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PRELIMINARY COMMUNICATION

Immunohistochemical evidence for the transport of dopamine-β-hydroxylase and a catecholamine binding protein in sympathetic nerves

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THE ORIGIN of the granular vesicles in nerve terminals which are involved in the synthesis, storage and release of noradrenaline is the subject of controversy.[1, 2] We describe immunohistochemical evidence which suggests that the protein components of the vesicles are synthesized in the soma of the neuron and then transported down the axon to the terminals.

The first evidence for this transport was that, after ligation of sympathetic nerves, endogenous noradrenaline[3] and various axoplasmic organelles, including granular vesicles,[4] accumulated on the proximal side of the constriction. Subsequently we showed that radioactive amino acids injected into the coeliac ganglion of the cat are incorporated into proteins, some of which are transported toward the terminals of the splenic nerves at the same rapid rate (5 mm/hr) as ^{14}C-labeled noradrenaline.[5] In order to identify these proteins and localize them *in situ*, we have used immunofluorescence techniques of high specificity and sensitivity.[6]

In the adrenal medulla, it is now known that at least two proteins are present in the granular vesicles which store and release catecholamines.[7] One of these binds catecholamines and the other, dopamine-β-hydroxylase, is the enzyme which catalyzes the last step in noradrenaline synthesis and is thought to be bound to the membrane of the granular vesicles.[8]

We have purified dopamine-β-hydroxylase and the catecholamine binding protein from sheep adrenal medullae on the assumption that these proteins would be immunologically compatible with those in sheep sympathetic nerves, as the common ontogenic origin of these tissues would suggest.

Preparation of antigens. Purified chromaffin granules, obtained by differential centrifugation,[9] were lysed in distilled water and the soluble proteins were chromatographed on DEAE cellulose.[7] The protein elution pattern was followed spectrophotometrically at 280 mμ and fractions were assayed fluorimetrically for dopamine-β-hydroxylase activity.[10] Those fractions exhibiting high dopamine-β-hydroxylase activity (Ag. DH) and those containing the catecholamine binding protein (Ag. CB) were pooled separately and each was pressure dialyzed to obtain a final protein concentration of 1–2 mg/ml. Polyacrylamide gel electrophoresis of both antigens[11] showed only one main band for each and their electrophoretic mobilities differed markedly. Preincubation of purified dopamine-β-hydroxylase with its antiserum abolished enzymic activity.

Production of antibodies. Rabbits were bled from an ear vein to obtain preimmune serum and were then immunized against either protein by injecting 1 mg protein, emulsified with complete Freund's adjuvant, in divided doses at four intramuscular sites. The injection was repeated after 6 weeks and the rabbits were bled 1 week later. The antibodies in their serum (As. DH and As. CB) were tested against the antigens by micro-immunodiffusion and electrophoresis in agarose. Only one precipitin line was observed for each immune serum and there was no cross reactivity between Ag. CB and As. DH or between Ag. DH and As. CB. Potent antisera were stored below −20° in the presence of 0·01% merthiolate.

Conjugation of serum. Both preimmune and immune sera were conjugated with Lissamine rhodamine B200-chloride[12] and a labeled γ-globulin fraction was prepared by adding an equal volume of saturated ammonium sulfate, centrifuging, and then dialyzing the precipitate against 0·1 M phosphate buffered saline, pH 7·2. Fluorescein-labeled goat anti-rabbit γ-globulin for use in the "sandwich" technique was obtained from Baltimore Biological Laboratories.

Immunofluorescence histology. Various sympathetic nerves in the sheep, including fibers from the superior cervical and coeliac ganglia, were ligated under anaesthesia and 24 hr later were removed together with the adrenals. Tissues were fixed in 98% alcohol, blocked in paraffin, sectioned, deparaffinized and then treated with the sera.[13]

The sections were examined using a Leitz Orthoplan fluorescence microscope with dark-field illumination.[6] Direct staining of the adrenal medulla with rhodamine conjugates of the rabbit sera demonstrated the presence of both antigens. The blue native fluorescence of the medulla and cortex was unaltered by the preimmune serum, but with immune sera the cells of the medulla showed a deep red fluorescence which contrasted with the blue color of the cortex. Indirect staining with the fluorescein-labeled goat anti-rabbit γ-globulin, using undiluted rabbit antisera as the middle layer of the "sandwich", likewise demonstrated the presence of dopamine-β-hydroxylase and the catecholamine binding protein in the adrenal medulla, but not in the cortex.

Immune sera to both antigens reacted strongly with the cytoplasm of cell bodies in the superior cervical and stellate ganglia and less strongly with sympathetic nerve fibers emanating from these ganglia. In constricted sympathetic nerves, there was a large increase in dopamine-β-hydroxylase fluorescence, which was red with rhodamine and applegreen with fluorescein conjugates. This was localized proximal to the constriction in the region where we have also observed specific catecholamine fluorescence. By contrast, the supporting connective tissue and the tissue at the constriction showed only the dark blue native fluorescence characteristic of the untreated tissue and that treated with preimmune sera. There was also a slight distal accumulation of the enzyme. The distribution of fluorescence attributable to the catecholamine binding protein in constricted sympathetic nerves was essentially identical to that of dopamine-β-hydroxylase.

These observations provide the first evidence that proteins involved in the synthesis and storage of noradrenaline in the adrenal medulla are immunologically cross reactive with those in sympathetic nerves. Although the granular vesicles in the two tissues differ in some morphological and biochemical respects,[14] both synthesize and bind noradrenaline. The finding that dopamine-β-hydroxylase and catecholamine binding proteins accumulate proximal to an axonal constriction is consistent with the concept that their site of synthesis is in the soma of the neuron and that they are transported to the axon terminals.

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