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THE EFFECT OF MONOAMINE OXIDASE INHIBITORS ON THE GANGLIONIC SEROTONIN AND CATECHOLAMINE LEVELS AND ON THE ACTIVITY IN THE FRESH WATER MUSSEL (*ANODONTA CYGNEA* L., PELECYPODA)

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The diurnal and seasonal variations of the monoamine levels in the central nervous system parallel with behavioural changes both in vertebrates (FRIEDMAN and WALKER, 1968; QUAY, 1963; 1967; UUSPÄÄ, 1963; SPAFFORD and PENGELLEY, 1971; HARRI, 1972 a, b) and in invertebrate animals (CARDOT, 1971; HIRIPI and SALÁNKI, 1973b; SALÁNKI et al., 1974) show that serotonin and catecholamines take part in the regulation of the activity and rest.

In the regulation of the activity of the adductor muscles being the effectors of activity, important role is attributed to serotonin and catecholamines in *Anodonta* (SALÁNKI and HIRIPI, 1970; SALÁNKI, 1972; SALÁNKI and LÁBOS, 1969). Concerning the pharmacological investigations the inhibition of serotonin synthesis or depletion of the stored serotonin resulted in the decrease of the activity. At the same time, the monoamine oxidase inhibitor (MAOI) tranylcypromine did not increase the serotonin level of the nervous system, although it influenced the activity markedly (HIRIPI, 1973).

MAO inhibitors increase the concentration of both the serotonin and the catecholamines in the nervous system of vertebrates (GARATTINI and VALZELLI, 1965), and they alter the activity, too.

As the effect of the tranylcypromine on *Anodonta* was similar to that of the serotonin at the begining of its action, while in the later phase to the effect of catecholamines, it seemed to be desirable to investigate the effect of the tranylcypromine and other MAO inhibitors on the level of serotonin and catecholamines as well as on the activity of the animal.

Methods

The pharmacological treatment was carried out as described earlier (HIRIPI, 1973) adding the drugs into 2—3 l Balaton water in which animals were kept. MAO inhibitors actomol, pargyline, nialamide, iproniazid and tranylcypromine were used in 10^{-4} mol concentration. Serotonin was estimated by the method of SNYDER et al. (1965) while noradrenaline and dopamine by the method of SHELLENBERGER and GORDON (1971), and the concentra-

tions of the monoamines were expressed in the percent of the control values. Control animals were stored in the same volume of Balaton water as the animals receiving the pharmacons.

The activity of the animals was recorded on actographs (SALÁNKI and BALLA, 1964) during a 10—14 days period before and after the treatment. The change of the activity was calculated from the records obtained before and after the treatment. The average length of active and rest periods, the percentual proportion of active and rest times and the frequency of the periodicity were taken into consideration (HIRIPI, 1973). The altered values of the parameters are expressed in the percent of the average values observed before the treatment.

Results

Treatment of the animals with MAO inhibitors did not significantly alter the serotonin level of the central nervous system *(Table 1)*. The values obtained after the treatment show a rather small increase reaching a maximum of only 18 per cent. The tranylcypromine decreased the serotonin level.

TABLE 1

Change of the 5HT level in the ganglia of Anodonta cygnea after treatment with MAO inhibitors. The 5HT level is expressed in percent of the control

MAOI	Duration after treatment (day)			
	1	2	4	7
Actomol	104.3	109.4	109.9	—
Pargyline	114.2	106.9	104.9	104.9
Nialamide	97.4	117.9	105.1	99.2
Iproniazid	106.3	116.2	105.0	115.8
Tranylcypromine	93.4	109.3	89.0	94.7

Similarly to the serotonin, the dopamine and noradrenaline levels of the nervous system were not changed significantly after the treatment *(Tables 2* and *3)*. Some differences were found between the changes of the dopamine and noradrenaline levels. The inhibitors generally increased the noradrenaline concentration. The nialamid and iproniazid increased while the others decreased the dopamine level, however, the changes were not higher than 25 per cent.

TABLE 2

Change of the DA level in the ganglia of Anodonta cygnea after treatment with MAO inhibitors. The DA level is expressed in percent of the control

MAOI	Duration after treatment (day)		
	1	2	4
Actomol	97.3	89.1	80.9
Pargyline	82.8	91.3	114.7
Nialamide	105.9	106.9	103.4
Iproniazid	110.2	122.2	89.9
Tranylcypromine	86.8	98.0	90.5

TABLE 3

Change of the NA level in the ganglia of Anodonta cygnea after treatment with MAO inhibitors. The NA level is expressed in per cent of the control

MAOI	Duration after treatment (day)		
	1	2	4
Actomol	103.2	103.5	96.7
Pargyline	108.4	110.9	119.8
Nialamide	95.8	123.7	121.0
Iproniazid	108.0	112.4	107.1
Tranylcypromine	89.7	133.3	99.7

In spite of the fact that MAO inhibitors influenced slightly the level of monoamines, marked alterations were found in the activity. Significant changes resulted in the average length of the periods and in the frequency of the rhythmic activity. All of the examined inhibitors decreased the average length of the active periods *(Table 4)*. The greatest decrease was caused by actomol

TABLE 4

Percentual change of the average length of active and rest periods compared to the control (100 per cent)

(+) = increase (−) = decrease

MAOI	Active periods	Rest periods
Actomol	−69.5	−11.1
Pargyline	−50.0	+68.7
Nialamide	−28.0	−32.8
Iproniazid	−17.4	+17.4
Tranylcypromine	−44.4	−12.3

while the effect of the iproniazid was the least. The average length of the rest periods increased often adding pargyline and iproniazid but it was decreased by actomol, nialamide and tranylcypromine. Only pargyline and nialamide caused a higher than 20 per cent change in the average length of the rest periods. Nialamide and iproniazid appeared to be the least effective. In the case of actomol the effect depended to a high degree on the type of the activity prevalent before the treatment. The duration of active periods decreased in all cases independently of the duration of active periods characterizing the activity before the treatment *(Fig. 1)*. The decrease of the duration of rest periods was caused, however, only in the case of those animals which had in control an activity characterized by short active and long rest periods *(Fig.1a)*. If the previous activity of the animals was characterized by long-lasting active and short rest periods, then the duration of the rest periods was slightly changed after the treatment *(Fig. 1b)*. The duration of active periods decreased and that of rest periods increased to pargyline independently from the previous activity *(Fig. 2)*.

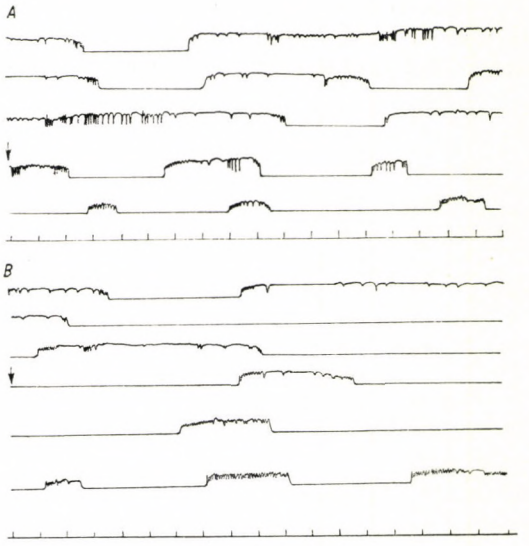

Fig. 1. Effect of actomol on the activity of the fresh water mussel. Continuous recording. Time scale: hours. ↓ injection of actomol

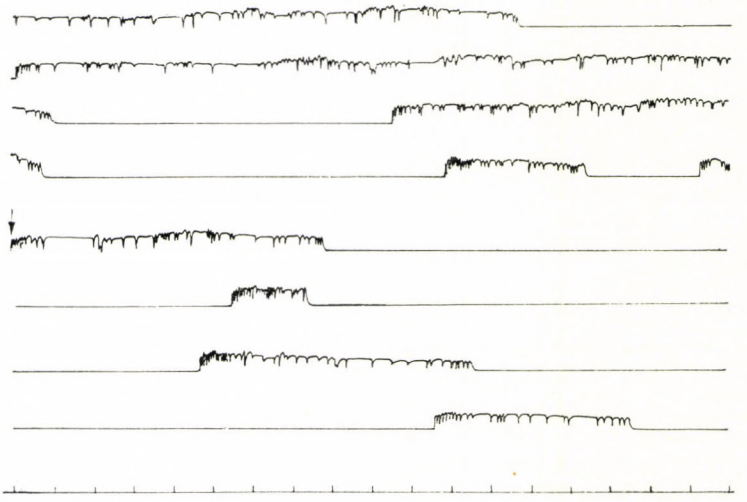

Fig. 2. Effect of pargyline on the activity of the fresh water mussel. Continuous recording. Time scale: hours. ↓ injection of pargyline

Two phases appeared upon the effect of tranylcypromine. Immediately after the treatment one or two long-lasting active periods appeared and during these periods the number of the fast rhythmic contractions increased markedly. This effect is very similar to the serotonin effect. After the long-lasting active period, however, the frequent alternation of very short rest and active periods was characteristic.

The frequency of the alteration of the active and rest periods changed also significantly parallel with the change of the average length of the periods *(Table 5)*. In the case of actomol and tranylcypromine where the duration of

TABLE 5

Change of the frequency of the periodicity after treatment with MAOI

MAOI	The frequency of periodicity		Changes (%)
	before treatment	after treatment	
Actomol	1.17	2.23	133
Pargyline	1.26	1.59	26
Nialamide	1.66	2.27	37
Iproniazid	1.44	1.56	8
Tranylcypromine	1.14	1.89	66

both active and rest periods decreased, the frequency of periodicity increased markedly. Pargyline and iproniazid altering the duration of active and rest periods in opposite directions changed the frequency slightly.

The percentual proportion of the active time decreased by 10—20 per cent after MAOI treatment, except after adding nialamide *(Table 6)*.

TABLE 6

Change of the percentual proportion of the active time after treatment with MAOI

MAOI	Percentual proportion of active time		
	before treatment	after treatment	decrease (%)
Actomol	63.4	52.3	17.5
Pargyline	77.8	58.4	24.9
Nialamide	68.6	68.4	0.?
Iproniazid	73.5	65.7	10.6
Tranylcypromine	69.5	58.2	16.2

After the pharmacological treatment 15—20 per cent of the animals perished. Higher concentrations of the pharmacons, as 5×10^{-4} mol, cannot be used, because at least 50 per cent of the animals perished when applying the actomol and pargyline treatment.

Discussion

In the nervous system of molluscs the role of MAO is not clear concerning the inactivation mechanism of the monoamines. In the nervous system of some species the acidic metabolite of the monoamines could be identified and the activity of MAO was measured in vitro (Osborne and Cottrell, 1970; Myers and Sweeney, 1972; Marsden, 1972; Jourio and Killick, 1972).

At the same time, in the other species neither the acidic metabolite could be identified nor the activity of MAO could be measured (CARDOT, 1964; 1966; MCCAMAN and DEWHURST, 1971; JOURIO and KILLICK, 1972).

Earlier we found that MAO is present in the different tissues of *Anodonta*. The presence of 5HIAA, DOPAC, and HVA in the nervous tissues (HIRIPI and SALÁNKI, 1971 and unpublished) show that MAO takes part in the enzymatic elimination of the serotonin and catecholamines. On the basis of these data it might be expected that the inhibition of MAO results upon the increase of monoamines in the nervous system. However, the investigated inhibitors did not increase significantly neither the serotonin nor the catecholamine level, although they altered the activity of the animal markedly. Our results agree with the data of KERKUT and COTTRELL (1963) and KERKUT et al. (1966) who found that iproniazid did not increase the serotonin and the dopamine levels in the nervous system of *Helix aspersa*. After the pargyline treatment neither did JOURIO and KILLICK (1972) observe any change in the monoamine level in the nervous system of *Helix*, but they did so in the *Octopus* brain.

The fact that MAO inhibitors did not increase the serotonine level in *Anodonta* ganglia may be explained by the following supposition: various tissues of *Anodonta* contain serotonin, though, its synthesis takes place almost exclusively in the nervous system (HIRIPI, 1968; HIRIPI and SALÁNKI, 1969). Serotonin is transported to the periphery, for example, to the adductor muscles, by axonal transport or through the circulation. In cases when in the nervous system the enzymatic break-down of the serotonin is inhibited by the inhibition of MAO, the circulation and the axonal transport can substitute the eliminating mechanism by transporting the excess of serotonin to the periphery.

According to pharmacological investigations (HIRIPI and SALÁNKI, 1973a) the increase in the serotonin level at the periphery stimulate the motor activity of the mussel. During the present investigations the increase of the activity appeared only in the first phase of the tranylcypromine effect. It may be supposed that the activity increase was produced as a side effect of tranylcypromine (KNOLL and MAGYAR, 1972) causing the liberation of the serotonin. The other MAO inhibitors decrease the activity, both the duration of active periods and the percentual proportion of the active time. Such type of effect is characteristic for the catecholamines (HIRIPI, 1973). The second phase of the tranylcypromine as well as the effect of the actomol and nialamide is similar to the dopamine effect while that of the pargyline and iproniazid is similar to the noradrenaline effect.

The slight effect of MAO inhibitors on the level of the catecholamines may be explained by the functional role of the COMT, which enzyme also takes part in the break-down of the catecholamines. In case of the inhibition of MAO both COMT and other mechanisms (circulation and axonal transport) can be responsible for the elimination of the catecholamines.

Earlier the decrease of the duration of the active periods and the increase of the duration of the rest periods were found after NA treatment (HIRIPI, 1973) and therefore the higher NA level observed after the pargyline, nialamide and iproniazid treatment may explain the decrease of the activity. After tranylcypromine treatment the changes in the level of the DA and NA were not unambiguous and so the two-phase in the effect of this pharmacon resulted rather in the mobilization of monoamines (KNOLL and MAGYAR, 1972) than in the inhibition of MAO.

The low changes found in the monoamine level after treatment with MAO raises another question. This is the problem of the high level of stored and the low level of functioning monoamines. In the experiments we are measuring the whole monoamine content of the brain and cannot differentiate the inactive pool from the active one. Since MAO exerts effect only on the liberated, functioning monoamines, in the case of MAO inhibition only this latter part will increase what does not cause a measurable rise in the total monoamine level. On the other hand, however, such a change may be explained by the enhancement of the activity of the animals.

Summary

The effects of five monoamine oxidase inhibitors—actomol, pargyline, nialamide, iproniazid and tranylcypromine—were investigated on the ganglionic level of serotonin, dopamine and noradrenaline as well as on the activity of the fresh water mussel *Anadonta cygnea* L.

MAO inhibitors did not increase significantly the ganglionic monoamine level, but they influenced markedly the activity. All the inhibitors decrease the average length of the active periods while in the length of the rest periods the pargyline and iproniazid caused no decrease but increase.

The frequency of the periodicity was increased and the percentual distribution of active time was decreased after treatement.

It is probable that MAO inhibitors influence the level of the active pool representing only a small part of the total monoamines and its change cannot be measured in the total amine level.

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MONOAMINOXIDÁZ GÁTLÓSZEREK HATÁSA AZ AKTIVITÁSRA, VALAMINT A GANGLIONÁRIS SZEROTONIN ÉS KATECHOLAMIN SZINTRE TAVIKAGYLÓN (*ANODONTA CYGNEA* L., PELECYPODA)

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Összefoglalás

A szerzők 5 monoaminoxidáz gátlószer — actomol, pargylin, nialamid, iproniazid és tranilcipromin — hatását vizsgálták a ganglionáris szerotonin, dopamin és noradrenalin szintre, valamint az aktivitásra tavikagylón (*Anodonta cygnea* L.).

A MAO gátlószerek nem okozták a ganglionáris monoamin szint növekedését, az aktivitást azonban jelentős mértékben befolyásolták. Az aktív periódus átlaghosszát minden egyes gátlószer csökkentette, a nyugalmi periódus esetén azonban a pargylin és az iproniazid nem csökkenést, hanem növekedést okozott.

A periodicitás frekvenciája növekedett, míg az aktivitásban eltöltött idő %-os aránya csökkent.

Valószínű, hogy a MAO gátlók az aktív pool szintjét befolyásolják, ami az össz monoamin tartalomnak csak kis hányada és ez a körülmény a szignifikáns különbséget elfedi.