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THE EFFECTS OF LOCAL ANAESTHETICS ON THE ACTIVITY GENERATION AND CHEMICAL SENSITIVITY OF GIANT NEURONES

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According to the literature the effect of local anaesthetics can be attributed at least to two different mechanisms. On the one hand they can stabilize the membrane similarly to Ca^{++} preventing the changes in Na^{+} and K^{+} permeability involved in the spike generation. On the other hand, their chemical structure is similar to that of ACh, so they can cause a competitive inhibition of the cholinergic transmission similarly to d-TC (ARIENS, 1964). According to NACHMANSSON's (1961) theory the membrane receptor protein taking part in the axonal spike propagation appears to be the same, that is involved in the cholinergic transmission, thus the site of the two actions essentially is the same. Recently MAUTNER et al. (1972) have a similar conception. However the examinations of some other authors have shown that sites, where the inhibition of neural activity and the blockade of synaptic transmission are produced are independent (BRYANT, 1958; USUBIAGA and STANDAERT, 1967), but they are dependent on the concentration of drugs (RIKER and KOSAY, 1970).

For the pharmacological investigations of localanaesthetics procain and various derivatives of lidocain were most frequently used. Under the influence of these drugs the abolition of spike generation on the giant axon of squid has been demonstrated (SHANES et al., 1959). This result has been interpreted as a consequence of a decrease in potassium and sodium conductances, which has been supported by some other works (BLAUSTEIN and GOLDMAN, 1966; NARAHASHI et al., 1967). The block of transmission has been investigated in particular on the end-plate potential and it has been shown, that procain influences mainly the K^{+}-chanel independently of the Na^{+}-chanel, while both are similarly affected by lidocain (MAENO et al., 1971).

It is known, that the mechanism as well as the conditions for generation of the spontaneous pacemaker activity are somewhat different from those of the evoked activity (ALVING, 1968; WAZIRI et al., 1965). Only a few investigations have been reported so far with respect to the effect of local anaesthetics on the pacemaker activity. On the Purkinje fibers of heart muscle lidocain inhibits the spontaneous potential generation (BIGGER and MANDEL, 1970). On spinal neurones procain blocks the generation of all kinds of action potential, while in the same concentration it does not affect the appearance of EPSP-s (CURTIS and PHILLIS, 1961.) WOOD (1972) proved in an indirect

manner, that some bursting neurons sending inhibitory impulses to the intestinal smooth muscle of the cat stopped generating activity in the presence of lidocain.

As far as we know the effect of local anaesthetics have not yet been examined on the giant neurones of Gastropods. As the neurones of *Lymnaea stagnalis* are suitable objects for studying the pacemaker activity generation and the chemical sensitivity of the soma simultaneously (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971), it appeared to be reasonable to investigate the effect of local anaesthetics on these cells. Beside the lidocain a compound marked as RG-1812 (1-piperidinopropanol,3-methoxybenzoate,hydrochloride) (KÁRPÁTI and SZPORNY, 1971; SZPORNY and KÁRPÁTI, 1972) was also tested. The main questions we wished to elucidate were as follows:

a) Are the effects similar to those described on other objects?

b) Whether the drugs exert their actions both on the spontaneous activity and on the chemical sensitivity by affecting the same membrane site or are there independent sites?

c) What are the qualitative and quantitative differences between the two local anaesthetics on the basis of the tests performed?

Material and methods

Examinations were conducted on the giant neurones of abdominal and right parietal ganglia of *Lymnaea stagnalis*. The thick connective tissue was removed from the dorsal surface of the ganglia, thus the cells became fairly visible through the thin tissue located under the thick one. The preparation was placed in a chamber *(Fig. 1)* containing 3 ml physiological saline (JULLIEN and RIPPLINGER, 1948). For changing the solutions a perfusion vessel and a pump were used. The chamber was fixed to the basis of a micromanipulator. A glass microelectrode filled with 2.5 M KCl was fixed to one arm of the micromanipulator, while an other arm was used for holding a capillar filled with ACh or 5HT solution, which could be placed instantaneously into the solution to the vicinity of the ganglionic surface.

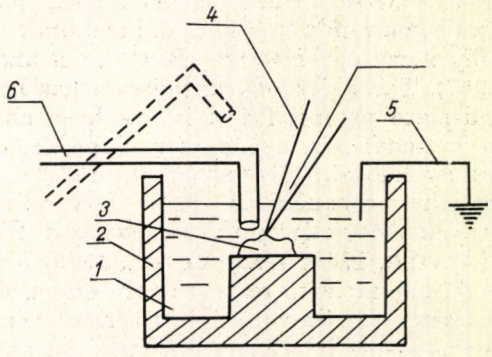

Fig. 1. Scheme of experimental arrangement
1 — physiological solution; 2 — experimental chamber ; 3 — preparation; 4 — microelectrode; 5 — reference electrode; 6 — capillar for the application of substances

During recording the glass microelectrode was connected with a FET negative capacitance high input impedance amplifier (VÉRÓ, 1971). In the course of the experiments the biological signals were fixed on a magnetic tape and the desired portions were registered later by means of a DISA Universal Indicator and photorecorder. The effects of lidocain and RG-1812 on the spontaneous activity were tested in such a way, that after recording the control activity the whole volume of the Ringer solution filling the experimental chamber was exchanged for a drug solution of appropriate concentration. For examining the chemical sensitivity first the control effects of ACh and 5HT were registered at concentration proved to be effective in our earlier work (KISS and SALÁNKI, 1971). Following this the preparation was incubated in drug solution for an appropriate time, then the effects of ACh and 5HT were tested again.

Results

Effects of lidocain and RG-1812 on the spontaneous activity of the neurones

$2\times10^{-3}-5\times10^{-4}$ g/ml concentrations of drugs were used. In several minutes marked changes occur becoming more pronounced in time and finally the spike generation ceases. This changes does not refer to the membrane potential, which was reduced by several mV-s at the most. The most pronounced effects have been shown on the following parameters:

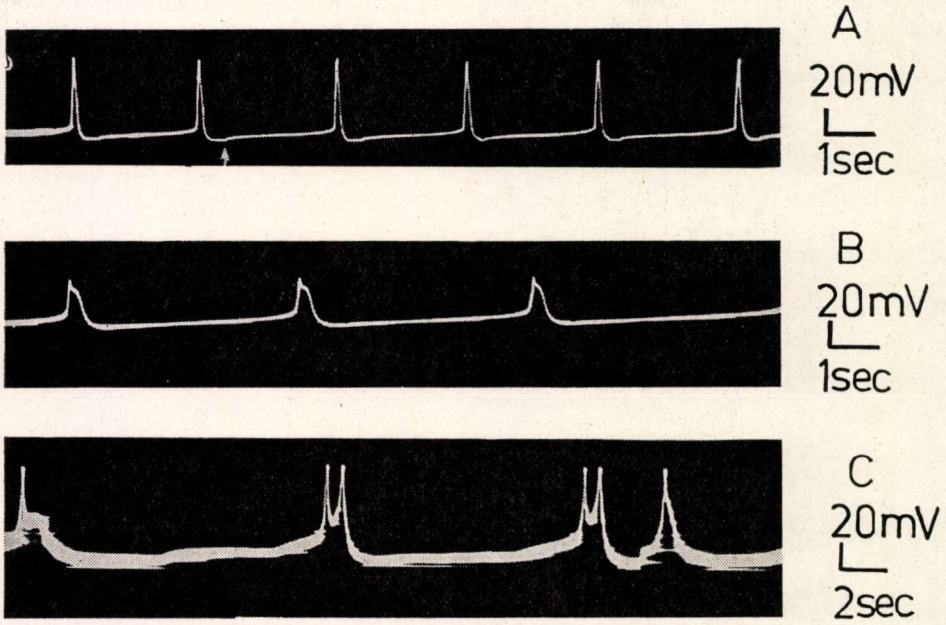

Fig. 2. Effect of lidocain and RG-1812 on the shape of the action potential *a)* arrow marks the application of 10^{-3} g/ml lidocain; *b)* 7th min. after the application of lidocain; *c)* effect of 10^{-3} g/ml RG-1812 showing the plateau formation very well

1. There is a 2—3-fold gradual increase in the spike duration *(Fig. 2)*. This increase appears to be the sum of two factors: an increased time course of the rising phase and a delayed repolarization. The latter tends to be similar to the falling phase of the action potential of heart muscle cells, in some cases a pronounced plateau is formed or during the delayed repolarization the membrane comes to be excited again resulting in a second or third peak *(Fig. 2.c)*.

2. The spike amplitude decreases. At a given concentration this reduction is proportional to the time, but the time-dependence is not linear, following abolition of the overshoot the curve becomes steeper *(Fig. 3)*. The most pronounced inflexion can be found in the case of RG-1812 of 5×10^{-4} g/ml concentration.

3. Hiperpolarizing afterpotentials tend to be reduced, at the same time, generally the rate of pacemaker depolarization decreases *(Fig. 4.b)*. After the action potential ceased, sometimes local potentials remain for a long time *(Fig. 4.c)*.

4. The spontaneous firing rate does not show any unequivocal change. Generally the effect of both drugs resulted in an increased frequency in the early period lasting for several minutes, but after 3—4 minutes there was a restoration of the control value, or a decrease to 50% of control level. In the latter case there is neither any excessive concentration-dependence nor pronounced difference between the two drugs *(Fig. 5)*. A complete abolition of spontaneous activity is caused in 5—21 min. by lidocain and in 5.7—27 min. by RG-1812. *(Fig. 6)*.

It must be noticed, that the standard deviation of data is large enough, which may be attributed to the existence of two groups of cell having different sensitivity to the drugs. The most sensitive neurones whose action potential

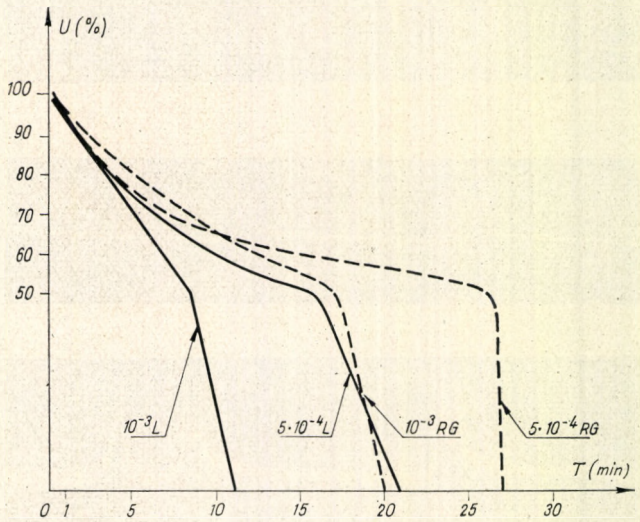

Fig. 3. Time dependence of the reduction of spike amplitude after the application of different concentrations of lidocain and RG-1812. Ordinate: diminished amplitudes as per cent of the control

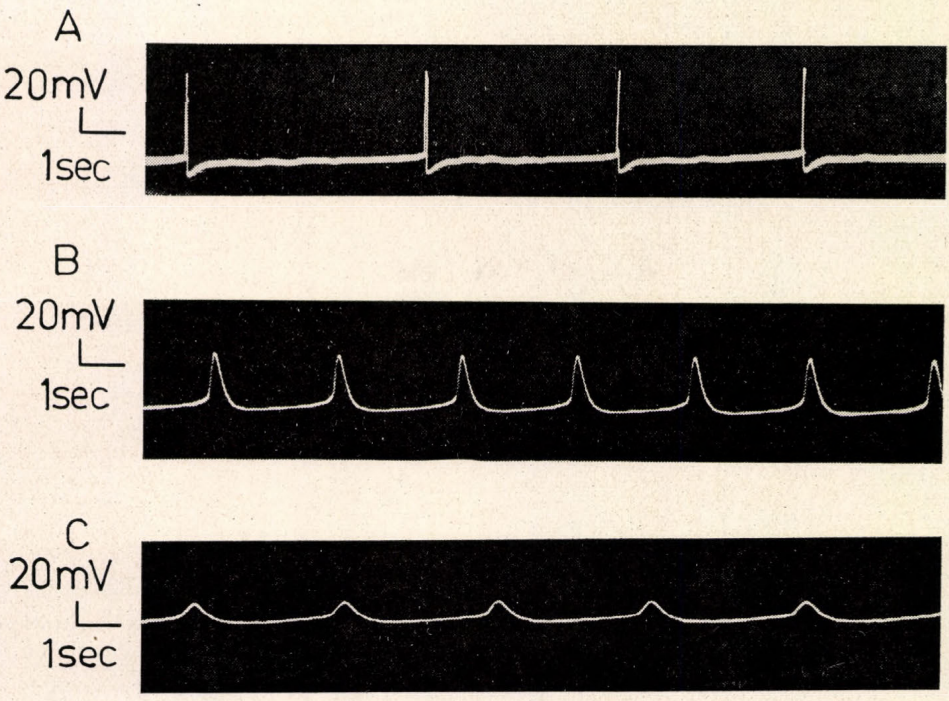

Fig. 4. Marked drug effect (RG-1812, 10^{-3} g/ml) causing a strong damage of the generation of action potential
a) control; *b)* 15th min. after the application of lidocain; *c)* in 20th min. only local potentials remain

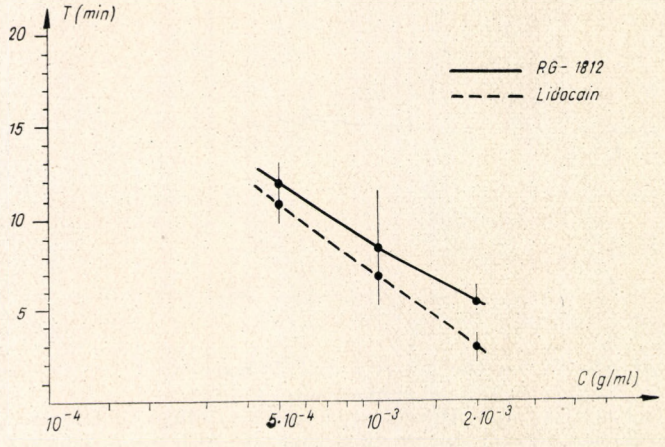

Fig. 5. Decrease in the firing rate of spontaneous activity vs. drug concentration

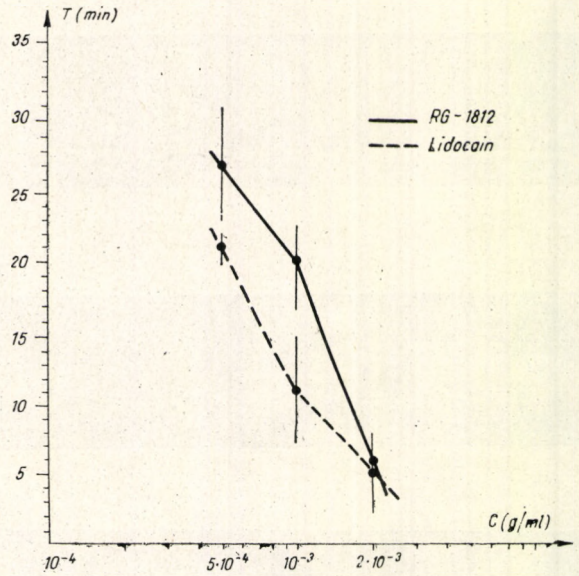

Fig. 6. Time of the complete inhibition of spontaneous activity vs. drug concentration

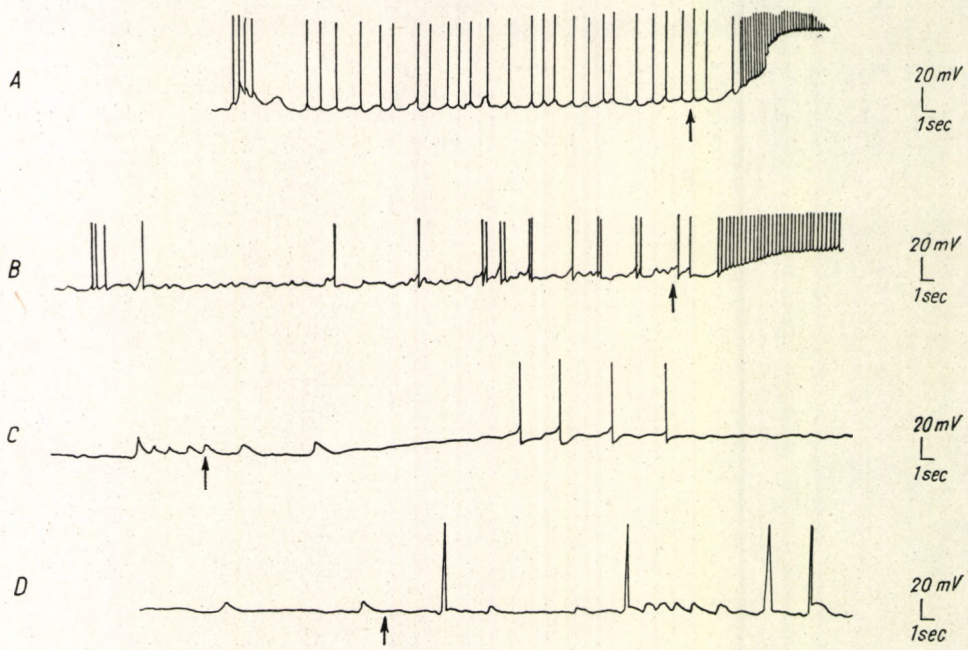

Fig. 7. Effect of lidocain on the activity and ACh-sensitivity of the neurons driven synaptically.
a) and *c)* control activity and ACh-effect; *b)* and *d)* effect of lidocain during which EPSP-s remain unchanged and the reactions given to ACh are influenced by the drugs in different ways. Arrow marks the application of ACh

can be most rapidly blocked appear to belong to the pure pacemaker cells discharging most regularly.

For making a comparison the behaviour of several synaptically driven neurones was examined. After the action potential was blocked by the drugs EPSP-s were found to remain unchanged *(Fig. 7)*.

Influence on the chemical sensitivity

For these examinations 10^{-3} and 5×10^{-4} g/ml lidocain and RG-1812 solutions were used.

a) Inhibition of the ACh-effect

The excitatory effect of ACh on D-cells is reduced by both drugs and it is completely prevented within 10 min. The effectiveness of lidocain and RG-1812 has been compared in two ways. On the one hand, the degree of inhibition has been calculated taking the increase in the firing rate caused by ACh under normal conditions as 100%. This degree is given by the mean value of data obtained within the first 9 min. following the drug application. 10^{-3} g/ml lidocain caused 56%, while the same concentration of RG-1812 96% inhibition.

On the other hand, the mean times required to prevent completely the excitatory effect of ACh by each of the drugs have been compared. In the case of RG-1812 this time is 8—9 min. *(Fig. 8 c, d)*, at the same time lidocain does not yet cause a complete disappearance of sensitivity to ACh *(Fig. 8 a, b)*.

As it has been mentioned, simultaneously with the inhibition of the spontaneous activity the examined drugs does not affect the EPSP-s. Consequently, the changes in the ACh-sensitivity cannot be so unequivocal on the driven neurones as they can be on the pacemaker ones. *Figure 7* shows two different effects of drugs on the neurones affected by ACh mainly through the postsynaptic membrane. In the "a—b" case there was a high frequency EPSP input to the postsynaptic neurone in the control state, and there is no significant reduction in the excitatory effect of ACh when lidocain is present. The figures "c—d" demonstrate the activity of a cell having a low frequency EPSP input in the control state. Following the application of lidocain the excitatory reaction of this cell given to ACh is greatly reduced.

b) Effect of 5HT in the presence of local anaesthetics

Until the 9th min. after the application of the examined drugs there is no significant change in the sensitivity to 5HT *(Fig. 9.)*. However, from the 9—10th min. 5HT is ineffective on the preparations incubated either in lidocain or in RG-1812 solution.

With respect to the influence on the serotonin-sensitivity there is no significant difference between the two drugs.

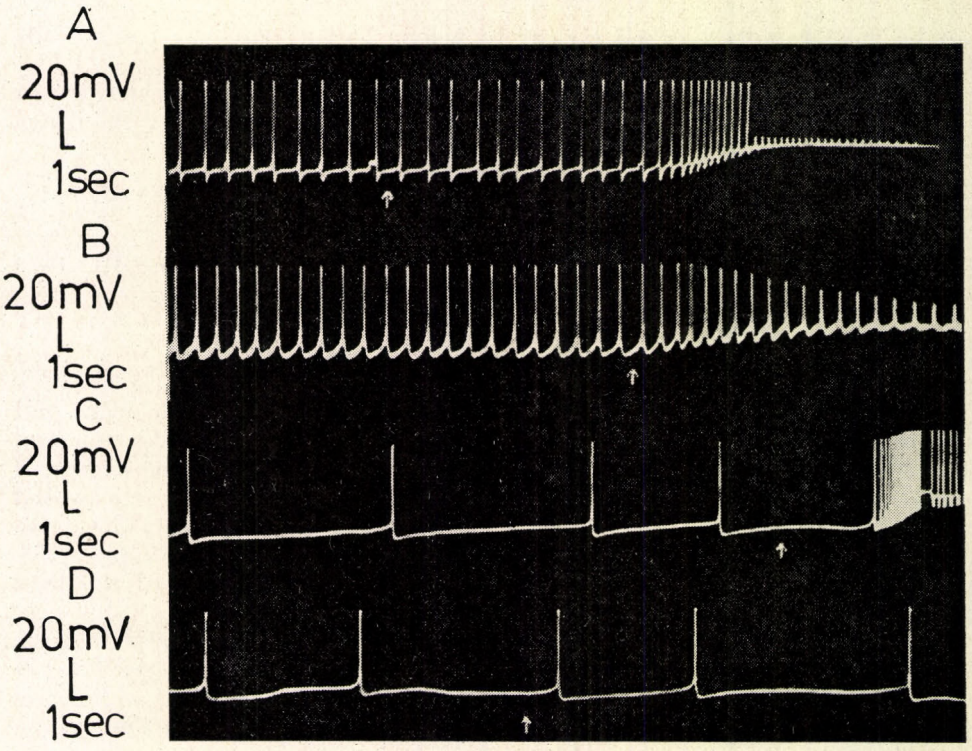

Fig. 8. Effects of drugs on the ACh-sensitivity of pacemaker neurones. *a)* and *c)* control activity and ACh-effect; *b)* in 8th min. after the application lidocain causes a decrease in the excitatory effect of ACh, but cannot completely prevent it; *d)* at the same time and concentration RG-1812 prevents completely the effect of ACh. Arrow marks the application of ACh

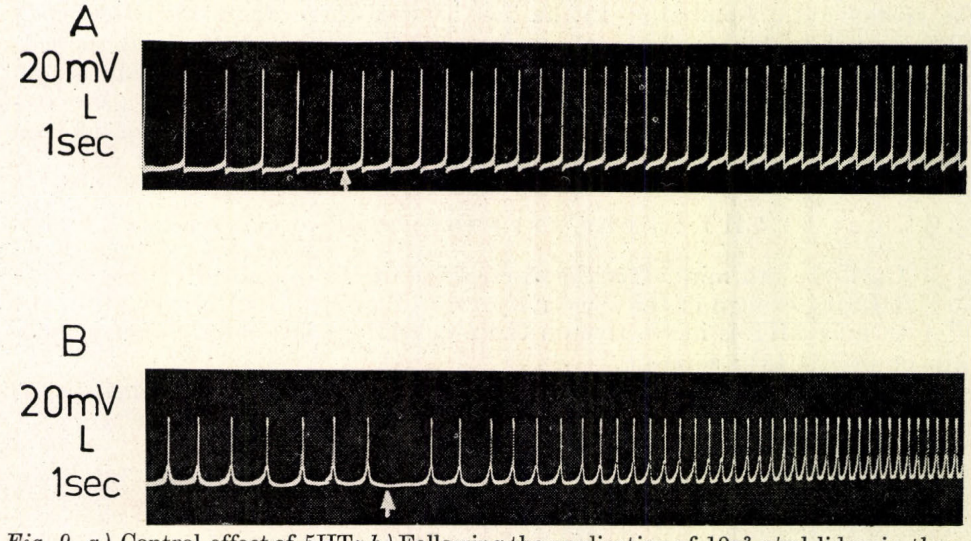

Fig. 9. a) Control effect of 5HT; *b)* Following the application of 10^{-3} g/ml lidocain there is no significant change in the effect of 5HT

Discussion

Our results show that the spontaneous firing of giant neurones reacts to lidocain and RG-1812 similarly to other excitable structures: a blockade of action potential and of the ACh-receptors of membrane is induced. There is a reason to suppose that inhibition of the spontaneous activity is resulted by a direct influence on the soma membrane, as if an inhibition of the axonal pacemaker locus occurred the somatic spike would not fall gradually, but it would be expected to disappear according to the "all or nothing" law. It is presumable again that the effect is not specific to the fundamental processes of pacemaker generation of potential as the spikes evoked synaptically are abolished too, on the other hand, the firing rate — a basic parameter of pacemaker activity — remains uneffected in a number of cases, as long as activity lasts. The oscillation in membrane potential underlining the spontaneous activity may also be maintained quite a long time after the cessation of the spike generation. It became clear that an inhibition both of the fast rising and the falling phase of an action potential occured. The decrease in amplitude as well as in steepness of the rising phase and the prolongation of repolarization take place at the same time, which indicate a simultaneous blockade of Na^+ and K^+ channels. This conclusion is in good agreement with the observations of MAENO et al. (1971) concerning lidocain.

In the present work the spontaneous activity of the soma was investigated, which was affected by ACh mainly through the receptors located on the soma membrane. Besides — as ACh was not applied by microiontophoresis — evidently the effects on the postsynaptic membrane may also be considered, but in the present case it is not the primary process, which becomes clear making a comparison with the cells driven synaptically — beside some other consideration. Namely on these driven cells one can continue to demonstrate EPSP-s of cholinergic origin after cessation of the spontaneous activity and ACh can cause an excitation similar to that obtained in the control state in spite of the presence of lidocain. This case is demonstrated in *Fig. 7a—b*, while in Fig. *7c—d* the difference between the control ACh-effect and that diminished by lidocain may be explained by a selective blockade of the soma receptors. Persistence of the synaptic input after the inhibition of spike generation can be explained by different pharmacological sensitivity of the somatic and synaptic regions. It is not surprising when one makes a comparison with some data in literature, which suggest that the Na^+-requirement for the production of axonal and somatic action potential is different (MAGURA et al., 1970), as well as that there are pharmacologically different ACh-receptors on the same individual neurone (KEHOE, 1972). In the investigations on some other objects RIKER and KOSAY (1970), CURTIS and PHILLIS (1961) and USUBIAGA and STANDAERT (1967) also found that the synaptic transmission sustained a loss later in comparison to the action potential.

If it is accepted that under the given experimental conditions the site of the action of drugs is located on the soma, the question at issue here is whether there is a common locus or there are separated loci for inhibition of the action potential and the ACh-sensitivity. Our results have demonstrated a reduction of ACh-effect, furthermore, even its complete prevention by RG-1812 occured when there was only an insignificant damage to the spike generation. These data showing the two kinds of effects to be realised at different

times support the idea of the existence of separate loci although does not verify it in se. It is rather confirmed by the results that the blockade of spontaneous activity by the drugs was more considerable at a concentration of 10^{-3} g/ml than at 5×10^{-4} g/ml, while the above difference has been failed to demonstrate for the inhibition of the ACh-sensitivity. Thus, an earlier appearance of the reduction of ACh-sensitivity might be explained as follows: After the application of the drugs more and more molecules interact with the given sites in the course of time. The ACh-receptors are sensitive even to a lower concentration, while for the receptors involved in the generation of action potential a higher concentration being reached later is required to cause a considerable inhibition. RIKER and KOSAY (1970) suggested a similar interpretation of two separated sites of lidocain action, however in their experiments the blockade of the ACh-receptors required a higher lidocain concentration because these investigations dealt with the synaptic transmission.

The prevention of 5HT-effect in 9-10th min. after the application of drugs cannot be unequivocally attributed to a specific blockade of the serotonine-receptors, since before it there was no significant inhibition, on the other hand, the spike generation is likewise considerably damaged by this time. Nevertheless, some kind of the drog-receptor interaction cannot be disregarded even so it is lower in extent compared to the inhibition of ACh. To clear up the question further investigations are required.

Comparing the effects of two examined drugs it can be established that the inhibitory effect of RG-1812 on the spike generation differs only quantitatively from that of lidocain which is shown by the dosage-effect curves, but there is qualitatively no difference, so this drug appears to be similar rather to lidocain, than to procain, regarded by MAENO et al. (1971) as a matter blocking the K^+-channel selectively.

The comparison has led to a surprising result. Lidocain, which has proved to be a stronger inhibitor of the spontaneous spike generation, is less effective on the ACh-sensitivity compared with RG-1812 having less influence on the spike generation. This fact can also be regarded, as an indirect evidence of the existence of separate sites of the action on the spontaneous activity and ACh sensitivity. Besides it is of interest to make a comparison with VARANKA's unpublished data showing on the CVC of *Anodonta* that the RG-1812 has the stronger anaesthetic effect.

Summary

Effects of lidocain and RG-1812 on the spontaneous activity and chemical sensitivity of the giant neurones of *Lymnaea stagnalis* were studied. Both drugs were found to inhibit the generation of action potential as well as to prevent the excitatory effect of ACh. It may be supposed that these two kinds of effect occur on separate receptors as their time- and concentration-dependence were not similar.

During the inhibition of action potential the rising phase and the repolarization were simultaneously damaged, which indicated a blockade of both Na^+ and K^+-channel.

The effect of RG-1812 differs from that of lidocain only quantitatively, it causes a smaller inhibition of the spontaneous spike generation, but has a more considerable effect on the ACh-sensitivity.

The effects of the drugs on the sensitivity to serotonin have not proved to be unequivocally specific.

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HELYI ÉRZÉSTELENÍTŐK HATÁSA AZ ÓRIÁS NEURONOK AKTIVITÁSGENERÁLÁSÁRA ÉS KÉMIAI ÉRZÉKENYSÉGÉRE

Kiss István és Vadász István

Összefoglalás

A lidocain és az RG-1812 jelzésű anyag hatását vizsgálták *Lymnaea stagnalis* óriás neuronjainak spontán aktivitására és kémiai érzékenységére. Mindkét drogra jellemző, hogy gátolja az akciós potenciál generálását és kivédi az ACh serkentő hatását. Feltételezhető, hogy a kétféle hatás különálló receptorokon érvényesül, mivel idő és koncentrációfüggésük nem egyezik meg.

Az akciós potenciál blokkolása folyamán a fel- és leszálló szár egyaránt sérül, amely a Na- és K-csatorna egyidejű blokkjára enged következtetni.

Az RG-1812 jelzésű anyag hatása csak mennyiségileg tér el a lidocainétól: gyengébben gátolja a spontán potenciál képzést, ugyanakkor erősebben hat az ACh-érzékenységre.

A drogok hatása a szerotonin-érzékenységre nem egyértelműen specifikus.