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REFLEX INVESTIGATIONS ON THE VISCERAL GANGLION OF THE FRESH WATER MUSSEL *(ANODONTA CYGNEA L.)*

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The central nervous system of the fresh water mussel consists of three pairs of ganglia from which the cerebral pair of ganglia is located far from each other, the pedal ganglia are arranged closely besides each other while the pair of visceral ganglia is entirely grown together. The visceral ganglion is found directly beside the posterior adductor muscle and is greatly involved in its innervation. It plays moreover a role in the afferent and efferent innervation of the posterior part of the mantle, in the nervous supply of the gill and heart as well as in the forwarding of impulses arriving from the cerebral ganglions or proceeding from the posterior half of the mussels towards the cerebral ganglia. The cerebro-visceral connectivum (CVC) serves to assure the connections with the latter (WOORTMANN 1926).

At the electrical stimulation of CVC the impulse through the visceral ganglion to the posterior adductor has a peculiar effect from the point of view of the action of the muscle since by changing of the parameters both tonic and phasic contraction or relaxation of the adductor can be brought about (PAVLOV 1855, ZHUKOV 1956, SALÁNKI and LÁBOS 1963). The mechanical stimulation of the posterior edge of mantle results in a lasting tonic contraction of the adductor. Similarly, in the way of a reflex an adductor response can be elicited by the mechanical stimulation of the pericardium (ZIKS and BOGDANOV 1956), foot and stomach wall (SALÁNKI 1962) and the chemical and electrical excitation of the heart (PÉCSI and SALÁNKI 1964). All this seems to point to the fact that the visceral ganglion as a reflex centre plays an important role in the life activities of the whole animal, in its responses to various actions and in the regulation of the activity of various organs. In the course of the physiological examinations conducted hitherto, however, it became not known which way the excitation from the posterior adductor proceeds through the visceral ganglion and whether the excitation of one of the paired CVC through the visceral ganglion gets back into the other branch and from there into the cerebral ganglion.

It is also questionable whether or not from the electrophysiological viewpoint by the investigation of the action potentials of the nerves running from the visceral ganglion into the posterior adductor the assumed double innervation of the muscle (PAVLOV 1885, SALÁNKI and LÁBOS 1963) can be supported.

Beyond the elucidation of this question we have set the objective in these investigations to obtain data to the issue which way the action potential that can be conducted from the CVC by electric excitation and includes several components (LÁBOS et al. 1963) changes after having run through the ganglion and/or to what extent it can be activated by other nerves arriving at the visceral ganglion.

We further investigated the effect of some pharmacons from which work we expected to obtain informations on the mediation of the transmission of nerve impulse.

Method

The tests were carried out on about 150 isolated visceral ganglion preparations in which we retained the nerves pertaining to the ganglion and in part of the preparations even a piece of the posterior adductor to be able to excite

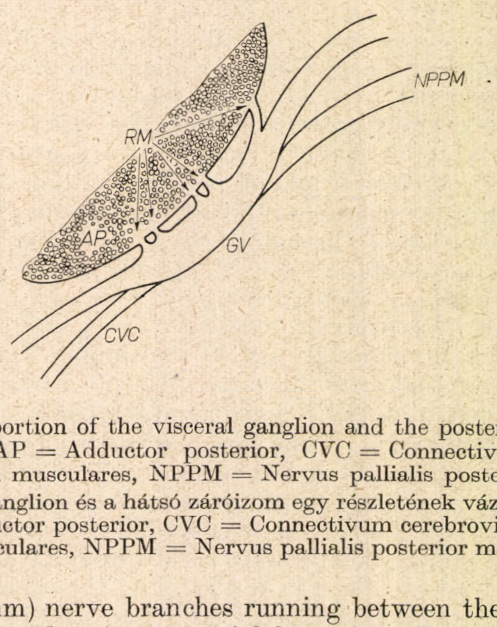

Fig. 1. Scheme of a portion of the visceral ganglion and the posterior adductor. GV = Ganglion viscerale, AP = Adductor posterior, CVC = Connectivum cerebroviscerale, RM = Rami musculares, NPPM = Nervus pallialis posterior maior

1. ábra. A viscerális ganglion és a hátsó záróizom egy részletének vázlata. GV = Ganglion viscerale, AP = Adductor posterior, CVC = Connectivum cerebroviscerale, RM = Rami musculares, NPPM = Nervus pallialis posterior major

the short (1 to 3 mm) nerve branches running between the ganglion and the muscle and/or to record action potential from them. To obtain these preparations we used 12 to 15 cm long *Anodonta cygnea* specimens which were kept previously in an aquarium with running water.

The morphological conditions are illustrated in *Fig. 1.* Accordingly we have dealt with the following possibilities of excitation and leading off

1. The excitation of one CVC and leading off from the same CVC,
2. The stimulation of one of the CVC-s and leading off from one of the short nerve branches running to the adductor,
3. The stimulation of a CVC and leading off from the n. pallialis posterior maior running to the posterior part of the mantle and to the syphon,
4. Stimulation of a CVC and leading off from the other CVC,
5. Muscle branch stimulation and recording from the one CVC,
6. Stimulation of pallial nerve and recording from the CVC.

Recording took place both at the homo- and contralateral sides of the stimulation.

In these experiments 5 to 20 Volt single square pulses with 4 msec duration were used and simultaneously with RC amplifier action potentials were lead off from an other nerve branch. In the course of leading the preparation was held in paraffin oil, partly to assure good leading (since the nerves in question were of a high resistance) and partly to prevent drying out. The technical conditions of stimulation and leading are demonstrated in *Fig. 2.*

In the investigation of pharmacon effects we proceeded so that we kept the isolated preparation for a definite period in the solution of given concentration and subsequently performed stimulation and leading. In part of the experiments leading took place also before incubation and several different incubation periods were applied in the case of a single preparation. In order, however, not to influence the diffusion of the substances into the ganglion by

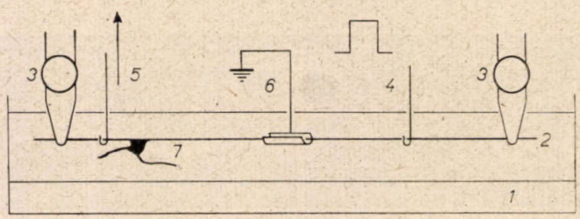

Fig. 2. The circumstances of excitation and leading off 1 = Physiological solution, 2 = paraffin oil, 3 = nippers, 4 = stimulating electrode, 5 = leading electrode, 6 = earth electrode, 7 = preparation

2. ábra. Az ingerlés és elvezetés módja. 1 = Fiziológiás oldat, 2 = paraffinolaj, 3 = befogó csipeszek, 4 = ingerlő elektród, 5 = elvezető elektród, 6 = földelektród, 7 = preparátum

the paraffin oil layer unavoidable in the electrophysiological test, in most cases we dispensed with self control prior to incubation and have drawn conclusions on the effects of the agents from controle carried out with a great number of other preparations. Of course exactly for this reason we could perform only qualitative observations.

The pharmacons applied in the course of the tests were atropin sulphate, chlorpromazine and iproniazide.

Results

In the course of investigations we succeeded in recording action potential in all excitation-leading variations referred to in the methodical part. The action potential obtained has, with increased stimulation tension or pulse width respectively increased in size up to a certain extent and also the number of the components was higher. In the present case we are dealing with the responses obtainable on supermaximal stimulation.

The values of the action potentials were rather varied and ranged from 2 to 14 mV. This can be explained with several factors. One of these is that depending on the character of the nerve and also on the size of the animals the thickness of the nerves and/or fibres is different, which is directly related to the value of the action potential. Another more essential cause is that the nerves of the mussel are leading the impulse with decrement (ZHUKOV 1946, SALÁNKI and LÁBOS 1964) and as a result the value of the action potential depends on the distance from the place of the stimulation or the ganglion respectively. In these experiments the most different distances occurred from 2 mm to 4 cm which explains the diversified values of the action potentials obtained.

The properties of action potentials elicited in the various nerves of the visceral ganglion

When in the course of the stimulation of a CVC we have lead off action potential from the same CVC before it had got into the visceral ganglion, we obtained four readily separable potentials from which three were more explicit while the fourth flattened, protracted.

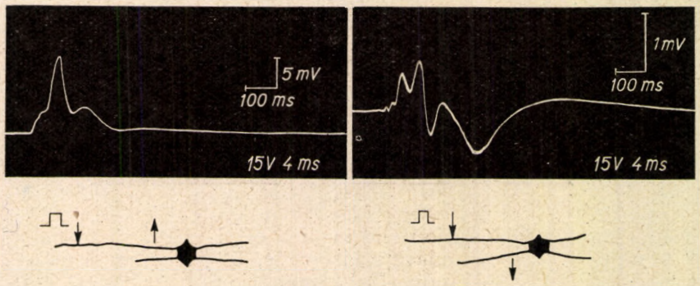

Fig. 3. The action potential lead off from the CVC at the stimulation of the CVC of the same and/or opposite side

3. ábra. A CVC-ről elvezetett akciós potenciál az ugyanazon, ill. ellenoldali CVC ingerlésekor

When at the stimulation of the CVC on the one side the lead took place from the other CVC so that the excitation passed through the visceral ganglion we obtained a picture somewhat different from the previous one (*Fig. 3*). The number of the components has apparently increased, five, possibly six waves can be recognized on the action potential. The period of the action potential has approximately doubled and its size diminished about $^1/_2$ to $^1/_5$. The increase of the number of components can be regarded as apparent because even in the leading off at the same side — if it took place at a distance great enough from the excitation — it appears that the first and second components break up into two components each which were marked with A_1, A_2 and/or B_1, B_2 (SALÁNKI, LÁBOS and NÁN 1964). Thus the greater pathway of the impulse explains the more significant separation of the components. Also the prolongation of the period of the action potential and the more explicit condition of the positive afterpotentials is related to the same cause. The reduction of the value of the potentials is probably connected with the decrement but perhaps still more with the properties of the ganglionic transfer.

Fig. 4 presents the action potential of one of the thin muscle branches leading to the adductor and/or the possibility of activation of the CVC by this nerve. It appears that in the case of the stimulation of the CVC two components can be lead off from the muscle branch. Also at the stimulation of the muscle branch two fibre groups can be activated in the cerebro-visceral connectivum, although on the first component of the potential sometimes a not significant secondary wave appears.

The action potential is significantly higher in the case when the leading takes place from the muscle branch which fact is probably connected with the proximity to the ganglion. The two components sharply separate from each other in the case of leading from the CVC.

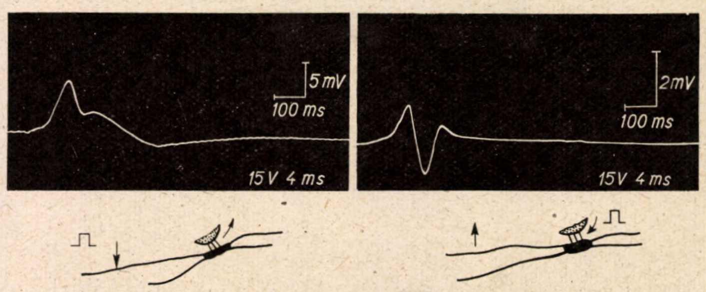

Fig. 4. The reflex connection of the CVC and the nerve branch running to the adductor
4. ábra. A CVC és a záróizomhoz menő idegág reflexkapcsolata

Fig. 5 demonstrates the action potentials that can be lead at the stimulation of the CVC from the n. pallialis posterior maior and at the excitation of the pallial nerve respectively from the CVC. It is seen that the number of components coincides, in each case 5 waves separate the conditions of size of which, however, are different. The first and third waves are well separated in both cases, the second wave comes in sight at the leading off from the pallial nerve while the fourth wave is very explicit at the leading off the CVC. The fifth component is also readily discernible in both cases while the positive after-potential after the fifth wave is very explicit.

This shows, consequently, that in the relationship of the CVC — pallial nerve in both directions identical fibre groups are activated but the proportion of the various fibres in the two nerves is visibly somewhat divergent.

The effect of atropin, chlorpromazine and iproniazide

Taking into account that no reliable knowledge is available on the nature of mediation processes occurring in the ganglion of Lamellibranchiata but

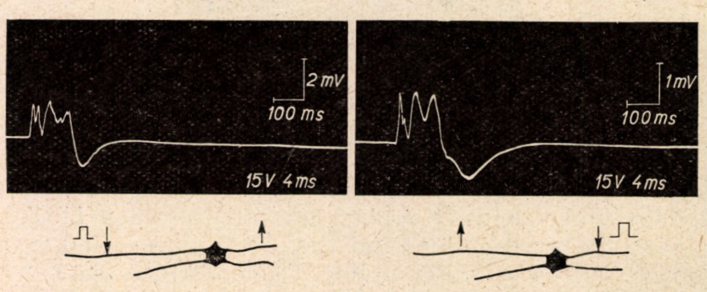

Fig. 5. The reflex connection of the CVC and the pallial nerve
5. ábra. A CVC és a palliális ideg reflexkapcsolata

according to some workers serotoninerg (Welsh 1958) while according to others cholinerg (Puppi 1962) mechanisms must be reckoned with, we conducted informatory tests to find out how far reflex connections can be paralysed

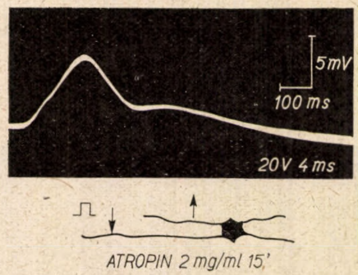

Fig. 6. The effect of atropin on the CVC—CVC reflex
6. ábra. Atropin hatása a CVC—CVC reflexre

with pharmacons. In these tests atropin was used for the inhibition of cholinerg transmission and chlorpromazine for the blocking of serotonin effect. Also the effect of iproniazide was investigated which by the inhibition of monoamino-oxidase may result in serotonin accumulation (Maas and Nimmo 1959).

We found that when the preparations were pretreated for 10 to 15 minutes with great doses of atropin some components of the action potential fell short. Complete blocking of the visceral ganglion could not be attained, however, though the shape also of the remaining components became distorted (*Fig. 6*).

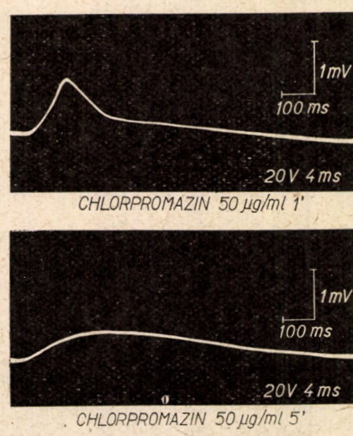

Fig. 7. The effect of chlorpromazine on the conduction of impulse in the CVC
7. ábra. Chlorpromazin hatása a CVC ingerületvezetésére

When chlorpromazine was applied not only the whole block of the transfer of nerve impulse was obtained but the conduction ceased that is the conductivity of the fibres was inhibited. *Fig. 7* demonstrates the block of the conduction of impulses in a CVC-piece after treatment for 1 and/or 10 minutes with 50 μg/ml chlorpromazine.

Upon the effect of iproniazide, when a 50 μg/ml concentration was applied after a 1 to 5 min. treatment 2 to 3 components are seen on the action potential *(Fig. 8a)* whereas after a treatment for more than 5 minutes only one component remained. In the case of a 10 min. treatment also the conduction is damaged if not to the same extent as with the application of chlorpromazine *(Fig. 8b)*.

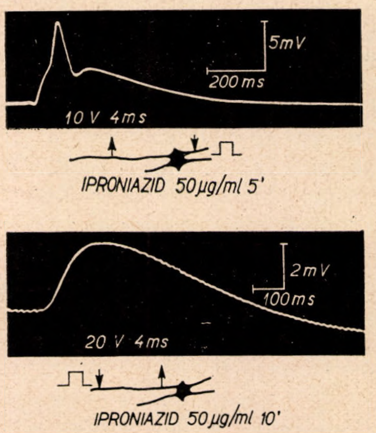

Fig. 8. The effect of iproniazide on the conduction of impulse in the CVC
8. ábra. Iproniazid hatása a CVC ingerületvezetésére

Discussion

The fact of the many sided activability of the nerves running through the visceral ganglion is in good agreement with the data found by HORRIDGE (1958) on a sea-lamellibranchiate *(Mya arenaria)*. At the stimulation of any nerve branch running to the visceral ganglion of *Anodonta* from any other nerve belonging to the visceral ganglion a potential of several actions (consisting of 2 to 6 components) can be lead off either on the homo- or on the contralateral side. This shows not only that the reflex connections of the visceral ganglion are exceedingly manifold, which corresponds to other physiological data (WOORTMANN 1926), but the two-way activability of all pathways seems to point out that the afferent impulses running into the visceral ganglion are forwarded towards the cerebrovisceral ganglion. This is particularly interesting and important in the case when in connection with the stimulation of one of the CVC an action potential of many components can be lead off also from the other CVC because this seems to indicate that the excitation proceeding from the cerebral ganglion towards the visceral ganglion in the form of feed-back can get back into the cerebral ganglion.

Very essential is the fact that from the nerve branch running from the visceral ganglion to the posterior adductor at the CVC stimulation an action potential of two components can be lead off because this points to the combined character of the nerve. This allows the assumption that the muscle fibres also from the functional point of view dispose of at least two kinds of innervation and essential processes of tonic regulation take place also on the neuromuscular level. The action potential on the other hand, that can be lead off the CVC when exciting the muscle branch calls the attention to the possibility of the afferentation of the posterior adductor.

It is extremely interesting that the action potential which can be lead off from the CVC is essentially different according to through which nerve the activation took place that is where the stimulation occured. In the case of the stimulation of the connectivum of the opposite side it consists of six components, in the case of the stimulation of the pallial nerve of five and when the muscle branches are excited only of two or three components and also its shape significantly differs from that which can be lead off without the insertion of the visceral ganglion at the excitation of the same CVC. This can be only in connection with the fact that the distance of the leading from the locus of the excitation and/or from the ganglion is different and the number of the fibre groups coming into excitement at the activation by different nerves and/or of the fibres belonging to the individual groups of fibres is different too. In this of course the visceral ganglion is involved and in the first place, the possibilities of the transmission of the nerve impulse and the fibres running possibly directly through the ganglion play a leading part. Beyond that, however, the possibility can not be excluded that the nervous paths contain not exclusively excitation-transfer loci through differentiated synapses but some elements of a neuron system preserving more primitive connections may play a certain role too.

The investigations conducted with pharmacons do not allow of drawing significant conclusions. At any rate the atropin effect shows that purely cholinerg mechanisms can be hardly assumed in the visceral ganglion. The effect of chlorpromazine and iproniazide on the other hand points to the fact that — if these specifically enough interfere with the serotonin metabolism — the serotonin may be involved not only in the transmission of nerve impulse but also in conduction.

Summary

The reflex connections of the visceral ganglion in *Anodonta cygnea* L. were examined by the stimulation of the pertaining nerves with single square impulses and the leading off of the action potential from an other nerve.

It has been established that at the excitation of the cerebrovisceral connectivum (CVC) of the one side, from the CVC of the other side and also from the nerves running to the adductor as well as from the n. pallialis posterior maior innerving the posterior part of the mantle an action potential consisting of several (2 to 6) components can be lead off. At the stimulation of the latter nerves an action potential consisting of several components can be gained also from the CVC.

The results obtained indicate that

1. the visceral ganglion disposes of a very manifold reflex connection,
2. the nerves running to the adductor include at least two kinds of fibres which is in agreement with the presumed double innervation of the muscles,
3. all informations reaching the visceral ganglion are forwarded also towards the cerebral ganglion,
4. the excitation reaching the visceral ganglion through one of the connectives gets through the other connective in the form of feed-back to the cerebral ganglion.

REFLEXVIZSGÁLATOK TAVI KAGYLÓ (*ANODONTA CYGNEA* L.) VISCERÁLIS GANGLIONJÁN

Salánki János és Lábos Elemér

Összefoglalás

Anodonta cygnea L. viscerális ganglionjának reflexkapcsolatait vizsgáltuk a hozzátartozó idegek egyes négyszögimpulzussal való ingerlése és akciós potenciál más idegről történő elvezetése útján.

Megállapítottuk, hogy az egyik oldali cerebroviscerális connectivum (CVC) ingerlésekor mind a másik oldali CVC-ről, mind a záróizomhoz menő idegekről, mind pedig a köpeny hátsó részét beidegző n. pallialis posterior maiorról több (2—6) komponensből álló akciós potenciál vezethető el. Utóbbi idegek ingerlésekor több komponensből álló akciós potenciál nyerhető a CVC-ről is.

A kapott eredmények azt mutatják, hogy

1. a viscerális ganglion igen sokrétű reflexkapcsolattal rendelkezik,

2. a záróizomhoz futó idegek legalább két rostféleséget tartalmaznak, ami összhangban van az izmok feltételezett kettős beidegzésével,

3. minden, a viscerális ganglionba jutó információ továbbítódik a cerebrális ganglion felé is,

4. az egyik connectivumon a viscerális ganglionba jutó ingerület a másik connectivumon át visszajelentés formájában eljut a cerebrális ganglionhoz.

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ИССЛЕДОВАНИЕ РЕФЛЕКСА ВИСЦЕРАЛЬНОГО ГАНГЛИЯ БЕЗЗУБКИ
(Anodonta cygnea L.)

Я. Шаланки и Э. Лабош

Были исследованы рефлексные связи висцерального ганглия беззубки при раздражении одиночными прямоугольными импульсами нервов, относящихся к названному ганглию, причем ток действия был регистрирован от другого нерва этого же ганглия.

Было установлено, что при одностороннем раздражении церебровисцерального коннктива можно отводить ток действия, состоящий из нескольких (2—6) компонентов с противоположного церебровисцерального коннектива, с нервов, идущих к запирательной мышце, и также с n. pallialis posterior maior, иннервирующего заднюю часть мантии. При раздражении последнего нерва можно отводить потенциал, состоящим из нескольких компонентов, и от церебро-висцерального коннектива.

Полученные данные показывают, что:

1. висцеральный ганглий обладает многочисленными рефлексными связями,

2. нервы, идущие к запирательной мышце, содержат по крайней мере два вида волокон, что соответствует предполагаемой двойной иннервации мышц,

3. всякая информация, идущая к висцеральному ганглию, передается и к церебральному ганглию,

4. возбуждение, идущее по одному коннективу к висцеральному ганглию, попадает по другому коннективу в виде обратной связи и в церебральной ганглий.