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ATROPINE AND RELATED COMPOUNDS ON THE METABOLISM OF ELECTRICALLY STIMULATED SECTIONS OF MAMMALIAN CEREBRAL CORTEX

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Several substances which act on the central nervous system produce large effects on the metabolism of the brain during their action *in vivo*, but have little effect on the same metabolic processes when these are studied in preparations from the brain *in vitro*. In recent studies, narcotics and convulsants were shown to behave in this way towards the respiration and lactic acid formation of mammalian brain. It was concluded from this that the effects of the drugs on metabolism *in vivo* were secondary to effects which they had on functional activities of the brain, such as the transmission of impulses, and were thus likely to be shown only in tissue in a comparable state of activity (Buchel and McIlwain, 1950 ; Anguiano and McIlwain, 1951).

Means have now been developed (McIlwain, 1951a) of stimulating portions of separated mammalian cerebral cortex electrically, under good metabolic conditions. After stimulation, the levels of respiration, glycolysis, inorganic phosphate, and phosphocreatine of the tissue all changed in the sense in which they change during increased activity of the brain *in vivo*. These methods should therefore be applicable to the study of agents such as the narcotics and convulsants. The first necessity, however, was to examine further the extent to which the increased metabolism was related to increased activity of a functional character, e.g., to spread of impulses. For this reason we have examined first the actions of agents which might block such spread, and report in the present paper the effects of a group of substances related to acetylcholine. The metabolic processes examined have been respiration and lactic acid formation. These are the main energy-yielding reactions in nervous tissues.

EXPERIMENTAL

Tissue metabolism.—Details are given elsewhere (McIlwain and Grinyer, 1950 ; McIlwain, Buchel, and Cheshire, 1951). The brain was removed from rats and guinea-pigs, the cortex sliced, and the slices examined in aerated, glucose-containing salines. Respiration was determined manometrically by three- or five-minute readings, which enabled its course to be followed closely. Lactic acid was estimated in the saline at the end of the experiments. Also, in a few instances, the course of its formation was followed by manometric measurements of the CO_2 evolution from bicarbonate salines. For electrical stimulation of slices, tissues were fixed in electrodes D in vessels " A "

of McIlwain (1951a), where full experimental details are given. Substances to be added during an experiment were placed first in the sidearm of a manometric vessel.

Chopped tissue and slice-fragments were examined while suspended in nutrient salines in the electrode vessels D and E described by McIlwain (1951b), where details of the preparation of the tissue are also given.

Stimulation.—Alternating current was of 50 cycles per second and at the root mean square voltages which are indicated. Condenser pulses were diphasic, from a circuit (McIlwain, 1951a) in which a condenser was alternately charged and discharged from a battery, through the experimental vessel, with a pulse frequency of 100/sec. unless indicated otherwise. Duration and peak voltage of the pulses were measured with an oscilloscope ; the duration given is the time-constant (msec. required for the pulse to fall to 1/3 of its peak value).

RESULTS

Atropine on normal and electrically stimulated metabolism

On respiration.—Atropine, when added in concentrations up to 10^{-3} M to media in which normal unstimulated brain slices were respiring, was without effect on their rate of oxygen uptake. Table I and parts of Fig. 1 show this. In Fig. 1, rates are given by the slopes of the lines ; line A, *a* (without atropine) is of very similar slope to lines A, *b* ; B, *d* ; and C, *a* and *c* (with atropine).

In marked contrast to this was the effect of atropine on the respiration of stimulated slices. The extent of the electrical stimulation in the absence of added substances can be seen by comparing curves A and D (Fig. 1) or the initial part of curves

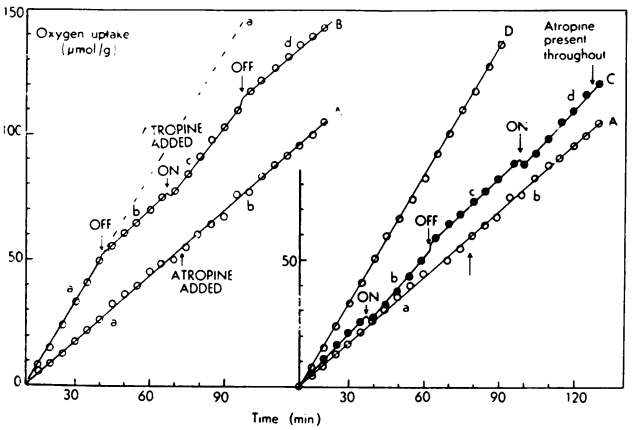

FIG. 1.—Atropine (final concentration 8×10^{-4} M) on respiratory response to stimulation. Cerebral cortex slices were fixed to electrodes in vessels containing glucose-phosphate saline saturated with O_2; CO_2 absorbed in NaOH-paper. Oxygen uptake was calculated from 5-min. pressure readings. Stimulation was by condenser pulses of 21–23V and time-constant 0.5 msec., applied as indicated. (A): unstimulated; atropine added at 66 min. (B): *a* and *c*, stimulated; atropine added at 66 min. (C) (closed circles): atropine present throughout; stimulated at *b* and *d*. (D): stimulated throughout.

A and B. The stimulation shown in the first part of B has been shown in other experiments to be maintained for long periods as indicated by D or the dotted line *a*. Table I shows that a typical increase on stimulation by the impulses used

was to 200 per cent of the normal rate. Condenser pulses of the characters quoted gave stimulation approaching the maximum of which the tissue was capable, with minimum applied voltage. Lesser voltage gave markedly less stimulation (Table I). The electrical energy which is applied in stimulating the tissue causes a very small rise in temperature of the experimental vessels (about 0.02° C.), which is seen in Fig. 1 as a small displacement of the lines at the points at which current has been started and stopped.

Stimulation in the presence of atropine gave a much smaller increase in respiratory rate. The contrast in Fig. 1 between lines D (without atropine) and C, *b* and *d* (with atropine) shows this. The action of atropine comes into effect quickly; the substance was added in B, *c* (Fig. 1) at the same time as stimulation was begun, and the stimulation is seen to be much less effective than in B, *a*. Moreover, the effect persisted for at least 1 to 2 hours after addition of the drug (C, *b* and *d*, Fig. 1).

TABLE I

ATROPINE AND RELATED COMPOUNDS ON RESPIRATION OF GUINEA-PIG CEREBRAL CORTEX

The experimental arrangement was similar to that of Fig. 1; the rates quoted below are the slopes of lines such as those shown in the Fig. As the action of atropine was found to be prompt and persistent, the experimental period during which the rates were measured has not been recorded; it varied between ½ and 2 hr. after the commencement of the experiment. Stimulation was always by condenser pulses, which were at the potential quoted and which were found by measurement on an oscilloscope to be of time constant 0.5–0.7 msec.

Preparation	Applied potential (V)	Drug added (M)	Rate of respiration (μmol./g./hr.)			
			Unstimulated		Stimulated	
			No drug	With drug	No drug	With drug
Slices in electrodes	24	Atropine 8×10^{-4}	60, 58, 60, 66, 61	62, 56, 60, 62, 61	130, 140, 124, 126 127	74, 82, 74, 81, 73
Slices in electrodes	24	Atropine 10^{-4}	60, 66, 61	61, 66, 63	124, 126, 127	124, 123, 120
Slices in electrodes	24	Atropine 10^{-5}	60	63	124	130
Slices in electrodes	12	Atropine 10^{-3}	61	61	98	61
Slices in electrodes	12	Atropine 10^{-4}	68	63	98	95
Slice-fragments, free	24	Atropine 8×10^{-4}	67	65	150	80
Chopped, free ..	24	Atropine 10^{-3}	68	64	109	68
Slices in electrodes	24	Hyoscine 10^{-3}	66	58	107	74
Slices in electrodes	24	Hyoscine 10^{-4}	72	69	127	109
Slices in electrodes	24	Hyoscyamine 10^{-3}	64	58	120	68
Slice-fragments ..	24	Cocaine 2×10^{-3}	63	61	125	66
Slice-fragments ..	24	Cocaine 2×10^{-4}	63	62	125	69

Several experiments are summarized in Table I in which the concentration of atropine, the preparation of the cortex, the stimulating potential, and the substances added were varied. The effective concentrations of atropine were found to be between 1 and 8×10^{-4} M (total concentration of both optical isomerides). Concentrations of 10^{-4} M and below had little effect, even with a relatively weak stimulus (12V) which gave an approximately 50 per cent increase in respiratory rate.

No marked difference was found when the effects of atropine on slices were compared with those on slice-fragments or on chopped cortex. Atropine has also

been found to act on some other parts of the brain (unpublished), but accurate comparisons have not yet been made. Throughout, the effects of the drugs on unstimulated respiration of the different preparations has been very small.

On glycolysis.—The stimuli effective in increasing respiration, without added drugs, have an even greater effect on the formation of lactic acid from glucose. Table II includes experiments in which the increase has been to over 300 per cent

TABLE II

ATROPINE AND COCAINE ON LACTIC ACID FORMATION

Slices of guinea-pig cerebral cortex were fixed to electrodes in a series of vessels containing glucose-phosphate saline saturated with O_2. Atropine, where indicated, was present throughout the experiment. Stimulation was by condenser pulses of 21–23V and 0.5–0.6 msec., applied to vessels with and without atropine; others were unstimulated. At the end of the experimental period, portions of the salines were taken for determination of lactic acid

Substance added (M)	Duration (min.) of		Lactic acid formation (μmol./exp.)			
	Whole exp.	Stimulation	Unstimulated		Stimulated	
			No drug	With drug	No drug	With drug
Atropine 8×10^{-4} ..	85	48	49	60	109	75
Atropine 10^{-4}, 10^{-5} ..	85	48	49	52	109	107, 109
Atropine 8×10^{-4} ..	92	50	34	38	109	58
Atropine 10^{-4} ..	92	50	34	34	107	85
Atropine 8×10^{-4} ..	125	90	31	—	87	39
Atropine 10^{-4} ..	125	90	31	—	87	78
Cocaine 2×10^{-4} ..	130	90	42	47	88	53
Cocaine 2×10^{-3} ..	130	90	42	55	88	65

of the initial rate. In calculating the percentage increase, use is made of the knowledge that the increased rate is regular, and that it begins promptly on starting the stimulus and ends promptly when stimulation is stopped. The active concentration of atropine was again between 10^{-3} and 10^{-4} M. Thus the mean stimulation without added substances, in the experiments of Table II, was to 390 per cent of the normal rate. The mean value in the presence of 10^{-4} M atropine was to 332 per cent, and with 8×10^{-4} M to 186 per cent. Control experiments showed these concentrations of atropine to have very little action on the normal glycolysis of unstimulated slices; lactic acid in the presence of 10^{-3} M atropine was found to be 106, 95, and 88 per cent and with 10^{-4} M 112 and 92 per cent of normal slices.

Manometric experiments showed the action of atropine to be prompt. These experiments, of which Fig. 2 gives examples, depended on the pressure changes observed in bicarbonate salines in equilibrium with O_2–CO_2 mixtures. Lactic acid displaces CO_2 from the saline, leading to increase in pressure, which is measured. Pressure is also affected by respiration, which absorbs O_2 and forms CO_2, but, as the respiratory quotient of brain cortex is nearly unity and remains so on stimulation, the respiratory exchange leads only to a small fall in pressure due to the greater solubility of CO_2 than O_2 in the saline. The pressure changes have been calculated as μmol. CO_2/g., which can be taken as approximately equivalent to the lactic acid formed per g. tissue; the measure is not exact, but has been shown

(McIlwain, Anguiano, and Cheshire, 1951) to be applicable to experiments of the present type.

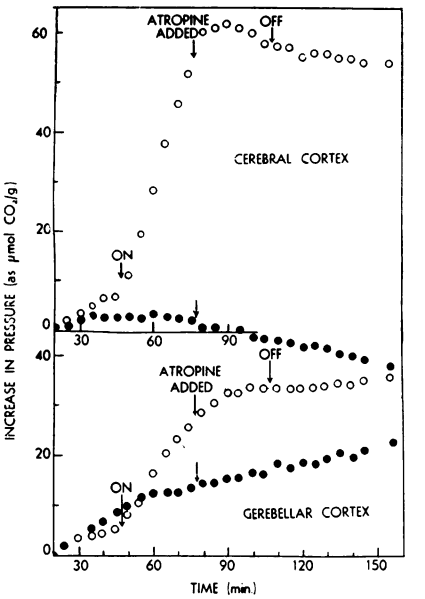

FIG. 2.—Atropine on glycolysis. Fragments of the tissues (average wt., 2 mg.) were floating freely in bicarbonate-glucose salines in equilibrium with O_2–5% CO_2. Open circles with both cerebral and cerebellar cortex: stimulation as described in Fig. 1 applied between 47 and 107 min.; closed circles: unstimulated. Atropine (to 8×10^{-4} M) added to all at 76–77 min.

In Fig. 2, atropine will be seen to have no effect on the course of acid formation by either cerebral or cerebellar cortex in the absence of stimulation. Stimulation greatly increased the rate of acid formation ; other experiments showed this increase to persist for over an hour. It was, however, promptly antagonized by atropine. The effect of atropine was seen within five minutes of its addition and became maximal within fifteen minutes. By this time the pressure change was becoming parallel in course to that of the unstimulated tissue, and little change took place on switching off the source of impulses.

Atropine on tissue stimulated by other means

The respiration and glycolysis of slices of cerebral cortex can be increased not only by electrical stimulation but also by the addition of certain substances to media in which they are metabolizing. In order to assess the relation between atropine and electrical stimulation, the effect of atropine on slices stimulated by these other means was examined. Experiments with 2 : 4-dinitrophenol and with high concentrations of potassium salts are shown in Table III. Potassium chloride was used at concentrations which gave a stimulation as great as that obtained electrically, and also at lower concentration giving a submaximal stimulation and presumably more scope for an antagonist. No antagonism was, however, shown by atropine to the action of KCl, when atropine was used at concentrations up to 10^{-3} M. These concentrations will be seen from previous Tables, and also from the parallel experiment of Table III, to have major effects in antagonizing the metabolic

results of electrical stimulation. The lack of antagonism to KCl extended to both respiration and glycolysis. Findings with 2 : 4-dinitrophenol were similar (Table III).

TABLE III

ATROPINE ON STIMULATION CAUSED BY VARIOUS AGENTS

Slices of guinea-pig cerebral cortex were used in glucose phosphate saline in a series of vessels. Atropine was present throughout in half of these, and the stimulating agents were added during the experiment; respiration was measured throughout and lactic acid determined at the end of the experiment. Electrical stimulation was by condenser pulses of 24V, and 0.5 μsec. duration

Stimulating agent	Respiration (μmol.O_2/g./hr.)				Lactic acid (μmol./g./exp.)		
	With no addition	With stimulating agent	With 10^{-3} M atropine	With stimulating agent and 10^{-3} M atropine	No addition	With stimulating agent	With stimulating agent and 10^{-3} M atropine
0.088 M-KCl ..	60	128	58	132	35	60	61
0.029 M-KCl ..	60	98	58	104	—	—	—
2×10^{-5} M-dinitrophenol ..	62	118	64	127	—	—	—
5×10^{-5} M-dinitrophenol ..	62	123	64	135	33	40	48
Electrical ..	62	117	64	66	33	66	31

Hyoscine, hyoscyamine, and cocaine

The concentrations of atropine active in the preceding experiments are relatively high (see Discussion), and information on their nature has been sought by examining some related compounds. Table I shows that, as would be expected, (-)-hyoscyamine was also active. (-)-Hyoscine was possibly slightly more active than atropine. Moreover, cocaine was as effective as atropine and acted in somewhat lower concentrations. The effect of cocaine extended to glycolysis also (Table II).

Eserine and acetylcholine

Eserine, in concentrations between 10^{-6} and 10^{-4} M, had no major effect on the respiration or glycolysis of unstimulated cerebral cortex (Table IV). Its effect on stimulated tissue was examined under conditions which gave a response approaching maximal, and also using stimuli which gave much smaller responses. Again, any effects were small. No consistent increase or decrease of the stimulation was observed, and atropine had its normal depressant effect in the presence of eserine. Submaximal stimuli were examined to see whether atropine caused any potentiation of the effect of electrical stimulation. The values of Table IV show small increases in some experiments but not in others, and in some experiments small comparable increases are shown in the absence of stimulation. None of these are considered significant.

In addition to the results quoted in Table III, experiments were run in bicarbonate saline and the course of excess acid formation followed manometrically. Again, no potentiation of submaximal stimuli was seen.

TABLE IV

ESERINE ON RESPIRATION AND LACTIC ACID FORMATION

Experiments used slices from the cerebral cortex of guinea-pigs, except with the last experiment, which was with rat tissue; they were with phosphate-glucose saline except where indicated. Stimulation was by condenser pulses at 100/sec. of the voltage and duration (time to fall to 1/3 of peak voltage) quoted. Vessels were unstimulated for two periods during the experiment, to obtain normal unstimulated rates of respiration; these were between 63 and 70 μmol./g./hr. The lactic acid formation refers to the whole experimental period

Preparation	Agent (M)	Stimulation		Respiration (% of normal controls)		
		V	Time-constant (μsec.)	Stimulated without drug	Stimulated with drug	Unstimulated with drug
Slices in electrodes	Eserine 10^{-4}	24	0.6	178	195	102
Slices in electrodes	Eserine 10^{-4}	24	0.6	170	164	94
Slices in electrodes	Eserine 10^{-4} + Atropine 10^{-3}	23	0.6	228	112	95
Slice-fragments, free	Eserine 10^{-4}	12	0.5	146	154	97
Slice-fragments, free	Eserine 10^{-4}	28	0.1	134	132	97
				Lactic acid formation (% of normal control)		
Slice-fragments, free	Eserine 10^{-4}	12	0.5	183	204	117
		28	0.1			
Slice-fragments, free (in bicarbonate saline)	Eserine 6×10^{-5} 6×10^{-5}	8	0.5	172	186	103
		20	0.1			

The effect of acetylcholine itself, in the presence or absence of eserine, was also examined. In these experiments, acetylcholine concentrations between 10^{-4} and 10^{-6} M were used, and these were sometimes present with eserine from the beginning of the experiment, and at other times added during the experiment. No stimulation (or depression) was caused by the acetylcholine, in respiration or glycolysis.

Discussion

The increased metabolic activity on stimulating electrically separated tissues of the central nervous system has only recently been observed (McIlwain, 1951a), and many aspects of its nature remain to be characterized. The present results contribute to this characterization. It is already known (McIlwain, 1951a, b, and unpublished exps.) that the stimulation *in vitro* is similar to that occurring during increased activity of the central nervous system *in vivo*, with respect to: (i) the nature and magnitude of the changes which take place in respiration, glycolysis, and in inorganic and creatine phosphates; (ii) the frequency, duration, and potential of the exciting impulses; (iii) the promptness and persistence of the responses. The present observations contribute to a fourth group of characteristics, namely those relating to added substances.

Atropine has been valuable in the present studies in giving a very clear example of a substance without effect on normal levels of respiration and glycolysis, but with a major effect on the increased respiration and glycolysis which result from electrical stimulation. Moreover, atropine is without effect on the increase in

respiration and glycolysis which is brought about by 2 : 4-dinitrophenol or by KCl. In magnitude, the effects of these agents are similar to that of electrical stimulation. A relatively specific relation of atropine to stimulation by electrical means is thus suggested.

Antagonism to electrical stimulation was found not only with atropine but also with hyoscine and cocaine. This action of cocaine may be compared to its action in blocking the passage of impulses in electrically stimulated peripheral nerves on local application (see, for example, Kato, 1936 ; Schaefer, 1942). The concentrations of atropine needed ($1 - 8 \times 10^{-4}$ M of the racemic compound) are much above those acting peripherally, and also are probably above those at which any specific central effects of atropine are observed after its general administration. It is doubtful whether even the 200 mg./day administered orally in some cases of Parkinsonism (Hall, 1937 ; Cohen and Craw, 1937), or the doses of up to 1 g. orally found to produce psychoses (see, for example, Alexander, 1946), would give concentrations of 10^{-4} M (29 mg./l.) in tissue fluids, though such values afford only an approximate basis for comparison. However, the concentrations found effective in the present experiments are certainly lower than those of 200 mg./l. (applied directly to the exposed optic lobes) found by Chauchard (1939–40) to alter the electrical response to stimulation of frog brain, and may be compared to the toxic intravenous dose of some 50 mg./kg. which shows central actions in dogs or cats (Koppanyi, 1939). Also, concentrations which are high in relation to those active *in vivo* may be necessary in our experiments on account of our means of stimulation or of the tissue chosen for the present work.

The present findings with atropine do not offer any unambiguous evidence with respect to the participation of cholinergic systems in electrical stimulation of the cortex. Findings with eserine and acetylcholine were also negative. With eserine, a range of concentrations was used, and submaximal electrical stimuli were applied in order to see possible augmentation of their effects, but none was found. As our findings are not exhaustive, they do not at all contradict the considerable evidence for cholinergic systems in the brain as a whole. Such systems appear much less important in some parts of the brain (Feldberg, 1945). Our observations do, however, illustrate properties of whatever systems are brought into action by the stimulation *in vitro*. In considering the neuronal elements which might be involved, observations on stimulation and recording at the cerebral cortex *in vivo* are also relevant. Spread of activity following such stimuli appears to involve dendritic connexions of cortical cells (Adrian, 1936 ; Chang, 1951 ; see also Burns, 1950) and can be inhibited by procaine. The sensitivity of the metabolic stimulation to other agents is being studied.

Summary

1. The increase in respiration and glycolysis of separated portions of cerebral cortex brought about *in vitro* by electrical stimulation was inhibited by atropine and by some structurally related compounds.

2. Atropine, hyoscine, hyoscyamine, and cocaine were effective in concentrations of about 10^{-4} M. When glycolysis had been increased to 300–400 per cent of its original rate, and respiration to about 200 per cent, these substances could reduce the processes to 100–150 per cent of their original rates.

3. The concentrations of the added substances which were effective as antagonists to the electrically stimulated metabolism had little or no action on normal respiration and glycolysis of the tissue.

4. Atropine was also ineffective in antagonizing similar increases in respiration and glycolysis brought about by potassium salts or by 2 : 4-dinitrophenol.

5. Eserine was without action on the normal or stimulated metabolism of the present preparations and acetylcholine with or without eserine did not produce the effects of electrical stimulation.

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