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PHARMACOLOGICAL INVESTIGATIONS ON THE ISOLATED PENIAL APPARATUS OF FRESH-WATER SNAIL LYMNAEA STAGNALIS L.

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Only few investigations are known concerning the pharmacology of the penial apparatus of Gastropoda. It was pointed out by KOSHTOYANTS (1936) that the genital apparatus of *Helix pomatia* L. behaves differently from the other organs of the vertebrates. It was established by JAEGER (1962, 1963) in course of his examinations on the penis retractor muscle of *Strophocheilos oblongus* that acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) produced antagonistic response of the preparation with respect to rhythm induction. As suggested by GODDARD (1962) only adrenaline is effective on the penial apparatus of *Helix aspersa* and ACh and histamine are ineffective at concentrations used. The response of the isolated penial complex of *Lymnaea stagnalis* on the application of various agents has been investigated recently by DUNCAN (1964). He observed that in the majority of cases 5-HT produced relaxation inducing frequent rhythmic activity, while the application of ACh resulted in an increase of frequency and decrease in amplitude of spontaneous activity. The aim of the present investigations is partly to examine the contradictory data on the effect of ACh and 5-HT and partly to obtain new data on the mechanism of the effect of these agents on penis preparations.

Method

The specimens of *Lymnaea stagnalis* L. used in these investigations were collected partly from Lake Balaton, partly from pond "Külső tó" at Tihany and were kept for several weeks in aquaria filled with Lake Balaton water before experimental use.

The penial preparation was made as follows: the shell of the animal was removed and the penial complex (penis, penis sheath, praeputium, flagellum) was exposed by cutting up the body wall. The penis retractor muscle and the nerves running to the preparation were cut through. Following this, the preparation was lifted out together with a small portion of the body wall, it was ligated at the end of the body wall and at the flagellum and hung up in a bath of 20 ml in volume. Physiological solution was prepared according to CARRIKER (1946). The activity of the preparation was registered by a light lever on a kymograph at a 10 : 1 lever ratio. After operation the preparations were left to

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stand for half an hour. The agents investigated were added to the bath with an injection syringe. The concentrations given are final concentrations expressed in g/ml. Air-flow was led through the bath producing a rapid mixing of the agents with the bath fluid.

The agents used were: 5-hydroxytryptamine creatinine sulphate (5-HT), acetylcholine chloride (ACh), methysergide (UML-491), 2-bromo-lysergic acid diethylamide (BOL-148), benzoquinonium chloride (mytolon), dibenamine, atropine sulphate, cocaine, morphine, d-tubocurarine chloride, and eserine.

The experiments were performed at room temperature (20—22 °C) in March, April and May.

Results

When the contraction subsequent to preparation ceased the preparation displayed, in the majority of cases, spontaneous rhythmic activity. This rhythmic activity consisted of small phasic contractions and of occasionally occurring interpolated large tonic contractions *(Fig. 1)*.

The effect of 5-hydroxytryptamine

The penial complex responded to 5-HT with contraction and with a subsequent rhythmic activity of increased amplitude and frequency *(Fig. 2)*, whereas on preparations in the resting period activity was induced. Depending on concentration two-phase effect was produced by 5-HT. It resulted in a small relaxation near threshold concentration (5×10^{-9} g/ml). At 10^{-8}—10^{-5} g/ml concentrations or above 5-HT never produced relaxation, the response produced was always tonic contraction and its duration increased at higher concentrations. If the preparation was subject only for a short period to the effect of 5-HT then after repeated washings it responded again to the same or to

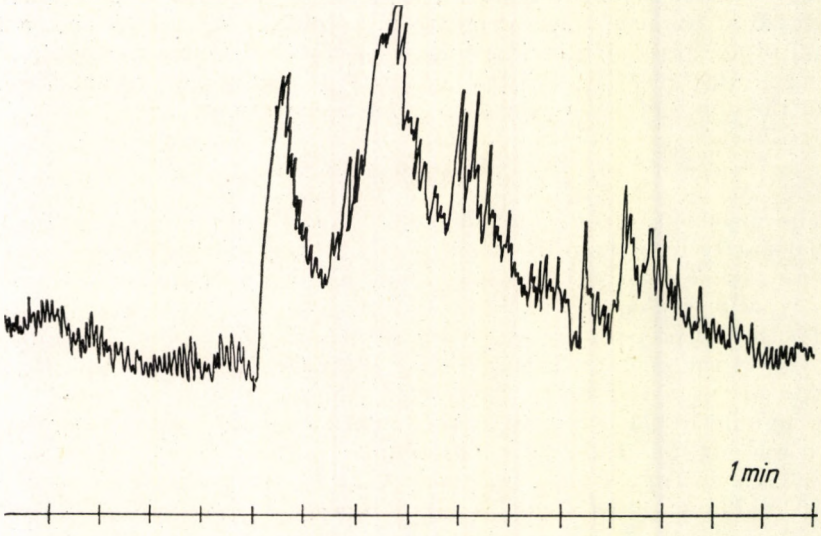

Fig. 1. Spontaneous rhythmic activity of isolated penis complex

1. ábra. Az izolált penis komplex spontán ritmikus aktivitása

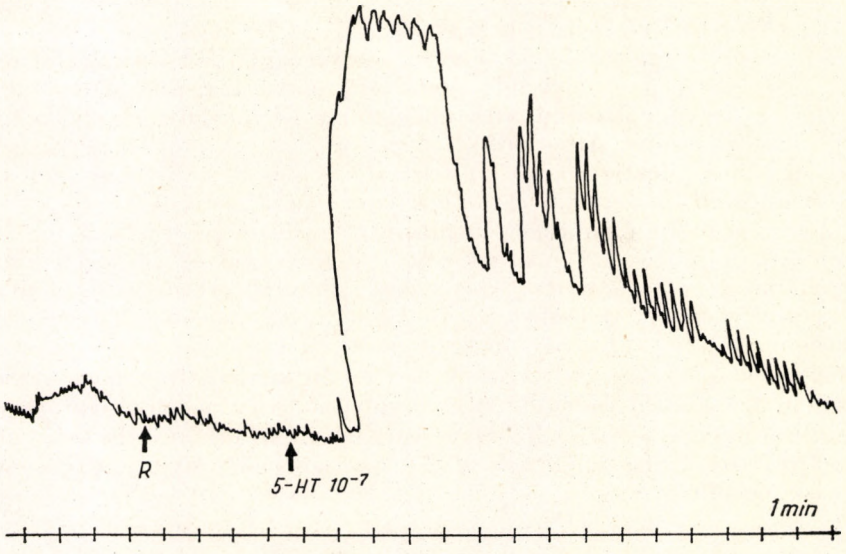

Fig. 2. Effect of 10^{-7} g/ml 5-HT on the isolated penis preparation of *Lymnaea*

2. ábra. 5-HT hatása *Lymnaea* izolált penis preparátumán 10^{-7} g/ml koncentrációban

higher concentrations of 5-HT. On the applications of high concentrations, however, tachyphylaxy was produced. For instance if 10^{-5} g/ml solution of 5-HT was left on the preparation for a longer period (1—2 hours) the original level of tonus from the initial contraction was restored without washing, the preparation, however, did not respond again to lower concentrations of 5-HT.

Effect of agents influencing the effect of 5-HT

The effect of serotonin was influenced by 5-HT and ACh-antagonists and narcotics.

BOL-148 applied by itself did not affect the activity of the penial apparatus. After pretreatment as long as 10—15 mins, 10^{-5}—10^{-6} g/ml concentrations of this agent completely inhibited the effect produced by 5×10^{-7} g/ml 5-HT *(Fig. 3)*. After repeated washings the preparation turned sensitive

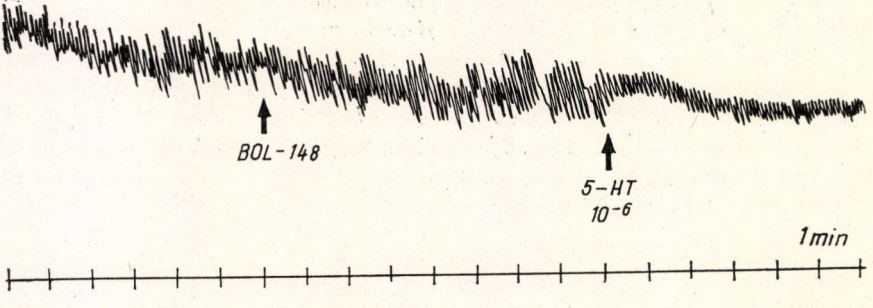

Fig. 3. Effect of 10^{-6} g/ml 5-HT on penis preparation pretreated with BOL-148 for half an hour

3. ábra. 10^{-6} g/ml 5-HT hatása a félóráig BOL-148-cal előkezelt penis preparátumon

again to 5-HT, and responded with contraction as usual. UML-491 produced a similar effect to BOL-148.

Dibenamine was also found to block the effect of 5-HT with the difference that it produced a more lasting contraction than 5-HT, and after about 25 mins the preparation treated with dibenamine became restored also to the original level of tonus. After pretreatment with 10^{-6} g/ml concentration of this agent the application of 10^{-6} g/ml 5-HT produced only small increase in tonus, it induced, however, a considerable rhythmic activity.

Atropine in 10^{-6} g/ml concentration strongly increased both frequency and amplitude of the rhythmic activity or if there was no rhythmic activity before induced typic activity. This agent inhibited strongly, even after 5 mins of pretreatment, the effect of 10^{-6} g/ml 5-HT solution which otherwise produced contraction *(Fig. 4)*. In some cases, however, an insignificant contracting effect persisted and was followed by an immediate and considerable relaxation. Subsequent to washing the original activity of the preparation was restored but upon applying 5-HT repeatedly it did not produce the usual effect, but increased only the amplitude of the spontaneous activity. Atropine produced lasting effect.

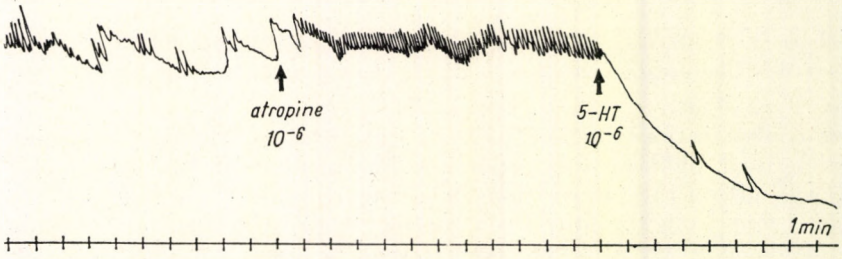

Fig. 4. Effect of 10^{-6} g/ml 5-HT on penis preparation pretreated with atropine (10^{-6} g/ml)

4. ábra. 10^{-6} g/ml 5-HT hatása atropinnal (10^{-6} g/ml) előkezelt penis preparátumon

Morphine at 10^{-5}—10^{-3} g/ml concentrations affected the penis preparations by itself and produced contraction of tonic character. After restoration to the original level of tonus the preparation responded after pretreatment with 10^{-5} g/ml morphine, to the application of 10^{-6} g/ml 5-HT either with unchanged or with slightly decreased contraction. Applying this agent, however, in 10^{-3} g/ml concentration the contracting effect of 10^{-6} g/ml 5-HT completely ceased and a frequent rhythmic activity was induced *(Fig. 5)*.

d-Tubocurarine and cocaine were ineffective in 10^{-5} g/ml concentration, when applied, however, in 10^{-3} g/ml concentration these agents inhibited completely the contracting effect of 10^{-6} g/ml 5-HT; after their application 5-HT produced relaxation *(Fig. 6)*. Further on, 5-HT when applied subsequent to the application of d-tubocurarine produced rhythmic activity of increased amplitude and frequency *(Fig. 7)*.

The effect of acetylcholine

The threshold concentration of this agent was about 10^{-10} g/ml. This and higher concentrations up to 10^{-5} g/ml produced alike contraction of the penial preparation. For a period rhythmic activity also ceased, it was however restituted by itself.

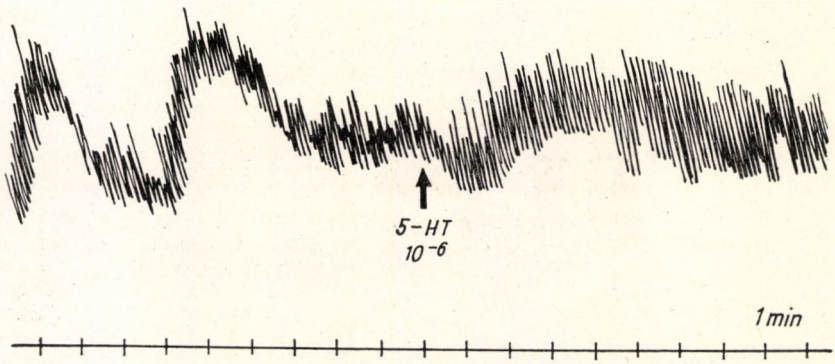

Fig. 5. Effect of 10^{-6} g/ml 5-HT after 20 mins pretreated with morphine (10^{-3} g/ml)

5. ábra. 10^{-6} g/ml 5-HT hatása morfinnal való (10^{-3} g/ml) 20 perces előkezelés után

Effect of agents influencing the effect of ACh

Mytolon solution at 10^{-6}—10^{-5} g/ml concentrations, which by themselves proved to be strongly stimulatory on the preparation, did not inhibit practically after 1 hour application the effect of 10^{-6} g/ml ACh and 5-HT. After a half an hour pretreatment with higher concentrations (10^{-4}—10^{-3} g/ml) of mytolon the stimulatory effect of ACh decreased only slightly. On the other hand, 10^{-6} g/ml concentration of atropine completely inhibited the contracting effect of ACh of same concentration *(Fig. 8)*. Rhythmic activity of very high frequency which was induced by atropine, however, ceased on the next application of ACh, it was, however, restored by itself also without washing within about 10 mins.

d-Tubocurarine, cocaine and morphine applied in 10^{-5} g/ml concentration did not inhibit the contracting effect of ACh (10^{-7}—10^{-6} g/ml), and d-tubocurarine even potentiated at the concentration applied the stimulatory effect of ACh. Rising the concentration of these agents further (10^{-4}—10^{-3} g/ml) the contraction-producing effect of ACh decreased, but was not completely blocked.

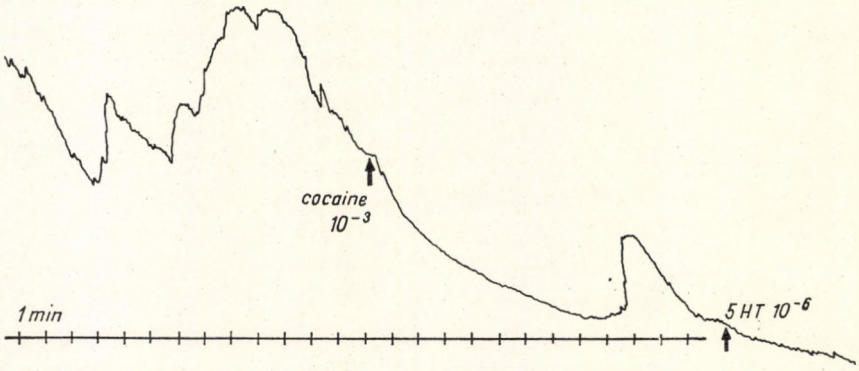

Fig. 6. Effect of 10^{-6} g/ml 5-HT on preparation pretreated with cocaine (10^{-3} g/ml)

6. ábra. 10^{-6} g/ml 5-HT hatása kokainnal (10^{-3} g/ml) előkezelt penis preparátumon

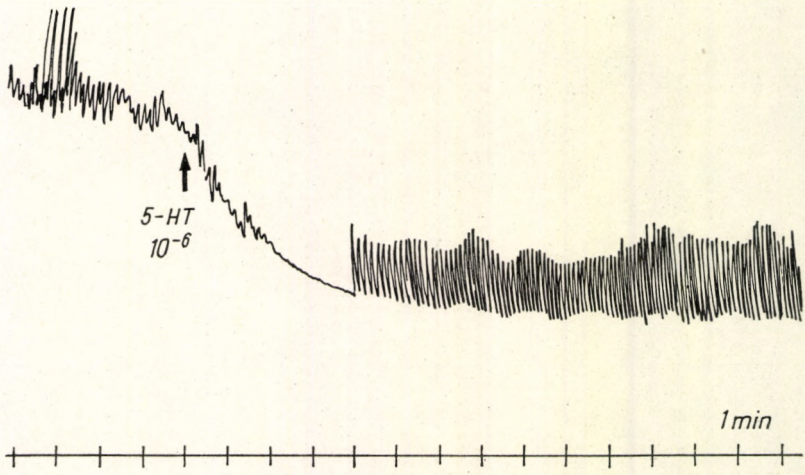

Fig. 7. Effect of 10^{-6} g/ml 5-HT on the application (15 mins) of d-tubocurarine (10^{-3} g/ml)

7. ábra. 10^{-6} g/ml 5-HT hatása d-tubocurarin (10^{-3} g/ml) (15 perc) adása után

Eserine (10^{-4} g/ml) increased strongly the tonus of the preparation. Which was restituted only after a longer interval, whilst a rhythmic activity of greater frequency and higher amplitude was induced as compared to the original. The contraction produced after subsequent application of ACh was similar to the reaction of untreated preparation, rhythmic activity, however, was completely inhibited.

Discussion

The rhythmic activity of the penial apparatus of *Helix aspersa* results from its unbroken contact with the central nervous system (GODDARD 1962). In opposition to this it was observed in conformity with previous observation by DUNCAN (1964) that in case of *Lymnaea stagnalis* the penial apparatus can produce spontaneous rhythmic activity also independently from the nervous system. As observed by us, the normal rhythmic activity consists of small phasic contractions and of occasional, interpolated great tonic contractions with superimposed phasic ones.

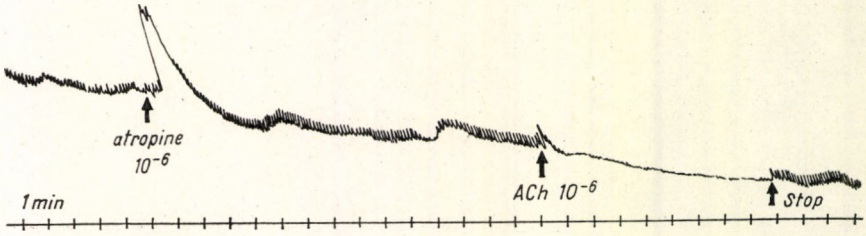

Fig. 8. Effect of 10^{-6} g/ml acetylcholine on penis preparation pretreated with atropine (10^{-6} g/ml)

8. ábra. 10^{-6} g/ml acetylcholin hatása atropinnal (10^{-6} g/ml) előkezelt penis preparátumon

The fact that the penial apparatus is richly supplied with nerves (ELO 1938, GODDARD 1962) renders the study of the effect of drugs more complicate, namely, not only the effect produced on the smooth muscle but that on the neurons must be also considered.

Concerning the effect of 5-HT only relaxation and a rhythmic activity of increased amplitude and frequency was observed by DUNCAN (1964) on the penial apparatus of *Lymnaea*. It was shown, however, by our experiments that 5-HT produced a two-phase effect. At low concentrations (5×10^{-9} g/ml) 5-HT produced weak relaxation, whereas at higher ones always a tonic contraction. Similar results were obtained previously by JAEGER (1963) applying 5-HT on the penis retractor muscle of *Strophocheilos oblongus*, when up to 10^{-8} g/ml concentration relaxation and at higher concentrations an increase of tonus was observed, further by GREENBERG and JEGLA (1963) on the rectum of *Mercenaria mercenaria* and by PHILLIS (1966) on the rectum of *Tapes watlingi*.

It was suggested by GREENBERG and JEGLA (1963) that 5-HT is effective on two sites on the isolated rectum of *Mercenaria:*

(a) it produces ACh release on cholinerg neurons,

(b) affects smooth muscles directly.

Using different inhibitors, it became evident, that in case of the preparation used by us in these studies both the neurons and the muscle itself are to be considered. In these studies the contracting effect produced by 5-HT was completely blocked by BOL-148, UML-491 and dibenamine and this was not accompanied by relaxation. The effect of 5-HT on the penial apparatus could also be effectively blocked by ACh antagonists, in that case, however, the application of 5-HT was always followed by a considerable relaxation. The two different types of blocking effect are presumably separated on the basis whether the agents eliminated the direct effect of 5-HT on the muscle or its neurotropic effect (GREENBERG and JEGLA 1963; PHILLIS 1966).

Out of the ACh antagonists investigated (atropine, d-tubocurarine, mytolon) atropine proved to be most effective as regards inhibition of the effect produced by 5-HT. This observation is in contradistinction to data obtained by GREENBERG and JEGLA (1963) on the rectum of *Mercenaria* in which case, namely, the increase of tonus produced by 5-HT was potentiated by atropine, and to those obtained by GRYGLEWSKI and SUPNIEWSKI (1963) according to which atropine did not influence the 5-HT sensitivity of the isolated stomach of *Helix*. d-Tubocurarine was at 1000 times greater concentration as effective as atropine, while mytolon was completely ineffective. Comparing data obtained with those on the inhibitory effect on ACh, it is seen that in this case atropine is the most effective antagonist produced complete inhibition of ACh effect. The contraction induced by ACh was only slightly diminished by d-tubocurarine and especially by mytolon. It was found previously that atropine is an ACh-inhibitor on the isolated stomach (GRYGLEWSKI and SUPNIEWSKI 1963) and intestine (MINKER and KOLTAI 1961) of *Helix*. The penial apparatus of *Lymnaea* behaves differently in this respect, from the genital apparatus of *Helix pomatia*, for in case of the latter the effect of ACh was not inhibited by atropine but by nicotine (KOSHTOYANTS 1936). It is known from studies on the rectum of *Mercenaria* (GREENBERG and JEGLA 1963) that atropine is more effective ACh antagonist than mytolon. Moreover, mytolon blocked on the rectum of *Tapes* only the inhibitory component of the ACh effect, whereby the excitatory component of ACh effect prevailed (PHILLIS 1966). In opposition

to this d-tubocurarine was the most effective ACh antagonist on the rectum of *Tapes*, it inhibited both synaptic and "non-synaptic" ACh receptors, while atropine had only small antagonistic effect (PHILLIS 1966). The same observation was made on the retractor muscle of isolated penis preparations of *Strophocheilos* (JAEGER 1962).

It is worth to note that eserine which normally potentiated the effect of ACh did not produce in the course of these investigations observable alterations.

From among the investigated agents only atropine induced at the concentrations used rhythmic activity of greater amplitude and frequency as compared to the control. This might be explained by the stimulatory effect of atropine on motor activity as observed also in case of preparations of other invertebrates (BARKER, BUEDING and TIMMS 1966).

Besides atropine other agents like morphine and cocaine which as observed by GADDUM and PICARELLI (1957) blocked the M-receptors on the ileum of guinea pig, produced also complete block of 5-HT effect and decreased the contracting effect of ACh on the penial apparatus of *Lymnaea*. It is suggested by GREENBERG and JEGLA (1963) that morphine inhibits the travel of nerve conduction produced by 5-HT, and possibly inhibits the ACh-release from cholinerg nerve endings. In view of the fact that 5-HT contracts the longitudinal muscles of penial preparations and that this effect is inhibitable by the above agents, it is thought probable that the contracting effect of 5-HT asserts itself by the excitation of cholinerg nerve fibers.

The fact that the penial apparatus of *Lymnaea* and the rectum of *Mercenaria* (GREENBERG and JEGLA 1963) and *Tapes* (PHILLIS 1966) respond similarly to the investigated chemical agents suggests that the preparations in question have similar pharmacological behaviour. The observation that in the case of the penial apparatus of *Lymnaea* the order of effectiveness of 5-HT-antagonists differed from that of preparations of other molluscs suggests the specificity of the preparation used. In view of the fact that ACh-antagonists which blocked in course of our experiments the contracting effect of 5-HT antagonized the effect of ACh, too, it is thought probable, as suggested by GREENBERG and JEGLA (1963), that this effect produced by 5-HT might be realized by way of ACh release. The relaxing effect produced by 5-HT, however, may assert itself only after the elimination of the above contracting effect of this agent.

Summary

The mechanism of the effect of 5-HT and ACh was examined on the isolated penial apparatus of a fresh-water snail, *Lymnaea stagnalis* L. The preparation responded with tonic contraction to the application of both 5-hydroxytryptamine and acetylcholine. On these two agents atropine proved to be the most effective antagonist. The blocking effect of BOL-148, UML-491, dibenamine, cocaine, d-tubocurarine, mytolon and morphine was studied on the effect produced by 5-HT and ACh.

The results emphasize besides the specificity of the preparation used the similarity between the isolated penial apparatus of *Lymnaea* and other molluscan preparations with regards to their pharmacological behaviour.

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FARMAKOLÓGIAI VIZSGÁLATOK EGY ÉDESVÍZI CSIGA, LYMNAEA STAGNALIS L. IZOLÁLT PENIS PREPARÁTUMÁN

Pécsi Tibor, H. Kuziemski és S.-Rózsa Katalin

Összefoglalás

A Lymnaea stagnalis édesvízi csiga izolált ivari apparátusán vizsgálták az 5-HT és ACh hatásmechanizmusát. A preparátum mind az 5-hydroxytryptaminra, mind az acetylcholinra tónusos kontrakcióval válaszolt. Mindkét vegyület leghatásosabb antagonistájának az atropin bizonyult. Az 5-HT, illetve az ACh hatás blokkolása céljából a BOL-148, UML-491, dibenamin, kokain, d-tubocurarin, mytolon és a morfin hatását vizsgáltuk. Az eredmények a preparátum specifikumának hangsúlyozása mellett a Lymnaea izolált ivari apparátusának más molluszka preparátumokhoz való hasonlóságát sugallják a farmakológiai viselkedést illetően.

ФАРМАКОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ НА ИЗОЛИРОВАННОМ ПОЛОВОМ АППАРАТЕ БОЛОТНОГО ПРУДОВИКА

Тибор Печи, Генрик Куземский и Каталин Ш.-Рожа

На изолированном половом аппарате болотного прудовика был изучен механизм действия серотонина и ацетилхолина. Препарат давал тонические контракции и на серотонин и на ацетилхолин. Атропин оказался самым эффективным антагонистом обоих веществ. С целью блокирования действия серотонина и ацетилхолина были применены БОЛ—148, УМЛ—491, дибенамин, кокаин, тубокурарин, митолон и морфий. Результаты показали, что изолированный половой аппарат болотного прудовика ведет себя подобно другим препаратам моллюсков.