ATPase activity of brain microsomes from DBA and C57 mice

| Strain | Age (days) | (Na, K)-ATPase activity (μmole/h/mg protein) | $P$ | Total ATPase activity (μmol/h/mg protein) | $P$ |
|---|---|---|---|---|---|
| DBA | 30 | 8.9 ± 0.8 | | 19.1 ± 1.8 | |
| C57 | 30 | 18.5 ± 1.2 | < 0.001 | 27.5 ± 2.6 | < 0.001 |
| DBA | 60 | 18.4 ± 0.8 | | 27.6 ± 1.2 | |
| C57 | 60 | 17.9 ± 2.8 | N.S. | 26.8 ± 0.9 | N.S. |

Mean values of 5–7 individual experiments are ± S.E. of the mean. $P$ gives the significance of the difference between DBA and C57 mice. N.S., not significant.

susceptible C57 mice. The animals had never had a convulsion and had never been subjected to loud sound or to heat.

In brains of 30-day-old animals, activity of (Na, K)-ATPase in DBA mice was half that in mice of the C57 strain and total ATPase was less by a third (Table). At 20 days, (Na,K)-ATPase activity was nearly the same in the two strains, and there was no difference at the age of 60 days. There was no difference between the strains in Mg-ATPase.

The method of assay is described in full[1]. Briefly, whole hemispheres were homogenized in isotonic sucrose containing 5.0 m$M$ phosphate buffer (pH 6.0) and 1.0 m$M$ EDTA. The supernatant after centrifugation at 10,000 $g$ for 20 min, was recentrifuged at 100,000 $g$ for 30 min and the sedimented microsomal membranes were washed and suspended in distilled water. (Na, K)-ATPase activity was determined as total ATPase activity minus ouabain-insensitive Mg-ATPase activity. The former was measured in an incubation medium containing 1.0 m$M$ $MgCl_2$, 120 m$M$ NaCl, 20 m$M$ KCl and 30.0 m$M$ histidine, HCl buffer (pH 7.0), and the latter in a corresponding medium prepared without NaCl and KCl and containing 1.0 m$M$ ouabain. 10 μg of microsomal membrane protein was preincubated (15 min, 30 °C), when ($\gamma$-$P^{32}$)ATP (specific activity 950 mCi/mmole) was added to give a final substrate concentration of 1.0 m$M$. Inorganic $P^{32}$ was extracted as previously described[4], and radioactivity counted by liquid scintillation. The specific enzyme activity is expressed as μmole of ATP hydrolyzed/h/mg protein.

Other neurochemical findings in brains of the same animals[1] may well be due to the deficit in (Na, K)-ATPase: lower concentration of $K^+$, of $K^+$-activated release of GABA, and of uptake of $O_2$ in brains of DBA mice at the age of susceptibility, but not before or after. The latter finding is also consistent with ABOOD and GERARD'S[2] report of a deficit in oxidative phosphorylation at the time of susceptibility to seizures.

In conclusion, the alterations in (Na, K)-ATPase activity point to a genetically determined age-dependent deficit in the brain with resultant ionic and metabolic changes that seem to account for susceptibility to seizures in mice[5].

*Zusammenfassung.* Nachweis, dass in Gehirnen 30 Tage alter für audiogene Krämpfe anfälliger Mäuse (Stamm DBA) die Aktivität der (Na, K)ATPase gegenüber nicht Anfälligen (Stamm C57) signifikant vermindert war.

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## Heart Tissue Acetylcholine in Chronically Exercised Rats[1]

The two divisions of the autonomic nervous system controling the activity of the heart were shown to have a modified tonus in the chronically exercised rat. TIPTON and TAYLOR[2] reported vagotony in the athletic heart and HERRLICH et al.[3] found an increase of acetylcholine concentration in the auricles of trained rats. Moreover, several authors have shown that chronic exercise also decreases the sympathetic nervous activity of the heart[4–6]. While previous evidence was presented by us that long-term exercise is related to a reduced heart catecholamine concentration[7,8], it now seemed appropriate to investigate further whether in our chronically trained rats the decreased catecholamine concentrations was accompanied by an increase of the cholinergic transmitter.

*Methods.* Male Wistar rats were exercised intermittently for a 3 month period at 500 m/h as described previously[7]. At the end of the training period, the animals were sacrificed by decapitation. Within 30 sec, the heart was excized, washed in icecold saline, blotted and plunged in liquid nitrogen. The frozen heart was weighed and ground

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Table I. Acetylcholine content of aliquots of a pooled heart extract following various treatments

| Treatment | Acetylcholine (μg) | Recovery (%) |
|---|---|---|
| None | 4.71 ± 0.15 | |
| + 0.8 μg Acetylcholine | 5.53 ± 0.26 | 102 |
| + 1.6 μg Acetylcholine | 6.15 ± 0.21 | 90 |
| + 2.4 μg Acetylcholine | 6.84 ± 0.14 | 89 |
| pH 10 and boiled | 0.43 ± 0.10 | |
| Acetylcholinesterase | 0.75 ± 0.15 | |
| Acetylcholinesterase alone | 0.70 | |

Values are means ± SEM

Table II. Body weight, heart weight and heart: body weight ratio

| | Exercised | Controls | $F$ ratio |
|---|---|---|---|
| No. of animals | 14 | 11 | |
| Heart weight (g) | 0.733 ± 0.014 | 0.756 ± 0.022 | $F < 1$ |
| Body weight (g) | 245 ± 5.6 | 241 ± 6.9 | $F < 1$ |

Values are means ± SEM

in liquid nitrogen. The extraction of acetylcholine was performed with a solution of 85% acetone and 15% 1 $N$ formic acid according to TORU and APRISON[9]. The extraction medium was evaporated to dryness within 15 min under reduced pressure at 40 °C. The residue was taken up in acetic acid-water at a pH of 3.8, centrifuged at 38,000 × $g$ for 1 h and ultracentrifuged at 140,000 × $g$ for another hour. The supernatant was ultrafiltrated in Ultra-Thimbles under reduced pressure (Schleicher and Schüll No. 100). In our hands, this step was found to be necessary in order to avoid aberrant high ethanol measurements. The ultrafiltrate was evaporated to dryness at 70 °C. The residue was dissolved in 0.1 ml of water and submitted to the borohydride reaction as described by STAVINOHA and RYAN[10], in order to reduce the acetylcholine to ethanol. The ethanol was assayed by gas chromatography. A Varian-aerograph gaschromatograph 1200 equipped with a flame ionization detector was used. The column was a 7-foot stainless steel $^1/_8$ o.d. tube packed with 150–200 mesh Porapak Q. The nitrogen carrier gas flow was 40 ml/min and the oven temperature was 150 °C. The specificity for the acetylcholine measurements was tested by boiling the sample before the borohydride reaction at pH 10 by addition of $NH_4OH$ or NaOH. The remaining activity, after this treatment, will be refered to as blank or background activity. Experimental values will be corrected for this and are reported in column 3 of Table III. In addition, some samples were treated at 37 °C for 30 min with acetylcholinesterase (Sigma) before the ultra filtration step. Statistical analysis of the data was performed according to SNEDECOR[11]. Differences were considered statistically significant for a $p$ value $< 0.05$.

*Results.* In Table I, we show the results obtained from aliquots of a pooled heart extract following various treatments. For 0.8 μg, 1.6 μg and 2.4 μg of acetylcholine added to a heart extract, the mean recovery was respectively of 101%, 90% and 89%. The test for specificity, as described above, is also indicated. As seen, the background activity or blank is much higher when treated with acetylcholinesterase. This phenomenon was previously reported by other investigators[10]. As seen in Table II, heart weights and body weights of exercised and control animals are identical. The correlation coefficient of the heart to body weight relationship is 0.998 in the controls and 0.999 in the exercised animals. When this relationship is analyzed by a covariance analysis, it is found that the heart weights are different not by slope ($F = 1.57$) but by elevation ($F = 5.28$). Regression lines for exercised and control animals are respectively $Y = 220.88 + 2.09\,X$ ($\sigma = 30$) and $Y = 43.54 + 2.95\,X$ ($\sigma = 41$). According to the values indicated in Table III, we may consider that long-term exercise produces a significant increase in the total acetylcholine content (μg) and acetylcholine concentration (μg/g). In Table III are also indicated the corrected values for the alkali boiled blank.

*Discussion.* As shown in Table II, the heart weights and body weights of control and experimental animals are identical. The heart to body weight relation was analyzed by a covariance analysis. The elevation being significantly higher in the exercised animals, we may conclude that the hearts of the exercised animals are relatively heavier when compared to the control animals. Although this differences must be very small, nevertheless the results observed are at variance with previous investigations in which no differences in the heart to body weight relation could be observed in the intermittently exercised animals[7,8]. When corrected for background activity after boiling sample in an alkali solution, the heart acetylcholine concentrations of the control animals as determined by gaschromatography can easily be compared

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Table III. Heart tissu acetylcholine content (μg) and concentration (μg/g)

| | Acetylcholine (μg) | Acetylcholine (μg/g) | Corrected values[a] acetylcholine (μg/g) |
|---|---|---|---|
| Exercised animals ($n = 12$) | 4.61 ± 0.22 | 6.27 ± 0.23 | 5.62 ± 0.23 |
| Control animals ($n = 11$) | 3.41 ± 0.15 | 4.48 ± 0.19 | 3.91 ± 0.19 |
| Variation (%) | + 35 | + 40 | + 43 |
| $F$ ratio | 18.95 | 33.63 | 33.63 |

Values are means ± SEM. $n$ = number of animals. [a] The values are corrected for the alkali boiled blank as indicated in Table I.

with recent bioassay measurements[12,13]. The slightly higher values obtained in our experiments could be explained by a more efficient extraction procedure[9]. As seen in Table III, chronic exercise induces an increase in the total acetylcholine content of the heart. Also, the heart tissue acetylcholine concentration as expressed by μg/g is significantly increased in the exercised animals. Very probably, and as shown by HERRLICH et al.[3] the increase of the acetylcholine concentration of the auricles is mainly responsible for these result. In addition, EKSTRÖM[14] reported recently that in chronically exercised animals, the choline acetyl transferase activity of the auricles was significantly increased. Although the existence of acetylcholine bound to the myocardium and independent from nerve terminals has been reported[15], it is tempting to suggest that the acetylcholine increase in the heart reflects an increase of the parasympathetic activity on the heart. Whether the activity of the heart in chronically exercised animals is mainly influenced by the sympathetic or parasympathetic system is still a controversial matter[3–6].

*Résumé.* Le taux de l'acetylcholine du myocarde a été dosé par chromatographie en phase gazeuse chez le rat. Chez l'animal exercé le taux de l'acetylcholine était plus élevé que chez son homologue sédentaire.

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## Neurotubules: Different Densities in Peripheral Motor and Sensory Nerve Fibres[1,2]

According to earlier findings in mouse sciatic nerve[3], which are substantially supported by other authors[4,5], a general rule of inverse relationship between fibre size and density of neurotubules is well established. In a previous paper[6], evidence was presented that – while the above-mentioned rule is fully valid – the density of neurotubules is significantly higher in ventral (motor) root fibres than in dorsal (sensory) root fibres of comparable diameter. The present investigation is concerned with the question of whether these differences in tubular density are also present in motor and sensory nerve fibres distal to the spinal ganglia and particularly in more distant parts of the peripheral nervous system.

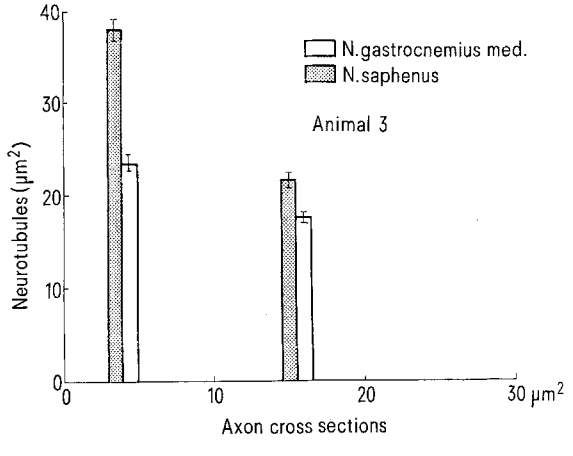


Fig. 1. Comparison of the densities of axonal neurotubules in samples of small and large nerve fibres of a cutaneous nerve (black bars) and a muscle nerve (white bars) of the rat. Ordinate: number of neurotubules per square micron of axon cross section (NT/μm²); the bars give the mean and the standard deviation of the mean. Abscissa: position of the bars according to the mean cross sectional area of the axons of each sample; axon cross sections in square microns (ACS/μm²) were determined by planimetry.

*Methods.* 3 albino rats (Sprague-Dawley/320–440 g) were fixed by perfusion with cacodylate-buffered 7% glutaraldehyde under nembutal anaesthesia. In 2 animals the dorsal roots of $L_5$ were excised in connection with the spinal ganglia, each with a short stump of the spinal nerve. In a third animal the saphenous nerve and the nerve to the medial head of the gastrocnemius muscle (MGN) were taken out as examples of a typical cutaneous and a typical muscular nerve. Postfixation in $OSO_4$; embedding in epon; double staining of ultrathin sections with uranyl acetate and lead citrate; Zeiss EM 9S electron microscope. Micrographs of complete nerve fibre cross sections were made at standard magnification of ×5000; for morphometric measurements prints at ×15,000 were used. Samples of 22–50 large as well as small diameter nerve fibres of each specimen were evaluated. The following parameters have been determined and subjected to statistical analysis: 1. area of cross section (ACS) in μm² (planimetry of the micrographs), and 2. total count of neurotubules per ACS and calculation of their density (number per μm² of ACS). For statistical analysis the Student *t*-test was applied.

*Results and discussion.* Nerve fibres of the saphenous nerve, a cutaneous nerve, were compared with those of the MGN, a mixed muscle nerve containing more than 50% of motor nerve fibres. The density of neurotubules was significantly higher in the axons of saphenous nerve fibres as compared with those of the MGN. These differ-

[1] This investigation was supported by the 'Fonds zur Förderung der wissenschaftlichen Forschung in Österreich'.
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