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RESPONSES OF CENTRAL NEURONES TO THE STIMULATION OF HEART CHEMORECEPTORS IN THE SNAIL, *HELIX POMATIA* L.

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It has been described that beside miogeneicity the heart of the snail *Helix pomatia* is controlled also by extracardial innervation (KRIJGSMAN and DIVARIS, 1955). On the other hand, well defined impulses evoked by the tactile, pressure, osmotic and chemical stimulation of the heart are running to the CNS through the intestinal nerve (S.-RÓZSA, 1972). We found that tactile stimulation of the heart can modify the activity of some central neurones by causing acceleration in some cases while inhibition in others, and also the efferent connection between some of the modulated neurones and the heart has been proved (S.-RÓZSA and SALÁNKI, 1973).

In our present work we wanted to elucidate whether there are special neurones in the central nervous system responding to chemical stimuli applied to the heart or not, and further, if they are present, what is their reaction at different modes of stimulation. Also the character of the respond evoked by the chemical stimulation of the heart was determined, and the reactions of neurones belonging to the heart-heart reflex were investigated.

Material and methods

Experiments were carried out on brain-heart preparation of *Helix pomatia* L. Snails kept in hibernation during winter were waken and activated by keeping them at room temperature and at increased humidity while they were fed. The heart rate of the animals activated by this way was regular and could be kept functioning in half-isolated conditions for long time (24 hours).

The shell of the animals was removed and the central nervous system was exposed. The circumoesophageal ganglionic ring was separated from the surroundings, and with the exception of the intestinal nerve all of the neural connections of the ganglia were cut. The intestinal nerve was cleared from the connective tissue and the blood vessels and side branches were transsected only the fine nerve running to the heart remained intact *(Fig. 1)*.

The heart was freed by opening the pericard. Cannula were inserted into the pulmonal vein and into the aorta near to the ventricle for perfusion. The conditions of the perfusion, assuring constant pressure, were described in details previously (S.-RÓZSA and GRAUL, 1964).

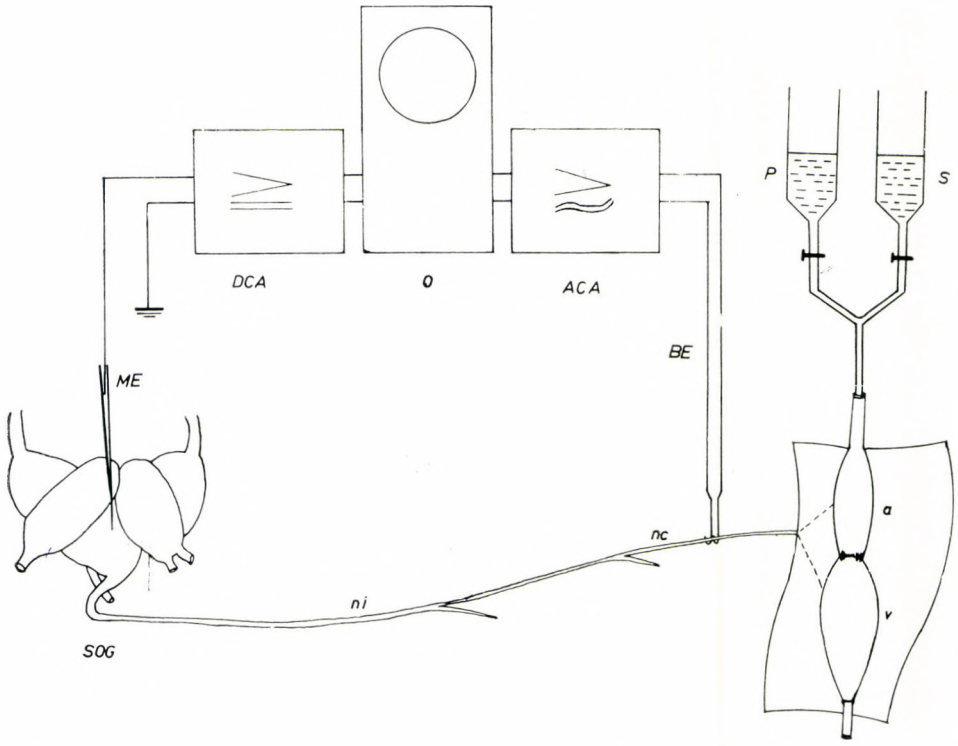

Fig. 1. Experimental arrangement. SOG — suboesophageal ganglionic mass; ni — n. intestinalis; nc — heart nerve; a — atrium; v — ventricle; ME — microelectrode; DCA — DC amplifier; BE — bipolar electrodes; ACA — AC amplifier; O — oscilloscope; p — perfusion chamber for physiological solution; S — chamber for substance (5HT)

The preparation consisting of the brain, intestinal nerve and heart was placed in a special chamber and the connection between the ganglia and the heart was maintained only through the nerve. The distance from the brain to the heart measured about 2 cm. Both heart and brain was protected from drying by physiological saline. For keeping the tissues wet and for solving substances MENG-solution (MENG, 1958) was used. The preparation and the experimental circumstances are given in *Fig. 1.*

For stimulating the heart chemoreceptors 5-hydroxytryptamine (5HT) was used. 5HT was perfused in 10^{-6}—10^{-3} mol concentration intracaridally with a pressure identical to the normal perfusion, using a turning cock. Tactile stimulation was performed at the atrio-ventricular area, with a fine brush.

The electrical activity of the intestinal nerve was recorded by bipolar Ag-AgCl electrodes, extracellularly. After heart stimulation the impulsation increases both in frequency and amplitude (S.-RÓZSA, 1972). The membrane and action potentials of the central neurones were recorded with conventional glass microelectrodes filled with KCl, and having resistance from 5 to 15 MOhms. In the experiments high input impedance amplifier (VÉRÓ, 1971) was used, and polarization could be performed with appropriate bridge circuit. For amplification and recording the potentials ALVAR instruments were used·

Neurones responding to the stimulation of the chemoreceptors of the heart were mapped using the method worked out previously on *Helix* brain (SAKHAROV and SALÁNKI, 1969). Part of these neurones were identical with cells responding to tactile stimulation (S.-RÓZSA and SALÁNKI, 1973).

Experiments were performed in autumn and winter at room temperature (20°—22 °C).

Results

Altogether 31 neurones from the visceral and right parietal ganglia of 19 preparations were investigated. Some of the cells could be identified in different preparations due to their constant localization. The numbering and location of the different neurones responding to the stimulation of the heart chemoreceptors with 5HT are shown in *Fig. 2.*

Most of the cells performed spontaneous activity during experiments, being the generation of action potentials endogenous (pacemaker cells) or evoked by synaptic potentials (driven cells). Several "silent neurones" were also investigated.

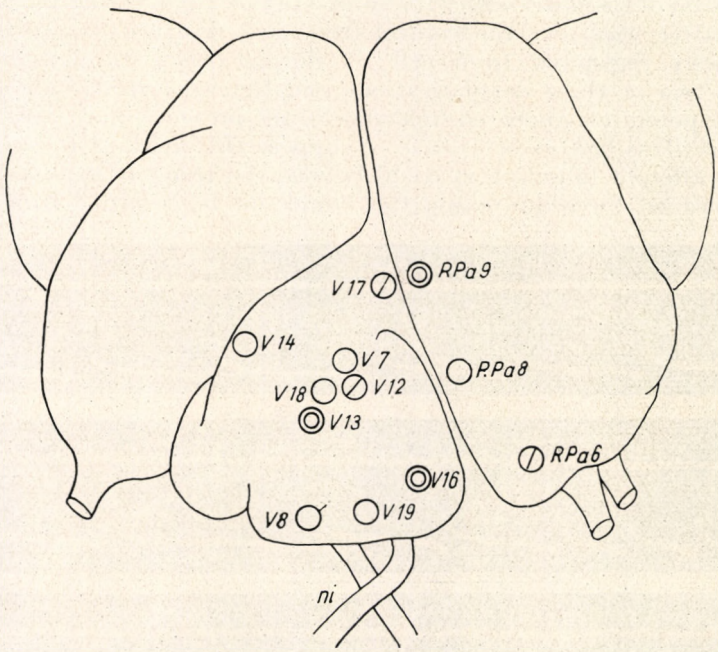

Fig. 2. Localization and reaction type of neurones responding to the stimulation of the heart with 5HT.

◎ — increase of activity,

○ — decrease of activity,

⊘ — biphasical reaction,

○ — no reaction

The characteristics of the afferent impulsation running from the heart to the CNS through the intestinal nerve were described previously (S.-Rózsa, 1972). In the present experiments both the stimulation and the recorded extracellular potentials were identical to that what was found earlier. All the neurones responding to the chemical stimulation of the heart were tested whether they give a response to the tactile stimulation of the heart or not.

1. *Central neurones responding to the stimulation of the heart chemoreceptors*

a) Neurones responding with the decrease of the activity

Five neurones have been found whose activity decreased due to the intracardially administered 5HT. These were V7, V14, V18, V19 and RPa8 neurones. The increased afferent impulsation recorded from the intestinal nerve was followed by the frequency decrease and further by elimination of the spike generation of the neurone recorded with intracellular electrode. As a rule, IPSP-s could not be recorded from the cell, and release from inhibition was not followed by increased activity. As it is shown in *Fig. 3* the perfusion of the heart with 5HT caused inhibition in the activity of neurone V7, and after wash out the control frequency returned only gradually. No IPSP-s were registered from cell V7 during heart stimulation, however, following two or three depolarizations and hyperpolarizations characteristic inhibitory potentials appeared, referring to the presence of synaptic influences on the cell. This fact gives a basis to suppose that inhibition occurring after increased afferent impulzation could be also the result of synaptic bombardment, however, for some reason this could not be recorded from the soma.

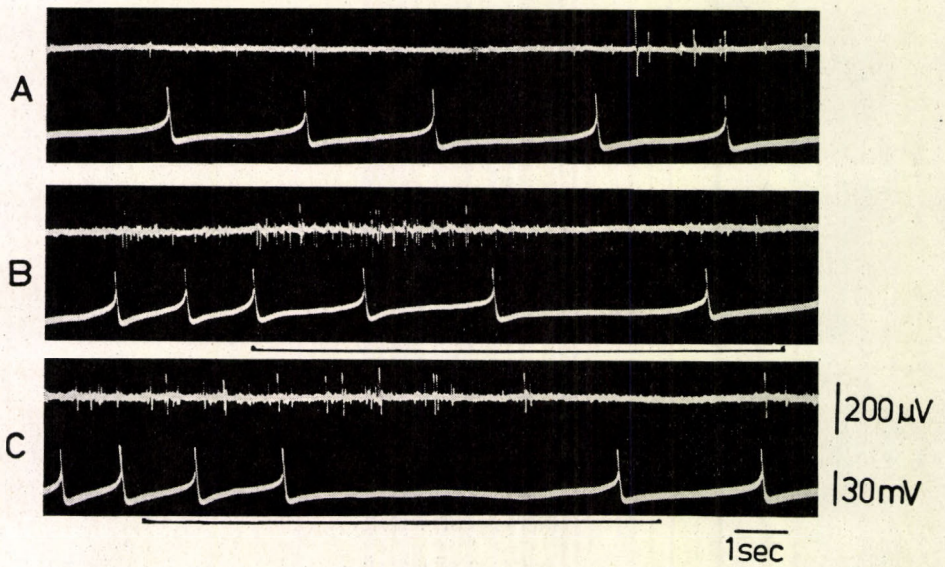

Fig. 3. Reaction of neurone V7 to the chemical stimulation of the heart. A — control; B and C — effect of stimulation. Here and in the following Figs: upper — extracellular recording from the heart nerve; lower: intracellular recording from the soma of the neurone

b) Neurones responding with the increase of the activity

Due to 5HT stimulation of the heart the activity of the neurones V13, V16 and RPa9 increased. This was preceded by the increased afferent impulsation recorded from the intestinal nerve *(Fig. 4)*. The increased activity of the neurones was observed until the elimination of the serotonin from the heart and the returning of the control activity of the intestinal nerve.

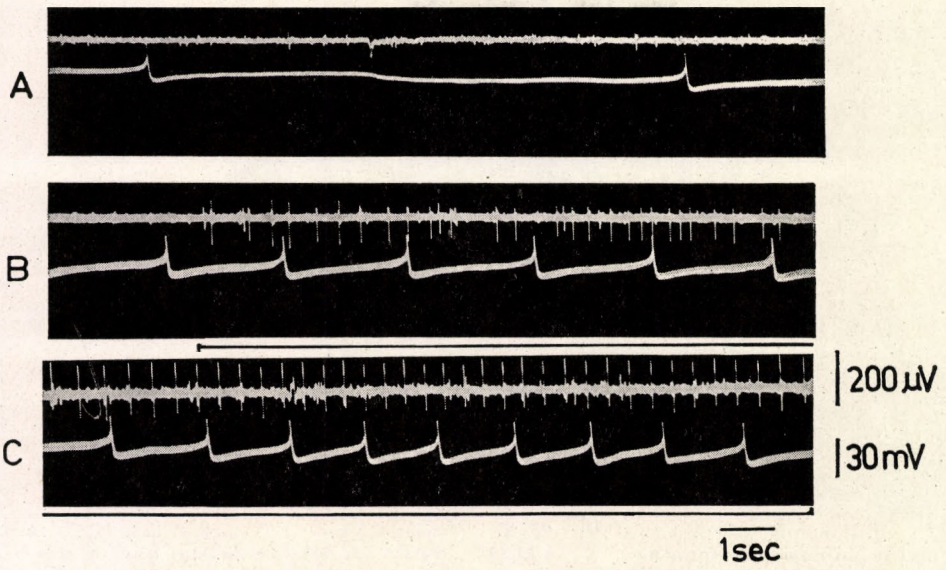

Fig. 4. Response of neurone V16 to the chemical stimulation of the heart. A — control; B and C — effect of continuous stimulation

Furthermore the activity of cell V13 was in close correlation with one of the components recorded from the heart nerve *(Fig. 5)*. As the intracellular spikes preceded the extracellular potentials, these latter can be considered as efferent impulses originating from the neurone and running towards the heart. This supposition is supported by the findings that the close correlation of the two signals could be observed also at the depolarization of the neurone *(Fig. 6)*.

Figure 5 shows that the chemoreceptors of the heart become active to 5HT not instantaneously, but there is at the beginning only a week signalization *(Fig. 5B)*. Also the elimination of the effect of the 5HT and the decrease of the activity of the intestinal nerve take place gradually. The activity of cell V13 remains enhanced until the activity of the intestinal nerve is higher than that of the control *(Fig. 5E, F)*.

No EPSP-s could be registered from neurones responding to the heart stimulation with the increase of the spike generation.

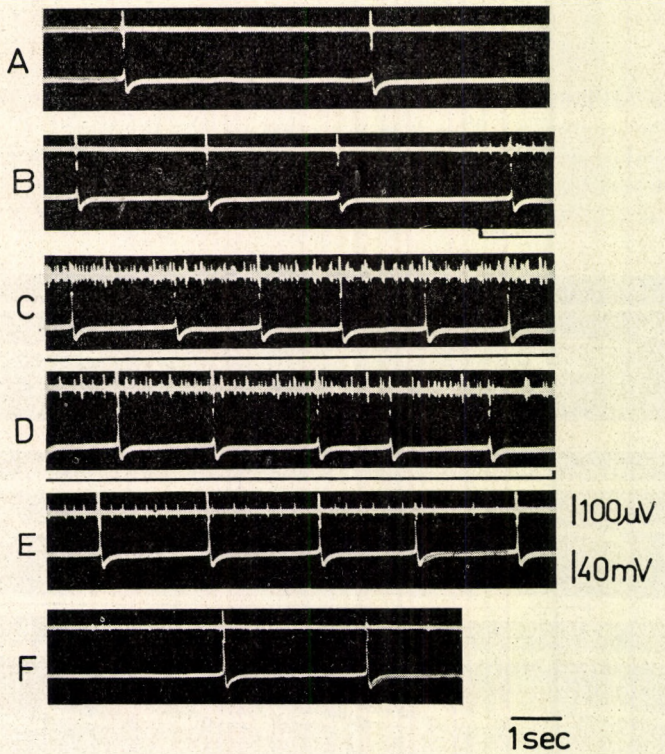

Fig. 5. Response of cell V13 to the chemical stimulation of the heart. A and B — control and beginning of stimulation; C — and D — during stimulation after 1 min; E and F — end of wash out after 3 min. It can be seen that the intracellular spike is followed by an extracellular one in each case

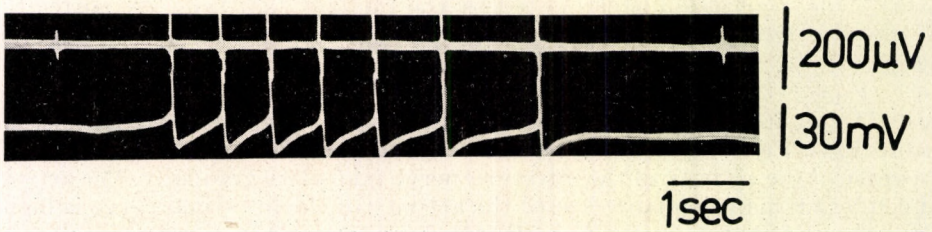

Fig. 6. Response of cell 13 to depolarization of the soma membrane. Note the correlation between the intra- and extracellular components

c) Neurones with biphasical response

There were cells responding biphasically to the chemo-stimulation of the heart. The frequency of the activity of cells V12, V17 and RPa6 increased at the beginning, than it decreased. The reaction of neurone V17 is demonstrated in *Fig. 7*. It can be seen that after perfusing the heart with 5HT both the amplitude and the frequency of the afferent impulsation increased and following this, the frequency of the action potentials recorded from neurone V17 was nearly doubled *(Fig. 7B)*. However, after 2 min. the activity of the

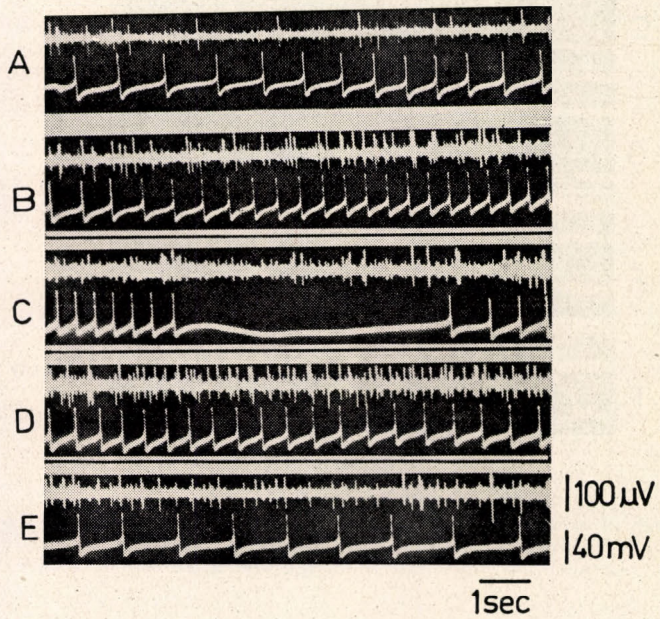

Fig. 7. Response of cell V17 to the stimulation of the heart with 5HT. A — control; B, C and D — continuous stimulation of the chemoreceptors; E — after washing

neurone was inhibited while first depolarization, than hyperpolarization occurred, and at the same time the frequency of the extracellular potentials with high amplitude was decreased *(Fig. 7C)*. Recovering from the phase of inhibition the cell is more active than was in the control state *(Fig. 7D)*, while after elimination of the 5HT it produced a lower activity compared to the control *(Fig. 7E)*. This type of reaction was very characteristic to this neurone and could be evoked repeatedly with very similar time curves in each case.

Cell V12 responds similarly to V17 *(Fig. 8)*, however, in this case the increase of the activity is only of short duration after heart stimulation *(Fig. 8B)*, and alteration of frequency increase and decrease was observed. This type of potential generation, resembling to a bimodal, bursting activity was observed also during the wash out of serotonin *(Fig. 8E,F)*. In some cases during the inhibitory phase IPSP-s were observed causing probably the in-

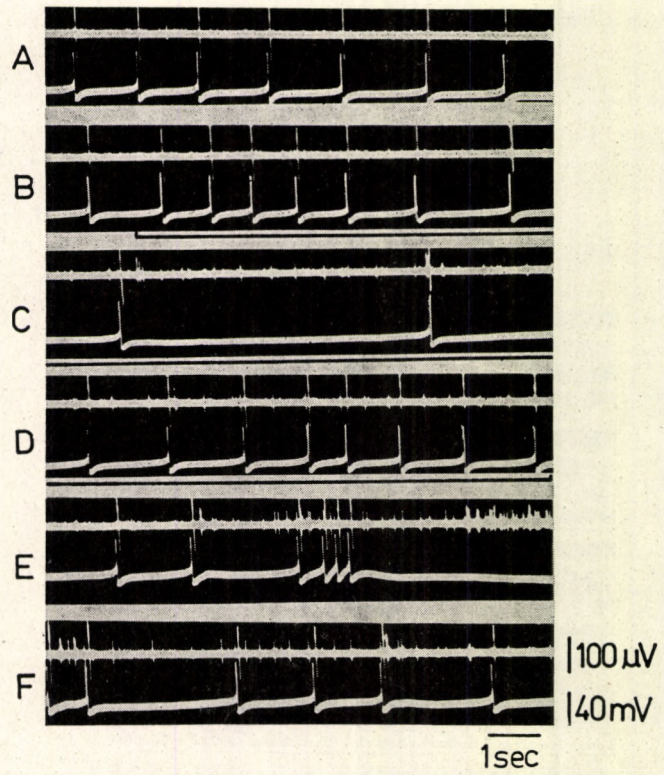

Fig. 8. Response of neurone V12 to heart stimulation. A — control; B, C and D — effect of stimulation; E — during wash out after 2 min; F — after 5 min. Note the synchronous extracellular potential following the intracellular spikes

hibition of the spike generation. In case of cell V12 also a close correlation was found between the intracellular spikes and a given extracellular potential type.

The initial increase of activity was followed by inhibition of the potential generation in case of the RPa6 neurone, too. The spikes of the cell could not be recorded in the extracellular registration from the heart nerve, nevertheless, the general impulzation of this nerve was lowered during the phase of inhibiton of the cell activity. This means that the RPa6 neurone is connected not to a particular axon in the heart nerve, but with several other neurones, sending their axon into this nerve. The generation of potentials in this neurone was clearly of synaptic origin driven by EPSP-s.

d) Effect of tactile stimulation of the heart on neurones giving reaction to chemo-stimulation

The reactions of neurones to chemical and tactile stimulation of the heart were compared in order to clear up the specificity of the central representation of the effect evoked with stimulation of different types. *Table I* summarizes the results.

TABLE I

Response of identified central neurones to the chemical and tactile stimulation of the heart

Neurone	Reaction to chemical stimulus	Reaction to tactile stimulus
V7	−	−
V8	0	−
V12	+,−	+
V13	+	−
V14	−	0
V16	+	+
V17	+,−	+,−
V18	−	+
V19	−	−
RPa6	+,−	−
RPa8	−	−
RPa9	+	−

+: increase of activity
−: decrease of activity
0: no effect

As can be seen, there were only two neurones responding only to one sort of stimulation. Cell V8 gave reaction only to tactile, while cell V14 to chemical stimulation of the heart. Cells V13, V18 and RPa9 gave antagonistic reaction to the chemical and tactile stimulation of the heart: V13 and RPa9 neurones became stimulated at chemical, and were inhibited at tactile stimulation. Cell V18 gave reversed response. Biphasic reaction was observed in the case of cell V17 to both types of stimulation, while cell V12 and RPa6 responded biphasically to chemo-stimulation of the heart. In these cases acceleration was the first phase, followed by a decrease and again by an increase of the spike generation.

Tactile stimulation caused on cell V12 only stimulation while on cell RPa6 only inhibition, however, one must take into consideration that the duration of the tactile stimulus is comparatively short, while chemical stimulation is long. Cells V7, V19 and RPa8 were inhibited and cell V16 was stimulated both at tactile and chemical stimulation of the heart.

e) Postsynaptic potentials on the central neurones at the stimulation of heart receptors

From the soma of neurones connected with heart receptors postsynaptic potentials could be registered only rarely. Cell RPa6 was the single neurone where EPSP-s were recorded in control conditions and from cell V7 could be recorded IPSP-s after repeated de- and hyperpolarization.

In two cells, however, where the effects of tactile and chemical stimulation were antagonistic, PSP-s occurred, and the effect was realized clearly through PSP-s. In cell V12 after chemo-stimulation of the heart depolarization took place at the beginning, without noticeable PSP-s. However, in the second phase the inhibition took place with the appearance of IPSP-s *(Fig. 9)*. On

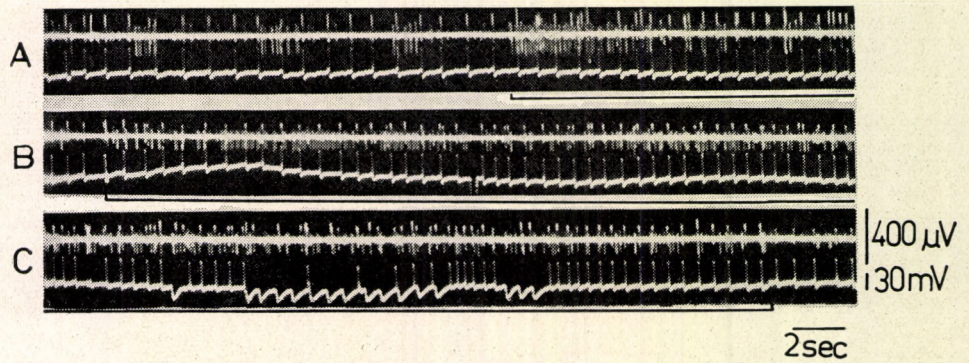

Fig. 9. Two phases of the response of neurone V12 in a case, when after the chemical stimulation of the heart ISPS-s were recorded. A — control and the effect of tactile stimulation of the heart; B — excitatory phase following the chemical stimulation of the heart; C — inhibitory phase during stimulation

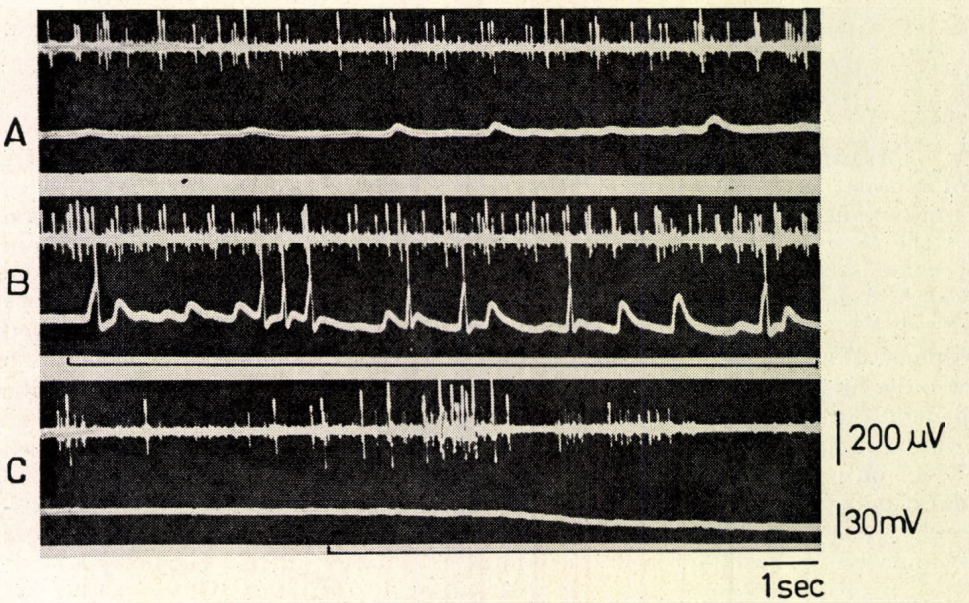

Fig. 10. Response of cell V18 to heart stimulation. A — control; B — effect of tactile stimulation of the heart. Note the increase of EPSP-s. C — effect of chemical stimulation of the heart

cell V12 the increase of activity appeared without EPSP-s also in case of tactile stimulation. On cell V18 the tactile stimulation of the heart resulted in the increase of EPSP-s and enhanced spike activity. On this cell the inhibition evoked by chemo-stimulation of the heart appeared without IPSP-s, however, the amplitude of the EPSP-s was drastically reduced, and membrane hyperpolarization was observed *(Fig. 10)*. In contrary to the fact that PSP-s were recorded only rarely these cases show that signalization arriving from the

heart can cause strong synaptic influence on the neurones. It seems probable that the modification of the cell activity occurs much more frequently through synaptic potential than we could observed, however, being the synapses far from the soma when recording with intracellular electrodes, these potentials do not become visible.

Discussion

It was shown earlier that the tactile stimulation of the heart modifies the activity of a number of central neurones (S.-Rózsa and Salánki, 1973). The present results give evidence that the chemoreceptors of the heart also have a similar central representation at cellular level. Our experiments were conducted on the visceral and right parietal ganglia, and these neurones were found not in groups, but in dispersed localization. It can be supposed, that neurones connected with heart chemoreceptors could be found in other parts of the ganglionic mass too.

According to these data neurones receiving signals from the heart at different modes of stimulation are mixed in the snail brain similarly to the central neurones of heart chemo- and baroreceptors in the brainstem of vertebrates (Miura and Reis, 1972; Spyer, 1972). Our earlier histological data also proved that the neurones innervating the heart of Gastropoda are in the ganglia scattered and do not form any definite group (Gubicza and S.-Rózsa, 1969).

Examining the neurones whether they can be influenced both from the chemoreceptors and tactile receptors we found that there is only a few of them responding only to one of the stimulus types. There were a few more cells responding antagonistically to the two different stimuli. The identical representation of different stimuli show that primary sensory neurones responding directly to stimulation can be present among the investigated cells only in a limited number. Such primary sensory neurones are the mechano- and stretch receptors of crabs, the chordotonal organ of arthropods, the muscle proprioceptors, while the central light sensisitive system of the arthropods and most of other sensory systems are composed of secondary sensory neurones (Grundfest, 1971). Most probably such secondary sensory neurones take part in the central representation of sensory areas of the *Helix* heart. The sensory character could explain that postsynaptic potentials were not registered from most of the neurones both at rest and after chemical or tactile stimulation of the heart. However, to explain the lack of PSP-s one can suppose too that the place of the synaptic influence and the location of the recording electrode are situated to a distance, and so the synaptic potentials can influence the spike generation, but cannot be recorded from the soma. If this supposition is true, one could record a more violent synaptic bombardment from the synaptic region than from the soma.

In case of biphasical responses sometimes PSP-s appeared clearly. This type of activity is based probably in each case upon synaptic influences, when the competition of excitatory and inhibitory inputs cause alteration in the cell activity. It can be supposed that the occurrence of biphasical responses would be more frequent after tactile stimulation if we apply this stimulus for minutes instead of seconds.

The occurrence of neurones responding differently to different modes of stimulation is an indirect proof showing that among receptors special chemo- and tactile receptors must exist, however, we are lack of any information about their morphological structure. As both type of stimulus cause an increase in the electrical activity of the intestinal nerve, the different neuronal reactions refer to the fact that specificity must exist also at neuronal level according to the sensory area. It cannot be excluded that this specificity is manifested in the form of the presence of primary sensory neurones. It can be supposed too that neurones influenced by heart receptors form a coupled system similarly to that described for the touch receptors of *Hirudo* (BAYLER and NICHOLLS, 1969). Such a coupling can give a basis not only for differentiation of various signals, but also for integration of different inputs and for the discrimination of the place of stimulus.

The functional role of some giant neurones is well known in the control of centrally triggered motor functions (WILLOWS and HOYLE, 1968) and in some reflex responses (KUPFERMANN and KANDEL, 1969; PERETZ, 1969; WEEVERS, 1971) in Gastropoda. In the visceral ganglion of *Limax* a neurone was described causing increase in the heart rate (MCKAY and GELPERIN, 1972). We have reported also about some neurones in the CNS of *Helix* taking part in the heart-heart reflex evoked by tactile heart stimulation (S.-RÓZSA and SALÁNKI, 1973). According to our present results the reflex responds occurring at the stimulation of chemo- and tactile receptors are very similar, and in some cases they are realized with the participation of the same neurones (cells V12 and V13). Similar common representation was described for the central neurones of the chemo- and baroreceptors in vertebrates (MIURA and REIS, 1972). Obviously, central neurones influenced by heart stimulation can take part in the functioning of other reflex pathways, too.

Our results give evidence for the central representation of the heart chemo-receptors at cellular level and prove on its basis the existence of such a heart-heart reflex which can take part in the extracardial regulation of the heart. The fact, that the activity of most of the neurones can be modulated by different types of stimuli calls the attention to the existence and functioning of regulatory systems distributed diffusely in the ganglia.

Summary

The responses of central neurones to chemical stimulation of the heart were examined in the visceral and right parietal ganglia of the snail *Helix pomatia* L. These responses were compared with reactions evoked by stimulation of the mechanoreceptors of the heart. The following results were obtained:

Twelve neurones were identified in the visceral and parietal ganglia responding to the stimulation of the heart with 5HT. The response was either an increase or a decrease in activity.

Four neurones, three from the visceral (V12, V13 and V17) and one from the right parietal ganglion (RPa9) gave biphasic reaction at chemical stimulation of the heart: the initial excitatory phase was followed by the inhibition of spike generation. In case of long lasting stimulation the alteration of the two phases was observed.

Cells V12 and V13 belong definitely to central neurones of the heart reflex. At stimulation of the chemoreceptors a close correlation was found between the spikes of these neurones and a particular component of the heart nerve activity. These neurones can be considered as central neurones of the extracardial heart regulation.

Most of the neurones investigated responded both to the chemical and tactile stimulation of the heart, however, in some cases the reaction was different to different modes of stimulation. Neurones, responding only to one type of stimulus can be considered as primary sensory neurones.

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KÖZPONTI IDEGRENDSZERI NEURONOK VÁLASZREAKCIÓI A SZÍV KEMORECEPTORAINAK INGERLÉSÉRE *HELIX POMATIÁN*

S.-Rózsa Katalin és Salánki János

Összefoglalás

Tanulmányozták a központi neuronok válaszreakcióit a szív kemoreceptorainak ingerlésére *Helix pomatia* L. viscerális és perietális ganglionjaiban. A kemoreceptorok ingerlésekor megfigyelt központi effektusokat összevetették a szív mechanoreceptorainak ingerlésekor leírt reakciótípusokkal. Az eredmények szerint:

1. A szív kemoreceptorainak ingerlésére 12 sejt reagált a viscerális és jobb parietális ganglionokban; a válaszreakció a spontán aktív sejteken gátlás vagy serkentés volt.

2. A viscerális ganglion több sejtje (V12, V13, V17), valamint a jobb parietális ganglion egyik sejtje (RPa9) kettősen reagált a szív kemoreceptorainak ingerlésére: a kezdeti serkentő hatást gátló fázis követte. Tartós ingerlés esetén a két fázis váltakozhat is.

3. A vizsgált sejtek közül a V12 és V13 bizonyítottan szív-szívreflex központi neuronja, mivel a kemoreceptorok ingerlésekor szoros korreláció mutatható ki a sejt aktivitása, valamint a szívidegről elvezetett aktivitás komponensei között. E korreláció törvényszerűen minden preparátumon kimutatható. E sejtek a szív extrakardiális szabályozásának központi neuronjai.

4. A vizsgált központi neuronok többsége egyaránt reagált a szív taktilis és kémiai ingerlésére, azonban az esetek egy részében a válasz a két ingerféleségre ellentétes volt. A kizárólag egyik ingerre reagáló sejtek elsődleges érző neuronok lehetnek.