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SENSORY INPUT CHARACTERISTICS AT THE CHEMICAL STIMULATION OF THE LIP IN THE SNAIL *HELIX POMATIA* L.

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The role of contact chemoreceptors in food detection, in escape reaction to predators and also in forming some other behavioural patterns was investigated both in terrestrial and aquatic gastropods (in: CHARLES, 1966). In experiments testing the reaction of the whole animal to various substances it has been shown that the receptors located around the mouth can differentiate among chemicals (KIECKEBUSCH, 1953). This finding is in correlation with earlier morphological data describing various receptor structures in the lip of the snail (SCHULZ, 1938).

Electrophysiological experiments on snail chemoreceptors have been done mainly on the osphradial nerve in order to elucidate the chemoreceptive characteristics of its peripheral organ. Although from the nerve itself no action potentials could be recorded (KOHN, 1961), when stimulating the osphradium with some chemicals the specific response of several central neurones was observed (BAILEY and LAVERACK, 1963; STINNAKRE and TAUC, 1969). Lately some special chemoreceptive ability of the heart and the central representation of the afferent inputes were demonstrated in *Helix* (S.-RÓZSA, 1972; S.-RÓZSA and SALÁNKI, 1973).

In our recent investigations we are dealing with the irritability of the receptive field of the mouth in the snail. We aim to explore the effect of different chemical substances and to clear up the peripheral and central mechanisms of the discrimination between them. In the present paper the methods applied and further, some characteristics of the afferent activity recorded from the lip nerve upon stimulating the chemoreceptors with various substances are described.

Material and method

Experiments were carried out partly on brain-lip preparations partly on isolated lip preparation of *Helix pomatia* L. When making the brain-lip preparation, these structures and the nerve connecting them were isolated, while in the case of the isolated lip only the radula, lips and a part of the lip nerve were included *(Fig. 1)*.

Using free moving animals, the shell of the snail has been removed, the coelom sack was cut and the central nervous system (CNS) with the nerves

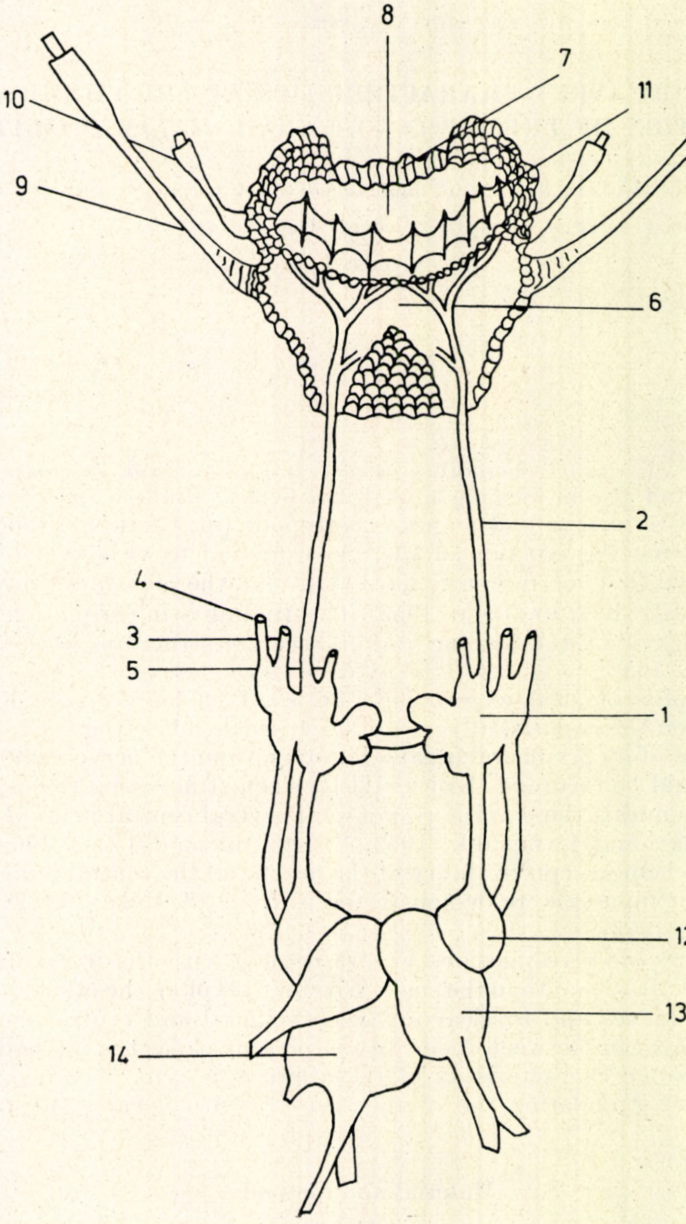

Fig. 1. Gross anatomy of the head and the CNS in *Helix pomatia* L. 1 — right cerebral ganglion; 2 internal lip nerve; 3 — medial lip nerve; 4 — external lip nerve; 5 — cerebro-buccal connective; 6 — upper lip; 7 — lower lip; 8— mouth; 9 — upper tentacle; 10 — lower tentacle; 11 — radula; 12 — right pleural ganglion; 13 — right parietal ganglion; 14 — visceral ganglion

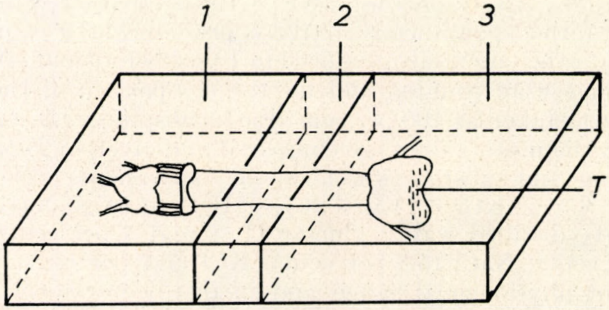

Fig. 2. Preparation placed in the experimental chamber. 1 — circumoesophageal ganglionic ring; 2 — lip nerves; 3 — mouth preparation; T = test zone

running towards the mouth were explored. Then the lip was isolated from the surroundings. The oesophagus was eliminated too, and so only the radula and the lips remained intact together with their nerve connections. Finally the CNS was nearly totally isolated: all its nerves were cut except the pair of lip nerves running to the upper part of the mouth.

The preparation was placed in a three parted plexiglass chamber *(Fig. 2)*. In the first the ganglionic ring, in the third the oral part, while between them, in the second, the lip nerves were located, the latter running through thin cuttings made on the two inner walls dividing the three sections. After placing the preparation in, the middle part was isolated from the others by filling up the cuttings with vaseline. The cut parts of the feeding organ, the nerve and the CNS were in physiological saline, while the lip was above the solution. When electrodes were placed under the nerve, the physiological solution was suctioned from the middle part and was replaced by paraffin oil, rendering recording possible and protecting the nerve from drying at the same time.

The isolated lip preparation was made similarly but the lip nerves were also cut at branching off from the CNS. Accordingly only the activity originating from the periphery was recorded. Both preparations remained in good accondition for hours at room temperature (20—24°C).

The method of testing the activity of the receptors was as follows: the electrical activity was recorded from one of the lip nerves by bipolar platinum electrodes fed into the input of an Alvar AC amplifier and registration took place from the screen of oscilloscope to film. It was cleared up in the preliminary experiments that upon lip stimulation the highest synchronous activity increase can be recorded from the internal lip nerves in isolated preparations. The exact area which gave the highest response on the nerve to tactile stimulation was determined by trials in every case *(Fig. 2,.T)* and this test zone was exposed locally to various chemicals during the investigations.

The application of substances was performed by using 5×5 mm filter paper soaked in solution prepared for testing, then it was put gently onto the test zone. At the first time for each preparation the piece of filter paper applied was soaked in physiological saline. This way the response to the tactile stimulus was tested. During the experiments the preparation was washed with physiological solution after each test, and the tests were carried out at 5—10 min intervals to assure the recovery of the receptor sensitivity.

The change of activity on the nerve at the appliation of test solutions was compared to the spontaneous activity preceding the application. At processing the data the potentials produced in 10 sec before and after the application of the drugs were counted and for the comparison of the values the control activity was taken as 100 per cent. Each substance was used in several preparations and the mean values are compared and given in *Table I*.

The solutions and substances used in the experiments were: physiological saline (NaCl 6.5 g, KCl 0.14 g, $CaCl_2 \cdot 2H_2O$, 0.12 g, $NaHPO_4$ 0.01 g, $NaHCO_3$ 0.02 g/l), distilled water; glucose (1, 2 and 5 per cent); saccharose (1, 2 and 5 per cent); NaCl (5.8 per cent); KCl (7.5 per cent). Sugars were dissolved both in physiological saline and in distilled water. The effect of several plant protecting agents which are used in agricultural practice against weeds, insects and molluscs, were also tested. These are: dazomet (3,5-dimethyl-tetrahydro-1,3,5-tiadiazin-2-tion) (10^{-2} and 10^{-3} M); dipterex (0,0-dimethyl 2,2,2-trichloro-1 hydroxyethyl phosphate) (10^{-2} and 10^{-3} M); malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate) (from 10^{-4} to 10^{-8} g/ml). The first two were dissolved in physiological solution while the third in distilled water.

The experiments were carried out throughout the year.

Results

A. *Brain-lip preparation*

Activity could be recorded from the nerve also in the case when no stimuli were applied to the test zone. This "resting" activity is composed of irregularly distributed fast components of various amplitudes (50–100 μV). The frequency varies in different preparations and changes also in the same preparation in time between 3–8 imp/sec. As a mean of 110 measurements it was found 4.6 spikes/sec *(Fig. 3)*. When touching the lip with a fine brush the

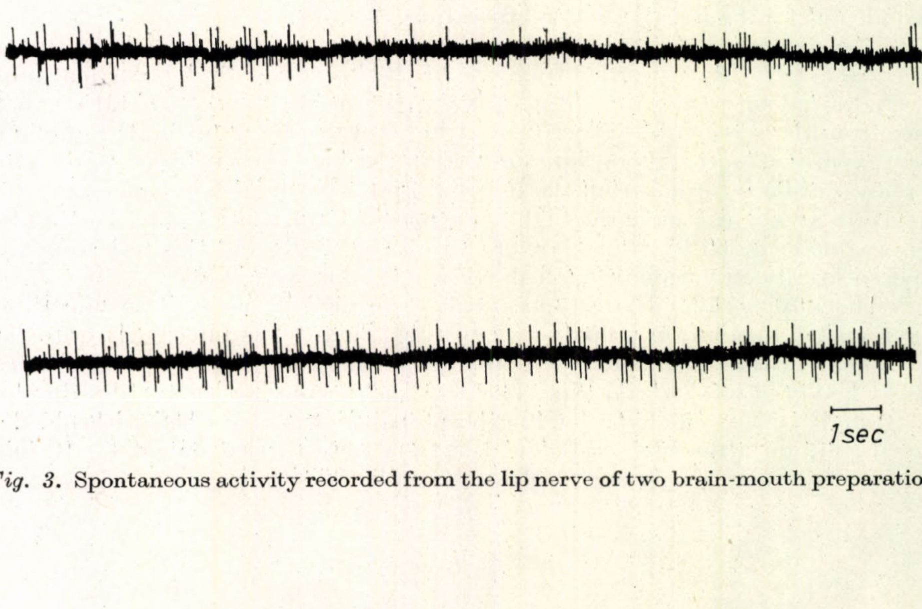

Fig. 3. Spontaneous activity recorded from the lip nerve of two brain-mouth preparations

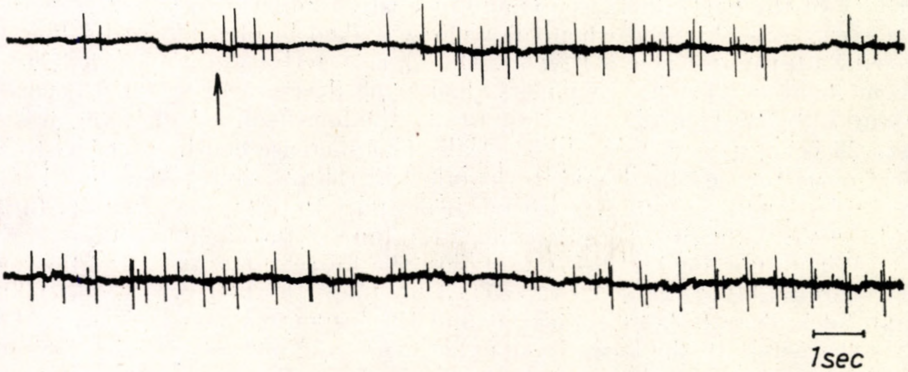

Fig. 4. Effect of filter paper soaked in physiological saline applied onto the lip. The second row is the continuation of the first one (in each further case too)

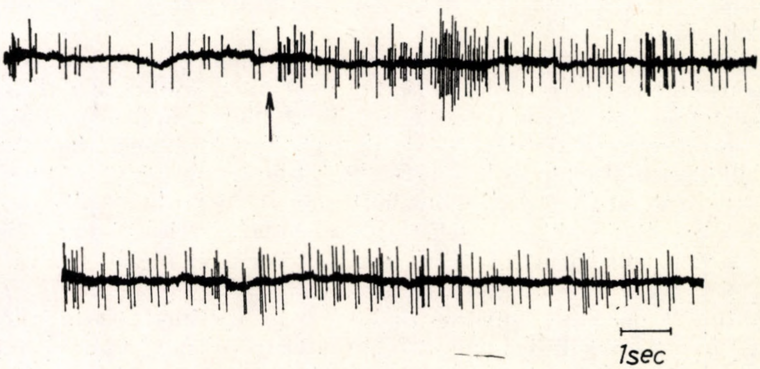

Fig. 5. Effect of distilled water

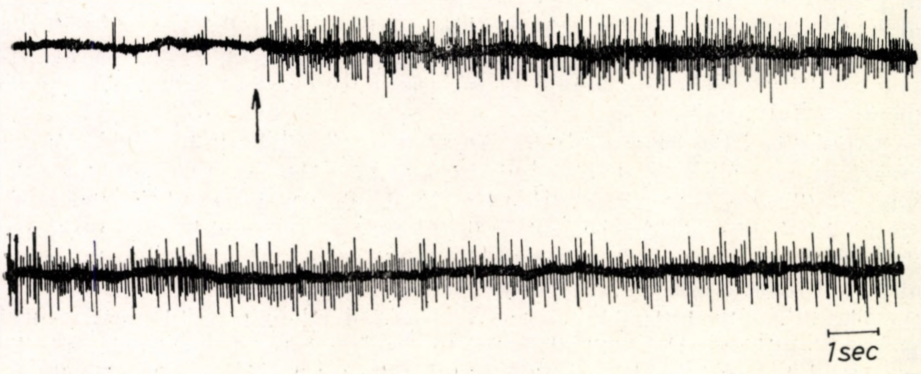

Fig. 6. Effect of 1 mol NaCl

activity of the nerve increased simultaneously. Similarly to this, the increase of activity was observed when filter paper soaked in physiological saline was placed onto the test zone *(Fig. 4)*. The highest activity was recorded just at the moment of placing the paper, then some decrease of activity occurred. Taking into consideration the first 10 sec, the increse of activity was in these cases 28 per cent as a mean of 17 trials. This increase can be ascribed to the effect of the tactile stimuli and to the effect of the physiological saline. Taking this into account, at applying various drugs only the change of activity differing from this value was considered as a repsonse of the chemoreceptors.

When applying distilled water onto the lip the activity of the lip nerve was considerably increased *(Fig. 5)*. The spikes, both of lower and higher amplitudes, became more numerous and the response was long-lasting, tonic in its character. In the mean of 10 experiments the increase of frequency was by 53 per cent above the control.

Applying 1 mol solution of NaCl onto the lip a very high increase in activity was detected *(Fig. 6)*. Both the lower and higher amplitudes became more frequent. The response is most expressed in the first seconds, decreasing somewhat after 6—10 sec but at continuous application it did not disappear within 1 min. In the mean of 8 experiments the increaase of the activity surpassed the control by 119 per cent.

The highest activity increase occurred at the application of KCl in 1 mol concentration. After a transient period of 1—1.5 sec, when the activity increase was only moderate, a very frequent and of high amplitude (150—200 μV) impulsation appeared. It decreased somewhat in 3—4 sec, then, in the case of continuous application of KCl these high frequency spikes reappeared again in the form of burst-like oscillations with somewhat lower amplitudes *(Fig. 7)*. The effect was tonic, mixed with these secondary series of burst-like spikes. Under the effect of KCl the activity was 147 per cent higher than that of the control.

Adding 1 per cent glucose dissolved in distilled water the activity response *(Fig. 8)* was differing in its character from that observed for distilled water. The increase of activity was not significant, it preceded the control only by 54 per cent, however, the newly appearing groups of spikes consisting at the beginning of 8—10, later on of 2—4 potentials of high amplitudes show a great difference both from the control and from the effect of distilled water. The effect of glucose was more expressed when it was solved in physiological saline and when different concentrations were used. 1, 2 and 5 per cent glucose caused 51, 68 and 78 per cent increase in activity, respectively, what is regarded to be significant. The effect of glucose gradually decreased but did not disappear within 1 min.

Applying 1 per cent solution of saccharose dissolved in distilled water an increase of activity was observed again, and also the frequency of potentials of high amplitudes is characteristic *(Fig. 9)*. Under the effect of saccharose the activity was increased by 41 per cent in the first 10 sec, and in the appearance of high potentials some periodicity could be observed. The response was tonic, and lasted over 1 min.

The effect of 1 per cent saccharose dissolved in physiological solution was also significant, the evoked increase of activity reached 89 per cent. The same effect was recorded for 2 per cent saccharose evoking a 89 per cent increase, while a 5 per cent solution of saccharose did not cause any increase,

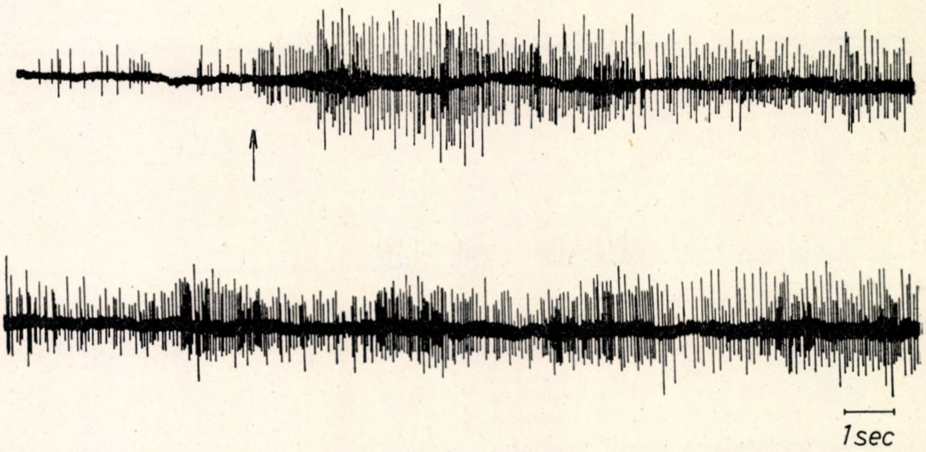

Fig. 7. Effect of 1 mol KCl

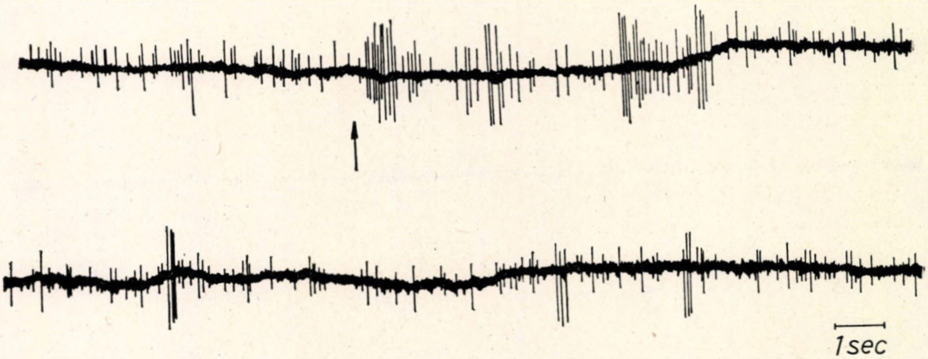

Fig. 8. Effect of 1 per cent glucose dissolved in distilled water

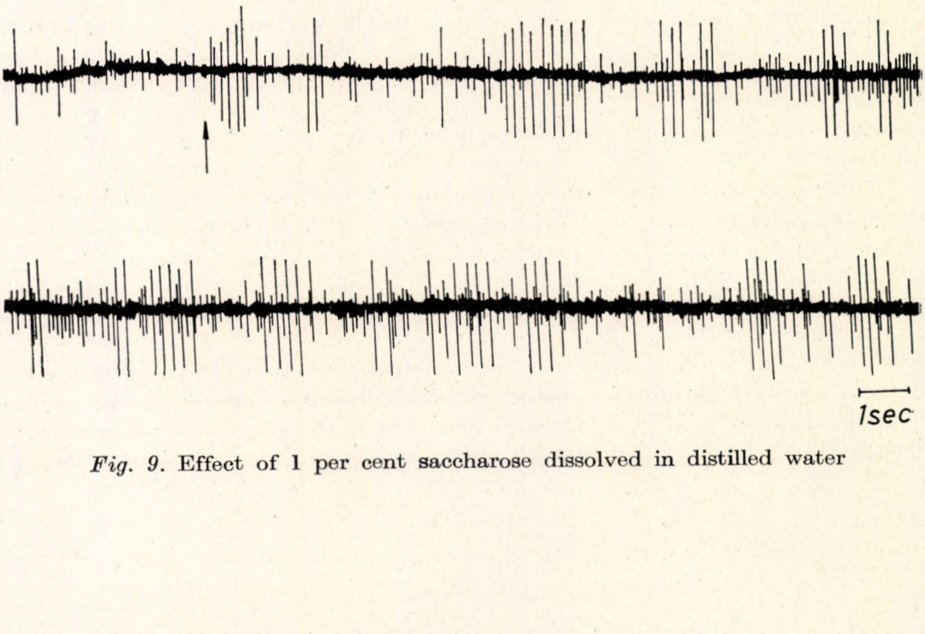

Fig. 9. Effect of 1 per cent saccharose dissolved in distilled water

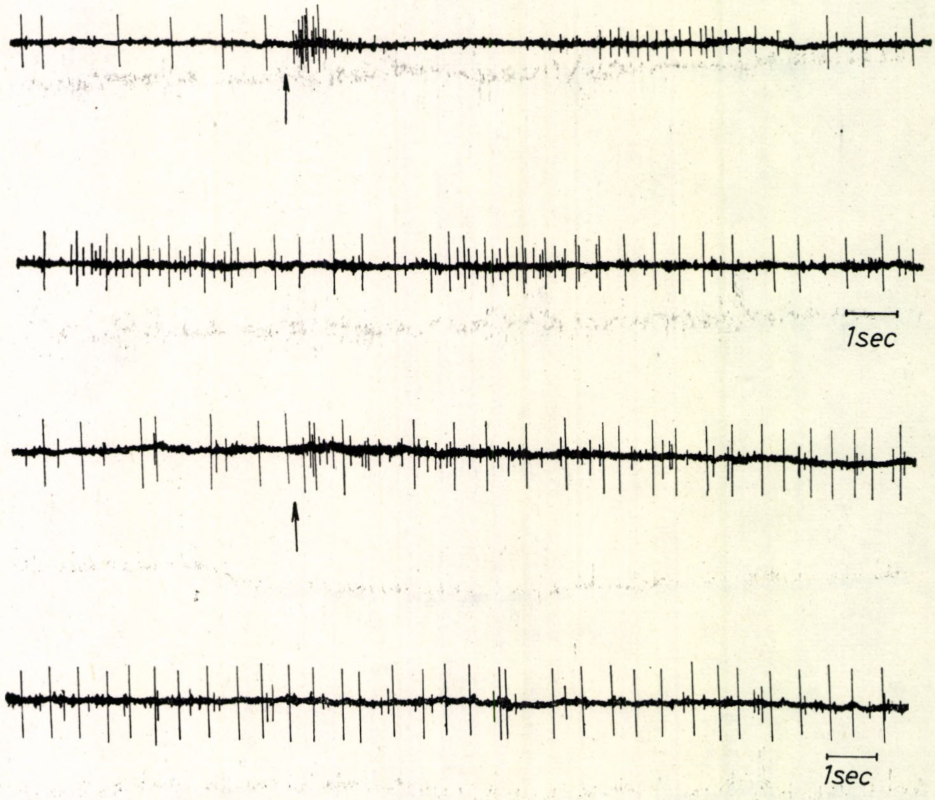

Fig. 10. Effect of dasomet 10^{-3} mol (upper) and 10^{-2} mol (lower)

activity corresponded only to 102 per cent of the control. Considering the effect of tactile stimulation (28 per cent increase), the effect of the 5 per cent saccharose must be interpreted as inhibitory.

Dasomet is a herbicide and belongs to the carbamat group, it evoked 70 and 55 per cent increase of activity in 10^{-2} and 10^{-3} mol concentrations, respectively. The effect was characteristic, dominating in the increase nearly regularly distributed single potentials of high amplitudes. In some cases the mixed appearance of higher and lower potentials, belonging probably to several axons was observed *(Fig. 10).*

Dipterex, being an organic phosphoric acid ester (insecticide), also increased the activity when applied onto the lip *(Fig. 11).* The increase of activity was tonic and surpassed the control by 92 per cent when used in 10^{-2} mol concentration, while by 41 per cent in 10^{-3} mol concentration. In 6–8 sec after application there was a decrease in activity, nevertheless, it was also at this time considerably higher than the control.

Malathion belongs also to organic phosphoric acid esters, it is used to kill molluscs. We tested its effect in a wide concentration range. At application of 10^{-4} g/ml the activity was 85 per cent higher than the control, while between 10^{-6}–10^{-8} g/ml it evoked only about 40–60 per cent increase, not differing much from the effect of the distilled water, used this case as a solvent. The increase both of lower and of higher spikes was observed *(Fig. 12)*, but the variation of amplitudes was less than when adding distilled water alone.

B. *Isolated lip preparation*

When the two lip nerves connecting the feeding organ and the CNS were cut at their origin from the cerebral ganglia, the control activity was suddenly increased. In about 10 min it decreased gradually than kept a constant level being somewhat higher, than before cutting off the ganglia. The activity varied between 2 and 11 imp/sec, yielding a mean of 5.3/sec (from 236 measurements). The amplitudes of the potentials measured $50-100$ μV.

It must be noted that spontaneous activity could be recorded from the nerve even if it was cut also at entering the lip, i.e. if an isolated nerve preparation was made. However, this activity ceased entirely within $8-10$ min, or very rarely occurring low ($20-30$ μV) potentials of long duration could be observed over ten minutes.

Applying the above substances onto the lip different activity increases could be observed, however, it was always less than we found on the brain-lip preparation. 15 per cent increase of activity caused by tactile stimulus was not

TABLE I

Electrical activity recorded from the lip nerve. The number of potentials occurring within 10 sec was counted. Values are expressed as percentage of the control

Substance	Brain-mouth preparation	Number of experiments	Isolated mouth preparation	Number of experiments
Physiological saline (control)	100		100	
Physiological saline applied on filter paper (tactile stimulus)	128	17	115	14
Distilled water	153	10	107	9
NaCl 5.8 per cent in dist. w.	219	8	145	12
KCl 7.5 per cent in dist. w.	247	7	166	16
Glucose 1 per cent in dist. w.	154	9	115	18
Glucose 1 per cent in physiol. saline	151	3	102	14
Glucose 2 per cent in physiol. saline	168	3	115	15
Glucose 5 per cent in physiol. saline	178	3	124	14
Saccharose 1 per cent in dist. w.	191	9	132	18
Saccharose 1 per cent in physiol. saline	189	3	132	15
Saccharose 2 per cent in physiol. saline	185	3	127	15
Saccharose 5 per cent in physiol. saline	102	3	141	15
Dasomet 10^{-3} mol in physiol. saline	155	9	112	8
Dasomet 10^{-2} mol in physiol. saline	170	8	123	10
Dipterex 10^{-3} mol in physiol. saline	141	8	122	9
Dipterex 10^{-2} mol in physiol. saline	192	7	122	8
Malathion 10^{-8} g/ml in dist. w.	142	7	145	8
Malathion 10^{-6} g/ml in dist. w.	160	7	140	6
Malathion 10^{-4} g/ml in dist. w.	185	7	111	6

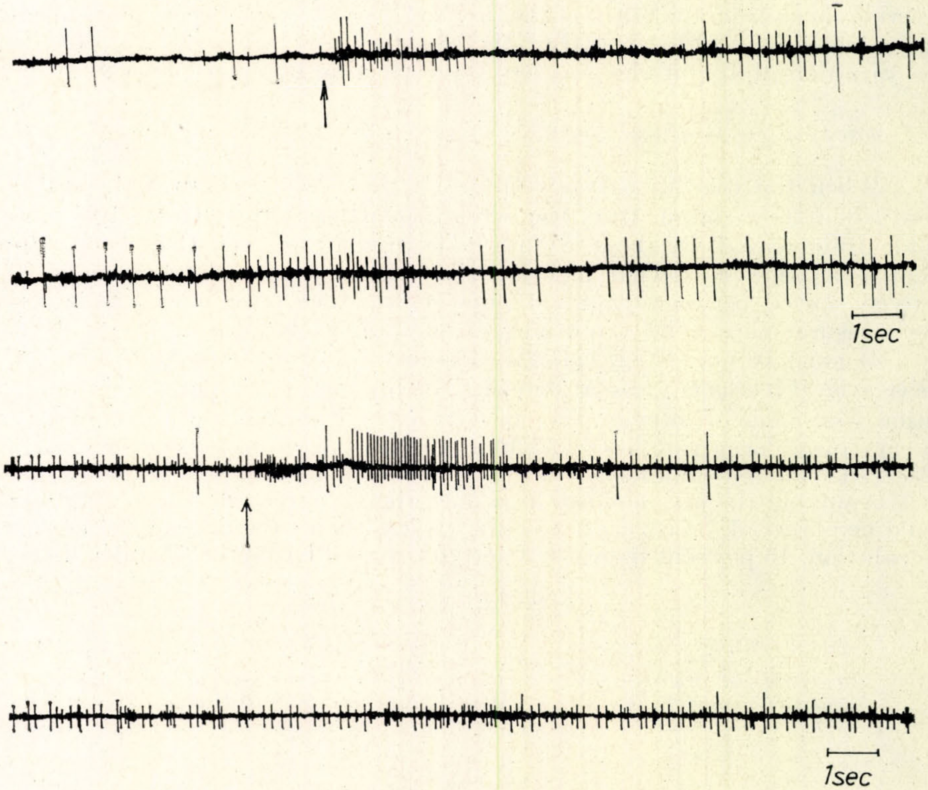

Fig. 11. Effect of dipterex 10^{-3} mol (upper) and 10^{-2} mol (lower)

surpassed upon applying distilled water, 1 and 2 per cent solutions of glucose solved both in distilled water or in physiological solution, dasomet in 10^{-3} mol and malathion in 10^{-4}—10^{-8} g/ml. In the other cases the increase of activity varied between 22 and 66 per cent. The mean values obtained at the application of substances is presented in *Table I*. For comparative purposes the values obtained on the brain-lip preparation are also presented.

Discussion

When investigating the feeding habits and food detection of several gatropods the specificity of receptors localized around the mouth was shown by several authors. KEICKEBUSCH (1953) carried out experiments on the behaviour of *Helix pomatia* offering various sugars and salts in solution. Based on the time of reaction he concluded that the chemoreceptors of the head, foot and mantle margin are different according both of sensitivity and type of the response. CARR (1967a, b) investigated the sensitivity of *Nassarius obsolatus* to the components of crab extract and found that the receptors of the proboscis are especially sensitive to several amino acids, amines and some other substances

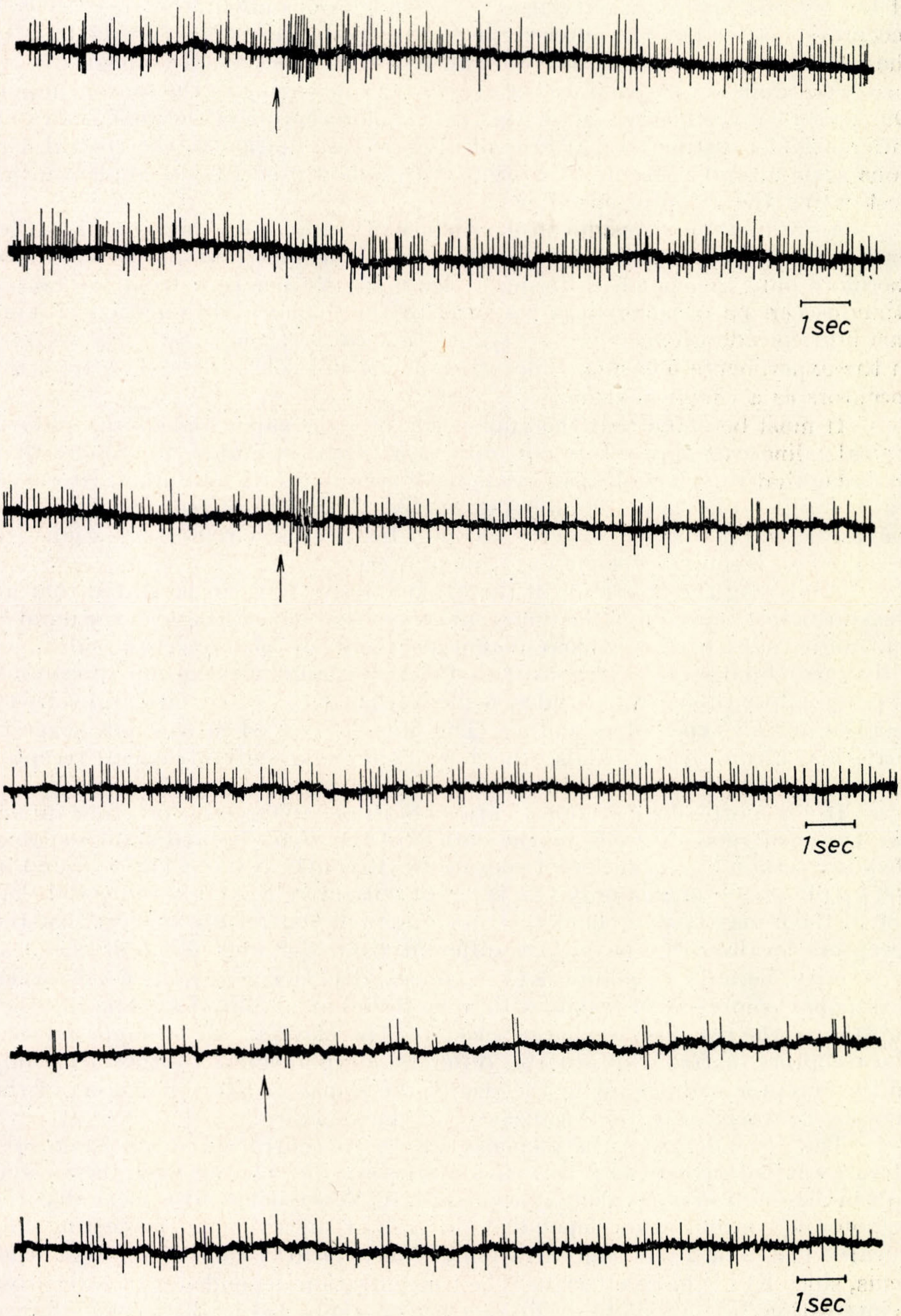

Fig. 12. Effect of malathion. Upper: 10^{-8} g/ml, middle: 10^{-6} g/ml, lower: 10^{-4} g/ml

of the extract. In these experiments the conclusions about the specificity of receptors localized on the outer surface of the body were drawn on the basis of the behavioural reaction of the animal, however, no electrophysiological analyses were done for clearing up the specificities occurring in the sensory input. Our present investigations show that lip chemoreceptors of the snail send well differentiable, distinctive patterns of afferent impulsation to the central nervous system, and it seems to us that the method used can be applied in investigating the receptor specificity.

According to our results on the surface of the lip both chemo- and tactile-receptors are present. The test method used includes also tactile stimulus, therefore only the values differing at least 15 – 20 per cent from the tactile stimulus can be considered as response to the chemical stimulation. Taking this into consideration, it can be stated that each of the substances we used in the experiments has such concentration which evokes a response from the receptors as a chemical stimulus.

It must be noted that the application of filter paper soaked into physiological saline can play a role not only as a tactile stimulus. It is more than probable that the physiological saline itself stimulates the receptors. However, for making and keeping the preparation alive the use of physiological saline is inevitable and it contacts also the receptor field. These circumstances must be accepted as a source of some experimental error.

Analyzing the character of the evoked activity it can be stated that all the substances caused long-lasting, tonic responses, nevertheless, in the number and amplitudes of the evoked potentials there are characteristic substance differences. So it was characteristic that sugars stimulate the group appearance of potentials with high amplitudes, while NaCl or KCl caused a general activity increase for all types of potentials. The effect observed in dasomet suggests that this substance causes also the activity increase only of several receptor types.

In his morphological investigations Schulz (1938) described four different types of receptor cells in the cuticle of the *Helix* lip and supposed that they are responding to different sensations. Recently Navoni (1973) found in the lip of opistobranchia only two types of sensory cells, and he supposed that one of them may have a chemoreceptor function. Our results suggest that the receptors localized in the lip are different from the wiev point of chemical sensitivity, because on adding KCl, a general depolarizing agent, we observed a response composed of spikes with very different amplitudes while in other substances the response was more selective. Therefore, it can be supposed that the receptors of *Helix* lip are also different morphologically either according to the receptor endings, or in the size of the somas or axon diameters of the sensory neurone, or in the localization of the somas.

The dependence of the response on the concentration of the same substance was not investigated this time systematically, neither were the various substances used in equivalent concentrations. Nevertheless, it is clear that the response is concentration-dependent (in case of sugars, plant-protecting chemicals). The same 1 mol concentration of NaCl and KCl caused different reactions, since KCl is more effective. The concentration-dependency of saccharose is interesting, because on brain-lip preparation there was no difference between the effect of 1 and 2 per cent solution, while 5 per cent saccharose was inhibitory in action. This may parallel the observation of Kieckebusch (1953) who found that *Helix* responds less to 5 per cent saccharose than to a 2 per cent solution of the same.

The activity increase observed on brain-lip preparation and on isolated lip preparation are differing mainly quantitatively, nevertheless, in several cases also qualitative differences occurred. So, in isolated lip preparation the control activity proved to be higher by about 15–20 per cent, but the increase of activity on the application of various substances was nearly always lower on this preparation. There seem to be two possible explanations for the latter. One possibility is that because of the higher control level the same activity increase results in a lower value if expressed in the percentage of the control. The other possibility is that the cutting of the nerve between the CNS and the lip causes the switch out of some receptors. The latter is more probable, since the sensory fibres having their soma inside the ganglion got eliminated by cutting their axons and they do not show spike activity any more.

The fact that the nerve of the isolated lip preparation gives a higher spontaneous activity than that of the brain-lip preparation refers to the well-known phenomenon that CNS has a regulatory role on the sensitivity and activity of the receptors. It seems that in sensory neurones relieved from central control there is a higher resting activity, but at the same time their sensitivity drops, too. Especially notable is the low sensitivity of receptors to distilled water and sugars in the isolated lip preparation, what can be explained by one of the above reasons.

The spontaneous activity observable in isolated nerve preparation for a short period of time can be ascribed to the firing of neurone somas located in this nerve, elicited by the injury of the axons. The presence of neuronal bodies in the intestinal nerve of *Helix* was shown by SCHLOTE (1955).

Summary

1. The chemical sensitivity of the lip receptors to various chemicals is different what can be detected by recording the electrical activity from the lip nerve both in brain-lip preparation and in isolated lip preparation.

Applying the general depolarizing agent KCl to the lip, the variation of spike amplitudes is very wide in the increased activity, the selectivity of the responding receptors is well expressed in the case of glucose and saccharose.

3. Distilled water seems to be a stimulus increasing the activity of the receptors differently than does physiological saline. The effects of NaCl, glucose, saccharose and of drugs used in plant-protecting chemistry (dasomet, dipterex and malathion) were also significant in some concentrations.

4. The basic activity of the lip nerve was higher on the isolated lip preparation than on the brain-lip preparation. This refers to the role of the CNS in the control of the activity of sensory neurones. Our electrophysiological records prove that there are neurones in the isolated lip nerve which function even after the isolation of the nerve.

5. Isolated lip preparations gave a somewhat lower response to the same chemical substance than did brain-lip preparations. This can be explained with the presence of a part of the sensory neuronal bodies in the ganglion itself.

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SZENZOROS BEMENET ELEKTROFIZIOLÓGIAI VIZSGÁLATA A SZÁJ KÖRÜLI RECEPTOROK KÉMIAI INGERLÉSE ESETÉN *HELIX POMATIA*-N

Salánki János és Truong Van Bay

Összefoglalás

1. Száj körüli receptorok kémiai érzékenysége különböző anyagokra eltérő, s ez az elektromos aktivitás ajakidegről történő elvezetése során agy-szájszerv preparátumon és izolált szájszerv preparátumon egyaránt jól detektálható.

2. Az általános depolarizáló hatású KCl ajakra való applikálásakor regisztrált aktivitásban az amplitúdóvariáció nagy, a glukóz, szacharóz esetén a szelektivitás jól kitűnik.

3. A desztillált víz a fiziológiás oldattól eltérő aktivitásfokozó ingert jelent az ajak receptoraira. Ugyancsak jelentős a NaCl, a glukóz, szacharóz, valamint a növényvédelemben használatos dasomet, dipterex és malathion hatása is.

4. Izolált szájszerv preparátum esetén az ajakideg alapaktivitása magasabb, mint agy-szájszerv preparátumnál. Ez a központi idegrendszer szenzoros sejtaktivitást szabályozó szerepére utal. Elektrofiziológiai adataink azt bizonyítják, hogy neuronok az izolált idegben is előfordulnak, melyek egyideig az ideg izolálása után is működnek.

5. Az izolált szájszerv preparátum ugyanazon kémiai ingerre kisebb aktivitással reagál, mint ami agy-szájszerv preparátum esetében megfigyelhető. Ez a központi idegrendszer receptorérzékenységet befolyásoló hatásával lehet összefüggésben ill. azzal, hogy a szenzoros neuronok egy része a központi idegrendszerben helyezkedik el.