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COMPARATIVE STUDIES ON THE EFFECT PRODUCED BY BIOLOGICALLY ACTIVE AGENTS ON THE ISOLATED HEARTS OF HELIX POMATIA L. AND ANODONTA CYGNEA L.

KATALIN S.-RÓZSA and TIBOR PÉCSI

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

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It is known that the heart of molluscs responds specifically to a series of biologically active agents. The type of the effect produced by the same agent on various representatives of Mollusca may often be different. Thus, for instance, the catecholamines are recorded to have inhibitory, stimulatory and mixed effects as well (PROSSER 1940, WELSH 1953, KRIJGSMAN and DIVARIS 1955, GREENBERG 1960, JAEGER 1965). Adrenaline was inhibitory or without effect on the hearts of *Helix* and *Anodonta* (JULLIEN and RIPPLINGER 1936, ERSPAMER and GHIRETTI 1951, FÄNGE 1955).

5-hydroxytryptamine was recorded (WELSH 1957, KERKUT and COTTRELL 1963, LOVELAND 1963, S.-RÓZSA and GRAUL 1964) to be stimulatory on various representatives of the Mollusca. Data concerning other biologically active amines are either contradictory or insufficient (PILGRIM 1954, GREENBERG 1960).

A review of the effects produced by acetylcholine on the molluscan hearts was given by GREENBERG (1965). The effect of this agent on different species is fairly varying it may be both inhibitory and stimulatory. In the majority of cases it may function as an inhibitory transmitter.

Previous data proved that the type of the effect produced by these agents on the hearts of different mollusc species are not comparable, for in many instances, the same species may respond differently depending on its habitat.

In view of this, it was considered, therefore, necessary to examine in detail the effect produced by these biologically active amines and amino acids on the hearts of *Helix* and *Anodonta*, since these animals were used for many years by us, as widely distributed species in Hungary, for purposes of biological testing because of the suitability of their size for physiological and pharmacological examinations.

Methods

The investigations were conducted on the isolated hearts of *Helix pomatia* L. and *Anodonta cygnea* L. Isolated snail heart preparations were made of unhibernating specimens. Before experimental use the fresh-water mussels were kept in an aquarium filled with Lake Balaton water.

Isolated snail heart preparation

The shell of the animal was removed, the visceral sac was opened along the helix and the heart exposed. Following this the pericardium was removed and a thin cannula was introduced into the auricle and ligated above the boundary of the auriculoventricle. Another ligation was made on the aorta and subsequent to this the heart was suspended by the cannula on a frame and cardiac action was registered on a kymograph. The level of the solution was kept constant in the cannula during the experiment.

In case of snail heart MENG's (1958) solution was used as a biological one and the agents investigated were also dissolved in the same.

Isolated mussel heart preparation

The valve on the dorsal side of the animal was removed and the heart was exposed by cutting open both the mantle and the pericardium. The two aortas and the left auricle were ligated and a cannula was introduced into the right side auriculoventricular opening. The ventricle was excised and suspended by the cannula on a frame and fastened by the binding at the left auricle to a light lever and cardiac action was registered on a kymograph.

To set isolated mussel's heart in action the physiological solution recommended by MARCZYNSKI (1959) was used.

The experiments were performed in spring months at room temperature (20—22 °C).

In the course of these experiments the following agents were used: acetylcholine, dopamine, adrenaline, noradrenaline, 5-hydroxytryptamine, 5-methoxytryptamine, tryptamine, tryptophan, L-tyrosine, tyramine, glutamine, γ-aminobutyric-acid (GABA), histamine, histidine, α-methyl-DOPA, DOPA, DL-phenylalanine, DL-serine, benzoquinonium chloride (mytolon) and 2-bromo-d-lysergic acid diethylamide (BOL).

Results

1. The effect of biologically active amines and amino acids

Acetylcholine (ACh) proved to be inhibitory on both the hearts of *Anodonta* and *Helix*. The heart of *Helix* was more sensitive to acetylcholine, at 10^{-9} M concentration and above the agent produced complete arrest of cardiac action. In case of lower concentrations this inhibition was eliminable after repeated washing out of the agent, at 10^{-3} M concentration, however, the inhibition was irreversible.

The threshold concentration of acetylcholine (10^{-6} M) for the heart of *Anodonta* produced a slight decrease of amplitude. Contrary to findings obtained by us previously (NISTRATOVA and PÉCSI 1966) the increase in concentration produced diastolic arrest. At 10^{-5} M and 10^{-4} M concentrations diastolic arrest lasted for 20 seconds and 1.5 mins, respectively, whereas in case of 10^{-3} M concentration for 4,5 mins. In these cases, however, the heart is delivered from inhibition by itself after a longer—shorter period and starts pulsating spontaneously. At high concentrations of ACh the amplitude of cardiac action decreased subsequent to the recovery of the heart from inhibition. ACh at 10^{-2} M concentration arrested cardiac action and the heart was

not delivered from this arrest of itself even after passing of 13 mins, and its activity was restored only by repeated washings with Ringer solution.

Acetylcholine solutions near the threshold were not stimulatory either on *Helix* or *Anodonta* hearts. It happened also that the irregular cardiac action of *Anodonta* registered before ACh treatment became regular after treatment in case the agent was removed by washings, and in some instances the amplitude increased, too. In the case of *Helix* heart no stimulation was noted following washing out.

The effect of catecholamines on *Helix* and *Anodonta* hearts is of opposite direction. On *Helix* heart, namely, dopamine (DA), adrenaline (A) and noradrenaline (NA) produced stimulatory effect, but on the heart of *Anodonta* these agents proved to be inhibitory.

Dopamine solutions at 10^{-9} M and higher concentrations produce positive inotropic and chronotropic effects on snail heart. At higher concentrations up to 10^{-3} M this agent is stimulatory. Its effect might be discontinued by repeated washings.

Helix heart responded with an increase of amplitude to 10^{-9} M and higher concentrations of adrenaline and noradrenaline. The degree of the increase of amplitude varied between 20—40% and was not dependent on concentration, but might much rather be depending on the individuum. The effect produced by adrenaline and noradrenaline might also be discontinued by repeated washings, and following this, the heart starts to respond again to these amines. In case of *Helix* heart no difference in effect between adrenaline and noradrenaline was observed. Both agents were stimulatory. In case of dopamine, however, not only an increase of amplitude was observable as compared with these agents, but that of frequency was observed, too.

The sensibility threshold of dopamine on the heart of *Anodonta* was about 10^{-7} M. This and 10^{-6} M concentrations of this drug produced an about 20% decrease of amplitude. To higher concentrations up to 10^{-4} M the heart responded with a sharp increase of tonicity. On the effect of concentration increase the inhibitory effect increased. After cessation of treatment with dopamine cardiac action is restored by itself and after some minutes the original ground level and amplitude characterized the pulsation of the heart. Following inhibition cardiac action was more regular and of a much greater amplitude than after application of either adrenaline or noradrenaline. Dopamine washed out easily and the heart responded again to further applications of dopamine, the reaction produced, however, was less intensive. On repeated application of this concentration the heart became adapted to dopamine and responded either with a very small decrease of amplitude only or did not respond at all. Following washing out of 10^{-4} M dopamine solution, the 10^{-5} M solution of this agent was practically without effect.

The threshold concentration of adrenaline on the heart of *Anodonta* proved to be 10^{-6}—5×10^{-6} M. At 5×10^{-5} M concentration a short systolic arrest took place, and after increasing the concentration further (10^{-4}—10^{-3} M) a sharp rise in tonicity was registrable and the heart was arrested in systole and after the lapse of some time cardiac action recovered itself without washing out the drug *(Fig. 1a)*. The reaction observed resembles strikingly that observed previously in the case of ACh (NISTRATOVA and PÉCSI 1966).

It is to be noted that in one single instance an increase of amplitude as compared to the control was observed after spontaneous restitution from in-

hibition caused by 10^{-4} M adrenaline solution (positive inotropic after-effect).

The effect produced by noradrenaline was similar to that of adrenaline and dopamine. In case of the heart of *Anodonta*, however, a 10—50 times greater concentration of the drug was necessary for producing the same effect.

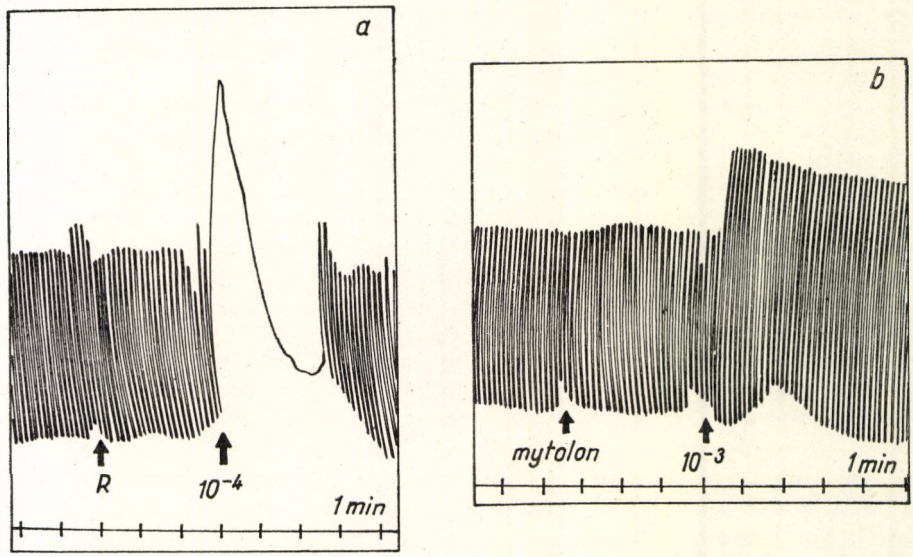

Fig. 1. — Effect of 10^{-4} M adrenaline on the isolated heart of *Anodonta* before (a) and b—after pretreatment with mytolon (10^{-3} M)

1. ábra. — Adrenalin hatása izolált *Anodonta* szíven 10^{-4} M koncentrációban (a) és b—ugyanaz 10^{-3} M mytolon előkezelés után

5-hydroxytryptamine (5-HT) was stimulatory on both *Helix* and *Anodonta* hearts. In case of both experimental objects the threshold concentration was the same (10^{-10}—5×10^{-10} M) and this produced positive inotropic and chronotropic effects. At 5×10^{-9} M and 5×10^{-8} M concentrations the increase of amplitude registered was 20% and 60%, respectively. The maximum stimulatory effect produced by 5-HT on *Helix* heart was not higher than 90%, whereas on *Anodonta* an increase of amplitude by more than 200% could often be observed depending on the initial amplitude. In agreement with data obtained previously by us neither *Helix* heart (S.-Rózsa and Graul 1964) nor *Anodonta* heart (Pécsi 1965) was arrested by high concentrations, and a slight increase in tonicity was observable, at most, when applying 10^{-3}—10^{-2} M concentrations.

Stimulatory effects produced on *Helix* heart by high concentrations (10^{-4}—10^{-3} M) of catecholamines and 5-HT are easily distinguishable on basis of the type of stimulation. The effect produced by 5-HT is characterized by an initial sharp increase of amplitude which is followed by a more stabile increase of amplitude. This initial effect is lacking from the stimulatory effect produced by catecholamines.

5-methoxytryptamine (5-MT) solutions of 10^{-10} M concentration and above produced on *Helix* heart positive inotropic and chronotropic effects but the amplitude never increased by more than 20%. This stimulation chang-

ed into inhibition at 10^{-6} M concentration and above, and the effect was easily discontinued by washing even at fairly high concentration (10^{-3} M). The effect produced by this agent on *Helix* heart is clearly discernible from that produced by 5-HT on basis of the characteristic two phases of its.

The threshold concentration of 5-methoxytryptamine on the heart of *Anodonta* was 10^{-9}—5×10^{-9} M. At this concentration both the amplitude and frequency of cardiac activity considerably increased. The effect produced by this drug on *Anodonta* heart is similar to that produced by 5-HT, namely, this drug does not inhibit cardiac action even at high concentrations. At 10^{-6} M concentration and above an increase in tonicity was produced. In case of this animal, too, the produced effect was easily washed out.

Tryptamine (TA) was stimulatory on both objects. At 10^{-10} M and higher concentrations it increased both amplitude and frequency of cardiac activity, in case of *Helix* heart. The effect of this drug resembles the reactions produced by 5-HT, but it is more easily discontinued by washings and the increase of amplitude is also smaller *(Table 1)*. In the majority of cases inhibitory effect was observed by repeated application of high concentrations (10^{-3} M).

On the heart of *Anodonta* tryptamine is less effectual than 5-HT. The threshold concentration of this drug varied between 5×10^{-8}—10^{-7} M. Car-

Table 1. — 1. Táblázat

Type of effect, threshold concentration and maximum effect produced by biologically active amines and amino acids on isolated heart preparations of *Helix* and *Anodonta*

Biológiailag aktív aminok és aminósavak hatástípusa, küszöbkoncentrációja és maximális effektusa izolált *Helix* és *Anodonta* szíven

Agent / Anyag	On heart of *Helix* / *Helix* szíven			On heart of *Anodonta* / *Anodonta* szíven		
	type of effect / Hatás típus	threshold in M / Küszöbkoncentráció M-ben	per cent of maximum effect / Maximális effektus %-ban	type of effect / Hatás típus	threshold in M / Küszöbkoncentráció M-ben	per cent of maximum effect / Maximális effektus %-ban
Acetylcholine	inhibition	10^{-9}	100	inhibition	10^{-6}	100
Dopamine	stimulation	10^{-9}	40	inhibition	10^{-7}	100
Adrenaline	stimulation	10^{-9}	40	inhibition	10^{-6}	100
Noradrenaline	stimulation	10^{-9}	40	inhibition	10^{-6}	100
5-hydroxytryptamine	stimulation	10^{-10}	90	stimulation	10^{-10}	100
5-methoxytryptamine	stimulation	10^{-10}	20	stimulation	10^{-9}	100
	inhibition	10^{-6}	20			
Tryptamine	stimulation	10^{-10}	30	stimulation	10^{-8}	100
Tryptophan	stimulation	10^{-9}	15	ineffectual	—	—
Tyramine	stimulation	10^{-9}	10	stimulation	10^{-8}	100
	inhibition	10^{-4}	50			
Glutamine	stimulation	10^{-9}	10	ineffectual	—	—
GABA	stimulation	10^{-9}	10	ineffectual	—	—
Histamine	stimulation	10^{-7}	8	ineffectual	—	—

diac action was not arrested even on the application of high concentrations (10^{-5}—10^{-4} M) of this drug, but responded with an increase of tonicity. Arrhythm was produced occasionally by tryptamine. This was never observed when using 5-HT. The effect produced by tryptamine is more easily discontinued by washing out than the effect produced by 5-HT.

Tryptophan (TRP) solutions at 10^{-9} M and higher concentrations produced a weak stimulation (10—15%) in case of *Helix* heart. Stimulation did not increase upon rising the concentration and ended, too, without washing out of the agent. Tryptophan proved to be ineffective on the heart of *Anodonta*.

The stimulatory effect produced on *Helix* heart by 10^{-9}—10^{-5} M concentrations of tyramine (TIR) was insignificant (5—10%), at higher concentrations (10^{-4}—10^{-3} M) this agent produced an about 50% inhibition. The effect of this drug is easily washed out and very often ceases by itself.

On the heart of *Anodonta* 10^{-8} M and higher concentrations of tyramine had positive inotropic and chronotropic effect. An increase in tonicity was observable also at concentrations beyond 10^{-5} M. The drug did not produce inhibition even at higher concentrations. The stimulatory effect may surpass even more than 100%.

Helix heart responds with a weak (10%) stimulation to low concentrations (10^{-9}—10^{-6} M) of glutamine (GLA). At higher concentrations (10^{-4} M) this drug is inhibitory or without effect. On the heart of *Anodonta* it is ineffective at every concentration.

GABA produced on *Helix* heart a nonspecific, small (10%) stimulation at 10^{-9} M and higher concentrations and was ineffective on *Anodonta* heart.

Histamine (HA) solutions at 10^{-7} M and above produced small stimulation (5—8%) in case of *Helix* heart, and were ineffective on *Anodonta* heart.

Histidine, tyrosine, α-methyl-DOPA, DL-phenylalanine and DL-serine proved to be ineffective on both *Anodonta* and *Helix* hearts.

Table 1. summarizes data on the type of effect, on sensitivity threshold and on the degree of effectiveness of biologically active agents investigated.

2. *Influencing of the effects of biologically active amines by ACh and 5-HT antagonists*

In the following we shall discuss the examinations performed on the mechanism of effect of agents that proved to be previously effective on both test objects and which interfere in neurohumoral regulation. Thus at first we attempted to obtain data on the susceptibility of the effects of acetylcholine, catecholamines, 5-hydroxytryptamine, tryptamine and tyramine to ACh and 5-HT antagonist, with the aim of discriminating the possible place of effect.

Pretreatment with mytolon and BOL was made at 10^{-5}—10^{-4} M and 10^{-4} M concentrations, respectively. These concentrations did not produce in themselves noteworthy effects either on *Helix* or on *Anodonta* hearts. A 30 mins pretreatment with 10^{-5} M mytolon solution resulted in a complete elimination of the inhibition produced by 10^{-8} M and 10^{-7} M acetylcholine solutions. The same pretreatment with mytolon, likewise, eliminates the stimulation caused by dopamine (10^{-7} M), adrenaline (10^{-7} M) and noradrenaline (10^{-7} M). The effect of these two latter agents may turn inhibitory on the heart of *Helix* pretreated with mytolon, and in particular following pretreatment of long duration (1—1.5 hour).

Table 2. — 2. Táblázat

Influence of mytolon and BOL on the effect produced by biologically active amines on the hearts of *Helix* and *Anodonta*

Mytolon és BOL hatása *Helix* és *Anodonta* szíven biológiailag aktív aminok effektusára

Agent / Anyag	On heart of *Helix* / *Helix* szíven		
	normal effect / normál hatás	after mytolon / mytolon után	after BOL / BOL után
ACh	inhibition	elimination	unchanged
Dopamine	stimulation	elimination	partial elimination
Adrenaline	stimulation	elimination or inhibition	partial elimination
Noradrenaline	stimulation	elimination or inhibition	partial elimination
5-HT	stimulation	unchanged	elimination
Tryptamine	stimulation	unchanged	elimination
Tyramine	stimulation inhibition	unchanged	stimulation is eliminated

Agent / Anyag	On heart of *Anodonta* / *Anodonta* szíven		
	normal effect / normál hatás	after mytolon / mytolon után	after BOL / BOL után
ACh	inhibition	elimination	unchanged or partial elimination
Dopamine	inhibition	elimination	elimination or stimulation
Adrenaline	inhibition	elimination or stimulation	elimination or stimulation
Noradrenaline	inhibition	elimination	elimination
5-HT	stimulation	unchanged	elimination
Tryptamine	stimulation	—	—
Tyramine	stimulation	elimination	elimination

Preincubation of *Helix* heart with mytolon, even if it lasted as long as 1.5—2 hours, did not influence the stimulatory effect of 5-HT, tryptamine and tyramine.

A pretreatment of 10—20 mins of *Anodonta* heart with mytolon (10^{-4} M) eliminated the inhibition produced by ACh, dopamine, noradrenaline and adrenaline. The inhibitory effect produced by 10^{-4}—10^{-3} M adrenaline solutions turned into definite stimulatory effect *(Fig. 1b)*. The positive inotropic and chronotropic effect produced by adrenaline after mytolon treatment was long-lasting without washing.

Mytolon did not influence the stimulatory effect produced by 5-HT on *Anodonta* heart, whereas it completely ceased the stimulatory effect of tyramine.

On *Helix* heart preincubated with BOL (10^{-4} M) a complete cessation of the stimulatory effect produced by 5-HT, tryptamine and tyramine solutions (10^{-6} M) occurred 20 mins following application. BOL decreased the stim-

ulatory effect of dopamine, adrenaline and noradrenaline (10^{-6} M) by approx. 50—60%, it did not affect, however, the inhibitory effect of ACh (10^{-8} M).

BOL (10^{-4} M) eliminated in case of *Anodonta* heart, too, the stimulatory effect caused by 5-HT and tyramine and also the inhibitory effect produced by dopamine, adrenaline and noradrenaline. Following pretreatment with BOL dopamine and adrenaline became slightly stimulatory.

BOL did not eliminate the effect produced by ACh on the heart of *Anodonta*, in some instances, however, decreased the degree of the effect produced.

To learn when the stimulatory effect produced by 5-HT and that obtained following mytolon treatment on the application of adrenaline is realized by the participation of the same receptors, we examined the effect produced by these two agents on the heart of *Anodonta* after pretreatment with mytolon and BOL. The effect of 5-HT remained invariably stimulatory also after pretreatment with mytolon, this effect could be stopped, however, completely by BOL. The effect of adrenaline, however, which was normally inhibitory turned strongly stimulatory following pretreatment with mytolon, but this stimulation was not influenced by BOL.

Table 2. summarizes the susceptibility of the investigated agents to various antagonists.

Discussion

The effect produced on *Helix* heart by the various amines investigated manifested itself, in general, in the increase or decrease of amplitude and frequency. Differences existed primarily not in the threshold concentrations, which was, in general, 10^{-10}—10^{-9} M in case of most agents but in the maximum effects produced. From among the amines only histamine had a higher sensitivity threshold (10^{-7} M). The sensitivity threshold of amines on the heart of *Anodonta* was, with the exception of 5-HT, higher by 2—3 order of magnitude than on *Helix* heart.

From among the factors examined only ACh was inhibitory on *Helix* heart at every concentration. ACh was found to be stimulatory on some Gastropoda, thus on the isolated heart of *Strophocheilos* (JAEGER 1961). The stimulatory effect of low concentrations of ACh was registered by CORDA (1955) on the heart of *Helix aspersa*, whereas the observations of JULLIEN et al. (1954) showed, as it was confirmed by our own findings, that on *Helix pomatia* ACh was never stimulatory whatever concentration it was used in. Concerning the lamellibranchs in the majority of cases ACh had a two-phase effect, it was partly stimulatory, partly inhibitory, depending on concentration (GREENBERG 1965). In our case the heart of *Anodonta* responded to ACh concentrations by 3 times greater order than *Helix* heart. ACh was found, however, in this case, too, definitely inhibitory at every concentration. In this respect a difference exist between the heart of *Anodonta cygnea* and the hearts of *Anodonta cataracta* and *Anodonta grandis*, namely, in the case of these two latter species ACh produced a two-phase reaction (inhibition and stimulation) (GREENBERG 1965). Data concerning other lamellibranchs are most varying and the comparison of theirs seems to be of no use (GREENBERG, 1965).

With respect to catecholamines *Helix* and *Anodonta* hearts behave differently. A positive inotropic and chronotropic effect was produced on *Helix* heart by 10^{-9} M and higher concentration, whereas, *Anodonta* heart responded in all instances with inhibition to all catecholamines examined by us.

The degree of the inhibition may, depending on concentration, range from a decrease of amplitude up to a complete arrest. In this respect, our data on the effect of adrenaline confirm those obtained by FÄNGE (1955).

Dopamine was found to have a two-phase effect on *Tapes* heart (CHONG and PHILLIS, 1965) and on *Mercenaria* heart (SPIRTES and JACOBOWITZ, 1966). This two-phase effect was not demonstrable by us on the heart of *Anodonta*. The degree and form of stimulation produced by each catecholamine applied on *Helix* heart was the same. It was not demonstrable, however, that stimulation was dependent on concentration. This finding of ours may be of some interest, for the catecholamines were regarded previously as either ineffective (ERSPAMER and GHIRETTI 1951) or inhibitory (OSTLUND 1954) factors on *Helix* heart.

Though several authors were (DAHL et al. 1966, PHILLIS, 1966) of the opinion that from among the catecholamines a special role is attributable to dopamine in the mediation of molluscs, in course of analysing the effect of adrenaline, noradrenaline and dopamine no data were obtained by us which would support the above suggestion. The effects produced by the three catecholamines did not differe considerably as regards the threshold concentration or the maximum effect produced.

Out of the amines investigated 5-hydroxytryptamine was the only that produced at the same threshold concentration (10^{-10} M) stimulation on both experimental objects. The only difference was that the maximum effect on *Anodonta* heart was greater *(Table 1)*. Contrary to data obtained by YOSHIHARA and KURIAKI (1957) and to those obtained by GREENBERG (1960) 5-HT did not produce inhibition even at very high concentrations. The type of the effect produced resembles that discribed by HILL (1958) and KUZIEMSKI (1962).

5-methoxytryptamine, tryptamine and tyramine have, too, produced stimulatory effect on both *Helix* and *Anodonta* heart. Threshold concentration on *Helix* heart was lower in case of these agents, too. Higher concentrations of both 5-methoxytryptamine and tyramine, however, were inhibitory on this object.

The effect of tryptophan, glutamine, GABA and histamine is stimulatory on *Helix* heart but does not produce, even at high concentrations a more than 5—15% increase of amplitude *(Table 1)*.

The order of the stimulatory agents on basis of the maximum effect produced is

on the heart of *Helix:* 5-HT > DA · A · NA > TA > 5-MT > TR >

TIR, GABA > GLA > HA,

on the heart of *Anodonta:* 5-HT > 5-MT > TA, TIR.

Much fewer amines produce stimulatory effect on *Anodonta* heart, than on *Helix* heart, and glutamine and histamine may be regarded as completely ineffectual, though 10^{-5} M glutamine solution was found stimulatory on *Tapes* heart (PHILLIS, 1966). GABA was ineffectual on *Tapes* heart, too. According to PHILLIS (1966) and PILGRIM (1954) histamine in high concentrations is unspecific agent on the hearts of other molluscs, too.

None of the amino acids investigated influenced cardiac action of the heart of *Anodonta*, and only tryptophan and GABA produced but negligible, nonspecific increase of amplitude on *Helix* heart.

Summing up, it may be concluded that amines can be taken for physiologically effective agents, and even the precursors of the effective amines, i.e. phenylalanine are insignificant in producing physiological effect. *Helix* heart is more sensitive to the agents investigated than *Anodonta* heart, consequently, it is more suitable for purposes of pharmacological investigations and biological testing. In view of the fact that in case of every agent but ACh stimulatory effects dominated, it is emphasized that in the realization of this effect identical mechanisms are involved, which produce on the effect of the various agents activity increases of different degree in the muscles. The uniformizing of responses to this degree is by all means indicative of the identity of the ground-mechanism.

On the other hand, the observation that the effect of catecholamines manifests itself in physiological effects of opposite direction on *Helix* and *Anodonta* hearts suggests that there is a great possibility for individual differences in case of different representatives. This may be elucidated only by detailed investigation of the properties of the receptors. It is not considered probable that in case of the two objects the catecholamines are interacting with different receptors, and it is thought much more possible that alterations taking place in other factors (receptor configuration, pattern of loading) are determining the different physiological effects. In case of some molluscs the accessibility of receptors may also influence the type of the response produced (JAEGER 1966). The fact that in some instances a stimulatory effect was observed on *Anodonta* heart following washing out of the agent indicates that the stimulatory effect of catecholamines is masked.

Data obtained with antagonist show that the effect produced by ACh takes place in case of *Helix* and *Anodonta* hearts in the same way as described previously on molluscs, namely, it was eliminated by mytolon, and not eliminated by BOL, or it was only slightly influenced by it (WELSH and TAUB 1951, WELSH and SLOCOMBE 1952, LUDUENA and BROWN 1952, CHONG and PHILLIS 1965). On the basis of our data it is inferred that the acetylcholine-receptors in these representatives of molluscs, too, are very similar.

Mytolon which inhibited the ACh receptors in molluscs eliminated also in both cases the effect of catecholamines. This agent decreases or discontinues the effect of dopamine, adrenaline and noradren line even if the effect produced is stimulatory on *Helix* heart, or inhibitory on the heart of *Anodonta (Table 2)*. Responses of opposite sign are produced in the majority of cases by catecholamines in hearts treated with mytolon, and accordingly, inhibition is produced on *Helix* heart and stimulation on the heart of *Anodonta*.

A reversion of response takes place in case of *Anodonta* heart on the application of adrenaline and in that of *Helix* heart on the application of adrenaline and noradrenaline. These facts suggest that the effect produced by catecholamine might be related to structures that are inhibitable by mytolon, they do not imply, however, that the effect takes place exclusively on these receptors. This latter statement is supported by the fact that BOL also influenced the effect of catecholamines, when, namely, this agent completely or partially eliminates it. BOL influenced the effect of catecholamines on *Helix* heart in a much less extent and never eliminated it completely. These facts

allow the supposition that there may exist another place of reaction for catecholamines the nature of which, however, needs further investigations.

The effect produced on *Helix* heart by 5-HT, tryptamine, and tyramine, and that produced by 5-HT on the heart of *Anodonta* were not influenced by mytolon. The effect of tryptamine on *Anodonta* heart is, however, inhibited by this agent. This involves, that the effect of 5-HT and tryptamine takes place on separate receptors and is completely eliminable by BOL in case of our two test objects. We have no exact knowledge as yet of the mechanism by which the effect of tyramine is realized on molluscs. In case of the vertebrates it is assumably connected to α-receptors (FARMER 1966).

It may be said on the basis of data obtained that the places of reaction suggested for ACh and 5-HT are isolated from each other, and the structure of the receptors of the other agents necessitates further studies.

Summary

1. Examining the effect of various amino acids and amines on the isolated hearts of *Helix* and *Anodonta* it was established that only amines are involved in the production of physiological effects. It was observed that the sensitivity of *Helix* heart on the agents tested was greater by 2—3 orders.

2. ACh produced on both *Helix* (10^{-9} M) and *Anodonta* (10^{-6} M) hearts negative inotropic and chronotropic effects.

3. Catecholamines (dopamine, noradrenaline, adrenaline) produced stimulation on *Helix* heart, and inhibition on the heart of *Anodonta*.

4. 5-HT produced on both objects stimulatory effect at the same threshold concentration (10^{-10} M) and inhibitory effect was not produceable even on the application of high concentrations.

5. Besides 5-HT other agents as tryptamine, 5-methoxytryptamine and tyramine were also stimulatory factors, the two latter, however, were inhibitory on *Helix* heart when applied at high concentrations. Tryptophan, glutamine, GABA and histamine produced an increase of amplitude on *Helix* heart, and were ineffectual on the heart of *Anodonta*.

6. The effect of ACh was inhibited on both objects by mytolon, and the effect of catecholamines was eliminated or was changed into the opposite effect by this agent. Mytolon does not influence the effect produced by 5-HT and tryptamine. It inhibits the effect of tyramine on the heart of *Anodonta*, but does not inhibit it on the heart of *Helix*.

7. BOL ceased the effect produced on the heart of *Anodonta* by the amines investigated except for ACh. On *Helix* heart it also did not influence the response produced by ACh, but eliminated the stimulatory effect of 5-HT, tryptamine and tyramine, and decreased to a small extent the effect of catecholamines.

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BIOLÓGIAILAG AKTÍV ANYAGOK HATÁSÁNAK ÖSSZEHASONLÍTÓ VIZSGÁLATA HELIX POMATIA L. ÉS ANODONTA CYGNEA L. IZOLÁLT SZÍVÉN

S.-Rózsa Katalin és *Pécsi Tibor*

Összefoglalás

1. Aminósavak és aminok hatásának vizsgálata alapján izolált *Helix* és *Anodonta* szíven megállapítást nyert, hogy fiziológiai effektus létrehozásában elsősorban az aminok vesznek részt. A *Helix* szív két-három nagyságrenddel érzékenyebb a tesztelt ágensekre.

2. Az ACh mind *Helix* ($1 \cdot 10^{-9}$ M), mind *Anodonta* ($1 \cdot 10^{-6}$ M) szíven negatív inotrop és kronotrop effektust vált ki.

3. Catecholaminok (dopamin, noradrenalin, adrenalin) *Helix* szíven serkentést, *Anodonta* szíven pedig gátlást hoznak létre.

4. Az 5-HT mindkét objektumon azonos küszöbkoncentrációban ($1 \cdot 10^{-10}$ M) hoz létre serkentő hatást, s gátló effektus nagy koncentrációk applikálásával sem váltható ki.

5. Az 5-HT-n kívül a triptamin, 5-methoxytriptamin és tiramin ugyancsak stimuláló faktorok, de a két utóbbi nagy koncentrációban *Helix* szíven gátol. A triptofán, glutamin, GABA és hisztamin *Helix* szíven amplitudónövekedést hoznak létre, *Anodonta* szíven hatástalanok.

6. Mytolon blokkolja az ACh hatást mindkét objektumon, ezenkívül kivédi vagy ellenkező előjelűvé változtatja a catecholaminok hatását. Nem befolyásolja az 5-HT és a triptamin hatást. A tiramin effektust blokkolja *Anodonta* szíven, de *Helix* szíven nem.

7. BOL *Anodonta* szíven az ACh kivételével valamennyi vizsgált amin hatását teljesen kivédi, *Helix* szíven nem befolyásolja az ACh hatást, de kivédi az 5-HT, triptamin serkentő hatását, míg a catecholaminok hatását kis mértékben csökkenti.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ДЕЙСТВИЯ НЕКОТОРЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ НА ИЗОЛИРОВАННОМ СЕРДЦЕ ВИНОГРАДНОЙ УЛИТКИ И БЕЗЗУБКИ

Каталин Ш.-Рожа и *Тибор Печи*

1. — В ходе исследования эффекта аминов и аминокислот на изолированном сердце виноградной улитки и беззубки было установлено, что амины являются более активными. Сердце виноградной улитки на 2—3 порядка чувствительнее сердца беззубки к изученным агентам.

2. — Ацетилхолин вызывает отрицательный инотропный и хронотропный эффект на сердце у виноградной улитки (1.10^{-9} М) и беззубки (1.10^{-6} М).

3. — Катехоламины (допамин, норадреналин, адреналин) оказывает усиливающее действие на сердце виноградной улитки, а на сердце беззубки — тормозящее.

4. — Серотонин вызывает стимуляцию на обоих объектах в одинаковых пороговых концентрациях (1.10^{-10} М) и в больших концентрациях тоже никогда не вызывает торможение.

5. — Помимо серотонина стимуляторный эффект оказывают триптамин, 5-метокситриптамин и тирамин, но из них последних два в больших концентрациях вызывают торможение на сердце виноградной улитки. Триптофан, глютамин, ГАБА и гистамин вызывают увеличение амплитуды на сердце виноградной улитки, а на сердце беззубки они неэффективны.

6. — Митолон снимает эффект ацетилхолина на обоих сердцах и предотвращает действие катехоламинов или же превращает его в противоположный знак. Митолон не влияет на эффект серотонина и триптамина, но блокирует действие тирамина на сердце беззубки но не на сердце виноградной улитки.

7. — БОЛ на сердце беззубки за исключением ацетилхолина снимает эффект всех изученных агентов, а на сердце виноградной улитки блокирует эффект серотонина, триптамина и тирамина, но не влияет на эффект ацетилхолина и снижает незначительно действие катехоламинов.