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CHARACTERIZATION OF THE FEED-BACK SYSTEM IN THE HEART OF *HELIX POMATIA* L.

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The heart activity is controlled by the regulatory influences of the central nervous system as a result of connections existing between the extracardial innervation and heart automatism. In the higher animal phyla both the central and peripheral parts of this reflex chain is well known. In Gastropoda mainly the efferent relations of the extracardial nerve were studied (CARDOT, 1909; RAFFO, 1929; SUZUKI, 1935; KRIJGSMAN, 1941; SCHLOTE, 1954; RIPPLINGER, 1957; S.-RÓZSA and GRAUL, 1964), however, the nature of the information originating from the reflex-areas of the heart and running to the nerve centre has not yet been cleared up. The sensitivity of the *Helix* heart muscle to tactile, pressure, osmotic and chemical stimuli was described on isolated hearts (KRIJGSMAN, 1955). The presence of a reflex arc was suggested only on the basis of coordination realized in the ganglia (ZUBKOV, 1935). In other Gastropoda species attempts were made to separate the afferent and efferent signals of extracardial nerve, in these studies mainly tactile receptor areas (NISBET, 1961; BAXTER and NISBET, 1963; DE VLIEGER, 1970), and the central representation of receptor areas were studied.

The aim of our present investigations was to determine the reflex areas of the *Helix* heart and to elucide the kind of environment factors which can evoke starting signals from the heart toward the central nervous system and the characteristic features of the feed-back signals.

Materials and methods

The experiments were carried out on the hearts of active snails, *Helix pomatia* L. in September-October at room temperature (20—22°C).

The heart was isolated together with the intestinal nerve as reported earlier (S.-RÓZSA and GRAUL, 1964). In some cases the connection of the intestinal nerve with the central nervous system was preserved. A thin glass cannula was introduced into the vena pulmonalis and into the aorta, then the heart was perfused by frog Ringer solution adapted for snail heart (TURPAEV et al., 1967). The substances studied were given to the perfusion fluid under constant pressure. In the course of experiments tactile, pressure, chemical and osmotic stimuli were applied. The tactile stimulus was produced by touching the heart surface with thin paint-brush while the pressure stimulus was applied by varying the level of the perfusion fluid. For the stimulation of osmoreceptors 5% NaCl and glucose, while for that of the chemoreceptors 5-hydroxytryptamine (5HT) was given onto the inner surface of the heart.

The electrical activity of the intestinal nerve was registered with bipolar Ag-AgCl electrodes. In the experiments ALVAR amplyfier, oscilloscope and fotorecorder were used.

Results

1. *Spontaneous electrical activity of the intestinal nerve*

On preparations containing also the central nervous system the electrical activity of the intestinal nerve is complex regarding its freuuency as well as the amplitude *(Fig. 1)*. The greatest variation in frequency and amplitude in the intestinal nerve was observed near to its origin, close to the abdominal ganglia, where all afferent and efferent signals are present. At the place of origin of the intestinal nerve 7—8 types of impulsations differing in amplitude can be distinguished. Here the value of the frequency varies between 12—21 imp/sec.

Following the exstirpation of cerebral and suboesophageal ganglia the pattern of the electrical activity turned more simple and simultaneously the 150—300 μV value of amplitude became dominant *(Fig. 1B)*. Removing the

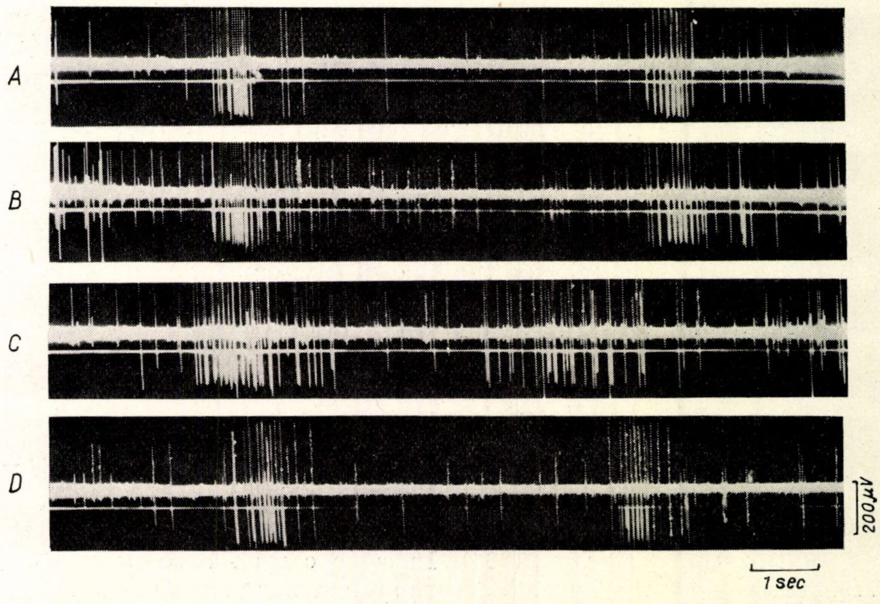

Fig. 1. Pattern of electrical activity of the intestinal nerve with intact connection between nerve and CNS as well as after discontinuity of CNS and heart.

A. — electrical activity of the nerve on its origin just after the ganglia
B. — the activity of the nerve after removing the two cerebral ganglia
C. — the activity of the intestinal nerve after complete dissection of the whole CNS
D. — the activity of the branch of the intestinal nerve innervating the heart

proximal part of the intestinal nerve the frequency of the potentials decreases. Just before the origin of the heart branch of intestinal nerve the electrical activity is less complex and the registered potentials according to their amplitudes can be divided into three groups: *1.* the values of the amplitudes reach 450—500 μV; *2.* it corresponds to 300 μV; *3.* the amplitude was only about 50 μV *(Fig. 1C)*. At this latter part of the nerve and nearer to the ganglia potentials originating from characteristic, simultaneously discharging neurons were registered, especially in the second type of potentials *(Fig. 1A,B,C)*. In some cases the distance between two spikes suggests that there is a delay corresponding to the transmission through only one synapse so that these potentials can originate from two coupled neurons. Such simultaneous activity cannot be registered when only the heart branch of the intestinal nerve is intact (Fig. 10).

When the potentials were registered from the heart branch of the intestinal nerve after losing its connections with the central nervous system the pattern of the activity showed further simplification. In this case we have a special preparation containing the heart and the extracardial nerve where all the effects influencing the afferent system of the heart can be traced on its afferent pathway. Under control conditions from the heart branch of the intestinal nerve three types of action potentials differing in amplitude can be registered *(Fig. 2B)*, among them the third type has much lower amplitude than the first and second one. In other cases action potentials were running to nerve center in the form of bursts *(Fig. 2C)*. Sometimes the bursts were

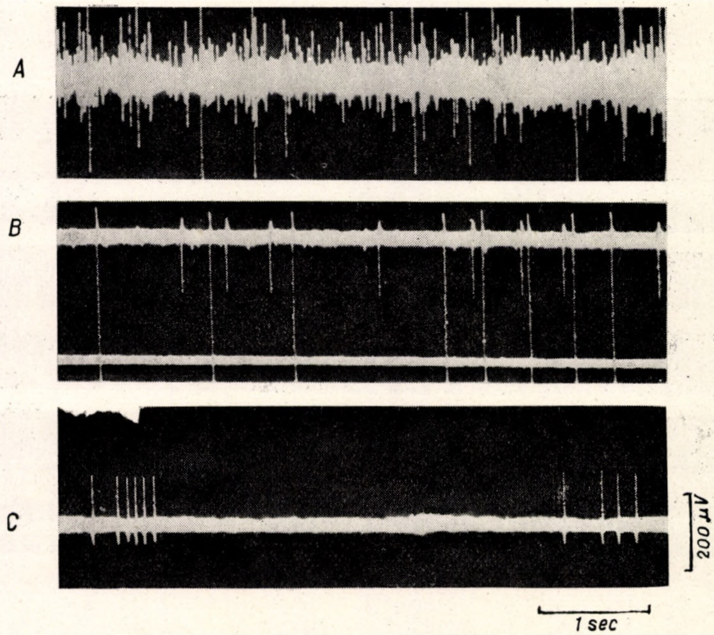

Fig. 2. The electrical activity of the intestinal nerve
A. — registration from the common root of the intestinal nerve near to the visceral ganglion
B. and C. — activity of the branch of the intestinal nerve innervating the heart

surrounded by action potentials of different amplitude *(Fig. 3A)*. It seems that the third type of the potentials ($\sim 50\ \mu V$) corresponds to the activity originating from the remains of the pericardium.

2. *Electrical activity of the intestinal nerve evoked by stimuli applied to the heart*

a) The effect of tactile stimulus and that of the change in pressure.

It was found that the heart of *Helix* has high sensitivity to the tactile stimulus applied on its surface. Under the influence of tactile stimulus an afferent signalization started towards the central nervous system. The most effective afferent impulzation was evoked in response to the stimulation of the border between the ventricle and auricle as well as that of the top of ventricle. As an answer to tactile stimulation the amplitude and frequency of the medium and the large type action potentials were increased *(Fig. 3)*. The impulzation upon tactile stimulation and the running to the nerve centre were present with unaltered intensity throughout the stimulation, moreover, the bursts accompanying the heart activity remained more frequent even after the stimulation compared to the control *(Fig. 3C)*.

Contrary to this, the changes in the pressure of the perfusion fluid lead only to changes in the pattern of the potentials appearing in the control with large amplitudes and showing the form of bursts *(Fig. 4)*. In this case no alteration was observed in the frequency and amplitude of the potentials of medium and low amplitudes. The burst form of potentials with large amplitude was eliminated under the influence of increased pressure, however, the frequency of electrical activity was increased and became more uniform *(Fig. 4)*.

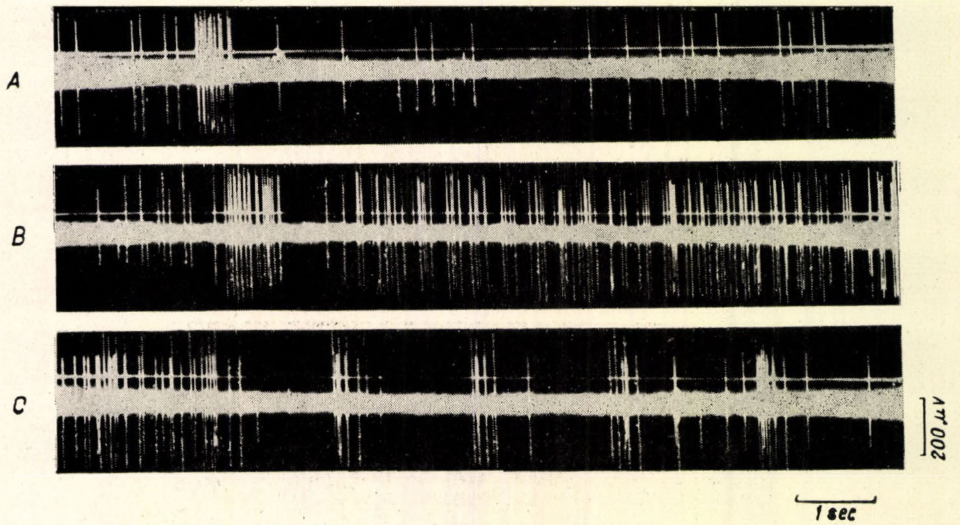

Fig. 3. Afferent reaction in intestinal nerve to tactile stimulation
A. — control activity
B. — beginning of tactile stimulus ↑
C. — end of applying tactile stimulus ↓, then the activity after stimulation

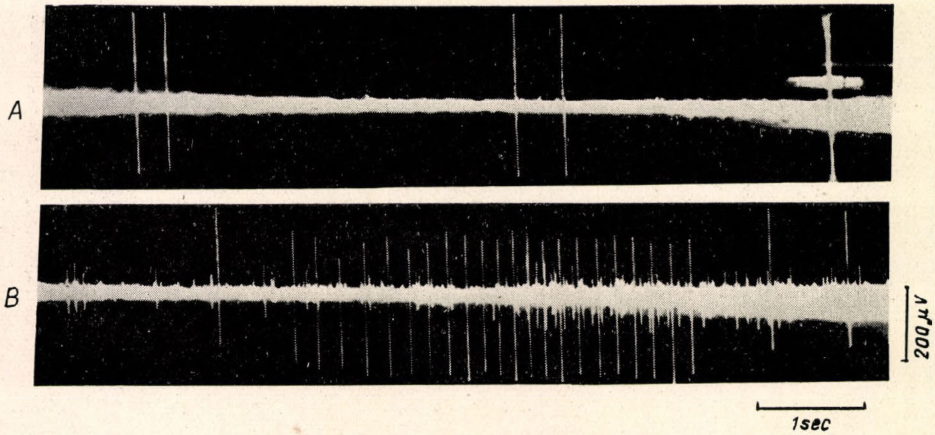

Fig. 4. Effect of changes in the pressure of perfusion fluid
A. — control, than at ↑ increase of the pressure of heart perfusion fluid two times
B. — continuation of A

Nevertheless this increase in frequency was not long-lasting for apparently the heart adapted quickly to the increased pressure and the activity was stabilized at a new level similar to te control.

b) The effects of osmotic and chemical stimulations.

The *Helix* heart showed high sensitivity to the osmotic changes in the perfusion fluid. The feed-back signalization became permanent and more intensive under the influence of increased osmotic pressure. The action potentials of nerve showed higher variation of amplitude *(Fig. 5B)*, while the increase in frequency was lower then after tactile stimulation. The changes accompanying osmotic effect were steady, and after stopping the stimulation they declined only slowly what could correspond to the course of recovering of normal osmotic conditions inside the heart. The presence of the osmoreceptors could be proved by NaCl as well as by glucose, and the feed-back signalisation was the same in both cases.

5-hydroxytryptamine (5HT), the most effective excitatory substance of the *Helix* heart, beginning from the concentration of 10^{-9} M evoked an intensive afferent impulsation of the branch of intestinal nerve innervating the heart. 5HT increased both the amplitude and frequency of the high and medium sized action potentials *(Fig. 6B)*. The effect of 5HT proved to be long-lasting and the afferent signalization remained higher for a long time even after washing out the 5HT compared to the control level *(Fig. 6D)*.

The time relations of the effects of tactile, osmotic and 5HT stimulations are demonstrated in *Fig. 7*. It can be seen that these three cases belong to the slowly adapting type of responds. The maximal increase in frequency was observed under the influence of tactile stimulus then according to their efficacy the chemical and osmotic stimuli followed. In these cases the afferent signalization running to the nerve centre had a character of "all-or-nothing" and the intensity of the reactions practically was not decreased in the first five seconds *(Fig. 7)*.

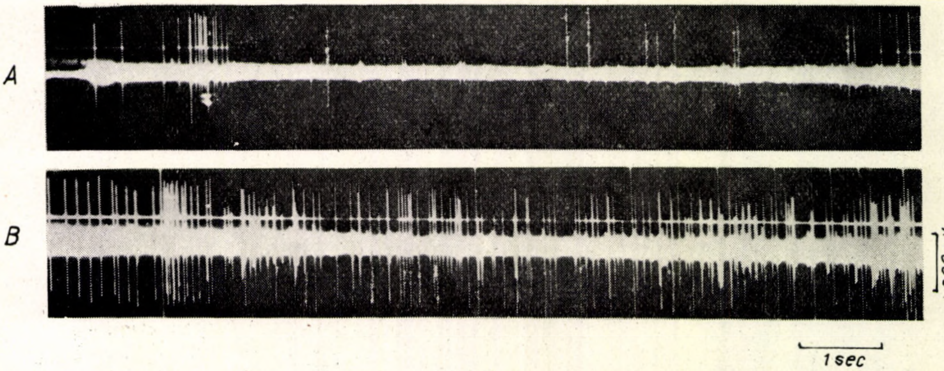

Fig. 5. Effect of 5% NaCl on the afferent signalization of intestinal nerve
A. — control
B. — effect of 5% NaCl

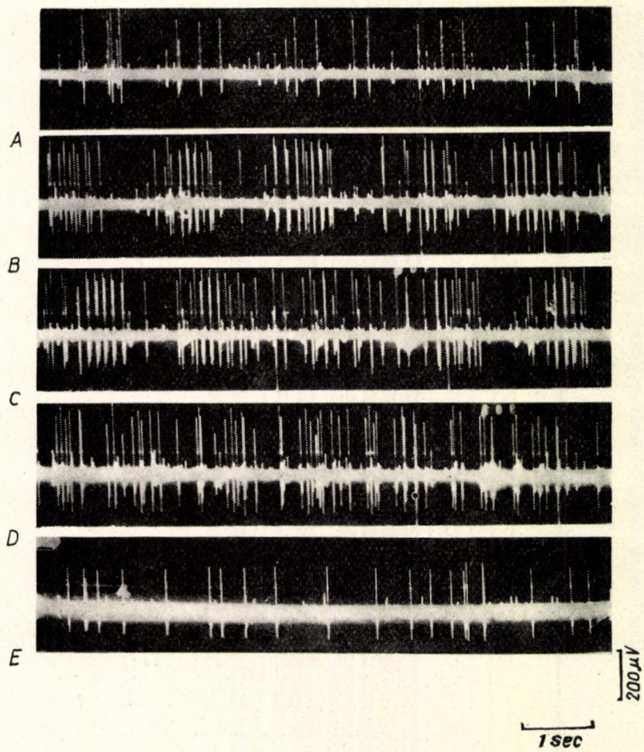

Fig. 6. Effect of 5HT on the heart at concentration 10^{-7} M
A. — control activity
B. — beginning of the effect of 5HT at concentration 10^{-7} M
C. — the same as in A after 2 minutes
D. and E. — activity of the intestinal nerve after long-lasting wash out of 5HT

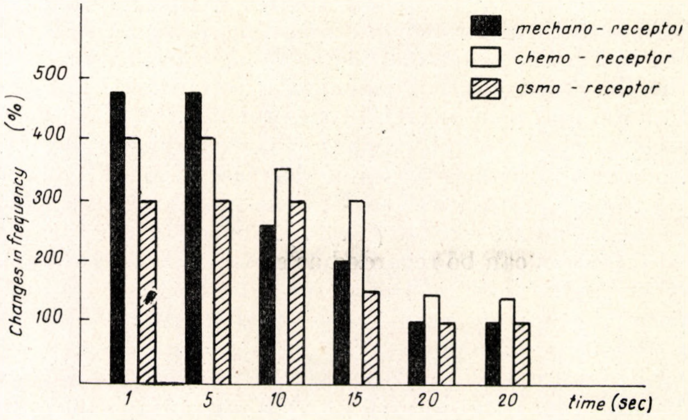

Fig. 7. The course of adaptation after the stimulation of tactile, chemical and osmotic receptors. The diagrams correspond to the mean value ±S.D. of five experiments

Then, according to the degree of adaptation, the intensity of the reaction decreased for mechano- and osmoreceptors comparatively quickly, while the sensitivity of chemoreceptors declined slowly. Using 5HT the increased afferent impulsation was registered even in 3—5 minutes after application and it could be eliminated only by repeated washing out.

It can be seen in all demonstrated *Figures*, that the electrical activity having irregular frequency and variable low amplitude hardly rising above the base line was not influenced by above effects. On the basis of this, it can be stated that this type of electrical activity originates not from the heart but belongs to the pericardium giving afferent fibres into the same branch of intestinal nerve as does the heart. This type of electrical activity was not changed under the influence of different stimuli applied to the heart.

Discussion

The results prove that the heart of *Helix* having diffuse myogenic pacemaker possesses the same receptor zones as the heart of higher animal phyla. It was found that the *Helix* heart contains receptors (end-organs) perceiving tactile, pressure, osmotic and chemical stimuli reacting to the changes of environmental factors. The reaction depends on the intensity and kind of stimulus. In our cases the type of reaction emphasized the presence of the primarily sensory neurons (GRUNDFEST, 1971) and showed all-or-nothing character.

The amplitude and frequency of the afferent signalization were increased under the influences of all applied stimuli. The character of the changes in amplitude proved to be similar applying tactile and osmotic or pressure and chemical stimuli. The increase in the amplitude was higher and more uniform when tactile or osmotic stimuli were amplied. The highest increase in frequency was observed with tactile stimulus, then chemical and osmotic stimulus followed according to the intensity of reaction elicited. The smallest increase in frequency was caused by changes in pressure. All investigated receptors except the pressure end-organ belong to the group showing a slow rate of adaptation. Among them the chemo-receptors showed the lowest degree of adaptation *(Fig. 7)*. The heart adapted to changes in pressure within 1—5 sec and then the increased afferent signalization finished *(Fig. 4)*, while the reaction of tactile and osmotic receptors prolonged during their application and the answer of chemoreceptors continued even during the washing out of the stimulatory agent. In these cases no decrease in amplitude and frequency depending on the adaptation was seen in the first 5 sec of application and it was negligible at the next 5 sec, too *(Fig. 7)*. Consequently, the tactile, osmotic and chemical receptors can be regarded as slowly adapting receptors on *Helix* heart. However on the basis of the above experiments it cannot be decided whether separated or common end-organs send the afferent signalization as a respond to different periferal signals. According to some suggestions there are not specialized receptor zones in the invertebrate hearts (see: KOSHTOYANTS, 1955), nevertheless, taking into account the different amplitude of the electrical activity of the intestinal nerve at least two different types of end-organ can be suggested. In the intestinal nerve the two types of action potentials different in magnitude show the presence of two different receptor zones in the *Helix* heart.

The results emphasize that the regulation of Gastropoda hearts is based upon a well developed afferent signalization which informs the central nervous system about the changes occurring in the environment of the heart, and subsequent to this the CNS modulates heart activity. In other molluscan species it was proved that "excitatory junctional potentials (EJPs)" take part in the modulation of heart activity and that of the generation of AP (KUWASAVA and MATSUI, 1970). On the *Helix* heart also the co-ordinated action of efferent and afferent components of extracardial innervation provides basis for this regulation. The peripheral part of this regulation circuit containes the same functional units for receiving and transmitting information similar to higher animal phyla. The comparatively simple structural organization of *Helix* CNS as well as a previous localization of central neurones giving direct branches to the heart (GUBICZA and S.-RÓZSA, 1969; 1971) could offer possibility to study also the central part of this regulatory circuit and in this way to contribute to the understanding of the regulation of heart activity.

Summary

The nature of the afferent signalization was studied in *Helix pomatia* L. on the preparation containing the heart and the extracardial nerve. It was found that under control conditions three types of afferent signalization differing in amplitude are running to the central nervous system through the branch of intestinal nerve innervating the heart among which two could be considered as having heart origin. If the heart is affected by tactile, pressure, osmotic or chemical stimulus afferent impulzation of all-or-nothing character starts on the intestinal nerve and runs to CNS. The highest increase in frequency was observed after stimulation of tactile receptors ($\sim$ 500%) then according to the intensity of reactions the excitation of chemoreceptors ($\sim$ 400%), osmoreceptors ($\sim$ 300%) and presso-receptors ($\sim$ 200%) followed. The tactile, chemical

and osmo-receptors can be categorized as slowly adapting receptors while the presso-receptors belong to those showing fast rate of adaptation. At least the presence of two different end-organs is suggested in *Helix* heart. The reflex areas of this heart are considered to be the analogue structures to that of the higher animal phyla.

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A VISSZAJELZÉSEK TERMÉSZETÉRŐL *HELIX POMATIA* SZIVÉBEN

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Összefoglalás

Helix szív-extrakardiális idegkészítményen vizsgálták az afferens jelzések természetét. Megállapították, hogy kontroll körülmények között háromféle amplitúdójú afferens jelzés halad a központi idegrendszer felé az intestinális ideg szívhez menő ágán, melyek közül kettő szíveredetű. Ha a szivet taktilis, nyomás, ozmotikus vagy kémiai inger éri, az intestinális idegen minden vagy semmi jellegű akciós potenciálsorok indulnak a CNS felé. Legnagyobbmérvű frekvencianövekedést a taktilis receptorok ingerlése esetén figyeltek meg (~ 500%), ezt követték a kemoreceptorok (~ 400%), az ozmoreceptorok (~ 300%), majd a nyomásreceptorok (~ 200%). A taktilis, kemo- és ozmoreceptorokat a lassan adaptálódó, a nyomásreceptorokat a gyorsan adaptálódó receptorok csoportjába sorolják. Legalább két eltérő felvevő végkészülék jelenlétét tételezik fel *Helix* pomatia szivében. E szív reflexogén zónáit magasabbrendűek hasonló képződményeivel analógnak tartják.