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METABOLIC AND OTHER EFFECTS OF RESERPINE UPON SMOOTH AND SKELETAL MUSCLE

BY

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Reserpine increased the concentration of potassium ions in the fluid bathing the isolated frog sartorius muscle. The effect of reserpine upon respiration and upon glycolysis in rabbit intestinal muscle has been investigated using the Warburg " Direct " method. After a latent period, reserpine (50 μg./ml.) depressed respiration but did not affect glycolysis in gut muscle. These results, together with previous observations, may point to an effect of reserpine upon carbohydrate metabolism.

Earlier work (Gillis and Lewis, 1956a, 1956b, 1957) indicated that reserpine affected metabolic processes which supplied energy for the contraction of smooth muscle. It was shown by Gillis and Lewis (1956b, 1957) that certain intermediates of carbohydrate metabolism antagonized the depression by reserpine of acetylcholine- and histamine-induced contractions of guinea-pig ileum. Intermediates of fat and protein metabolism were ineffective. If reserpine had a metabolic site of action it seemed reasonable to investigate whether this was reflected in a decreased ability of the muscle to utilize glucose. The potassium ion (K^+) is known to participate in the activation of certain important enzyme systems involved in carbohydrate metabolism (Sheppard, 1951). Changes in the extra- and intra-cellular concentrations of K^+ might be observed if reserpine interfered with the intermediary metabolism of carbohydrate. It is well established that contractions of smooth (Born and Bülbring, 1956), cardiac (O'Brien and Wilde, 1952) and skeletal (Fenn, 1940; Hahn and Hevesy, 1941; Noonan, Fenn, and Haege, 1941) muscle are associated with an increase in the K^+ concentration of the fluid bathing the tissue. This appears to be due to an increased efflux of potassium ions rather than to a decreased influx, since adrenaline-induced relaxation in intestinal smooth muscle is accompanied by an increased inward movement of K^+ (Born and Bülbring, 1956). Reserpine causes a contraction of the isolated frog rectus abdominis muscle (Gillis and Lewis, 1956a; Barrett, Baker, and Plummer, 1956) and of the isolated rabbit duodenum (Gillis and Lewis, 1957). The time-characteristics of the contraction of the frog rectus muscle led us to consider the possibility that reserpine was causing an increase in the concentration of K^+ in the extracellular fluid.

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METHODS

Preparation of Solutions.—For experiments on the frog sartorius muscle, reserpine was dissolved in an aqueous solution of 10% ascorbic acid to give a concentration of 2 mg./ml. The *p*H was about 2.5, but was adjusted, by the addition of small volumes of 5% $NaHCO_3$ solution, to 4.5 immediately before use. The mixture was diluted with frog Ringer solution to give a final concentration of reserpine of 1 mg./ml. The control solution was prepared by treatment of 10% ascorbic acid solution in the same way.

For the determination of respiration and glycolysis, a 5 mg./ml. solution of reserpine was prepared in 5% aqueous tartaric acid. The *p*H of a 2 ml. aliquot was adjusted to 4.5 with $NaHCO_3$ and the mixture diluted to 7.4 ml. Addition of 0.1 ml. of this solution from the side arm gave a final flask concentration of 10 μg./ml. reserpine.

Isolated Frog Sartorius Muscle.—The radioactive material was obtained as a 1.15% solution of ^{42}KCl. Frogs received 1 ml. of this solution by injection into the dorsal lymph sac.

After a 2 hr. equilibration period the frogs were decapitated and pithed, the skin over the thigh removed and the rectus abdominis muscle carefully freed at the point of insertion into the pelvic girdle. The upper tendinous attachment of the sartorius muscle was thus exposed and the proximal and distal attachments then freed from underlying tissues. Cotton threads were tied around both ends of the sartorius muscle, which was freed from the femur and pelvic girdle and finally from the underlying muscles. Two types of experiment were performed. In the first, the muscle was suspended in a test-tube containing 10 ml. frog Ringer solution (NaCl, 0.65%; KCl, 0.014%; $CaCl_2$, 0.012%; $NaHCO_3$, 0.02%; dextrose, 0.2%) at room temperature and through which O_2 was bubbled. Two parallel series of nine tubes were set up. One series contained 50 μg./ml. reserpine in frog Ringer solution; the other contained an equivalent volume of the control solution. One muscle from each pair was used in the reserpine series, the other in the control series. The muscles were suspended by a thread from a wire support at the top of the tube. They were stretched with a 2 g. weight tied to the threads at their lower ends. At 20 min. intervals each muscle was transferred to the next tube in the series. The ^{42}K in the bathing fluid was measured with a Geiger-Müller liquid counter (type M.6). The results in counts/min. were corrected for background and lost counts. Allowance was made for decay and the final value for the activity converted to parts/million of ^{42}K. In the second type of experiment, the muscle was suspended in a 50 ml. bath containing frog Ringer solution. The bath fluid was circulated continuously by a pump, through an F.M.6 flow counter, cooled, and thence returned to the bath. A continuous integrated record of the ^{42}K in the bathing fluid was obtained with a "Labgear" recording count ratemeter. An increase in ^{42}K in the bathing fluid was recorded as an increased gradient of the tracing. Muscle tone was recorded simultaneously by conventional methods on a smoked surface.

Respiration and Glycolysis in Isolated Rabbit Intestine.—Rabbits weighing between 1.5 kg. and 2.1 kg. and between 4 and 9 months old were used. About 60 cm. of small intestine was rapidly removed. For experiments on respiration, it was moistened with ice-cold modified Krebs-Hensleit solution (NaCl, 0.692%; KCl, 0.035%; $CaCl_2$, 0.029%; KH_2PO_4, 0.016%; $MgSO_4.7H_2O$, 0.029%), or, for experiments on glycolysis, with Krebs-bicarbonate solution (NaCl, 0.692%; KCl, 0.035%; $CaCl_2$, 0.029%; KH_2PO_4, 0.016%; $NaHCO_3$, 0.21%; $MgSO_4.7H_2O$, 0.029%; dextrose, 0.2%). The segment of intestine was opened along the line of attachment of the mesentery, stretched gently and, with the mucosa upwards, pinned at its four corners upon a dissecting board. The mucosa was removed as completely as possible by scraping gently with a scalpel blade. Two incisions were made about 2 mm. from and parallel to both outer edges of the strip. The tissue remaining between the incisions was cut into pieces, each weighing about 100 mg. These were used in the experiments to be described. Experiments on oxygen consumption and on glycolysis were carried out by means of the Warburg "Direct" method, using either an atmosphere of O_2 (respiration) or 95% N_2 and 5% CO_2 (glycolysis). *For respiration*, the main chamber of each Warburg flask contained 2.2 ml. modified Krebs-Hensleit solution. The centre well contained 0.2 ml. 20% KOH. Since the addition of saline/reserpine or saline/control mixtures from the side arm would cause a slight change of the final salt concentration in the main compartment, the composition of the physiological saline in the side arms was suitably adjusted to avoid any change. *For glycolysis*, the main chamber of the flask contained 2.4 ml. of Krebs-bicarbonate solution. Similar precautions to those outlined above were taken to avoid alteration in the final ionic concentration. Reserpine was added from the side arm to give final concentrations of 10 or 50 μg./ml. In control experiments, an equivalent volume of tartaric acid control solution was added from the side arm. After tipping, the total volume of the reaction mixture together with the piece of tissue was 3 ml.

RESULTS

Frog Sartorius Muscle.—In all experiments reserpine, in doses of 20 and 50 μg./ml., caused a marked increase in the ^{42}K content of the bath fluid ($^{42}K_b$) (Fig. 1, Table I). A slow contraction of the muscle accompanied this phenomenon (Fig. 2). Less than 20 μg./ml. reserpine did not consistently produce a contraction of the muscle.

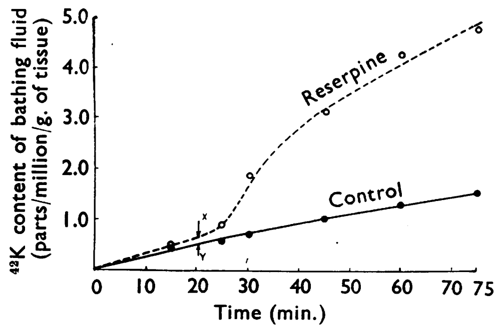

FIG. 1.—The effect of reserpine upon the ^{42}K concentration of the fluid bathing an isolated frog sartorius muscle. At X, reserpine (50 μg./ml.) was added. At Y, the control solution was added.

TABLE I

^{42}K CONTENT OF FLUID BATHING THE FROG SARTORIUS MUSCLE WHEN RESERPINE OR THE CONTROL SOLUTION WAS ADDED

All values (±S.D.) are in parts/million and were calculated from tracings obtained with a "Labgear" recording count ratemeter.

	Time after Addition of Reserpine or Control Solution (min.)					
	15	25	30	45	60	75
Reserpine 50 μg./ml. (mean of 9 experiments) ..	0·49 (±0·25)	0·90 (±0·39)	1·81 (±1·09)	3·14 (±1·69)	4·28 (±1·92)	4·88 (±1·96)
Control (mean of 10 experiments)	0·41 (±0·17)	0·59 (±0·30)	0·70 (±0·36)	1·00 (±0·48)	1·29 (±0·53)	1·55 (±0·71)

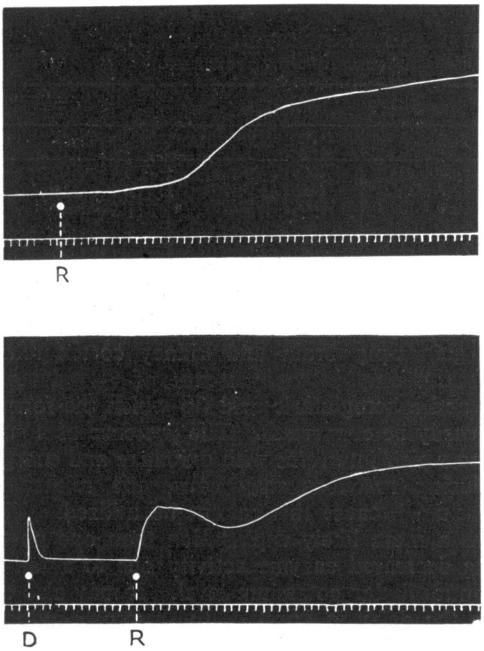

FIG. 2.—The stimulant action of reserpine upon the isolated frog sartorius muscle. Upper record, reserpine 50 µg./ml. alone at R; lower record, reserpine 50 µg./ml. at R after 20 µg./ml. decamethonium at D. The biphasic response to reserpine shown in the lower record was not always seen. Time, 30 sec.

Both the appearance of the contraction and the increased $^{42}K_b$ were preceded by a latent period of 4 to 11 min. The contraction was maintained only so long as reserpine remained in contact with the muscle. (+)-Tubocurarine (25 µg./ml.) increased the latent period by as much as 100%. Decamethonium (20 µg./ml.) reduced or abolished it (Fig. 2). Neither the magnitude of the contraction nor the increased $^{42}K_b$ was altered by tubocurarine or decamethonium. Neither drug had any effect upon an established reserpine contraction. 2:4-Dinitrophenol (0.2 mM), a concentration shown by Maizels (1951) to cause a release of potassium from frog skeletal muscle, was ineffective when ordinary frog Ringer solution was used, but caused an increase in the $^{42}K_b$ when a potassium-free solution was substituted. Thus there appears to be some difference in the mechanism of potassium release between reserpine and 2:4-dinitrophenol.

When test and control muscles were suspended in parallel series of nine tubes (see Method), a marked increase in $^{42}K_b$ was seen in the test series. The release of potassium by the ascorbic acid solution in the control series was not greater than that seen in experiments in which the muscles were suspended in frog Ringer solution. The increase in $^{42}K_b$ was seen only when the muscle was immersed in a solution containing reserpine: it fell to control values on transferring to the reserpine-free solution.

Warburg Experiments.—The addition of 0.2% dextrose did not influence the $Q_{O_2}^{O_2}$ values over the first, second, or third hours of the experiment. This presumably indicated that the tissue had sufficient endogenous glucose to support aerobic metabolism. Dextrose was therefore omitted from the physiological salines in these experiments.

Reserpine (50 µg./ml.) reduced significantly (P=0.05) the oxygen uptake of pieces of rabbit intestinal muscle when compared with the control values for tissue incubated in modified Krebs-Hensleit solution. There was a latent period of 20 min. before a difference was observed between flasks containing reserpine and those containing the control (Fig. 3, Table II).

Reserpine (10 µg./ml.) and the control solution of tartaric acid, equivalent to 10 µg. and 50 µg./ml. of reserpine, had no demonstrable effect upon respiration.

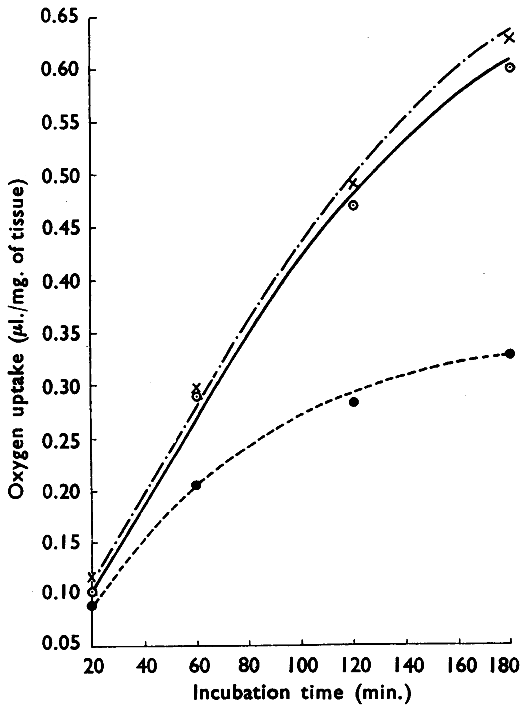

FIG. 3.—Warburg experiments. Measurements of oxygen uptake by isolated rabbit intestinal muscle. In modified Krebs-Hensleit control solution X–·–X; in tartaric acid control solution (equivalent to 50 µg./ml. of reserpine) ⊙———⊙; and in reserpine (concentration of 50 µg./ml.) ●- - -●.

TABLE II

THE EFFECT OF RESERPINE UPON RESPIRATION IN ISOLATED RABBIT INTESTINAL MUSCLE

The oxygen uptake in µl. (±S.D.)/mg. of tissue at each time is the average of eight experiments.

	Incubation Time (Min.)			
	20	60	120	180
Modified Krebs-Hensleit solution ..	0·116 (±0·062)	0·296 (±0·114)	0·491 (±0·145)	0·629 (±0·219)
Reserpine 50 µg./ml.	0·090 (±0·021)	0·206 (±0·086)	0·283 (±0·129)	0·330 (±0·143)
,, 10 ,, ...	0·114 (±0·055)	0·264 (±0·140)	0·460 (±0·278)	0·550 (±0·291)
Tartaric acid solution equivalent to				
(a) 50 µg./ml. reserpine	0·105 (±0·004)	0·292 (±0·108)	0·469 (±0·158)	0·600 (±0·231)
(b) 10 ,, ,, ...	0·097 (±0·045)	0·306 (±0·120)	0·511 (±0·217)	0·618 (±0·218)

Reserpine (10 and 50 µg./ml.) had no significant effect (P=0.05) upon the glycolytic activities of isolated rabbit intestinal muscle.

DISCUSSION

Reserpine has been shown to depress both spontaneous and drug-induced activity in isolated skeletal, visceral, and cardiac muscle (Gillis and Lewis, 1956a, 1956b, 1957). Previous evidence points to a relationship between reserpine and carbohydrate metabolism in the smooth muscle of guinea-pig ileum (Gillis and Lewis, 1956b, 1957). Under suitable conditions of bath *p*H, certain intermediates of carbohydrate metabolism antagonized the depression by reserpine of drug-induced contractions, whilst intermediates of fat and protein metabolism were inactive.

Anoxia has been shown to cause either a reduction in, or complete inhibition of, spontaneous tone and rhythmic activity in isolated rabbit intestinal muscle (West, Hadden, and Farah, 1951), and in the taenia coli muscle of the guinea-pig (Born, 1956). At the same time the ability of the tissue to maintain electrically-induced (Born, 1956) or drug-induced (West *et al.*, 1951) tone was completely abolished.

The effects of reserpine upon spontaneous and drug-induced tone are similar in many respects to those of experimentally produced anoxia. It is possible that reserpine in effect renders the intestinal smooth muscle of the guinea-pig ileum anoxic by virtue of its ability to interfere with the energy-producing reactions of carbohydrate metabolism.

An effect upon the oxidative metabolism of carbohydrate is supported by our observations that reserpine, after a latent period, decreased oxygen-uptake in isolated rabbit intestinal muscle and caused an increased release of potassium from frog skeletal muscle.

Krebs, Eggleston and Terner (1951) have shown that guinea-pig brain and kidney cortex retain potassium only in the presence of (−)-glutamic acid and α-ketoglutaric acid (to which the former can easily be converted). The presence of a substance which interferes with oxidative and energy-producing reactions may cause the release of potassium because this cannot be utilized. This seems a more likely explanation than one postulating decreased enzyme activity due to a fall in the intracellular potassium level. It may also explain the delay in onset of the reserpine-induced increase in $^{42}K_b$. That is to say that existing stores of metabolites must be exhausted before the reserpine effect becomes manifest.

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