

of histamine<sup>17-19</sup> can increase the levels of CAMP in the slices of brain in the presence of histamine<sup>1</sup>.

Classic  $\beta$ -adrenergic blocking agents were potent blockers of noradrenaline-stimulated CAMP accumulation in the cerebellum of the guinea-pig<sup>16</sup>, as well as the blockers of glycogenolytic influence of noradrenaline and dopamine<sup>9</sup>, but not of histamine<sup>9</sup>, while classical  $\alpha$ -adrenergic blocking agents had no ability to block the phenomena mentioned<sup>9,16</sup>. On the other hand, anti-histaminic agents blocked the accumulation of CAMP by histamine<sup>16</sup>; the results of this work show that antistine also prevented the glycogenolytic influence of histamine, but not that of other tested monoamines, neither of CAMP or db-CAMP. Therefore it seems apparent, that receptors for histamine and noradrenaline and/or serotonin in cerebral cortex, caudate and thalamus of rat are separate and that some blockers are capable of blocking one site without having an effect on the other. The data obtained suggested that histamine by accumulation of CAMP produces glycogenolysis in rat brain and that there must be different subunits of adenyl cyclase for histamine and other monoamines which are involved in the process of glycogenolysis in rat cerebral cortex, caudate and thalamus.

**Résumé.** On a démontré que l'antistine empêche in vitro l'effet glycogénolytique de l'histamine, bien qu'elle n'ait pas d'influence sur les actions glycogénolytiques de la noradrénaline, de la dopamine, de la sérotonine, du 3', 5'-AMP cyclique et de son dérivé dibutirique. Ces résultats suggèrent l'existence de récepteurs particuliers pour les amines biogéniques dans le tissu du cortex, du nucleus caudatus et du thalamus. De ces résultats on a conclu que, dans le cerveau du rat, l'adénocyclase pourrait être le récepteur histaminique.

B. B. MRŠULJA

*Institute of Biochemistry, Laboratory for Neurochemistry, Faculty of Medicine, Yu-11001 Beograd 7 (Yugoslavia), 19 June 1972.*

<sup>17</sup> M. MEDINA and P. A. SHORE, *biochem. Pharmac.* 15, 1627 (1966).

<sup>18</sup> H. GREEN and R. W. ERICKSON, *Arch. int. Pharmacodyn. Thé.* 166, 121 (1967).

<sup>19</sup> J. P. GREEN, *Handbuch für Neurochemie* 4, 221 (1970).

## Axoplasmic Organelles: Quantitative Differences between Ventral and Dorsal Root Fibres of the Rat<sup>1,2</sup>

Based on electro-physiological investigations of single peripheral nerve fibers, several parameters for discriminating motor and sensory fibers have been described<sup>3</sup>. Morphology has so far no means for distinguishing different types of nerve fibres within a mixed nerve, except within certain limits by cholinesterase histochemistry<sup>4</sup>. The results presented below suggest a new possibility for differentiating motor and sensory components in peripheral nerves by means of electron microscopy.

In the present investigation, the contents of neuroplasmic organelles (mitochondria, axoplasmic reticulum, neurotubules, and neurofilaments) in comparable caliber classes of rat ventral and dorsal root fibres have been determined by morphometric methods<sup>5</sup>. The statistical significance of the results has been examined by a *t*-test. The demand for the use of nerve fibers of comparable diameters is based on the fact that, according to previous investigations<sup>6,7</sup>, the relative number of axon organelles is dependent on fiber size.

In accordance with these previous results<sup>7</sup>, the amount of axonal organelles was found related to the axon diameter as follows (see Table and Figure 1): The percentual amount of mitochondria and axoplasmic reticulum of the cross-sectional area of an axon is higher in small diameter nerve fibres. Likewise, the number of neurotubules related to cross-sectional area is higher in the small nerve fibres of both roots. However, the density of the neurofilaments in areas devoid of other organelles is rather constant.

In addition we found regular differences between ventral and dorsal root fibres of the same caliber class:

There are significantly higher amounts of neurotubules and of axoplasmic reticulum in the ventral root fibres than in the dorsal root fibres of comparable size (Figures 1c and 1b).

Less consistent results were found with respect to axoplasmic mitochondria. As shown in Figure 1a, the amount of mitochondria in small ventral root fibres is higher than in small dorsal root fibres, but the reverse situation is seen in the large fibres. The cross-sectional

Morphometry of ventral and dorsal root fibres of the rat

	Very small Dorsal root fibres	Small		Large			
		Ventral root fibres (A- $\gamma$ )	sign.	Dorsal root fibres	Ventral root fibres (A- $\alpha$ )	sign.	Dorsal root fibres
<i>n</i>	30	30		30	30		30
Cross sectional area of axon in $\mu\text{m}^2$ (ACS)	3.55 $\pm$ 0.27	10.05 $\pm$ 0.77	ns	10.98 $\pm$ 0.69	51.49 $\pm$ 2.03	++	41.85 $\pm$ 2.12
Mitochondria (% of total ACS)	2.24 $\pm$ 0.39	1.68 $\pm$ 0.21	+	1.01 $\pm$ 0.16	0.51 $\pm$ 0.04	+	0.70 $\pm$ 0.06
Axoplasmic reticulum (% of total ACS)	1.99 $\pm$ 0.35	2.39 $\pm$ 0.20	+	1.71 $\pm$ 0.18	1.27 $\pm$ 0.05	+++	0.89 $\pm$ 0.06
Neurotubules (number/ $\mu\text{m}^2$ of ACS)	16.24 $\pm$ 1.12	17.04 $\pm$ 0.86	+	14.13 $\pm$ 0.70	10.50 $\pm$ 0.27	+++	7.64 $\pm$ 0.29
Neurofilaments (number/ $\mu\text{m}^2$ of ACS)	149 $\pm$ 4.4	143 $\pm$ 3.2	++	161 $\pm$ 4.2	141 $\pm$ 2.6	++	155 $\pm$ 2.8

The values give the mean and the standard deviation of the mean. The values of comparable caliber classes of ventral and dorsal root fibres were statistically examined by the Student *t*-test. sign., significance; ns, not significant; +,  $p < 0.05$ ; ++,  $p < 0.01$ ; +++,  $p < 0.001$ .

areas of the large fibres of ventral and dorsal roots, however, do not correspond exactly; the mean diameter in the ventral roots is about 20% higher than in the dorsal roots. Thus, the above-mentioned influence of the fibre caliber

on the distribution of organelles has to be considered and could in fact be the prevailing component here. So it remains uncertain whether there are real differences in the mitochondria content of ventral and dorsal root fibres of equal size or not.

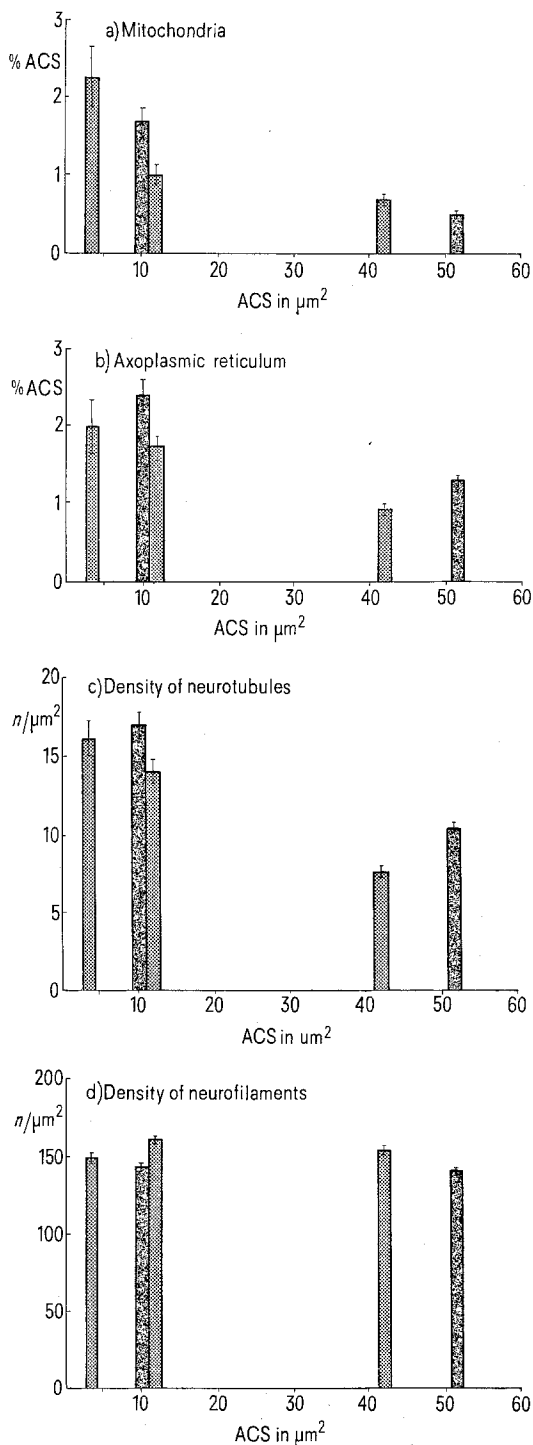
The density of the neurofilaments (number per  $\mu\text{m}^2$ ) in regions free of other organelles is somewhat lower in the ventral root fibres, though the values are in the same order of magnitude irrespective of fibre size (Figure 1d). The small differences have to be interpreted with some reservation, as they could result from difficulties in finding areas devoid of neurotubules for filament counts; the higher number of neurotubules in the ventral root fibres would in this case result in a lower filament count in the areas examined.

The data presented give clear evidence that there are significant differences in the amount of axoplasmic organelles between motor and sensory nerve fibres on the level of the spinal roots. Especially the density of the neurotubules and the development of the axoplasmic reticulum have turned out to be reliable criteria for the discrimination of ventral and dorsal root fibres of the same size. Further investigations in this department will be concerned with the question whether these quantitative differences of axonal organelles are also present in motor and sensory fibres distal from the spinal ganglia and in more distant parts of the peripheral nervous system.

**Zusammenfassung.** Motorische und sensible Nervenfasern lassen sich im Bereich der Rückenmarkswurzeln (Ratte,  $L_6$ ) aufgrund der Mengenverteilung der Axonorganellen unterscheiden. Bei vergleichbarem Faserkaliber haben Vorderwurzelfasern signifikant mehr Neurotubuli und axoplasmatisches Retikulum als Hinterwurzelfasern.

W. ZENKER, R. MAYR and H. GRUBER

2. Anatomisches Institut der Universität Wien,  
Währingerstrasse 13, A-1090 Wien (Austria),  
8. June 1972.



Amount of axonal organelles in ventral and dorsal root fibres of the rat. Ordinates in a) and b) indicate the percentage of the axon cross-sectional area (% ACS), in c) and d) the number per square micron ( $n/\mu\text{m}^2$ ) of axon cross section (ACS). Black bars, ventral root fibres; dotted bars, dorsal root fibres. The bars give the mean and the standard deviation of the mean for  $n = 30$  (i.e. 3 animals, 10 fibers per animal). Abscissa: position of the bars according to the mean cross-sectional area of the small and large axons.

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<sup>3</sup> H. SCHMIDT and R. STÄMPFLI, *Helv. physiol. Acta* 22, C143 (1964).

<sup>4</sup> W. ZENKER, 66. Vers. Anat. Ges., Zagreb 1971, *Anat. Anz.*, in press.

<sup>5</sup> The ventral and dorsal roots of the 5th lumbar segment of 3 albino rats (Sprague-Dawley/300–350 g) were used. Fixation by perfusion with phosphate-buffered glutaraldehyde under nembutal anaesthesia; preparation of the roots and postfixation in  $\text{OsO}_4$ ; embedding in epon; double staining of the ultrathin sections with uranyl acetate and lead citrate; Zeiss EM 9S electron microscope. Micrographs of complete nerve fibre cross sections were made at standard magnifications of  $\times 5,000$ ; for morphometric measurements prints at  $\times 15,000$  were used. Samples of 10 nerve fibre cross sections from both the A- $\alpha$  and the A- $\gamma$  peaks of all 3 ventral roots and from comparable diameter classes of the 3 dorsal roots, as well as from a class of very small myelinated fibres of the dorsal roots, were used for evaluation. The following parameters have been determined and subjected to statistical analysis: 1. area of axon cross sections in  $\mu\text{m}^2$  (planimetry of the micrographs), 2. percent amount of mitochondria on axonal cross sections (morphometry by differential point counting), 3. percent amount of axoplasmic reticulum (differential point counting), 4. total counts of neurotubules per axon cross section and calculation of the densities (number per  $\mu\text{m}^2$ ), and 5. counts of neurofilaments on the prints in 3 areas of  $1 \text{ cm}^2$  per axon cross section and calculation of the neurofilament densities (number per  $\mu\text{m}^2$  of axon cross section devoid of other organelles).

<sup>6</sup> R. L. FRIEDE and T. SAMORAJSKY, *Anat. Rec.* 167, 379 (1970).

<sup>7</sup> R. MAYR, 66. Vers. Anat. Ges., Zagreb 1971, *Anat. Anz.*, in press.