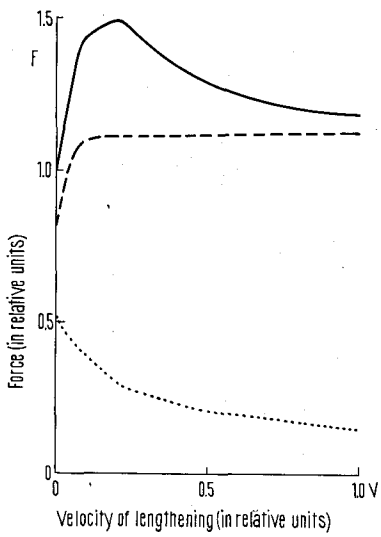
isometric tension value. A similar effect can also be deduced from figure 2 of ABBOTT, AUBERT and HILL[5].

Using different velocities of lengthening, a characteristic set of force-velocity data could be recorded (Figure). To obtain force-velocity curves comparable to the isotonic experiments reported in the literature, in the case of muscle twitches the maximal tension values characteristic for any given velocity of lengthening have been used for the construction of the curves; at this point there exist truly isotonic conditions as dF/dt is zero.



The solid curve has been constructed from the true steady state tension values recorded under tetanic conditions for a variety of isovelocity stretches. The isovelocity ramp stretches were applied for 100 msec to an isometrically contracted muscle (after 3 sec of stimulation at a rate of 12 pulses/sec). The dashed curve corresponds to force-velocity values obtained upon applying an 100 msec isovelocity ramp input to a muscle contracting isometrically at t = 175 msec, following a single pulse stimulus. The pack tension value developed by a particular muscle at a given velocity of lengthening was used for the construction of the curve. When the isovelocity stretches were applied at t = 300 msec during the time course of the twitch, the relation between peak tension values and velocities is indicated by the dotted curve. Each of the 3 curves is the computer-averaged mean least-square fit of experiments on 20 muscles. To be able to compare different experiments, the data have been normalized against the tetanic isometric force (F = 1.0) developed by each muscle (between 50 to 60 mg). For the same reason the velocities of lengthening have been expressed as a fraction of the maximal shortening velocity (V = 1.0) characteristic for each muscle, which ranged from 4.5.–4.9 cm/sec. Each muscle has been tested for at least 6 different velocities.

The results for constant velocities of lenthening shown in the Figure differ from those recorded by KATZ[1] for tetanically stimulated frog sartorius under supramaximal loads. There no longer exists a unique force-velocity relation for negative velocities, as for different velocities of lengthening the same force is developed by the muscle.

Further, it became apparent that the force-velocity relation depends critically on the activation conditions prevailing within the muscle. If isovelocity stretches were applied towards the end of the rising phase of an isometric twitch, the maximal force which can be developed by the muscle remains essentially constant at velocities above 0.1 V. Applying the same stretches, however, during the relaxation phase of an isometric twitch, a hyperbolic force-velocity curve was recorded again, which was almost the inverse of the force-velocity hyperbola characteristic for muscle shortening.

A likely explanation is that the shape of the force-velocity curve reflects the number of active myosin cross-bridges. Under tetanic conditions the high Ca^{2+} level may provide for maximal activation. The postulate is then that the tension rise at very low velocities of lengthening results from an actual compression of the cross-bridges attached to the actin filaments as the 2 sets of filaments are displaced against each other contrary to their normal sliding direction. At the higher velocities this effect will be masked as the number of cross-bridge links decreases. In the relaxation phase, where the Ca^{2+} concentration is already low[6], even very low velocities of lengthening will tend to reduce the fraction of attached cross-bridges.

Zusammenfassung. Für Muskelverlängerungen zeigt die Kraft-Geschwindigkeit-Beziehung des Frosch-Sartoriusmuskels bei konstanten Geschwindigkeiten der Verlängerung eine Doppeldeutigkeit, wobei der Aktivierungsgrad des Muskels einen wesentlichen Einflussfaktor darstellt.

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[5] B. C. ABBOTT, X. M. AUBERT and A. V. HILL, Proc. R. Soc. B *139*, 86 (1951).
[6] R. A. CHAPLAIN and E. PFISTER, Experientia *26*, 505 (1970).

Influence of Electrical Stimulation on the Subcellular Distribution of Dopamine-β-Hydroxylase in the Dog Spleen

In previous studies it has been shown that two populations of noradrenaline (NA) containing vesicles occur in sympathetic nerve endings[1,2]. In the dog spleen these two kinds of vesicles are characterized by their equilibrium densities in sucrose gradients ($NA_{1.125}$; $NA_{1.178}$). The latter type of vesicle contains the biosynthetic enzyme dopamine-β-hydroxylase (DβH)[2], approximately 80% of which is firmly bound to the vesicular membrane, the remaining 20% being in a soluble form within the particle[3–5].

The partially soluble property of DβH has been made use of when showing that DβH and another protein, chromogranin A, also partially soluble within the vesicle, were released from the spleen by stimulation of the splenic nerves[6–8]. From this evidence it would seem clear that the $NA_{1.178}$ vesicle is directly involved in the release of NA, but the contribution of the other ($NA_{1.125}$) vesicle in the release process was still unknown.

The present experiments were undertaken to obtain additional evidence for an exocytotic release mechanism from sympathetic nerves by making use of the membrane bound property of DβH. Additionally, the experiments were designed to show any changes in NA-distribution in the spleen after electrical stimulation.

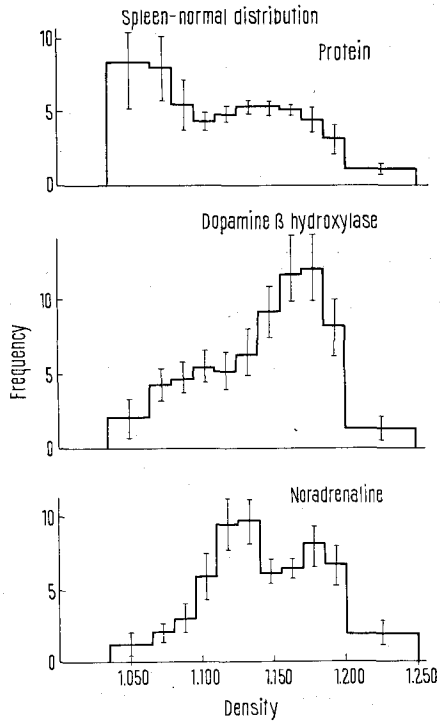

Fig. 1. Control distribution of NA, DβH and protein after isopycnic gradient centrifugation (for details of centrifugation see ref.[2]). The recovery of all 3 components measured was in the range of 85 to 95%. Data were obtained from 12 spleens.

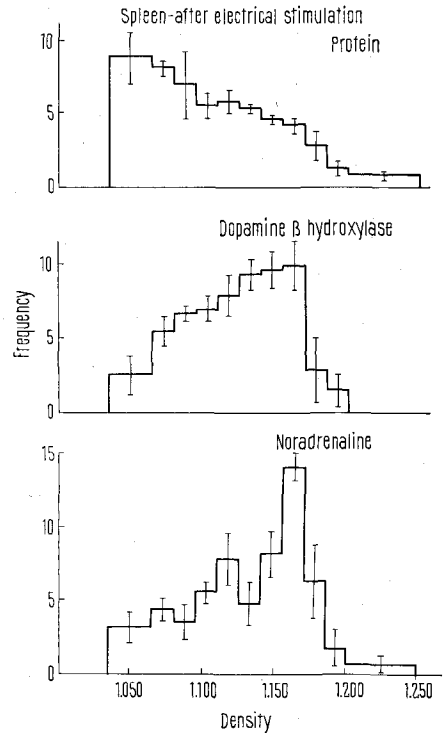

Fig. 2. Distribution of NA and DβH after density gradient centrifugation of electrically stimulated spleens. Data were obtained from 6 spleens.

Materials and methods. The abdomen of young mongrel dogs under pentobarbital anaesthesia was opened by midline incision. The splenic nerves were then stimulated in situ with bipolar platinum electrodes supramaximally with monophasic rectangular pulses of 2 msec duration at a rate of 10/sec. Stimulation was carried out non-stop for 30 min.

At the end of the stimulation period the spleens were taken from the animal and prepared and centrifuged as previously described[2]. Isopycnic density gradient centrifugation was used in order to differentiate the NA containing particles and vesicular and membranous DβH[2].

NA, DβH and protein and density were estimated as previously described[5]. The results of the density gradient distributions are expressed exactly as has been described by BEAUFAY et al.[9] Results are expressed as means ± S.E.M.

Results. The distribution patterns, in control spleens, of DβH, NA and protein are shown in Figure 1. As has been previously reported, of the two NA-containing vesicles only one, $NA_{1.178}$, is reflected by DβH activity[2], although there is also some enzyme activity at a lower density range (1.107–1.115). As shown in Figure 2, there is a relatively larger amount of DβH activity at this low density level after electrical stimulation. It is also apparent from Figure 2 that there is a relatively greater loss of NA from $NA_{1.125}$ than from the other particle. The distribution pattern of the total protein pattern is essentially unchanged. In terms of absolute amounts, there was a small but significant decrease in NA content (36.9 ± 6.1 ng/mg protein) in the electrically stimulated as compared to the control spleen (50.1 ± 3.8, $P < 0.05$). For DβH there was no significant change (54.1 ± 5.0 units/mg protein as compared to 51.5 ± 4.2).

Discussion. The most obvious differences between the density gradient distribution patterns of the normal and stimulated spleens are the relative decrease in the NA content of $NA_{1.125}$ and the increase in DβH activity at the lower density range (Figure 2).

In terms of absolute amounts, there was a significant decrease in the splenic NA content but not in the DβH level. Since it has been shown that electrical stimulation releases DβH from the splenic nerve[6–8], it could be expected that there would be a decrease in the absolute amount of the enzyme in stimulated spleens. Experimentally, however, this would be difficult to show. Even if every vesicle lost its soluble DβH, then the maximum losses of the enzyme could only amount to some 20% of the total. Such decreases would not be significantly

[1] R. H. ROTH, L. STJÄRNE, F. E. BLOOM and N. T. GIARMAN, J. Pharmac. exp. Ther. *162*, 203 (1968).
[2] I. W. CHUBB, W. P. DE POTTER and A. F. DE SCHAEPDRYVER, Nature, Lond. *228*, 1203 (1970).
[3] L. STJÄRNE, R. H. ROTH and F. LISHAJKO, Biochem. Pharmac. *16*, 1729 (1967).
[4] H. HÖRTNAGL, H. HÖRTNAGL and H. WINKLER, J. Physiol., Lond. *205*, 103 (1969).
[5] W. P. DE POTTER, A. D. SMITH and A. F. DE SCHAEPDRYVER, Tissue Cell *2*, 529 (1970).
[6] L. B. GEFFEN, B. G. LIVETT and R. A. RUSH, J. Physiol., Lond. *204*, 58 P (1969).
[7] A. D. SMITH, W. P. DE POTTER, E. J. MOERMAN and A. F. DE SCHAEPDRYVER, Tissue Cell *2*, 547 (1970).
[8] G. P. GEWIRTZ and I. J. KOPIN, Nature, Lond. *227*, 406 (1970).
[9] H. BEAUFAY, D. S. BENDALL, P. BAUDHUIN, R. WATTIAUX and C DE DUVE, Biochem. J. *92*, 184 (1964).

measurable due to tissue variation of enzyme concentration.

The fact that there is essentially no change in the absolute amount of the enzyme indicates that the increased activities recovered in the lower density range comes from the $NA_{1.178}$ particle. If this is the case, and since 80% of the DβH is bound to the vesicular membrane, then the vesicle must be transformed into lighter units by nerve stimulation – these lighter units possibly being fragments of the vesicular membrane itself.

When a vesicle containing pellet from the spleen was lysed by hypo-osmotic shock, and then subjected to density gradient centrifugation, the DβH activity was almost entirely confined to the 1.107–1.115 density range. This apparently membrane-bound DβH has the same density characteristics as some of the DβH present in the spleen after electrical stimulation, i.e. sedimentation to the density range 1.107–1.115. Additionally, when a vesicular pellet was incubated at 37°C for 30 min to empty it of its NA, the density of the DβH of these 'empty' vesicles was 1,150. These results show that it is not merely a loss of NA which causes the marked DβH density changes after electrical stimulation but that the vesicle is fragmented so that only the membrane remains.

It is apparent that $NA_{1.125}$ has lost more of its NA than the other particle (Figure 2). While it seems obvious from previous experiments[6–8] that $NA_{1.178}$ directly releases its NA (and DβH), the results reported here imply that both particles are involved in the release process. Whether they both release their NA directly into the extracellular space remains unresolved, however. It is equally possible that $NA_{1.125}$ acts as an emergency store of transmitter which is used to fill the other vesicles under stress conditions.

Of the several modes of NA release which have been suggested[10], the one to gain the most recent support has been that of exocytosis[6–8]. The present series of experiments offer additional support for this release mechanism in that it is the only one which could increase the amount of DβH containing membranes. Whether the release is by a true exocytotic mechanism i.e. a fusion of the vesicular and axonal membranes for a finite time, or by a reversed pinocytosis where the vesicle is incorporated into the axonal membrane, cannot be resolved by these experiments. In either case, however, the resultant increase in vesicular membrane in the nerve endings indicates that once having fused, the vesicle is replaced and not reused[11].

Résumé. Une stimulation électrique in situ de la rate du chien provoque l'apparition de membranes contenant de la dopamine-β-hydroxylase. Les membranes sont très probablement le résultat d'une fusion de la membrane axonale et de la membrane vésiculaire, pendant le processus de décharge. Une telle formation de membranes indique que les particules chargées de noradrénaline ne peuvent être utilisées qu'une seule fois.

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[10] L. B. Geffen and B. G. Livett, Physiol. Rev. *51*, 98 (1971).

[11] This work was supported by a grant from the Fund for Interdisciplinary Research, Belgium. We thank Misses L. Feys, H. Geffens and M. Vanneste for valuable technical assistance.

Effects of Noradrenaline and Thyroxine on Cerebral Metabolism

It is well known that thyroid hormones regulate growth, differentiation and oxidative metabolism in various animals. There are many interrelationships between catecholamines and thyroid hormones[1,2]. The aim of this study is to show what effect noradrenaline, thyroxine and both together have on respiration of the rat cortex slices during ontogeny. Because it has been observed that the concentration of 10^{-4} *M* noradrenaline increases the oxygen consumption of some parts of the brain[3], only this concentration was used. Thyroxine-doses at the different stages of ontogeny were given approximately according to Tirri et al.[4].

Materials and methods. Sprague-Dawley rats were used as experimental animals. Litters were reduced to 8 animals. Half of them were injected s.c. with thyroxine once a day and the other half, injected with 0.9% NaCl, served as controls. Thyroxine was dissolved first in 0.1 *N* NaOH and then diluted with 0.9% NaCl saline. The animals were killed by decapitation, the brains were excised at 0°C and 0.3–0.4 mm thick slices were cut from the cortex. The oxygen consumption of the brain cortex slices was measured by conventional Warburg technique at 37°C using Krebs-Ringer phosphate saline (pH 7.2) with glucose (6 m*M*) as a medium. Pure oxygen was used as gaseous phase. Noradrenaline used in half of the experiments was tipped into the medium after the first manometric reading. Usually less than 120 sec elapsed from decapitation to cooling of the brain with saline and after 15–20 min the Warburg vessels were in the thermostat. The results were calculated on the basis of dry weight of the tissues and expressed as QO_2 (μl O_2 per mg dry weight per h) for the first three 30-min periods after the gassing and equilibration periods of 5 min and 10 min, respectively.

Results and discussion. It can be seen from Table I that noradrenaline (10^{-4} *M*) had no effect on the respiration of rat brain cortex slices during ontogeny. In an earlier study, it was observed that noradrenaline does not affect the respiration of the adult rat cortex slices[3]. However, noradrenaline increased the respiration of cortex slices in age groups of 20, 40 and 120 days in rats treated with thyroxine. It was generally observed that the stimulation of oxygen consumption caused by noradrenaline decreases as a function of time. Because 10 days was the only age

[1] G. Krishna, S. Hynie and B. B. Brodie, Proc. Natn. Acad. Sci., USA *59*, 884 (1968).

[2] N. Hangaard and M. E. Hess, Pharmac. Rev. *18*, 197 (1966).

[3] L. Nieminen and K. Y. H. Lagerspetz, Annls Med. exp. Biol. Fenn. *49*, 93 (1971).

[4] R. Tirri, M. Pantio and H. Tarkkonen, Experientia *24*, 365 (1968).