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EFFECT OF PHENCYCLIDINE ON OXYGEN CONSUMPTION OF RAT BRAIN MITOCHONDRIA *IN VITRO* AND *IN VIVO*

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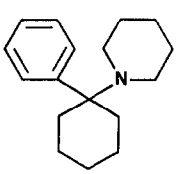
Abstract—Addition of phencyclidine, 1-(1-phenylcyclohexyl)piperidine, to rat brain mitochondria, enhances oxidation of succinate, glutamate, α-ketoglutarate and pyruvate + malate. Oxygen uptake increases with rising drug concentration up to a maximum of about 0·6 mM, beyond which it again decreases in all substrates but pyruvate + malate. Administration of phencyclidine to rats (10 mg/kg) results in reduced rates of oxygen uptake, in mitochondria isolated from their brains at 20 and 50 min after injection, with a minimum at 30 min. The reduction is more marked with pyruvate than with succinate. Oxygen uptake is gradually restored to normal by 70 min. The effect of some phencyclidine analogues on oxygen uptake *in vitro* is found to parallel the pharmacological activity of the drugs.

PHENCYCLIDINE has mixed stimulant and depressant properties in a large variety of animals.[1,2] Its effect is species-dependent and also varies with route of administration and dosage. Phencyclidine affects the respiratory rate of men and rats *in vivo*,[1–3] causing it to increase at small doses and to decrease at very large ones. Lees[4] has demonstrated that, in liver homogenate and mitochondria *in vitro*, oxygen consumption is augmented by low doses of phencyclidine, reaching a maximum at 0·8 mM for



Phencyclidine

α-ketoglutarate and 0·5 mM for succinate. At higher doses, it gradually decreases again. Lees[4] has also reported that pretreatment of rats with phencyclidine *in vivo* does not cause conclusive changes in the *in vitro* oxidation of succinate by liver homogenates.

In view of these studies and of the fact that phencyclidine is a drug principally affecting the central nervous system, we have considered it worthwhile to examine its effect on brain mitochondria *in vitro* as well as following its injection *in vivo*. The corresponding effects of some phencyclidine analogues have also been investigated.

MATERIALS AND METHODS

Male albino rats of our own stock, weighing about 150 g each, were used. For each *in vivo* experiment, the animals were divided into four groups, each group consisting of six animals. The first three groups were injected intraperitoneally (i.p.) with 2·5 and 10 mg phencyclidine/kg body wt, respectively. The fourth group served as controls and were injected with an equivalent volume (0·2 ml) of phosphate buffer 0·1 M. All the rats were used at predetermined times after injection.

For the preparation of mitochondrial fractions, the animals were killed by a blow on the head and bled. Their brains and livers were then removed immediately and placed in cold 0·33 M and 0·44 M sucrose solutions, respectively. Mitochondrial fraction was isolated from the brain according to the method of Løvtrup and Zelander[5] and suspended in 0·22 M sucrose. Liver mitochondrial fraction was isolated according to the method of Myers and Slater[6] in 0·33 M sucrose.

Oxygen uptake was determined manometrically by the conventional Warburg technique. The incubation medium employed consisted of: 150 μmole KCl; 6 μmole $MgSO_4$; 40 μmole K-phosphate buffer (pH 7·4); 3 μmole ATP, substrate as indicated in the results and 0·5 ml of mitochondrial suspension (corresponding to 1·5 mg protein) in 0·22 M sucrose solution. Sucrose was added up to isotonicity. The final reaction volume was 3 ml. The duration of each experiment (after a 10 min temperature equilibration) was 20 min, since, during this period, a straight line for the oxidation uptake was obtained. Incubation temperature was 37°.

For the determination of the P/O ratios, each flask contained, in one of its side arms, 60 μmole glucose and 0·5 mg hexokinase (Sigma type III). The other side arm contained 0·5 ml of 20% trichloracetic acid (TCA). After an equilibration period of 10 min, the contents of the first side arm was poured into the main chamber (for details of its contents see Results) and mixed. At the end of the incubation period, the TCA was added in order to terminate the reaction. In all cases, the center well contained 0·2 ml of 20% KOH on filter paper.

Phosphorus was measured by a modification of the method of Fiske and Subbarow.[7] Protein was determined according to the method of Lowry *et al.*[8]

All the chemicals used were of reagent grade. Phencyclidine and its analogues were synthesized and provided by Dr. A. Kalir *et al.*[9,10] The following compounds were tested:

(1) 1-[1-(2 Thienyl)cyclohexyl]piperidine,
(2) Phencyclidine-1-(1-phenylcyclohexyl)piperidine,
(3) *N*-Ethyl-1-phenylcyclohexylamine,
(4) 1-(1-Phenylcyclohexyl)pyrrolidine,
(5) 1-(1-Phenylcyclopentyl)piperidine,
(6) *N*-Methyl-1-phenylcyclohexylamine,
(7) 1-(1-Allylcyclopentyl)piperidine,
(8) 1-(1-Phenylcyclohexyl)piperidine methiodide.

The first seven compounds are tertiary amines, while the last one is a quaternary amine. Compounds 1–4, 6 and 7 are cyclohexylamine derivatives while compound 5 is a cyclopentylamine.

The drugs in the form of hydrochlorides were dissolved in water, while dilute

hydrochloric acid served for dissolving the free bases. Phosphate saline buffer (pH 7) was added to the solutions until its final concentration reached 0·1 M.

RESULTS AND DISCUSSION

The oxidation rates of succinate, α-ketoglutarate, glutamate and pyruvate, as a function of phencyclidine concentration in brain mitochondria, are presented in Fig. 1. It may be seen that, with rising concentration of phencyclidine, oxidation rates increase, reaching a maximum at about 0·6 mM. Further increase in drug contents causes a gradual decrease in oxygen uptake, except in the case of pyruvate + malate in which no significant further change occurs.

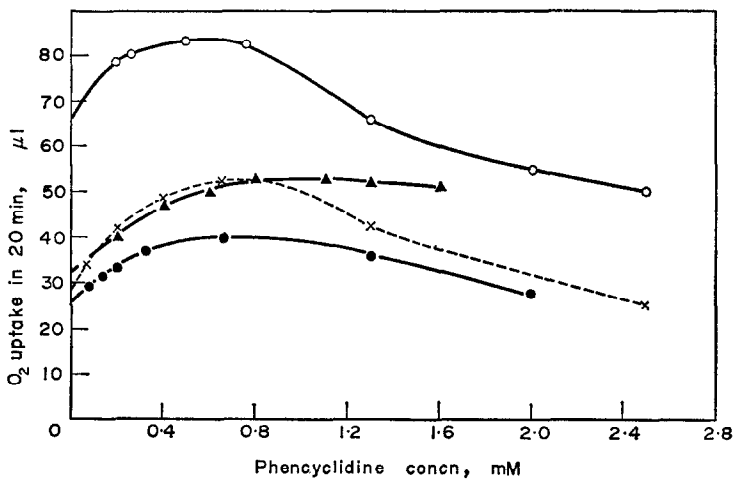


FIG. 1. Effect of phenocyclidine on the oxidation of various substrates by brain mitochondria *in vitro*. ○——○, Succinate, 50 µmole; ×– – –×, α-ketoglutarate, 60 µmole; ▲——▲, pyruvate + malate, 60 µmole + 5 µmole; ●——●, glutamate, 60 µmole.

The effect of the drug on the P/O ratios is presented in Table 1. P/O ratios decrease with increasing phencyclidine concentrations indicating that oxidative phosphorylation is uncoupled by the drug in the presence of succinate and all the DPN-linked substrates examined.

In order to verify whether the observed oxidative effects of phencyclidine *in vitro* could also be demonstrated after injecting it to the animal, *in vivo* experiments with different doses of the drug were performed. In accordance with the findings of Lees,[4] no conclusive results were obtained with liver mitochondria even at doses up to 10 mg/kg using succinate or α-ketoglutarate as substrates. Nor were any significant changes observed with brain mitochondria at doses up to 5 mg/kg, using succinate or pyruvate + malate. However, at a dose of 10 mg/kg, a distinct depression of oxygen uptake in the brain mitochondria was observed at 20–50 min after injection (Fig. 2), with a minimum at about 30 min. The effect was clearly more pronounced with pyruvate + malate than with succinate. These two substrates were chosen because of their being affected differently by increased concentration of phencyclidine added *in vitro* (Fig. 1).

TABLE 1. EFFECT OF PHENCYCLIDINE ON PHOSPHORYLATION

Substrate	Concn phencyclidine (mM)	$-\Delta Pi$ (μmoles)	ΔO (μatom)	P : O
Succinate	None	7·3	13	1·8
	0·5	4·8	7·4	1·5
	0·8	4·8	5·2	1·1
Glutamate	None	3·8	9·3	2·5
	0·5	3·9	6·3	1·6
	0·8	3·6	4·3	1·2
α-Ketoglutarate	None	5	12	2·4
	0·5	3·5	7·7	2·2
	0·8	2·7	4·6	1·7
Pyruvate + malate	None	5·2	12·5	2·4
	0·5	4·8	10	2·1
	0·8	4·4	7·9	1·8

Flask contents in 3 ml: 40 μmole phosphate buffer (pH 7·4); 150 μmole KCl; 10 μmole $MgCl_2$; 4 μmole ATP; 0·5 mg hexokinase and 60 μmole glucose. Substrates: 50 μmole succinate, 60 μmole glutamate; 60 μmole α-ketoglutarate, 60 μmole pyruvate + 5 μmole malate. 0·5 ml mitochondrial suspension (corresponding to 1·5 mg protein) in 0·22 M sucrose. Sucrose added up to isotonicity. Incubation temperature 30°.

The P/O ratios of various substrates in brain mitochondria isolated from injected animals were not affected by phencyclidine even at doses up to 10 mg/kg.

The respiratory depression observed by Chen *et al.*[1] in animals subjected to large doses of the drug, conforms with our findings *in vivo* regarding the changes in oxygen uptake. On the other hand, the increased respiratory rates at smaller doses of the drug, as reported by Domino,[3] have not been obtained in our system. The observed restoration of oxygen uptake at 70 min after drug administration (Fig. 2), which

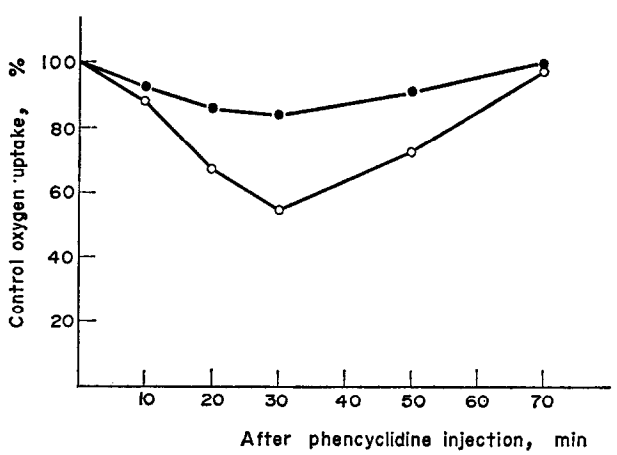


FIG. 2. Oxidation of succinate and pyruvate by brain mitochondria of rats injected with 10 mg/kg phencyclidine. ●——●, Succinate, 50 μmole; ○——○, pyruvate + malate, 60 μmole + 5 μmole. Each experimental point represents an average value obtained from six animals.

constitutes a relatively short duration of action for phencyclidine, points to its being rapidly metabolized. This indication agrees with the quick recoveries usually observed 2–3 hr after phencyclidine administrations (Domino[3]).

In order to clarify whether a parallelism exists between the pharmacological potency and the effect on oxygen uptake *in vitro*, the action on brain mitochondria of phencyclidine and a series of its analogues have been compared (Fig. 3), with pyruvate serving as substrate. The data seem to indicate that: (1) introduction of thienyl instead

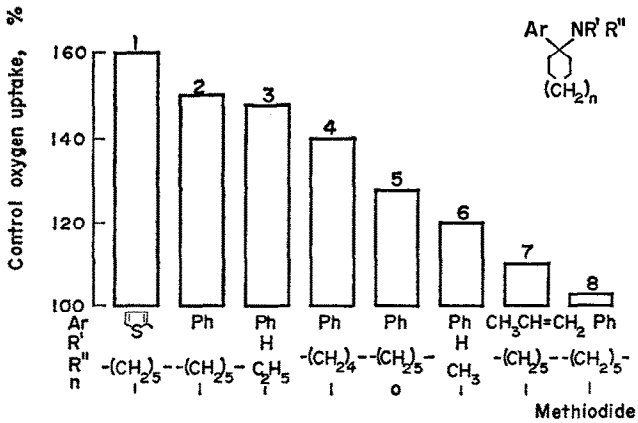


FIG. 3. Comparison of the effects of phencyclidine and its analogues on the oxidation of pyruvate by brain mitochondria.

of phenyl increases activity; (2) the cyclohexyl derivative is more active than the cyclopentyl one; (3) the ethyl derivative is more active than the methyl compound; (4) the quaternary amine is inactive. Obviously, the data represented in Fig. 3 is not sufficient for drawing definite conclusions as to what groups or combinations are responsible for the potency of the drug. Comparison of our results with the pharmacological data of Kalir *et al.*[6] points to a parallelism between pharmacological and biochemical activity. The only exception is the ethyl derivative; though, pharmacologically, it is the most active compound in the series, it ranks only third with respect to biochemical activity.

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