ANNAL. BIOL. TIHANY	33	125—133	HUNGARIA 1966

ADAPTATION OF THE HEART OF *HELIX POMATIA L.* TO THE INHIBITORY EFFECT PRODUCED BY THE EXTRACARDIAC NERVE

KATALIN S.—RÓZSA

Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

Received: March 29th 1966

As earlier investigations show (S.—Rózsa and Graul 1964) it is possible to produce by the stimulation of the extracardiac nerve of *Helix pomatia L.* both inhibitory and stimulatory effects in the heart. The effects depend on the stimulation parameters applied. It has been also established that serotonine and a ninhydrine-positive second factor standing close to arginine chromatographically (S.—Rózsa and Perényi 1966) is demonstrable by chemical methods in the perfusate collected from the heart stimulated.

The objective of the study reported here was to establish the conditions of the realization of these nervous effects, with special regard to the earlier observation that beside stimulation parameters seasonal changes may also be involved in these inhibitory and stimulatory effects. The observations obtained may be explained either by the alterations occurring in the synthesis of transmitter substances or by sensitivity changes of excitable structures through which the effect of chemical agents released during stimulation of the nerve is realized.

Method

The experiments were performed on *Helix pomatia L.* Activation of the inactive animals before the experiments was conducted by providing the animals with water and food. The calcareous shell of the animals was removed together with the foot and the central nervous system. Thereafter the visceral bag was opened and a thin canule was inserted into the aorta through which Meng's solution and the agents investigated were perfused through the heart in a way described previously (S.-Rózsa and Graul 1964). In every case the direction of perfusion was identical with the natural direction of the hemolymph i. e. the physiological solution was being circulated from the pulmonal vena towards the aorta.

Special care was taken that the hydrostatic pressure weighing on the heart should not change during the experiment. The heart-innervating intestinal nerve was exposed and placed on stimulating electrodes and stimulated with a square-wave stimulator. At the beginning of the experiment the parameters necessary for producing stabile inhibition and stimulation were determined for every preparation and they were used during the experiment in question. Heart activity was registered by a kymograph. Under the given experimental conditions the perfused hearts were capable of functioning for 1—2 days.

As a physiological solution MENG'S solution (1960) was used and the agents examined were similarly diluted in it. In these experiments the following substances were used: acridine orange, neutral red, adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). The adenosine preparates were REANAL products.

Experimental results

1. Inhibiting and stimulating effects produced by lasting and repeated stimulation of the intestinal nerve

a) *Inhibitory effect.* — A gradual cessation of the inhibitory effect produced by lasting excitation of the intestinal nerve was observable during the experiments. Despite continued stimulation the heart thus liberated from the inhibitory effect continues to operate rhythmically for longer or shorter periods, during which a rhythmic alternation of periodic activity and rest periods is observable. In case of repeated and lasting stimulation the duration of inhibition periods gradually decreases and the restored activity of the heart dominates. This is well demonstrated in *Fig. 1* which illustrates also the typical responses of the heart in case of stimulation at parameters producing inhibition. In this case the stimulation of the intestinal nerve was performed at the following parameters: frequency: 5/sec; duration of the impulses 1 msec; amplitude: 10 V. The duration of stimulation was 2—3 minutes. The liberation of the heart from the inhibitory effect of the nerve at the 20th—25th minute after the onset of stimulation is well visible *(A* in *Fig. 1).* At that time, namely, the heart begins to move rhythmically with an increasing amplitude, which is still followed here by a new inhibition. Further on a nearly regular alternation of active and rest periods is observable during the stimulation of the nerve. At unchanged parameters the duration of inhibition decreases to its half after repeated stimulation and thereafter the heart is more infrequently inhibited than in the case of the first stimulation *(B* in *Fig. 1).* — After the fourth- fifth stimulation *(C* in *Fig. 1)* the duration of inhibition is even shorter and does not practically return again despite repeated stimulation. Moreover, about the first minute after the onset of stimulation the frequency of cardiac action does not differ either from that observed before stimulation. If the parameters were left unchanged it was achieved by further repeated stimulation that the heart ceased to respond. The longer the duration of the single stimulations the sooner the inhibitory effect disappears.

b) *Stimulatory effect.* — Fundamental difference exists between inhibitory and stimulatory effects induced by lasting and repeated stimulation of the heart of the snail. In the case of parameters producing stimulatory effect adaptation never occurs after lasting or repeated stimulation, and the heart is not liberated from the stimulating effect during the time of excitation. The stimulatory responses to repeated excitation remain practically unchanged for 4—5 hours at unchanged parameters. Of course intervening rest periods between the single stimulations are important for the preparations.

In that period of the year when the experients were performed it was found in accordance with previous experiments (S.-Rózsa and Graul 1964) that increased activity stopped immediately after the cessation of stimulation.

Figure 2 illustrates the above finding obtained at 8/sec frequency, 1 msec duration of impulse and 15 V. This figure presents the lst, 5th and 10th

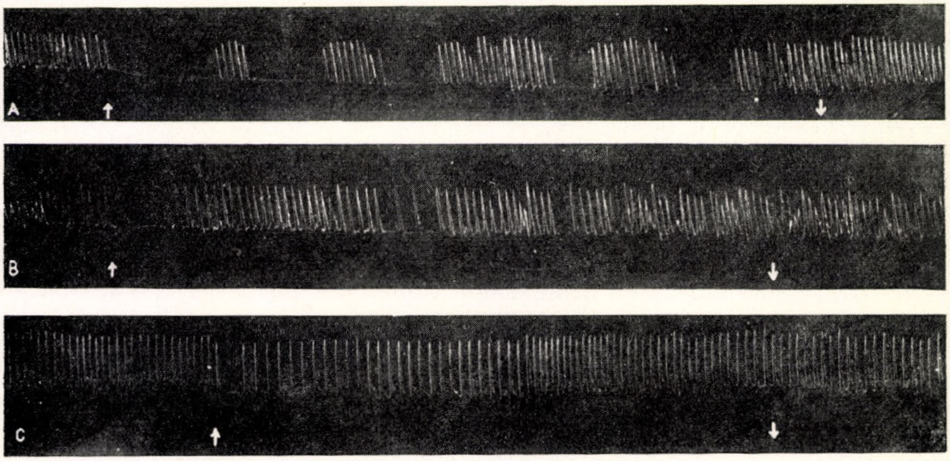

Fig. 1 Inhibitory effects produced on the heart of *Helix pomatia Z.* by long lasting and repeated stimulation of the intestinal nerve. Parameters of stimulus: frequency: 5/sec; duration of the impulses: 1 msec; amplitude: 10 volt. A — stimulation of intestinal nerve on the first occasion, B — second stimulation, C — fifth stimulation

1. ábra Gátló hatások *Helix pomatia L.* szívén az intestinális idegének hosszan tartó és ismételt ingerlése esetén. Ingerparaméterek: frekvencia 5/sec, impulzusszélesség 1 msec, feszültség 10 V. A — az intestinális ideg első alkalommal történő ingerlése — B — másodszori ingerlés — C — ötödik ingerlés

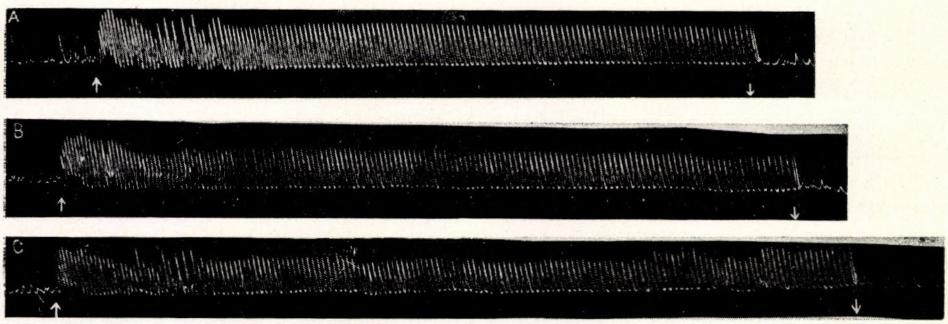

Fig. 2. Stimulatory effects produced by long lasting and repeated stimulation of the intestinal nerve in the heart of *Helix pomatia L.* Parameters of stimulus: 8/sec, 1 msec, 15 V. A — first stimulation of the intestinal nerve, B — fifth stimulation, C — tenth stimulation

2. ábra Stimuláló hatások *Helix pomatia L.* szívén az intestinális ideg hosszantartó és ismételt ingerlése esetén. — Ingerparaméterek: frekvencia 8/sec, impulzusszélesség 1 msec, feszültség 15 V. — A — az intestinális ideg első alkalommal történő ingerlése — B — ötödik ingerlés — C — tizedik ingerlés.

stimulations performed at unchanged parametrs. It is visible that after an initial, somewhat greater increase in amplitude the heart is stabilized to an activity of higher amplitude which remains practically unchanged till the end of the stimulation *(A, B, C* in *Fig. 2)*. When stimulation stops the amplitude rapidly decreases to the original level. In case of repeated stimulation the effect produced remains in its entire course the same as at the first stimulation. During the experiment MENG's solution was perfused through the heart and thus the released mediators could not accumulate in the heart.

The course of inhibitory and stimulatory effects produced by lasting and repeated excitation suggests that a basic difference may exist in the sensitivity of structures through which these effects are realized. The heart becomes easily adapted to the kind of stimulation which produces inhibition and consequently stimulation becomes ineffectual hereafter. In case of stimulatory effect this kind of adaptation was not observed.

The studies on the different behaviour of inhibitory and stimulatory influences experienced in case of lasting and repeated stimulation are based on the suggestion that this phenomenon cannot be due to the exhaustion of inhibitory mediator reserves, for there is no reason to assume that they exist in a lesser amount than the stimulating transmitters. It was assumed, therefore, that the adaptation to inhibitory stimulation is due to the fact that the heart becomes insusceptible to the inhibitory substances released during stimulation because the released chemical substances change those surface structures through which the effects of the inhibiting nerve are realized. Accordingly, studies were performed to investigate the course and realization of inhibitory influence by agents that produce alterations in surface structures by changing the proportion between free and bound nucleotides and nucleic acids.

2. Effect of agents influencing the structure of nucleotides and nucleic acids on the course of the inhibitory effect.

Dyes were used (acridine orange and neutral red) as agents influencing nucleotides and nucleic acids. It is known that free nucleic acids and nucleotides present on the surface of the cell are adsorbed by acridine orange and that in the resulting chemical bonds adenyl groups are involved (SZENT—GYÖRGYI 1957). Neutral red, on the other hand, enters into interaction with RNS and influences extracellular nucleic acid content (KOSHTOYANTS 1963).

In the presence of acridine orange the inhibition of the heart ceases and at constant parameters the effect produced was only stimulatory. These observations are demonstrated in *Fig. 3*. The inhibitory effect stops when $1 \cdot 10^{-4}$ g/ml acridine orange is perfused and from the fifth minute of perfusion stimulating influence was observed at the parameters which produced inhibition previously *(C* in *Fig. 3)*. The original inhibitory effect was not restituted by washing with physiological solution. Neither could AMP or ADP restitute the inhibition eliminated by acridine orange. Perfusion of the heart with $1 \cdot 10^{-4}$ g/ml ATP, however, effectuates again complete inhibition within 10 minutes during nerve stimulation *(E* in *Fig. 3)*.

Neutral red did not influence essentially the course of the inhibitory effect. Inhibition is not realized, nor was the transition from inhibitory to stimulatory effect observable at the parameters left unchanged.

If snail heart was perfused with $1 \cdot 10^{-4}$ g/ml acridine orange before stimulation for 20—30 minutes inhibition never took place, only stimulating effect was observed. Similar phenomena due to seasonal changes were reported earlier (S.-Rózsa and Graul 1964). It was observed namely that in certain periods of the year only stimulatory effects could be produced. It is inferred on basis of the present data that alterations in structure of nucleotides and nucleic

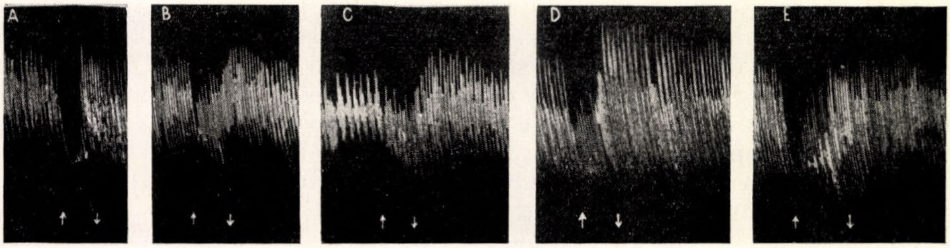

Fig. 3. Effect of acridine orange and ATP on the response reaction produced by the stimulation of the intestinal nerve. Parameters of stimulus: 5/sec, 1 msec, 10 V. A — initial inhibitory effect produced on the heart by stimulation; B — reaction of the heart on stimulation at the same parameters 3 minutes after the perfusion of $1 \cdot 10^{-4}$ g/ml acridine orange; C — the same after 5 minutes; D — reaction of the heart after 5 minutes long perfusion of $1 \cdot 10^{-4}$ g/ml ATP; E — the same after 10 minutes

3. ábra Acridine orange és ATP hatása a szív intestinális ideg ingerlésére fellépő válaszreakcióra. — Ingerparaméték: frekvencia 5/sec, impulzusszélesség 1 msec, feszültség 10 V. — A — kezdetben a sziven ingerléssel kiváltott gátló effektus — B — a szív reakciója ugyanazon ingerparaméterekkel történő ingerlés esetén 3 perccel $1 \cdot 10^{-4}$ g/ml acridine orange perfuzálása után — C — ugyanaz 5 perc mulva — D — a szív reakciója $1 \cdot 10^{-4}$ g/ml ATP 5 percig tartó perfuzálása után — E — ugyanaz 10 percig tartó perfuzió után.

acids are influencing the receptors of the inhibitory transmitter, and by means of this influence the heart becomes insensitive towards inhibitory influences.

Isolated snail heart does not respond practically to AMP and ADP introduced from outside. Its response to ATP cannot be taken for physiological either, for this agent produces only small increase in amplitude and only at a high concentration ($1 \cdot 10^{-3} - 1 \cdot 10^{-2}$ g/ml). Nevertheless, this kind of increase in amplitude is similar to that induced by most amines and amino acids at similar concentrations.

Discussion

The liberation from the inhibiting influence produced by stimulating the extracardiac nerve is a phenomenon known long ago in the heart of vertebrates. The inhibition produced by lasting stimulation of n. vagus in the heart of the frog is not continuous and active periods are observable periodically (Poskonova 1961, Beregszászi et al 1957). It has been demonstrated by Poskonova (1961) also that uridine and uracyl change the inhibitory effect on frog heart to stimulatory effect. Putintseva and Turpaev (1959), Putintseva (1961) suggest that a chemical agent producing stimulatory effect is released during long lasting stimulation in frog heart on the influence of acetylcholine. It is belived by these authors that the chemical agent released is identical with uridinediphosphate in opposition to the suggestion of BEREGSZÁSZI et al (1957) that this stimulatory effect is attributable to the presence of a contraregulatory mechanism and to the liberation of an adrenaline-like substance.

Of Molluscs *Venus mercenaria* was studied by PROSSER (1940) from this point of view, who observed the liberation of the heart of this animal from the effect of inhibitory nerve and attributed it to the exhaustion of transmitter reserves. SAKHAROV and NISTRATOVA (1963) observed an adaptation to acetylcholine in the heart of *Anodonta,* and explained this by the presence of ATP. The ATP which is released by acetylcholin in the heart of *Anodonta* becomes involved, as suggested by these authors, in the elimination of inhibitory effect because they act as competing antagonists on the acetylcholine receptors and displace acetylcholine from them. They believe that cholinesterase is not present in the heart of *Anodonta,* and the heart is liberated from the influence of the inhibitory transmitter in the above discussed manner. In special studies considerable amount of cholinesterase was demonstrated in the heart of both *Helix* and *Anodonta* especially in the area of the auricle (unpublished data) and thus, it is considered more probable that released ATP might much rather be involved in the energy turnover and in the regulation of ion migration (CALDWELL et al 1964). It seems improbable that the cessation of inhibitory influence on the heart of *Helix* by continuous stimulation is due to the complete consumption of the inhibitoray transmitter (PROSSER 1940), because a stimulating mediator is practically inexhaustible in preparations of normally operating heart nerve.

It is suggested that the above discussed effects are due to structural alterations occurring on the receptors. This explains the different behaviour of both inhibitory and stimulatory effects in case of lasting stimulation, because the acetylcholine receptor is a proteinaceous substance (TURPAEV 1962), whereas the serotonine receptors are considered lipid-like substances (VANE et al, 1961, WOOLLEY 1966). A smaller change in the protein receptors may take place more easily also on the influence of the transmitter substance released under natural conditions. If, however, simultaneously with the transmitter another substance is also liberated, then this may have a role in the alteration of the sensible structure which turns it insusceptible towards the liberated transmitter. This might explain why snail heart is set free despite continuous stimulation from the inhibitory effect of intestinal nerve.

The observation that nervous stimulation becomes of opposite sign on the influence of dye-stuffs forming complexes with nucleic acids and nucleotides, further that this effect is restorable by the administration of ATP indicate that perhaps adenyl groups are being inactivated on the influence of acridine orange. Under this condition inhibitory effect cannot be realized either because of the fixation of adenyl groups or because of their structural alterations. The observation that instead of inhibitione a stimulating effect is produced makes two suppositions probable:

a) The effect produced by acetylcholin released by means of nerve stimulation does not take place in the usual manner, and not inhibition but stimulation is produced by the excitation of the altered structure.

b) It is possible that the release of stimulatory mediator takes place also under inhibitory effect, if, however, the inhibitory effect predominates this mediator cannot manifest itself. It is prevailing only if inhibition is eliminated.

The fact that ATP restores the inhibitory effect produced by nerve stimulation after the addition of dye stuffs suggests that structural alterations of certain proteins are responsible for this transition from inhibition to stimulation. Thus it is inferred that ATP might promote the restitution of structures that are involved in stimulation transfer between nerve-terminations of the intestinal nerve and cardiac muscle.

On basis of these considerations it may be concluded that the effect of transmitter agents depends on the condition of the surface structures of cells. In case of snail heart this is closely related to the localization of nucleotides and of nucleic acids and in case of inhibitory effects primarily to the adenyl groups of these substances.

Conclusions

1. The long lasting stimulation of the intestinal nerve of *Helix pomatia L.* liberates the heart from inhibitory influences. By repeated stimulation the time of inhibition periods is shortened and finally ceases completely.

2. In case of lasting and repeated excitation stimulating effects occur with unchanged intensity.

3. It is assumed that the release from the inhibitory effect is due to the adaptation of acetylcholine receptor protein. A similar adaptation was not observable in case of serotonine receptors involved in stimulatory effects of lipid nature.

4. Acridine orange which influences surface structures stops the inhibitory effect produced by the intestinal nerve and produces an effect of opposite sign (stimulating).

5. In the heart treated with acridine orange ATP ($1 \cdot 10^{-4}$ g/ml) restores the eliminated inhibitory effect of the intestinal nerve. This fact emphasizes the importance of adenyl groups in the realization of inhibitory effects.

6. In the realization of the inhibitory effect of intestinal nerve on snail heart an important role is ascribed to nucleotides and nucleic acids.

Summary

Author investigated the responses of the heart of *Helix pomatia L.* to long lasting and repeated stimulation of intestinal nerve. It was established that in case of long lasting and repeated nerve stimulation with inhibitory parameters the heart becomes liberated from the inhibiting effect, whereas at other parameters stimulating effect is demonstrable which is of unchanged intensity even in case of long lasting and repeated stimulation. The liberation of the heart from the inhibitory effects might be explained by the adaptation of acetylcholine receptors of protein nature. In case of 5-HT receptor of lipid nature an adaptation of this kind was not observed. Acridine orange which forms complexes with nucleotides and nucleic acids stops the inhibition produced by the intestinal nerve and makes it stimulatory even if the parameters are left unchanged. ATP restores the inhibitory effect. It is suggested that in the realization of inhibition produced by the intestinal nerve surface structures and primarily structural changes in the adenyl groups might be of importance.

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EXTRAKARDIÁLIS IDEG GÁTLÓ HATÁSÁHOZ VALÓ ALKALMAZKODÁS VIZSGÁLATA *HELIX POMATIA L.* SZÍVEN

S.-Rózsa Katalin

Összefoglalás

Szerző vizsgálta *Helix pomatia L.* szivének viselkedését az intestinális ideg hosszantartó és ismételt ingerlése esetén. Megállapítást nyert, hogy gátlást kiváltó paraméterekkel történő hosszantartó és ismételt idegingerlés esetén a szív kiszabadul a gátlás alól, míg más paraméterekkel kiváltott stimuláló hatások változatlan intenzitással jelentkeznek ugyancsak hosszantartó és ismételt ingerlés mellett is. A szív gátló hatások alól való kiszabadulása a fehérjetermészetű acetylcholin-receptor adaptációval magyarázható. A lipid-természetű 5-HT-receptor hasonló jellegű adaptációja nem figyelhető meg. A nukleotidokkal és nukleinsavakkal komplexet képező acridine orange megszünteti az intestinális ideg gátló hatását és változatlan ingerparaméterek mellett stimulálóvá alakítja azt. A gátló hatást az ATP helyreállítja. Az intestinális ideg gátló hatásának realizálódásában a felületi struktúráknak, elsősorban az adenyl-csoportok struktúraváltozásainak van jelentőségük.

ИССЛЕДОВАНИЕ АДАПТАЦИИ СЕРДЦА ВИНОГРАДНОЙ УЛИТКИ К ТОРМОЗНЫМ ВЛИЯНИЯМ ЭКСТРАКАРДИАЛЬНОГО НЕРВА

Каталин Ш.-Рожа

Изучено поведение сердца при длительном и повторяющемся раздражении интестинального нерва виноградной улитки. При применении параметров раздражения, вызывающих тормозной эффект, происходит выход сердца из торможения в условиях продолжающегося раздражения. При применении параметров раздражения, вызывающих стимуляцию, уменьшения реакции сердца не наблюдается. Выход сердца из-под тормозящих воздействий объясняется адаптацией холинорецепторов, имеющих белковую природу. Сходной адаптации не удается наблюдать на 5-ОТ-рецепторах, имеющих липидный характер. Акридиноранж, образующий комплекс с нуклеотидами и нуклеиновыми кислотами, снимает тормозное влияние интестинального нерва и при неизменных параметрах раздражения превращает тормозной эффект в стимулирующий. Предполагается, что в реализации тормозного эффекта интестинального нерва имеют значение поверхностные структуры, прежде всего изменения адениловых групп, так как добавление АТФ восстанавливает тормозной эффект, подавленный акридиноранжем.