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THE ACTION MECHANISM OF RESERPINE IN THE NERVOUS SYSTEM OF INVERTEBRATES

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BEIN (1955), BRODIE, SHORE, SILVER (1955) were the first to establish similarity of the effects elicited in the nervous system of Vertebrates by reserpine and serotonin or 5-hydroxytryptamine (5HT). Later, on account of the identical action of reserpine and 5 HT a number of authors proved the assumption that reserpine exercises its action by the release of 5HT (PEETSCHER, SHORE, BRODIE 1956, PAASONEN, VOGT 1956, BRUNE, HIMWICH 1961 and others).

According to the concept of BRODIE and SHORE (1957) 5HT is present in the organism in a bound form and this protects it from the decomposing effect of monoaminoxidase. Reserpine reduces the 5HT binding capacity of the neurons, 5HT is released and decomposes on the action of monoaminoxidase present in the tissues. Following the action of reserpine, a long time is needed until the nerve cells regain their original 5HT binding capacity. It has been also demonstrated (SHORE, BRODIE 1957, ERSPAMER 1957) that reserpine does not act on serotonin synthesis in the brain and thus the final concentration of 5HT in the brain after the reserpine effect only depends on the relation of the reaction velocity of 5HT synthesis and decomposition to each other.

The concept of BRODIE encountered a series of objections. It appeared that reserpine promotes not only the release of 5HT but also of the catecholamines (HOLZBAUER, VOGT 1956, SHEPPARD, ZIMMERMAN 1960). SILVER and BRODIE (1960) though proved that the catecholamines do not play a part in the realization of the reserpine effect, but other chemical agents may enter into consideration. It is well known that the biologically active amines assume an inactive form in the organism, that is they enter into reaction with some sort of compound. They form such complexes most frequently with nucleotides (BLASCHKO, BORN, D'JOIRO, EADE 1956, PRUSOFF 1960). Some data seem to point out that at the release of serotonin upon the action of reserpine simultaneously with 5HT also those nucleotides are released that had hold it bound (HILLORP 1960, BURACK, HAGEN 1960). Nucleotides, however, themselves can be involved in the realization of nervous effects in the nervous system (KOSHTOYANTS 1958) and therefore their action must be largely taken into account when evaluating the reserpine effect.

The data of literature referred to indicate that for the action of reserpine not only 5HT is responsible but a number of factors must be reckoned with

in this process. In our experiments we attempted to elucidate whether as to reserpine effect in the nervous system of Invertebrates some nucleotides have to be taken into consideration.

Method

The experiments were conducte don the central nervous system of *Helix pomatia*. The calcareous shell of the animal was removed together with the visceral bag and the visceral organs. The musculous foot of the animal was fastened to a wax board and the central nervous system exposed. The bioelectric activity of the various ganglia was recorded as described in a previous communication (KOSHTOYANTS and RÓZSA 1961). 0.7 per cent LOCKE solution was applied as physiological solution. In this were dissolved also the substances examined and the constancy of the osmotic pressure has been attained by the simultaneous reduction of sodium ions in equimolar amount. The substances examined were applied on the pleural ganglion of the snail and the lead off and recording of the bioelectric activity was done from the same ganglion.

Results

1. *The effect of reserpine on the bioelectric activity of the pleural ganglion of the snail.*

In the experiments reserpine in a $1 \cdot 10^{-4}$ M concentration significantly increased the bioelectric activity of the pleural ganglion in the snail. Similar results were obtained in all experiments. Taking the mean values, the activity of ganglions upon the action of reserpine increases to its 4 to 5 fold. The activity of the ganglions remains very intensive also after the removal of the applied reserpine and repeated rinsing with LOCKE solution. These are demonstrated in *Fig. 1.I.*

The long lasting activity observed subsequently to the reserpine effect supports the view that the nerve cells after the administration of reserpine do not recover their 5HT binding capacity for a long time. In the nervous system of the snails the stimulating effect elicited by reserpine exceeds even the maximum stimulating effect elicited by serotonin brought in from outside. The effect of serotonin solution of $1 \cdot 10^{-7}$ M concentration is presented for comparison in *Fig. 1.II.* According to our earlier investigations under the given experimental conditions this concentration of serotonin elicits maximum stimulating effect (RÓZSA 1961) in the central nervous system of the snail. This difference between the effect of serotonin brought in from outside and reserpine may imply that in the reserpine effect besides serotonin also other substances are involved or that serotonin brought in from outside is not able to fully reproduce the endogenous serotonin effect.

The assumption according to which reserpine releases also catecholamines which too take part in bringing about the reserpine effect can not be maintained in our case. Our earlier experiments (KOSHTOYANTS and RÓZSA 1961) prove that the catecholamines which can enter into consideration: noradrenaline and adrenaline, inhibit the spontaneous bioelectric activity of the central nervous system of the snail in all concentrations and therefore can not be involved in the realization of the stimulating effect of reserpine, not even in the case that they actually are released upon the action of reserpine.

2. The responsiveness of seserpine effect on the application of various pharmacons

In the following, to elucidate the question whether the substance releasefi in the nervous system of the snail can be identical with serotonin, the action os substances of antiserotonin character and of nucleotides on the above described phenomenon was studied.

The effect of chlorpromazine which compound is known as a serotonin antagonist (PAGE 1958). According to our earlier data chlorpromazine wards

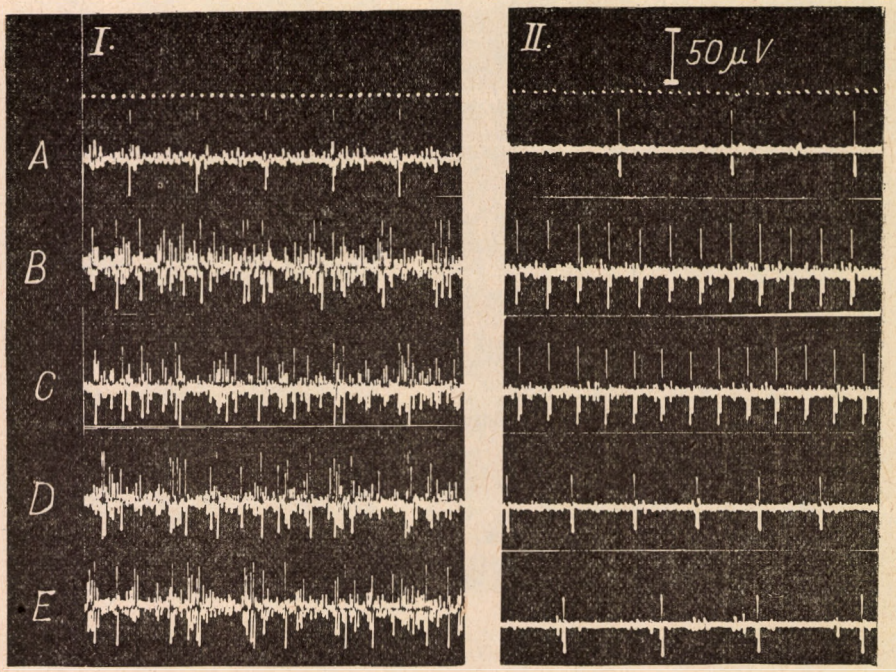

Fig. 1. Bioelectric activity of the ganglion of *Helix pomatia*
I. Effect of reserpine on the bioelectric activity of the ganglion of the snail
A = basic activity of pleural ganglion; *B* = activity of ganglion 1 minute after the application of $1 \cdot 10^{-4}$ reserpine; *C* = the same after 3 minutes; *D* = the same after 8 minutes; *E* = activity of the ganglion after removal and washing out of reserpine
II. The effect of serotonin on the bioelectric activity of pleural ganglion
A = basic activity of the ganglion; *B* = activity of the ganglion 1 minute after the application of $1 \cdot 10^{-7}$ M serotonin to the ganglion; *C* = the same after 3 minutes; *D* = removal of serotonin; *E* = washing out of serotonin
Time signal: 0.05 sec.

1. ábra. Helix pomatia ganglionjának bioelektromos aktivitása
I. Reserpin hatása az éticsiga ganglionjának bioelektromos aktivitására
A = pleurális ganglion alapaktivitása; *B* = a ganglion aktivitása 1 perccel $1 \cdot 10^{-4}$ M reserpin applikálása után; *C* = ugyanaz 3 perc múlva; *D* = ugyanaz 8 perc múlva; *E* = a ganglion aktivitása a reserpin eltávolítása és kimosása után
II. Serotonin hatása pleurális ganglion bioelektromos aktivitására
A = a ganglion alapaktivitása; *B* = a ganglion aktivitása 1 perccel $1 \cdot 10^{-7}$ M serotonin ganglionra való applikálása után; *C* = ugyanaz 3 perc múlva; *D* = a serotonin eltávolítása; *E* = a serotonin kimosása
Időjelzés: 0,05 sec.

off the stimulating effect of serotonin brought in from outside in the ganglions of the snail (RÓZSA 1961). In the present tests reserpine ($1 \cdot 10^{-4}$ M) and chlorpromazine ($1 \cdot 10^{-4}$ M) were applied together after reserpine stimulation. It was found that in the case of combined application the stimulating effect arising upon the action of reserpine gradually weakens *(Fig. 2.II)* and 5 to 10 minutes after the application the basic activity of the pleural ganglion is

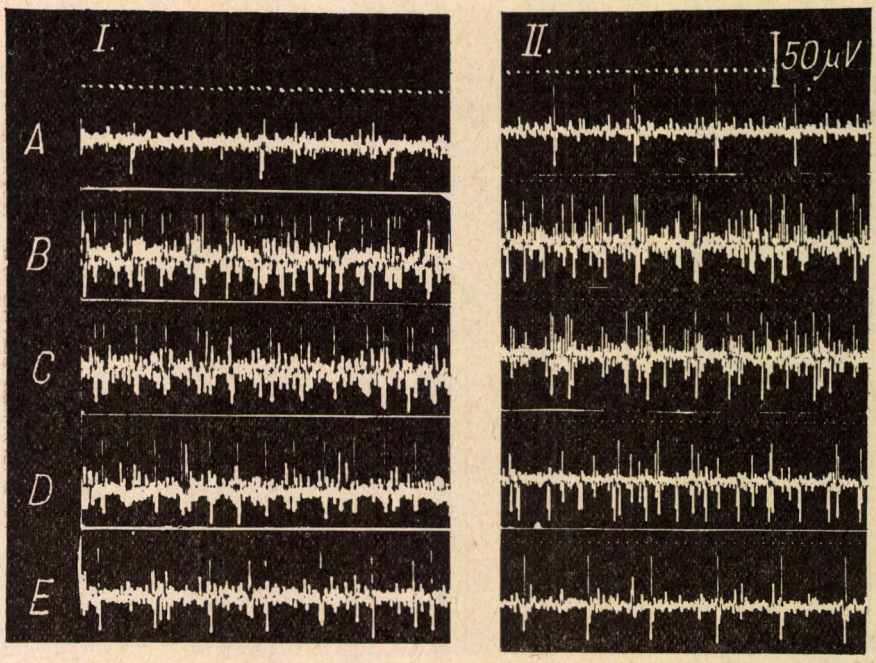

Fig. 2. Bioelectric activity of the ganglion of *Helix pomatia*
I. The effect of uridinediphosphate on the bioelectric activity of the ganglion of the snail
A = basic activity of the pleural ganglion; *B* = activity of the ganglion 1 min. after application of $1 \cdot 10^{-6}$ M uridinediphosphate to the ganglion; *C* = the same after 3 minutes; *D* = the same after 5 minutes; *E* = after removal and washing out of the uridinediphosphate
II. The action of chlorpromazine on the reserpine effect
A = basic activity of the pleural ganglion; *B* = activity of the ganglion 2 minutes after application to the ganglion of $1 \cdot 10^{-4}$ M reserpine; *C* = the same after 3 minutes; *D* = the activity of the ganglion 3 minutes after the combined application of $1 \cdot 10^{-4}$ M reserpine and $1 \cdot 10^{-5}$ chlorpromazine; *E* = the same after 5 minutes
Time signal: 0.05 sec.

2. ábra. Helix pomatia ganglionjának bioelektromos aktivitása
I. Uridindifoszfát hatása az éticsiga ganglionjának bioelektromos aktivitására
A = a pleurális ganglion alapaktivitása; *B* = a ganglion aktivitása 1 perccel $1 \cdot 10^{-6}$ M uridindifoszfát ganglionra történő applikálása után; *C* = ugyanaz 3 perc múlva; *D* = ugyanaz 5 perc múlva; *E* = az uridindifoszfát eltávolítása és kimosása után
II. Chlorpromazin hatása a reserpin effektusra
A = a pleurális ganglion alapaktivitása; *B* = a ganglion aktivitása $1 \cdot 10^{-4}$ M reserpin ganglionra történő applikálása után 2 perccel; *C* = ugyanaz 3 perc múlva; *D* = a ganglion aktivitása 3 perccel $1 \cdot 10^{-4}$ M reserpin és $1 \cdot 10^{-5}$ M chlorpromazin együttes applikálása után; *E* = ugyanaz 5 perc múlva
Időjelzés: 0,05 sec.

restored *(Fig. 2.II.E)*. By washing out the reserpine applied with LOCKE's solution no such rapid restoration can be obtained *(Fig.1.I.E)*.

The data obtained with this serotonin antagonist is in good agreement with the view on vertebrates according to which the reserpine effect can be brought into connection with the effect of the released serotonin. It can be well imagined that chlorpromazine figures as an antagonist of the active substances released by reserpine and thus wards off the stimulating effect of reserpine.

The fact, however, that reserpine in the ganglions of the snail brings about a more intensive increase of activity than serotonin brought in from outside prompted us to test the effect also of other, in the first place of nucleotide substances on the effect referred to above, because presumably these are also released simultaneously with serotonin (BURACK, HAGEN 1960). In various tissues of the snail the presence of uridinediphosphate was demonstrated (WHEAT 1960) and therefore we assumed that this substance may be involved in the binding of 5HT and consequently might be released upon the action of reserpine, together with 5HT. This agent figures as stimulating factor for the heart of amphibians (PUTINTZEVA 1961, KOSHTOYANTS 1961).

In our tests uridinediphosphate elicited a very strong stimulating effect in the central nervous system of the snail. $1 \cdot 10^{-8}$ M uridinediphosphate proved to be threshold concentration but already a 10^{-6} M concentration can increase the basic activity of the pleural ganglion to its 9 to 15 fold *(Fig. 2.I)*. Reserpine frequently elicits a very strong intensification of activity of the same type *(Fig. 1.I)*.

The increased activity elicited by uridinediphosphate is warded off by chlorpromazine and acridine-orange. The latter acts presumably by the binding or inactivation of the released substances. According to KOSHTOYANTS (1961) acridine-orange plays a part in the binding of free nucleotides.

Further experiments are needed to decide the question which nucleotides may be released, in addition to uridinediphosphate, simultaneously with serotonin, on the action of reserpine. Our preliminary experiments indicate that ATP, ADP and AMP did not bring about similar major increase of activity as uridinediphosphate and reserpine under the given experimental conditions.

Discussion

Our experiments prove that reserpine in a $1 \cdot 10^{-4}$ M concentration substantially increases the basic activity of the ganglions in the edible snail. The fact that this activity is warded off by chlorpromazine seems to indicate that serotonin may be involved in the realization of reserpine effect. Since, however, the action of reserpine exceeds the maximum stimulating effect elicited by serotonin, we arrived at the conclusion that other substances may be responsible too for the phenomenon mentioned above.

Most probable seemed the assumption that simultaneously with serotonin those nucleotides are released which under normal conditions serve to bind, to inactivate serotonin. Similar views can be encountered in literature concerning other amines (BLASCHKO 1958, BURACK, HAGEN 1960). According to our experiments uridinediphosphate may be involved in the realization of the stimulating effect of reserpine in the central nervous system of the snail. This is indicated by our results obtained with acridine-orange.

On the grounds of these experiments it can not be finally decided what part the nucleotides play in the realization of the reserpine effect. It is possible that the nucleotides themselves serve as mediator (KOSHTOYANTS 1958, 1961), but it is also imaginable that their action is connected with the facilitating effect on serotonin.

The results obtained from the study of reserpine effect indicate that in the realization of nervous effects a whole series of physiologically active substances may be involved. Reserpine, by releasing serotonin, changes the proportion and amount of free and bound nucleotides which then themselves can partake in the appearance of the reserpine effect and can elicit long lasting activity of the neurons.

Summary

According to the study reserpine in a $1 \cdot 10^{-4}$ M concentration can bring about in the central nervous system of the snail a lasting activity of the nerve cells which activity exceeds the stimulating effect of serotonin brought in from outside. The increased activity elicited by reserpine can be warded off by adding chlorpromazine and acridine-orange. A phenomenon similar to the reserpine effect is caused by uridinediphosphate in a $1 \cdot 10^{-6}$ M concentration. On the grounds of the experimental results obtained it may be assumed that in the realization of reserpine effect on the nerve cells of snails also those nucleotides participate which simultaneously with serotonin are released upon the action of reserpine.

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A RESERPIN HATÁSMECHANIZMUSÁRÓL GERINCTELEN ÁLLATOK IDEGRENDSZERÉBEN

S. Rózsa Katalin

Összefoglalás

A vizsgálatok szerint reserpin $1 \cdot 10^{-4}$ M koncentrációban éticsiga központi idegrendszerében az idegsejtek hosszantartó aktivitását hozza létre, mely aktivitás felülmúlja a kívülről bevitt serotonin stimuláló hatását. A reserpin által kiváltott aktivitásfokozódás kivédhető chlorpromazin és acridin-orange adásával. A reserpin-effektushoz hasonló jelenséget hoz létre az uridindifoszfát $1 \cdot 10^{-6}$ M koncentrációban. A kapott kísérleti eredmények alapján feltehető, hogy a reserpinhatás megvalósulásában éticsiga idegsejtjein részt vesznek azok a nukleotidok is, amelyek a serotoninnal egyidejűleg szabaddá válnak reserpin hatására.

О МЕХАНИЗМЕ ДЕЙСТВИЯ РЕЗЕРПИНА В НЕРВНОЙ СИСТЕМЕ БЕЗПОЗВАНОЧНЫХ ЖИВОТНЫХ

Каталин Ш. Рожа.

По экспериментальным данным резерпин в концентрации $1 \cdot 10^{-4}$ М вызывает продолжительную активность нервных клеток центральной нервной системы виноградной улитки, превышающаяся стимуляторного влияния введенного извне серотонина. Увеличенная активность нервных клеток, вызванная резерпином, снимается при применении хлорирмазина и акридинаранжа. Сходный к действию резерпина эффект вызывает уридиндифосфат в концентрации $1 \cdot 10^{-6}$ М. На основе полученных данных можно предположить, что в осуществлении эффекта резерпина на нервных клетках виноградной улитки участвуют и те нуклеотиды, которые высвобождаются вместе серотонином под влиянием дезерпина.