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EFFECTS OF PSYCHOPHARMACOLOGICAL AGENTS ON BRAIN METABOLISM—I.

EFFECT OF IMIPRAMINE AND PROTHIADENE UPON CONSUMPTION OF OXYGEN AND THE UPTAKE AND INCORPORATION OF L-PHENYL-ALANINE INTO PROTEINS AND LIPIDS OF BRAIN SLICES

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Abstract—Imipramine and Prothiadene causes an inhibition of oxygen consumption in slices of cerebral cortex at concentrations of 5×10^{-3}M and 5×10^{-4}M, but do not change oxygen consumption at a concentration of 10^{-4}M. At the same concentration Imipramine and Prothiadene reduce the uptake of L-phenylalanine by brain slices. Chlorpromazine at 5×10^{-3}M acts similarly to Imipramine and Prothiadene both on the oxygen consumption and uptake of L-phenyl-alanine.

Imipramine and Prothiadene at 5×10^{-3}M and 5×10^{-4}M significantly increases the incorporation of L-phenyl-alanine into proteins of cerebral cortex slices whilst at 10^{-4}M there is a non-significant decrease.

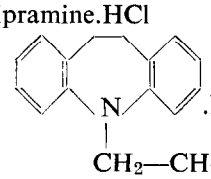
Chlorpromazine at 5×10^{-3}M does not affect the incorporation of L-phenyl-alanine into proteins.

Imipramine and Prothiadene, and to a lesser extent Chlorpromazine, at 5×10^{-3}M increase the incorporation of L-phenyl-alanine into the lipid fraction of cerebral cortex *in vitro*. At lower concentrations of Imipramine and Prothiadene the incorporation of L-phenyl-alanine into lipid fraction is not changed.

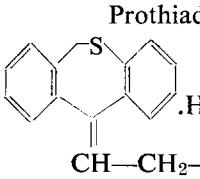
INTRODUCTION

IN AN effort to find further compounds with psychotropic effects Protiva *et al.* synthesized 11-[-3-dimethyl-amino propylidene]-6,11-dihydro-dibenz(b, c)-thiepin-hydrochloride [Prothiadene].[1, 2] This compound, together with the antidepressive drug 5-[3′-dimethylaminopropyl]-10, 11-dihydro 5 H-dibenz (b,f)-azepine (Imipramine, Tofranyl), has a similar antireserpine effect which is characteristic of thymoleptics.[3] Preliminary clinical tests[4] have also shown an antidepressive effect of Prothiadene.

Imipramine.HCl



Prothiadene.HCl



In experiments *in vitro* we tried to compare certain metabolic effects of Imipramine and Prothiadene with those of Chlorpromazine, which is structurally related.

EXPERIMENTAL

Animals. White rats, weighing 200–250 g and fed on standard Larsen diet, were used.

Chemicals. All chemical reagents employed (r.g.) were used without any further purification. Prothiadene and Imipramine were kindly supplied by Dr. M. Protiva of the Research Institute for Pharmacy and Biochemistry in Prague. U-^{14}C-L-phenyl-alanine of specific activity 5·02 mc/mmole was used (as provided by the Institute for the Production of Radioisotopes in Prague).

Oxygen consumption. Brain cortex slices were prepared with a hand microtome from whole brains washed in a Krebs-Ringer buffer solution (Medium II)[5] according to the method of Stadie and Riggs.[6] Slices weighing 30–40 mg were transferred to Warburg vessels which each contained 2 ml Krebs-Ringer buffer solution (Medium II). Into the main space Imipramine, Prothiadene, or Chlorpromazine in 0·2 ml water was added. The side-arm contained 2 μc U-^{14}C-L-phenyl-alanine (0·39 μM) in 0·2 ml, and the centre well contained 0·1 ml M KOH. The flasks were filled with oxygen, incubated at 38° and shaken at 110 oscillations per min. The oxygen consumption was measured after 10, 20, 30, 45 and 60 min.

Uptake of L-phenyl-alanine by brain cortex slices. After the incubation (60 min) the slices were removed, washed in a Krebs-Ringer buffer solution (Medium II), homogenized in 3 ml 5% trichloracetic acid (teflon pestle, glass test-tube) and the homogenate centrifuged. 0·3 ml of the supernatant were placed on an aluminium disc of 20 mm diam. and after drying the activity was determined with the aid of a GM window tube (1–1·5 mg/cm^2).

A phenyl-alanine standard ($10^{-2}\mu$c = 1·96 mμ mole) was simultaneously measured and the quantity of free phenyl-alanine (PHE) which had penetrated into the slices was determined according to expression:

$$\text{Uptake of PHE}[\text{m}\mu\text{ mole/g}] = \frac{\text{cpm} \times 1000 \times 10}{\text{cpm/m}\mu\text{ mole PHE} \times \text{mg slice (wet wt.)}}.$$

Incorporation of L-phenyl-alanine into proteins. The sediment obtained after centrifuging the slice homogenate in TCA was washed (always with 5 ml) 4 times in 5% TCA, twice in 96% ethanol, twice in an ethanol-diethyl ether mixture of 3 : 1 at 60° for 10 min, once in a chloroform-methanol mixture of 2 : 1 at 50° for 10 min, once in a petrolether-diethyl ether-acetone mixture of 6 : 3 : 0·5, again with 5% TCA for 15 min at 90°, once in an ethanol-diethyl ether mixture of 1 : 1 and once in diethyl ether.

Proteins obtained in this way were dissolved in concentrated formic acid and each sample was transferred in equal quantity onto two weighed aluminium discs of a 20 mm diameter. After the evaporation of formic acid, the discs, each bearing $\sim$ 1 mg of proteins were weighed, and the activity measured similarly to the measurement of the penetration of L-phenylalanine into the slices.

From the measured activities the relationship of mμ moles of L-phenylalanine incorporated in 100 mg of proteins per hr were calculated.

Incorporation of L-phenyl-alanine into lipids. Extracts obtained with ethanol and other lipid solvents were collected from each slice and evaporated at 50–70°. The residue was dissolved in a chloroform–methanol mixture of 2 : 1 and was transferred

to weighed aluminium discs. After drying and weighing the activity was measured and the results were calculated as mμ moles of L-phenyl-alanine incorporated into 100 mg lipids of cerebral cortex.

RESULTS

In agreement with published observations[7, 8] Imipramine and Chlorpromazine inhibit oxygen consumption at relatively high concentrations in brain mitochondria. Prothiadene acts similarly to Imipramine on oxygen consumption of brain cortex slices (Table 1).

TABLE 1. EFFECT OF IMIPRAMINE, PROTHIADENE AND CHLORPROMAZINE ON OXYGEN CONSUMPTION (RESULTS EXPRESSED AS μM/100 MG SLICE WEIGHT/HR ± SD)

Drug Concentration	Control	Imipramine	Prothiadene	Chlorpromazine
	15·78 ± 2·34 (28)*			
5×10^{-3} M		2·6 ± 0·59 (8)	3·0 ± 0·75 (7)	6·2 ± 1·13 (8)
5×10^{-4} M		6·5 ± 1·29 (8)	6·7 ± 0·61 (8)	—
10^{-4} M		17·3 ± 2·09 (12)	16·8 ± 1·7 (10)	
		p 1 : 2 ≪ 0·01	p 1 : 2 ≪ 0·01	p 1 : 2 ≪ 0·01
		p 1 : 3 ≪ 0·01	p 1 : 3 ≪ 0·01	
		p 1 : 4 ≫ 0·3	p 1 : 4 ≫ 0·3	

* Numbers in parenthesis indicate the number of experiments (usually 2 slices from one animal)

TABLE 2. UPTAKE OF L-PHENYL-ALANINE BY SLICES OF BRAIN CORTEX AND THE EFFECT OF IMIPRAMINE, PROTHIADENE AND CHLORPROMAZINE (RESULTS EXPRESSED AS MμM PHENYL-ALANINE/G/HR/± SD).

Concentration of Drug	Control	Imipramine	Prothiadene	Chlorpromazine
	169·3 ± 58·1 (30)	37·4 ± 17·8 (8)	35·2 ± 12·3 (8)	29·7 ± 12·5 (8)
5×10^{-3} M		31·3 ± 18·7 (8)	35·9 ± 17·7 (8)	—
5×10^{-4} M		101·5 ± 29·8 (12)	106·6 ± 25·6 (12)	—
10^{-4} M		p 1 : 2 ≪ 0·01	p 1 : 2 ≪ 0·01	p 1 : 2 ≪ 0·01
		p 1 : 3 ≪ 0·01	p 1 : 3 ≪ 0·01	
		p 1 : 4 < 0·01	p 1 : 4 < 0·01	

0·392 μM Phenyl-alanine (U-^{14}C) in 2·4 ml Medium.
Other data as in Table 1.

With decreasing concentrations of Imipramine and Prothiadene there is a similar decrease in the inhibition of oxygen consumption.

The penetration of free L-phenyl-alanine into slices was significantly reduced by Imipramine, Prothiadene and by Chlorpromazine at all concentrations used (Table 2).

The uptake of L-phenyl-alanine from the medium into the cells is obviously not primarily influenced by oxygen consumption of the slices as can be seen by comparing Tables 1 and 2.

The incorporation of L-phenyl-alanine into proteins is influenced by Imipramine and Prothiadene in an unexpected manner. Whereas at high concentrations of these drugs an increased incorporation takes place, at a lower concentration a significant decrease occurs. Further reduction of the Imipramine and Prothiadene concentration to 10^{-4} M causes the incorporation of L-phenyl-alanine to equal the values of the control (Table 3).

Chlorpromazine at a concentration of 5×10^{-3} shows a non-significant tendency to decrease the incorporation of L-phenyl-alanine into proteins.

TABLE 3. EFFECT OF IMIPRAMINE, PROTHIADENE AND CHLORPROMAZINE ON L-PHENYL-ALANINE INCORPORATION INTO PROTEINS. (RESULTS EXPRESSED AS M μM PHENYL-ALANINE /100 MG PROTEINS/HR/$\pm$ SD.)

Concentration of Drug	Control	Imipramine	Prothiadene	Chlorpromazine
	$5{\cdot}27 \pm 1{\cdot}4$ (28)	$6{\cdot}61 \pm 1{\cdot}33$ (8)	$7{\cdot}3 \pm 2{\cdot}8$ (8)	$4{\cdot}6 \pm 1{\cdot}61$ (8)
5×10^{-3} M		$2{\cdot}3 \pm 0{\cdot}81$ (7)	$2{\cdot}3 \pm 0{\cdot}84$ (8)	—
5×10^{-4} M		$5{\cdot}08 \pm 2{\cdot}7$ (10)	$4{\cdot}3 \pm 2{\cdot}35$ (10)	—
10^{-4} M		p 1 : 2 $<$ 0·05 p 1 : 3 $\ll$ 0·01 p 1 : 4 $>$ 0·3	p 1 : 2 $<$ 0·05 0 1 : 3 $\ll$ 0·01 p 1 : 4 $>$ 0·3	p 1 : 2 $>$ 0·1

Data as in Tables 1–2

TABLE 4. EFFECTS OF IMIPRAMINE, PROTHIADENE AND CHLORPROMAZINE ON L-PHENYL-ALANINE INCORPORATION INTO LIPIDS OF BRAIN CORTEX SLICES. (RESULTS EXPRESSED AS M μM PHENYL-ALANINE/100 MG LIPIDS/HR $\pm$ SD).

Concentration of Drug	Control	Imipramine	Prothiadene	Chlorpromazine
	$7{\cdot}63 \pm 2{\cdot}05$ (30)	$19{\cdot}7 \pm 4{\cdot}7$ (8)	$21{\cdot}9 \pm 5{\cdot}21$ (8)	$11{\cdot}4 \pm 4{\cdot}25$ (8)
5×10^{-3} M		$6{\cdot}52 \pm 1{\cdot}53$ (7)	$7{\cdot}2 \pm 1{\cdot}68$ (8)	—
5×10^{-4} M		$8{\cdot}21 \pm 2{\cdot}56$ (10)	$9{\cdot}52 \pm 3{\cdot}38$ (10)	—
10^{-4} M		p 1 : 2 $\ll$ 0·01 p 1 : 3 $>$ 0·1 p 1 : 4 $\gg$ 0·3	p 1 : 2 $\ll$ 0·01 p 1 : 3 $>$ 0·3 p 1 : 4 $>$ 0·3	p 1 : 2 $<$ 0·02

Data as in Tables 1–2

The incorporation of L-phenyl-alanine into lipid fraction is similarly affected. At a concentration of 5×10^{-3}M Imipramine as well as Prothiadene causes a marked increase ($\doteq$ 250 per cent) of L-phenyl-alanine incorporation into the lipid fraction while changes at lower concentrations of antidepressives are not statistically significant. (Table 4).

DISCUSSION

Metabolic effects of Imipramine and Prothiadene *in vitro* are parallel. This, and also the antireserpine action reported by Metyšová *et al.*[3] and the clinical observations of antidepressive action of Prothiadene,[4] supports the claims that Prothiadene is antidepressive.

In previous experiments on brain cortex slices we observed a significantly higher incorporation of ^{14}C into proteins when using U-^{14}C-L-phenyl-alanine instead of U-^{14}C-glucose, L-glutamic acid or L-aspartic acid. For this reason we used U-^{14}C-L-phenyl-alanine for studies on the effect of antidepressives upon the metabolic activity of proteins.

The influence of Imipramine upon the uptake of glycine in brain slices was studied by Abadom *et al.*[7] who also observed a decrease of Imipramine of 89 per cent at 10^{-3}M and 72 per cent at 5×10^{-4}M. Our observation when using L-phenyl-alanine instead of glycine was similar. At the same time the incorporation of glycine into proteins was significantly decreased. At 10^{-3}M of Imipramine it was entirely inhibited. On the other hand phenyl-alanine used under our conditions was more intensively incorporated into proteins at a concentration of the antidepressive of 5×10^{-3}M, although simultaneously oxygen consumption was almost entirely suppressed and the uptake of phenyl-alanine by slices was small. The incorporation of glycine described by Abadom *et al.*[7] differs from our observations with phenyl-alanine, in as much as a drop in the Imipramine concentration caused an increase of glycine incorporation.

The effect of Imipramine and Prothiadene on the lipid metabolism is very characteristic. We verified the assumption that L-phenyl-alanine is incorporated unchanged into the lipid fraction (into lipopeptides or lipoaminoacids) by means of paper chromatography of the lipid fraction hydrolysate. After the action of 6 N hydrochloric acid at 105° for 20 hr on the lipid fraction, amino acids were separated using Partridge mixture of n-butanol-acetic acid-water. The radioactivity of lipids was localized on the chromatograms in the place of phenyl-alanine. For this reason we calculated the results as mμ moles of phenyl-alanine incorporated into the lipid fraction.

The effect of Imipramine upon the metabolism of lipids is known from the work of Fumagalli *et al.*[9] At 10^{-3}M of Imipramine the synthesis of fatty acids of neutral lipids in brain slices was increased when ^{14}C-acetate was used.

On the other hand, at a lower Imipramine concentration (10^{-4}M) fatty acids of phospholipids were synthesized more intensely. Our finding of increased phenyl-alanine incorporation into lipids of brain slices shows that Imipramine and Prothiadene may act in a different metabolic pathway for brain lipids.

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