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THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE HEART OF FRESH WATER MUSSEL *(ANODONTA CYGNEA* L.) IN THE CASE OF DIFFERENT MODES OF APPLICATION

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A number of studies are known concerning the effect of 5-hydroxytryptamine (5-HT) on the molluscan heart. Within molluscs the lamellibranchiates occupy a significant position because their isolated heart serves as a suitable test object for the bioassay of 5-HT. In the experiments known up to now mainly the isolated heart of the marine lamellibranchiates was used (WELSH 1953, 1956; GADDUM—PAASONEN 1955; YOSHIHARA—KURIAKI 1957; GREENBERG 1960a, b; LOVELAND 1963), while from the fresh water mussel species few were examined.

FÄNGE (1955) was the first to suggest 5-HT bioassay using the heart of *Anodonta cygnea* L. on which the effect of 5-HT and its related compounds was examined in some detail by MARCZYNSKI (1959). More recently KUZIEMSKI (1962) made use of fresh water mussel species *(Unio pictorum* and *Anodonta cygnea)* for the examination of 5-HT effect on the heart of the mussel.

In literature two methods of the preparation and use of the isolated mussel's heart (ventriculus) are known. According to the first method a cannula was inserted in the heart and thus the 5-HT injected into the cannula influenced directly the inner wall of the heart. According to the other method the heart was placed into a bath full of physiological liquid and the 5-HT solution given into the latter acted on the external wall of the heart.

Concerning the comparison of the sensitivity of the external and internal wall of isolated mussel's heart up to now some data are known mainly in connection with acetylcholine (ACh). According to the examinations conducted by WELSH and TAUB (1948) the external wall of the heart of *Mercenaria (Venus) mercenaria* is as sensitive to ACh as the inner wall. Other authors (TEN CATE—REESINCK 1954; FLOREY—MERWIN 1961) demonstrated a difference in the sensitivity to ACh of the interior and exterior sides of the isolated mussel's hearts. Similarly a difference was demonstrated in the effect of some alkaloids (CARLSON 1922) and salts (MOTLEY 1934) on the mussel's heart according to whether they were introduced into the bath liquid or through a cannula in the interior of the heart. Therefore the question of the dependence on the locus of the sensitivity effect may well arise also in connection with the 5-HT. To be able to make consistent the 5-HT sensitivity data referred to in literature — which were obtained in mussel heart prepared with the above method — with each other, comparative examination of the sensitivity of the two methods became necessary. As a further comparison also the 5-HT sensitivity of the *in situ* mussel heart was investigated. Thus in the course

of our experiments the 5-HT effect on the heart of *Anodonta* was examined in the case of four different methods of application:

a) into the interior of the isolated heart,
b) into the bath liquid of the isolated heart,
c) to the *in situ* heart or pericardial liquid respectively and
d) into the auricle of the in situ heart.

Method

The experiments were conducted on *Anodonta cygnea* L. hearts in the winter months at 20—22° C room temperature. Isolation and suspension of mussel's hearts placed into bath liquid was carried out with slight modification according to the method of WAIT (1943) and/or WELSH and SLOCOMBE (1952) described in a previous paper (PÉCSI—SALÁNKI 1964). In a perfectly similar way were prepared the isolated hearts provided with a cannula with the only difference that their suspension and the recording of the cardiac action was carried out in the air. The latter fact of course rather restricted the period of usability of the thus prepared hearts for testing. The method of obtaining in situ preparations was also described earlier (PÉCSI—SALÁNKI 1964).

To be able to obtain normal or nearly normal cardiac action immediately after preparation in the case of isolated heart preparations, attention had to be paid to the blood volume of the hearts. Methods used by us made it possible to change the pressure within the heart and to adjust it to a value at which the heart was enabled to normal activity. Thus, in contrast to published data (FÄNGE 1955; MARCZYNSKI 1959; KUZIEMSKI 1962; SAKHAROV—NISTRATOVA 1963) we never experienced a shorter or longer standstill of the hearts subsequently to preparation.

Recording of cardiac action was carried out with the aid of a light lever on a smoked drum.

To set in action the isolated hearts, as a bath liquid and for the preparation of the 5-HT creatinine sulphate solutions a mussel physiological solution (MARCZYNSKI 1959) was used.

In the case of isolated hearts placed into a bath the whole heart was in the 5-HT solution of adequate concentration while in the three other cases the 5-HT solutions were added to the heart in an amount of 0.2 ml with the aid of a syringe. In these latter cases we always had to reckon with a considerable (minimum 10 fold) dilution.

Results

A) *Isolated heart preparations*

To 5-HT applied in two different ways the isolated hearts did not respond with the same sensitivity. The more sensitive method proved to be that in which the 5-HT solution was injected into the cannula *i.e.* the interior of the heart *(Fig. 1)*. In this case the 5-HT sensitivity threshold of the heart was observed at the dosage of 0.2 ml 10^{-9} M solution *(Fig. 1a)* to which the heart responded with increased amplitude and frequency. Since the amount of 5-HT introduced into the heart was diluted to about its 10-fold by the liquid within the heart, the threshold of these hearts can be determined around

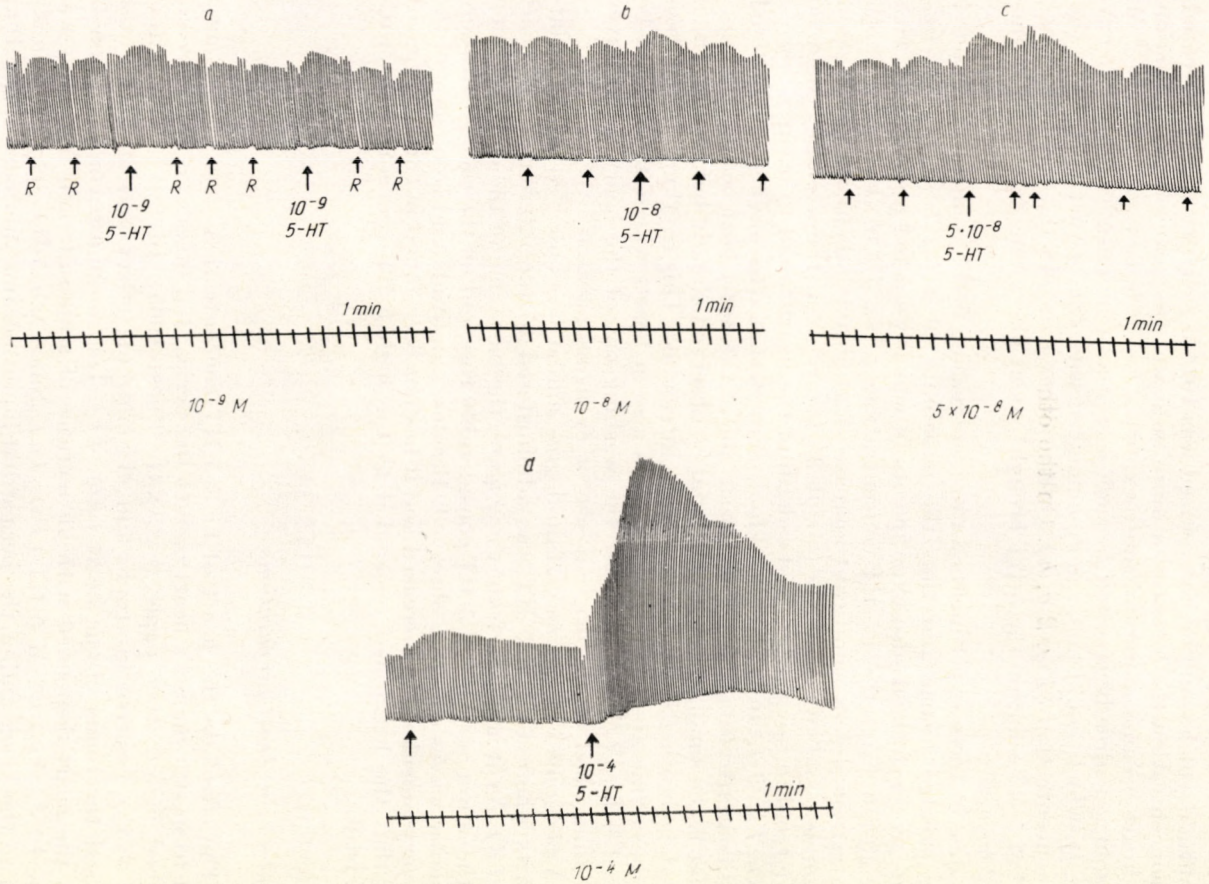

Fig. 1. The effect of 5-hydroxytryptamine in case of administering into the cannula of the isolated heart
1. ábra. Az 5-hydroxytryptamin hatása az izolált szív kanüljébe adása esetén

10^{-10} M. With the increase of the amplitude was always more manifest than that of frequency *(Fig. 1 b, c)*.

The sensitivity to 5-HT of the hearts placed into the bath was lesser than in the former case. The minimum 5-HT concentration resulting in an increase of amplitude which could be just noticed was 10^{-8} M *(Fig. 2a)*. With increased 5-HT concentration the increase in amplitude and frequency of the pulsation of the heart became more and more explicit *(Fig. 2 b, c, d, e, f)*. 5×10^{-6} M 5-HT solution already resulted in such increase of amplitude and frequency *(Fig. 2 f)* which could be no more enhanced with the further increase of the 5-HT concentration *(Fig. 2 g, h)*. On the other hand, in the case of the use of 10^{-4} M 5-HT solution the 5-HT brought about also an increase in the tonus *(Fig. 2 h)*.

Since in these experiments an ever increasing 5-HT concentration acted on the isolated hearts and thus the possibility of a 5-HT adaptation was present, we performed also experiments in which we acted immediately with very high (10^{-5}—10^{-4} M) 5-HT concentrations *(Fig. 3)*. In the case of both concentrations a very significant increase of amplitude (about 200 per cent on the average) and of frequency (about 25 per cent on the average) could be observed and in no case was a standstill of the heart found to occur.

On *Fig. 3* it can be seen in all four pictures that after having administered a great concentration of 5-HT the basic line of the heart beat became undulating, that is the small waves which could be observed already before and which followed each other in regular intervals, intensified. This rhythmic fluctuation of the basic line appeared in many cases also in hearts where it could not be observed before *(Fig. 3 b)*. After the washing out of the heart these waves either diminished to the original size or even disappeared.

A phenomenon up to a certain degree similar was observed also in isolated hearts where 10^{-4} M 5-HT was administered to the interior of the heart *(Fig. 1 d)*. As it appears from the *Figure*, the basic line of the cardiac action after the administration of 5-HT considerably rose and on the rising shaft here too smaller waves could be observed. Besides, amplitude and frequency of the heart very considerably increased and it has not been found in this experiment either that the heart had responded to the high 5-HT concentrations with a standstill.

B) *In situ heart preparations*

The effect on the heart of high 5-HT concentrations (10^{-4} M) administered to in situ mussel's heart or into the pericardial liquid has been already described in an other paper (Salánki—Pécsi 1965). In this concentration 5-HT had a positive inotropic and chronotropic effect and as a rule also increased the tonus of the heart *(Fig. 4 c)*. The minimum concentration to which the heart responded with an increase of amplitude and frequency was 0.2 ml 10^{-6}—5×10^{-6} M 5-HT *(Fig. 4 a)*. Since the 5-HT solution administered to the heart and/or the pericardial liquid became diluted to more than 10-fold, the threshold of the 5-HT thus applied was around 10^{-7}—5×10^{-7} M. A drawback of the method is that the exact place of the 5-HT effect can not be controlled and delimited because there is a possibility for the 5-HT solution given to the *in situ* heart to spread.

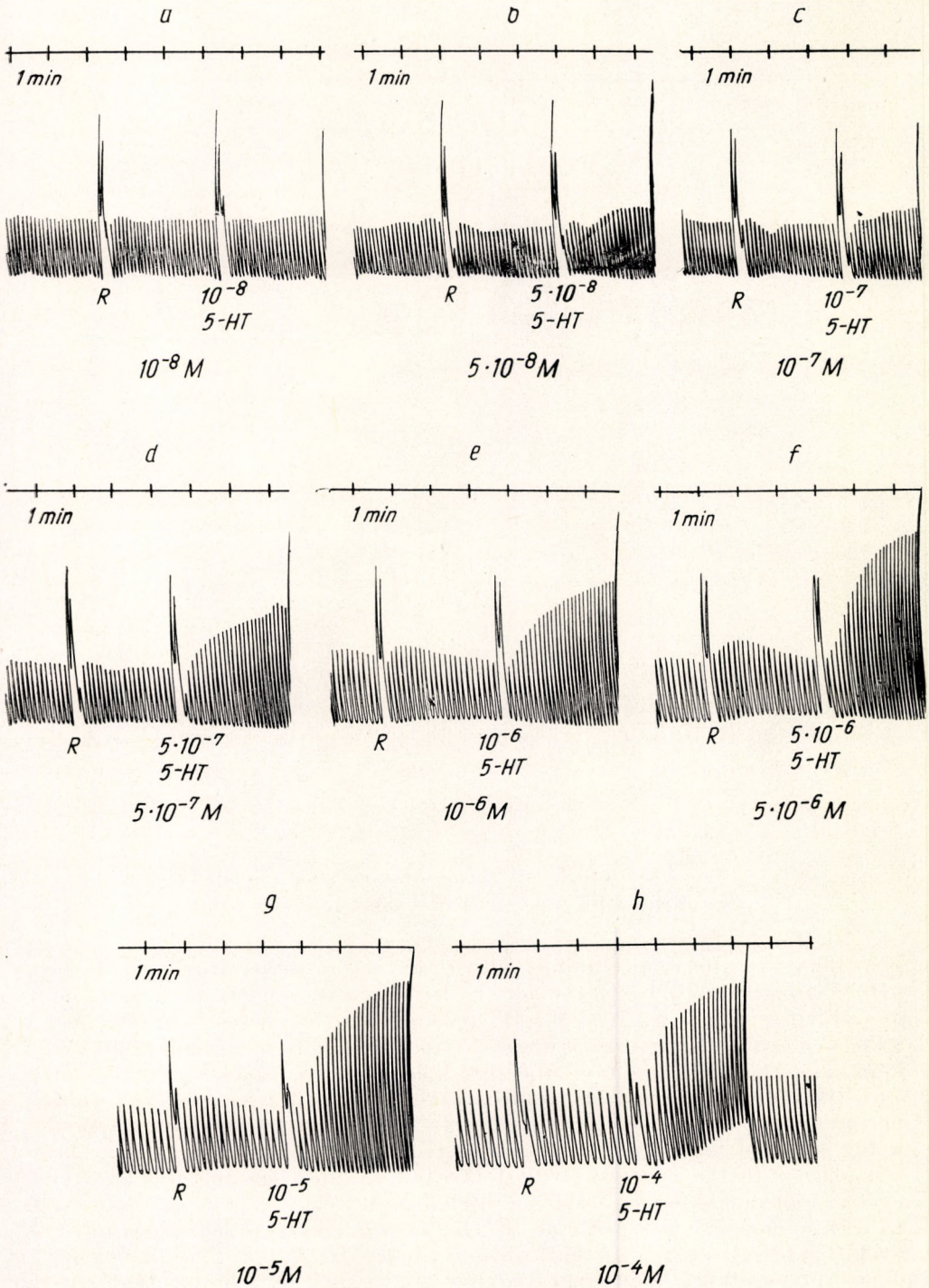

Fig. 2. The action of 5-HT solutions applied as bath liquids on the heart
2. ábra. Fürdőfolyadékként alkalmazott 5-HT oldatok hatása a szíven

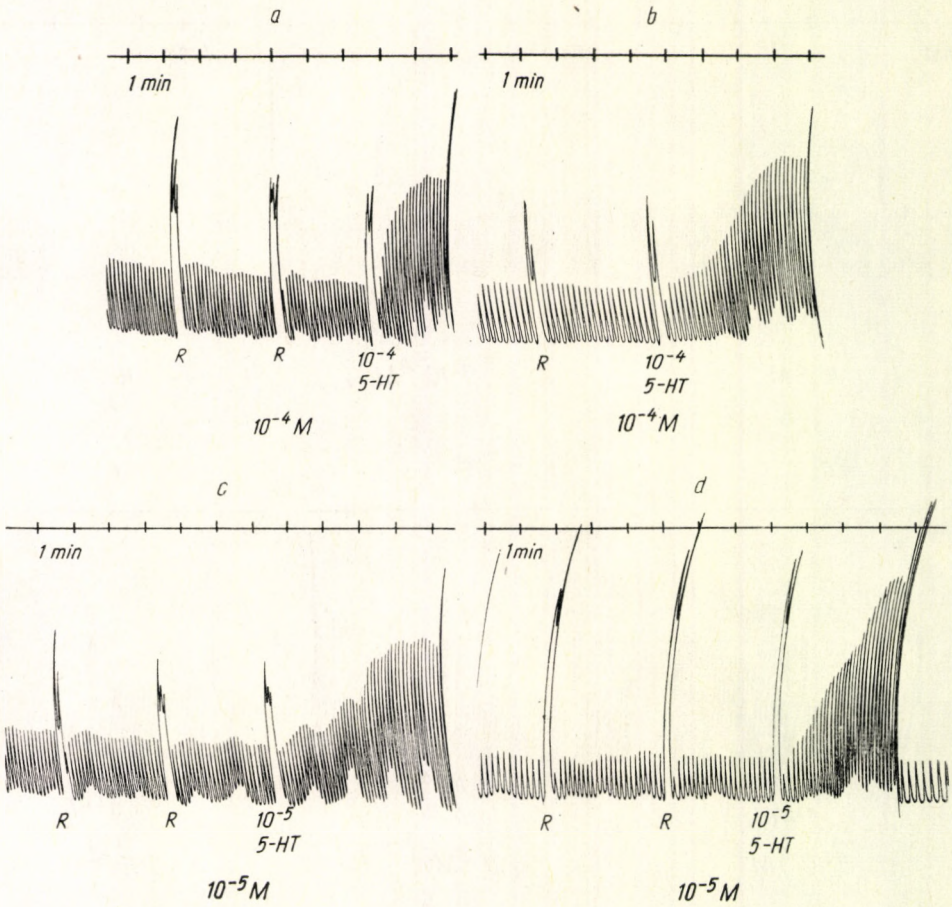

Fig. 3. The effect of high 5-HT concentrations on the isolated heart
3. ábra. Nagy 5-HT koncentrációk hatása az izolált szívre

Better results were obtained with those *in situ* preparations where the 5-HT has been injected into the auricle. In these experiments already 0.2 ml 10^{-7} M but especially 5×10^{-7} M 5-HT resulted in an explicitely positive effect *(Fig. 5 a)*. Since also here it must be reckoned with a minimum 10-fold dilution of the 5-HT solution, the threshold was around 10^{-8}—5×10^{-8} M. With the increase of the 5-HT concentration amplitude frequency and tonus increased *(Fig. 5 b, c, d, e)*. Those hearts which were immediately exposed to the effect of high (10^{-4}—10^{-3} M) 5-HT concentrations responded in the first place with the increase of the tonus but within a few minutes also the positive inotropic effect of 5-HT manifested itself *(Fig. 5 d, e)*.

Difference in the sensitivity of the *in situ* heart preparations to the 5-HT applied in two different ways is shown in *Fig. 6*. The deficiency of such preparations — although they are giving good results — is that after the various 5-HT concentrations the heart can not be washed out, that is

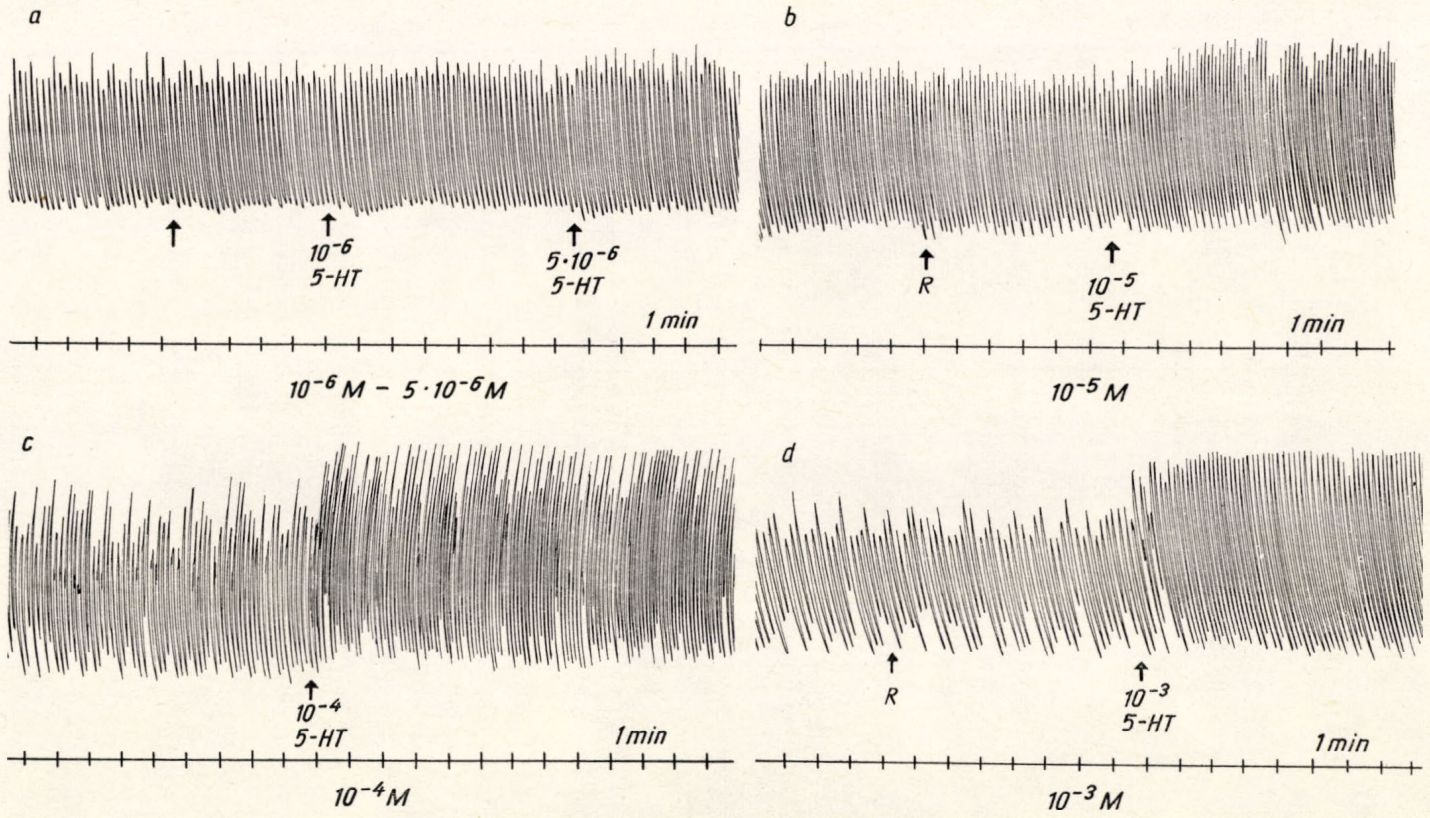

Fig. 4. The effect of 5-HT given to the *in situ* heart
4. ábra. Az in situ szívre adott 5-HT hatása

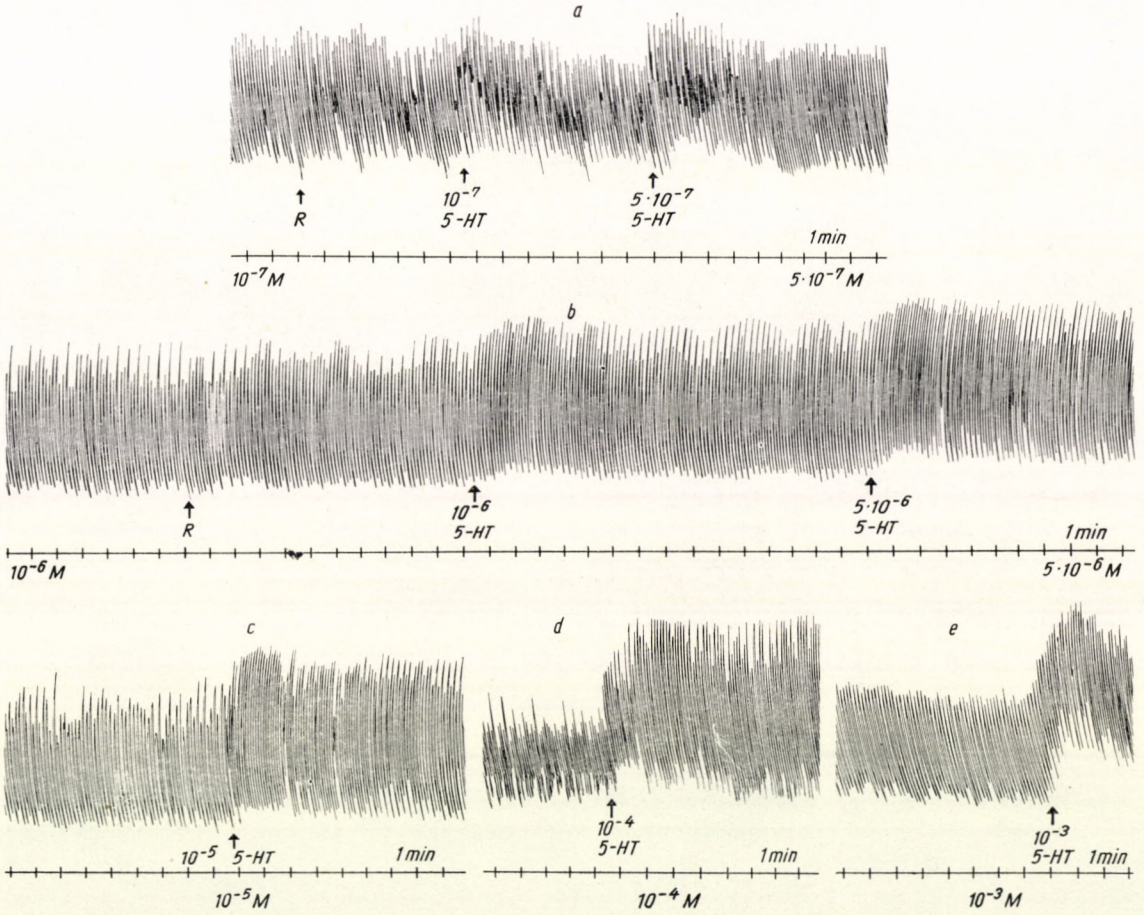

Fig. 5. The effect of 5-HT in case of injecting into the auricle of the *in situ* heart
5. ábra. Az 5-HT hatása az in situ szív pitvarába való injekciózás esetén

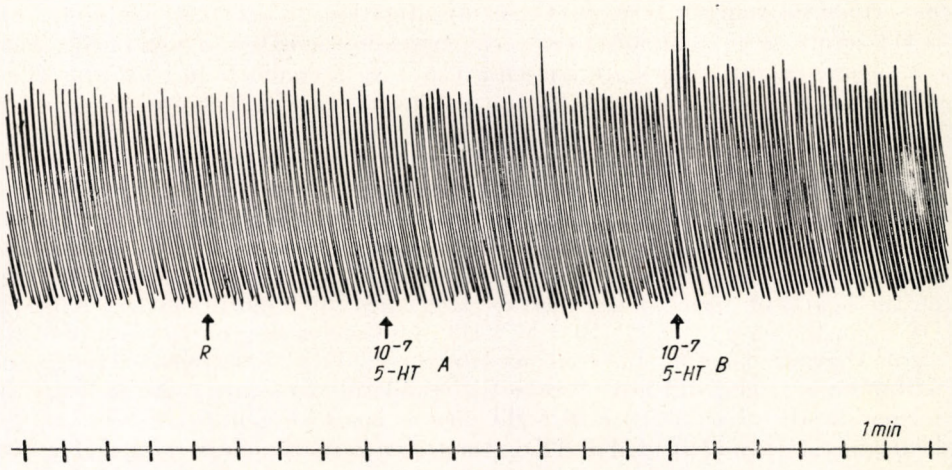

Fig. 6. Comparison of the sensitivity to 5-HT given on and/or into the heart on *in situ* preparation

6. ábra. A szívre, illetve a szívbe adott 5-HT-re való érzékenység összehasonlítása in situ preparátumon

the 5-HT from the heart with the haemolymph spreads in the whole organism and on the effect of 5-HT such reactions appeared (contraction of the adductors, then relaxation, stretching of the foot etc.) which as mechanical excitations adversely influenced the reaction of the hearts to further 5-HT.

Discussion

As well known from earlier works (Fänge 1955; Marczynski 1959) and corroborated by our own data the isolated *Anodonta* heart preparations may serve as suitable test objects for the determination of low 5-HT concentrations. However the response of isolated hearts to 5-HT was obviously influenced by whether 5-HT was given into the cannula or the bath liquid, that is whether 5-HT acted on the interior or exterior wall of the ventricle. The fact found during the experiments that in case 5-HT was given into the cannula the threshold was around 10^{-10} M while in the case of 5-HT acting as bath liquid the heart responded perceptibly only to the 10^{-8} M solution, permitted to conclude that the mussel's heart is more sensitive to 5-HT acting in the interior of the heart. Since this finding was supported also by our experiments conducted in mussel's hearts *in situ*, we concluded from our data that the 5-HT sensitivity of the inner wall of the ventricle is greater than that of the external wall.

Our results show a close agreement with the thresholds observed up to now in *Anodonta* (Fänge 1955; Marczynski 1959), *Unio* (Kuziemski 1962), *Venus* (Welsh 1953, Welsh—McCoy 1957) and *Cyprina* (Welsh 1956) hearts. Newer data were obtained on the isolated heart of *Unio* by Kuziemski (1962) when using parallel with the kymographic examinations also photoelectric recording method finding thresholds of 10^{-12}—10^{-16} M 5-HT depending on

experimental temperature. In these investigations KUZIEMSKI employed the 5-HT solutions as bath liquid that is he acted on the external wall of the heart.

Insufficient studies are known up to now in connection with the effect of high 5-HT concentrations on the molluscan hearts. The results found in literature are contradictory which may be explained by the fact that the examinations were conducted on the hearts of different Mollusca species. The examinations of JAEGER (1962) resulted at the 5-HT 10^{-6} g/ml concentration in a systolic standstill on the heart of the snail *Strophocheilos oblongus*. Similar results were obtained in the case of 10^{-5}—10^{-4} M solutions by GREENBERG (1960a) and of 5×10^{-6}—10^{-5} M 5-HT solutions by LOVELAND (1963) acting on the hearts of *Mercenaria (Venus) mercenaria*, by YOSHIHARA and KURIAKI (1957) in the case of 10^{-4}—10^{-5} M 5-HT solutions acting on the hearts of the clam *Meretrix lusoria*. In contrast to these data KUZIEMSKI (1962) and GREENBERG (1965) did not observe the standstill of pulsation of the heart the former in the case of 10^{-5} M 5-HT on the heart of *Unio pictorum* and the latter in the case of 10^{-4} M 5-HT on the heart of *Anodonta grandis* and according to the investigations of HILL (1958) the ventricle of the snail *Busycon canaliculatum* does not show systolic standstill even on the action of 10^{-2} M 5-HT. Both in the isolated and *in situ Anodonta* hearts our experience was that the hearts did not come to a standstill in any case upon the action of 10^{-5}—10^{-4} M 5-HT. Thus the isolated hearts of the marine *Venus* and of the fresh water *Anodonta* although they responded similarly to low 5-HT concentrations gave different responses to high (10^{-5}—10^{-4} M) concentrations.

Since in the course of our experiments the hearts were hung up by bindings made on the aorta, for the undulation of the basic line of the heart's activity which can be observed in some cases, the intestine located in the axis of suspension should be considered responsible. As it appears from *Fig. 3* the spontaneous rhythmic activity of the intestine became manifest in the form of small and comparatively infrequent waves which at the use of the high (10^{-5}—10^{-4} M) 5-HT doses as bath liquids increased and in cases when the activity of the intestine could not be observed earlier even rhythmic activity was induced upon these doses *(Fig. 3 b)*. Therefore several authors, to avoid the interaction between the heart and the intestine proceeding in the longitudinal axis of the heart either hung up the ventricle by bindings applied at the auricles (WAIT 1943; WELSH—TAUB 1948; WELSH—SLOCOMBE 1952; KUZIEMSKI 1962; LOVELAND 1963; SAKHAROV—NISTRATOVA 1963) or had removed the intestine from the heart (TEN CATE—REESINCK 1954; YOSHIHARA—KURIAKI 1957; SAKHAROV—NISTRATOVA 1963).

According to examinations conducted by NYSTROM (1963) on *Spisula solidissima* and GREENBERG and JEGLA (1963) on *Mercenaria (Venus) mercenaria* the 5-HT up to 10^{-6} M resulted in a stimulating effect on the rectum while the higher concentrations (5×10^{-6}—10^{-5} M) inhibited the rhythmic activity of the intestine and considerably increased the tonus of the intestine (GREENBERG—JEGLA 1963). Taking these results into consideration we can conclude from our data that from the 10^{-5}—$10-^{4}$ M bath liquid 5-HT diffuses into the interior of the heart and the 5-HT concentration within the heart attains such value which beside increasing the rhythmic activity of the intestine probably enhances the tonus of the intestine too. When we administered 0.2 ml 10^{-4} M 5-HT this meant even after dilution in the liquid within the heart such concentration resulted in increase of tonus in the intestine

stretch within the heart *(Fig. 1 d)*. Thus in our opinion the intestine passing through the heart although it is by the binding applied on the two aortas in close interaction with the ventricle does not specially disturb the response of the heart to 5-HT doses around the threshold; its role becomes manifest more in the case of high 5-HT dosage rates. This of course excludes in no way the possibility that under physiological conditions the sensitive intestine movement of the 5-HT may influence the activity of the heart also in the case of low concentrations.

Summary

The effect on the heart of the fresh water mussel *(Anodonta cygnea* L.) of 5-hydroxytryptamine (5-HT) was examined with four modalities of application:

a) given into the interior of the isolated heart,

b) given into the bath liquid of the isolated heart,

c) administered to the *in situ* heart or the pericardial liquid respectively and

d) into the auricle of the *in situ* heart.

It has been established that both in the case of isolated and *in situ* heart preparations the interior wall of the ventricle is more sensitive to 5-HT than the outer wall which must be absolutely taken into consideration when titrating 5-HT on the heart of lamellibranchiates and/or comparing data obtained with different methods.

Under the experimental conditions, examining the interaction of the heart and of the rectum passing through it we demonstrated that the rectum does not disturb the response of the heart to low 5-HT concentrations, its effect appeared in high concentrations.

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AZ 5-HYDROXYTRYPTAMIN HATÁSA A TAVI KAGYLÓ *(ANODONTA CYGNEA* L.) SZIVÉN KÜLÖNBÖZŐ ALKALMAZÁSI MÓDOK ESETÉN

Összefoglalás

Pécsi Tibor

Az 5-hydroxytryptaminnak (5-HT) a tavi kagyló *(Anodonta cygnea* L.) szívén való hatását vizsgáltuk négyféle applikálási mód mellett:

a) izolált szív belsejébe adva,
b) izolált szív fürdőfolyadékába adva,
c) in situ szívre, illetve a pericardiális folyadékba adva, és
d) in situ szív pitvarába adva.

Megállapítottuk, hogy mind az izolált, mind az in situ szívpreparátumok esetén a ventriculus belső fala érzékenyebb az 5-HT-ra mint a külső fal, amit az 5-HT Lamellibranchiata szíven való titrálásakor ill. különböző módszerrel nyert adatok összehasonlításakor feltétlenül figyelembe kell venni.

Kísérleti körülményeink között a szív és a rajta áthaladó rectum egymásrahatását vizsgálva kimutattuk, hogy a rectum nem zavarja a szívnek a kis 5-HT koncentrációkra való reagálását; hatása a nagy koncentrációknál jelentkezett.

ЭФФЕКТ СЕРОТОНИНА НА СЕРДЦЕ БЕЗЗУБКИ (Anodonta cygnea L.) В ЗАВИСИМОСТИ ОТ СПОСОБА ЕГО ПРИМЕНЕНИЯ

Т. Печи

Изучался эффект серотонина на сердце беззубки в зависимости от разных способов его применения: серотонин давался

а) внутри изолированного сердца,

б) в раствор, отмывающего изолированного сердца,

в) на сердце in situ т. е. в перикардиальную жидкость,

г) в предсердие сердца in situ.

Было установлено, что внутренняя стенка желудочка изолированного сердца и также сердца in situ чувствительнее к серотонину чем его внешняя стенка, и это необходимо принимать во внимание при титрировании серотонина на сердцах пластинчатожаберных и при сопоставлении данных полученных разными методами.

Изучая взаимодействие сердца и проходящейся через него прямой кишки, было установлено, что прямая кишка не препятствует реакцию сердца к малым дозам серотонина, но оказывает свое действие при применении больших концентраций этого агента.