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CYTOCHROME *b*-561 IN SYMPATHETIC NERVE TERMINAL VESICLES FROM RAT VAS DEFERENS

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Summary

The spectral properties of sympathetic nerve vesicles isolated from the vas deferens of the rat are similar to those of the bovine chromaffin granule membranes and bovine nerve trunk vesicles, indicating the presence of the specific cytochrome *b*-561. The cytochrome occurs only in the fractions containing nerve vesicles, thus suggesting usefulness as a marker enzyme.

Membrane preparations of chromaffin granules from bovine adrenal medulla [1,2] and nerve trunk from bovine splenic nerve [3] contain a specific *b*-type cytochrome with an absorption maximum around 561 nm [4]. A fraction enriched in sympathetic nerve terminal vesicles has been isolated from rat vas deferens, and because of the supposedly close relationship of nerve trunk vesicles and nerve terminal vesicles it was of great interest to establish whether cytochrome *b*-561 also occurs in nerve terminals. In view of the difficulty of obtaining pure preparations of nerve terminal vesicles, it was also of interest to see if the cytochrome could be used as a marker for terminal vesicle membranes.

Nerve terminal vesicles were isolated from the vas deferens of the normal rat according to Fried, Lagercrantz, and Hökfelt (Neuroscience, in press). Seven fractions were obtained after gradient centrifugation in sucrose 0.25—0.8 M. Nerve trunk vesicles were isolated from bovine splenic nerve according to Lagercrantz [6,7]. After freezing and thawing the vesicles were dialyzed against 50 mM potassium phosphate buffer, pH 6.5. Spectra were recorded in cuvettes of 10 mm light path with an Aminco UV/VIS double beam/split beam spectrophotometer at room temperature. Reduced-oxidized spectra were obtained through reduction of the sample with a few grains of sodium dithionite. Protein was determined according to Lowry et al. [8].

Reduced-oxidized difference spectra from fraction 4 of vas deferens preparation and from nerve trunk vesicles can be seen in Fig. 1, a and b, respectively. Fraction 4 reveals absorption maxima at 427 and 561 nm, which corresponds to the absorption maxima for the nerve trunk vesicles. This fraction is also the one which is most enriched in nerve terminal vesicles according to biochemical and morphological evidence (Fried, Lagercrantz and Hökfelt, Neuroscience, in press). No other fraction contained exclusively cytochrome *b*-561, thereby indicating the possibility of using this cytochrome as a marker for nerve vesicles.

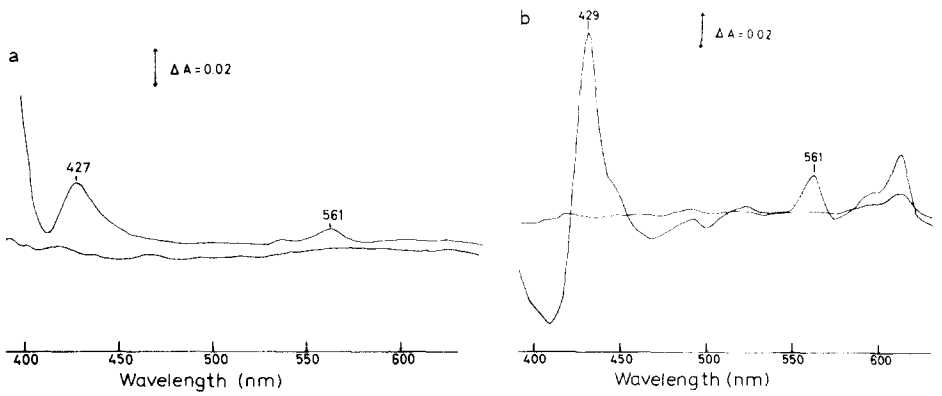

Fig. 1. Reduced-oxidized spectra from fraction 4 of the vas deferens preparation (a) and nerve trunk vesicles from bovine splenic nerve (b). The cuvettes contained 50 mM potassium buffer at pH 6.5 and varying amounts of membrane preparation. The amount of protein per sample is 0.16 mg/ml (a) and 0.43 mg/ml (b).

Fractions 3 and 5 contained absorption maxima at 420—422 nm in the Soret-band and at 550—551 nm in the α-band. Fraction 7, which is the resuspended pellet from gradient centrifugation (i.e., the particles sedimenting at a higher density than 0.8 M sucrose) contained absorption maxima at 555, 563 and 605 nm, respectively. Combined absorption in the Soret and α-bands gave peaks at 429 and 537 nm. The *b*-type cytochrome in this fraction (absorption maximum 563 nm) could be of mitochondrial origin as it appears together with cytochrome *a* (absorption maximum 605 nm). It might also be a mixture of mitochondrial *b*-cytochrome and cytochrome *b*-561 from large vesicle membranes.

The amount of cytochrome *b*-561 in fraction 4 was 0.41 nmol/mg protein as compared to 0.44 nmol/mg protein in the nerve trunk vesicles. These values are based on a molar extinction coefficient for cytochrome *b*-561 of $1.8 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ [5]. We estimate the purity of the nerve terminal vesicles in fraction 4 to be about 5—10%, and the purity of the nerve trunk vesicles to be about 70—80%.

NADH-oxido-reductase (EC 1.6.99.3) activity has been reported to occur in both chromaffin granules [1] and nerve trunk vesicles [3]. All fractions of the vas deferens preparations show rotenone-insensitive NADH-cytochrome *c*-reductase activity, with maximal values in fraction 2 (Fried, G., unpublished). This enzyme is generally considered to be a marker of microsomal membranes, and in the present preparation it does not seem possible to

distinguish a true NADH-cytochrome *c*-reductase activity of the nerve vesicles, if it exists, from a microsomal contamination.

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