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Effect of 5-Hydroxytryptamine on Phosphorylase and Glycogen Levels in Muscle Tissue.* (25828)

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Although the isolated uterus of the estrous rat is frequently employed for bio-assay of certain psychopharmacological agents and hormones, little is known concerning the effects of these substances, particularly the former, on glycogen stores and enzyme phosphorylase in uterine muscle. Epinephrine and oxytocin, however, induce a rapid decrease in both uterine glycogen(1) and phosphorylase activity(2). The effects of serotonin (5-OH-tryptamine), lysergic acid and reserpine on these 2 constituents in uterine smooth muscle were investigated. Since serotonin alone produced changes in smooth muscle, its effect was studied also on skeletal, cardiac, and diaphragm muscle.

Methods. Adult female rats were spayed, 7-9 days later injected with 50 μg of estradiol benzoate (Schering Co.) subcutaneously and 48-56 hours later given an intraperitoneal injection of the test compound. Uteri were removed 30-60 minutes later from the anesthetized rats and were frozen and stored as de-

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TABLE I. Phosphorylase Activity in Uterine Smooth Muscle, Diaphragm and Heart.

Treatment*		Exp. P-lase *a*	Exp. P-lase *t*	Exp. *a*/*t* ×100	Control P-lase *a*	Control P-lase *t*	Control *a*/*t* ×100
			Uterus				
Serotonin	(12)	35 ± 2	96 ± 3	36.4 ± 1.0	74 ± 2	116 ± 2	64.0 ± 1.1
Reserpine	(9)	44 ± 2	136 ± 7	33.6 ± .8	46 ± 3	134 ± 5	34.7 ± 2.1
LSD	(12)	62 ± 3	130 ± 2	47.9 ± 2.9	69 ± 3	128 ± 4	54.3 ± 1.7
			Diaphragm				
Serotonin	(10)	119 ± 5	150 ± 3	79.4 ± 1.8	119 ± 3	164 ± 7	72.9 ± 1.4
" & electr. stim.	(8)	33 ± 6	94 ± 7	35.0 ± 5.5	36 ± 4	94 ± 4	39.2 ± 3.6
Epinephr. & " "	(8)	58 ± 5	96 ± 5	60.7 ± 2.7	44 ± 3	108 ± 5	40.5 ± 1.2
			Heart				
Serotonin	(16)	126 ± 9	194 ± 4	64.6 ± 3.2	126 ± 7	189 ± 6	66.4 ± 2.0

* Details in text. Figures in parentheses = No. of rats.

scribed(2). Diaphragms were obtained from male rats, cardiac muscle from both sexes, and all tissues were quickly frozen upon removal. To produce tetanic contraction of the diaphragms prior to removal, electrodes were placed on posterior surface of half of exposed diaphragm of the anesthetized rat while passing a current from electronic stimulator for 20 seconds (frequency 60/sec, duration 1 msec, 10 volts). Serotonin creatine sulfate (California Corp. for Biochem. Res), reserpine (Ciba Pharm. Co.), lysergic acid diethylamide (LSD, Sandoz Pharm. Co.), and l-epinephrine-HCl (Parke Davis Co.), were diluted in water and given in 1 ml volume, intraperitoneally, in doses/100 g body weight. Details of methods of analysis are given; for glycogen(1,3), for uterine (smooth muscle) phosphorylase(2) and for striated muscle phosphorylase(4). Active phosphorylase *a* and total phosphorylase *t* (with added adenylic acid) are reported as μg P liberated from glucose-1-PO_4 in 10 minutes at 37°C by 40 mg of uterine tissue, 2.5 mg of diaphragm or 10 mg of cardiac tissue. Results are presented as means ± standard error.

Results. Doses of serotonin sufficient to alter either glycogen or phosphorylase levels were considerably in excess of those used for *in vitro* pharmacological testing. Groups of 4 female rats, pretreated as described, were injected with the following doses of serotonin: 0.1, 0.25, 0.5, 1.0 mg and, after 30 minutes, uterine glycogen levels in mg % were respectively, 291 ± 15, 217 ± 16, 207 ± 17 and 153 ± 40 as compared to 307 ± 7 mg % in controls. To determine if a change in phosphorylase activity would accompany this decrease in glycogen, similarly prepared rats were given 0.5 mg of serotonin and, after 30 minutes, the tissues assayed. Table I shows that a marked decrease in active phosphorylase a and activity ratios occurred. When reserpine and LSD (0.5 mg dose each) were injected into the prepared female rats, within 1 hour, uterine glycogen level was unaltered and phosphorylase activity was unchanged as noted (Table I). The relatively low activity ratios in controls of experiments with reserpine indicate again the variability encountered between successive experiments(2). Phosphorylase a levels in these controls are about twice that found if estrogen had not been given and sufficiently high to permit a lowering of enzyme activity under the proper conditions.

Examination of leg muscles in several rats for changes in either glycogen or phosphorylase revealed that these 3 substances were without effect. A report that serotonin increased uptake of P^{32} in the rat diaphragm (5) suggested a study of this muscle. Serotonin (0.5 mg dose) lowered glycogen levels of diaphragms in male rats from 314 ± 18 to 246 ± 19 mg % in 30 minutes (12 rats, previously starved for a day). When 1 mg doses of serotonin were injected into fed male rats and diaphragms assayed 1 hour later, glycogen decreased from control level of 626 ± 28 to 391 ± 16 mg % (12 rats). There was no change in active phosphorylase and only a

slight rise in activity ratios in diaphragms of these rats (Table I).

Except for negative results on phosphorylase levels in the diaphragm, the effects of serotonin were suggestive of those of epinephrine. A more sensitive method for demonstrating epinephrine effects on skeletal muscle phosphorylase requires that muscles of experimental and control animals be electrically stimulated prior to removal(6). To further ascertain that serotonin had no effect on phosphorylase in the diaphragm, the muscle was subjected to this more sensitive assay procedure. Preliminary experiments were made in which doses of 20 μg of epinephrine were given to rats 30 minutes before placing the electrode on the center of the right half of the diaphragm and stimulating for 20 seconds. While the right half was in tetanic contraction, the left half almost always continued its normal respiratory movements (only the right portion assayed). Control rats were treated similarly but without epinephrine. Results in Table I show that epinephrine inhibited the expected decrease in active phosphorylase and maintained activity ratios 50% higher than in controls. When rats were injected with 1 mg doses of serotonin and stimulated as above, phosphorylase activity was not maintained at a level higher than that in controls.

In studying heart muscle, 1 mg doses of serotonin were given to 8 fed rats (4 of each sex) and after 1 hour, the rats were autopsied. An equal number of controls of both sexes were used in the determinations. No sex difference was noted and the results were pooled. Table I shows that serotonin did not alter phosphorylase activity but, surprisingly, heart glycogen increased from a control level of 465 $\pm$ 27 to 728 $\pm$ 47 mg %.

Discussion. Serotonin, but not reserpine or LSD, lowered uterine glycogen and phosphorylase, both of which are located essentially in the myometrium(7). Although LSD *in vitro* antagonizes serotonin induced uterine contractions(8) and produces overt behavior in the rat, neither smooth nor skeletal muscles were affected. LSD affects carbohydrate metabolism in other ways and in other target organs(9) but there is little information on *in vivo* effects in muscle. The same may be said for serotonin(10) although it produces hyperglycemia without affecting skeletal muscle glycogen(11). High levels of serotonin in the uterus have been produced *in vivo* by administering the precursor, 5-hydroxytryptophan, without inducing abortion or altering uterine activity(12) but it is likely that much higher levels of serotonin are required to affect the glycogen.

Induced changes in uterine smooth muscle glycogen and phosphorylase have so far always been positively correlated(1,2). Acute changes in glycogen in other tissue, (liver, skeletal muscle, heart) have been correlated with changes in phosphorylase but not necessarily in the same direction(6,13,14,15). However, the effect of serotonin was unique, it lowered diaphragm glycogen, elevated cardiac glycogen and in both instances failed to alter phosphorylase activity. Phosphorylase activity may not be rate limiting in all instances of acute changes in tissue glycogen(13). The increase in heart glycogen following serotonin may be the result of increased mobilization and utilization of fatty acids. Preferential utilization of fatty acids over glucose would permit rapid accumulation of glycogen without necessarily requiring a concurrent increase in phosphorylase activity (summary ref. 16). Epinephrine increases cardiac glycogen and phosphorylase activity(14) thus differing from serotonin. Serotonin is also reported to increase phosphorylase and glycogenolysis in the sheep liver fluke(17).

Summary. Serotonin, but not reserpine or lysergic acid (LSD), when given intraperitoneally to rats, decreased uterine (smooth muscle) glycogen levels and phosphorylase activity. Serotonin rapidly decreased glycogen concentration in the diaphragm, rapidly elevated glycogen in heart muscle and in neither instance altered phosphorylase activity. Thus it is possible to produce acute changes in glycogen stores in some tissues without evoking alterations in phosphorylase activity.

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