BBA Report

BBA 41183

Electron carriers of the noradrenaline storage vesicles from bovine splenic nerves

T. FLATMARK, H. LAGERCRANTZ, O. TERLAND, K.B. HELLE and L. STJÄRNE

Departments of Biochemistry and Physiology, University of Bergen, Bergen (Norway) and Department of Physiology I, Karolinska Institutet, Stockholm (Sweden)

(Received July 5th, 1971)

SUMMARY

The spectral properties of the noradrenaline storage vesicles of bovine splenic nerves are essentially the same as those recently reported for the adrenal chromaffin granules (T. Flatmark, O. Terland and K.B. Helle, *Biochim. Biophys. Acta*, 226 (1971) 9) indicating the presence of flavoprotein(s) and a high-potential *b*-type cytochrome termed cytochrome b_{561}. The specific activities of NADH : (acceptor) oxidoreductase (EC 1.6.99.3) were higher than those previously found in the adrenal granules. Based on the assay of these enzymic activities, the flavoprotein(s) also appear to be more stable in the nerve granules than in the adrenal granules upon prolonged storage of the organelles at 0°.

Recent studies[1,2] on chromaffin granule membrane preparations of bovine adrenal medulla have revealed the presence of at least two electron carriers in addition to dopamine β-hydroxylase (3,4-dihydroxyphenylethylamine : oxygen oxidoreductase (hydroxylating), EC 1.14.2.1), *i.e.* flavoprotein(s)[1] and a unique high-potential *b*-type cytochrome (termed b_{561})[1,2]. These electron carriers appear to be integral parts of the granule membrane structure and are probably linked to the main biosynthetic function of these organelles, *i.e.* the formation of noradrenaline[3]. The noradrenaline storage vesicles from the bovine splenic nerve trunk have recently been isolated at a high degree of purification[4] and great chemical similarities have been observed between these vesicles and the chromaffin granules of the adrenal medulla. Their lipid pattern is very similar[5], and both organelles contain dopamine β-hydroxylase and a specific protein termed chromogranin A[6]. It was therefore considered of great interest to look for other possible similarities in their chemical and enzymic organization and in the present paper we have focused our interest on the electron transfer pigments and functions previously detected in highly purified bovine adrenal chromaffin granules[1,2].

Noradrenaline storage vesicles were isolated from bovine splenic nerves as described[4,7]. The chromaffin granules were isolated from bovine adrenals as previously

reported[8]; the pellet P_c was used. Membrane preparations were obtained by extensive dialysis[2,9] of the isolated granules against 50 mM potassium phosphate buffer, pH 6.5. All spectra were measured in a Shimadzu multipurpose recording spectrophotometer (Model MPS-50 L) calibrated with a mercury lamp. This spectrophotometer is specially designed for the measurement of turbid samples and has attachments for low-temperature spectrophotometry. Spectra at liquid-nitrogen temperature (77°K) were performed essentially as described by Kawai[10]. NADH(NADPH) : (acceptor) oxidoreductase activities were measured as described[1]. The measurement of reduction and oxidation of cytochrome b_{561} was performed in cuvettes of 10 mm light path using an Aminco-Chance dual-wavelength spectrophotometer with the reference wavelength set at 550 nm and the measuring wavelength set at 561 nm[1]. Protein concentration was determined according to the method of Eggstein and Kreutz[11], using bovine serum albumin from the Sigma Chemical Co., U.S.A., as a standard[1].

The reduced–oxidized absorption spectrum of a suspension of noradrenaline storage vesicles from bovine splenic nerves at 25° revealed absorption maxima at around 429 nm and 561 nm as compared to 430 nm and 561 nm for the adrenal chromaffin granules[1]. However, due to the low yield of highly purified nerve granules, the amplitude of the peak in the visible part of the spectrum ($A_{561\ nm} - A_{550\ nm}$) did not exceed 0.006. Therefore, low-temperature spectra were carried out.

The reduced–oxidized difference spectra of the two types of chromaffin granules at −190° are shown in Figs. 1A and 1B. The α- and β-absorption bands of the *b*-type cytochrome are intensified (about 10 times as compared to room temperature spectra[1]), and they are shifted by about 3 nm toward the blue. The Soret band is also intensified and shifted to a shorter wavelength. It is seen that nearly identical spectra were obtained for the nerve granules (Fig. 1A) and the adrenal granules (Fig. 1B), and the content of *b*-type cytochrome was very similar when determined on a protein basis. A slight difference was, however, observed in the flavoprotein region, *i.e.* the broad negative peak around 450–460 nm, of the two types of granules. This difference may be related to the difference in stability of the flavoprotein(s) present in the two types of membranes (see below).

Cytochrome b_{561} of the nerve vesicles was readily reduced by ascorbate, *i.e.* about 75% reduction occurred in less than 1 min at an ascorbate concentration of 1.2 mM. This value is in good agreement with the extent of the aerobic reduction of the high-potential cytochrome b_{561} of adrenal chromaffin granules[2].

The membrane fraction of the adrenal storage granules has been shown to contain NADH : (acceptor) oxidoreductase (EC 1.6.99.3) activities[1] with ferricyanide, 2,6-dichlorophenol and bovine heart ferricytochrome *c* as acceptors. From Table I it is seen that high oxidoreductase activities were also found in the nerve granule preparations; in fact, the specific activities were 2–4 times higher than previously found in adrenal granules[1]. No NADPH-dependent activities were, however, found in any of our preparations. This result is similar to the situation in the adrenal granules. On the other hand, the NADH : (acceptor) oxidoreductase activities are more stable in the nerve granules upon prolonged storage at 0° than in the adrenal granules.

No significant contamination by mitochondrial cytochromes could be detected in the spectra at −190° (Fig. 1A) indicating a high degree of purification of the nerve granules, as recently suggested by Lagercrantz[7].

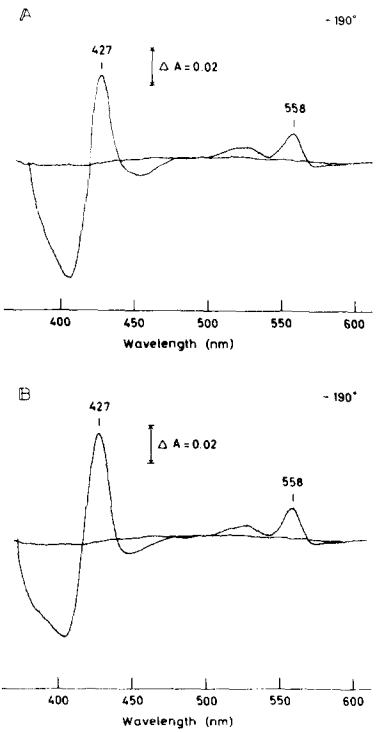

Fig. 1. The spectrophotometric detectable electron transfer pigments of noradrenaline storage vesicles from bovine splenic nerve (A) and of adrenal chromaffin granules (B). These changes of absorbance occur when the oxidized pigments (ferricyanide) become reduced upon addition of dithionite. The cuvettes contained: 50 mM potassium phosphate buffer at pH 6.5; glycerol, 50% (v/v); membrane preparation, 0.25 mg of protein per ml (A) and 0.24 mg of protein per ml (B). Optical path of the cuvettes was 2 mm; -190°.

TABLE I

NADH(NADPH) : (ACCEPTOR) OXIDOREDUCTASE ACTIVITIES OF THE NORADRENALINE STORAGE VESICLES FROM BOVINE SPLENIC NERVES

Experimental conditions were as described in the text; 50 mM phosphate buffer (pH 6.5); 25°. Activities are expressed as μmoles of NADH(NADPH) oxidized and cytochrome *c* reduced per min per mg of protein. Mean values of at least three experiments.

Electron donors	*Electron acceptors*	
	Ferricyanide (75 μM)	*Ferricytochrome c*★ *(10.4 μM)*
NADH (27 μM)	1.07	0.122
NADPH (27 μM)	0	0

★In the presence of $3 \cdot 10^{-4}$ M KCN.

The experimental work described in this paper was supported in part by grants-in aid from the Norwegian Council on Cardiovascular Diseases to T.F. and from the Swedish Medical Research Council (B71-14X-2479-04B) to L.S. and H.L., as well as by a Student Scholarship from the Norwegian Research Council for Science and the Humanities to O.T.

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