PHYSIOLOGY

Release of Tritiated Epinephrine following Sympathetic Nerve Stimulation

IT has been previously demonstrated that *dl-nor*-epinephrine-^{3}H is bound by the isolated perfused dog gracilis muscle and that nerve stimulation causes a release of the bound *nor*epinephrine-^{3}H. Both *nor*-epinephrine-^{3}H and epinephrine-^{3}H are bound in dense core vesicles of sympathetic nerve endings[1]. We therefore examined the possibility that epinephrine-^{3}H taken up from the circulation could also be released by stimulation of sympathetic nerves.

The dog gracilis muscle was isolated and perfused, and 80–100 μc. *dl*-epinephrine-^{3}H was infused into the vascular bed, as described previously[2]. Thirty minutes after the labelled catecholamine was administered, phenoxybenzamine was given to block vascular responses to nerve stimulation, and the changes in transcapillary transport accompanying these responses[2].

Epinephrine-^{3}H and its metabolites were measured in effluent blood[3]. Before nerve stimulation, about 40 per cent of the total radioactivity found in the effluent was epinephrine, 30 per cent was metanephrine, and only 4 per cent was 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol. This is in contrast to *nor*epinephrine[2], where the deaminated metabolites represented about 45 per cent of the effluent radioactivity.

Following nerve stimulation (8 impulses/sec) there was a marked rise in total tritium in the venous effluent, with a 2-fold increase in epinephrine-^{3}H (Fig. 1). In addition there was a 50 per cent increase in the amount of metanephrine, with only a slight increase in the deaminated products. The results shown are typical of a total of eight periods of sympathetic nerve stimulation in three separate experiments.

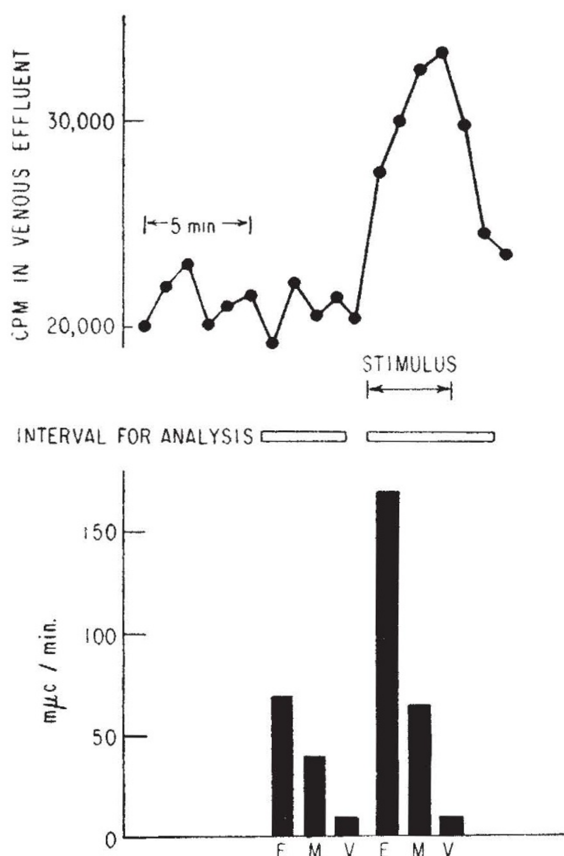

Fig. 1. Liberation of epinephrine-^{3}H and its metabolites from gracilis muscle before and during sympathetic nerve stimulation. *E*, epinephrine; *M*, metanephrine; *V*, 3-methoxy-4-hydroxymandelic acid + 3-methoxy-4-hydroxyphenylglycol

In similar experiments with *nor*epinephrine, sympathetic nerve stimulation resulted in a rise only of *nor*epinephrine, with no increase in any of the metabolites[2]. At the end of the experiment, 2 h after the end of the epinephrine infusion, about 60 per cent of the recovered radioactivity found in the muscle was present as epinephrine, about 30 per cent as metanephrine, and about 10 per cent as deaminated products.

From these results, it can be concluded that epinephrine can be taken up from the circulation into the sympathetic nerve endings and discharged by sympathetic nerve stimulation. The increase in metanephrine could be due to a release of the *O*-methylated metabolite, *O*-methylation of the released epinephrine, or both.

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Isolation and Purification of Hormones of the Crustacean Eye-stalk

DURING the past three decades, investigations of neurosecretory eye-stalk hormones among crustaceans have revealed a variety of physiological effects produced by eye-stalk ablation as well as by replacement experiments involving injection of extracts of eye-stalk tissue[1,2]. Resolution of the number of hormones involved in such physiological responses cannot be readily made with the crude extracts so far used. Progress along this line of crustacean endocrinology will evidently depend on isolation and purification of the different hormones, determination of their chemical nature and structure, and testing such purified preparations for the specificity of their physiological effects. Some attempt in this direction with the pigmentary effector hormones indicates that they may be peptide in nature[3-5]. On the basis of physiological[6] and specificity tests[7], the light-adapting retinal pigment hormone and the hormone which concentrates pigment in erythrophores appear to be different entities; less-complete separation of retinal pigment hormone and erythrophore-dispersing substance by paper electrophoresis has also been reported[8].

Large-scale purification of crustacean eye-stalk hormones in order to permit their chemical characterization was therefore undertaken in this laboratory. We can now report isolation and purification of the retinal pigment hormone and the erythrophore-concentrating hormone. The prawn, *Palaemon adspersus*, was used as the assay animal for both hormones, the methods being similar to ones previously described for these pigmentary effectors[5,9]. Eye-stalks collected from living *Pandalus borealis* were kept frozen until subsequent lyophilization.

Extraction of the powdered eye-stalks is started by heating the aqueous suspension at 100° for 2 min to denature an inactivating enzyme occurring in the tissue[5]. Both hormonal activities are obtained in one of 19 separated peaks following chromatography on a column of 'Dowex 50-X2'; this step results in a 200-fold purification when related to the total absorption at 280 mμ. Further purification is accomplished by chromatography on a column of 'DEAE-Sephadex *A*-25'. The separation

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