EFFECTS OF LOCAL ANAESTHETICS ON RESPIRATION OF RAT BRAIN CORTEX *IN VITRO*

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THE presence of narcotics, at their narcotizing concentrations, produces inhibitions of the rate of respiration of brain cortex slices, but when these slices are bathed in a glucose-Ringer medium at 37 C., the inhibitions are usually small, about 6 to 32 per cent (1). When the respiration of the isolated brain tissue is stimulated by the addition of potassium ions or by electrical means, these inhibitions are greatly enhanced (2, 3). With such stimulated material, narcotics, at concentrations which, in the organism, produce deep narcosis, produce considerable inhibitions of respiration. These results (3) indicate that narcotics, at pharmacologically active concentrations, exercise marked inhibitory effects on the total respiration of the *stimulated* nerve cell by suppressing that aspect of the respiration which is responsive to the presence of high concentrations of potassium ions and which is concerned with carbohydrate (and pyruvate) oxidation in brain cortex. With the resting, unstimulated, nerve cell the respiration consists of processes which are largely neither highly narcotic sensitive nor stimulated by potassium ions. It is suggested (3) that this is the reason why pharmacologically active concentrations of a narcotic have so little effect *in vitro* on the diminished respiration of the unstimulated neurone. With stimulated nerve, however, the potassium-responsive, narcotic-sensitive aspect of respiration becomes the dominant phase. With all substrates where the presence of excess potassium brings about increased rates of respiration with intact brain cortex slices, narcotics at low concentrations exercise marked inhibitions of oxygen uptake. This effect may be so great as to cause a virtual disappearance of potassium stimulation. With both succinate and glutamate as substrates of rat brain cortex, there is no stimulatory effect of potassium and no increased sensitivity to narcotics. Apparently, a specific phase of respiration in brain, presumably one closely involving the operations of the citric acid cycle, is affected by narcotics at low concentrations. Evidence also indicates that narcotizing drugs (as exemplified by Amytal® and Chloretone®) are highly effective inhibitors of endogenous DPNH oxidation and its associated phosphorylations (4, 5, 6). In view of these conclusions, it was of considerable interest to discover whether local anaesthetics would affect brain cortex respiration, both in the stimulated and resting condition. There is already evidence to indicate that local anaesthetics affect

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neurone respiration. Sherif (7) has pointed out that procaine and cocaine inhibit respiration of the sciatic nerve of the rabbit. Watts (8) has studied the effects of local anaesthetics on brain metabolism and has shown a correlation between the relative ability of the drugs to inhibit aerobic utilization of various substrates and their relative potencies as local anaesthetics. Cocaine, like narcotics, suppresses respiration and aerobic acetylcholine synthesis in the brain (9) and it is claimed that it acts by blocking the entry of active acetate in the citric acid cycle. Synthetic local anaesthetics are considered to behave in the same way (10).

It is known that nerve conduction may be impeded by cocaine in concentrations which do not affect total respiration of the resting nerve (11). Experiments were carried out, therefore, to ascertain whether local anaesthetics affect the oxygen consumption of stimulated nerve, the stimulation being effected by exposure of brain tissue to relatively high concentrations of potassium ions.

Method

The experimental work was carried out using the conventional Warburg manometric apparatus. Rat brain cortex was used throughout. The animals were killed by decapitation and brain cortex sections were cut with a Stadie-Riggs slicer. The slices were weighed wet immediately and placed in Warburg manometric flasks containing a medium of the following composition: NaCl, 128 m*M*; KCl, 5 m*M*; $CaCl_2$, 0.6 m*M*; $MgSO_4$, 1.3 m*M*; KH_2PO_4, 1.3 m*M*; Na_2HPO_4, 10 m*M*. The medium was brought to pH 7.4 with *N* HCl. The medium contained glucose (0.01 *M*). Respiration took place at 37 C. in oxygen, and the liberated carbon dioxide was absorbed by 0.2 ml. of potassium hydroxide solution placed in the centre wells of the manometric vessels.

Potassium chloride solution, the anaesthetic solution, or the mixture of potassium chloride and the anaesthetic, was placed in the side tube of the manometric vessel. It was tipped into the medium containing the brain cortex slices when the normal respiratory rate had been measured for thirty minutes. The final volume in the Warburg vessel was 3.2 ml. The new respiratory rates, following the tipping of the solutions in the side arm into the main vessels, were carefully followed, and frequent readings were taken.

Results

Adding potassium chloride (0.1 *M*) solution to rat brain cortex respiring in a glucose-Ringer medium brings about an immediate increase of respiratory activity. This phenomenon was shown many years ago (12, 13) to take place with glucose, pyruvate, or lactate as substrates of the brain tissue. Respiratory activity was increased as much as 100 per cent, and this was confirmed in experiments by Ghosh

and Quastel (3). Effects of potassium on respiration, however, are variable, and Webb and Elliott (14) have rarely obtained increases of respiratory activity greater than 40 per cent. Much of the variability depends upon the preparation of the brain cortex slice. If brain tissue is minced, the accelerative effect of potassium on the respiration disappears. (In fact, inhibitory effects of potassium on the respiration of brain suspensions have been recorded (13).) If the brain cortex slice is prepared too thin, it tends to break up during the shaking in the Warburg flasks, and the potassium accelerative effect is likely to disappear; if the brain cortex slice is prepared too thick, its respiratory activity is not optimal owing to lack of full oxygenation in the

TABLE 1

EFFECTS OF LOCAL ANAESTHETICS ON RAT BRAIN CORTEX RESPIRATION AT 37 C. IN PRESENCE OF GLUCOSE

Anaesthetic Added	Number of Experiments	Average QO_2* Without Added KCl	Number of Experiments	Average QO_2* With Added KCl (0.1 *M*)
None	20	10.8	50	15.04
Procaine				
10 m*M*	13	9.8	6	13.9
20 m*M*	7	10.7	8	14.1
Lidocaine				
5 m*M*	9	10.7	8	12.7
Tetracaine				
1 m*M*	7	10.4	7	12.1
10 m*M*		Complete inhibition		
Dibucaine				
0.5 m*M*	5	10.2	6	12.2
1 m*M*		Complete inhibition within 20 minutes		

* Respiratory activity (QO_2) refers to the period of 30 minutes following the addition of KCl (0.1 *M*) or of the mixture of KCl (0.1 *M*) and anaesthetic or of the anaesthetic alone to the respiring brain tissue.

interior of the slice, and again the activity of added potassium is affected. It is, therefore, important to prepare brain cortex slices of suitable thickness to observe optimal potassium effects. Possibly other factors are also involved (10). Because of these circumstances, it is necessary to carry out a relatively large number of experiments when testing the effects of drugs on potassium-stimulated brain cortex respiration.

We have compared the effects of the local anaesthetics, procaine, lidocaine, tetracaine, and dibucaine. A minimum of six experiments has been carried out with each anaesthetic, and the results have been compared with those of control experiments (over fifty) in which no anaesthetic has been present with the brain tissue.

TABLE 2

EFFECT ON RESPIRATORY ACTIVITY OF RAT BRAIN CORTEX (IN GLUCOSE-RINGER-PHOSPHATE MEDIUM AT 37 C.) IN PRESENCE OF ANAESTHETIC, KCl (0.1 *M*), OR MIXTURE OF BOTH

Anaesthetic Added*	QO_2†		Percentage Fall in Respiration	QO_2†		Percentage Increase in Resp.	Net Percentage Increase in Resp. Due to KCl
	During First 30 Min. of Experiment	During Second 30 Min. of Experiment No KCl Added		During First 30 Min. of Experiment	During Second 30 Min. of Experiment After KCl* (0.1 *M*) Added		
None	11.8 ± 1.11	11.3 ± 0.76	4.3	11.8 ± 1.11	15.04 ± 1.85	30.8	35.1
Procaine							
10 m*M*	11.6 ± 1.22	9.8 ± 0.96	15.6	12.3 ± 1.45	13.9 ± 1.24	13.0	28.6
20 m*M*	12.1 ± 0.79	10.7 ± 0.34	11.5	12.6 ± 0.46	14.1 ± 0.54	11.9	23.4
Lidocaine							
5 m*M*	11.7 ± 0.77	10.7 ± 0.90	8.5	11.5 ± 0.52	12.7 ± 1.15	9.8	18.3
Tetracaine							
1 m*M*	11.7 ± 1.46	10.4 ± 1.08	11.1	12.0 ± 1.39	12.2 ± 1.62	1.7	12.8
Dibucaine							
0.5 m*M*	11.0 ± 0.68	10.2 ± 0.56	7.3	12.0 ± 0.46	12.2 ± 0.62	1.7	9.0

† Added at the beginning of the second 30 minutes of the experiment.
* All values of QO_2 (respiratory activity) are averages of at least six experiments. Standard deviations are given with each value of QO_2.

It soon became clear that with the potent anaesthetics, tetracaine or dibucaine, relatively high concentrations such as 10 m*M* brought about complete cessation of brain respiration. Hence, smaller concentrations were used, in quantities that were of pharmacological significance.

Results given in table 1 show that the average increase of rat brain cortex respiration brought about by addition of 0.1 *M* KCl was diminished by the presence of procaine (20 m*M*), lidocaine (5 m*M*), tetracaine (1 m*M*) or dibucaine (0.5 m*M*). These drugs, at the concentrations quoted, have little or no effect on the unstimulated brain cortex respiration.

An analysis of the results in this study of respiratory activities of rat brain cortex in the presence of anaesthetics and of potassium chloride is shown in table 2. The respiration of brain cortex normally falls (about 4 per cent) during the second half hour of the experiment

TABLE 3

INHIBITORY EFFECTS OF LOCAL ANAESTHETICS ON RAT BRAIN CORTEX RESPIRATION (IN PRESENCE OF GLUCOSE) STIMULATED BY ADDITION OF KCl (0.1 *M*)

Anaesthetic Added		Percentage Inhibition of Potassium Stimulation
Procaine	10 m*M*	18
	20 m*M*	33
Lidocaine	5 m*M*	48
Tetracaine	1 m*M*	63
Dibucaine	0.5 m*M*	74

and this fact is noted in the third column of table 2. The amount of this normal drop in respiration should be added to the increase in respiration secured by potassium addition, noted in the seventh column, in order to give a proper assessment of the net increase in respiration brought about by potassium addition (eighth column).

Results given in table 3 show the percentage inhibition of potassium stimulation owing to the addition of the local anaesthetic. They show clearly that the order of potency of inhibition of potassium stimulation is dibucaine > tetracaine > lidocaine > procaine. This order is similar to that existing between the relative anaesthetic activities of these drugs.

The results indicate that, within the limits of experimental variation in these experiments, local anaesthetics exert more powerful inhibitions of brain cortex respiration stimulated by potassium ions than of the unstimulated brain cortex respiration. Moreover, they show that, at pharmacologically active concentrations, local anaesthetics are capable of bringing about marked inhibitions of the stimulating action of potassium ions on nerve cell respiration. In this respect they resemble narcotizing drugs, such as the barbiturates or Chloretone (3). The extent to which they affect stimulated respiratory activity of peripheral nerve cells is still a matter for investigation.

Summary

The local anaesthetics, procaine, lidocaine, tetracaine and dibucaine, at pharmacologically active concentrations, inhibit the potassium-stimulated respiration of rat brain cortex in presence of glucose, though they have little or no effect at these concentrations on the resting, or unstimulated, brain cortex respiration. The decrease of stimulation of brain cortex respiration owing to the addition of potassium chloride (0.1 *M*) amounts to 74 per cent with dibucaine (0.5 m*M*), 63 per cent with tetracaine (1 m*M*), 48 per cent with lidocaine (5 m*M*) and 33 per cent with procaine (20 m*M*). The potencies of these drugs as inhibitors of potassium stimulation of brain cortex respiration parallel their anaesthetic activities.

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