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Noradrenaline storage particles in splenic nerve

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Our present work is aimed at obtaining information about noradrenaline storage particles in splenic nerve—both of axonal and terminal derivation. These experiments have been carried out using differential and density gradient centrifugation techniques. Because of difficulties in obtaining an absolutely pure preparation of noradrenaline vesicles (Stjärne 1966) we have not attempted a purification of the particles but have used the other possible alternative as first proposed by de Duve (1964). This method involves the localization of a particle, in fractions obtained by centrifugation, by estimation of an internal constituent; the degree of contamination of a particulate fraction can be assessed by estimation of constituents of other known particles. By this method it is then possible to determine which constituents belong to any one particle.

Non-terminal axons of splenic nerve

Figure 1 shows results obtained by differential centrifugation experiments performed on bovine splenic nerve homogenates.

From figure 1 it can be seen that the distribution of noradrenaline is closely paralleled by that of dopamine β-hydroxylase activity and by that of the protein chromogranin A. One can argue from these results that the three constituents are contained in one and the same particle. It can also be argued that this particle is different from any other known particles as shown by the distribution of characteristic marker enzymes for those particles (see de Potter, Smith & de Schaepdryver 1970). Perhaps the next most interesting point to be seen in figure 1 is the distribution of ATP. It can be seen that the ATP closely, but not exactly, follows the distribution of the mitochondrial marker enzyme cytochrome oxidase. If an allowance is made for mitochondrial contamination of the fraction enriched in noradrenaline, a molar ratio of noradrenaline to ATP of 7.5 to 12 is obtained for the noradrenergic vesicles. This result is somewhat different from the ratios 3.0 to 3.8 previously obtained, but in those experiments no allowance was made for mitochondrial contamination of the noradrenaline containing particles (Schümann 1958; Stjärne 1964; Banks, Helle & Mayor 1969). In our experiments a similar ratio was obtained only when the total particulate fraction was used for estimation of both ATP and noradrenaline (de Potter *et al.* 1970).

Knowing the distribution of dopamine β-hydroxylase, noradrenaline, chromogranin A and ATP, a comparison can then be drawn between the splenic nerve storage particles and adrenal chromaffin granules. The chromaffin granules can be readily purified and hence a direct and quantitative measure of their constituents can be obtained (see Smith 1968). The results of such a comparison are summarized in table 1.

Table 1 shows that the ATP concentrations, relative to catecholamines, in the two particles are markedly different, much less being present in the splenic nerve particles. Perhaps this result means that ATP does not play such an important role in the binding of noradrenaline within the storage particles of sympathetic nerves as has generally been believed. The other striking

difference between the two particles is the difference in dopamine β-hydroxylase activities. This may reflect differences in turnover rates of catecholamines between the two organs. Chromogranin A is present in the same order of magnitude in both particles.

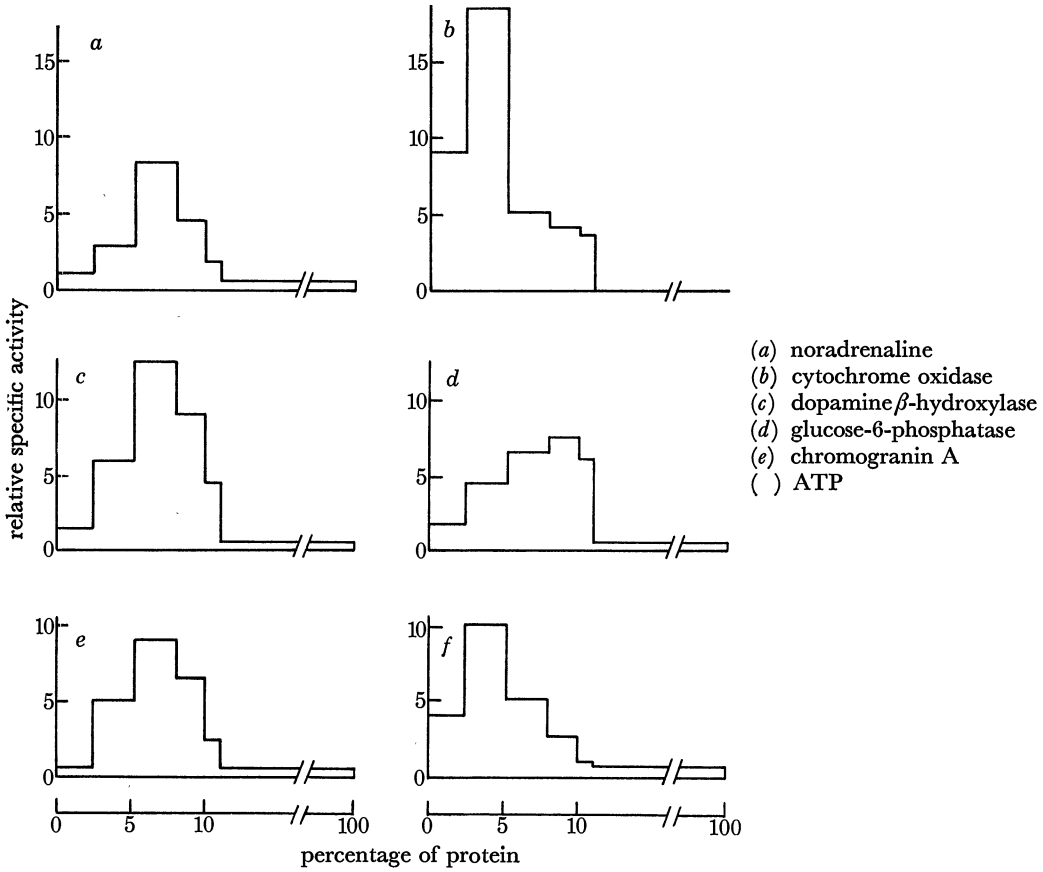

FIGURE 1. Differential centrifugation of bovine splenic nerve homogenate. Relative specific activity is the percentage in each fraction divided by the percentage of protein in each fraction. (From de Potter, Smith & de Schaepdryver 1970.)

TABLE 1. COMPOSITION OF FRACTION 3 OBTAINED BY DIFFERENTIAL CENTRIFUGATION OF SPLENIC NERVE HOMOGENATES

constituent	units	fraction 3 from bovine splenic nerve	chromaffin granules from bovine adrenal medulla
catecholamine	nmol	1	1
ATP	nmol	0.132	0.22
dopamine β-hydroxylase	unit	443	7.25
chromogranin A	μg	0.26	0.10

The composition of each particle is expressed per nanomole of catecholamine. The data for adrenal chromaffin granules were calculated from data given in the review by Smith (1968), with the exception of the activity of dopamine β-hydroxylase which was determined with the same incubation mixture (substrate [^{3}H]tyramine at 3.8 μmol/l) as used for the splenic nerve. (From de Potter *et al.* 1970.)

The results of density gradient centrifugation experiments (figure 2) give additional evidence that dopamine β-hydroxylase and noradrenaline are contained within the same particle. Hörtnagl, Hörtnagl & Winkler (1969) also found a closely parallel distribution of noradrenaline and dopamine β-hydroxylase on their sucrose gradients of splenic nerve fractions.

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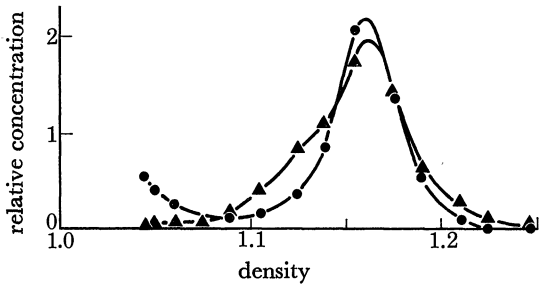

FIGURE 2. Isopycnic gradient centrifugation of a particulate fraction from bovine splenic nerves. Relative concentration is the amount in each fraction relative to the total amount applied to the gradient. ●, noradrenaline; ▲, dopamine β-hydroxylase activity. (From Chubb, de Potter & de Schaepdryver 1970.)

TERMINALS OF THE SPLENIC NERVE

To study the noradrenaline-containing particles in splenic nerve terminals, we have used homogenates of the spleens of young mongrel dogs. Figure 3 shows the distribution of noradrenaline and dopamine β-hydroxylase obtained by density gradient centrifugation of a particulate fraction of the dog spleen.

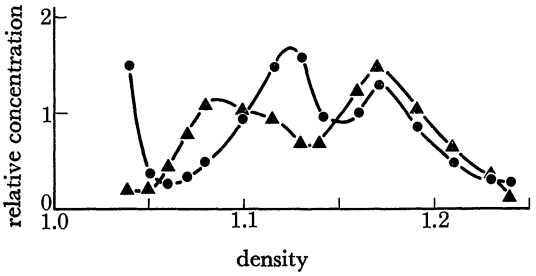

FIGURE 3. Isopycnic gradient centrifugation of a particulate fraction from dog spleen. ●, noradrenaline; ▲, dopamine β-hydroxylase activity. (From Chubb *et al.* 1970.)

It can be seen from this figure that the noradrenaline distribution in the spleen is considerably different from, and more complicated than, the distribution for the splenic nerve axons (cf. figure 2). Two peaks of noradrenaline are obtained but only one is reflected by a peak of dopamine β-hydroxylase activity, the other having little or no enzymic activity. The densities of the two noradrenaline peaks are (means ± S.D.) 1.126 ± 0.004 and 1.178 ± 0.011. The less dense peak of dopamine β-hydroxylase activity has recently been shown to be due to membrane bound dopamine β-hydroxylase (I. Chubb & W. P. de Potter, unpublished observations). A similar bimodal distribution of noradrenaline was found by Roth, Stjärne, Bloom & Giarman (1968) in density gradients of rat heart homogenates: these authors called the particles 'light' and 'heavy' noradrenaline storage particles, respectively.

This bimodal distribution of noradrenaline in splenic nerve terminals has been confirmed on gradients using properties other than the density of the particles for separation. Figure 4*a* shows the results of differential sedimentation gradient experiments. There is clearly a bimodal distribution of noradrenaline and, again, only one of the noradrenaline peaks is reflected in dopamine β-hydroxylase activity. Figure 4*b* further confirms the bimodal noradrenaline distribution, in this case on discontinuous sucrose gradients. This noradrenaline distribution has been shown to be unaffected by the addition of KCl, Mg^{2+} or buffers to the sucrose (Chubb, de Potter & de Schaepdryver 1970). The noradrenaline-containing particles are absent in the

chronically denervated spleen, and do not appear when exogenous noradrenaline is added to the homogenate of the denervated spleen (Chubb *et al.* 1970).

The question that now arises from these results is, what relationship do the axonal noradrenaline-containing particles have to those particles in the nerve terminals?

The well-documented phenomenon of axonal flow is the most probable source of the 'heavy' particle in the terminals (Dahlström 1967). Both the axonal vesicle and the 'heavy' vesicle in the terminal contain dopamine β-hydroxylase. The apparent density differences between the two (cf. figures 2 and 3) are most probably due to species difference. We have now found that the dog splenic nerve particles have approximately the same density as the 'heavy' vesicle from the dog spleen.

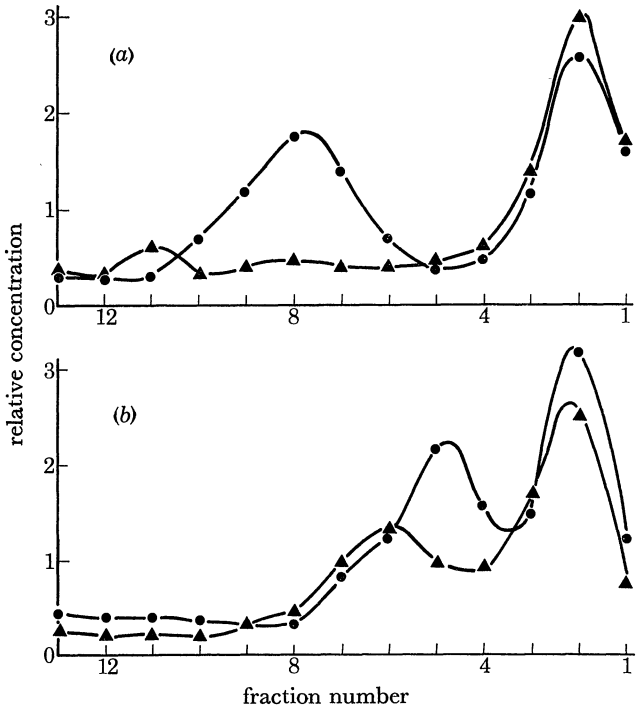

FIGURE 4. Gradient centrifugation of a particulate fraction from dog spleen. (*a*) Differential sedimentation gradient; (*b*) discontinuous gradient; ●, noradrenaline; ▲, dopamine β-hydroxylase activity. (For methods, see Chubb *et al.* 1970.)

An interesting point from these experiments on the dog nerves was the relative concentrations of noradrenaline in the vesicles from the nerve and the spleen. Dopamine β-hydroxylase has been shown to exist, at least for the most part, in a form firmly bound to the vesicular membrane (Hörtnagl *et al.* 1969; de Potter *et al.* 1970). The amount of dopamine β-hydroxylase should, therefore, be approximately proportional to the size of the vesicular population. Accordingly, the amount of noradrenaline relative to that of dopamine β-hydroxylase should give us an estimate of the noradrenaline concentration within the vesicles. If such a calculation is made for the vesicles in axons of the dog splenic nerve and for the 'heavy' spleen vesicles (as determined after gradient centrifugation) it is found that there is of the order of ten times more noradrenaline in the terminal, as compared to the axonal vesicle (I. Chubb & W. P. de Potter, unpublished observations). This result indicates that the major role of axonal transport is to supply the vesicles and not the noradrenaline itself to the terminal.

The origin of the 'light' particle, which is found in the terminals, is less clear. It does not

appear to be present in the axon, or at least, if it is present it does not contain noradrenaline. Since the 'light' vesicle contains only small amounts, if any, of dopamine β-hydroxylase, it is unlikely to be derived from the 'heavy' axonal particle. Experiments are in progress to determine the origin of the 'light' particle as well as to determine the roles played by each particle in the neurotransmission process.

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