Effect of Triperidol on Carbohydrate and Amino Acid Metabolism in Rat Brain-Cortex Slices

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1. The effect of triperidol on the metabolism of glucose, pyruvate, glutamate, aspartate and glycine was studied with rat brain-cortex slices, U-¹⁴C-labelled substrates and a quantitative radiochromatographic technique. 2. Triperidol at a concentration of 0.2 mM decreased the oxygen uptake and the ¹⁴CO₂ production by about 30% when glucose, pyruvate and glutamate were used as substrates, whereas no effects were observed with aspartate and glycine. 3. The drug did not alter qualitatively the metabolic pattern of the substrates. 4. Quantitatively, triperidol decreased the incorporation of ¹⁴C from [U-¹⁴C]glucose and [U-¹⁴C]-pyruvate into glutamate, glutamine and γ -aminobutyrate but not into lactate, alanine and aspartate. The overall utilization rates of glucose and pyruvate were decreased. The relative specific radioactivities of glutamate and aspartate were also decreased. 5. Triperidol increased the rate of disappearance of U-¹⁴C-labelled glutamate, aspartate and glycine from the incubation medium, and altered the distribution of their metabolites between medium and tissue. 6. No appreciable effect of triperidol on [1-¹⁴C]galactose disappearance was found.

It is well known that a large number of basic butyrophenones have a strong neuroleptic activity (Janssen *et al.* 1959; Janssen & Niemegeers, 1959; Janssen, Jageneau & Schellekens, 1960; Janssen, Niemegeers & Schellekens, 1960; Boissier, Pagny, Mouillé & Forest, 1960). Their pharmacological properties are generally similar to those of chlorpromazine and related phenothiazines (Janssen & Niemegeers, 1961; Janssen, Niemegeers & Schellekens, 1965), though some studies indicate that their profile is in a certain way intermediate between that of neuroleptics and that of anti-depressants (Goldwurm, 1961; Gatti, 1967).

The biochemical effects of triperidol, 1-[3-(4-fluorobenzoyl)propyl]-4-(3-trifluoromethylphenyl)-piperidin-4-ol, have not yet been established. This paper presents the results of a study of this compound on the intermediate metabolism of carbohydrates and amino acids in brain-cortex slices. Preliminary results have been reported by Gatti, Michalek & Pocchiari (1966).

MATERIALS AND METHODS

Animals. Male Wistar rats weighing approx. 150 g. were used.

Materials. [U-¹⁴C]Glucose, [U-¹⁴C]pyruvate, [U-¹⁴C]-glutamate, [U-¹⁴C]aspartate, [U-¹⁴C]glycine and [1-¹⁴C]-galactose were obtained from The Radiochemical Centre,

Amersham, Bucks.; triperidol was kindly supplied by the Janssen Research Laboratories, Beerse, Belgium.

Incubation of rat brain tissue. The conditions of incubation were the same as those described by Beloff-Chain *et al.* (1955a) and Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari (1955b, 1959).

Approx. 100 mg. of brain cortex sliced 0.35 mm. thick was incubated in 1.2 ml. of a phosphate-buffered medium (Elliott & Henderson, 1948), pH 7.4. The substrate concentration was 5.5 mM-glucose, 11.4 mM-pyruvate, 5.4 mM-glutamate, 7.5 mM-aspartate, 13.3 mM-glycine or 5.5 mM-galactose; the radioactivity was approx. 15 μ C/vessel for glucose, glutamate and aspartate, 10 μ C/vessel for pyruvate and 1 μ C/vessel for glycine and galactose. The triperidol concentration was 0.2 mM (Gatti *et al.* 1966). It was taken into solution in an excess of lactic acid and subsequently neutralized; the same amount of lactic acid (0.5 mM) added in control experiments was without effect of the characteristics measured.

In some experiments a phosphate-buffered medium without K⁺ ions was used, KCl and KH₂PO₄ being replaced respectively by NaCl and NaH₂PO₄.

In all experiments brain-cortex slices from the same rat were incubated with and without the drug, each animal thus acting as its own control.

Preparation of samples for tissue-radioactivity measurements. The methods of preparation of tissue extracts after incubation and the methods of chromatography were those described by Beloff-Chain *et al.* (1955a,b, 1959). The brain-cortex slices were removed from the Warburg vessels, blotted on filter paper and rapidly washed in three lots of

Table 1. *Effect of triperidol on oxygen uptake and $^{14}\text{CO}_2$ production in rat brain-cortex slices incubated with various $\text{U-}^{14}\text{C}$ -labelled substrates*

Tissue (100 mg.) was incubated in 1.2 ml. of phosphate buffer (Elliott & Henderson, 1948), pH 7.4, at 37° in O_2 for 60 min. The substrate concentration was 5.5 mM-glucose, 11.4 mM-pyruvate, 5.4 mM-glutamate, 7.5 mM-aspartate or 15.7 mM-glycine. The radioactivity was approx. $15 \mu\text{C}$ /vessel for glucose, glutamate and aspartate, $10 \mu\text{C}$ /vessel for pyruvate and $1 \mu\text{C}$ /vessel for glycine. The concentration of triperidol, when present, was 0.2 mM. Mean values $\pm$ S.E.M., with the numbers of experiments in parentheses, are given.

Substrate	Uptake of O_2 ($\mu\text{moles/g. of tissue/hr.}$)		Substrate converted into CO_2 ($\mu\text{g./100 mg. of tissue/hr.}$)	
	No triperidol	With triperidol	No triperidol	With triperidol
Glucose (6)	120 ± 5	72 ± 2	228.5 ± 15.4	129.6 ± 6.0
Pyruvate (6)	112 ± 3	79 ± 3	335.7 ± 19.7	230.3 ± 15.9
Glutamate (6)	81 ± 2	65 ± 2	173.4 ± 8.8	120.5 ± 2.9
Aspartate (3)	58 ± 4	51 ± 1	89.8 ± 3.3	83.1 ± 3.3
Glycine (3)	60 ± 2	52 ± 0.2	4.1 ± 0.4	3.3 ± 0.1

0.9% (w/v) NaCl and blotted on filter paper after each washing. The slices were then transferred to 1.5 ml. of 60% (v/v) ethanol, homogenized in a small Potter homogenizer and transferred to a centrifuge tube; the homogenizer was washed with 1 ml. of aq. 60% (v/v) ethanol and then again with 0.5 ml. The washings were added to the brain homogenate. The latter was centrifuged and the ethanolic extract collected, the insoluble residue being re-extracted with three 1 ml. portions of 60% (v/v) ethanol. The extracts were pooled and evaporated to dryness in a rotatory evaporator (Buchler Instruments, New York, N.Y., U.S.A.). The dried extract was dissolved in 0.5 ml. of water.

Paper chromatography. Samples ($50 \mu\text{l.}$) of the extract and samples ($20 \mu\text{l.}$) of the medium were chromatographed on Whatman no. 1 paper. The following solvent mixtures were used: solvent 1, butan-2-ol-acetic acid-water (40:11:25, by vol.); solvent 2, butan-2-ol-formic acid (85%, w/v)-water (15:3:2, by vol.); solvent 3, phenol-water-aq. NH_3 soln. (sp.gr. 0.88) (80:20:1, by vol.).

Lactate was detected by one-dimensional chromatography with solvent 1, and the amino acids were separated by two-dimensional chromatography with solvent 2 followed by solvent 3.

Quantitative radioactivity measurements. The radiochromatograms were scanned by the automatic scanning device developed in this Laboratory (Frank, Chain, Pocchiari & Rossi, 1959).

$^{14}\text{CO}_2$ was trapped on a filter-paper strip wetted with 0.2 ml. of 2.5 N-NaOH. At the end of the incubation period the $^{14}\text{CO}_2$ was immediately collected from the centre well of the Warburg vessel and precipitated with BaCl_2 as described by Vilee & Hastings (1949). $\text{Ba}^{14}\text{CO}_3$, after a washing, was transferred to aluminium planchets, dried under an infrared lamp and counted under a mica-window Geiger counter.

Enzymic determination of glutamate and aspartate in the tissue after incubation. For enzymic analysis of glutamate and aspartate the methods were essentially those described by Bernt & Bergmeyer (1963) and by Pfeleiderer (1963) respectively. We adapted them for determinations over smaller ranges.

RESULTS

Glucose and pyruvate metabolism. Triperidol at a concentration of 0.2 mM decreased oxygen uptake of brain-cortex slices incubated with either glucose or pyruvate by about 30%. [It was demonstrated (Gatti *et al.* 1966) that the effect of triperidol on oxygen uptake was maximal at a concentration of 0.1 mM and remained constant up to 0.4 mM.] The production of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]$ glucose or from $[\text{U-}^{14}\text{C}]$ pyruvate was influenced in the same way (Table 1).

The inhibitory effect on oxygen uptake occurred from the beginning of the incubation period and remained constant throughout the whole experiment. This is in contrast with the effect of chlorpromazine, which decreased oxygen uptake only after 30 min. of incubation (Lindan, Quastel & Sved, 1957). However, if K^+ ions in the incubation medium were replaced by Na^+ ions, triperidol had no effect on oxygen uptake (Fig. 1). Similar results were obtained previously with certain narcotics (Jowett & Quastel, 1937; McIlwain, 1953).

Triperidol did not alter the qualitative pattern of glucose and pyruvate metabolism in rat brain-cortex slices. As was previously shown (Beloff-Chain *et al.* 1955b; Tower, 1958) the disappearance of radioactive substrates from the incubation medium was accounted for as aspartate, glutamate, GABA,* alanine, lactate and carbon dioxide. When glucose was the substrate, glutamine was also found (Tower, 1958; Kini & Quastel, 1959; Chain, Chiozzotto, Pocchiari, Rossi & Sandman, 1960). Quantitatively, triperidol decreased the glucose or pyruvate utilization by about 20% (Table 2). Production of radioactive lactate, aspartate and

* Abbreviation: GABA, γ -aminobutyric acid.

Fig. 1. Effect of triperidol on uptake of O_2 in rat brain-cortex slices incubated with glucose in the presence or absence of K^+ ions. Tissue (100mg.) was incubated in 1.2ml. of phosphate-buffered medium, pH 7.4, at 37° in O_2 . The concentration of glucose was 5.5mM and that of triperidol, when present, was 0.2mM. ●, Medium with K^+ , no triperidol; ○, medium without K^+ , no triperidol; ▲, medium with K^+ , with triperidol; △, medium without K^+ , with triperidol.

noteworthy that in our experiments the amount of glucose converted into glutamate was almost unchanged in the incubation medium, changing only from 12.2 to 11.4 $\mu\text{g.}/100\text{mg.}$ of tissue whether triperidol was present or not, whereas in the tissue extracts the presence of the drug decreased the conversion of glucose into glutamate from 72.6 to 43.5 $\mu\text{g.}/100\text{mg.}$ of tissue.

To elucidate the difference in the effect of the drug on the incorporation of glucose radioactivity into glutamate and aspartate the specific radioactivities of both amino acids were determined. It was found that the relative specific radioactivities (Table 4) of aspartate and glutamate were decreased similarly by about 30% in the presence of triperidol.

Glutamate, aspartate and glycine metabolism. Triperidol decreased the oxygen uptake of brain-cortex slices incubated with glutamate by about 20%. This decrease was reflected in the $^{14}\text{CO}_2$ production (Table 1). Triperidol did not significantly affect oxygen uptake or $^{14}\text{CO}_2$ formation (Table 1) when the incubation medium contained aspartate or glycine. It is well known that these amino acids do not cause an increase of tissue respiration above the endogenous value (Weil-Malherbe, 1936).

The qualitative pattern of glutamate and aspartate metabolism in rat brain-cortex slices was not affected by triperidol. As previously shown (Sellinger, Catanzaro, Chain & Pocchiari, 1962), the disappearance of radioactive glutamate from the incubation medium was accounted for as aspartate, glutamine, GABA and carbon dioxide, whereas the disappearance of radioactive aspartate was accounted for as glutamate, GABA, citrate, malate and carbon dioxide. Glycine was practically not metabolized, as had been demonstrated by Abadom & Scholefield (1962). When slices were incubated with glutamate or aspartate as substrate the amount of either amino acid found in the tissue was increased by the presence of triperidol. This penetration of the amino acids from the incubation medium into the tissue seemed to be independent of oxygen uptake or subsequent metabolism of the amino acids. The swelling of the tissue slices that occurs during incubation was not responsible for the increased amount of amino acids found in the tissue. This swelling was the same whether triperidol was present or not. In addition, when galactose was the radioactive substrate, its penetration from the medium into the tissue was not affected by the drug (Table 5).

When glutamate was used as substrate, triperidol increased the formation of radioactive GABA and glutamine in the tissue, and decreased the overall concentration of aspartate in the tissue and medium. With aspartate as substrate, glutamate and GABA formation was increased (Table 6).

Table 2. Effect of triperidol on substrate utilization in rat brain-cortex slices incubated with various $U\text{-}^{14}\text{C}$ -labelled substrates

Experimental conditions were as described in Table 1. The methods of preparation of tissue extracts after incubation and the methods of chromatography were those described by Beloff-Chain *et al.* (1955a). The substrate utilization was calculated as the sum of unchanged substrate found in the tissue extract and all metabolites found in the medium and in the tissue extract. Mean values $\pm$ S.E.M., with the numbers of experiments in parentheses, are given.

Substrate	Substrate utilization ($\mu\text{g.}/100\text{mg.}$ of tissue/hr.)	
	No triperidol	With triperidol
Glucose (6)	1007 $\pm$ 34	814 $\pm$ 31
Pyruvate (6)	735 $\pm$ 19	597 $\pm$ 20
Glutamate (6)	462 $\pm$ 9	524 $\pm$ 5
Aspartate (3)	271 $\pm$ 13*	342 $\pm$ 10*
Glycine (3)	124 $\pm$ 8	190 $\pm$ 10

* Some radioactivity present in citrate and malate was included.

alanine was not affected by the drug. The formation of radioactive glutamine from glucose was slightly diminished. However, triperidol decreased considerably the formation of radioactive glutamate and GABA from both substrates (Table 3). It is

Table 3. *Effect of triperidol on glucose and pyruvate metabolism in rat brain-cortex slices*

Experimental conditions were as described in Table 1. Mean values of six experiments $\pm$ S.E.M. are given for the conversion of glucose or pyruvate into various metabolites. GABA, γ -aminobutyric acid.

Metabolite	Glucose converted ($\mu\text{g./100 mg. of tissue/hr.}$)		Pyruvate converted ($\mu\text{g./100 mg. of tissue/hr.}$)	
	No triperidol	With triperidol	No triperidol	With triperidol
Aspartate	10.9 $\pm$ 0.2	10.1 $\pm$ 1.0	26.4 $\pm$ 1.8	25.7 $\pm$ 1.2
Glutamate	84.8 $\pm$ 3.9	54.9 $\pm$ 1.7	79.6 $\pm$ 2.5	59.2 $\pm$ 3.3
Glutamine	11.5 $\pm$ 2.8	9.1 $\pm$ 0.8	0	0
GABA	14.3 $\pm$ 0.9	8.2 $\pm$ 0.7	9.4 $\pm$ 0.6	5.1 $\pm$ 0.7
Alanine	9.3 $\pm$ 1.0	10.1 $\pm$ 0.6	31.0 $\pm$ 0.4	29.0 $\pm$ 1.0
Lactate	647.9 $\pm$ 24.2	592.4 $\pm$ 29.0	252.0 $\pm$ 6.6	251.0 $\pm$ 8.6
CO ₂	228.5 $\pm$ 15.4	129.6 $\pm$ 6.0	335.7 $\pm$ 19.7	230.3 $\pm$ 15.9

Table 4. *Effect of triperidol on the concentrations of glutamate and aspartate and on their relative specific radioactivities in rat brain-cortex slices incubated with [U-¹⁴C]glucose*

Experimental conditions were as described in Table 1. Mean values of five experiments $\pm$ S.E.M. are given. Relative specific radioactivity is here defined as ratio of the specific radioactivity (counts/min./g. atom of C) of amino acid present in the tissue after incubation to the specific radioactivity (counts/min./g. atom of C) of the [U-¹⁴C]glucose initially present in the medium.

Metabolite	Amino acid in the tissue ($\mu\text{moles/g. of tissue}$)		Rel. sp. radioactivity	
	No triperidol	With triperidol	No triperidol	With triperidol
Glutamate	7.78 $\pm$ 0.38	7.35 $\pm$ 0.21	0.63 $\pm$ 0.08	0.40 $\pm$ 0.04
Aspartate	1.57 $\pm$ 0.12	1.94 $\pm$ 0.09	0.58 $\pm$ 0.08	0.43 $\pm$ 0.11

Table 5. *Effect of triperidol on the penetration of U-¹⁴C-labelled amino acids and of [1-¹⁴C]galactose from the medium into the tissue and on the swelling of rat brain-cortex slices*

Experimental conditions were as described in Table 1. The tissue swelling was calculated as the percentage increase of tissue weight after incubation. Mean values $\pm$ S.E.M., with the numbers of experiments in parentheses, are given.

Substrate	Substrate found in the tissue ($\mu\text{g./100 mg. of tissue/hr.}$)		% increase of tissue wt. after incubation	
	No triperidol	With triperidol	No triperidol	With triperidol
Glutamate (6)	76 $\pm$ 3	192 $\pm$ 3	36 $\pm$ 2	30 $\pm$ 5
Aspartate (3)	98 $\pm$ 8	156 $\pm$ 5	33 $\pm$ 5	30 $\pm$ 4
Glycine (3)	120 $\pm$ 9	187 $\pm$ 9	37 $\pm$ 2	29 $\pm$ 3
Galactose (4)	126 $\pm$ 3	114 $\pm$ 2	32 $\pm$ 3	33 $\pm$ 4

Table 6. *Effect of triperidol on glutamate and aspartate metabolism in rat brain-cortex slices*

Experimental conditions were as described in Table 1. Mean values $\pm$ S.E.M. of six and three experiments for glutamate and aspartate metabolism respectively are given. GABA, γ -aminobutyric acid.

Metabolite	Substrate glutamic acid converted ($\mu\text{g./100 mg. of tissue/hr.}$)				Substrate aspartic acid converted ($\mu\text{g./100 mg. of tissue/hr.}$)			
	Medium		Tissue extract		Medium		Tissue extract	
	No triperidol	With triperidol	No triperidol	With triperidol	No triperidol	With triperidol	No triperidol	With triperidol
Glutamate	—	—	76.0 $\pm$ 3.0	192.0 $\pm$ 3.0	45.0 $\pm$ 1.5	54.9 $\pm$ 4.1	8.0 $\pm$ 0.9	16.9 $\pm$ 1.4
Aspartate	132.9 $\pm$ 7.9	70.0 $\pm$ 5.8	43.3 $\pm$ 2.6	71.6 $\pm$ 4.0	—	—	98.0 $\pm$ 8.0	156.0 $\pm$ 5.0
Glutamine	24.6 $\pm$ 2.5	40.4 $\pm$ 1.8	3.9 $\pm$ 0.1	13.5 $\pm$ 1.6	0	0	0	0
GABA	0	0	6.9 $\pm$ 0.3	16.3 $\pm$ 1.1	0	0	0.4 $\pm$ 0.1	2.0 $\pm$ 0.2

DISCUSSION

The results reported in the present paper show that triperidol acts on the brain tissue *in vitro*. The effect on oxygen uptake and on $^{14}\text{CO}_2$ production, from labelled glucose, pyruvate or glutamate (Table 1), suggests that triperidol enters the cell and acts at the mitochondrial level. In this respect its action is similar to that of other central-nervous-system depressants, their activity being as respiratory inhibitors or inhibitors of oxidative-phosphorylation processes (Richter, 1961). The inhibitory effect of triperidol on oxygen uptake in the presence of glucose in the incubation medium depends on the presence of K^+ ions (Fig. 1). It is noteworthy that McIlwain (1964) found that haloperidol, belonging to the same butyrophenone group, partially inhibited the respiratory response of guinea-pig brain-cortex slices only when electrically stimulated.

The formation of lactate from glucose, on the other hand, is unchanged in the presence of triperidol (Table 3). One of the possible explanations for this occurrence is that the cytoplasmic concentration of ATP is sufficient for glycolysis in spite of decreased oxygen uptake.

In addition to its effect on oxygen consumption, triperidol increases the penetration of amino acids into the tissue, when these are the substrates. This increase is almost threefold for glutamate (Table 5); the oxygen uptake is decreased by about 30% (Table 1). If we accept that ATP is essential in the transport mechanism of the amino acids, the present findings are difficult to explain. It has been shown (de Almeida, Chain & Pocchiari, 1965) that cations influence oxygen uptake and L-histidine active transport in brain-cortex slices in opposite directions, demonstrating that active transport of amino acids is not necessarily correlated with oxygen uptake.

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