PHARMACOLOGICAL INVESTIGATIONS ON THE REGULATION MECHANISMS OF THE PERIODIC ACTIVITY OF THE FRESH WATER MUSSEL (*ANODONTA CYGNEA* L.)

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The synthesis, storage, depletion, inactivation and the pharmacological effects of the monoamines in the central nervous system indicate that these substances perform a complex function in the brain. During the last decade an attempt has been made to reveal the connection between the changes of the monoaminergic systems and the behavioural responses. One of the most useful ways for this work proved to be the alteration of the monoaminergic systems by different pharmacons and the analysis of changes of the behavioural responses. Nevertheless, the quantitative evaluation of these responses encounters significant difficulties and only the general aspects could be followed such as the increase or decrease of responsiveness. The situation has become more complicated due to some recent observations according to which many pharmacons are able to induce significant changes without affecting the monoamine level, only altering their turnover. The results, however, called attention to the role of monoamines, first of all, to that of serotonin (5HT) in the regulation of the activity. Thus e.g. seasonal changes of adrenaline, noradrenaline (NA) and 5HT parallel with the EEG activity have been described in frog by SEGURA et al. (1967). On the basis of experiments carried out also on frogs, the role of 5HT in activity regulation and temperature acclimatization processes has been emphasized in amphibia (HARRI, 1972).

Significant, however not unequivocal changes were found in the brain 5HT level during hibernation (UUSPÄÄ, 1963; SPAFFORD and PENGELLEY, 1971; DRASKOCZY and LYMAN, 1967). At the same time, seasonal changes were detected in mice kept under the same conditions (VALZELLI and GARATTINI, 1968).

The daily change of acitivity is followed by a significant change of 5HT level in rat and turtle (FRIEDMANN and WALKER, 1968; QUAY, 1963; 1967) connected with the conditions of illumination. The most convincing evidence for the regulatory role of 5HT in the activity has been presented by JOUVET (1968; 1969) who demonstrated a higher 5HT level of the brain during sleep than wakefulness.

The nervous systems of invertebrates contain a significant amount of monoamines (WELSH and MOOREHEAD, 1960; DAHL et al., 1966; SWEENEY, 1963), and although the stimulatory transmitter function of 5HT has long

been accepted, unfortunately its role in the activity regulation has hardly been investigated.

CARDOT (1971) described the seasonal change of 5HT level in *Helix pomatia*, whereas our investigations proved that the seasonal change of 5HT level regulates the initiation and maintenance of hibernation (HIRIPI and SALÁNKI, 1972).

The activity of the fresh water mussel *(Anodonta cygnea)* is characterized by a distinct periodicity. This involves a regular alternation of active and rest phases realized in different functioning of the adductor muscles and other organs (SALÁNKI and LUKACSOVICS, 1967; MORTON, 1969). The period of rest is characterized by a prolonged tonic contraction of the adductors, whereas during the active period the adductors are relaxed and perform quick, rhythmic contractions. According to previous investigations, 5HT plays a significant role in the relaxation of the adductors (SALÁNKI, 1963; SALÁNKI and LÁBOS, 1969), and one can assume that the regulation of the mechanisms maintaining the activity takes place through the serotoninergic system.

It has been shown during our previous investigations that the central nervous system of fresh water mussel contains $40-70$ $\mu g/g$ wet weight of 5HT (HIRIPI, 1968), $10-20$ $\mu g/g$ wet weight of dopamine (DA) and $1-2$ $\mu g/g$ wet weight of NA (HIRIPI, 1972). It has also been proved that 5HT may have connection with the regulation of the periodic activity (SALÁNKI, 1963). Further evidences were given by our earlier investigations for that role of 5HT. It is known that the stimulation of the cerebro-visceral connective (CVc) using suitable parameters is of relaxing effect on the adductors in the majority of cases, and the relaxation is especially of expressed degree upon the influence of repeated stimulation (SALÁNKI and LÁBOS, 1963). We determined the 5HT content in both adductors during relaxed state. After two hr of stimulation, the 5HT content of the anterior and posterior adductors was higher than that of the control muscles. The stimulation induced an increase of 33 percent in the anterior and 25 percent in the posterior adductor in the 5HT content, being significant in both cases.

The 5HT content was analyzed in the ganglia and the adductors at the beginning of the active and rest periods, i.e. in the opposite phases of the spontaneous periodic activity.

When measuring the 5HT content of the ganglia, only that of the visceral one changed at the beginning of the active and rest periods. It was 23 percent lower at the beginning of the active period in the visceral ganglion.

Measurements of 5HT content in the anterior and posterior adductors separatedly, revealed that the 5HT level is twice as high in the former than in the latter one. The 5HT contents were higher in both adductors at the beginning of the active state than those in the period of rest. The increase amounted to 30 percent in the anterior adductor and 25 percent in the posterior one, being significant only in the former.

Since the activity of this bivalve can quantitatively be followed on the basis of the characteristic periodicity, it represents a good object for investigating of the connections between the changes of the monoamine level and the activity pattern. The fact that the activity can be recorded not only under laboratory but also under natural conditions, represents a significant contribution to the analyses of the behavioural responses. This way one can

control the effects of the laboratory circumstances on the natural environmental conditions.

The present investigations were intended at answering the question, how far the pharmacons affecting the monoamine metabolism do alter the monoamine level and the activity, and how can we interpret those alterations from the point of view of regulatory mechanisms of the periodic activity?

Methods

Determination of 5HT

The 5HT content of 100—200 mg ganglion tissue was measured after homogenization in 0.1 n HCl using the method of KUNTZMAN et al. (1961).

Determination of catecholamines (CA)

About 200 mg of ganglion tissue was homogenized in acidified buthanol (CHANG, 1964), then centrifuged (5 min, 2000 rpm). The CA content of the supernatant was transferred to 1 ml of 0.1 n HCl in the presence of heptane by shaking (MAICKEL et al., 1968). The pH of this acidic phase was modified to 8.5 by addition of 10 vol Tris-HCl buffer (0.5 M, pH 8.5), whereas the isolation of CA using Al_2O_3 and determination was carried out by the method of ANTON and SAYRE (1962; 1964).

Treatments with pharmacons

Mussels of 150 g body weight were used. Their activity was recorded on actographs (SALÁNKI and BALLA, 1964).

The acitivity had been recorded for 3—5 weeks before the treatment took place, and for 2—5 weeks subsequent to the treatment depending on the effect. The treatments were carried out as follows: the pharmacons were dissolved in filtered Balaton-water, then 4 mussels were placed in 2 l of water containing the pharmacons: the animals were kept in the solutions for 24 hr, except that of tranylcypromine, where the treatment had only a 10 hr duration. After the treatment, the solutions were changed to normal running water. In the cases of control animals the running of the Balaton-water was stopped for the same period of time as that of the treatments. At different points of time, the 5HT and CA contents of the nervous systems of the animals were measured. The pharmacons used were as follows:

5HT-creatinine sulphate	10	μmole/l
NA-bitartarate	10	,,
DA-HCl	10	,,
p-Chlorphenylalanine (pCPA)	250	,,
α-methyl-metathyrosine (α-MMT)	512	,,
Reserpine (Inj. Rausedyl)	2.05	,,
Trans-2-phenyl-cyclopropylamine		
(tranylcypromine)	188	,,
Para-bromo-metamphetamine (V-111)	19	,,

Evaluation of parameters of the activity

The duration (T_A and T_R) as well as number (n_A and n_R) of active and rest (A and R) periods were measured in each case before and after the treatments *(Fig. 1)*. The sums of durations of active and rest periods were calculated (ΣT_A and ΣT_R) and so was the total duration of the investigation ($\Sigma T_A + \Sigma T_R$). The total duration of active and rest periods was expressed as a percentage of the total duration of investigations:

$$\frac{\Sigma T_A}{\Sigma T_A + \Sigma T_R} \times 100 \text{ and } \frac{\Sigma T_R}{\Sigma T_A + \Sigma T_R} \times 100.$$

The frequency of periodicity was:

$$\frac{n_A}{\Sigma T_A + \Sigma T_R} = \frac{n_R}{\Sigma T_A + \Sigma T_R}.$$

The average length of active and rest periods:

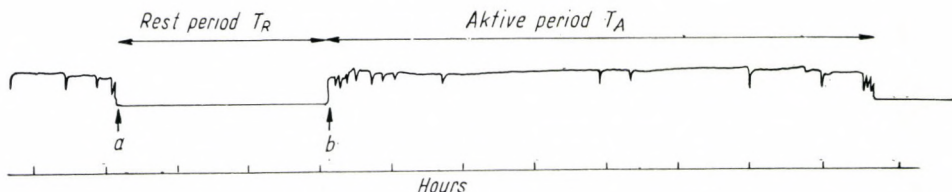$$\frac{\Sigma T_A}{n_A} \text{ and } \frac{\Sigma T_R}{n_R}.$$

Fig. 1. The periodicity of the activity. a: beginning of the period of rest; b: beginning of the active period
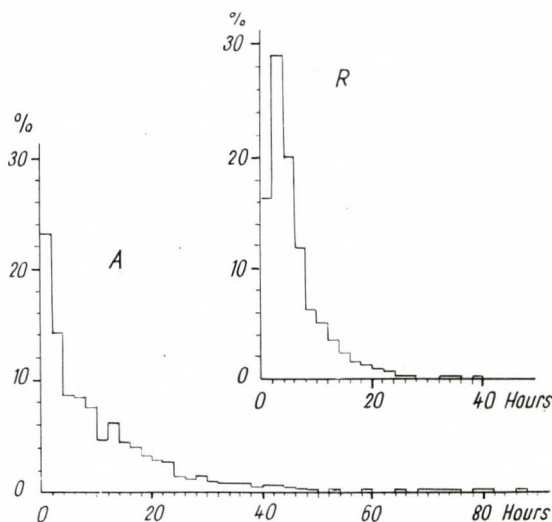

Fig. 2. The frequency distribution of the periods before the treatments

Considering the activity of all mussels before the treatment, we calculated the frequency distribution of the active and rest periods *(Fig. 2)*, and on the basis of activities of mussels after the treatment, the same parameter was determined.

The investigations were carried out in the months of April, May and June. The frequency distributions of the active and rest periods were calculated from 1400 periods before the treatment and from 300—350 periods after the treatment. The thin lines on the figures indicate this parameter before the treatment, whereas the thick ones do the same after the treatment.

Results

The effect of pharmacons affecting the monoamine level

In the first step we investigated whether the activity and monoamine level of the mussels are influenced by stopping of water current for 24 hr. The effect is shown in *Table I* based on the examination of 5 mussels. The parameters of the activity were calculated on the basis of 15 days before and 15 days after the stopping of water current, and the differences are expressed as percentages.

The results show unanimously that the stopping of water current induced no change in the periodic activity. The only change apparently significant (+18 percent) occurred in the average length of the periods of rest. However, this change can be explained by the wide dispersion of the average

TABLE I

The changes of the parameters of the periodic activity.
The changes are expressed in percent of the average values before treatment
(+) = *increase*   (−) = *decrease*

	Frequency of the periodicyt	Active time	Average length of active period	Average length of rest period
Control	− 0.7	− 4.2	− 1.5	+ 18.0
pCPA	+ 12.5	−12.5	−30.8	+ 26.9
α-MMT	+ 7.3	−12.4	−20.4	+ 51.5
Reserpine	+ 17.9	−30.3	−42.9	+147.9
Tranylcypromine	+ 56.0	− 2.3	−30.7	− 27.3
V-111	+ 36.3	+ 2.7	−20.9	− 11.7
5HT	− 4.7	+ 6.6	+81.2	− 18.2
DA	+114.5	− 0.1	−27.1	− 37.6
NA	+ 44.0	− 4.2	−43.8	+ 50.7

lengths of the periods (the S.D. value amounts to 100—150 percent of the average), and extreme values occurring in certain cases may distort unreally the average length. This was the situation in this case, too, since the change was not reflected in the other parameters of the activity.

The analysis of monoamine concentrations revealed the same result. The concentrations of 5HT, DA and NA showed only less than 10 percent change as compared to the animals kept in running water. In the case of 5HT even 3—5 days of anoxia induces no higher alteration.

Effects of 5HT, NA and DA

The animals become active within a few minutes after the administration of 5HT. If the adductor muscles are in tonic contraction, the 5HT relaxes them, and the frequency as well as amplitude of the quick, rhythmic contractions increase. This activity pattern persists during the treatment, then after the change of the water, the original level of activity is only slowly restored. The treatment and the slow cessation of the effect result in an active period of 20—60 hr. The rest periods following this long active one are of short duration. Already before the first period of rest one can observe short rest states when the tonic contraction of the adductors is of lower level than before the treatment and even later. This effect is more expressed when the monoamino-oxidase is inhibited by tranylcypromine *(Fig. 3)*. The increase of the frequency of rhythmic contractions is also of considerable extent during the active period. The change of distribution of active and rest periods *(Fig. 4)* indicates a decrease in the percentual rate of the short, active periods of 2—4 hr duration in favour of the longer ones. In the case of the rest periods the situation is reversed, namely the percentual rate of the periods of rest shorter than 2 hr significantly increases and the longer ones become considerably less frequent.
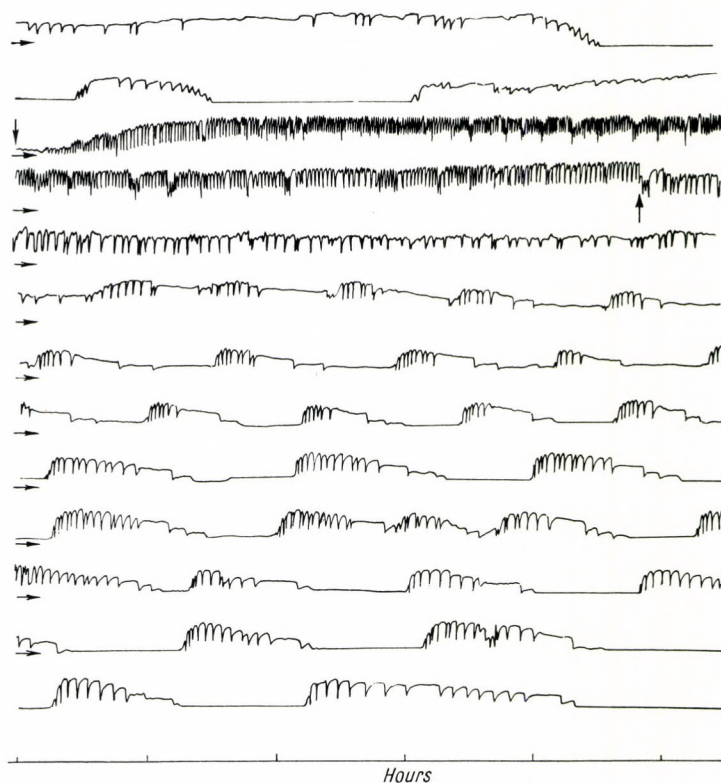

Hours

Fig. 3. Effect of tranylcypromine on the activity. Continuous recording. ↓ beginning of the treatment; ↑ end of the treatment; → level of tonic contraction before the treatment
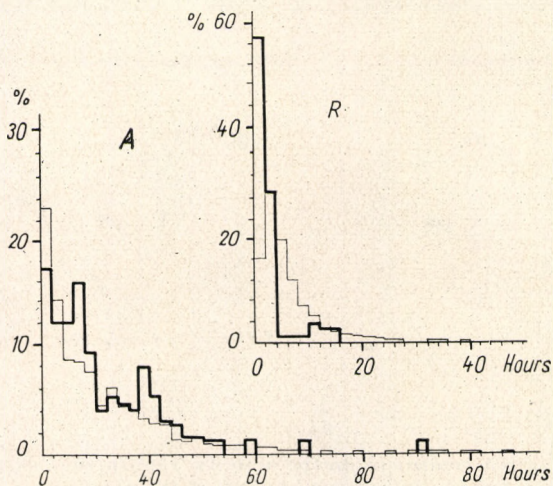
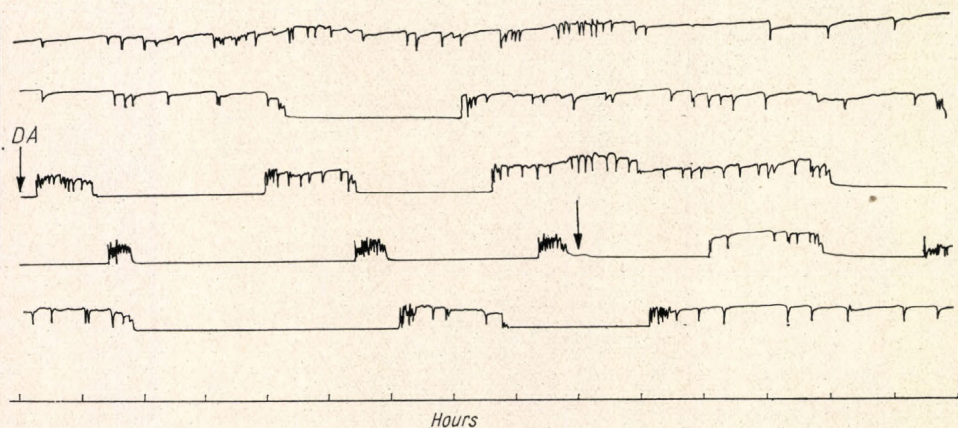*Fig. 4.* The frequency distribution of the periods after 5HT treatment

Fig. 5. The effect of DA treatment on the activity. Continuous recorping. DA beginning of the treatment; ↓ end of the treatment ↓

The effects of NA and DA can be observed practically only during the treatments. Both monoamines seem to interrupt the prolonged active periods by inducing short rest ones of 1—2 hr duration *(Figs. 5 and 6).* This way the frequency of periodicity significantly increases, nevertheless the percentual rate of the rest time remains unchanged *(Table I.).* This effect manifests itself in the percentual distribution of the active and rest periods *(Figs. 7 and 8),* in the increase of rate of the short active periods and decrease of that of the longer ones. At the same time, the rate of the rest periods shorter than 2 hr increases to a significant extent and that of the longer ones decreases.

It is of interest that while the DA decreases the average lengths of both active and rest periods, the NA does only that of the active ones, and increases that of the rest periods *(Table I.).*
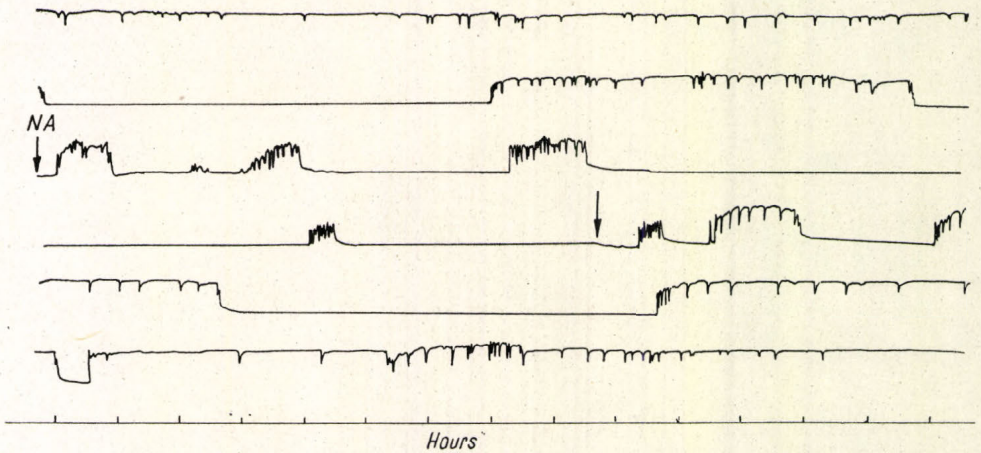
Fig. 6. The effect of NA treatment on the activity. Continuous recording. NA beginning of the treatment; ↓ end of the treatment ↓
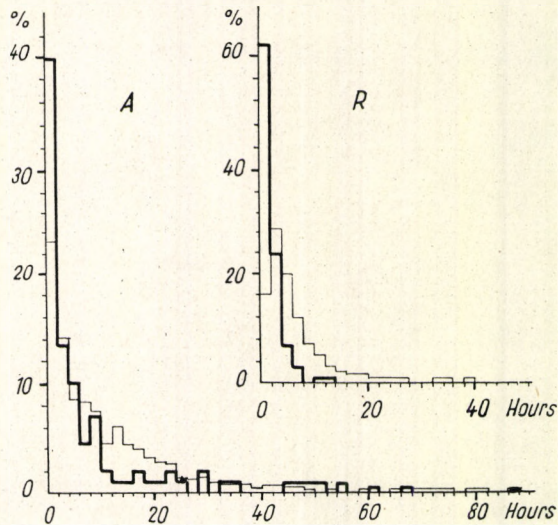

Fig. 7. The frequency distribution of periods after DA treatment

Effect of parachlorphenylalanine (pCPA)

The frequency of the periodicity, the average length of the rest periods as well as their total duration increase, whereas the total time of the active periods as well as the average lengths of them decrease (*Table I.*) upon the effect of the treatment.

The distribution of the active periods after the treatment shows a slight increase of active periods of mean duration (8—10 hr), while both the shorter and longer ones display a lower percentual rate. At the same time, the rate of rest periods shorter than 6 hr decreases, whereas that of the rest periods
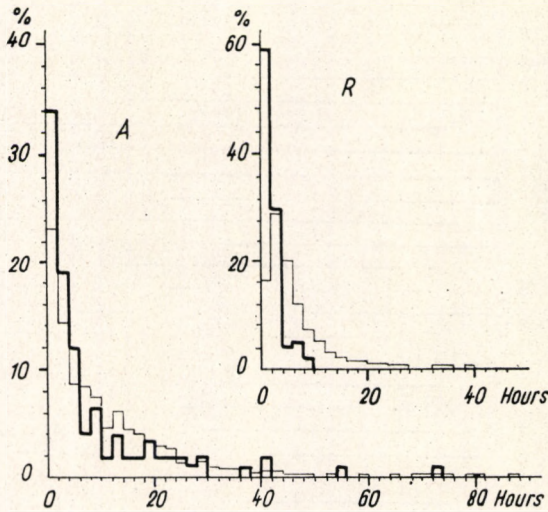
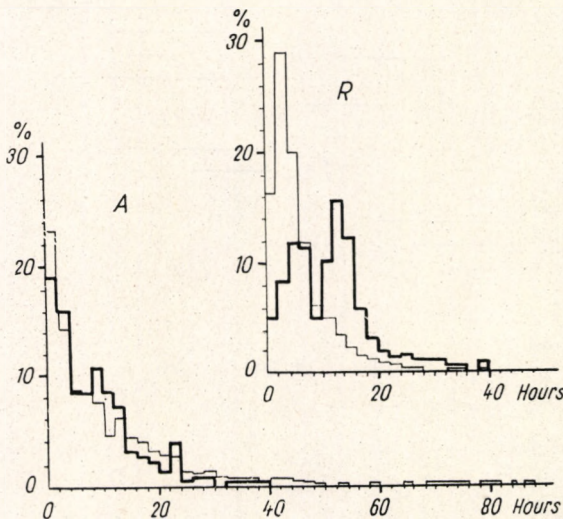*Fig. 8.* The frequency distribution of periods after NA treatment

Fig. 9. The frequency distribution of the periods after pCPA treatment

of 10—40 hr duration significantly increases *(Fig. 9)*. The decrease of the average length of the active periods apparently can be attributed to the total disappearance of the active periods longer than 40 hr. At the same time, the increase of the average length of the rest periods originates in the extension of the short periods up to 10—20 hr. The activity of the animals gradually decreases upon the influence of the treatment *(Fig. 10)*. The 5HT content of the ganglia also shows a gradual diminution *(Fig. 11)*.
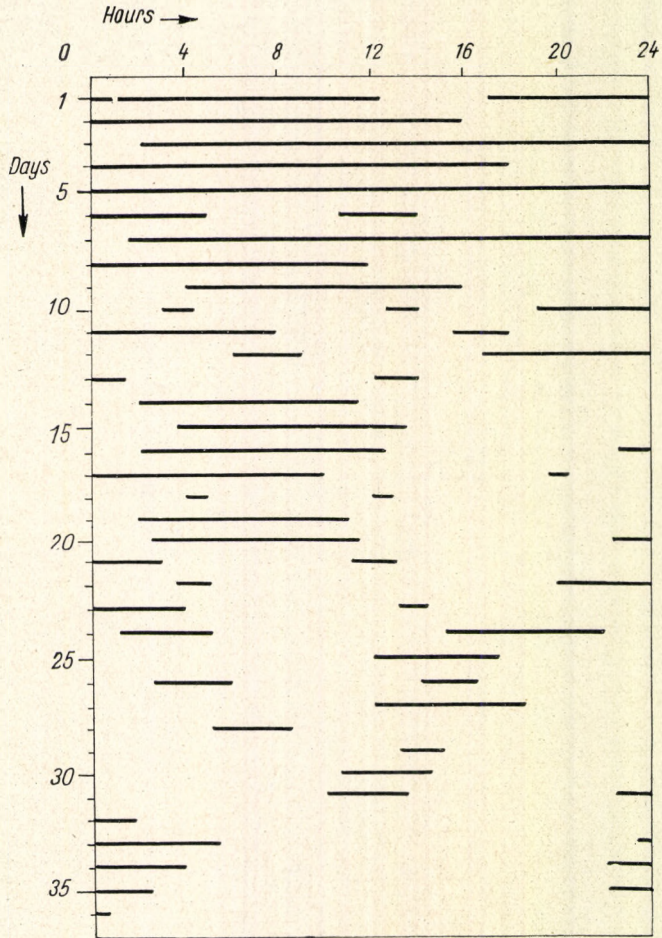
Fig. 10. The gradual decrease of the activity after pCPA treatment. The treatment took place on the 1st day
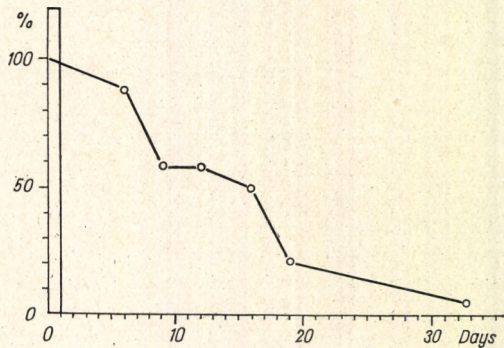

Fig. 11. The decrease of 5HT after pCPA treatment. The 5HT content of the treated animals is shown as a percentage of the control ones

The effect of α-methyl-meta-tyrosine (α-MMT)

The frequency of periodicity and the total duration of the rest periods slightly increased after the treatment, while the average length of the rest periods was 50 percent longer and the total active time and the average length of the active periods decreased *(Table I.).* The rate of the active periods
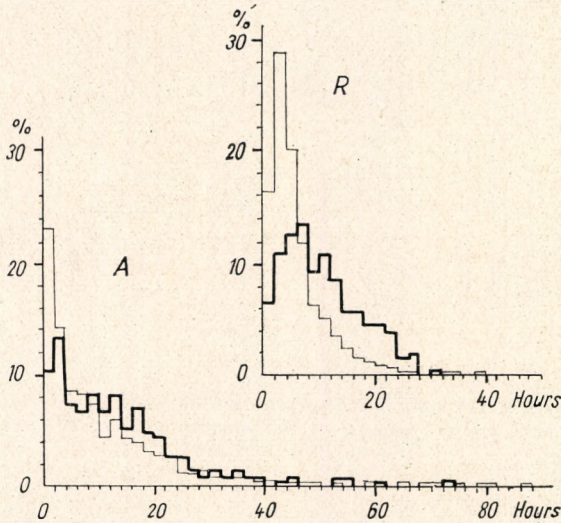

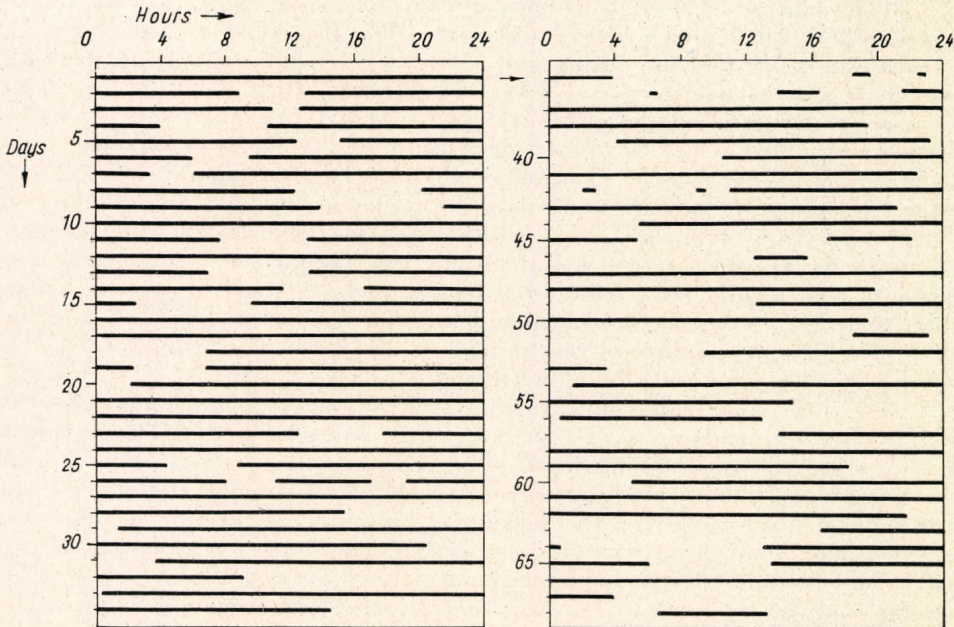*Fig. 12.* The frequency distribution of periods after α-MMT treatment

Fig. 13. The effect of α-MMT on the periodic activity. 1—34 days: activity before the treatment; 35th day: treatment; 35—68th days: activity after the treatment

shorter than 10 hr decreases, that of the longer ones (10—30 hr) increases. The distribution of the rest periods shows a more significant change. The rest periods shorter than 8 hr extend and result in a significant increase of the rate of rest periods of 10—30 hr *(Fig. 12)*. The effect of this treatment on the periodic activity is shown by *Fig. 13*. The alteration of CA is presented in *Fig. 14*. At the concentration of α-MMT used, the CA level decreased about 50 percent.
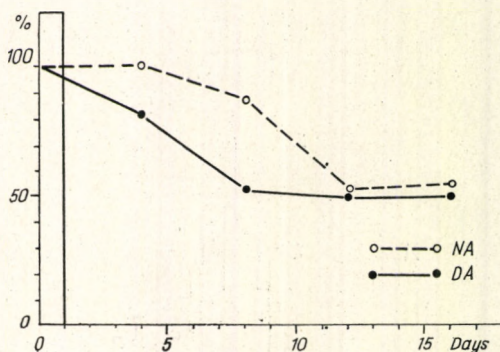

Fig. 14. The decrease of DA and NA after the α-MMT treatment. The concentrations of monoamines are given as percentages of the normal values. The treatment took place on the 1st day

Effect of reserpine

The changes induced by reserpine are more significant than those observed after pCPA and α-MMT treatments. The frequency of periodicity increases almost 20 percent during and after the reserpine treatment of 24 hr. The total active time decreases about 30 percent, while the total rest time increases to the same extent. The decrease of the average lengths of the active periods originates in the significant decrease of the active periods longer than 25 hr *(Fig. 15)*. Active periods longer than 25 hr occur mainly during and after the treatment, and in some cases the prolonged rest state is interrupted by an activity of 1—2 days 3—4 weeks after the treatment *(Fig. 16, 63—64—65th days)*. The average length of the rest periods increases nearly 150 percent. This should be attributed to the increase of rate of periods longer than 15 hr as well as to the decrease of rate of the periods of short duration *(Fig. 15)*. The appearance of rest periods of 40—60 hr is of significance, this has never been observed in the controls.

The reserpine treatment causes significant and quick changes in the ganglionic concentrations of all the three monoamines *(Fig. 17)*. The depletion of DA and 5HT is of the highest speed. By the end of the treatment, the concentrations of both amines decrease to about 50 percent. Ninety percent of DA becomes depleted by the 4th day, whereas the same rate of depletion is reached by the 6th day in the case of 5HT. The depletion of NA is slower. Fifty percent depletion is to be measured after 3.5 days, while 90 percent is reached only after 15 days. The concentrations of monoamines remain permanently low after the treatment and neither the monoamine level nor the activity seem to be restored during the first month subsequent to the treat-
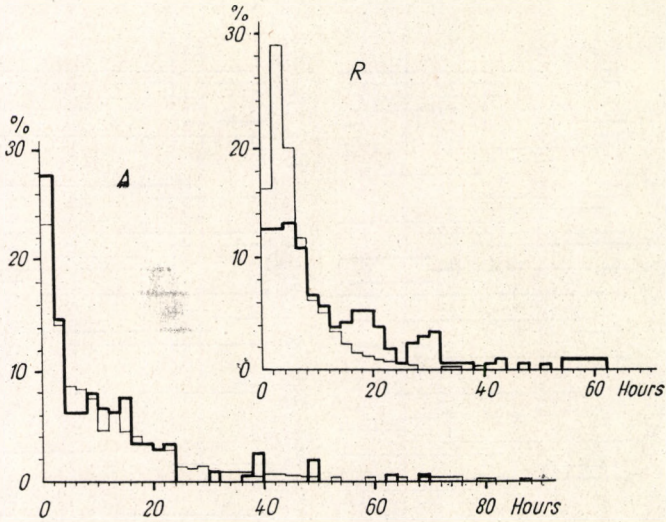
Fig. 15. The frequency distribution of periods after reserpine treatment

ment. What is more, the activity of the animal demonstrated in Fig. 16 remained at a low level even 2.5 months after the treatment.

Although systematic investigations concerning the quick, rhythmic adductor functions within the active periods have not been carried out, it deserves interest that the frequency of the quick, rhythmic activity increases to a high extent during the treatment as it was observed in the case of 5HT treatment, too. This frequency, however, strongly decreases during the 2nd and 3rd weeks following the treatment and is of lower value than before the treatment.

The effect of tranylcypromine

This treatment induces a very significant alteration of the activity of the animals resembling the effect of 5HT during the initial phase. This pharmacon increases the frequency of the periodicity to a very high extent, by 56 percent, whereas the rates of total active and rest times remain unchanged. The average lengths of both the active and rest periods decrease uniformly about 30 percent. The percentual distributions of both periods significantly change. The rates of short active and rest periods (less than 2 hr) markedly increase, while the rates of those longer than 6 hr become minimal *(Fig. 18)*. The response of the animals resembles to the effect of 5HT even from the point of view that during the treatment and cessation of the effect, prolonged active periods appear (between 30—70 hr), representing, however, only 0.1—0.2 percent of the number of periods. Similarly as after the administration of 5HT, the frequency of quick, rhythmic contractions greatly increases during the active periods appearing in the course of the treatment *(Fig. 3)*. The 5HT concentration of the nervous system is altered only to a low extent by the treatment *(Fig. 19)*.

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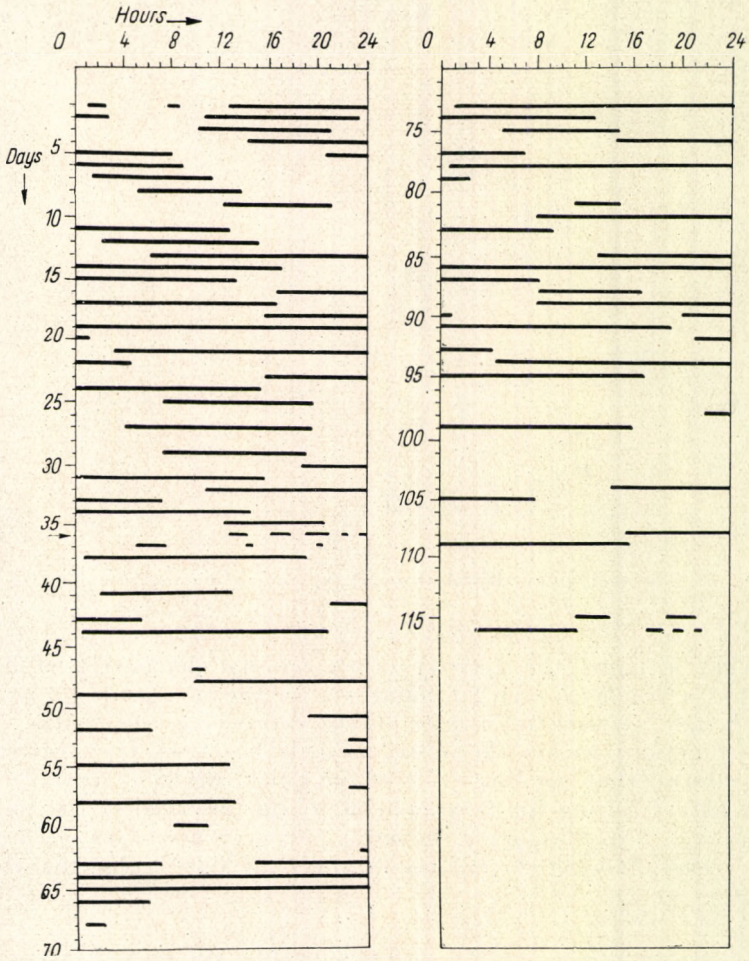
Fig. 16. The effect of reserpine on the periodic activity. 1—35th days: activity before the treatment; 36th day: treatment; 36—115th days: activity after the treatment. The lines following each other indicate the duration of the active periods, the interruptions do that of the passive ones
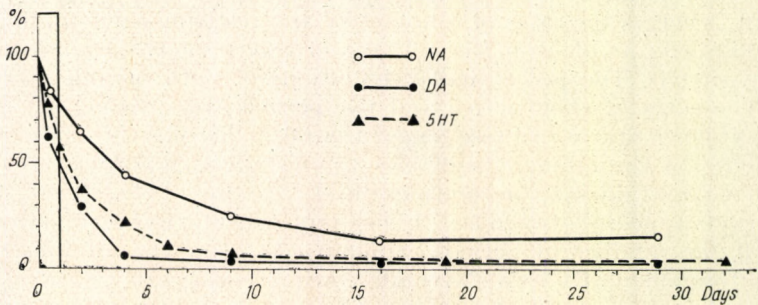
Fig. 17. The decrease of 5HT, DA and NA after reserpine treatment. The treatments took place on the 1st day. The concentrations of monoamines are expressed as percentages of those of normal ones
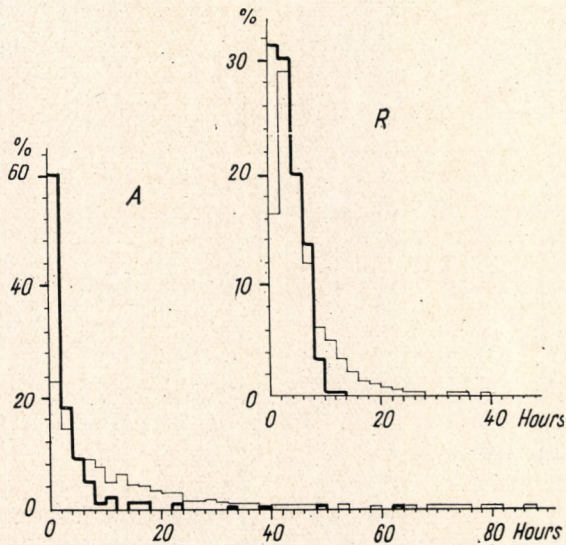
Fig. 18. The frequency distribution of periods after tranylcypromine treatment
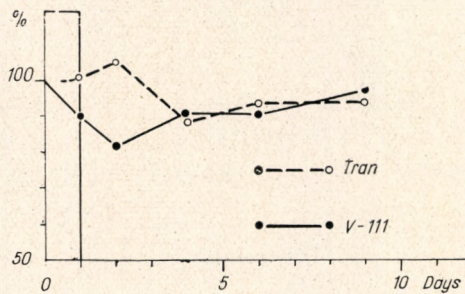
Fig. 19. The decrease of 5HT after tranylcypromine and V-111 treatments. The treatment took place on the first day. The concentrations of monoamines are given as percentages of those of the control animals

Para-bromo-metamphetamine treatment

The frequency of periodicity increases 36 percent after the treatment, the average lengths of both periods decrease, whereas the percentual rates of total active and rest times show only a minimal alteration *(Table 1)*. The percentual distribution of duration of both the active and rest periods displays a considerable change. The rate of active periods shorter than 8 hr increases, while that of the longer ones decreases. The rate of the rest periods shorter than 4 hr also increases, whereas that of the longer ones significantly decreases *(Fig. 20)*. The 5HT level shows an unequivocal decrease (maximally 18 percent), restored by the 8th day *(Fig. 19)*
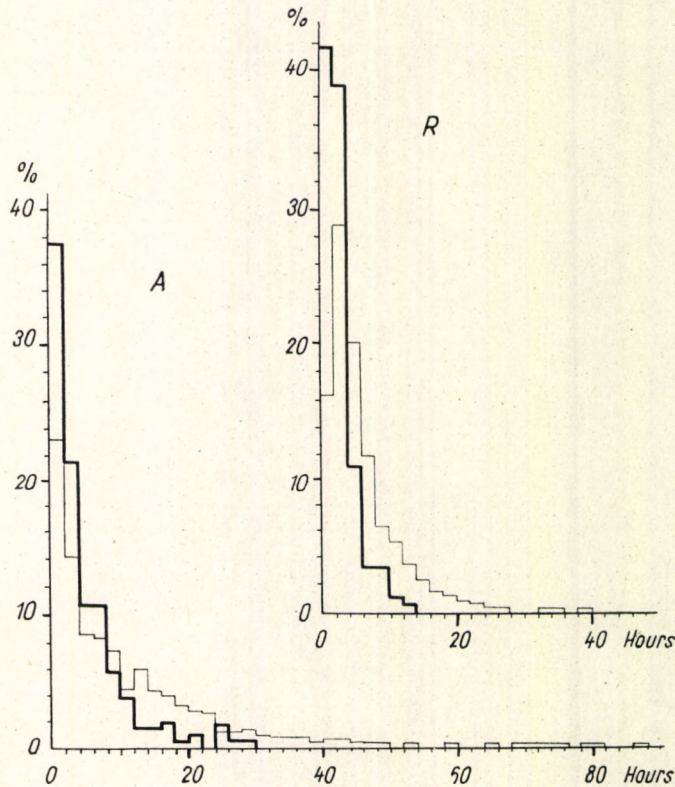
Fig. 20. The frequency distribution of periods after V-111 treatment

Discussion

The analysis of the possible mechanism of rhythm regulation revealed that centres of "activity" and "rest" localized in the central nervous system are responsible for its determination and maintenance (SALÁNKI, 1970). These centres function through the transmitter system or systems.

Earlier investigations have rendered probable that in the fresh water mussel the transmitter system of the centre of "activity" is of serotoninergic nature, since 5HT relaxes the adductor muscles on both ganglionic (SALÁNKI, 1963) and muscular (SALÁNKI and LÁBOS, 1969) levels, furthermore, significant concentrations of it are present in both the nervous system and the adductors (HIRIPI, 1968).

The functional presence of this system in the relaxation of the adductors from the tonic contraction is supported by the earlier findings when stimulating the CVc (SALÁNKI and HIRIPI, 1970). The hypothesis was obvious that if the 5HT caused relaxation not only applied to the ganglia but also at the level of the adductors (SALÁNKI and LÁBOS, 1969), the stimulation of the CVc resulting in the relaxation, too, (SALÁNKI and LÁBOS, 1963) should influence the 5HT level of the adductors. The increase of 33 and 25 percent in the 5HT

concentration of the adductors upon the influence of the stimulation proved the correctness of that hypothesis. Since the adductors do not synthesize 5HT, this process takes place only in the nervous system (HIRIPI and SALÁNKI, 1969) and mainly so does the storage of it (HIRIPI, 1968; HIRIPI et al., 1972), the 5HT demand of the relaxation of the adductors as well as the increase of the 5HT level should originate in the ganglia. The 5HT from the ganglia reaches the adductors probably by means of an active transport independent from the concentration, where it takes part in the relaxation of the adductors depleting at the neuromuscular level.

Since the periodic activity of the mussel consists of an alternation of tonic contraction and relaxation of the adductors, it seemed to be reasonable to investigate, whether any change of the 5HT level can be observed in the adductors during the spontaneous, physiological active and passive periods, being in accordance with those observed after the stimulation of the CVc. According to our results, the 5HT level was higher in the adductors at the beginning of the active period, i.e. at the spontaneous relaxation of the adductors, than at the beginning of the rest state (SALÁNKI and HIRIPI, 1970). The increase of 5HT level (30 percent in the anterior and 25 percent in the posterior adductor) is practically identical with the values observed after the stimulation of the CVc, although this increase was not significant statistically in the posterior adductor at the beginning of the spontaneous relaxation. The increase of 5HT level of the adductors was followed by a change of 5HT level only in the visceral ganglion among the three ganglia. Nevertheless, the change amounting to 23 percent in the visceral ganglion was of opposite direction as compared to that of the adductors. If according to the chain of ideas mentioned above, the 5HT content of the adductors originates in the ganglia, a significant difference should exist between the cerebral and visceral ganglia as regards their regulatory mechanism toward the anterior and posterior adductors, respectively, connected with the alteration of 5HT level. Namely, the decrease of 5HT level of the visceral ganglion at the beginning of the activity can be interpreted in the way that the higher 5HT demand of the adductor relaxation is satisfied from the visceral ganglion at an unchanged rate of synthesis even during the active transport, resulting in a decrease of the ganglionic 5HT level. At the same time, in the cerebral ganglion a periodic change of the synthetic speed may assure a constant 5HT level independently from the periodic activity. This way the cerebral ganglion possesses a primary role in the regulation of the periodicity of the activity, which is in accordance with the conclusions drawn from other experiments that the cerebral ganglion regulates primarily the relaxation of both adductors (PAVLOV, 1885; VERESCHAGIN, 1960; SALÁNKI et al., 1968).

Our results prove the hypothesis with a high probability that the serotoninerg system functions as a chemical regulatory system of the centre of "activity".

The question arises what system regulates the centre of "rest"? The results of the pharmacological analysis of the serotoninergic system as well as the independence of the 5HT level from the state of activity in the cerebral ganglion exclude the possibility that the decrease of 5HT in itself can induce the appearance of rest period. Pharmacological data show that among the CA NA and adrenaline are able to induce tonic contraction of the adductors at the muscle level of *Anodonta* (SALÁNKI and LÁBOS, 1969), however, at the

ganglionic level they induce the relaxation of the adductors similarly to the effect of 5HT (SALÁNKI, 1963). Nevertheless, this effect is produced by concentrations of them an order of magnitude higher than that of 5HT. At the same time electrophysiological results revealed that both DA and NA cause prolonged inhibition of certain nerve cells of Gastropoda (GERSCHENFELD and TAUC, 1964; GLAIZNER, 1968; KISS and SALÁNKI, 1971; WALKER et al., 1971), and what is more, the NA blocks the activity of the Br-cell showing a characteristic rhythm in the nervous system of *Aplysia* (BOISSON and CHALAZONITIS, 1972). The different chemical sensitivity as well as the different effects of the same transmitter on various neural pathways have also been demonstrated in the ganglia of *Anodonta* (SALÁNKI and VARANKA, 1971). In the light of these pharmacological results one cannot exclude the catecholaminergic system as being the transmitter system of the centre of "rest", so much the more as significant concentrations of DA and NA are present in the nervous system of the fresh water mussel (HIRIPI, 1972).

The pharmacological investigations influencing the function of the above transmitter systems proved to be suitable to draw further conclusions concerning the existence and function of the centres of "activity" and "rest".

The prolonged active state, induced by exogenous 5HT can be interpreted that the increase of 5HT level significantly altered the dynamic balance of centres of "activity" and "rest" and the effect of the former became realized. The 5HT acts probably both periphperically at the level of the adductors and centrally on the ganglia. The effect manifests itself even after the treatment when the 5HT acting peripherically is not present any more. We assume that the effect is prolonged by the part of 5HT picked up and stored by the nervous elements. The subcellular localization of 5HT showed that a significant part of the endogeneous 5HT is bound to synaptosomes (HIRIPI et al., 1972). It has also been shown by our earlier investigations that 5HT injected into the foot musculature is taken up by the nervous system, and apart from the increase of activity, this 5HT maintains a higher 5HT level of the ganglia for several days (HIRIPI and SALÁNKI, 1971). The exogeneous 5HT is distributed among the subcellular fractions to the rates identical with those of the endogeneous one (unpublished observations). This way the exogeneous 5HT taken up mainly by the synaptosomes shifts the balance toward the centre of "activity" for several days. The effect of the centre of "rest" becomes limited in time so that it can maintain only short periods of rest besides the long active periods. This is also indicated by the change of distribution of durations of the active and rest periods, namely by the increase of the rate of the short, rest periods of 2 hr as well as that of the more prolonged active ones.

The serotoninergic system is probably one of the regulatory factors of the quick, rhythmic contraction activity, too. Namely, all pharmacological treatments flooding the neuromuscular and ganglionic serotoninergic receptors with 5HT, increased the frequency of the quick rhythm to a high extent. This effect appeared during the treatment with 5HT, the MAO-inhibitor tranylcypromine and the reserpine.

The results of DA and NA treatments indicate that the dopaminergic and noradrenergic systems functioning in the centre of "rest" are not able to overcompensate the serotoninergic one permanently to such a degree that the rest period would be able to get a significant predominance. The DA can

activate the function of the centre of "rest" by inducing short rest periods interrupting this way the function of the centre of "activity". This results in the shortening of both the active and rest periods as well as the 100 percent increase of the frequency demonstrated well by the change of distribution of the periods.

The NA stimulates the "rest centre" by interrupting the prolonged active periods with more prolonged rest ones than existed before, increasing this way the frequency and, as against to the DA, also the average length of the rest periods. This difference in the effects indicates that DA acts not only as a precursor increasing the concentration of NA, but also as an independent dopaminergic component. The justification of this assumption is supported by the pharmacological investigations cited formerly, evidencing the specific, prolonged inhibitory effect of DA on certain neurons. Notwithstanding, the two systems cannot be completely separated, since DA as the precursor of NA may also act through the alteration of concentration of NA. Since there are no data at our disposal concerning the change of the CA level at the spontaneous change of periods, any further analysis of the mechanism would only be mere speculation. However, on the basis of our results one can assume that the catecholaminergic system is able to function as a transmitter system of the centre of rest.

In the further experiments we attempted to reveal the mechnaisms of the two systems mentioned above by means of pharmacological influences. The function of the centre of "activity" was attempted to be inhibited through the blocking of synthesis of its transmitter.

It has been evidenced by KOE and WEISSMANN (1968) that pCPA inhibits specifically the synthesis of 5HT in vertebrates without any considerable influence on the catecholamines. The slow but significant decrease of 5HT appears even in the fresh water mussel. The interpretation of the alterations of activity fits well the model constructed for the explanation of regulations.

The analysis of the pCPA effects leads also to the former conclusion that 5HT plays a decisive role in the function of the centre of "activity". However, it is conspicuous that even in spite of the considerable decrease of 5HT level, the centre of "rest" does not reach that degree of predominance which could be expected on the basis of the decrease of 5HT level. This may be explained by assuming that 5HT is present in two pools in the nervous system. One of them is an active pool stored mainly in the synapses and easily mobilized, the other one is of inactive state and forms a reserve. The balance of them assures the level of the active pool even in cases of wide variations of the reserved 5HT. Probably, the ganglionic 5HT is stored in the reserve pool in a greater concentration where the decrease of 5HT level does not alter the function of the active pool. Of course, this is true only for a critical level.

The decrease of synthesis distorts the balance of the active and reserve pools to such a degree that the alteration of the active pool will damage the serotoninergic mechanism. This way the centre of activity seems to be exhausted and the effect of centre of rest becomes predominant. This is why the average length of the active periods decreases and that of the passive ones increases.

The p-brom-metamphetamine, as KNOLL and MAGYAR (1971) described, specifically affects the serotoninergic system so that it does not inhibit the

synthesis but does the uptake of 5HT and causes 5HT liberation. The concentration used altered the 5HT level only to a slight extent, namely decreased it. Notwithstanding, a characteristic change appeared in the activity. The increase of the frequency and decrease of the active and rest periods are reflected even in the significant change of the percentual distribution of the periods. This effect of V-111 differs from that of pCPA and the difference can be explained just by the various mechanisms of effect. While the effect of pCPA inhibiting the synthesis is prolonged and appears to be considerable at the critical level of the reserve pool, that of V-111 appears quickly. The inhibition of incorporation into the synaptosomes may result in the damage of both pools, thus the active transport may be influenced through the stabile pool. The effect of V-111, however, induces no prolonged passive period, since both pools quickly regenerate during the rest, resulting in the disconnection of the effect of the centre of rest.

It is of interest that the alteration of the catecholaminergic system assumed to be the transmitter system of the centre of "rest" by α-MMT induces an effect like the pCPA. However, it increases the frequency only to a lower extent, the decrease of the average length of the active periods is smaller, but the increase of average length of the rest periods is far more significant. While in the case of pCPA treatment the rest periods being shorter than 8 hr are extended to 15—20 hr at the expense of the active ones longer than 15 hr, in the case of α-MMT treatment the rest periods shorter than 8 hr increase to 15—25 hr so that the duration of the active periods hardly changes. This effect of α-MMT supports further the hypothesis that the catecholaminergic system might be the transmitter of the centre of rest. CARLSSON and his group demonstrated (CARLSSON, 1964) in vertebrates that the depletion of NA caused by α-MMT is mediated by a corresponding decarboxylated compound of this pharmacon. Namely, after α-MMT treatment one can measure both the α-methyl-metatyramine formed by decarboxylation and metaraminol formed by dopamine β-hydroxylase from the latter compound. The binding of these compounds is stronger than that of NA in the brain.

The competition plays important role in the depletion mechanism of NA caused by α-MMT, since the appearance of the α-methylated amine is of the same order of magnitude as that of the disappearing NA. However, the inhibition of the synthesis may also contribute to this process.

The effect of α-MMT in *Anodonta* may well be interpreted in the light of the data obtained in vertebrates and fits well the basic hypothesis.

The catecholaminergic system assures the predominance of the centre of rest so that the depleted NA stimulates the inhibitory neurons. The decrease of concentration caused by the depletion, however, results in no damage of the centre of rest, since the methylated products substitute the NA and are able to maintain the noradrenergic transmission (CARLSSON, 1964; SHORE et al., 1966).

Comparing the effects of α-MMT and the two catecholamines investigated, the extension of the rest periods caused by α-MMT seems to be realized through the effect of endogeneous NA, which may be modulated by the decrease of dopamine level by means of a yet unknown mechanism. It is of interest to compare the effect of chlorpromazine observed in vertebrates and mussels from the view-point of the role of catecholamines. Besides other effects, it induces a supersensitivity toward CA in vertebrates by blocking

the inactivation performed by binding, therefore, a higher concentration of the free CA is present at the receptor sites (AXELROD, 1964).

If the mechanism of the effect is the same even in the nervous system of *Anodonta*, the prolonged tonic contraction induced by chlorpromazine (SALÁNKI, 1963) and the effects of CA, mainly the extension of the passive periods caused by NA may be the consequences of increase of CA-saturation of the inhibitory neurons.

When analyzing the effects of reserpine, the conclusion can be drawn that the effects are brought about by common influences of both serotoninergic and catecholaminergic systems, however, the change of the former is of decisive role. Upon the influence of reserpine the depletion of 5HT and DA is quick and nearly exponential, whereas that of NA is much more prolonged. Comparison of the effects and the depletion shows that during the treatment the prolonged active periods and the quick rhythmic activity are caused first of all by the quick depletion of 5HT. This effect predominates so that the centre of rest is only rarely able to sustain the activity during the treatment. The reserpine causes depletion of the labile 5HT pool with the inhibition of incorporation in that pool (COSTA and BRODIE, 1964; CARLSSON, 1964). However, the depletion is so quick during the treatment that the liberated 5HT cannot be inactivated by MAO completely, therefore the ganglionic level of free 5HT increases. This may stimulate the neuronal activity inducing the active transport and may increase even the speed of the passive diffusion toward the effector organs. Because of the short distance between the ganglia and the adductor muscles, the diffusion of 5HT can be so intense that it may substitute even the active transport. The receptors of the adductors become greatly saturated the effect of which resembles that caused by 5HT and tranylcypromine. After the quick depletion of 5HT, the activity of the centre of rest is induced not only by the significant concentration of NA but also by the exhaustion of the centre of activity. After the extensive decrease of 5HT level, the labile pool drops below the critical level and becomes unable to maintain the function of the stabile pool. The prolonged rest periods appear and at minimal levels of the amine concentration and not infrequently periods of rest occur of even several days, duration. This is indicated by a 150 percent increase of length of rest periods and by the change of the percentual distribution of the periods. In this state the limiting factor of appearance of an active period is the speed of recovery of the stabile pool. This way from the point of view of reserpine effects not the level of depletion but the speed of it is of decisive role, which has been evidenced in vertebrates by BRODIE and RAID (1968).

This is indicated also by our observations that two different doses may induce the same level of depletion at different points of time, and the larger one causing a much quicker depletion increases the frequency of the quick rhythm to a high extent, whereas the smaller one shows only minimal effect of this type. It has earlier been mentioned that the MAO-inhibiting tranylcypromine induces an effect resembling those of 5HT and reserpine. The administration of it causes a prolonged active period during which even the rhythmic activity increases, meanwhile the 5HT level of the ganglion remains practically unchanged. It has been shown in vertebrates that the inhibition of MAO increases the 5HT level (GARATTINI and VALZELLI, 1965). The presence of MAO has been demonstrated in the nervous system of *Anodonta* and it

takes part in the inactivation of 5HT (HIRIPI and SALÁNKI, 1971). However the investigations of different tissues of *Anodonta* led to the conclusion that most part of 5HT is not inactivated in the ganglia but in the kidney. The first step of this way of inactivation is the depletion of 5HT from the ganglia toward the kidney taking place through diffusion and the participation of the circulatory system. This agrees with the results obtained on other objects (GERSCHENFELD and STEFANI, 1968; CARDOT, 1964; MIROLLI, 1968), indicating at the same time that in some species the MAO does not take part in the inactivation of 5HT in the ganglia at all. This has also been evidenced by the investigations demonstrating that MAO-inhibition induces no increase in the ganglionic 5HT level (KERKUT and COTTRELL, 1963).

The inhibition of the enzymatic inactivation, however, results in the increase of the 5HT level only in the case if that is the single way of elimination. The inhibition of MAO in *Anodonta* resulted in the increase of 5HT content in the peripheral organs. The 5HT content of the ganglia does not increase, since the 5HT uninactivated by MAO is eliminated by diffusion and circulation. The rate of increase may be much more higher in the adductors and the saturation of 5HT receptors of adductors may come into being. Reaching the critical level, the adductors relax. However, the 5HT not inactivated in the ganglia may increase also the active axonal transport by stimulating the neurons and this further increases the 5HT concentration of the adductors. The restitution, i.e. the appearance of periods of rest depends on the rate of regeneration of MAO. The 5HT inactivation increases depending on the speed of regeneration of the enzyme molecules and below a critical 5HT level, the adductors may again show a tonic contraction.

The administration and effect of MAO-inhibitors can be interpreted in a different way in case of CA as in that of 5HT. Since the catechol-o-methyltransferase is able to inactivate the CA not inactivated by MAO, the unchanged CA level indicates that the centre of rest is not affected by the MAO-inhibition.

The pharmacological investigations revealed at the same time that apart from the change of ganglionic 5HT level, first of all, the 5HT transport toward the adductors plays a decisive role in the activity.

According to our assumption, the fast axonal transport existing in the nerves of *Anodonta* (HESLOP and HOWES, 1972) may represent the active transport. According to the data of the above authors the axonal transport slightly differs from that of vertebrates. This transport is controlled and independent from the pressure and concentration gradients. It is inhibited by much lower concentrations of dinitrophenol and cianid, indicating its metabolic dependence. The most striking difference manifests itself in the effect of temperature, since it is independent from the temperature between 4 and 12° C, and linearly increases with the further increase of the temperature. The temperature dependence of the axonal transport agrees with that of the activity of the fresh water mussel. The investigation of seasonality and temperature dependence of the activity as well as of 5HT level (unpublished results) indicates that under natural circumstances the activity observed at low temperatures, begins to be normalized at 8—10° C, after which the axonal transport linearly increases with the temperature.

Summary

On the basis of an earlier working hypothesis we investigated whether the pharmacological influences on the transmitter systems of the assumed centres of "activity" and "rest" responsible for the regulation of the rhythm of fresh water mussel (*Anodonta cygnea* L.) act on the monoamine levels and the periodicity of the activity. The serotoninergic system of the centre of activity was influenced by inhibition of synthesis (pCPA) and the inactivating enzyme (tranylcypromine) by depletion of 5HT (Reserpine, V-111) as well as by exogeneous 5HT.

The catecholaminergic system of the centre of rest was altered by exogeneous dopamine and noradrenaline, α-methyl-metatyrosine, reserpine and tranylcypromine.

Investigating the periodic activity and the monoamine levels, it was found:

1. The 5HT level plays a decisive role in the function of the centre of activity. The prolonged activity caused by exogeneous 5HT is a consequence of prolonged increase of 5HT level, manifesting itself primarily in the increase of the average length of active periods.

2. The ganglionic 5HT level was not increased by the MAO-inhibiting tranylcypromine, however, prolonged active periods and great increase of rhythmic contractions were induced by this drug through the absence of the peripheric inactivation. After the treatment the frequency of rhythmicity was increased by decreasing the average lengths of active and rest periods.

3. The decrease of 5HT level caused by pCPA and reserpine, resulted in the decrease of average length of active periods and the increase of that of the rest ones. The p-brom-metamphetamine increases mainly the frequency of rhythmicity through a slight decrease of 5HT level.

4. The dopamine decreases the average length of both the active and rest periods, whereas the noradrenaline increases that of the inactive ones. The effect of α-MMT is probably realized through the effect of NA and the significant increase of the rest periods is caused by the liberation of NA.

5. After reserpine treatment the predominance of the centre of rest is induced partly by the significant decrease of 5HT level and partly by the considerable NA level persisting because of the slower depletion of NA.

6. The 5HT level in itself cannot determine the function of the centre of activity. The active transport toward the adductors takes also part in the relaxation of the adductors, i.e. in the regulation of the active period. The decrease of 5HT level influences the active transport only below a certain critical level.

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A PERIODIKUS AKTIVITÁS SZABÁLYOZÁSI MECHANIZMUSÁNAK—FARMAKOLÓGIAI VIZSGÁLATA TAVI KAGYLÓN (*ANODONTA CYGNEA* L.)

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Összefoglalás

Egy korábbi munkahipotézisünkből kiindulva vizsgáltuk, hogy a tavikagyló (*Anodonta cygnea* L.) ritmusának szabályozásáért felelős „aktivitási" és „nyugalmi" központok feltételezett transzmitter rendszerének farmakológiai befolyásolása hogyan hat a monoamin szintre és az aktivitás periodicitására. Az aktivitási központ szerotoninerg rendszerét a szerotonin szintézis gátlásával (pCPA), a lebontó enzim gátlásával (tranilcipromin), a szerotonin kiürítésével (reserpin, V-111) és exogén szerotoninnal befolyásoltuk.

A nyugalmi központ katecholaminerg rendszerét exogén dopaminnal és noradrenalinnal, α-metil-meta-tirozinnal, reserpinnel és tranilciprominnal befolyásoltuk.

A periodikus aktivitást és monoamin szintet vizsgálva azt találtuk, hogy:

1. A szerotonin szint meghatározó szerepet játszik az aktivitási központ működésében. Az exogén szerotonin által okozott tartós aktivitás a szerotonin szint növekedésének következménye, mely elsősorban az aktív periódusok átlaghosszának növekedésében jelentkezik.

2. A monoamino-oxidáz gátlószer tranilcipromin nem növeli a ganglionáris szerotonin szintet, de a perifériás inaktiváció hiányán keresztül tartós aktív periódust és a ritmikus kontrakciók nagymértékű növekedését eredményezi. A kezelés után pedig a ritmicitás frekvenciáját növeli az aktív és nyugalmi periódusok átlaghosszának csökkentésén keresztül.

3. A szerotonin szint csökkenése pCPA és reserpin hatására az aktív periódusok átlaghosszának csökkenését és a nyugalmi periódusok hosszának növekedését eredményezi A p-bróm-methamphetamin a szerotonin szint kismértékű csökkentésén keresztül elsősorban a ritmicitás frekvenciáját növeli.

4. Míg a dopamin mind az aktív, mind a nyugalmi periódusok átlaghosszát csökkenti, addig a noradrenalin a nyugalmi periódust növeli. Az α-MMT hatása valószínű-

leg a NA hatásán keresztül realizálódik és a felszabaduló NA okozza a nyugalmi periódus jelentős növekedését.

5. A reserpin kezelés alkalmával a nyugalmi központ túlsúlyát a szerotonin szint jelentős csökkenésén túl a NA lassúbb kiürülése folytán még meglevő jelentős NA szint is okozza.

6. A szerotonin szint önmagában nem meghatározója az aktivitási központ működésének. Elsősorban a záróizom irányába történő aktív transzport az a folyamat, amely a záróizom relaxációjában, azaz az aktív periódus szabályozásában részt vesz. Az 5HT szint csökkenése csak bizonyos kritikus szint alatt befolyásolja az aktív transzportot.