EVIDENCE FOR A LOCALIZATION OF DOPAMINE-β-HYDROXYLASE WITHIN THE CHROMAFFIN GRANULES

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1. Introduction

Early studies [1] indicate that dopamine-β-hydroxylase was formerly believed to be associated with catecholamine-containing granules in the adrenal medulla. However, this could only be demonstrated by centrifuging a large mitochondrial fraction through a sucrose density gradient [2–4]. More recently it was claimed that dopamine-β-hydroxylase was located partly on the exterior of the chromaffin granule membrane. This suggests that β-hydroxylation might occur outside the granules which would mean that it is not necessary for the dopamine to penetrate the granules [5]. As these results are completely at variance with the concept of the structure-linked latency of dopamine-β-hydroxylase [6], the present experiments were undertaken.

In order to determine whether or not an enzyme exists in a latent form, two conditions must be fulfilled [7]. Firstly, the enzyme must be contained within a given subcellular particle. The second, and perhaps the most important condition is that the membrane of this particle must be impermeable to the substrate, thus preventing its penetration towards the enzyme. Several enzymes, such as monoamine oxidase and cytochrome oxidase (which, however, are mitochondrial) have no latency because of the high permeability of mitochondrial membranes to their substrates.

2. Methods

Fresh bovine adrenal glands were obtained from the local slaughterhouse and immediately chilled in ice-cold 0.25 M sucrose. The medullae were dissected from the total gland, minced and then homogenized in 10 vol of 0.25 M sucrose with a Duall homogenizer. An M + L fraction was prepared as previously described [6]. After two washings, this fraction was carefully suspended in 20 vol of 0.25 M sucrose.

Purified chromaffin granules were obtained by centrifuging an M + L fraction layered on a 1.6 M sucrose solution according to the method of Smith and Winkler [8].

The free activity of dopamine-β-hydroxylase was measured in isotonic conditions as previously described [6] except that, here, catalase and tranylcypromine were omitted. The total activity was assayed in the presence of 0.05% Triton X-100.

3. Results and discussion

The structure-linked latency of dopamine-β-hydroxylase was estimated biochemically as the difference between the activity assayed under isotonic conditions, which tend to respect the integrity of the particles (free activity), and the activity determined in the presence of Triton X-100, which disrupts the particle (total activity). Fig.1 shows both activities as obtained by increasing the enzyme concentration in an M + L fraction from bovine adrenal medulla. At low enzyme concentration, *N*-ethylmaleimide had no effect on the total dopamine-β-hydroxylase activity. However, the latter was only found to increase linearly as a function of enzyme concentration when *N*-ethylmaleimide was added to the incubation mixture. Even under such conditions, more than 90 per cent of the total dopamine-β-hydroxylase was present in a latent form since the total activity was practically 10 times greater than the

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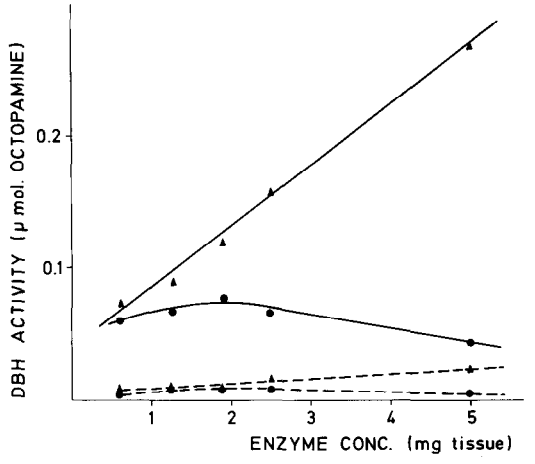

Fig.1. Influence of increasing enzyme concentration on the free (– – –) and total activity (——) of dopamine-β-hydroxylase in an M + L fraction from bovine adrenal medulla in the presence (▲) and the absence (●) of 10^{-2} M *N*-ethylmaleimide.

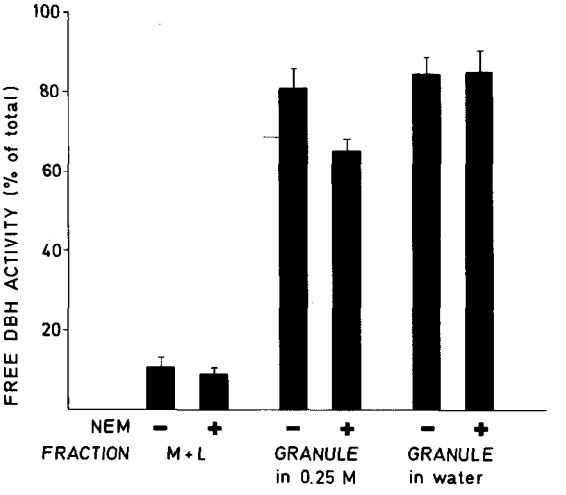

Fig.2. The free activity of dopamine-β-hydroxylase as a percentage of total activity, in an M + L fraction and in purified granules which were suspended in 0.25 M sucrose or distilled water. The enzymic activities were assayed in the presence or absence of 10^{-2} M ethylmaleimide. Results are the mean of six determinations with standard deviation.

free activity. Consequently, the structure-linked latency of dopamine-β-hydroxylase was not due to the presence of endogenous inhibitors, but to the fact that the membrane of chromaffin granules prevents the penetrations of the substrate into the granules where dopamine-β-hydroxylase is normally located. The existence of a residual free activity (about 10% of total activity) must be due to damage to the granules during homogenization or even caused by the suspension pellet corresponding to the M + L fraction.

As reported earlier [6] the structure-linked latency of dopamine-β-hydroxylase disappeared after various activating procedures such as osmotic shock. A similar phenomenon was also observed when granules were purified by centrifuging through a 1.6 M sucrose solution. Fig.2 shows that the free activity of dopamine-β-hydroxylase increased tremendously as compared with an M + L fraction, when purified granules were suspended in distilled water as well as in isotonic sucrose. Identical results were obtained by suspending the granules in 0.16 M or 1 M KCl. This clearly indicates that in the course of purification, the granules were disrupted by osmotic shock, and also virtually rules out the suggestion of Thomas et al. [5] that dopamine-β-hydroxylase might be bound to the exterior of chromaffin granule membrane. The establishment of the dopamine-β-hydroxylase latency appears a valuable tool, and is probably the most sensitive means of assessing the degree of integrity or damage in a granule preparation. In this respect, granules from splenic nerves were found to be much more damaged (free activity ranging from 30 to 40% of total activity) than chromaffin granules as nerve tissues are far more difficult to homogenize [9]. For in vitro studies, the isolation procedure using Ficoll gradients in isotonic conditions [10] is therefore preferable to using sucrose gradients [8].

In conclusion, the foregoing data confirm our previous finding that dopamine-β-hydroxylase is a latent enzyme and is thus readily contained on the inside of chromaffin granules, a feature which has been recently confirmed by immunological techniques [11,12]. The endogenous inhibitors are not responsible for such structure-linked latency which may be attributed to the absence of penetration of tyramine and even dopamine [6] into granules. Consequently, the latency concept does not support the view, still a classical one, however, that catecholamines may diffuse from the cytosol into granules [13]. On the contrary, it supports indirectly the idea that catecholamines [14] and specifically dopamine [4] are taken up into the

granules by an active process, the rate of which nevertheless is too low to influence the latency of dopamine-β-hydroxylase. Conversely a diffusion of catecholamines from the granules to outside which certainly occurs under in vitro conditions presumably owing to the abnormal external medium, is quite unlikely to occur in vivo because if it were so, then the granules or vesicles would lose almost their entire catecholamine content through the axonal flow or cytoplasmic transport, without concomitantly releasing dopamine-β-hydroxylase. Consequently, the chromaffin granules which behave as osmometers, may be considered as subcellular entities relatively independent and allowing the precursors of noradrenaline or adrenaline to cross the granule membrane only in one direction and by means of an active process. This view is entirely compatible with the structure-linked latency of dopamine-β-hydroxylase

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