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SEROTONIN (5-HYDROXYTRYPTAMINE): A POSSIBLE REGULATOR OF GLYCOGENOLYSIS IN PERFUSED MUSCLE SEGMENTS OF ASCARIS SUUM

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Summary: When isolated muscle segments from the parasite Ascaris suum were perfused with varying concentrations of serotonin (5-hydroxytryptamine), the level of cyclic AMP increased three-fold. Maximal levels of cyclic AMP were produced by the muscle at serotonin concentrations of 50 μM. Perfusion of saturating levels of serotonin through isolated muscle segments for various time periods resulted in an inactivation of glycogen synthase and an activation of phosphorylase with a concomitant rise in cyclic AMP values. Collectively, the results support the concept that serotonin may be functioning as a regulatory hormone of carbohydrate metabolism in this worm.

During the past few years there has been a considerable effort to elucidate the control mechanism of glycogenolysis in the muscle of the porcine parasitic roundworm, Ascaris suum (1-5). Since this muscle contains up to 20% glycogen by wet weight (2), the regulatory enzymes for glycogen synthesis, glycogen synthase (E.C. 2.4.1.11), and mobilization, glycogen phosphorylase (E.C. 2.4.1.1) appeared to be logical control steps. While these enzymes in the parasite have been identified and implicated in this regulation (2-4), no control hormone has been found in this worm nor has any been shown to regulate these enzymes in vitro (1). Recently, a muscle perfusion system has been developed for the parasite (4) and this system is now available for study of possible regulatory substances.

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In mammalian skeletal muscle systems, control of the glycogen metabolizing enzymes has been directly linked to epinephrine via cyclic AMP (6,7). Circulating levels of epinephrine cause increased muscle cyclic AMP levels which initiate a cascade of reactions that activates phosphorylase and inactivates glycogen synthase. This report concerns the identification of a putative hormone in Ascaris suum which functions by raising cyclic AMP levels, activating phosphorylase and inactivating glycogen synthase in perfused muscle segments.

METHODS

The maintenance and in vitro perfusion systems were essentially the same as that described previously by Donahue et al. (4). Briefly, the worms were cut posterior to the genital pore and anterior to the intestinal attachment to the muscle wall. The intestinal and reproductive tracts were dissected from the worms leaving a hollow muscle preparation, approximately 10 cm long. This 10 cm long segment of muscle was cut in half generating two 5 cm hollow tubes. One tube served as the experimental tissue and the other as the control. The muscle preparation was cannulated and ligated to polyethylene tips which were mounted on glass tubes inserted through rubber inlet and outlet stoppers in the perfusion apparatus. The system was continually gassed with 95% N_2 and 5% CO_2 to maintain a saturated anaerobic system. The perfusion system was maintained in a water bath at 37 C. Each muscle preparation was connected by a surgical thread to a muscle transducer (myograph B-4178) in which the contractions were recorded on a Narco Bio-systems physiograph model IFour-A. The muscle segments were freeze-clamped and stored in liquid nitrogen until analysis could be carried out (4). Glycogen synthase was assayed in homogenates of the muscle by the method of Thomas et al. (8). Apparent Ka values of glycogen synthase for glucose 6-phosphate (Glc-6-P) were obtained from Hill plots (7,9). The activity of glycogen phosphorylase was determined according to Gilboe et al. (10). The phosphorylase activity ratio was expressed as the ratio of the activity in the absence of AMP to that in its presence. Cyclic AMP was assayed by the radioimmunoassay of Brooker et al. (11) and protein was assayed by the Bradford method (12).

RESULTS AND DISCUSSION

Isolated segments of obliquely-striated ascarid muscle were perfused with biogenic amines to establish if any were functioning as a regulator of glycogenolysis in Ascaris muscle. Of the compounds tested, none elicited muscle contraction. In muscle segments perfused with 50 μM epinephrine no significant increase in the Ka value of glycogen synthase for Glc-6-P was observed, nor was there any change in the phosphorylase activity ratio (Table I). Several other biogenic amines which have been found to have some effect on glycogenolysis in either vertebrates or invertebrates (13) were also per-

TABLE I
Effect of Perfusing Some Biogenic Amines on Glycogen Synthase and Phosphorylase in Muscle Segments of Ascaris suum.

Drug	Glycogen Synthase Ka(mM)		Phosphorylase Activity Ratio	
	Control	Exptl.	Control	Exptl.
Epinephrine	0.46	0.65	0.47	0.48
Norepinephrine	0.58	0.54	0.49	0.43
Synephrine	0.58	0.56	0.48	0.45
Octopamine	0.54	0.55	0.48	0.43
Dopamine	0.54	0.40	0.54	0.40
Histamine	0.58	0.52	0.42	0.47
Serotonin	0.50	1.51	0.40	0.79

Muscle segments were perfused for 10 min with Ascaris saline plus 50 μM drug (Exptl.) or saline alone (Control). All values are averages ($N \geq 3$) and standard deviation is $\leq$ 10%.

fused through these muscle segments. None of these significantly effected the Ka values or phosphorylase activity ratio except serotonin (5-hydroxytryptamine). Perfusion of serotonin evoked a marked increase in Ka value from a control of 0.5 mM to 1.51 mM (Table I). There was also a significant increase in the phosphorylase activity ratio from 0.40 to 0.79 in those segments perfused with serotonin.

Isolated segments of muscle were perfused with varying concentrations of serotonin for 10 minutes and assayed for cyclic AMP levels (Figure 1). The initial levels of cyclic AMP were approximately 0.71 $\pm$ 0.05 nmoles/g muscle and this level increased to 2.61 $\pm$ 0.1 nmoles/g after perfusion with increased levels of serotonin. No further increase in cyclic AMP accumulation was observed with concentrations greater than 50 μM serotonin in the muscle perfusion.

Muscle segments were perfused for various time periods with saturating levels of serotonin and the muscle was assayed for phosphorylase and glycogen synthase activity and cyclic AMP accumulation. At zero time the glycogen syn-

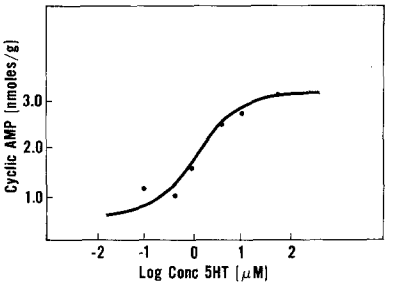

Figure 1. Cyclic AMP accumulation in muscle segments perfused with serotonin. Muscle segments were perfused for 10 minutes with various concentrations of serotonin. The muscle was freeze-clamped in liquid nitrogen, homogenized in 10% trichloroacetic acid, and assayed for cyclic AMP as described in Methods. Each value is $N \geq 3$ and standard deviation was $\leq 10\%$. Control values remained unchanged at 0.71 ± 0.05 nmoles/g muscle.

thase Ka value for Glc-6-P was 0.5 mM and this value increased to 1.72 mM after perfusion of 50 μM serotonin for 20 minutes (Figure 2A). During this time period there was an increased phosphorylase activity ratio from control values of 0.50 to 0.77 (Figure 2B). Levels of cyclic AMP (Figure 2C) increased to approximately 3.0 nmoles/g muscle in these same muscle segments. Perfusion for longer periods of time did not significantly increase the cyclic AMP levels.

Serotonin (1), as well as other biogenic amines, including synephrine and octopamine, have been identified as neurotransmitters which mediate contraction in other invertebrate muscle. No change in the rate of muscle contraction or relaxation was observed in Ascaris muscle preparations perfused with serotonin concentrations ranging from 1 to 50 μM.

Collectively, the data support the concept that serotonin may be functioning as a hormonal regulator of glycogenolysis. In Ascaris suum muscle serotonin appeared to function similarly to the manner in which epinephrine functions in mammalian skeletal muscle by increasing the cyclic AMP levels. The correlation between cyclic AMP accumulation in serotonin-perfused muscle segments and activation of phosphorylase and inactivation of glycogen synthase strongly supports the evidence that glycogenolysis is regulated, at least in part, by a hormone-mediated cascade mechanism involving protein phosphoryla-

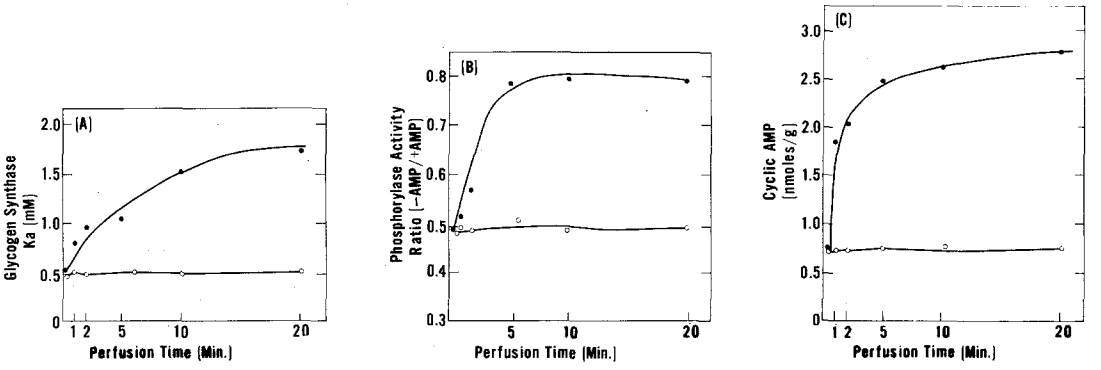

Figure 2. Effects of serotonin perfusion on glycogen synthase, phosphorylase and cAMP levels in Ascaris muscle. (A) Apparent Ka of glycogen synthase for Glc-6-P. Muscle segments were perfused for various time periods with saline plus 50 µM serotonin (•) or saline alone (o). The tissues were freeze-clamped in liquid nitrogen, homogenized and assayed for glycogen synthase activity as described in Methods. Each value is N ≥ 3 and standard deviation was ≤ 10%. (B) Phosphorylase activity ratio in muscle segments perfused with serotonin. Saline + 50 µM serotonin (•); saline alone (o). Phosphorylase activity ratio was calculated as described in Methods. (C) Cyclic AMP levels in muscle segments perfused for various times with serotonin. Saline + 50 µM serotonin (•); saline (o). Tissues were homogenized and assayed as in Figure 1.

tion. This is further supported by the observation that this muscle contains a cyclic AMP-dependent protein kinase which is similar to that found in mammalian muscle (5). Hormonal regulation of various mammalian systems is well documented (6,7). However, basic knowledge concerning many aspects of hormonal regulation of glycogen metabolism in invertebrate systems is still fragmentary and inadequate. Some of the naturally occurring biogenic amines (Table I) have been shown to exist as neurotransmitters but their role, if any, as hormones is not established in invertebrates (13). Evidence is presented here that serotonin may be functioning as a hormone which regulates glycogen metabolism in the parasite muscle.

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