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ELECTROPHYSIOLOGICAL AND ELECTRON MICROSCOPIC STUDIES ON THE FIBRE COMPOSITION OF THE CEREBROVISCERAL CONNECTIVE OF *ANODONTA CYGNEA* L.

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PAVLOV (1885) was the first to study in detail the function of the adductor muscles in *Anodonta cygnea*. By analysis of muscle responses to electric stimulation of the cerebrovisceral connective (CVC) he arrived at the conclusion that at least two different — stimulatory and inhibitory — types of fibrils run in the connective between the cerebral and visceral ganglia. SALÁNKI and LÁBOS (1962) corroborated PAVLOV's assumption in a study on the responses of the posterior adductor to stimuli with different parameters applied to the CVC, further using different drugs. A double innervation of molluscan smooth muscles in other species was observed and interpreted in different manner by several authors (WINTON 1937, FLETCHER 1937, HOYLE and LOWY 1956).

Action potentials from *Anodonta* CVC were first recorded by ZHUKOV (1946). The potentials were composed of several waves pointing to the existence of more fibre types. In spite of these findings ZHUKOV and associates (1956) did not ascribe the inhibition of the posterior adductor to the action of separate tonus inhibiting fibres.

In the present work authors endeavoured to obtain a more precise picture of the fibre composition and conducting properties of the CVC by comparison of results obtained with electrophysiological and morphological methods. The results may contribute to a further analysis of the adductor function.

Material and methods

The animals were kept in running tap water. To isolate the CVC glass instruments were used. Care was taken to avoid traumatization or desiccation of the preparations. The connective usually had a diameter of 0.2 to 0.4 mm.

Electrophysiological methods

The velocity and decrement of conduction were studied by leading off the action potentials in different distances from the stimulating electrode in the same preparation. An impulse generator supplied the quadrangular pulses.

An RC amplifier with a time constant of 0.75 sec was used for the registration of the action potentials. The potentials were lead off through 0.2 mm thick silver electrodes from isolated nerves which were immersed into paraffin oil. Both the stimulation and the registration were unipolar.

Electron microscopic methods

An approx. 0.5 cm long piece of the isolated CVC was fixed in 1 per-cent osmium tetroxyde buffered to pH 7.2 for one hour at 4 C°. Dehydration was performed with ethanol. The material was stained with a saturated uranyl acetate solution in 70 per cent ethanol for 20 minutes, after treatment with 70 per cent ethanol. The material was embedded into a 9 : 1 proportion mixture of butyl-methyl-metacrylate sectioned on an LKB Ultrotome and viewed in a Zeiss D 2 electron microscope with direct magnifications of 6000 ×, 12000 ×, and 20000 ×. Fibre analysis was performed by random cutting of 519 axon sections from not overlapping photographic prints (final magnificaion: 66 000 ×). The cuttings were weighed with an accuracy of 0.2 mg. Knowing the weight of the unit surface of the printing paper the diameters in μ of circles having the same surface as the individual axon cross sections were calculated on the basis of the obtained values. The prints used in fibre analysis represented different areas of the same nerve.

Results

The action potential evoked by a single rectangular pulse comprises several individual components whose number varies in individual cases. Three main components are clearly discernible as a rule. The presence of further components is indicated by frequent appearance of inflections on the main waves. A fourth and even fifth wave may also appear on certain tracings. The latter may be due either to slow fibres or represent after-potentials of fast fibres.

The velocity of conduction was determined by changing the distance between stimulation and leading off. The results revealed that the individual components correspond to different groups of fibres conducting the impulses with velocities of 0.60 to 1.40 m/sec., 0.20 to 0.50 m/sec or 0.05 to 0.25 m/sec, respectively *(Fig. 1)*.

By increasing the distance between stimulation and leading off (this distance varied in the present experiments from 18 to 36 mm) the discrimination in time of individual components became more pronounced and, at the same time, the decrement of impulses could be demonstrated *(Fig. 2)*.

Figs 3 and *4* show the electron microscopic picture of the CVC. The fibres are not myelinated and lack a Schwann's sheath. Groups of larger numbers of fibres are surrounded by glial plasm. A part of these fibres contains neurosecretum granules of 0.1 to 0.4 μ diameter. *Fig. 5* presents the results of the fibre analysis. The cross section of fibres, expressed as the diameter of discs having the same surface, lies between 0.15 and 1.35 μ. The histogram has two maxima at 0.25 to 0.30 and at 0.45 to 0.50 μ.

The voltage of the action potential and the speed of conduction are proportional in first approximation to the cross section and diameter of the

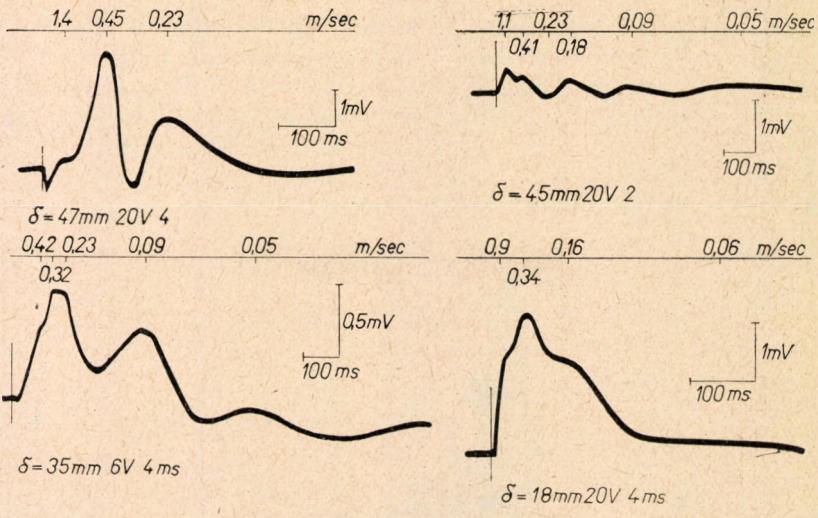

Fig. 1. Velocity of conduction in individual components of the CVC (m/sec). All tracings from different nerves. ∂ — distance of stimulation and lead, ms — duration of the pulse

1. ábra. A CVC rostkomponenseinek ingerületvezetési sebessége (m/sec). Minden akciós potenciál más idegről lett elvezetve; ∂ — az ingerlő és elvezető elektródák egymástól való távolsága; ms — az ingerimpulzus időtartama

1. рис. Скорость проведения разных групп волокон церебро-висцерального коннектива (м/сек). Каждый ток действия отведен от разных нервов: δ — расстояние между раздражающими и отводящими электродами: ms — продолжительность нервного импульса в мсек

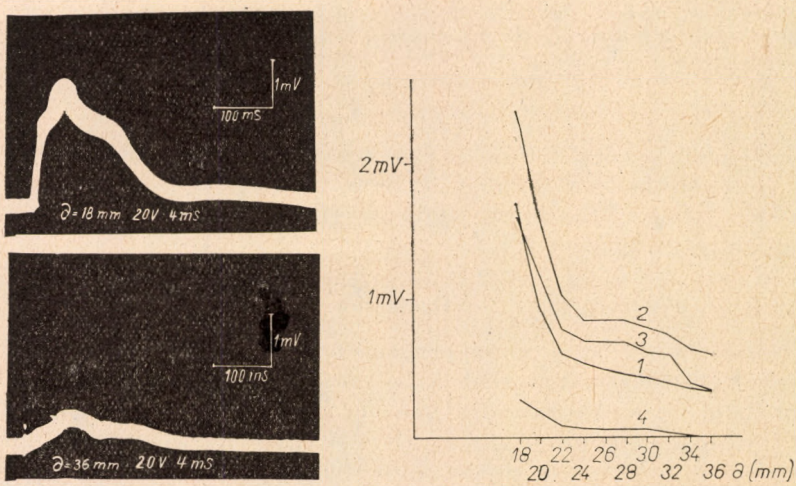

Fig. 2. Decrement of the conducted impulses. Dependency of the amplitude of the individual components (1 to 4) on the distance of stimulation and lead. The action potential nature of the 4th component is not certain

2. ábra. Az ingerületvezetés dekrementes jellege. A CVC egyes komponensei (1—4) amplitúdójának függése az ingerlő és elvezető elektródák távolságától. A 4. komponens akciós potenciál jellege vitatható

2. рис. Декрементный характер проведения возбуждения. Зависимость амплитуды отдельных компонентов (1—4) церебро-висцерального коннективаот расстояния между раздражающими и отводящими электродами. Характер 4-ого компонента, как тока действия, спорный

axon, respectively (GASSER and GRUNDFEST, 1939). In view of this fact the ratio of total cross section of fibres belonging to individual diameter intervals to total determined cross section was calculated *(Fig. 6)*. A comparison of *Figs 5* and *6* reveals that the sum of cross sections of thick fibres comprises a

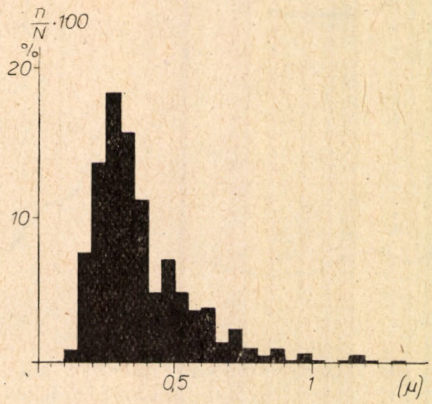

Fig. 5. Histogram of diameter distribution of fibres in the CVC. Abscisse: equivalent fibre diameter in μ, ordinate: per cent frequency of fibres in the mm individual grups. N 519 (total number of fibres measured)

5. ábra. A CVC különböző átmérőjű rostjainak gyakoriság-eloszlása. Abszcissza: ekvivalens rostátmérő μ-ben, ordináta: az egyes átmérőintervallumba eső rostok %-os aránya. N = 519 (az összes vizsgált rostok száma)

5. рис. Частотное распределение волокон разных диаметров церебро-висцерального коннектива. Абсцисс: эквивалентный диаметр волокна в μ. Ординат: процентное соотношение волокон, находящихся в отдельных промежутках диаметра. N = 519 (общее число изученных волокон)

larger portion of the total determined cross section than the ratio of the number of thick fibres to the total number of fibres.

A calculation of the total number of fibres in the CVC gave a value of the order of magnitude of 10^5.

Discussion

The results of fibre analysis performed both by electrophysiological and by morphological methods revealed the presence of fibre components having different diameter and conducting velocity in the CVC. An assymetric histogram of fibre diameters would already show the existence of several components. This conclusion, however, is further supported by the appearance of two maxima on the histogram. With due regard to GASSER and GRUNDFEST's data concerning the relationship between voltage, conduction velocity and axon size, our *Fig. 6* allows the conclusion that the less numerous thick fibres which belong to one diameter interval, contribute in a greater extent to the formation of the action potential of the nerve than it would be expected on the basis of their number alone.

The dependence of the posterior adductor responses and of the action potentials on the parameters of the stimuli applied to the CVC (SALÁNKI and

LÁBOS, 1962) may suggest that the thinner and slower fibres with higher tresholds are tonus inhibitory in their function whereas the thicker and faster fibres with lower tresholds are responsible for tonic and phasic contractions.

In accordance with ZHUKOV 3 main fibre groups were demonstrated in the CVC by electrophysiological methods. These groups, however, may be further divided. The slow waves following the three main waves might be either action potentials of further fibre components or after potentials. This

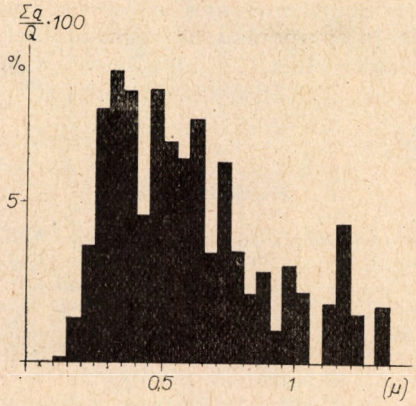

Fig. 6. Histogram of summarised cross section (q) of fibres belonging to individua groups expressed in per cent of the total cross section (Q) of all fibres measured. Abscisse: diameter in μ, ordinate: relative frequency weighted in regard to the cross section

6. ábra. A CVC egy-egy átmérőintervallumba tartozó rostjainak összkeresztmetszete (q) az összes vizsgált rost összkeresztmetszetének (Q) %-ában feltüntetve. Abszcissza: átmérő μ-ben, ordináta: keresztmetszettel súlyozott relatív gyakoriság

6. рис. Суммарный поперечный срез волокон, относящихся к разному промежутки диаметра (q), дан в процентах суммарного промежуточного среза всех исследуемых волокон (Q). Абсцисс: диаметр в μ. Ординат: относительная частота разных поперечных срезов

problem is not yet settled. Conduction velocities observed in this work show a good agreement with the values obtained by HORRIDGE (1958) on the CVC of *Mya arenaria*. ZHUKOV (1946) found a maximal velocity of 2 cm/sec, this value corresponds to our slowest waves. Decrement of conducted impulses was demonstrated by KAHN and KUSNETZOV (1938) in the CVC of *Anodonta cygnea*. *Fig. 2* shows that the amplitude of action potentials is not in linear relationship with the distance between stimulation and lead, but an increase in distance results in a decreased decrement. All fibre components conduct with decrement. This property of *Anodonta* fibres may be due to the lack of myelin sheath.

No thick axons were found in *Anodonta* which would be similar to the thick fibres in the CVC of *Aplysia californica* (BATHAM, 1961). The fibres of *Anodonta* CVC have no myelin or Schwann's sheath. The fibres are arranged in groupings surrounded by a single process of a glial cell. Only 100 to 250 Å. wide clefts separate the axolemm of the fibres belonging to the same group. This picture strikingly resembles the arrangement of fibres lacking both types of sheath in the optic nerve of the frog (MATURANA, 1960).

The neurosecretory substance found in fibres originates most probably from the secretion observed in the ganglia (FÄHRMAN 1961, BARANYI and SALÁNKI 1962).

Summary

An electrophysiological and electron microscopic analysis of the fibre composition of the cerebrovisceral connective in *Anodonta cygnea* L. revealed that the fibres have no neurilemm and are not myelinated. The connective contains at least tree main components as regards the fibre caliber and speed of conduction. The fibres conduct the impulses with decrement. The different components most probably differ in their functional significance.

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ELEKTROFIZIOLÓGIAI ÉS ELEKTRONMIKROSZKÓPOS ADATOK AZ *ANODONTA CYGNEA* L. CEREBROVISCERÁLIS CONNECTIVUMÁNAK ROSTÖSSZETÉTELÉHEZ

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Összefoglalás

Az *Anodonta cygnea* L. cerebro-visceralis connectivumának elektrofiziológiai és elektronmikroszkópos rostanalízise alapján megállapítható, hogy a rostok velő- és Schwann-hüvelymentesek, vastagság és vezetési sebesség szempontjából legalább három fő összetevőt tartalmaznak, az ingerületet dekrementesen vezetik és feltehető, hogy a különböző rostok eltérő funkcionális jelentőséggel is bírnak.

ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЕ И ЭЛЕКТРОННОМИКРОСКОПИЧЕСКИЕ ДАННЫЕ К СОСТАВУ ВОЛОКОН ЦЕРЕБРО-ВИСЦЕРАЛЬНОГО КОННЕКТИВА БЕЗЗУБКИ (Anodondta cygnea L)

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Резюме

На основании электрофизиологического и электронномикроскопического анализов волокон церебро-висцерального коннектива беззубки установлено, что волокна лишены миэлиновой и Швановской оболочек. С точки зрения диаметров и скорости проведения возбуждения они содержат по крайней мере три вида волокон, проводящих возбуждение с декрементом. Можно предположить, что различние волокна обладают различной функцией.

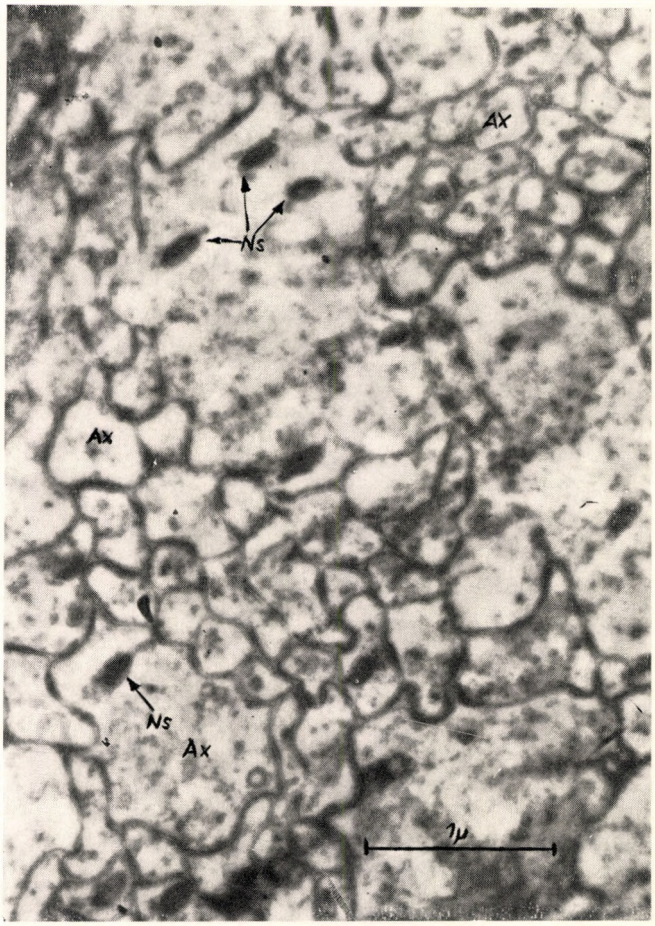

Fig. 3. Cross section of the CVC. Ax: axon, Ns: neurosecretory substance in the axon. Magnification: 36 000 ×

3. ábra. CVC keresztmetszete. Ax: axon, Ns: neurosecretum az axonban. Nagyítás: 36 000-szeres

3. рис. Поперечный срез церебро-висцерального коннектива. Ax — аксон. Ns — нейросекрет в аксоне. Увеличение в 36 000

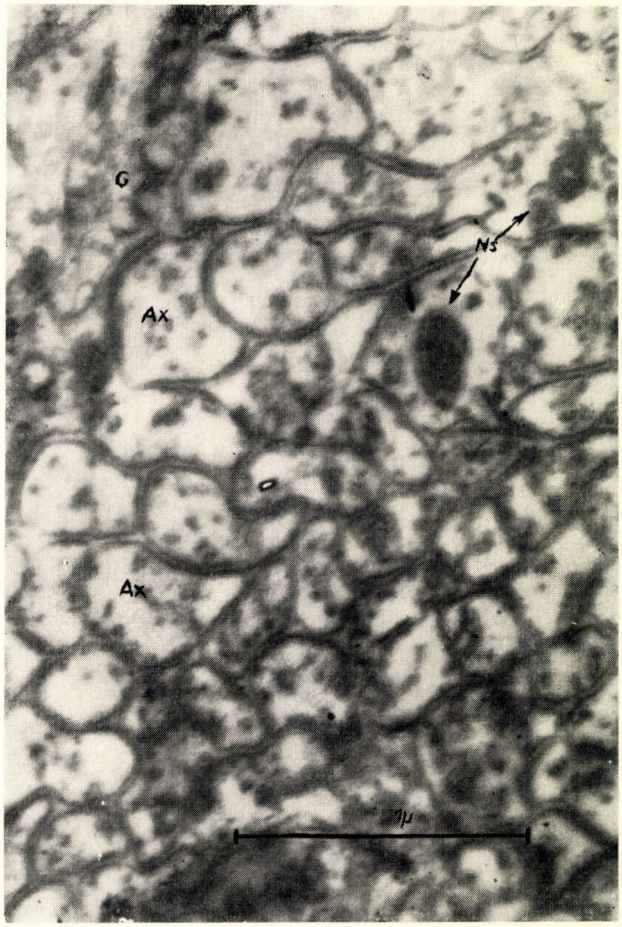

Fig. 4. Cross section of CVC. Ax: axon, Ns: neurosecretory substance in the axon, G: part of a glial cell. Magnification: 60 000 ×

4. ábra. CVC keresztmetszete. Ax: axon, Ns: neurosecretum az axonban. G: gliasejt részlete. Nagyítás: 60 000-szeres

4. рис. Поперечный срез церебро-висцерального коннектива. Ax — аксон, Ns — нейросекрет в аксоне, G — участок глии. Увеличение в 60 000