The Turnover Rate of Noradrenergic Vesicles

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The rate at which noradrenergic vesicles turn over in the peripheral nervous system has been studied by using noradrenaline as a vesicular marker. Based on the assumption that the axonal vesicle has the same, or nearly the same, content of noradrenaline as the terminal particle, a life-span of some 3-5 weeks has been postulated (Dahlström, 1970). In a series of experiments on the storage of noradrenaline in the splenic nerve, it has been shown that the noradrenaline content of the axonal vesicle may be as low as one-tenth that of the terminal particle (De Potter, 1971). A re-evaluation of the vesicular life-span is therefore required by using a vesicular marker specific for the particle but, at the same time, not synthesized in the periphery. Such a marker could be the enzyme dopamine β -hydroxylase (EC 1.14.2.1). This enzyme has been shown to be vesicular in origin, for the most part (80%) firmly attached to the vesicular membrane, the remainder being soluble within the vesicle (Hörtnagl, Hörtnagl & Winkler, 1969; De Potter, Smith & De Schaepdryver, 1970), and to flow down adrenergic axons, at a linear rate with respect to time, at about the same rate as noradrenaline (Laduron & Belpaire, 1968).

Our approach therefore has been to determine the amount of dopamine β -hydroxylase that flows down the axons into the spleen and, assuming a steady concentration of the enzyme within the spleen itself, the rate at which the enzyme, and hence the vesicle, turns over can be calculated.

The abdomen of young mongrel dogs (5-7 kg) under Nembutal anaesthesia was opened by midline incision. The splenic nerve axons going to one side of the spleen were dissected free from the artery, gathered and constricted by means of a final silk ligature. At 2.5, 4 or 18 h after constriction, the abdomen was again opened and a 1 cm length of nerves was taken, from the point of constriction, on both the proximal and distal sides. A 1 cm length of nerves was also taken from the intact axons innervating the other side of the spleen. This section was taken to serve as a control. The axonal segments were then homogenized in 0.25 M-sucrose and samples taken for assay of dopamine β -hydroxylase. The amount of this enzyme in the spleen was measured by using a pellet that was sedimented after 60 min at 100 000 g_{av} , after an

initial centrifugation at 10 000 g_{av} for 10 min. The enzyme was assayed either directly on the pellet or after isopycnic-density-gradient centrifugation (Chubb, De Potter & De Schaepdryver, 1970). Dopamine β -hydroxylase activity was measured as previously described with [3H]tyramine (4 μM ; 500 $\mu Ci/\mu mol$; New England Nuclear, Frankfurt/Main, Germany) as substrate (Viveros, Arqueros & Kirshner, 1968).

From the results shown in Table 1 it can be calculated that, with 116 units of dopamine β -hydroxylase flowing into the spleen/h, the turnover rate of the enzyme, and hence of the vesicle, must be of the order of 40 h.

Table 1 also shows that there is no accumulation of the enzyme distal to the constriction, hence excluding the possibility of a retrograde flow of the enzyme.

Assuming a similar concentration of noradrenaline in axonal and terminal particles, a vesicular life-span of 22 days has been proposed (Dahlström, 1970). Although this value has been corrected for a noradrenaline loading in different parts of the nerve, the actual calculations were made from particles that were of axonal origin and not those of the nerve terminals themselves. It would appear from recent calculations, however, that a local synthesis of noradrenaline may load the terminal vesicles with up to ten times more noradrenaline than is contained in the axonal particle (De Potter, 1971). The net result of this would be that a measure of the noradrenaline is not an estimate of the number of vesicles and hence would result in an underestimate of the rate at which the vesicles turn over. In our experiments it is most unlikely that dopamine β -hydroxylase is also synthesized peripherally, and therefore the estimated life-span of 1.5 days is based only on the assumption that a steady activity of this enzyme is maintained within the spleen.

There are two main sources for error in our calculations, both of which would give an overestimate of the vesicular turnover rate. First, there is the possibility of enzyme inhibitors giving an artificially low activity of dopamine β -hydroxylase in the spleen. For this reason, experiments using sucrose-density-gradient techniques were carried out. In these experiments a dilute purified sample of the adrenal-medullary enzyme was added to the

Table 1. *Accumulation of dopamine β -hydroxylase at the constriction in a 1 cm segment of the nerves*

Results are expressed as total units minus controls, and are means $\pm$ S.E.M. of the numbers of experiments in parentheses. One unit of enzyme activity is defined as 1 pmol of the measured product formed/20 min. Units on the proximal side of the constriction represent the units accumulated in one of the two nerve branches/h at all time-intervals studied.

Accumulation (units/h)	
Proximal	Distal
58 ± 9 (8)	0 (8)
Total enzyme units in spleen (mean wt. 16.0 ± 2.0 g)	
4600 ± 190 (21)	

gradient fractions after determination of the splenic enzyme activity. A re-determination of the fractions showed that there was no inhibition of the enzyme under the assay conditions used. The second possible source for error is that significant amounts of enzyme were in fractions from the spleen not included in the calculation. We did not detect any activities in other particulate fractions, whether on density gradients or not, and only some 15–20% of the total activity was recovered in the final supernatant. Since the error introduced by this amount is small, we have confined our calculation to the particulate fraction, which contains the noradrenaline-storage particles and 85% of the sedimentable dopamine β -hydroxylase. On the other hand it is also possible that this estimate of the life-span of the vesicles is still too high. Experiments in our laboratory have shown that not all of the sedimentable dopamine β -hydroxylase in normal spleens is associated with noradrenaline and hence is not in a vesicular form. This extravascular enzyme is probably associated with the membranes of the vesicles, which have lost both their nor-

adrenaline and their vesicular form (I. W. Chubb, W. P. De Potter & A. F. De Schaepdryver, unpublished work). By taking into account only the vesicular dopamine β -hydroxylase, the calculated life-span of the vesicle would be of the order of 26 h, and thus from our calculations it would appear that the vesicular life-span must be between 1.0 and 1.5 days.

The relatively short life-span of the vesicles, which is of the same order as the estimated turnover rate of the noradrenaline itself (see Geffen & Livett, 1971), is taken as support for the hypothesis of exocytosis as being the release mechanism in the adrenergic nerves. It is possible to interpret this result as meaning that, when a vesicle has fused with the neuronal membrane, it loses its functional integrity and, rather than being used over again, it is destroyed and replaced by a new vesicle. Experimental evidence for the destruction of used vesicles has been obtained (I. W. Chubb, W. P. De Potter & A. F. De Schaepdryver, unpublished work).

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