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The Action of Some Ergot Derivatives, Mescaline and Dibenamine on the Metabolism of Separated Mammalian Cerebral Tissues

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The substances examined (Fig. 1) are related structurally in so far as mescaline and dibenamine are phenylalkylamines and this type of structure is discernible in lysergic acid; they act to varying degrees on the central and sympathetic nervous systems. Their effects on the metabolism of separated cerebral tissues were examined to see whether a metabolic basis for the central actions could be detected, and also in order to explore the different ways in which the separated, electrically stimulated tissue responds to added agents.

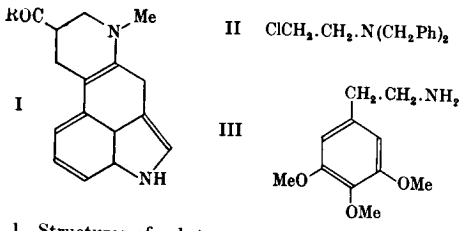

Fig. 1. Structures of substances examined. Lysergic acid diethylamide: I, R = NEt_2. Ergotoxine: a mixture of lysergic acid derivatives. Dihydroergotamine: dihydro derivative of I, R = $C_{17}H_{22}O_4N$. Dibenamine: II. Mescaline: III.

EXPERIMENTAL

Procedure

Guinea pigs were stunned by a blow on the back of the neck, bled, the brain removed, and for most experiments slices were cut as described by McIlwain (1951*a*). The dry wt. of such slices was 0·14 of their moist wt., and the moist wt. was used as basis for expressing metabolic rates. In some experiments of Table 2, tissue wa weighed while fresh (dry wt./fresh wt., ratio, 0·19) and chopped into prisms 0·35 × 0·35 × 2 mm. (McIlwain & Buddle, 1953). The slices used in most experiments were either trimmed into rectangles and floated on to silver grid electrodes type *H*, which were fitted into vessels type *A*, as described by Ayres & McIlwain (1953); or cut into a number of small pieces with dissecting scissors for use in manometric electrode vessels type *E*, with ring electrodes (McIlwain, 1951*b*).

Electrical stimulation was by condenser pulses (McIlwain, 1951*a*) at a frequency of 100/sec., time constant 0·3 msec., and a peak voltage of 18 v, except in certain instances described in the text when the voltage was altered or the series of pulses interrupted (McIlwain, 1954).

Respiration was measured manometrically in Krebs–Ringer phosphate–saline (Krebs & Henseleit, 1932) in O_2, the vessels being placed in the bath at 37·5° approx. 25 min. after the death of the animal and equilibrated for 10 min. Experiments were normally of 120 min. duration, consisting of a 30 min. unstimulated period, followed by 60 min. stimulation either at one voltage throughout, or two consecutive 30 min. periods at two different voltages, the lower voltage being used first; and finally a 30 min. unstimulated period. At the end of the experiments, samples of the fluid were taken for determination of lactic acid according to the method of Barker & Summerson (1941).

Materials

Mescaline sulphate was from Roche Products, ergotoxine ethanesulphonate, B.P. (1948), from Burroughs Wellcome, lysergic acid diethylamide and dihydroergotamine methanesulphonate from Sandoz Products and dibenamine hydrochloride from L. Light and Co. Ltd. They were dissolved in saline containing neither glucose nor calcium salts, at such a concentration that addition of 0·35 ml. to the 3·15 ml. of fluid in the vessels produced the desired final concentration.

α-Oxoglutaric acid and L-glutamic acid were titrated to pH 7·2 with NaOH; lactic acid was boiled with glass-distilled water, and pyruvic acid distilled, before neutralizing. These substrates were used at a final concentration of 20 mM; glucose was at 10 mM.

RESULTS

Effects on glucose metabolism

During the present series of experiments with glucose as substrate and in the absence of applied agents the tissue respired at about 54 μmoles O_2/g. moist wt./hr., and the lactic formed during incubation for 2 hr. was about 43 μmoles/g. Applied electrical pulses increased these values to about 109 μmoles O_2/g. moist wt./hr. and 84 μmoles/g., respectively, and the agents were all found in varying concentrations to antagonize the effect of the pulses. Fig. 2 shows a typical experiment.

Greater sensitivity of stimulated metabolism. A major finding of the present experiments is that all the agents examined markedly affected the metabolism of the separated tissue in the presence of applied electrical pulses at concentrations without

effect in the absence of pulses. Figs. 2 and 3 show this effect. With most agents the respiration in the presence of pulses was sensitive to one-thirtieth of the concentration of drug needed to affect the unstimulated tissue. Action with and without pulses was particularly well differentiated in mescaline, lysergic acid diethylamide and ergotoxine. With 10^{-3}M dihydroergotamine a small inhibition of respiration occurred in the absence of pulses and increased during the experiment.

Parallel effects on respiration and glycolysis. A second generalization which can be made from the results of Fig. 3 is that the agents examined affect respiration and glycolysis in parallel. Thus mescaline at 10^{-4}M is without effect on either process, while at 3×10^{-3}M respiration is inhibited by 68% and glycolysis by 71% in the presence of applied pulses. Similar phenomena are shown by lysergic acid diethylamide between 3×10^{-6}M and 10^{-4}M, by ergotoxine between 10^{-5}M and 10^{-3}M, and by dihydroergotamine except at 3×10^{-6}M.

Dibenamine presented a somewhat different picture in that its effect increased markedly during the progress of incubation, respiration falling progressively throughout the experiments. At 10^{-5}M the initial percentage increase on stimulation (Fig. 3) was 88% but during the 60 min. stimulation period the respiration fell until it represented only 32% increase over the unstimulated rate. At 4×10^{-4}M and 6×10^{-4}M no increased respiration was seen on the passage of pulses and the respiration fell markedly until it was only 40 and 30%, respectively, of the unstimulated rate in the absence of drug.

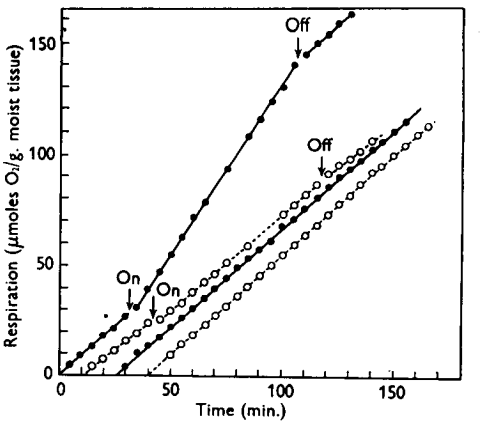

Fig. 2. Respiration of guinea pig cerebral cortex slices in the presence and absence of applied pulses (100/sec., peak potential 18 v and time-constant 0·3 msec.) with (○) mescaline (10^{-3}M) and without (●). Electrical pulses applied in two cases as indicated by the arrows. Abscissae displaced for clarity in the diagram.

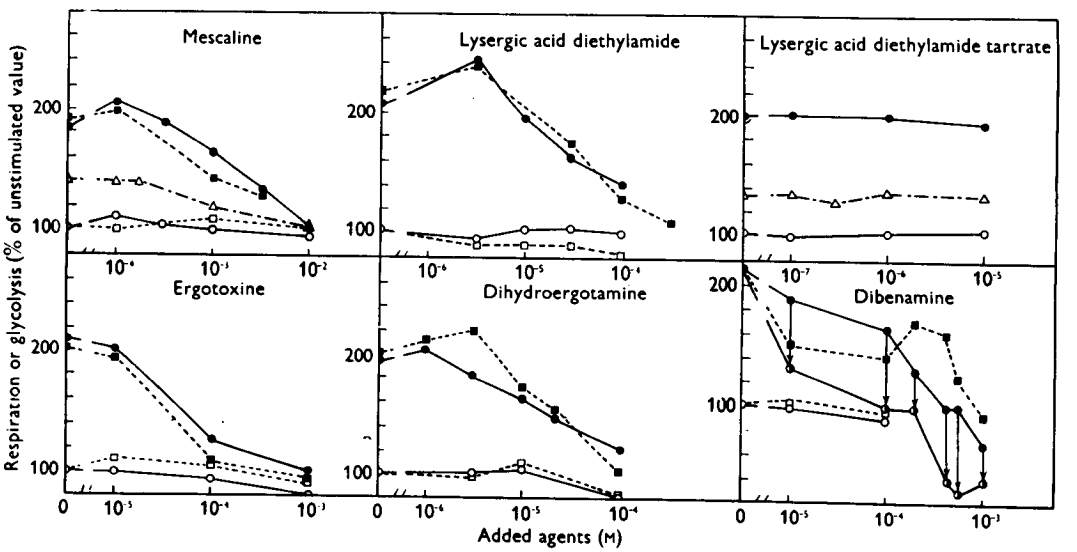

Fig. 3. Metabolism of guinea pig cerebral cortex: respiration with (●) and without (○) electrical pulses applied continuously; with interrupted pulses, △. Concomitant formation of lactic acid with (■) and without (□) pulses applied continuously. Pulses are described as continuous when applied at 100/sec., peak potential 18 v and time-constant, 0·3 msec.; and interrupted when applied in cycles of twenty such pulses followed by an interval of 2 sec. Ordinates give respiratory rates as percentages of the rate in unstimulated tissues without added agents, and the amount of lactic acid formed, again as percentage of the quantity in unstimulated controls. The arrows in the diagram with dibenamine run from points giving the result during the first 30 min. of stimulation, to those giving results during the second 30 min. Abscissae give the molar concentrations of added agents on logarithmic scales.

Lactic acid formation also showed a less regular relationship to the concentration of added inhibitor than was the case with other agents examined.

Types of electrical pulse. In the experiments described stimulation has been by pulses of exponential time–voltage relationship, applied in a continuous series. Greater sensitivity has been found to anticonvulsants (Forda & McIlwain, 1953) and to veratrine (Wollenberger, unpublished; McIlwain, unpublished), with interrupted series of pulses or with sine-wave currents. Fig. 3 shows the effects on respiration of lysergic acid diethylamide tartrate both with continuous and interrupted series of pulses. At concentrations between 10^{-5}M and 10^{-7}M, this substance was found to have little effect on the response to pulses whether continuous or interrupted. Experiments shown in the graph are with a stimulation period of 0·2 sec. every 2 sec. Other experiments (some of which are quoted in Table 2) with a stimulation-time of 0·2 sec. every 0·5 or 4 sec. similarly showed little effect. Experiments with lysergic acid diethylamide (Fig. 3) under slightly different conditions show a rise then a fall with concentrations of drug up to 10^{-5}M; this difference in response could arise due to the different conditions but does not affect the main conclusions. Mescaline also (Fig. 3) had no greater effect with interrupted pulses. Ergotoxine at concentrations of 10^{-5}M, 10^{-4}M and 10^{-3}M was found to have similar effects on respiration stimulated by sine-wave alternating currents of 50 cyc./sec. and by condenser pulses.

Sensitivity with different substrates

Table 1 shows the response of cerebral tissues with and without dihydroergotamine to applied pulses with substrates other than glucose. No response to pulses was seen when α-oxoglutaric acid or glutamic acid were substrates (McIlwain, 1951*c*; Kratzing, 1952); respiration fell throughout the experiment and no significant effect of the drug was seen. With pyruvic or lactic acids as substrates, pulses increased respiration, and dihydroergotamine almost completely prevented this increase at a concentration comparable with that acting when glucose was substrate.

Lysergic acid diethylamide was examined for its effect on stimulated metabolism of guinea pig cerebral cortex with glucose as substrate, at 2 mM as well as at the normal 10 mM, but no greater sensitivity to the drug was found. Dihydroergotamine (10^{-4}M) and ergotoxine (10^{-4}M) were without effect on the hexokinase activity of suspensions of guinea pig cerebral hemispheres, determined according to Long (1952).

Possible antagonists to ergot derivatives

Certain interactions between effects of dihydroergotamine and adrenaline, and of lysergic acid diethylamide and 5-hydroxytryptamine (serotonin) exist in the whole animal and in separated organs (Gaddum, 1953; Shaw & Woolley, 1953; and see below). Experiments to show possible interactions in separated cerebral tissues are summarized in

Table 1. *Metabolic response to electrical pulses with substrates other than glucose, in the presence of dihydroergotamine methanesulphonate*

Fragments of slices from guinea pig cerebral cortex (65–80 mg.) were in 3·5 ml. Krebs–Ringer phosphate medium in electrode vessels type *E* in O_2. Condenser pulses were applied at 100/sec., peak potential 18v and/or *10v and time-constant 0·3 msec. The drug, where present, was at 10^{-4}M in the medium before the tissue was added. All substrates were adjusted to pH 7·2 with NaOH. Results are quoted as mean values of μmoles O_2/g. moist wt./hr. with the standard error where applicable.

Substrate	No. of observations	Drug	No pulses (1st 30 min.)	With pulses (2nd and 3rd 30 min.)	No pulses (4th 30 min.)	Change with pulses (%)
α-Oxoglutaric acid	2	+	58	**52**	25	−11
	1	−	60	**48**	24	−20
	1	+	60	.	36	.
	2	−	58	.	40	.
Glutamic acid	2	+	77	**63**	40	−16
	1	−	80	**72**	52	−10
	1	+	81	.	57	.
	2	−	70	.	51	.
Pyruvic acid	2	+	51	**52**	42	+ 1
	2	−	59	**100**	55	+69
	3	+	54	.	54	.
	4	−	50	.	50	.
Lactic acid	8	+	70 ± 3·8	**83** ± 6·0	67 ± 5·0	+19
	12	−	67 ± 2·3	**128** ± 5·1	69 ± 2·8	+91
	6	+	65 ± 3·1	.	.	.
	11	−	63 ± 1·6	.	.	.
	2	+	59	**66***	58	+12
	1	−	65	**96***	70	+41

Table 2. *Absence of antagonism of dihydroergotamine by adrenaline, and of lysergic acid diethylamide by serotonin*

Slices from guinea pig cerebral cortex were used in Expt. *A*, in electrodes *H* in vessels *A* (Ayres & McIlwain, 1953) and the chopped tissue in Expt. *B* in vessels *E* (McIlwain, 1951*b*) in each case in 3·5 ml. phosphate-buffered glucose saline. The added substances were present together in the medium before the tissue was added. Condenser pulses were alternating of time-constant 0·3 msec., at intervals of 10 msec. and at the peak voltages quoted; when interrupted, the sequence was: 2 pulses applied, 50 pulses omitted, repeated throughout the 'interrupted' period. Rates are mean values from the number of experiments indicated in parentheses, and are followed by the S.E.M. where applicable.

Experiment *A*

Adrenaline (M)	Dihydroergotamine (M)	Respiratory rates: Without pulses (μmoles/g. wet wt./hr.)	Increase with pulses (%): Pulses at 8–10 v	Increase with pulses (%): Pulses at 18 v
0	0	57 ± 2 (5)	81 (2)	95 (3)
10^{-4}	0	56 ± 1·1 (7)	89 (2)	113 ± 8 (7)
0	10^{-4}	58 ± 1·9 (6)	27 ± 3 (5)	21 (3)
10^{-4}	10^{-4}	59 ± 1·9 (13)	25 ± 13 (6)	23 ± 6 (7)
0	2×10^{-5}	55 (2)	33 (2)	42 ± 3 (5)
10^{-4}	2×10^{-5}	56 ± 1·3 (6)	20 (3)	35 ± 7·9 (4)
10^{-5}	2×10^{-5}	54 (1)	.	32 (1)
10^{-6}	2×10^{-5}	56 (1)	.	40 (1)

Experiment *B*

Serotonin (M)	Lysergic acid diethylamide (M)	Respiratory rates: Without pulses (μmoles/g. fresh wt./hr.)	Increase with pulses (%): Interrupted	Increase with pulses (%): Continuous
0	0	75 (4)	33 (4)	76 (4)
10^{-5}	0	75 (2)	31 (2)	72 (2)
10^{-5}	3×10^{-5}	70 (2)	14 (2)	50 (2)
0	3×10^{-5}	72 (2)	12 (2)	57 (2)
0	8×10^{-6}	74 (2)	13 (2)	67 (2)
10^{-4}	0	72 (2)	30 (2)	74 (2)
10^{-4}	3×10^{-5}	70 (2)	17 (2)	42 (2)
10^{-4}	8×10^{-6}	73 (2)	16 (2)	36 (2)

Table 2. Passage of electrical pulses in vessel *E* with either gold-plated or molybdenum-ring electrodes was found to catalyse the oxidation of adrenaline, possibly to adrenochrome. However, with electrodes *H* which were of silver wire, the comparatively slow oxidation of adrenaline which occurred in oxygenated salines was not affected by applied pulses. These electrodes were used in the experiments shown in Table 2. In the absence of adrenaline and dihydroergotamine the respiration of the separated tissue was increased by 95% over the unstimulated value. The presence of 10^{-4} M adrenaline did not alter this value significantly. Dihydroergotamine (10^{-4} M) diminished the increase in respiration due to the passage of pulses, to 21%. Addition of 10^{-4} M adrenaline did not greatly influence this value. The above results were obtained on application of pulses at a peak potential of 18 v. Similarly, no evidence for antagonism between adrenaline and dihydroergotamine was found when the voltage was lowered and the concentrations of dihydroergotamine and adrenaline were varied.

Serotonin itself had little effect on respiration of cerebral tissues or on their respiratory response to pulses, and did not decrease but tended to increase the effect of lysergic acid diethylamide.

DISCUSSION

Present findings have given several further instances in which electrically stimulated metabolism of separated cerebral tissues is more sensitive to added agents than is their metabolism as ordinarily studied. In some cases the sensitivity of the stimulated tissue approaches that necessary to explain the effects of the drugs *in vivo*, though assessment of the tissue concentration under the two conditions remains necessary. Thus dibenamine has been used as an adrenergic blocking agent at 0·5–2 m-moles/kg. (Nickerson & Goodman, 1947) and rapid intravenous injection of 2–3 m-moles/kg. can produce convulsions. Solutions of 1 mM dibenamine had appreciable effect in the present experiments. Mescaline induces hallucinations in man when

10–50 μmoles/kg. are given intravenously or orally (Guttman, 1936; Salomon, Gabrio & Thale, 1949). Present effects are seen in guinea pig tissues at 300 μM mescaline.

The ergot derivatives are active *in vivo* in man and in laboratory animals at extremely low concentrations; dihydroergotamine and components of ergotoxine affect sympathetic responses and glucose tolerance tests at intravenous doses of 10^{-3}–10^{-2} μmole/kg. and show LD_{50} doses of 0·5 and 0·02 μmole/kg., respectively (see Rothlin, 1946–7). Lysergic acid diethylamide induces hallucinations in man and changes in blood glucose, hexose monophosphate, and adrenaline at about 2×10^{-4} μmole/kg. (Stoll, 1947; Mayer-Gross, McAdam & Walker, 1952; Liddell & Weil-Malherbe, 1953). Action of the amide in the present experiments was seen at 10 μM. The amide was also examined at lower glucose concentrations in view of its effects on glucose metabolism, but no greater sensitivity observed.

Considering the effects of the present agents only on the separated electrically stimulated tissue, interesting relationships emerge which are in contrast to the effects of several other added substances. Different concentrations of the ergot alkaloids and of mescaline either inhibited both the respiratory and glycolytic responses to pulses, or affected neither. With the many different concentrations and substrates and pulse types examined, this relationship held true, whereas a different group of substances has given the opposite result (Heald, 1953). Iodoacetate and fluoride depressed glycolysis at concentrations with little action on respiration; malonate decreased stimulated respiration while increasing glycolysis. Moreover, effects of iodoacetate, fluoride and malonate on respiration changed also with change of substrate from glucose to lactate. This latter contrasting group of inhibitory agents are known to act at fairly defined points in intermediary metabolism of the unstimulated tissue. By contrast, the present agents which affect similarly the different metabolic sequelae of applied pulses, may be considered to prevent as a whole the change in level of activity normally caused by applied pulses, and thus to be acting at points more immediately connected with the tissue's response to them than the energy-yielding reactions of glucose catabolism.

SUMMARY

1. Dihydroergotamine methanesulphonate, lysergic acid diethylamide, mescaline sulphate, ergotoxine ethanesulphonate and dibenamine hydrochloride have been examined for effect on respiration and formation of lactic acid by preparations of guinea pig cerebral cortex in glucose-saline media.

2. These processes in the normal tissue were not very sensitive to the added agents, but on stimulation by electrical pulses became much more sensitive, inhibition being observed with mescaline at 10^{-3}M and with the ergot derivatives and dibenamine at 10^{-5}M, these concentrations in several cases being one-thirtieth of those which affected metabolism in the absence of pulses. Dependence of inhibition on the type of pulse applied was not demonstrated.

3. Agents within the range of concentrations in which they were effective, inhibited to similar degrees both respiration and glycolysis. No difference in the sensitivity of respiration to added agents was observed when glucose was replaced as substrate by lactic acid, pyruvic acid, α-oxoglutaric acid or glutamic acid.

4. The effect of dihydroergotamine was not prevented by the simultaneous presence of adrenaline, nor that of lysergic acid diethylamide by 5-hydroxytryptamine.

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