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INHIBITION OF STIMULATED CEREBRAL RESPIRATION *IN VITRO* AND OXYGEN CONSUMPTION *IN VIVO* IN RATS TREATED BY CHOLINOLYTIC DRUGS

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Abstract—The effects of different doses of *N*-methyl-3-piperidyl benzilate hydrochloride (JB-336/3), *N*-methyl-4-piperidyl benzilate hydrochloride (JB-336/4) and 3-quinuclidinyl benzilate hydrochloride (HNB-3) *in vivo* and at a range of concentrations of these cholinolytics *in vitro*, on oxygen consumption *in vivo* and on tissue respiration of rat cerebral cortex and medulla oblongata *in vitro*, have been studied.

The three cholinolytic drugs are shown to block stimulated respiration of rat cerebral cortex and medulla oblongata (at a concentration of 5×10^{-4} M) without demonstrable effects on unstimulated tissue. Qualitatively similar effects were observed *in vivo*.

A relation between central cholinergic synapses and energy metabolism in excitable tissues is suggested from the results described.

CHOLINOLYTIC drugs are generally esters of a heterocyclic amino alcohol and a glycolic acid, and possess strong anticholinergic properties. Many of these esters are potent anticholinergics, but some of them are 10- to 100-times more potent than atropine or scopolamine in their action on the central nervous system. Cholinergic blockade might be a criterion of predictability for psychomimetic potency but is not the mechanism of action.[1]

At present the psychomimetic and specially hallucinogenic mechanisms of action of these substances are not known. O'Neill *et al.*[2] have shown that six cholinolytic drugs, including Ditran (JB-339) blocked stimulated respiration of rat and guinea-pig cerebral cortex *in vitro*, without demonstrable effects on unstimulated tissues.

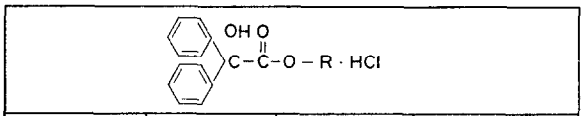
Much attention has recently been devoted to the actions of psychotropic drugs and the numerous reviews[3] and symposia reflect an intensive effort to find satisfactory explanations for their diverse actions at the biochemical level.[4]

In this laboratory three cholinolytic drugs which were previously known as psychomimetics[5] have been studied. This paper describes their selective inhibition of stimulated respiration *in vivo* and *in vitro*.

EXPERIMENTAL

The cholinolytic compounds used in this investigation are shown in Fig. 1.

Measurement of tissue respiration. Rats were stunned by a blow to the back of the neck and were exsanguinated by cutting across the carotid arteries and decapitated. The brain was removed and two slices (about 0·35 mm thick) were cut from each hemisphere and the medulla oblongata.[6] Each slice was weighed (30–50 mg) and



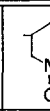
	R	Mp, °C	Soluble in water	LD_{50} (mg/kg, s.c.)
1		219–21	20 mg/ml	>1000
2		210–12	>2000 mg/ml	989·2 ± 42·9
3		238–40	535 mg/ml	644·4 ± 31·2

FIG. 1. (1) *N*-Methyl-3-piperidyl, benzilate hydrochloride (JB-336/3). (2) *N*-Methyl-4-piperidyl benzilate hydrochloride (JB-336/4). (3) 3-Quinuclidinyl benzilate hydrochloride (HNB-3).

immediately placed into Warburg vessels containing 3·2 ml of a medium of the following composition; NaCl, 135 mM; KCl, 5 mM; $MgSO_4$, 1·3 mM; $CaCl_2$, 0·5 mM; glucose, 12 mM and phosphate buffer, pH 7·4, 0·01 M. The medium was equilibrated before use with carbogen (95% O_2 + 5% CO_2). Respiratory rates were measured by the conventional Warburg technique[7,8] at 37°. Vessels were gassed for 5 min and then equilibrated for 10 min at 37°, before readings were taken at 10 min intervals for 60 min. When potassium-stimulated respiration was being measured readings were taken at 10 min intervals to establish the unstimulated respiratory rate before KCl (final concentration of 100 mM) was added from the side-arm. Readings were then taken for a further 50 min.

Results have been expressed in terms of μmole O_2/g wet wt of tissue/hr, and statistical analysis was by Student's *t*-test.

In experiments in which the effects of the cholinolytics were to be examined *in vitro*, the compound was added in 0·2 ml of 0·9% NaCl at the start of the experiment.

Treatment of animals. Male Wistar albino rats, weighing 244 ± 10·2 (S.E.)g, were used in all *in vivo* experiments. Control animals were injected subcutaneously with 1 ml 0·9% (w/v) NaCl solution, whereas experimental animals were injected with the required dose of the cholinolytic dissolved in 1 ml of physiological saline. Animals were killed 60 min after injection.

Measurement of oxygen consumption in vivo. Oxygen consumption in male rats was determined using apparatus for determining the oxygen uptake in small experimental animals as described by Harvey[15] and simplified by Spioch and Rozmus.[9] In each animal oxygen consumption was measured 60 min before and 60 min after injection of different doses of the cholinolytics used in this study. Each animal acted as its own control in this series.

Results have been expressed in terms of ml O_2/kg/hr and statistical analysis was by the Student's *t*-test.

RESULTS

Effects of cholinolytic compounds in vivo *and* in vitro *on the respiration of isolated slices of rat cerebral cortex and medulla oblongata.* In initial experiments *in vitro* we have repeated a study on the effects of JB-compounds and HNB-3 on the oxygen uptake in potassium-stimulated and unstimulated slices of cortex and medulla oblongata of control rats, similar to that reported by O'Neill *et al.*[2] The concentration of JB-compounds and HNB-3 used was 5×10^{-4} M in each experiment. Results are shown in Table 1.

TABLE 1. INHIBITORY EFFECT OF CHOLINOLYTICS ON POTASSIUM-STIMULATED RESPIRATION OF CEREBRAL CORTEX AND MEDULLA OBLONGATA

Drug	QO_2 (μmole O_2/g tissue/hr)		Inhibition (%)*
	Unstimulated	Stimulated	
1. Cerebral cortex			
Control	76·3 ± 2·3	121·5 ± 5·6	
JB-336/3	78·6 ± 1·9	95·3 ± 4·7	63·0
JB-336/4	81·2 ± 2·2	96·1 ± 3·9	67·0
HNB-3	86·7 ± 2·8	100·8 ± 3·3	68·8
2. Medulla oblongata			
Control	63·4 ± 1·3	89·5 ± 2·4	
JB-336/3	67·8 ± 1·7	73·8 ± 2·5	77·0
JB-336/4	65·4 ± 1·2	69·3 ± 1·8	84·7
HNB-3	61·1 ± 2·0	63·3 ± 1·9	91·5

For each value eight to ten animals were used. Results are expressed as mean ± S.E.M.
* Inhibition:

$$\frac{\text{Control (stimulated} - \text{unstimulated)} - \text{Drug (stimulated} - \text{unstimulated)}}{\text{Control (stimulated} - \text{unstimulated)}}$$

As can be seen, the respiratory rate was depressed to a significant extent ($P < 0{\cdot}001$) at concentrations used. All the drugs studied depressed stimulated respiration, from 63·0 to 68·8 per cent in cortex and from 77·0 to 91·5 per cent in medulla oblongata. Especially noteworthy are the apparent selective effects on respiration, since there was no demonstrable change in unstimulated respiration with any drug studied. In fact the drugs seemed to increase unstimulated respiration in the cortex by about 10 to 22 per cent, but these differences are not statistically significant ($P = 0{\cdot}5$).

Further experiments were carried out *in vivo*. Dose levels employed were (mg/kg): 5, 10, 15 and 20·0 of JB-336/3, JB-336/4 and HNB-3, injected subcutaneously.

Tissue respiration in slices of cerebral cortex and medulla oblongata were measured in the absence and in the presence of added KCl as described in Methods.

It can be seen, from results presented in Fig. 2, that the drugs studied depressed stimulated respiration in the range from 35 to 64 per cent of the control rate, but there were no demonstrable changes in unstimulated respiration with any drug studied.

Effects of JB-compounds on oxygen consumption in rats in vivo. Effects of different doses of JB-compounds on oxygen consumption in rats are shown in Table 2.

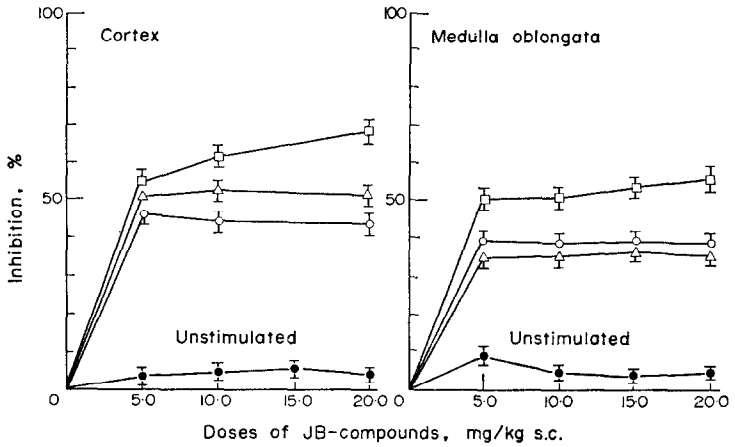


FIG. 2. Inhibitory effects of different doses of JB-compounds on the oxygen uptake in slices of rat cerebral cortex and medulla oblongata. The animals were killed 60 min after injection. Tissue respiration was measured manometrically in the absence (●——●) and in the presence of added KCl as described in methods. Each point represents the mean value of ten experiments (mean ± S.E.M.). (JB-336/3 ○——○; JB-336/4 △——△ and HNB-3 □——□).

The control rate of oxygen consumption was measured in each experimental animal during 60 min and then the animal was injected with one of the doses of JB compounds. The oxygen consumption was then measured under the influence of the cholinolytics during a further 60 min.

As can be seen there was no significant change in oxygen consumption in the rats treated by JB-compounds *in vivo*.

In the rat a small dose of soman (pinacolyl-methylphosphonofluoridate) caused a marked increase in oxygen consumption.[10] We have used this dose (0·025 mg/kg, s.c.) to produce stimulation of oxygen consumption in animals *in vivo* and to study the

TABLE 2. EFFECTS OF DIFFERENT DOSES OF JB-COMPOUNDS ON THE OXYGEN CONSUMPTION IN THE RATS

Compound	Dose (mg/kg)	QO_2 (ml O_2/kg/hr) Before JB (A)	QO_2 (ml O_2/kg/hr) After JB (B)	Effect (%; A/B × 100)
JB-336/3	4·0	732·5 ± 32	775·0 ± 31	+7·1
	8·0	732·2 ± 27	773·8 ± 28	+5·1
	15·0	719·3 ± 21	702·9 ± 22	−1·2
JB-336/4	4·0	741·0 ± 28	757·4 ± 26	+2·1
	8·0	702·5 ± 13	687·5 ± 30	−2·1
	15·0	690·4 ± 27	653·6 ± 25	−5·3
HNB-3	4·0	680·8 ± 23	588·4 ± 27	−13·5
	8·0	698·9 ± 28	620·0 ± 17	−11·2
	15·0	725·5 ± 32	640·3 ± 27	−11·7

Each value is the mean of the results from twelve animals. Results are expressed in ml-O_2/kg/hr as mean ± S.E.M.

TABLE 3. INHIBITORY EFFECTS OF JB-COMPOUNDS ON SOMAN-STIMULATED RESPIRATION IN THE RAT *in vivo*

Compound*	QO_2 (ml-O_2/kg/hr) Unstimulated	QO_2 (ml-O_2/kg/hr) Stimulated†	Inhibition (%)
Control	738·5 ± 24·2	986·2 ± 34·5	
JB-336/3	739·2 ± 34·3	756·8 ± 26·4	93·6
JB-336/4	738·5 ± 31·7	753·9 ± 26·2	93·7
HNB-3	742·6 ± 33·9	757·6 ± 30·8	94·3

* Doses of all compounds were 15 mg/kg, subcutaneously.
† Stimulation by 0·025 mg/kg of soman subcutaneously.
Each value is the mean from twenty animals. Results are expressed as mean ± S.E.M. Calculation of inhibition in per cent as in the legend to Table 1.

effects of JB-compounds on such stimulated respiration. Results of these experiments are shown in Table 3.

It may be seen from the results that respiratory activity *in vivo* can be increased by a small dose of soman injected subcutaneously. All the drugs studied depressed this stimulated respiration in the same range from 93·3 to 94·3 per cent of control. It could be seen that there was no demonstrable change in unstimulated respiration with any drug studied. The results show soman-stimulation to be somewhat more sensitive to the action of these drugs than potassium-stimulation since there is greater depression of the extra respiration *in vivo* than *in vitro* (Fig. 2 and Table 1).

DISCUSSION

The marked depression of stimulated respiration by JB-compounds *in vitro* noted earlier[2,11] is accompanied by a significant decrease in glucose utilization and lactic acid production. There are no significant effects on these parameters in unstimulated tissue. Our results *in vivo* and *in vitro* are in good agreement with these findings.

It was demonstrated that certain atropin-like spasmolytics interfere with acetyl–co-enzyme A-dependent system. This may be relevant to the proposal of Kini and Quastel[12] that potassium-stimulated processes are associated with pyruvate utilization via the Krebs cycle. It is possible that drug-sensitive enzymes in major or alternative metabolic pathways remain undetected, unless a shift in steady-state levels caused by stimulation *in vitro* or *in vivo* makes them rate limiting. In this situation inhibition would lower metabolic activity to the unstimulated condition and could thus explain why there is no depression of unstimulated tissue metabolism *in vitro* and the whole animal *in vivo*, when these enzymes are no longer rate limiting.

It is recognized that much work needs to be done before such speculations can be confirmed by experimental data. The value of results given in this paper lies in the fact that we obtained significant changes in respiration *in vivo* with JB-compounds in spite of the great number and heterogenicity of cells participating in overall metabolic activity.

One possible explanation of the effects of cholinolytic drugs on soman-stimulated respiration is that the drugs are interfering with soman itself to prevent its action. This possibility is being investigated.

In summary it can be said that cholinolytic drugs JB-series, known to cause mental disturbance and hallucination in human subjects, are without apparent effects on unstimulated respiration *in vitro* and *in vivo*. In rat cerebral cortex and medulla oblongata, potassium ions cause marked increases in rates of respiration.[13] The same effect has been obtained using small doses of soman (which did not produce the muscular exertion involved in fasciculations) given *in vivo*. JB-compounds and HNB-3 depress stimulated respiration to varying degrees depending upon the drug and the nature of the stimulus.

A tentative explanation is either a blockade of cholinergic central synapses[14] or inhibition of a drug-sensitive enzyme[12,13] which is only apparent when a change in steady-state makes the enzyme rate limiting, i.e. during stimulation by potassium ions *in vitro* or by small doses of soman *in vitro*.

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