

PROTECTING AND REACTIVATING EFFECT OF SEROTONIN ON THE HEAT-INACTIVATED HEART OF THE FRESH WATER MUSSEL

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The significance of serotonin as a mediator in molluscs is generally known. Many facts seem to point out that beside the mediator function this substance plays an important part also as a local hormone which is necessary for the endogenous excitation of the corresponding cells. Thus referring to the effect of serotonin exciting the cilia there is ample evidence that this substance is involved in the regulation of the automatic ciliary activity in lamellibranchiates (AIELLO 1960, GOSSELIN 1961, GOSSELIN—MOORE—MILTON 1962). The localization of serotonin in the neurons of Anodonta on the other hand points to its possible role in the process of excitation induced by neurons (ZS.-NAGY—S.-RÓZSA—FÖLDES—PERÉNYI—SALÁNKI—DEMETER 1965).

In the present study we attempted to clarify the significance of the endogenous serotonin in the automatism of the cardiac muscle in the mussel. As experimentally demonstrated, the heart subjected to the effect of high temperature which thus has stopped automatic pulsation repeatedly regained this capacity when treated with serotonin.

Method

To the experiments isolated hearts of the fresh water mussel (*Anodonta cygnea* L.) were used for whose activation and as a bath fluid a proper physiological solution was utilized (MARCZYNSKI 1959). The method of preparation has been described earlier (PÉCSI—SALÁNKI 1964). For the heat treatment of the heart hot physiological solutions were used the temperature of which was measured both before and during use. In this paper double temperature values were given: the first value indicates the temperature of the liquid at the moment of introducing into the ventricle containing the heart while the second (in parenthesis) shows the temperature to which the liquid cooled down while acting on the heart.

The period of application of the hot liquid depended on the temperature. In the case of 50 (44)—55 (47) °C the heart was kept under heat treatment for 1 minute while in the case of 70 (59) °C for 15 sec only. The hot liquid after

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its effect was over has been always changed for a solution of room temperature (20–22 °C).

In the course of experiments the following agents were used: serotonin creatinine sulphate, serotonin hydrogen oxalate, 5-hydroxy-*N*'-dimethyl-tryptamine (bufotenin), 5-methoxytryptamine oxalate, 5-methoxy-*N*'-acetyl-tryptamine (melatonin), tryptamine hydrochloride, 1-tryptophan, 5-hydroxy-dl-tryptophan, tyramine hydrochloride, 1-noradrenaline, histamine. The solutions made of the individual agents were applied as bath liquids.

Experiments were carried out in the months of October to January at room temperature.

Results

When the bath liquid of the isolated mussel's heart was changed for hot physiological solution a characteristic reaction appeared in the cardiac activity: frequency and tonus of pulsation increased in each case rapidly and strongly, while the amplitude as a rule decreased. With the increase of the temperature of the hot solution and of the period during which it acted on the heart the effect increased. In a liquid of 40 (36) °C the heart still could be kept without major thermal damages. The liquid of 50 (44) °C in most cases resulted already in systolic standstill of the heart which only ceased after a shorter or longer period (*Fig. 1*). The liquid of 55 (47)–57.5 (49.5) °C still more rapidly and intensively damaged cardiac activity. When the heart's activity was restored, amplitude and frequency of pulsation considerably decreased as compared to the condition previous to the heat treatment (*Fig. 1*).

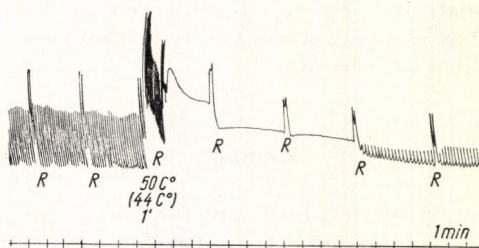


Fig. 1. Effect of 50° C physiological liquid applied for 1 minute on cardiac action
1. ábra. Az 1 perces 50 C°-os fiziológias folyadék hatása a szívnyűködésre

Treatment of the heart for 1 minute with 50 (44) °C hot physiological solution led to the reduction of the amplitude of cardiac action by 50 per cent, of the frequency by 30 per cent. At treatment for 1 minute with 55 (47) °C solution the amplitude of pulsation decreased by 65 per cent, the frequency by 30 per cent. When temperature was further increased, both the reduction of amplitude and frequency was still more explicit, until at a certain temperature maximum final standstill of the heart was obtained. The maximum temperature action after which the cardiac activity was still spontaneously restored was 67 (56) °C solution applied for 30 sec or 70 (59) °C solution applied for 15 sec.

Serotonin restored the original amplitude and frequency of the cardiac action weakened by heat treatment. Furthermore in those cases when the hearts did not begin to beat from themselves, in the case of definite temperature limits serotonin was able to induce pulsation. Serotonin concentration necessary to restore cardiac action depended on temperature and period of application of the solution used for heat treatment. The threshold of sensitivity to serotonin of the hearts was normally around 5×10^{-9} – 10^{-8} M. After heat treatment for 1 minute with 50 (44) °C bath liquid 10^{-7} M while after 55 (44)–57.5 (49.5) °C liquid application 5×10^{-7} M serotonin was needed to start again the cardiac action that came to a standstill as a consequence of the heat treatment. After a heat treatment for 15 sec with 67 (56)–70 (59) °C the heart responded with a start of pulsation to 10^{-6} M serotonin only.

During these experiments the interesting phenomenon was observed that if before the application of the hot physiological solution the heart was treated with serotonin, depending on temperature and period of application amplitude and frequency of pulsation increased, did not change or hardly, or at least suffered less damage than in the cases when the same heat treatment was applied without giving serotonin previously. Thus, when prior to the treatment with 50 (44) °C solution for 1 minute the heart was kept for a few minutes in 5×10^{-7} M serotonin solution the heat treatment except for an initial transitory phase did not stop the pulsation and in such cases even frequency and in some cases also amplitude of pulsation increased (Fig. 2).

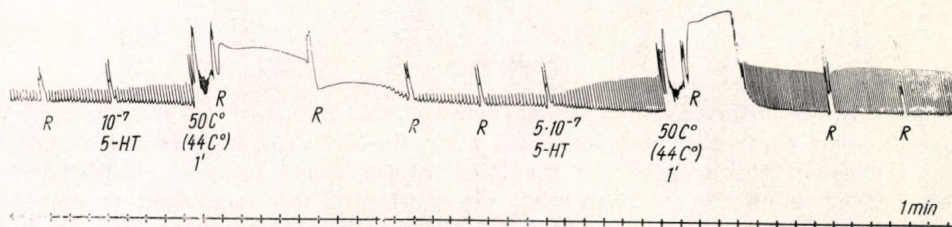


Fig. 2. Effect of 5×10^{-7} M serotonin given before the application of 1 minute 50° C physiological liquid

2. ábra. Az 1 perces 50 C°-os fiziológias folyadék applikálása előtt adott 5×10^{-7} M. serotonin hatása

Such protection from heat treatment of the cardiac action was in the overwhelming majority of cases not afforded by 10^{-8} – 10^{-7} M serotonin where as pretreatment with 5×10^{-7} – 10^{-6} M serotonin already resulted in the above mentioned protective effect.

Parallel with the investigation of the specificity of the above serotonin effects we examined also the effect of other biologically active substances. These experiments were of an informatory character.

An effect similar to the above action of serotonin was found in the case of two related compounds: tryptamine and 5-methoxytryptamine. From the point of view of effectivity, however, both compounds were about 10 times less

active than serotonin. At the same time noradrenaline, tyramine, histamine, tryptophan, 5-hydroxytryptophan, 5-hydroxy-N'-dimethyltryptamine and 5-methoxy-N'-acetyltryptamine were found to be completely ineffective even in high 10^{-4} M-concentration.

Discussion

The results obtained indicate that the pulsation of the mussel's heart brought to a standstill with proper heat treatment can be started with the application of serotonin as a bath liquid. It is evident that the action of high temperature damages the contractile apparatus of the heart muscle and the serotonin receptors to a lesser degree than the generation of rhythmic excitations. Since the latter mechanism is restored by serotonin it may be concluded that in natural generation of rhythmic impulses endogenous serotonin is deeply involved and that the standstill of the heart induced by heat treatment may be engendered among others by the disturbance of the biochemical system responsible for serotonin synthesis. On the basis of this conception the significance of the serotonin pretreatment of the heart becomes comprehensible which leads to the accumulation of serotonin in the cardiac tissue and as a result to the resistance of the heart to high temperature effects.

It may be assumed that the known effect of serotonin as a mediator of the stimulatory nervous actions exercised on the heart of molluscs (WELSH 1957, KOSHTOYANTS 1957, LOVELAND 1963, S.-RÓZSA—GRAUL 1964) is in a close connection with the function of this substance as a local hormone, since in both cases serotonin, acts on the same system, the excitable structure of the cardiac tissue.

Summary

Serotonin within certain limits restores the pulsation of the isolated mussel's heart that came to a standstill upon the action of high temperature. Heat treatment resulting in the standstill of the heart becomes ineffective if the heart is kept previously in serotonin solution. These facts seem to point out that endogenous serotonin which acts here as a local hormone may have an important part in the automatism of the mussel's heart.

Tryptamine and 5-methoxytryptamine have a similar protective effect from the heat inactivation of the heart which is, however, about 10 times less active than that of serotonin. On the other hand no such effect was observed, even in the case of high concentrations, when noradrenaline, tyramine, histamine, tryptophan, 5-hydroxytryptophan, 5-hydroxy-N'-dimethyltryptamine and 5-methoxy-N'-acetyltryptamine were applied.

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A SEROTONIN VÉDŐ ÉS REAKTIVÁLÓ HATÁSA AZ ÉDESVÍZI KAGYLÓ HÓINAKTIVÁLT SZÍVÉN

Összefoglalás

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A serotonin bizonyos határokon belül helyreállítja a magas hőmérséklet hatása révén megállt izolált kagyló szív verését. A szív leállítását eredményező hőkezelés hatásatlanná válik, ha a szívet előzetesen serotoninoldatban tartjuk. Ezen tények arra mutatnak rá, hogy az endogén serotonininak, amely itt mint lokális hormon szerepel, lényeges szerepe lehet a kagyló szív automatizmusában.

A szív hóinaktivációjával szemben hasonló, de a serotoninénál kb. 10-szer kevésbé aktív védőhatással rendelkezik a tryptamin és az 5-methoxytryptamin. Viszont nem tapasztaltunk ilyen hatást még nagy koncentrációk esetén sem a noradrenalin, tyramin, histamin, tryptophan, 5-hydroxytryptophan, 5-hydroxy-N'-dimethyltryptamin és az 5-methoxy-N'-acetyltryptamin alkalmazásakor.

ЗАЩИТНОЕ И ВОССТАНОВИТЕЛЬНОЕ ДЕЙСТВИЕ СЕРТОНИНА ПРИ ТЕМПЕРАТУРНОЙ ИНАКТИВАЦИИ СЕРДЦЕБИЕНИЙ БЕЗЗУБКИ

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Серотонин восстанавливает биения изолированного сердца беззубки, остановленного действием высокой температуры. Температурная обработка, приводящая к остановке сердца, становится неэффективной после предварительного выдерживания сердца в растворе серотонина. Эти факты рассматриваются как указание на то, что эндогенный серотонин имеет существенное значение в автоматизме сердца беззубки, выступая здесь в роли локального гормона.

Сходным защитным действием против температурной инактивации обладают триптамин и 5-метокситриптамин, но они примерно в 10 раз менее активны, чем серотонин. Не обладают защитным действием, даже в высокой концентрации, норадреналин, тирамин, гистамин, триптофан, 5-окситриптофан, 5-окси-N'-диметилтриптамин и 5-метокси-N'-ацетилтриптамин.