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Action of Protoveratrine on the Metabolism of Cerebral Cortex

2. ELECTRICALLY STIMULATED CEREBRAL-CORTEX TISSUE

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(Received 29 December 1954)

In the preceding communication (Wollenberger, 1955) it was reported that addition of small amounts of protoveratrine to freshly cut sections of guinea pig cerebral cortex causes, amongst other metabolic changes, an acceleration of oxygen con-

sumption. The metabolic conditions which were required for this acceleration to take place were similar to those which had previously been found in this laboratory to be essential for acceleration of the metabolism of cerebral-cortex slices by means of electrical pulses. The present experiments were started in order to see whether these pulses and

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protoveratrine acted synergistically on cerebral-cortex respiration. When such synergism was not at first found, cerebral-cortex tissue was treated with the veratrum alkaloid and stimulated by brief electrical pulses spaced widely apart. A large rise in respiratory activity ensued which was reminiscent of the metabolic response of veratrinized nerve axons to brief stimuli (Hill, 1933; Schmitt & Gasser, 1933).

METHODS

Adult guinea pigs were decapitated, and slices of cerebral cortex 0.35 mm. thick prepared and handled for electrical stimulation and measurement of oxygen consumption in glucose-phosphate-saline as described by McIlwain (1951). Silver-wire grid-electrodes *H* (Ayres & McIlwain, 1953) and electrode vessels *A* (McIlwain, 1951) were used. Electrical stimuli were generated by condenser discharge with an instrument (Ayres & McIlwain, 1953) delivering alternating pulses at a rate of 100/sec. to up to four vessels simultaneously. As shown on the screen of a cathode-ray oscillograph the pulses had time-constants of 0.45–0.5 msec. and the peak voltages indicated below. For intermittent stimulation the current to the vessels was periodically interrupted with a relaxation oscillator device designed and constructed by P. J. W. Ayres (see McIlwain, 1954). The interrupter was set to allow current to flow for up to 16 msec. between intervals lasting 2.6–2.9 sec. Thus, since the frequency of discharge was 100/sec., 1–2 pulses were delivered every 2.6–2.9 sec.

Following a preliminary incubation of 30 min. during which the rate of oxygen consumption of the unstimulated tissue was determined, impulses were applied and protoveratrine was added from the side arms of the vessels. The sample of protoveratrine used, which was kindly donated by Professor Kraye, consisted of 60% of protoveratrine A and 40% of protoveratrine B. Vessels not connected to the pulse generator and/or not containing protoveratrine served as controls.

RESULTS

Electrical stimulation at 100 pulses/sec.

Voltage-response relation in normal slices. Under present experimental conditions the threshold voltage for eliciting a rise in the rate of respiration of cerebral cortex by means of uninterruptedly applied pulses lies between 1.5 and 2.0 v (Table 1).

As the applied potential is increased above 2 v the respiratory rise becomes progressively larger until a maximum increase of about 80% is reached at or probably somewhat below 10 v. That the rate of respiration did not increase by 100%, as it did in other experiments where stimulating electrodes of similar geometrical characteristics were used (e.g. McIlwain, 1951), may have been because the present slices were not quite sufficiently covered by the electrode grid, leaving the outer fringes out of the reach of the pulses.

Tissue treated with protoveratrine. Table 1 shows that the stimulating effects of protoveratrine and of high-frequency condenser pulses on cerebral-cortex respiration are not additive. This is best illustrated by the experiment carried out at an applied peak potential of 2 v. Pulses of this peak potential normally raise the rate of respiration by about 20%. In the presence of 1.4×10^{-6} M protoveratrine, which accelerates respiration by about 33%, they do not cause uptake of additional oxygen. The difference in the rate of O_2 uptake of slices exposed to 2.8×10^{-6} M protoveratrine at 0 and at 2 v is likewise not significant. At a peak potential of 10 v this concentration even lowers the rate of electrically stimulated metabolism, as does 1.4×10^{-6} M protoveratrine at 20 v.

Intermittent electrical stimulation

Results of experiments in which single pulses or pairs of pulses were delivered at intervals of 2.6–2.9 sec. are recorded in Table 2. The peak potential was 20 v. In contrast to peripheral nerve (Schmitt & Gasser, 1933), cerebral-cortex slices normally respond to this type of stimulation with an appreciable acceleration of oxygen consumption, which amounts to about 20%. The same acceleration is obtained, according to McIlwain (1954), when cerebral-cortex slices are exposed to bursts of condenser pulses of similar characteristics for one-fifth of the experimental time, with intervals between bursts greater than 8 sec.

In the presence of a concentration of protoveratrine (1.4×10^{-6} M) which increases the rate of oxygen uptake of electrically unstimulated cerebral-

Table 1. *Effect of protoveratrine on the metabolic response of cerebral-cortex slices to electrical pulses delivered at 100/sec.*

Each figure represents the mean $\pm$ standard error of five to eight individual determinations during the period of 5–45 min. after the addition of protoveratrine.

Protoveratrine (μ M)	Increase in respiratory rate (rate without drug and pulses = 100) at peak potentials of					
	0	1.5 v	2.0 v	3.5 v	10 v	20 v
0	—	4 ± 3.1	19 ± 3.7	47 ± 9.4	79 ± 5.9	75 ± 2.3
1.4	33 ± 4.0	30 ± 4.1	31 ± 4.1	—	75 ± 8.6	51 ± 4.4
2.8	55 ± 6.5	—	62 ± 3.0	—	53 ± 4.0	—

cortex tissue by about 30 % the tissue responds to the present mode of intermittent electrical stimulation with an increase amounting to 75 % (Table 2). Thus, simultaneous exposure to protoveratrine and to brief electric currents applied at long intervals produces stimulation of metabolism which is greater than the sum of the individual effects of each of these two agencies. Comparison with Table 1 indicates that in cerebral-cortex slices treated with protoveratrine delivery of pulses with a peak potential of 20v at a rate of 1-2 every

Table 2. *Effect of protoveratrine on the response of respiring slices of cerebral cortex to intermittent electrical stimulation*

One or two condenser pulses of peak potential 20v were delivered at intervals of 2.6-2.9 sec. Experimental period: 45 min. Rates are mean values followed by the standard error of the mean.

Protoveratrine (μ M)	Increase in respiratory rate (untreated tissue = 100)	
	No pulses	With pulses
0	0	22 $\pm$ 1.3
1.4	29 $\pm$ 4.3	75 $\pm$ 8.8

2.6-2.9 sec. brings about greater acceleration of respiration than delivery at a rate of 100 per sec. The acceleration approaches the maximum rise obtainable by means of the uninterruptedly delivered pulses. Reckoned in terms of the percentage increment of the oxygen uptake due to one impulse, delivery of 20v pulses at a frequency of 1-2 per 2.6-2.9 sec. is between 38 and 85 times as effective in normal cerebral slices as delivery at 100 per sec. In slices treated with 1.4×10^{-6} M protoveratrine the effectiveness of the low-frequency stimulation is between 344 and 766 times as great.

Response of white matter of brain

Kratzing (1951), who investigated the effect of electrical currents on the respiration of various isolated guinea-pig tissues, reported that only 'excitable' tissues responded with an increase in respiratory activity. In the preceding investigation (Wollenberger, 1955) the metabolic action of protoveratrine was found to be characterized by similar tissue specificity. In neither of the two studies, however, had the white matter of brain been included among the tissues tested. It seemed of interest in connexion with the present series of investigations to determine the effect of electrical impulses and of protoveratrine on the respiration of white matter of brain. For this purpose the corpus callosum of guinea-pig brain, which consists almost exclusively of white matter, was dissected out

(I am indebted to Dr J. B. Brierley for performing this dissection). It was freed from adhering grey matter, and treated in the same manner as were cerebral-cortex slices in the experiments described above. The tissue responds to both electrical impulses and to protoveratrine with marked rises in the rate of respiration (Table 3). However, the rise due to electrical pulses, which had a peak potential of 10v and were delivered at a rate of 100 per sec., was less than in grey matter (cf. Table 1). Pulses of higher potential were not applied. It may be noted that the rate of oxygen uptake of unstimulated corpus callosum ranged between 25 and 29 μ moles/g./hr. Pieces of unstimulated cerebral cortex of the guinea pig respire at about twice this rate. In isolated ox brain Dixon & Meyer (1936) had found a tenfold difference between the rates of oxygen consumption of cerebral cortex and of corpus callosum.

Table 3. *Stimulation of respiration of white matter of brain (corpus callosum) by electrical pulses and by protoveratrine*

Alternating condenser pulses of peak potential 10v were delivered at 100/sec. Experimental periods were of 40 min., with protoveratrine at 2.8×10^{-6} M.

Stimulating agent	O ₂ uptake (μ moles/g./hr.)	
	Before exposure to agent	During exposure to agent
Electrical pulses	26	39
	29	39
Protoveratrine	25	36
	27	40

DISCUSSION

Schmitt & Gasser (1933) reported that application of strong electrical shocks to isolated veratrinized nerve produced slight to moderate acceleration of respiration. The rate of oxygen uptake of such nerve, stimulated twice per sec., was greater than that of non-veratrinized nerve stimulated 120 times per sec. In the present experiments analogous changes were found in cerebral cortex. The acceleration caused in this tissue by protoveratrine jointly with strong condenser pulses delivered at a frequency of 1-2 per 2.6-2.9 sec., exceeded the acceleration caused by continual delivery of these pulses at 100 per sec. in the absence of the alkaloid. The effect was much more pronounced than the corresponding one observed by Schmitt & Gasser, although it did not approach in magnitude the extraordinary rise in heat production which Hill (1933) had found in veratrinized nerve on electrical stimulation at frequencies below 1 per sec.

Schmitt & Gasser (1933) were able to correlate the increase in metabolism, brought about in nerve by electrical stimulation in the presence of veratrine, with an increase of the negative after-potential. It is not unlikely that the potentiation of the metabolic effect of brief electrical stimuli, brought about in sliced cerebral cortex by protoveratrine, is attended by a corresponding electrical change. In the nerve fibre, protoveratrine produces a lesser, but longer-lasting augmentation of the negative after-potential than does veratrine (Graham & Gasser, 1931). However, nothing is known as yet about the action of these or of other veratrum alkaloids on electrical activity in the higher parts of the nervous system. This also makes it all the more difficult to explain why the accelerating effects of protoveratrine and of high-frequency electrical stimuli do not sum up, although (see Wollenberger, 1955) they are strikingly similar in many respects. It seems that, as in the case of the veratrine effect on heat production in nerve (Hill, 1933), a period of rest between electrical stimuli is required in order that the protoveratrine effect can take its course.

SUMMARY

1. Simultaneous exposure of cerebral-cortex slices to protoveratrine and to brief condenser pulses, delivered at a rate of 100 per sec., did not

bring about a greater increase in the rate of respiration than did exposure to the drug or to the pulses alone.

2. Simultaneous exposure of cerebral-cortex slices to protoveratrine and to brief condenser pulses, delivered at a rate of 1–2 every 2.6–2.9 sec., brought about an increase in the rate of respiration which was greater than the sum of the increases caused by the drug and by the pulses individually.

3. The rate of respiration of isolated white matter of brain (corpus callosum) was increased both by electrical pulses and by protoveratrine.

I wish to express my thanks to Dr H. McIlwain for his hospitality and for his help and advice during this work which was undertaken during the tenure of a Life Insurance Medical Research Fund Fellowship.

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The Resynthesis of Glycogen by Guinea Pig Cerebral-Cortex Slices

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(Received 29 December 1954)

Some time ago Kerr (1936) showed that the glycogen in the brain is very labile and that its determination during *in vivo* experiments required special quick-freezing techniques. This lability has since been confirmed by others (Chesler & Himwich, 1943; Albaum, Tepperman & Bodansky, 1946; Klein & Olsen, 1947), and recently Chance & Yaxley (1950) showed that the brain glycogen of mice dropped markedly within 1 min. after death and had effectively disappeared in 4–5 min.

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Consequently, as prepared for *in vitro* experiments, brain slices, unlike liver slices or hemidiaphragm preparations, contain practically no glycogen, and to the best of our knowledge resynthesis of glycogen by such slices has not previously been demonstrated.

In the studies of labile brain metabolites conducted in this laboratory, conditions for resynthesis of glycogen by surviving guinea pig cerebral-cortex slices were sought and are now reported, together with some studies of the effects of changes in concentration of potassium and phosphate ions, of added 2:4-dinitrophenol, and of electrical pulses, on the resynthesis.