ANNAL. BIOL. TIHANY | 38 | 87–96 | HUNGARIA 1971

DIFFERENCES IN THE CHEMICAL SENSITIVITÉ OF NERVE PATHWAYS IN THE CENTRAL NERVOUS SYSTEM OF FRESH WATER MUSSEL (*ANODONTA CYGNEA* L.)

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Received: 26th January, 1971

The functional differentiation of the central nervous system is connected in most of the cases with morphological dissimilarities of various parts of the brain, however, there can be functional differences between nervous structures which seem to be identical according to their morphological structure. It is well known, e.g. especially in lower animals, that neurones being similar to one another in structure are often characterized by different chemical and pharmacological properties.

Because of the high degree of similarities in the morphological picture of neurones the question about the functional differentiation in the CNS of Pelecypoda did not come into the foreground of investigations. Even the functional relations between the ganglia are hardly known, and we have practically no information as to the nature of physiological mechanisms maintaining the contact between various ganglia and how they influence each other. Both the neurones of the cerebral and visceral ganglia being connected with the cerebro-visceral connectives (CVc) and of the pedal ganglia are similar in structure in *Anodonta cygnea* L. (GUBICZA, 1965; GUBICZA and ZS.-NAGY, 1965). Neither of them contains any special type of neurones. All the three pairs of ganglia are characterized by corticalization, one could only find an intraganglionic cluster of neurones in the visceral ganglion (GUBICZA and ZS.-NAGY, 1966), but no special function can be attributed to this structure. Investigations carried out for localizing neurones in the CNS during regeneration of axons referred to the central representation of several neural pathways (SALÁNKI and GUBICZA, 1969), however, this method was not suitable to trace functional differences between different paths. The neurones of the various ganglia did not show differences also according to their histochemical characteristics and ultrastructural organization (BARANYI, 1966; ZS.-NAGY, 1967 a; 1967 b).

Investigating the concentration of serotonin by biochemical methods HIRIPI (1968) found significant differences between the ganglia, and also the different, special physiological role of each ganglia is well known (PAVLOV, 1885; BARNES, 1955; VERESCHAGIN, 1960; SALÁNKI et al. 1968). On the basis of electrophysiological and pharmacological experiments HORRIDGE (1961) admitted the supposition that in *Mya* the various nerve paths can be activated with different chemical substances, and really, PUPPI (1963) described some

differences between the cerebral and visceral ganglia in their reaction to acetylcholine and atropin. Nevertheless, up to now there are no systematic, comparative studies on the chemical mediation, activation and inhibition of various ganglia and neural pathways in Pelecypoda.

In the present investigations we tried to check the chemical sensibility of the cerebral and visceral ganglia and of the pathways originating from them. We performed parallel registrations of electrical activity of several nerves and on the basis of the results we tried to make conclusions about the chemical differentiation supposed in the CNS of the *Anodonta cygnea*.

Material and methods

For the experiments large specimens (18—22 cm in length) of *Anodonta cygnea* L. were used. After removing one of the shells the visceral ganglion (VG), one of the cerebral ganglia (CG), the posterior pallial nerves (nppm), one of the anterior pallial nerve (npa I), the cerebro-visceral connective (CVc) and the cerebro-cerebral commissura (CC) were revealed as described earlier (Salánki and Varanka, 1969). For recording the electrical activity the nerves were laid on bipolar platinum electrodes (diameter 0.5 mm). The substances to be tested were applied directly to the ganglia with a piece of cotton wool. In different experiments either the cerebral or the visceral ganglion was treated, but the electrical activity was recorded in all cases from the following four nerves: nppm, CVc, npa I, CC *(Fig. 1)*. The efferent fibres of the nppm and npa I are motoric fibres, while most of the axons in the CVc and CC belong to interganglionic pathways, however, these latter contain also efferent fibres. The experiments began after 4—6 hours following the preparation. Substances were applied after checking the control activity, and further the activity was recorded in the first two minutes, and also in the 5th, 10th, 20th and 30th minutes using an 8-channel electroencephalograph (Typ EMG MB 5206). The input impedance was 2 MΩ. During the evaluation of the data the changes were compared to the own control activity.

The effect of the following substances was tested: acetylcholine (ACh), serotonin (5-HT) and dopamine (DA). According to some above mentioned

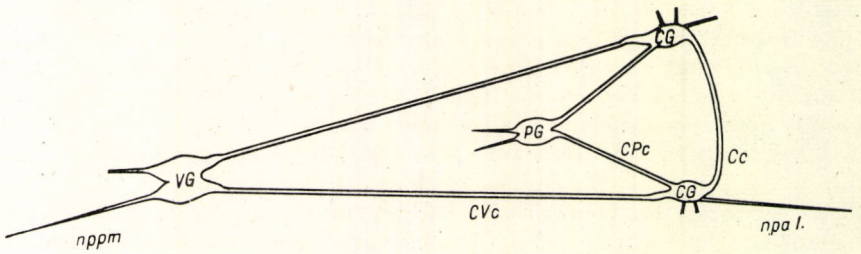

Fig. 1. Topographical arrangement of the investigated neurones and their connections in the nervous system of *Anodonta cygnea* L. CG — ganglion cerebrale; VG — ganglion viscerale; PG — ganglion pedale; CVc — connectivum cerebro-viscerale; CC — commissura cerebro-cerebrale; CPc — connectivum cerebro-pedale; nppm — nervus pallialis posterior maior; npa I — nervus pallialis anterior I

data these substances are present or may function in the CNS of *Anodonta* in physiological circumstances. The substances were dissolved in physiological solution (MARCZYNSKI, 1959). Each type of the experiments was carried out 4—6 times.

Results

The preparation was considered intact and suitable for experiment if from all the tested nerves activity could be recorded with lower or higher frequency. The amplitude of the potentials varied between 10—40 microvolts, while the frequency fluctuated between 5 and 40 cps.

On the CVc and CC there appeared sometimes also potentials of higher amplitude (up to 60—80 μV). The control corresponded as usual to that what was described earlier in details (SALÁNKI and VARANKA, 1969). On the control registrations differences were found between the activities of the CVc, CC and npa, originating all the three from the cerebral ganglia as well between the activities of the CVc and nppm being in direct contact with the visceral ganglion. Using this method we could not find any definite relations between the activity level of nerves belonging to the same ganglion, and there were also various levels of activity if compared the control activity of the same nerves in different preparations.

Application of drugs onto the ganglia was followed by characteristic to the type of the experiment changes in the electrical activity of one or more nerves, being different sometimes even on nerves originating from the same ganglion. There were, furthermore, differences in the activity of the CVc connected with both the cerebral and visceral ganglion, depending on whether the application of the drug took place on the cerebral or on the visceral ganglion.

The effect of the drugs was tested in 10^{-4} M and 10^{-5} M concentrations. As we did not find significant differences between the effects of the two concentrations, the results are described and discussed collectively.

The effect of acetylcholine (ACh): application of ACh to the cerebral ganglion resulted in different effects on different nerves *(Fig. 2)*. On the CVc, in most of the cases there was stimulation in the first 5—10 minutes, than the effect disappeared and the control activity was restored. In one case out of five we did not notice any change in the activity. On the CC, connecting the two cerebral ganglia, in contrary to the former, inhibition was observed in a few minutes after application; it appeared in one case after short, initial stimulation. There was only one case, when stimulation was recorded without inhibition, this was that case, when the applied ACh did not cause any effect on the CVc. On the npa I the effect was similar to that found on the CC. On the nppm, in most of the cases, there was no change in the activity.

If ACh was applied onto the visceral ganglion, the activity of the CVc was not changed in the first minutes, afterwards it was unanimously inhibited. Contrary to this, on the CC stimulation occurred in the first 5—10 minutes. There was also increase of activity on the npa I while on the nppm no definite effects were registered.

The effect of serotonin (5-HT): after applying 5-HT to the cerebral ganglion, increase of activity appeared on the CVc, and in some cases on the CC, too. At the same time there was not observed any effect on the pallial nerves in most of the cases. Applying the 5-HT to the visceral ganglion resulted

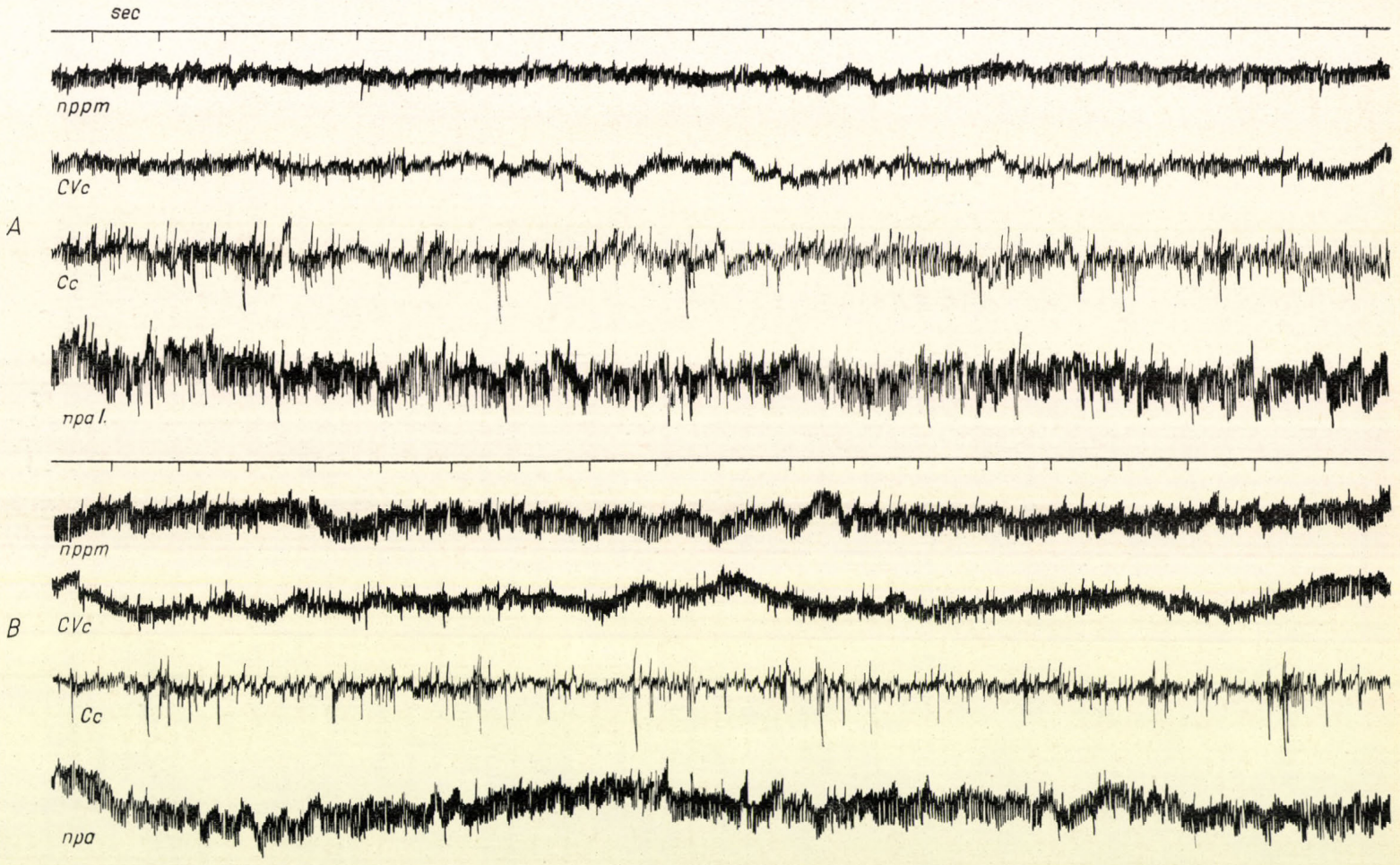

Fig. 2. Effect of acetylcholine (10^{-4} M) applied to the cerebral ganglion. A — control, B — 5 min after application. Abbrev.: see in *Fig. 1*

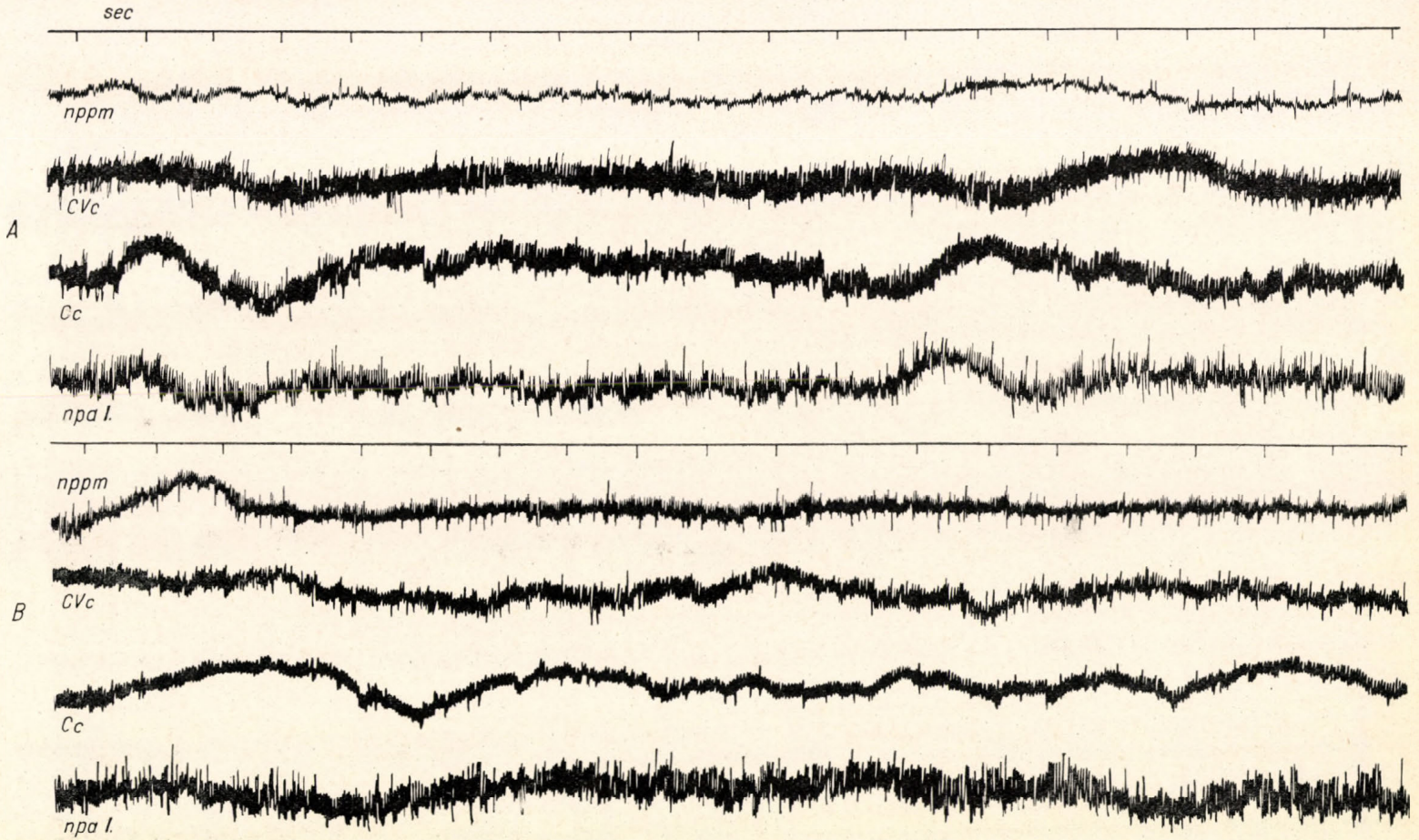

Fig. 3. Effect of serotonin (10^{-4} M) applied to the visceral ganglion. A — control, B — 10 min after application

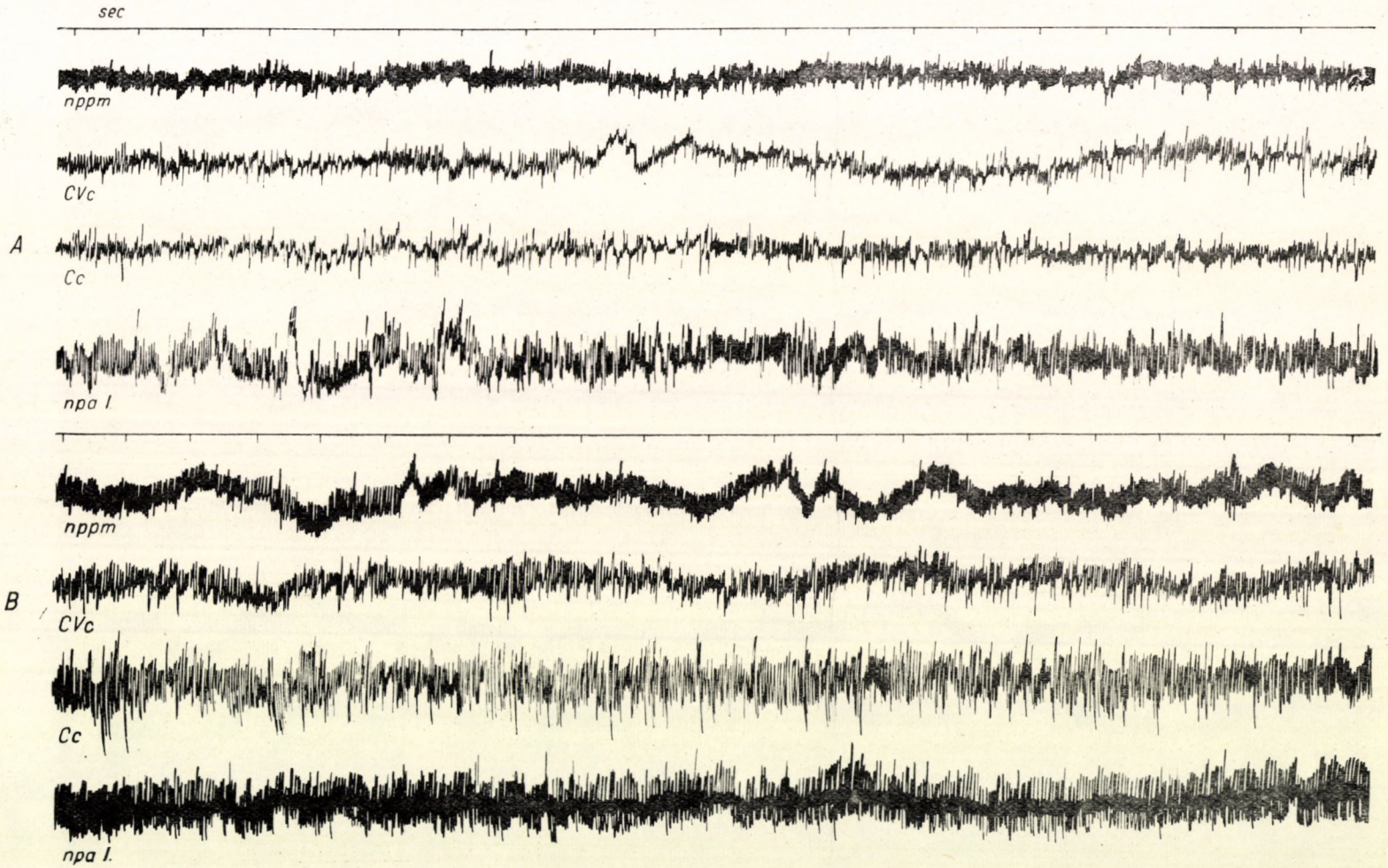

Fig. 4. Effect of dopamine applied to the cerebral ganglion. A — control, B — 10 min after application

in inhibition on the CVc and CC, while on the nppm the increase of activity was registered. No definite effect was recorded from the anterior pallial nerve (npa I) *(Fig. 3)*.

The effect of dopamine (DA): the effect of the DA applied both to the cerebral and visceral ganglion manifested itself in most of the cases in stimulation *(Fig. 4)*. Treating the cerebral ganglion resulted in the increase of the activity of the CVc, and in most of the cases of the npa I. No effect was observed in most cases, on the CC, however, in one-one case increase and also decrease occurred. On the anterior and posterior pallial nerves the increase of activity was noticed in half of the cases, in the other experiments no change was observed. In case DA was applied to the visceral ganglion, in most cases increase of activity was observed both on the CVc and CC, while on the pallial nerves either no change was recorded or it was not unequivocal.

Discussion

The integrative functioning of the nervous system is based mainly on the differences between nerve cells, on the differentiation of the interneuronal connections and neural pathways. One of the mode of differentiation is the heterogenous chemical sensitivity of neurones and interneuronal connections, meaning that they operate with different mediators (Eccles, 1964). Our results show that the central nervous system of the *Anodonta* is characterized by chemical differentiation referring to the operation of such principles, and explain the unidentical effects of various chemical substances.

In most of the cases ACh, 5-HT and DA applied to the cerebral ganglion influenced differently the activity of the nerves originating from the treated ganglion. The reaction of the nerves originating from the visceral ganglion was also different. In the interpretation of these facts there may be several possibilities: the applied drug *(a)* can influence the activity of various nerves differently (stimulation, inhibition, ineffective); *(b)* it influences primarily the activity of a special group of neurones and the latter causes stimulation or inhibition in the activity of other groups; *(c)* it does not affect directly the membrane of the neurones or synapses, but causes the mobilization of the transmitters. With the present method we cannot decide which of the possibilities exists in reality. Nevertheless, on the basis of the fast appearance of the effects one can assert that the change of the activity increasing in one case and decreasing in the other after applying ACh and 5-HT originates from direct neuronal action. The same may be valid in case of the stimulating effect of the DA. Taking into consideration that the presence of 5-HT and DA was demonstrated in the CNS of *Anodonta* (Dahl et al. 1962; Zs.-Nagy, 1967 a), and presumably also cholinergic mediation exists in the ganglia (Salánki et al. 1967) one can state that the observed effects reflect physiological mechanisms.

It was rather unexpected, that ACh and 5-HT caused increase of the activity of the CVc when applied to the cerebral ganglion, but caused decrease of it when they were applied to the visceral one. This seems to refer to the different chemical sensitivity of the soma of axons leading to opposite directions in the CVc, however, in the establishment of the activity of the CVc one has

to take into consideration also synaptic connections and influences. The results refer also to the fact, that neither in the CNS of *Anodonta* exist unambiguous stimulating or inhibiting substances, but the quality of the effect depends on the differentiated chemical sensitivity of the neurones in action.

Our results refer also to some specificities of the interconnections between neural pathways. Treatment of the visceral ganglion with 5-HT caused decrease of the activity not only in the CVc, but also in the CC. Contrary to this, simultaneously with the inhibitory effect of ACh on the CVc, stimulation was found on the CC. The effect of 5-HT may be connected with the fact, that in the CC there are also fibres originating from the visceral ganglion (SALÁNKI and GUBICZA, 1969). However, the number of these fibres is low, therefore the results may be interpreted better supposing that 5-HT inhibits in the visceral ganglion the activity of the neurones, which suppress the activity of nerve cells responsible for the activity of the CC. The existence of pathways of different directions and functions detected in the CVc (PAVLOV, 1885; VERESCHAGIN, 1960; SALÁNKI et al. 1968) allows the supposition of the chemical differentiation of such a degree.

The complexity of connections between neurones and groups of neurones and further the different activity levels at the time of the application of the drugs may explain the differences found in the summed neuronal activity of different preparations, what cause difficulties in the evaluation of the chemical sensibility of the paths. Notwithstanding, on the basis of our results one can state that there is a path in the CVc in cerebrovisceral direction and also in the npa I, which can be stimulated with ACh, while the viscero-cerebral paths of the CVc are inhibited by ACh. 5-HT stimulates the cerebro-visceral pathways of the CVc, and also the paths of the CC and the nppm. According to earlier data (SALÁNKI, 1963; SALÁNKI and HIRIPI, 1970) the relaxing paths supplying the adductor muscles belong also to this latter category. At the same time 5-HT inhibits the viscero-cerebral pathways of the CVc.

The present results support also the observations described earlier concerning *Anodonta* showing that some drugs cause different effects on different nerves (PUPPI, 1963; SALÁNKI and VARANKA, 1969). The biphasic effects (PUPPI, 1963) can be explained also by the disproportional activation of neurone groups influencing each other. It is very probable that using further active substances and specific drugs and also by investigating other nerve trunks the scheme of the differentiated, heterogeneous chemical sensitivity of the central nervous system of *Anodonta* can be completed and so the chemical basis of the integrative functions of the nervous system can be given in a positive form up to neurone groups and neural pathways.

Summary

On the basis of experimental results obtained after applying drugs onto the cerebral and visceral ganglia during simultaneous recording of electrical activity from several nerves it was stated that the same substance causes different effects on the different nerves.

Acetylcholine stimulates the neurones of the cerebro-visceral pathways and that of the anterior pallial nerve, and inhibits the cerebro-cerebral and viscerocerebral paths. Serotonin activates the cerebro-visceral and cerebro-cerebral

pathways and also the neurones of the posterior pallial nerve, but inhibits the viscero-cerebral paths. Dopamine caused either the stimulation of the investigated pathways or was ineffective.

The stimulated or inhibited pathways influence secondarily the activity of other groups of neurones in the same or in an other ganglion, playing important role in the functional differentiation of the central nervous system connected with the heterogeneous chemical sensitivity of neurones and neural pathways.

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IDEGPÁLYÁK ELTÉRŐ KÉMIAI ÉRZÉKENYSÉGE TAVI KAGYLÓ (*ANODONTA CYGNEA* L.) KÖZPONTI IDEGRENDSZERÉBEN

Salánki János és *Varanka István*

Összefoglalás

A cerebrális, ill. viscerális ganglionok kezelése és több idegről történt egyidejű elvezetése során megállapítást nyert, hogy a különböző idegpályák elektromos aktivitása ugyanazon kémiai anyaggal eltérő módon befolyásolható.

Acetylcholin serkenti a cerebro-visceralis pályák, valamint az elülső palliális ideg neuronjait, és gátolja a cerebro-cerebrális és a viscero-cerebrális pályákat. Szerotonin serkenti a cerebro-viscerális, valamint a cerebro-cerebrális pályákat, és a hátsó palliális ideg neuronjait, és gátolja a viscero-cerebrális pályákat. Dopamin a vizsgált pályákat serkentette, vagy hatástalan volt.

A serkentett, ill. gátolt pályák másodlagosan újabb neuroncsoportok aktivitását befolyásolják ugyanazon vagy másik ganglionban, ami a neuronok eltérő kémiai érzékenységének a központi idegrendszer funkcionális differenciáltságának meghatározásában játszott szerepét mutatja.