthesis is greatly increased in the presence of glucose or sodium pyruvate, being intimately connected with the carbohydrate metabolism in the tissue.[2–4] In this respect the cerebral tissue is one of the most active among various rat tissues studied.[2]

The synthesis of acetylcholine is strictly dependent on the availability of some metabolites of the tricarboxylic acid cycle, the citrate being a precursor of the acetyl moiety of acetylcholine in the translocation of mitochondrially generated acetyl-CoA to the cytosol[5] where the choline acetyltransferase is localized.[6]

It would then be expected that triperidol, by reducing the availability of some metabolites of the tricarboxylic acid cycle in the oxidative metabolism of glucose and pyruvate, would have an inhibitory effect on the synthesis of acetylcholine.

This paper presents the results of a study of the effect of triperidol on the synthesis of free acetylcholine in brain cortex slices and on the activity of some enzymes directly linked with the synthesis and the hydrolysis of acetylcholine in rat brain *in vitro*. The same study was extended to other regions of rat brain as the rate of synthesis and the activity of the above mentioned enzymes vary considerably in different regions of central nervous system.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing approximately 180 g were used.

Materials. Acetylcholine chloride Roche and eserine base Merck were used. Triperidol was kindly supplied by the Janssen Research Laboratories (Beerse, Belgium).

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All reagents for the determination of acetylcholine esterase activity were obtained from Boehringer (Mannheim, Germany).

Synthesis of free acetylcholine in fresh brain tissue. The synthesis of free acetylcholine in slices of cerebral cortex and in other regions of rat brain was studied in the conditions of incubation described by McLennan and Elliott.[7] Approximately 100 mg of brain cortex slices 0·35 mm thick were incubated in 2·0 ml of saline medium containing 0·098 M NaCl, 0·027 M KCl, 0·024 M $NaHCO_3$, 1·2 mM $MgSO_4$, 1·3 mM $CaCl_2$, 0·4 mM KH_2PO_4, 0·01 M glucose and 0·4 mM eserine (dissolved in HCl and subsequently neutralized), pH 7·4. The approximate weight of other parts of rat brain incubated in each vessel was about 100 mg for caudate nucleus and thalamus, 75 mg for hypothalamus and 200 mg for pons and cerebellum. In all experiments brain tissue from the same rat or from three pooled rats was incubated with and without the drug, except for the hypothalamus because of its little weight. The triperidol was taken into solution in an excess of lactic acid and subsequently neutralized. The same concentration of lactate was present in control experiments without the drug. The incubation proceeded in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide for 60 min at 37°. At the end of the incubation period, after removing the tissue, the incubation medium was centrifuged and brought to pH 4·0.

Determination of free acetylcholine in the medium. The free acetylcholine content in the medium after incubation was bioassayed within 48 hr by means of a preparation of the isolated *rectus abdominis* muscle of *Rana temporaria*[8] in several experiments with acetone sensitization.[9] The muscle was bathed in frog–Ringer solution containing 0·01 mM eserine. The effect of triperidol used for incubation, present in the medium, was carefully examined on the *rectus abdominis* preparation in order to exclude any influence on the response of the muscle to acetylcholine. In a few experiments *d*-tubocurarine was found to antagonize the effect of an active medium sample to the same extent as shown on an equipotent dosis of a standard solution of acetylcholine.

Choline acetylase system activity. The activity of the choline acetylase system was measured in extracts of acetone powders prepared from isolated brain regions pooled from 15 rats. The incubation proceeded in 1·0 ml of the medium described by Giarman and Pepeu[10] (0·007 M phosphate buffer, 0·01 M choline chloride, 0·01 M potassium acetate, 0·027 M KCl, 0·005 M $MgCl_2$, 0·2 M cysteine chloride, 0·003 M ATP, 0·2 mM CoA and 0·25 mM eserine) at pH 6·8 in air at 37°. The acetone powders were extracted with a neutralized solution of 0·021 M cysteine chloride in 0·9% NaCl in proportion from 1:40 to 1:10. The quantity of acetone powder in an incubation medium was 7·5 mg for cerebral cortex, thalamus (30 min incubation) and hypothalamus (60 min incubation); 15 mg for cerebellum and pons (60 min incubation); 3·75 mg for caudate nucleus (30 min incubation). The triperidol was added at concentrations varying from 0·001 mM to 0·2 mM in experiments with cerebral cortex and 0·2 mM in experiments with other parts of rat brain. The reaction was stopped by adding 0·25 ml of 0·33 N HCl and centrifuged. The samples were diluted 1:4 and brought to pH 4·0 with 0·33 N NaOH just before bioassay of acetylcholine on frog isolated *rectus abdominis* preparation.

Acetylcholine esterase activity. The hydrolysis of acetylcholine in various parts of rat brain was measured according to the procedure of Ellman *et al.*[11] with a Beckman DK-2 spectrophotometer. The concentrations of the reagents were 0·039 M phosphate buffer, pH 7·2, 5·6 mM acetylthiocholine iodide and 0·21 mM 5,5′-dithiobis-2-

nitrobenzoic acid. As a source of enzyme were used the centrifugated homogenates of various parts of rat brain (pooled from at least three rats) in 1:7 buffer according to McLennan and Elliott.[7] The reaction was initiated by adding the enzyme preparation to a cuvette containing all the reagents with or without triperidol (total volume 1·4 ml). The values of extinction at 412 nm were measured once a minute from the second to the sixth minute of the reaction. The difference in extinction between the second and the third minute was used to calculate the enzyme activity. The nonenzymatic hydrolysis of acetylthiocholine was found to be negligible.

RESULTS

Synthesis of free acetylcholine. Triperidol at a concentration of 0·2 mM decreased by about 80 per cent the synthesis of free acetylcholine in brain cortex slices incubated with glucose. This decrease was proportional to the drug concentration and was statistically significant even at a concentration of 0·002 mM (Table 1). It is important to note that this effect was found in an incubation medium containing 0·027 M KCl, that is in conditions of large increase in free acetylcholine formation provoked by K^+.[3,4,12]

TABLE 1. EFFECT OF TRIPERIDOL ON THE SYNTHESIS OF FREE ACETYLCHOLINE IN RAT BRAIN CORTEX SLICES

Conc. of triperidol (mM)	No. of experiments	Free acetylcholine synthesis (μg of Ach*/g of tissue/hr)		Inhibition (%)	P
		None	With triperidol		
0·200	10	16·9 ± 0·6	3·5 ± 0·3	80	< 0·001
0·050	10	24·6 ± 1·2	8·4 ± 0·4	66	< 0·001
0·025	6	16·6 ± 1·6	10·6 ± 2·0	38	< 0·001
0·010	10	23·5 ± 1·0	16·9 ± 2·0	28	< 0·001
0·005	10	19·8 ± 1·0	17·0 ± 0·7	14	< 0·005
0·002	9	18·8 ± 1·3	16·0 ± 0·7	15	< 0·05
0·001	5	12·7 ± 1·5	12·3 ± 0·6	3	N.S.

The tissue was incubated in 2·0 ml of medium[7] pH 7·4, at 37° in 95% O_2–5% CO_2, for 60 min. The pH of medium was then brought to 4·0. The acetylcholine content was bioassayed on frog isolated *rectus abdominis* muscle preparation. Mean values ± S.E.M.

* Acetylcholine, expressed as acetylcholine chloride.

In order to confirm that the effect of triperidol was really on the synthesis of acetylcholine and not on its release from the tissue, we measured in one experiment in triplicate the total acetylcholine, extracted according to McLennan and Elliott,[4] formed in 1 hr incubation in the presence and absence of the drug. The values of total acetylcholine were 19·3 ± 0·6 and 5·0 ± 0·3 μg/g/hr in the absence and presence of 0·2 mM triperidol, respectively. In the same experiment, the value for the synthesis of free acetylcholine in the absence of triperidol was 14·7 ± 1·2 μg/g/hr. This means that under the conditions of incubation used 76 per cent of the total acetylcholine may be found in the medium, as demonstrated by McLennan and Elliott[4] and Sharkawi and Schulman.[12] The inhibitory effect of triperidol on the synthesis of total acetylcholine is in the same range as that on free acetylcholine.

In order to compare the inhibitory effect of triperidol in various regions of rat brain, we used a drug concentration of 0·025 mM which causes an inhibition of about 40 per cent in cerebral cortex. Triperidol at this concentration decreased the free acetylcholine synthesis by about 75 per cent in hypothalamus and by 55 per cent in thalamus. The inhibitory effect of triperidol in caudate nucleus, cerebellum and pons did not differ from that found in cerebral cortex (Table 2).

TABLE 2. EFFECT OF TRIPERIDOL ON THE SYNTHESIS OF FREE ACETYLCHOLINE IN VARIOUS REGIONS OF BRAIN

Region of brain	No. of experiments	Free acetylcholine synthesis (μg of Ach/g of tissue/hr) None	With triperidol (0·025 mM)	Inhibition (%)	P*
Cerebral cortex	6	16·6 ± 2·6	10·6 ± 2·0	38	
Caudate nucleus	5	55·8 ± 5·6	31·8 ± 5·1	41	< 0·02
Thalamus	6	8·9 ± 1·2	4·0 ± 0·4	55	< 0·01
Hypothalamus	6	7·5 ± 1·0	2·0 ± 0·5	73	< 0·001
Pons	6	8·2 ± 0·6	5·7 ± 0·2	31	< 0·01
Cerebellum	6	1·6 ± 0·2	1·0 ± 0·1	35	< 0·05

See the legends for Table 1.
* P between per cent inhibition in cerebral cortex and the respective region of brain.

To elucidate whether there is a direct action of the drug on the choline acetylase system, the effect of triperidol on the enzymes activity in cell free extracts was studied. It is well known that the enzyme system can be extracted from acetone dried brain tissue and that this procedure yields more active preparations than homogenates.[10,13,14]

The enzyme system synthetizing acetylcholine in extracts from acetone powders of cerebral cortex was not altered at triperidol concentrations varying from 0·001 mM

TABLE 3. EFFECT OF TRIPERIDOL ON THE ACTIVITY OF THE CHOLINE ACETYLASE SYSTEM IN CELL FREE EXTRACTS OF RAT BRAIN CORTEX

Conc. of triperidol (mM)	No. of experiments	Activity of choline acetylase system (μg of Ach/g of acetone dried tissue/hr)
None	4	596 ± 34
0·200	6	616 ± 19
0·050	3	559 ± 27
0·010	3	614 ± 28
0·005	2	609 ± 6
0·001	3	605 ± 12

Extracts of acetone powder were incubated in 1·0 ml of medium,[10] pH 6·8, at 37° in air for 30 min. To the medium was then added 0·25 ml of 0·33 N HCl. The pH was brought to 4·0 before bioassay on frog isolated *rectus abdominis* muscle. Mean values ± S.E.M. P between values without triperidol and each value with triperidol are not significant.

to 0·2 mM (Table 3). There was no effect of the drug even at the highest concentration used, which in slices caused an inhibition of about 80 per cent in the synthesis of free acetylcholine.

The activity of this enzyme system varies widely in different regions of animal brain in relation to the number of cholinergic neurons.[15,16] In extracts of acetone powders prepared from various regions of rat brain triperidol at a concentration of 0·2 mM, the highest used, did not show any effect on the activity of the choline acetylase system (Table 4), even in hypothalamus and thalamus, where its effect in fresh tissue was the most pronounced.

TABLE 4. EFFECT OF TRIPERIDOL ON THE ACTIVITY OF THE CHOLINE ACETYLASE SYSTEM IN CELL-FREE EXTRACTS OF VARIOUS REGIONS OF RAT BRAIN

Region of brain	No. of experiments	Activity of choline acetylase system (μg of Ach/g of acetone dried tissue/hr) None	With triperidol (0·2 mM)
Cerebral cortex	5	596 ± 34	616 ± 19
Caudate nucleus	6	1172 ± 30	1145 ± 23
Thalamus	3	472 ± 18	528 ± 24
Hypothalamus	2	108 ± 9	112 ± 3
Pons	4	22 ± 3	17 ± 3
Cerebellum	6	224 ± 5	220 ± 4

See the legends for Table 3.
All P values are not significant.

Hydrolysis of acetylcholine. In order to obtain some information about the effect of triperidol on other cholinergic mechanisms in brain tissue, we studied the effect of the drug on acetylcholine esterase. Because of marked regional variations in enzyme activity,[17] the effect of triperidol was investigated in homogenates of different parts of rat brain.

TABLE 5. EFFECT OF TRIPERIDOL ON THE HYDROLYSIS OF ACETYLCHOLINE IN HOMOGENATES OF VARIOUS REGIONS OF RAT BRAIN

Region of brain	Acetylcholine hydrolysis (mg of Ach/g of tissue/hr) None	With triperidol (mM) 0·2	0·05	0·01	0·005	0·001
Cerebral cortex	11·1 ± 0·4	9·7 ± 0·8	10·7 ± 0·5	11·3 ± 0·5	11·1 ± 0·5	10·6 ± 0·6
Caudate nucleus	37·9 ± 3·1	32·9 ± 2·5	36·9 ± 3·7	37·1 ± 5·3	39·3 ± 3·8	44·9 ± 1·0
Thalamus	15·4 ± 1·5	11·8 ± 0·9	14·1 ± 1·4	14·2 ± 1·0	14·8 ± 0·7	16·5 ± 2·2
Hypothalamus	30·0 ± 2·9	23·9 ± 2·1	28·2 ± 2·0	31·9 ± 3·2	29·7 ± 1·0	30·7 ± 3·2
Pons	14·2 ± 0·3	13·9 ± 0·5	14·1 ± 0·4	14·9 ± 1·4	15·7 ± 0·4	16·3 ± 0·4
Cerebellum	16·6 ± 0·9	13·2 ± 1·0	15·1 ± 1·0	16·6 ± 1·3	15·4 ± 0·6	17·4 ± 1·1

Homogenates of various regions of brain were incubated in 1·4 ml of medium.[11] Mean values ± S.E.M. of at least four experiments.

There was virtually no effect of triperidol at concentrations varying from 0·001 mM to 0·2 mM (Table 5). Only a slight inhibition of enzyme activity was observed in thalamus and hypothalamus at a concentration of 0·2 mM. The concentration of triperidol which produced a 50 per cent inhibition (I_{50}) in cerebral cortex was about 1 mM. It was difficult to obtain an exact value of I_{50} because of the scanty solubility of triperidol at pH 7·2 at concentrations over 1 mM.

DISCUSSION

The results reported in the present paper show that triperidol acts on the processes involving acetylcholine in rat brain *in vitro* by inhibiting its synthesis in fresh tissue, under conditions of large increase of free acetylcholine formation due to 0·027 mM KCl. It must be remembered that triperidol decreases the oxygen uptake in rat brain cortex only in the presence of K^+ ions (0·031 mM) and is without effect when K^+ ions on the incubation medium are replaced by Na^+ ions.[1]

The inhibition of free acetylcholine synthesis in brain cortex slices by triperidol at higher concentrations (range of 0·1 mM) resembles that of other central nervous system depressants of the barbiturate group.[7] At lower concentrations (range of 0·01 to 0·001 mM) triperidol has not the stimulant effect on free acetylcholine synthesis, which was demonstrated by those authors for the barbiturates; even at a concentration of 0·002 mM triperidol always shows some inhibitory effect.

The activity of the choline acetylase system in cell free extracts is not influenced by triperidol. In this respect it behaves as some narcotics.[18–20]

The effect of triperidol on the synthesis of acetylcholine may be explained by a decreased availability of acetyl-CoA, directly connected with the oxidative metabolism, the oxygen uptake being decreased under the same experimental conditions. In fact the reduction of the oxidative metabolism diminishes the availability of citrate, precursor of acetyl-CoA[5] and thus of acetyl-CoA for the synthesis of acetylcholine.

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