isometric tension value. A similar effect can also be deduced from figure 2 of Abbott, Aubert and Hill.

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 isometric conditions as \(dF/dt\) is zero.

The results for constant velocities of lengthening shown in the Figure differ from those recorded by Katz 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, 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.

R. A. Chaplain

Department of Biocybernetics,
Advanced College of Technology,
DDR-301 Magdeburg (DDR),
24 August 1971.

---

**Influence of Electrical Stimulation on the Subcellular Distribution of Dopamine-\(\beta\)-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. In the dog spleen these two kinds of vesicles are characterized by their equilibrium densities in sucrose gradients (\(NA_{A1,125}; NA_{A1,178}\)). The latter type of vesicle contains the biosynthetic enzyme dopamine-\(\beta\)-hydroxylase (D\(\beta\)H), approximately 80% of which is firmly bound to the vesicular membrane, the remaining 20% being in a soluble form within the particle.

The partially soluble property of D\(\beta\)H has been made use of when showing that D\(\beta\)H and another protein, chromogranin A, also partially soluble within the vesicle, were released from the spleen by stimulation of the splenic nerves. From this evidence it would seem clear that the \(NA_{A1,125}\) vesicle is directly involved in the release of NA, but the contribution of the other (\(NA_{A1,178}\)) 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 D\(\beta\)H. Additionally, the experiments were designed to show any changes in NA-distribution in the spleen after electrical stimulation.
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. Isopycnic density gradient centrifugation was used in order to differentiate the NA containing particles and vesicular and membranous DβH. NA, DβH and protein and density were estimated as previously described. The results of the density gradient distributions are expressed exactly as has been described by BEAUFAY et al. 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, NA1.125, is reflected by DβH activity, 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 NA1.125 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 ± 5.6 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 NA1.125 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 nerves, 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

---