Topical application to the pedal ganglia resulted in an immediate high local concentration with slow and minimal distribution to other tissues.

We believe that within the 1st 30 min, and probably the 1st day, the total radioactivity represents mainly 5-HT and not its metabolites for the following reasons. Nonlabeled 5-HT was applied to the pedal ganglia of 8 mussels and the ganglia extirpated after 30 min from 4 and after 24 h from the other 4, extracted and assayed fluorimetrically for 5-HT. After subtracting the control value for 4 untreated mussels (23–27 ng) it was found that 32–40% of the original 1 µg dose was retained after 30 min and 4–7% after 24 h. The approximate 8fold decrease in 5-HT corresponds roughly to the 6fold decrease in total radioactivity retained by the ganglion over the same time span and suggests that at least 75% of the label represents 5-HT. Fluorescence analysis of 5-HT in all 3 ganglia was also measured after intracardiac injection of nonlabeled 5-HT and again, the relative distribution and change with time in the amount found compared favorably with the total radioactivity data. The rate of metabolic degredation of endogenous 5-HT is unknown although Stefano and Aiello[4] found that inhibition of monoamine oxidase enhanced histofluorescence within 24 h.

The increase in radioactivity in the ABRM, innervated by 5-HT fibres[13], between 2 and 24 h can be accounted for by axonal transport from the pedal ganglia at a rate of 42.2 mm/days as calculated by the method of Dahlström[14]. Experiments in progress suggest that most declines in radioactivity are due to excretion into the bathing medium with insignificant reabsorption. The amount of 5-HT estimated to be accumulated by various tissue is well within physiological limits and is presumably being metabolized normally. For example, the 2 h reading for the visceral ganglia after i.m. injection represents less than 2% of the estimated content and that in the pedal ganglia 30 min after topical administration represents less than 20% of the content, calculated from present and previous data[15]. In summary, intracardiac injection gives proportionately higher and earlier peak concentrations in tissues that specifically accumulate it, i.m. injection gives slower but longer lasting accumulations, and topical application to a ganglion essentially localizes the drug in that structure.

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Changes in cholinesterase activity of muscle after crushing the sciatic nerve of rats[1]

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Summary. The subcellular distribution of cholinesterase (ChE) was studied in the gastrocnemius muscle of rats after strong or weak nerve crushing. The ChE activities of muscle were decreased to a greater extent by strong crushing than by weak crushing. In particular, the ChE activity of the fraction containing sarcoplasmic reticulum was most greatly decreased. These results suggest that the change in the ChE activity of the microsomal fraction most finely reflects the strength of nerve crushing.

It is well known that the ChE activity of muscle decreases after denervation. Many authors, however, have reported the changes in the level of homogenate[2,4] and histochemical staining[3–5]. It has been reported that under normal conditions ChE was distributed on the sarcolemma membrane, and to a considerable extent on the sarcoplasmic reticulum[6–8]. Therefore, this paper describes the distribution of the ChE activity in rat gastrocnemius muscle after strong or weak nerve crushing.

Methods. Male Wistar-Imamichi rats weighing 110–130 g were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The left sciatic nerve was crushed over a length of 2 mm under constant pressure at the level of the thigh for 5 min with Péan's forceps whose contact surfaces had been flattened. The details of the method of crushing the nerve will be described elsewhere[9]. The contralateral muscle served as a control. The muscle was prepared as follows. The gastrocnemius muscle was rapidly removed, minced at 0 °C and homogenized in 0.6 M KCl-10 mM Tris-maleate buffer (pH 7.4). After incubation at 37 °C for 30 min, the homogenate (3.2%, w/v) was centrifuged at 1,000 × g for 5 min. The resulting precipitate was called fraction *a*. The supernatant was diluted 4fold with distilled water, and centrifuged at 100,000 × g for 60 min. The resulting precipitate was called fraction *b*, and the supernatant, fraction *c*. Fraction *a* and *b* was suspended in modified Krebs Ringer's solution (final Ca^{2+} concentration, 1 mM). No solution was added to fraction *c*. Fraction *a*, *b* and *c* contained mainly sarcolemma ChE, sarcoplasmic reticulum ChE and solubilized ChE, respectively. On the following day the ChE assay was performed. The ChE activity, determined by the slightly modified method of Chuang[10], was calculated from the ^{3}H-acetate extracted into toluene-isoamylalcohol (5:1, v/v) produced from ^{3}H-acetylcholine hydrolyzed in 10 min. 1 unit is defined as the hydrolyctic activity which will produce 10^{-9} moles of acetylcholine per 1 min. Protein concentrations were estimated by the method of Lowry et al.[11].

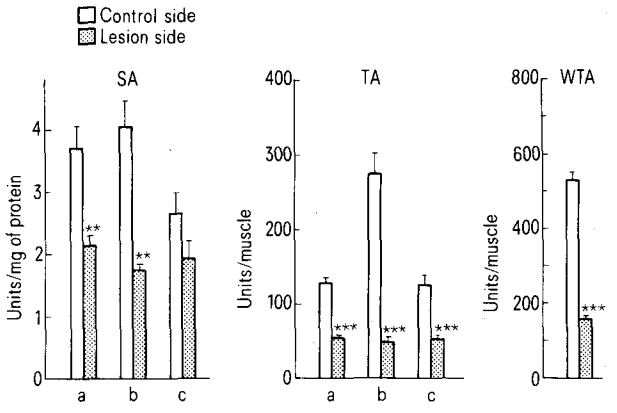

Fig. 1. The ChE activities in each fraction of gastrocnemius muscle on the 7th day after strong nerve crushing. SA, specific activity; TA, total activity (activity of each fraction per muscle); WTA, whole tissue activity (sum of total activity of each fraction); *a* 1,000×g precipitate; *b* 100,000×g precipitate; *c* 100,000×g supernatant. Unit, 10^{-9} moles of acetate per 1 min. Column with bar, mean±SEM. Number of rats, 5; *p < 0.05; **p < 0.01; ***p < 0.001 when compared with control value (Student's t-test).

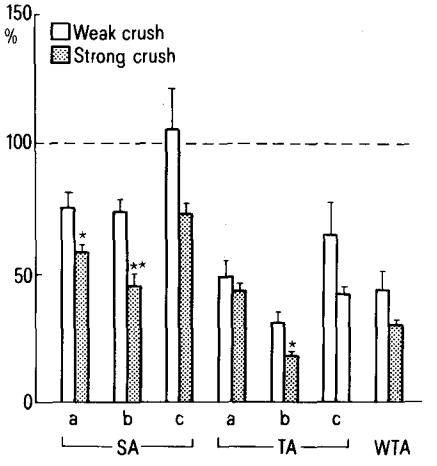

Fig. 3. The percentage change of ChE activities in each fraction of gastrocnemius muscle on the 7th day after nerve crushing. The ChE activities are expressed as a percentage in comparison with those of intact side. *p < 0.05; **p < 0.01 when compared between the values after strong nerve crushing and those after weak nerve crushing. Other remarks on data are the same as in figure 1.

Results. The total activities of ChE in each fraction decreased to a minimum on the 7th day after nerve crushing, subsequently returning very gradually to near the control value. ChE activities on the 7th day after operation are shown in figure 1 and 2. The specific activities of fraction *a* and *b*, the total activity of all fractions and whole tissue activity decreased significantly with strong nerve crushing. The decrease in fraction *b* was greater than that of other fractions. The total activities of fraction *a* and *b* and whole tissue activity decreased significantly with weak nerve crushing. Since the ChE activity of the control side was not affected significantly by the strength of nerve crushing, the percentage values relative to the control side are shown in figure 3. The specific and total activities of each fraction, and the whole tissue activity of ChE decreased more remarkably by strong crushing than by weak crushing. There was a significant difference between strong and weak crushing in the specific activities of fraction *a* and *b*, and the total activity of fraction *b*. Denervation affects not only the ChE activity in the sarcolemma but also that in the sarcoplasmic reticulum. The ChE activity of sarcoplasmic reticulum was most greatly decreased by nerve crushing, and best reflected the strength of nerve crushing.

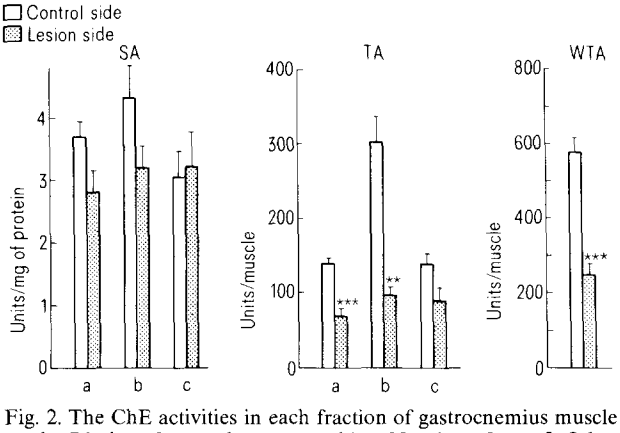

Fig. 2. The ChE activities in each fraction of gastrocnemius muscle on the 7th day after weak nerve crushing. Number of rats, 5. Other remarks on data are the same as in figure 1.

Discussion. The ChE activities of muscle were decreased to a greater extent by strong crushing than by weak crushing. This suggests a close connection between the nerve and the muscle ChE activity. Guth et al.[12] reported that the ChE of muscle homogenate was roughly proportional to the number of innervated muscle fibers. On the other hand, Smith et al.[7], Namba[6] and Liu et al.[8] reported that the highest activity of ChE was found in the microsomes. In this paper it was found that the muscle ChE activity reflected the degree of axonal degeneration and that the fraction containing mainly sarcoplasmic reticulum ChE was most sensitive to the nervous disorder. Furthermore, in our results with soleus muscle after nerve crushing, the decrease of microsomal ChE activity was significantly greater than that of muscle homogenate[13]. These results suggest that the decrease of ChE activity of muscle, particularly of that in the microsomal fraction, may be a good index of nervous disorder.

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