Inhibition of Insect Nerve Cord Phosphorylase Activity by 5-Hydroxytryptamine

In a study on the mode of action of the hyperglycaemic hormone contained in the corpus cardiacum of the cockroach, *Periplaneta americana*, it was shown that the only tissue other than the fat body affected by the hormone was the nerve cord[1]. Extracts of the gland were shown to have a powerful glycogenolytic effect on this tissue. The work of BARTON-BROWNE et al.[2,3] indicated that the corpus cardiacum contained one or more adrenergic-like compounds. Substances belonging to this group have been shown to induce glycogenolysis in mammalian liver and muscle as a result of the activation of the enzyme phosphorylase[4]. The studies of COLHOUN[5] have shown that another amine, 5-hydroxytryptamine (5-HT), not only occurs in the cockroach nerve cord but is synthesized by it. He has also shown that 5-HT is contained in the corpus cardiacum[5]. Furthermore 5-HT has been shown to activate phosphorylase in the liver fluke[6]. Because there appears to be no record of hormonal regulation of phosphorylase in nervous tissue the investigation of possible effects of epinephrine and 5-HT on insect nerve cord phosphorylase seemed warranted.

The cockroaches used in this study were raised in the laboratory. The nerve cords were removed under Ringer's solution buffered at pH 6.5 with 0.005 *M* *Tris*-HCl buffer[7]. Dissections were made from the ventral surface and only the thoracic and abdominal portions of the ventral nerve cord were used. Great care was taken to ensure that the nerve cords were free from fat body tissue. The nerve cords were incubated with or without hormone in small beakers in a shaking water bath at 30 °C for 1 h. Epinephrine was used as the bitartrate salt and 5-HT as the creatine sulfate complex. Following the period of incubation the nerve cords were transferred to a Potter-Elvehjem homogenizer containing 1 ml of a chilled solution of 0.1 *M* sodium fluoride in 0.01 *M* ethylenediamine tetraacetic acid and homogenized for 1 min. The homogenates (2 nerve cords/sample) were then centrifuged for 10 min at 27,000 *g*. The resulting supernatants were transferred to test tubes and kept on ice until required for the assay.

Active phosphorylase was determined using the method of STEELE[1]. For the determination of total phosphorylase 5′-AMP was included in the phosphorylase reagent at a concentration of 0.0025 *M*.

The data given in the Table show that epinephrine was without an effect on phosphorylase activity at a concentration of $1 \times 10^{-4} M$ whereas 5-HT at the same concentration showed an unexpected although interesting inhibition of activity. This inhibition was greatly augmented when the concentration of 5-HT was increased to $1 \times 10^{-3} M$. The reduction in phosphorylase activity is due to a decrease in the active form of the enzyme.

The failure of epinephrine to activate phosphorylase in the nerve cord was unexpected, not because it has this effect in mammalian tissues but because it has been shown that the insect fat body enzyme will respond to this hormone[8]. It may be that epinephrine is unable to enter the nerve cord. On the other hand it is possible that the enzyme system in the nerve cord is different from that of mammals or even that of the insect fat body and therefore will not respond to epinephrine.

The inhibitory effect of 5-HT was unexpected, particularly in view of the observation by MANSOUR[6] that it had an activating effect on liver fluke muscle phosphorylase. However, it is possible that the mode of action of 5-HT in muscle is quite different from that in nervous tissue, and there may be no simple relationship between the effects of this compound in the 2 tissues. The only other report of inhibition of phosphorylase by 5-HT is that of LEONARD and DAY[9] who used uterine smooth muscle. The observations reported here are all the more interesting in view of the study by COLHOUN[5] showing that nerve cord contains, and is capable of synthesizing, 5-HT. It is obvious that the metabolic role of 5-HT is still poorly understood and many details require further resolution. Nevertheless a possible neurohumoral role of this substance in the cockroach nerve cord suggests that nervous control may be of considerable importance in the regulation of nerve cord metabolism.

Zusammenfassung. Die Phosphorylaseaktivität der in vitro mit $1 \times 10^{-4} M$ 5-Hydroxytryptamine inkubierten Insektennervenstränge war bedeutsam verringert. Der Effekt schien konzentrationsabhängig zu sein, da er sich mit steigender 5-HT-Konzentration verstärkte. 5-HT verminderte die aktive Form des Enzyms im Verhältnis zu dessen inaktiver Form. Epinephrin hatte keine Wirkung.

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The effect of epinephrine and 5-hydroxytryptamine on nerve cord phosphorylase activity

% of phosphorylase active			
Control	Hormone		Change
62.8 ± 2.7[a] (5)	Epinephrine $1 \times 10^{-4} M$	63.1 ± 4.9 (5)	None
58.0 ± 3.7 (10)	5-HT $1 \times 10^{-4} M$	42.0 ± 2.6 (9)	−27.6% ($P < 0.005$)
57.7 ± 3.8 (5)	5-HT $1 \times 10^{-3} M$	23.8 ± 3.6 (5)	−58.8% ($P < 0.005$)

[a] Mean ± standard error of the mean. The number in brackets refers to the number of observations.

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