IN VITRO OXYGEN UPTAKE BY TISSUES IN THE PRESENCE OF TRANQUILIZERS AND ANTIDEPRESSANTS

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ABSTRACT. The oxygen uptake by rat brain slices at 37° was evaluated by Warburg criteria before and after the introduction of various tranquilizers, antidepressants, phenobarbital and the antihistaminics, cyclizine and chlorcyclizine hydrochlorides, at levels of up to 100 μg and the differences analyzed for statistical significance. Reserpine chlorpromazine, prochlorperazine dimaleate, azacyclonol, benactyzine, hydroxyzine, chlordiazepoxide, diazepam, oxanamide, chlormezanone, chlorprothixene and doxepin and the antidepressants, imipramine, protriptyline and nialamide in addition to phenobarbital, depressed brain oxygen uptake. The *in vitro* respiration of brain and liver slices from rats injected s.c. 1 hr previously with several of the drugs, was in the range of the respective saline-injected controls.

Introduction

Few systematic studies are available on the action of tranquilizers and antidepressants on the *in vitro* oxygen uptake by brain and other tissues, except for the phenothiazines, notably chlorpromazine (CPZ). The latter has been investigated in regard to blood sugar (Gupta *et al.*, 1960; Jori *et al.*, 1964; Susten *et al.*, 1971) and has been shown to inhibit cytochrome oxidase and adenosine triphosphatase (Abood, 1955; Bernsohn *et al.*, 1956; Dawkins *et al.*, 1959, 1960; Williams *et al.*, 1963), succinoxidase (Helper *et al.*, 1958), alcohol dehydrogenase (Wollemann and Keleti, 1962; Khouw *et al.*, 1963), brain phosphorylase (Iriye and Simmonds, 1967), brain cyclic 3'5'-adenosine monophosphate (Uzinov and Weiss, 1971) and liver mitochondrial NADH-cytochrome c reductase (Dawkins *et al.*, 1959), among others. Sodium and potassium-activated adenosine triphosphatase of beef brain was also depressed by hydroxyzine, haloperidol and diazepam (Ueda *et al.*, 1971). An uncoupling of oxidative phosphorylation of brain was viewed as a prominent feature in the pharmacological action of CPZ (Abood, 1955; Bernsohn *et al.*, 1956) and an inhibition of oxidative phosphorylation also occurred with liver mitochondria (Gallagher *et al.*, 1965).

With respect to the oxygen uptake by animal brain, low to moderate levels of CPZ proved inhibitory (Courvoisier *et al.*, 1953; Peruzzo and Forni, 1953; Finkelstein *et al.*, 1954; Kok, 1956; Lindan *et al.*, 1957a,b; McIlwain and Greengard, 1957; Kozák *et al.*, 1958; Yanagawa, 1958) as was likewise the case with imipramine (Abadom *et al.*, 1961). According to Bernsohn *et al.* (1956), the aerobic respiration of brain was inhibited while the anaerobic metabolism was insensitive to CPZ. The latter was without effect on the respiration of such tissues as liver (Peruzzo and Forni, 1953; Grenell *et al.*, 1955). The distribution and concentration of several tranquilizers in the brain have been investigated. Thus, CPZ was found to inhibit the metabolism of the hypothalamus and the posterior lobe of the pituitary gland (Wase *et al.*, 1956; Larsson, 1961) and the accumulation of phenothiazines in rat brain following their injection has been reported by Mahju and Maickel (1969). Amitriptyline readily enters the brain (Hucker and Porter, 1961) and its distribution has been demonstrated in rabbit and cat brain (Corona *et*

al., 1971; Cassano *et al.*, 1965a,b). Reports are also available on the concentration of diazepam in the newborn monkey brain (van der Kliin and Wijffels, 1971 and the uptake of imipramine by rat brain (Schneider and Schneider, 1969).

In the present study, a number of tranquilizers and antidepressants were investigated in relation to their action on the oxygen uptake by rat brain slices. The experiments were so implemented as to afford relatively similar conditions for the comparison of the effects of the agents. For additional correlations, phenobarbital and the antihistaminics, cyclizine and chlorcyclizine hydrochlorides were included. In yet another series, high levels of several of the agents were injected s.c. into rats and the oxygen uptakes by liver and brain slices compared with those of the respective controls.

MATERIALS AND METHODS

The drugs were bulk powders of USP or good grade and unless otherwise stated, where salts were employed, these comprised the hydrochlorides. The dosages are based on the latter. The agents and sources were as follows: captodramine (Ayerst); phenyltoloxamine citrate (Bristol); cyclizine and chlorcyclizine (Burroughs Wellcome); lyophilized Ritalin® hydrochloride or methylphenidate (Ciba); imipramine (Geigy); amitriptyline, chlorprothixene, chlordiazepoxide and diazepam (Hoffman-La Roche); phenaglycodol (Lilly); benactyzine, emylcamate and protriptyline (Merck Sharpe & Dohme); azacyclonol, oxanamide and pipradrol (Merrell); reserpine (Nutritional Biochemicals); doxepin, hydroxyzine and nialamide (Pfizer); CPZ, prochlorperazine dimaleate and tranylcypromine sulfate (Smith Kline & French); mephenesin carbamate (Squibb); chlormezanone (Sterling-Winthrop); ectylurea (Upjohn); meprobamate (Wallace) and promazine (Wyeth). In a few experiments, parenteral Valium® (Roche) and Serpasil® (Ciba) were used. Male Charles River rats of 250-300 g in weight comprised the experimental animals throughout. They were administered Teklad rat feed and water *ad lib.*

Each animal was sacrificed by swift decapitation, the brain removed and the cortex sliced in its entirety, the slices from 2-3 rats being pooled in chilled saline for each Warburg run. Portions of the randomized slices were gently blotted, weighed and transferred to the main chamber of the flask together with 1.8 ml of Locke-Ringer solution. The test drug as a solution or a very fine suspension in the same medium was introduced at a volume of 1.0 ml into the sidearm; 0.20 ml of 20% potassium hydroxide and fluted filter paper were inserted into the center well. A total of 2-4 drugs was investigated per run which involved 17 flasks in addition to the thermobarometer. The system was gassed with 100% oxygen and readings taken over an interval of 20 min following equilibration at 37°. The test solution was then tipped into the main chamber and after 10 min, readings were again recorded over a period of 20 min. The differences in oxygen uptake were determined and the significance tested by calculation of the Fisher *t*-values. The above procedures have been discussed previously in greater detail (Spencer *et al.*, 1964; Umbreit *et al.*, 1964).

With the injection series, the drug solution or saline was administered s.c. and the animals sacrificed 1 hr later. Brain as well as liver were sliced and processed as in the above except that 2.8 ml of Krebs-Ringer-phosphate solution comprised the medium and readings were taken over a period of 30 min; no fluid was used in the sidearm.

RESULTS AND DISCUSSION

The differences in Qo_2 or $\mu l\ O_2$/mg dry tissue/hr, before and after introduction of the drugs together with the respective standard errors (SE) and Fisher *t*-values are presented in Table 1. The agents were screened at 1.0, 10 and 100 μg, the 10 μg-level being the more prominent one. Of the major tranquilizers, reserpine as a fine suspension at 1.0 μg/ml depressed the oxygen uptake of rat brain slices as was also the case with the parenteral product and with the two phenothiazines, the well-known depressant, CPZ and prochlorperazine; the difference with promazine was not significant. The triphenylmethane tranquilizers, azacyclonol, benactyzine and hydroxyzine, markedly suppressed brain oxygen uptake at 10 μg but captodramine, even at 100 μg, proved ineffective. Both diazepines diminished the oxygen consumption but the propanediol derivatives, mephenesin carbamate, meprobamate and phenaglycodol were without action at the levels screened. In the miscellaneous group of tranquilizers, ectylurea and emylcamate comprised inactive members, whereas chlormezanone, doxepin and chlorprothixene inhibited brain respiration. In this respect, the latter phenothiazine analog simulates the activity of CPZ.

Of the seven antidepressants listed in Table 1, the hydrazide, nialamide, depressed brain oxygen uptake in addition to the N-heterocyclic compound based on phenothiazine, imipramine. The latter finding is in agreement with prior studies (Abadom *et al.*, 1961). On the other hand, the homocyclic derivative, amitriptyline, was without effect at a comparable level but the closely allied protriptyline, caused an inhibition and at the 5% level of probability. In line with earlier reports, phenobarbital depressed the oxygen uptake when introduced at a level of 10 μg. Cyclizine and chlorcyclizine which were included with the idea of correlating greater inhibitory activity with the presence of the nuclear chloro-group as noted for the pair, promazine and CPZ, were without effect on the differences. In fact, possibly a change in the direction of a stimulation might be construed for cyclizine.

Oxygen uptake findings for brain and liver slices from 12 controls and groups of 10 rats each injected s.c. with very high dosages of meprobamate, Serpasil®, CPZ, Valium®, methylphenidate and hydroxyzine and sacrificed 1 hr later appear in Table 2. One value was obtained per tissue pool from each rat. Without exception, the mean values of the drug-treated rats were in the range of the respective controls. Presumably, the cellular changes or the extent of deposition of the drugs in the organs are not too great under these conditions. The negative findings with liver are also apparent from earlier studies (Peruzzo and Forni, 1953; Wase *et al.*, 1956). In this conjunction, Gey *et al.* (1965) reported that rats injected i.p. with a single dosage of CPZ, phenobarbital or reserpine underwent a depression in both glucose-6-phosphate and fructose-6-phosphate and in line with earlier accounts, brain glycogen accumulated in the presence of phenobarbital and reserpine but not with CPZ. A similar effect had been noted with a single injection of CPZ by others (Máthé *et al.*, 1961) but on repeated administration over protracted periods of time, the glycogen level rose.

Differences among a few of the psychotropic drugs are also evident from other types of *in vitro* metabolic studies. Saha and Ghosh (1972) reported an inhibition of glucose oxidation by yeast cells with several phenothiazine tranquilizers in addition to phenothiazine dima-

TABLE 1. Differences in Oxygen Uptake of Brain Slices in the Presence of Drugs[a]

Drug	Level 100 μg	10 μg	1.0 μg
Tranquilizers			
Rauwolfia Drugs			
Reserpine[b]		1.03 ± 0.26 (22)**	1.18 ± 0.18 (18)**
Serpasil®		1.25 ± 0.13 (18)**	
Phenothiazines			
CPZ		0.62 ± 0.06 (15)**	
Prochlorperazine dimaleate		1.49 ± 0.20 (18)**	
Promazine		0.63 ± 0.39 (17)	
Diphenylmethane Derivatives			
Azacyclonol[b]		1.73 ± 0.15 (21)**	
Benactyzine	3.10 ± 0.52 (17)**	1.25 ± 0.17 (12)**	
Hydroxyzine	1.81 ± 0.44 (15)**	1.05 ± 0.34 (15)**	
Captodramine	—0.17 ± 0.39 (16)	0.28 ± 0.31 (16)	
Propanediol Derivatives			
Mephenesin carbamate		0.28 ± 0.29 (22)	
Meprobamate	—0.63 ± 0.24 (18)	0.32 ± 0.31 (16)	
Phenaglycodol[b]			0.32 ± 0.49 (19)
Benzodiazepines			
Chlordiazepoxide		1.19 ± 0.40 (17)*	
Diazepam		0.84 ± 0.02 (15)**	
Miscellaneous			
Ectylurea	0.26 ± 0.23 (14)	—0.13 ± 0.41 (12)	
Oxanamide		0.54 ± 0.20 (19)*	
Emylcamate	—0.43 ± 0.27 (15)	0.11 ± 0.11 (13)	
Chlormezanone		1.01 ± 0.26 (20)**	
Chlorprothixene[b]			1.07 ± 0.06 (11)**
Doxepin		0.77 ± 0.27 (19)*	
Antidepressants			
Imipramine		1.14 ± 0.35 (19)**	
Protriptyline		0.57 ± 0.22 (19)*	
Amitriptyline		0.71 ± 0.36 (18)	
Pipradrol		0.24 ± 0.22 (19)	
Methylphenidate	—0.20 ± 0.34 (14)	0.32 ± 0.33 (17)	
Nialamide		1.05 ± 0.20 (14)**	
Tranylcypromine sulfate		0.46 ± .0.56 (13)	
Phenobarbital		0.58 ± 0.21 (18)*	
Cyclizine	—1.09 ± 0.54 (13)	—0.32 ± 0.50 (14)	
Chlorcyclizine	0.72 ± 0.72 (15)	0.57 ± 0.51 (21)	

[a] All values are Q_{O_2} differences (± SE), a positive one denoting a depression in respiration. A figure in parentheses refers to the number of flasks employed. The weight of tissue per vessel ranged up to 100 mg.

[b] Due to the low solubility of the drug, a fine suspension in saline was used.

*$P < 0.05$.

** $P < 0.01$.

TABLE 2. Respiration of Brain and Liver Slices from Rats Injected S.C. with Various Drugs and Sacrificed 1 Hr. Later

Drug	Number of rats	Dosage, mg/kg	Brain $Q_{O_2} \pm SE$	t	Liver $Q_{O_2} \pm SE$	t
Control (saline)	12		7.44 ± 0.19		4.48 ± 0.25	
Meprobamate	10	128	7.40 ± 0.34	0.62	4.43 ± 0.32	0.12
Serpasil®	10	4.00	7.99 ± 0.33	1.53	4.44 ± 0.29	0.10
CPZ	10	30.0	8.10 ± 0.30	2.00	4.55 ± 0.16	0.23
Valium®	10	10.0	7.10 ± 0.55	0.46	4.85 ± 0.13	1.19
Methylphenidate	10	20.0	7.19 ± 0.34	0.68	4.61 ± 0.26	0.35
Hydroxyzine	10	48.0	7.26 ± 0.27	0.56	5.06 ± 0.13	1.93

leate, reserpine and mephenesin, the activity being less with the last three agents. Of a group of drugs screened on the succinoxidase system, the phenothiazines displayed the strongest depressive activity, followed by reserpine and oxanamide was relatively inactive (Helper *et al.*, 1958). Brain succinoxidase was less sensitive than the liver system and the action of the agents was thought to be unrelated to the mechanism of tranquilizing action. In yet another investigation (Gershbein, 1966), CPZ in amounts of up to 0.50 mg, did not affect the oxygen uptake by the isolated rat diaphragm although both the glucose utilization and glycogen content were depressed. The phenothiazine, promazine, inhibited only the glucose uptake; barbituric acid, meprobamate and reserpine were without effect on any of these parameters. Accordingly, the above data do not parallel the current brain oxygen uptake findings but rather complement or amplify many of the physiological characteristics of these diversified psychotropic agents.

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