Medicina Experimentalis
Editor: R. DOMENJOZ, Bonn
S. KARGER – BASEL/NEW YORK (Printed in Switzerland)
SEPARATUM

Med. exp. *6:* 169–172 (1962)

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New Evidence for the Localization of Noradrenalin in the Adrenergic Nerve Terminals

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von Euler and his co-workers have produced strong evidence to support the view that the adrenergic transmitter is noradrenalin and that this amine is accumulated and stored in the terminals of the adrenergic nerves (cf. *von Euler*, 1956, 1961). However, it has hitherto been impossible to demonstrate directly, *e.g.*, histochemically, the presence of noradrenalin in these terminals. The importance of this problem has recently become pronounced, since it has been questioned whether the noradrenalin in peripheral tissues is stored in the nerves themselves, or, *e.g.*, in chromaffin cells (*Burn and Rand*, 1960; *Brandon and Rand*, 1961).

Several ways have been tried in these laboratories to obtain sensitive histochemical methods for catecholamines (*Carlsson* et al., 1961; *Falck* et al., 1962; *Falck and Torp*, 1962a). Perhaps the most promising method is based on the principle that the primary amines can be transformed into intensely fluorescent products by condensation with formaldehyde. Despite the noradrenalin cells in the adrenal medulla showing an intense fluorescence when treated thus, the noradrenalin in other peripheral tissues could not be demonstrated.

It seemed probable that the chief problem was to prevent extraction or diffusion of the very minute quantities of the amine before or during the condensation reaction. Since the fluorescent products were found to be indissoluble by hot paraffin or xylene, condensation was attempted under nearly dry conditions *before* the paraffin embedding by exposing freeze dried tissue to water-poor formaldehyde vapor. Experiments with models and adrenal medulla surprisingly showed that a condensation easily took place, accompanied by the development of an extremely intense

Received: January 4th, 1962.

fluorescence without significant diffusion. The details of the method will be described elsewhere (*Falck and Torp*, 1962b).

A large number of tissues from several different mammals have been studied. In all tissues where adrenergic nerves are known to be present (*e.g.*, heart, iris, submaxillary gland, blood vessels in some organs) the formaldehyde treatment developed a strong yellow-green fluorescence in a network of fine fibres which stood out brilliantly against a dark or weakly fluorescent background. The fibres had the same characteristic morphology and topography (a typical network around the smooth muscle layer in small blood vessels, for instance) as those of the axons running in the autonomic groundplexus (cf. *Hillarp*, 1946, 1959). This, as well as the fact that the fluorescence reaction disappeared after degeneration of the postganglionic—but not the preganglionic—nerves (see below), leaves no doubt that the fibres represent postganglionic preterminal or terminal axon ramifications. Especially the round or elongated enlargements of the axons, the varicosities, dispersed along the fibres showed a high fluorescence. In complete contrast to these fibres the axons in the larger nerve bundles which are finally distributed to the groundplexus showed only a faint fluorescence. This could be seen especially clearly in preparations of unsectioned thin tissues mounted as a whole (*e.g.*, iris and mesentery) which showed the very rich innervation apparatus just as in preparations with excellent methylene blue staining (cf. *Hillarp*, 1946, 1959).

The following facts support the view that the fluorescence reaction demonstrates the presence of noradrenalin in adrenergic nerve fibres.

1. An intense fluorescence develops in the same manner and under the same conditions on formaldehyde treatment of the nerves, the noradrenalin cells of the adrenal medulla, and the pure amine in models (for details see *Falck and Torp*, 1962b).

2. In all peripheral tissues examined, only the autonomic nerve fibres—besides some granular epitheloid cells (*inter alia*, the enterochromaffin cells)—develop a strong fluorescence. Of the biogenic monoamines only noradrenalin is known to occur in significant amounts in some of these tissues (*e.g.*, heart). These facts considered together with the results in a study of the chemistry, specificity, and sensitivity of the reaction (*Falck* et al., 1962) support the view.

3. An intense fluorescence develops only in the preterminal or terminal axon ramifications where the adrenergic transmitter is probably accumulated in concentrations very much higher than in the more proximal parts of the adrenergic fibres (cf. *von Euler*, 1956).

4. In the iris, only the nerves in the dilator muscle, but not those in the sphincter (if one ignores the vessel innervation itself) which are solely or mainly sympathetic adrenergic and parasympathetic cholinergic, respectively, show the fluorescence reaction. The reaction cannot be induced after degeneration of the postganglionic cervical sympathetic nerves (2 days or more after section of the nerves) but preganglionic denervation which does not influence the organ content of noradrenalin (*Rehn*, 1958) does not alter the reaction.

5. The reaction can be prevented by treating the animals with reserpine, which causes a disappearance of the noradrenalin in peripheral tissues, probably by depleting the adrenergic nerves of their transmitter (*Carlsson* et al., 1957; *Bertler* et al., 1956).

The results obtained so far agree completely with *von Euler*'s conception of the adrenergic transmitter and *Hillarp*'s conception of the autonomic innervation apparatus.

It seems possible that the fluorescence method, by modifications of the conditions for the formaldehyde condensation, may permit the cellular localization not only of noradrenalin and 5-hydroxytryptamine but also dopamine and adrenalin. A systematic study along these lines is being pursued in these laboratories.

Summary

By means of an essential modification of a previously developed fluorescence method (*Falck* et al., 1962) it now seems possible to demonstrate directly the adrenergic transmitter, noradrenalin, in the adrenergic nerve terminals. The method is based upon the principle that the amine in freeze dried tissues can be condensed with formaldehyde under nearly dry conditions to intensely fluorescent products.

Zusammenfassung

Mit Hilfe einer wesentlichen Abänderung der kürzlich beschriebenen Fluoreszenzmethode (*Falck* et al., Med. exp. 1962) erscheint es uns möglich, den adrenergischen Überträgerstoff Noradrenalin direkt im adrenergischen Nervenende nachzuweisen. Die Methode basiert auf dem Prinzip, daß das Amin in gefriergetrockneten Geweben unter nahezu trockenen Bedingungen mit Formaldehyd zu einem intensiv fluoreszierenden Produkt kondensiert werden kann.

Résumé

Aux moyens d'une modification importante de la méthode fluorescente précédemment décrite par *Falck,* il semble possible de démontrer directement le transmetteur adrénergique noradrénaline dans les terminaisons nerveuses adrénergiques. La méthode est basée sur le principe que l'amine dans les tissus congelés peut être condensée avec la formaldéhyde dans des conditions particulières pour conduire à des produits intensément fluorescents.

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This work has been supported by grants from the Medical Faculty, University of Lund; the Air Force Office of Scientific Research and Development Command, United States Air Force; and by a grant (B-2854) from the United States Public Health Service.

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