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# ON THE MODULATING EFFECT OF SOME BIOLOGICALLY ACTIVE SUBSTANCES IN THE CENTRAL NERVOUS SYSTEM OF MOLLUSCS

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It is known that the sensibility of the elements in the central nervous system to the afferent signalization may play an important role in the reflex processes and in addition also in the behaviour of the animals. It has been recently generally considered that the influence of some chemical agents, primarily that of the so called sedatives is based on the fact that these substances are able to change, "to modulate" the sensitivity of the central nervous elements to afferent stimuli (KILLAM 1960, KOELLA and others 1960, ABDULIAN et al. 1960). Thus, in that case one has to recon not with the direct effect of certain substances, but only with the amplification or reduction of signals arriving from the periphery. The nervous system of molluscs seems to be especially suitable for investigating the nature of modulating effects, because it is of relatively simple organization and the single nerve centers are fairly well isolated. Just for this reason it was intended by making use of previous studies on osmoeffect (KOSHTOYANTS & RÓZSA 1961 b) to clear up, whether biologically active agents may produce modulating effects in the central nervous system of molluscs at concentrations which are ineffective in themselves, or not.

## Material and method

The experiments were conducted on the nervous system of edible snail (*Helix pomatia* L.). After removing the calcareous shell and the visceral organs the suprapharyngeal and subpharyngeal ganglia were exposed and their bioelectric activity was registered with the method described previously (KOSHTOYANTS & RÓZSA 1961 a). Peripheric stimulus was applied by the excitation of the osmoreceptors located on the surface of the foot. On their excitation, in agreement with previous data, changes were observable in the bioelectric activity of the nervous centers (KOSHTOYANTS & RÓZSA 1961 b).

At the beginning of the experiments the spontaneous bioelectric activity of the pedal ganglion was registered, thereafter the surface of the foot placed into a special chamber was moistened with water and the increase in bioelectric activity occurring in the pedal ganglion was registered simultaneously. Then the water was removed from the chamber and the chemical agents investigated were applied at a concentration below threshold on the pedal ganglion and the peripheric osmoreceptors were excited again and the central effect obtained was registered. The applied chemical agents were removed by

washing with 0.7% LOCKE-solution containing NaCl and thereafter the surface of the foot was excited again and the responses of pedal ganglion was registered.

In the course of these experiments the modulating influence of serotonin, uridindiphosphate, noradrenaline and chlorpromazine was studied. The threshold concentration of these substances has been determined previously (KOSHTOYANTS & RÓZSA 1961 a, RÓZSA 1964 a). In every case care was taken that the applied substances should not change the activity of the pedal ganglion, but only interfere in the realization of afferent stimuli arriving from the periphery.

0.7% LOCKE-solution was used as physiological solution and the substances investigated were diluted in the same solution.

## Results

The results show that the response of the pedal ganglion to the afferent stimulus is modulated by the substances applied at a concentration below threshold. This modulating effect may either be inhibiting or stimulating depending on the properties of the chemical agent used.

*Serotonin*

This substance in concentrations below threshold ($1 \cdot 10^{-11}$—$5 \cdot 10^{-11}$) increases the response on the pedal ganglion evoked by the stimulation of the peripheric osmoreceptors by water. In this concentration serotonin does not change the spontaneous activity of the pedal ganglion. *Figure 1* illustrates the responses obtained on the application of serotonin: at the beginning of the experiment the spontaneous activity of the ganglion pedale *(Fig. 1 A)*, later the increase in activity after the peripheric stimulation *(Fig. 1 B, C)* was registered. After this serotonin in $1 \cdot 10^{-11}$ M concentration was applied on the pedal ganglion and the peripheric osmoreceptors were repeatedly stimulated *(Fig. 1 E, F)*. In that case the central effect produced by the excitation of osmoreceptors was more intensive: this consisted of a 25% increase in amplitude. After washing out serotonin the osmoeffect decreased below the original level, and the response produced by the excitation of osmoreceptors became gradually reestablished *(Fig. 1 I)*.

*Uridinediphosphate*

This substance below threshold concentration increases the stimulating effect produced in the pedal ganglion by the excitation of the osmoreceptors without affecting spontaneous activity. Uridinediphosphate was applied in $1 \cdot 10^{-10}$ M concentration. On its influence an increase in osmoeffect by 30% took place which lasted for a long period even after the washing out of the substance.

*Noradrenaline*

Contrary to the previous two agents this substance reduces the reaction of pedal ganglion to the previously mentioned peripheric stimulus. In *figure 2* the effect produced by $1 \cdot 10^{-13}$ M noradrenaline is illustrated. At the start

of the experiment the spontaneous activity of the ganglion pedale was registered *(Fig. 2 a)*. Later on changes in activity produced by the excitation of peripheric osmoreceptors were studied *(Fig. 2 B, C)*. After this noradrenaline in the above concentration was applied to the pedal ganglion and the peripheric osmoreceptors were repeatedly excited. Comparing to the previous effect the considerable smaller increase in the response is well observable *(Fig. 2 E, F)*. In some cases the stimulating influence of the stimulation of peripheric osmoreceptors becomes completely omitted under the effect of noradrenaline and in spite of repeated washings is not completely restored.

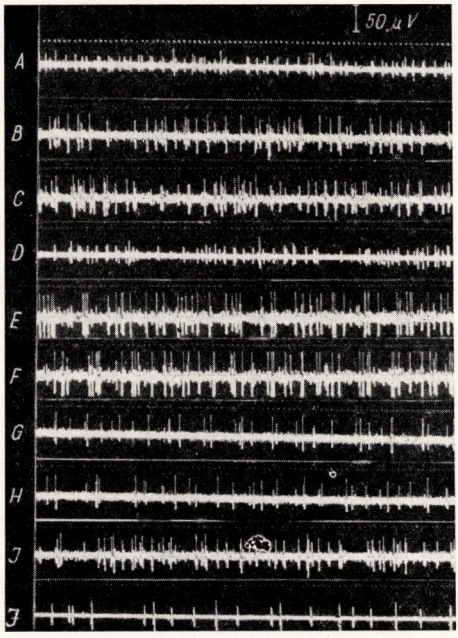


*Figure 1.* The modulating influence of serotonin on the central effect produced by the excitation of osmoreceptors. *A* — ground activity of pedal ganglion. *B* — the activity of ganglion after the first minute of the excitation of the osmoreceptors by water. *C* — the same after two minutes. *D* — the activity of pedal ganglion after the stimulation of the osmoreceptors and after the application of $1 \cdot 10^{-11}$ M serotonin solution on pedal ganglion. *E* — activity of ganglion after the first minute of the repeated excitation of osmoreceptors. *F* — the same after 2 minutes. *G* — activity of ganglion after the cessation of stimulation of osmoreceptors and the removal and washing out of serotonin. *H* — activity of ganglion after the first minute of the repeated stimulation of osmoreceptors. *I* — the same after 2 minutes. *J* — activity of ganglion after the cessation of stimulation of osmoreceptors
Time: in 0.05 sec.

*1. ábra.* A serotonin moduláló hatása az osmoreceptorok ingerlésére fellépő központi effektusra. *A* — a pedális ganglion alapaktivitása. *B* — a ganglion aktivitása 1 perccel az osmoreceptorok vízzel történő ingerlése után. *C* — ugyanaz 2 perc múlva. *D* — a pedális ganglion aktivitása az osmoreceptorok ingerlése után és a serotonin $1 \cdot 10^{-11}$ M koncentrációjának a pedális ganglionra való applikálása után. *E* — a ganglion aktivitása az osmoreceptorok ismételt ingerlése után 1 perccel. *F* — ugyanaz 2 perc múlva. *G* —a ganglion aktivitása az osmoreceptorok ingerlésének megszűnte és a serotonin eltávolítása és kimosása után. *H* — a ganglion aktivitása az osmoreceptorok ismételt ingerlése után 1 perccel. *I* — ugyanaz 2 perc múlva. *J* — a ganglion aktivitása az osmoreceptorok ingerlésének megszűnése után. Időjelzés: 0,05 sec.

*Chlorpromazine*

This agent in a concentration below threshold ($1 \cdot 10^{-9}$ M) reduced similar to noradrenaline the response of pedal ganglion induced by the stimulation of peripheric osmoreceptors. In single cases the central effect is completely

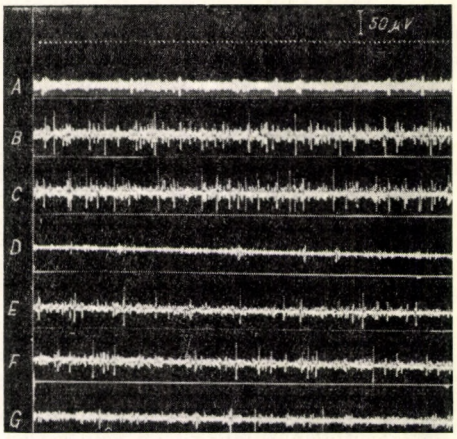


*Figure 2.* Modulating influence of noradrenaline on the central effect produced by the excitation of osmoreceptors. *A* — ground activity of pedal ganglion. *B* — activity of ganglion after the first minute of the excitation of osmoreceptors by water. *C* — the same after two minutes. *D* — activity of pedal ganglion after the excitation of osmoreceptors and after the application of $1 \cdot 10^{-13}$ M noradrenaline solution on pedal ganglion. *E* — activity of ganglion after the first minute of the repeated excitation of osmoreceptors. *F* — the same after 2 minutes. *G* — activity of ganglion after the cessation of excitation of osmoreceptors and the removal and washing out of noradrenaline. Time: 0.05 sec.

*2. ábra.* A noradrenalin moduláló hatása az osmoreceptorok ingerlésére fellépő központi effektusra. *A* — a pedális ganglion alapaktivitása. *B* — a ganglion aktivitása 1 perccel az osmoreceptorok vízzel történő ingerlése után. *C* — ugyanaz 2 perc múlva. *D* — a pedális ganglion aktivitása az osmoreceptorok ingerlése és noradrenalin $1 \cdot 10^{-13}$ M koncentrációjának a pedális ganglionra történő applikálása után. *E* — a ganglion aktivitása az osmoreceptorok ismételt ingerlése után 1 perccel. *F* — ugyanaz 2 perc múlva. *G* — a ganglion aktivitása az osmoreceptorok ingerlésének megszűnése és a noradrenalin eltávolítása és kimosása után. Időjelzés: 0,05 sec.

omitted, but the process of restoration takes place more quickly than in the case of noradrenaline.

## Discussion

It is evidenced by the results that the realization of stimuli arriving from the periphery is decidedly dependent on the sensitivity of the nervous cells of the center: the realization of stimuli arriving from the periphery are facilitated by some agents as in the present case by serotonin and uridinediphosphate, or are rendered more difficult by others (noradrenaline, chlorpromazine).

Comparing the results obtained with previous data on the role of serotonin as a mediator (Koshtoyants & Rózsa 1961a, Rózsa 1964a) it may be said that the same agent may serve in the molluscs as mediator and modulator

depending on the degree of the concentration used. The modulating influence produced by these agents is of similar character as that one resulted by their higher concentrations on the spontaneous activity of the ganglion. According to data published previously the spontaneous activity of the ganglia of edible snail is stimulated by serotonin and uridinediphosphate, and inhibited by noradrenaline and chlorpromazine at concentrations above threshold (KOSHTOYANTS & RÓZSA 1961a, RÓZSA 1964b). The modulating effect of these substances is of the same direction (facilitation or depression).

The results show that the modulators may display their effect by way of changes occurring in the threshold sensitivity of the central nervous system, whereby the same peripheric stimulus may induce a greater or smaller effect respectively than that observable in the absence of the modulating agent. It is assumable that the modulators are interfering into processes involved in electrogenesis under normal circumstances. It seems probable that the chemical agents investigated are influencing membrane potential but the changes induced are only local. The realization of peripheric stimulus on the membrane thus modified by the modulators depends on the given conditions, namely the original stimulating effect may increase or decrease. This depends as to which degree and in which direction was the polarization of cell membrane changed by the modulating substances. Intracellular registration of biopotencials is needed to clear up the more intimate mechanism of modulating influences. Nevertheless, the results obtained show that the nervous system of the molluscs may be successfully used for elucidating the mechanism of modulating influence.

## Conclusions

1. The modulating effect of biologically active agents is demonstrable in the central nervous system of the molluscs. This effect is reflected by changes in the sensitivity of nerve centers produced by stimuli arriving from the periphery.

2. The realization of stimuli arriving into the pedal ganglion from the osmoreceptors are facilitated by serotonin and uridinediphosphate in a concentration below threshold and inhibited by noradrenaline and chlorpromazine.

3. In the nervous system of molluscs the same agent may serve as mediator and modulator depending upon the concentration applied.

4. The nervous system of edible snail is most suitable medium for investigating the mechanism of modulating influences. This influence is presumably closely related to electric phenomena taking place on the surface of the cell membrane.

## Summary

The modulating effect of some biologically active substances (serotonin, uridinediphosphate, noradrenaline, chlorpromazine) on the realization of stimuli arriving from the periphery was investigated in the central nervous system of *Helix pomatia*. The bioelectric activity of the pedal ganglion was registered; the agents were applied to the same ganglion at a concentration below threshold. The stimulation by water of the osmoreceptors located on

the surface of the foot served as peripheric stimulus. It was established, that the realization of stimuli arriving from the periphery to the nerve center was facilitated by serotonin ($1 \cdot 10^{-11}$ M) and uridinediphosphate ($1 \cdot 10^{-10}$ M) and depressed by noradrenaline ($1 \cdot 10^{-13}$ M) and chlorpromazine ($1 \cdot 10^{-9}$ M). Coclusions are drown as to the identity of mediators and modulators.

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## NÉHÁNY BIOLÓGIAILAG AKTÍV ANYAG KÖZPONTI MODULÁLÓ HATÁSÁRÓL PUHATESTŰEK IDEGRENDSZERÉBEN

### Összefoglalás

*S. Rózsa Katalin*

A szerző vizsgálta néhány biológiailag aktív anyag (serotonin, uridindifoszfát, noradrenalin, klórpromazin) moduláló hatását molluszkák központi idegrendszerében a perifériáról befutó ingerek realizálódására. Regisztrálta a pedális ganglion bioelektromos aktivitását; a vizsgált ágensek küszöbalatti koncentrációinak applikálása ugyanerre a ganglionra történt. Perifériás ingerként a talp felületén elhelyezkedő osmoreceptorok vízzel történő ingerlése szolgált. Megállapítást nyert, hogy a serotonin ($1 \cdot 10^{-11}$ M) és az uridinfoszfát ($1 \cdot 10^{-10}$ M) megkönnyítik, míg a noradrenalin ($1 \cdot 10^{-13}$ M) és klórpromazin ($1 \cdot 10^{-9}$ M) megnehezítik a perifériáról az idegközpontba befutó ingerek realizálódását. Következtetést von le a mediátor és modulátor anyagok azonosságára vonatkozóan molluszkákon. A moduláló anyagok hatását összefüggésbe hozza az idegsejtek membránpotenciáljának változásaival, ezen ágensek applikálásának hatására.

## О ЦЕНТРАЛЬНОМ МОДУЛЯТОРНОМ ВЛИЯНИИ НЕКОТОРЫХ БИОЛОГИЧЕСКИ АКТИВНЫХ АГЕНТОВ В НЕРВНОЙ СИСТЕМЕ МОЛЛЮСКОВ

Каталин Ш.-Рожа

Автором было изучено модуляторное влияние в центральной нервной системе моллюсков некоторых биологически активных агентов (серотонин, уридиндифосфат, норадреналин, хлорпромазин) в реализации импульсов, приходящих с периферии. Регистрировалась биоэлектрическая активность педального ганглия виноградной улитки;

аппликация исследованных веществ в подпороговых концентрациях произошла на этот же ганглий. Периферическим возбуждением служило раздражение водой осморецепторов, расположенных на поверхности подошвы. Было установлено, что серотонин ($1.10^{-11}$ М) и уридиндифосфат ($1.10^{-10}$ М) облегчают, а норадреналин ($1.10^{-13}$ М) и хлорпромазин ($1.10^{-9}$ М) затрудняют реализацию импульсов, идущих с периферии в нервный центр. Высказывается предположение об идентичности медиаторов и модуляторов у моллюсков. Автор связывает модуляторное влияние изученных агентов изменением мембранного потенциала нервных клеток, наступающим после аппликации перечисленных выше химических веществ.