Quantitation of Noradrenaline Fluorescence in the Superior Cervical Ganglion of the Rat and the Effect of Postganglionic Axotomy

The superior cervical ganglion of the rat contains two main types of sympathetic neuron. Using the method of FALCK et al.[1], it may be shown that these neurons display specific fluorescence due to the NA[2] content of the perikarya of sympathetic noradrenergic neurons and to the DA[2] content of small, intensely fluorescent cells[3-6]. The noradrenergic perikaria contain variable fluorescence and by inference different NA concentration. ERÄNKÖ and HÄRKÖNEN[7] proposed that high fluorescence intensity was correlated with high ACHE[2] activity, although HÄRKÖNEN[8] had also suggested an inverse relationship between a positive reaction for non-specific cholinesterase and a high fluorescence intensity. A different and more plausible explanation for the heterogeneity of ganglion cells with respect to their NA content was advanced by K. FUXE (cit. [4]) who assumed that monoaminergic neurons with short axons exhibited a higher amine concentration in their non-terminal axons and in their cell bodies than neurons with long axons.

In general, the perikaryon volume of the neuron is proportional to the neuronal territory, i.e. to the sum of the volumes of the neuronal processes[9,10]. Assuming that the total NA content per cell is about constant, it may be expected that the large NA perikarya in the superior cervical ganglion belong to neurons with long axons and a low average NA concentration per unit area and that the small NA perikarya belong to short neurons and therefore have a high apparent NA concentration.

In order to test this hypothesis, the intensity of fluorescence was evaluated microfluorimetrically[1]. The superior cervical ganglia were taken from male albino rats (Sprague-Dawley derived, weighing 270–350 g), frozen in liquid nitrogen, freeze-dried, treated with formaldehyde gas at 80 °C for 75 min and embedded in paraffin. The formaldehyde gas was generated from paraformaldehyde powder equilibrated at 50% relative humidity. Longitudinal sections of the ganglia, 5 μm in thickness, were examined in a Zeiss-Cytofluorometer equipped for fluorescence combined with phase contrast. For excitation a HBO-200 mercury lamp was used with a BG 12 filter. The emission, limited by the barrier filter to 470–650 nm, was measured in a circular area of 1.7 μm in diameter. This spot was placed in between the cell nucleus and the perikaryon surface. The galvanometer readings were taken as a measure of the average cytoplasmic fluorescence intensity of a particular perikaryon and expressed in arbitrary units (AU). These units were based on a gelatine standard[11] containing up to 100 μg/g of NA. Since there is no strict linear relationship over the whole range between the readings of the standard and the concentration of amine in situ, the units reflect only relative values obtained under comparable conditions of mesurement. Only the perikarya that possessed a distinct nucleus with nucleolus were measured and 1 random value of the fluorescence of each perikaryon was taken. In the phase contrast image, the length and width of each perikaryon was determined by means of an ocular micrometer and the approximate perikaryon volumes were estimated, the shape of perikarya being considered to be close to a sphere or a revolution ellipsoid[10]. The small, intensely fluorescent cells were purposely omitted.

In order to determine the normal fluorescence intensity, a total number of 772 NA-containing perikarya from 3 left and from 4 right superior cervical ganglia were measured. The perikarya were grouped into different classes according to their estimated volumes and an average fluorescence intensity was computed for each class. The results showed an inverse relationship between the average fluorescence intensity and the volume of noradrenergic perikarya (Figure 1). This relation is statistically significant according to SPEARMAN's rank correlation ($R = 0.943$ and $2P < 0.05$). It is concluded from these results that large and small NA perikarya of the superior cervical ganglion of the rat could contain comparable total amounts of NA. In addition, it was found that the thickness of the sections (i.e. 5, 10 and 15 μm) did not influence this relation. In the cytoplasm of the small perikarya, the fluorescence was homogeneously distributed, whereas in the large perikarya it was usually more intense near the cell surface. This peripheral fluorescence of some perikarya was originally noticed by ERÄNKÖ and HÄRKÖNEN[3]. To investigate the NA concentration of perikarya under the conditions of a chromatolytic reaction, a postganglionic axotomy was performed. 4 rats were used in this experiment. Axotomy was carried out on the right side and the contralateral ganglia served as controls. 800 perikarya (100 per ganglion) were measured (Figure 2). 1 week after the postganglionic nerve dissection the chromatolytic reaction was very marked and at this stage the cell nuclei were eccentrically placed[8]. If such chromatolytic perikarya were measured at the level of their nuclei, their profile fields appeared smaller than in the controls and the estimated volumes therefore decreased. These apparently small perikarya revealed a significant decrease in the average fluorescence intensity when compared with control cells of the same size (Figure 2). In the intermediate size classes, no difference was seen but there was

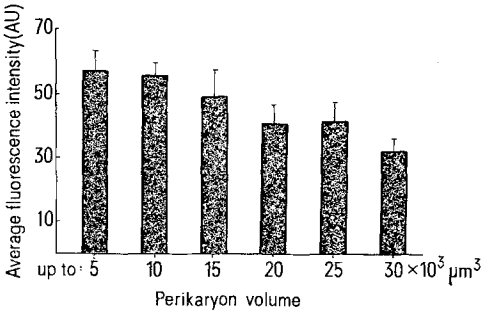

Fig. 1. In the rat superior cervical ganglion, the average intensity of NA fluorescence is inversely correlated to the estimated size of the perikarya.

[1] B. FALCK, N. HILLARP, G. THIEME and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962).
[2] Following abbreviations are used: NA, noradrenaline; DA, dopamine; ACHE, acetylcholinesterase.
[3] O. ERÄNKÖ and M. HÄRKÖNEN, Acta physiol. scand. *58*, 285 (1963).
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an apparent increase in fluorescence intensity above control level in those perikarya that had remained large.

The effect of axotomy on the still large perikarya could possibly be explained by a stagnation of NA axonal transport under conditions of intact catecholamine synthesis. In the apparently small chromatolytic cells, the catecholamine synthesis may be assumed to be decreased. This view is in accord with a number of qualitative observations[8, 12–14].

The relationship of cell size to ACHE activity remains an open question. Histological studies of autonomic ganglia in the rat revealed that the parasympathetic (i.e. highly cholinergic) perikarya of the ganglion nodosum were on the average larger than the sympathetic (i.e. noradrenergic) perikarya of the superior cervical ganglion[15]. By using the acetylthiocholineiodide method of KARNOVSKY and ROOTS[16], we found in the rat superior cervical ganglion both small and large perikarya to exhibit a highly positive reaction for ACHE activity. In conclusion, our investigation furnished no evidence that the size of the perikaryon determines the total catecholamine content or the apparent ACHE activity of the sympathetic neuron.

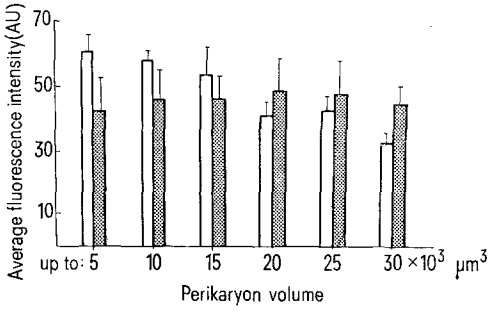

Fig. 2. 1 week after postganglionic axotomy, there is a decrease in NA fluorescence in the chromatolytic perikarya, which appear to be smaller (volume classes up to 5 and $10 \times 10^3 \mu m^3$). The large perikarya exhibit an increase in average NA fluorescence (volume class up to $30 \times 10^3 \mu m^3$).

□, control ganglia; ▨, ganglia 1 week after postganglionic nerve dissection.

Zusammenfassung. Die mikrofluorimetrische Messung der spezifischen Katecholaminfluoreszenz von sympathischen Neuren im Ganglion cervicale superius der Ratte ergab eine reziproke Beziehung zwischen apparentem Noradrenalingehalt und Perikaryonvolumen. Der totale Katecholamingehalt der Neuren sowie die qualitativ erfasste Azetylcholinesteraseaktivität erschienen von der Zellgrösse unabhängig. Diese Beziehung war nach postganglionärer Axotomie verändert.

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[17] Acknowledgment. The technical assistance of Miss D. HÄSLER is gratefully acknowledged.

Recovery of Cholinesterase Activity in the Cerebrospinal Fluid, Brainstem and Plasma of the Unanesthetized Cat After Irreversible Cholinesterase Inhibition

The apparent recovery of cholinesterase (ChE) activity following administration of an irreversible ChE inhibitor, such as Soman (pinacolyl methylphosphonofluoridate), has not been reported for cerebrospinal fluid (CSF). As several studies have dealt with the effect of ChE inhibition on brain-stem centers related to basic functions such as respiration[1, 2], we were curious as to whether ChE activity in CSF might be used as a measure to reflect the level of ChE activity in brainstem. To study this, the time course of recovery of ChE activity in brainstem, CSF, and plasma were compared following Soman intoxication in cats.

Materials and methods. Samples of CSF (1.0 ml) were obtained daily from the cisterna magna of unanesthetized cats previously implanted with cisterna cannulae, as described elsewhere[3]. Blood was simultaneously collected from the brachial vein using a sterile, heparinized syringe. Plasma samples were obtained immediately after withdrawal by spinning the whole blood sample at 1000 *g* for 10 min in a refrigerated centrifuge. The total ChE activity of each sample was then measured using the radiometric method of SIAKOTOS et al.[4] and protein levels obtained using LOWRY's method[5]. These assays were performed on pure CSF while plasma was diluted 1:3 with distilled water and 1:100 for protein determinations. Brainstem samples were obtained by sacrificing other animals with an overdose of sodium pentobarbital and perfusing the brain through the descending aorta with 0.9% NaCl for 3–5 min, or until clear of blood. The brainstem, that tissue, exclusive of cerebellum, lying between the inferior colliculus and C-1, was then removed and washed in ice cold saline. An homogenate was then prepared by dispersing the tissue in a glass homogenizing tube with a teflon pestle in 10 volumes of an ice cold solution consisting of 0.1*M* sodium phosphate buffer at pH 7.8, 0.3*M* NaCl and 1% Lubrol WX (I.C.I. Organics Inc., Stamford, Connecticut). This homogenate was then diluted to 1% with additional volumes of the same buffer. Protein determinations as well as ChE assays were performed with the 1% homogenates.

After the establishment of a stable baseline for 3 days, each animal was pretreated with a peripheral antimuscarinic compound, atropine methyl nitrate (0.5 mg/kg, i.m.). 1 h later, Soman (27 µg/kg; 27 µg/ml), or an equal volume of saline, was injected s.c. in a shaved region between the scapulae. CSF and blood samples were taken and assayed in duplicate as described above. Statistical comparisons were made using the Student's *t*-test statistic.

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