

CHARACTERISTICS OF THE RESTING AND ACTION POTENTIALS OF THE HEART MUSCLE FIBRES IN SOME INSECTA SPECIES

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The basic mechanisms of the generation of biopotentials have not been elucidated in Insecta hearts. Investigations using microelectrodes were carried out only on the heart of a few species, particularly on *Bombyx mori* (ISHIKAWA, 1959), *Hyalophora cecropia* (McCANN, 1963; 1965), *Musca domestica* (FOURCROY, 1967), *Periplaneta americana* (SNEFF, 1967; MILLER, 1969), and *Sarcophaga bullata* (BRUEN and BALLARD, 1970). Neither these results nor macroelectrophysiological investigations (TENNEY, 1953; ZWICKY and HODSON, 1965; SNEFF, 1967; MILLER and METCALF, 1968) gave a basis for generally acceptable conclusion as the data described in these species are contradictory regarding the shape of the potentials as well as the mode, place and origin of heart rhythm. For the solution of the contradictions parallel studies of the underlying mechanisms on several species were proposed (McCANN, 1970), which may offer possibility to draw general conclusions regarding the myogenic or neurogenic nature of the rhythm in Insecta hearts, as well as regarding the properties of the physiological phenomenon known as reversal-phenomenon (GEROULD, 1933; TENNEY, 1963; McCANN and WIRA, 1967; McCANN, 1970).

Material and method

Experiments were carried out on the hearts of *Locusta migratoria migratorioides*, *Mantis religiosa*, *Phaneroptera nana*, *Ephippigera ephippiger*, *Tettigonia viridissima* and *Gryllotalpa vulgaris*. *Locusta* was obtained from our breeding house kept at 32–36 °C temperature with 12 hours photoperiodism. The other species were collected on Tihany peninsula.

The exposing of the hearts was done as reported elsewhere (S.-RÓZSA and V.-SZŐKE, 1970). In the experiments physiological saline was used, prepared for *Periplaneta* heart according to LUDWIG et al. (1957). The experiments were performed at room temperature (22–24 °C).

For the recording of the resting and action potentials conventional glass microelectrodes were used filled with 3 M KCl. The resistance of the microelectrodes was between 10–20 MΩ and their tip-potential never exceeded –7 mV. The biological signal was sent through a compensation unit to a negative capacitance electrometer (VÉRÓ, 1970) and was displayed on a dual beam oscilloscope (EMG Typ. TR 4602) and the records were made by a photorecorder. All records are positive upward.

Results

1. Resting potential

The values of the resting potentials varied between 12–40 mV in the investigated species. The lower values (12 mV) were registered from the heart of *Phaneroptera* and *Tettigonia*, the highest ones (40 mV) from *Locusta*, *Mantis* and *Gryllotalpa* hearts. The results are summarized in the Table I.

TABLE I
Characteristics of the resting and action potentials in the heart muscle fibers

Species	Resting potential (mV)	Action potential			
		amplitude (mV)	duration (msec)	overshoot (mV)	frequency (cycle/min)
<i>Locusta migratoria</i>	20–40	28–50	30–60	1.1–11.6	12–60
<i>Mantis religiosa</i>	15–40	20–50	60–140	5–15	18–32
<i>Phaneroptera nana</i>	12–35	15–50	40–80	3–15	30–45
<i>Ephippigera ephippiger</i>	15–30	20–40	60–120	5–10	30–50
<i>Tettigonia viridissima</i>	12–35	25–45	60–100	10–13	16–24
<i>Gryllotalpa vulgaris</i>	12.5–40	15–45	89–167	2.5–5	12–28

The value of the resting potentials differed from one another not only in different species but also in the different individuals of the same species; moreover, in the same heart one myocardial cell could be characterized by low and the other by high value of the resting potential.

Data in Table I show the lowest and the highest values registered in the course of investigations.

2. Action potential

The amplitudes of the action potentials varied between 15–50 mV. The lower values (15 mV) were registered from *Phaneroptera* and *Gryllotalpa* hearts, the higher values from *Locusta*, *Mantis* and *Phaneroptera* (50 mV), from *Tettigonia* and *Gryllotalpa* (35 mV) and from *Ephippigera* (40 mV).

The shape and the duration of the potentials had also some variances in the different species.

From *Locusta* heart the potentials with different shape and amplitude could be registered. Action potentials of pacemaker type were found to be the prevailing form (Figs 1, 2, 3) which can be registered from all the segments. No special site was found to be characterized by the generation of one or another form of potentials. All the potentials but the one demonstrated in the Fig. 2B was characterized by an initial slow depolarization of different degree. The initial depolarization can vary from the hardly noticeable (1–2 mV) (Fig. 1A) to the value running to one third of the action potential (Fig. 1C, Fig. 2A). Depending on the extent of the initial depolarization the ascending phase of the action potential was abrupted or more elongated (Fig. 1A, C). On the whole, at lower initial depolarization, the ascending phase of the action potentials was more abrupted (Fig 1A). The value of the initial depolarization

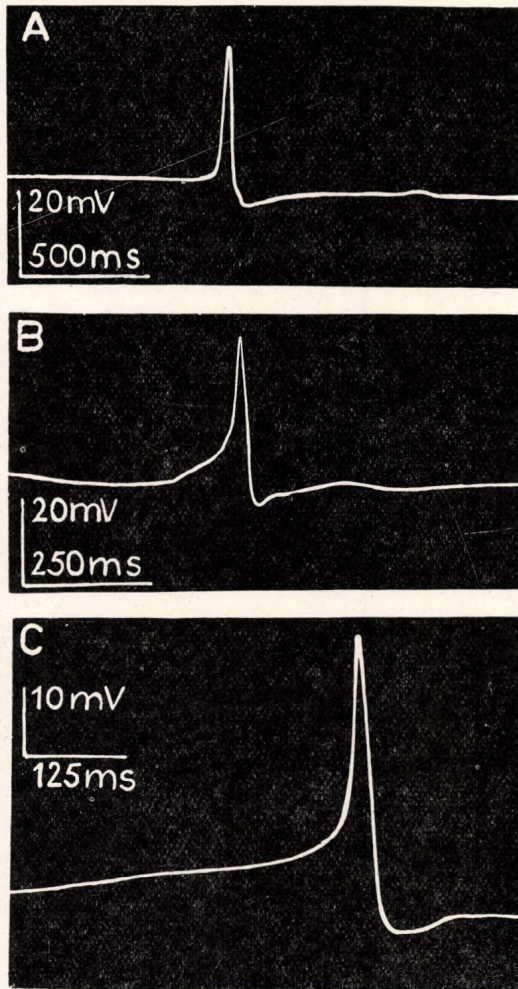


Fig. 1. Action potentials registered from *Locusta* heart

influenced also the duration of the action potentials: at lower initial depolarization the duration of the action potentials was shorter. The duration of the action potentials occurred between 30–60 msec in *Locusta* heart (Table I).

In some cases the resting potential showed changes similar to the initial depolarization, however, it did not lead always to spike generation. The action potentials were not followed always by positive afterpotentials but when it occurred, it turned smoothly to the initial depolarization phase (Fig. 2A, B). From *Locusta* heart the action potential with delayed repolarization could be registered comparatively rarely, although it is one of the characteristic forms of the action potentials in vertebrate hearts. Such type of the potentials is characterized by the initial fast phase of repolarization, followed by a plateau indicating delayed repolarization. From *Locusta* heart

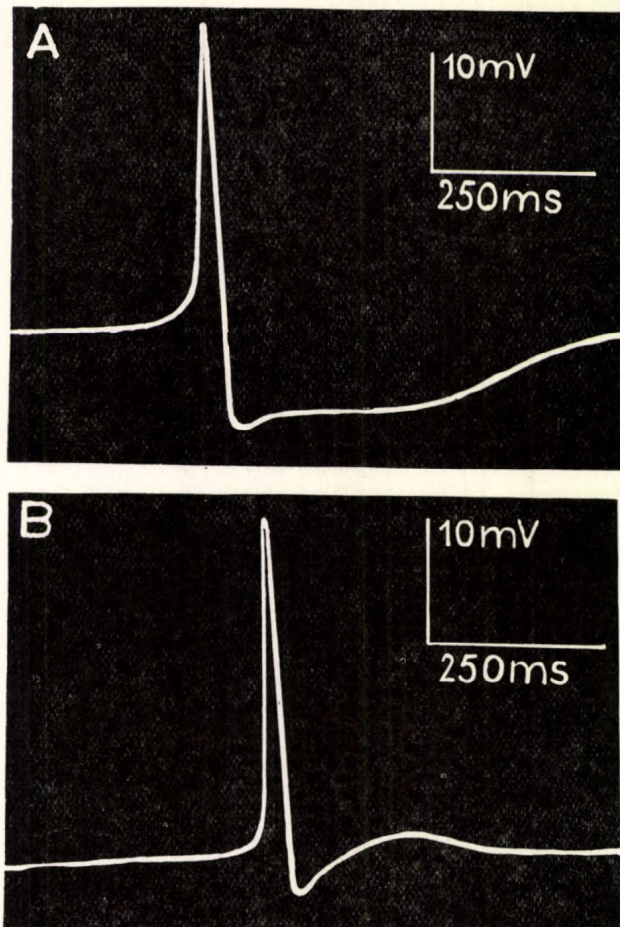


Fig. 2. Action potentials registered from *Locusta* heart

this type of potential can be registered only after the reversal-phenomenon which is to be discussed further on. In the same manner, action potentials without initial depolarization could also be registered after reversal (Fig. 3B). On the muscle fibers of *Locusta* heart the action potentials characterized as dominant pacemaker type are the most frequent, where is no stationary phase between two action potentials and the prolonged repolarization phase turned immediately to the initial depolarization (Fig. 3C).

In *Mantis religiosa* the action potentials of the heart muscle cells differ from that of *Locusta* so far as the potential with delayed repolarization is more frequent (Fig. 4B) and the action potential with abrupt ascending phase arising after negligible initial depolarization can be registered independently from the reversal-phenomenon (Fig. 4A). The operation of the dominant pacemaker is characterized in this case also by the absence of the stationary period between two action potentials (Fig. 4C). Regarding the

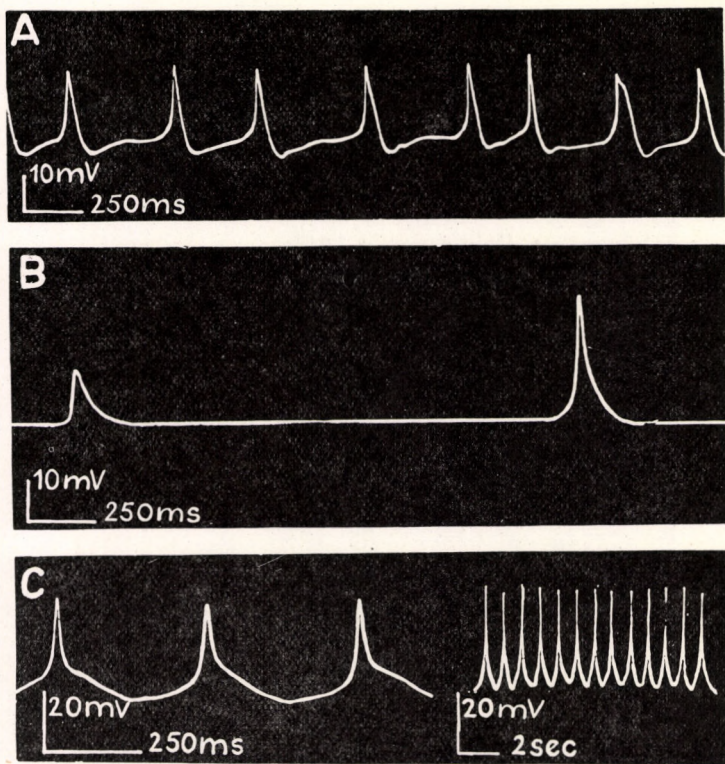


Fig. 3. Action potentials registered from *Locusta* heart

amplitude of the action potentials there were no significant differences between *Locusta* and *Mantis* hearts, however, the duration of the potential in *Mantis* heart exceeded more than twice that of the potential recorded from *Locusta*.

On *Phaneroptera* heart the action potential on the whole was similar to the above two species but the afterpotential was higher and appeared more frequently, moreover, the overshoot was more definitive (Fig. 5A). The duration of the action potentials extended until 80 msec in *Phaneroptera* heart. From the investigated species the plateau type of the action potentials occurred more frequently than the spike ones only in *Ephyppigera* heart (Fig. 6).

Junction potentials registered from *Ephyppigera* heart (Fig. 6) can be seen on the basic line between the spikes. This was observed occasionally also in the case of other species.

On the heart of *Gryllotalpa* and *Tettigonia* the shapes of the action potentials were identical with that of the other investigated species but *Gryllotalpa* excelled the others for the duration of the potentials was the longest. *Tettigonia* displayed no plateau (Fig. 6). The values of different parameters of the action potentials is summarized in Table I.

It can be seen in the Table I that the magnitude of the resting and action potentials ranged within wide limits. Neither different species nor individuals showed constants values and the action potentials of different

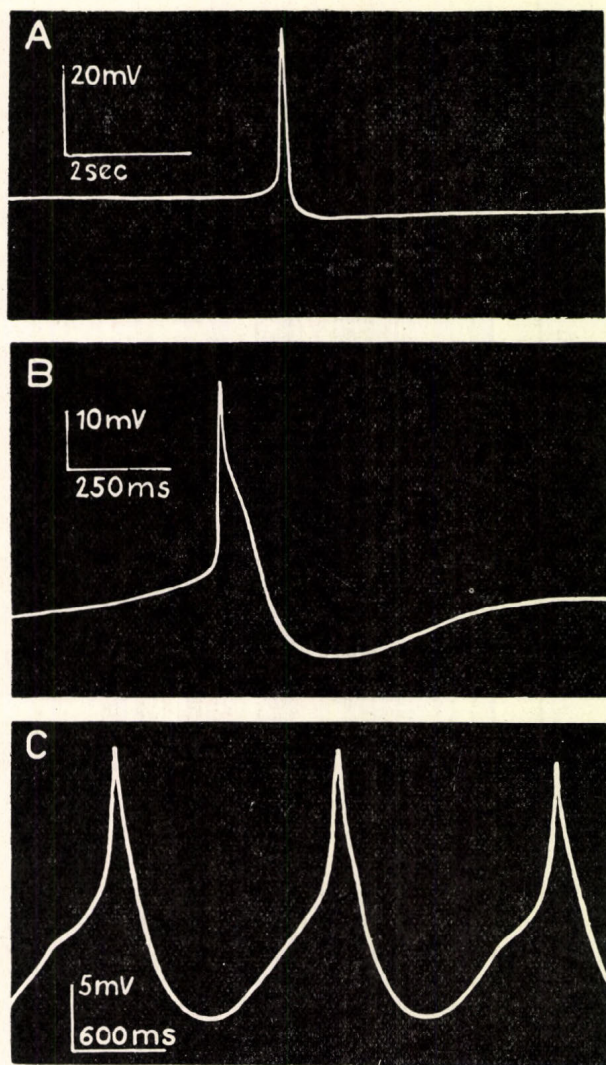


Fig. 4. Action potentials registered from *Mantis* heart

value can be registered from the heart of the same animal. Considerable variability was observed also in the duration and rate of the action potentials where more than twofold differences occurred. The action potential showed an overshoot varying also within wide range.

3. Characteristics of the action potentials in different heart chambers and the reversal-phenomenon.

On *Locusta* heart we tried to clear up, whether the variability in the values of the action potentials depend on the segmental location of the heart

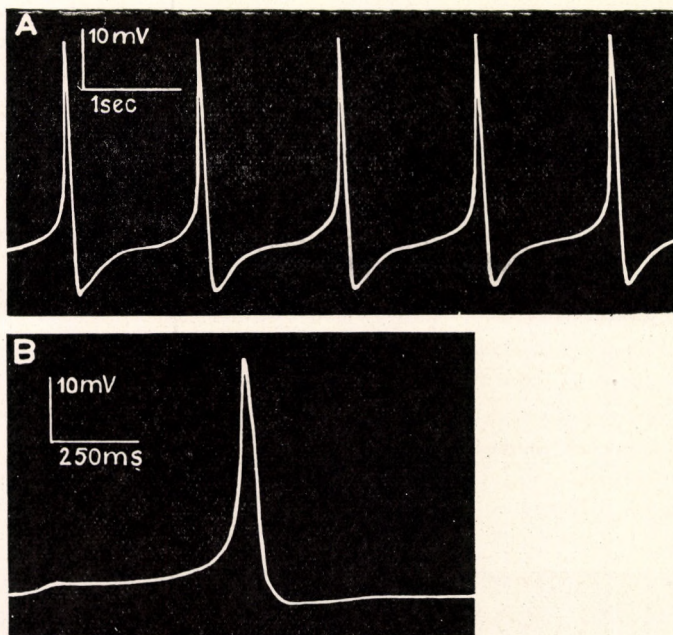


Fig. 5. Action potentials registered from *Phaneroptera* heart

chambers, i.e. the different heart chambers are characterized by differing values of resting and action potentials or not.

As it can be seen from the *Table II* there is no significant increase or decrease in the magnitude of resting and action potentials according to the caudal or apical localization of the heart chambers.

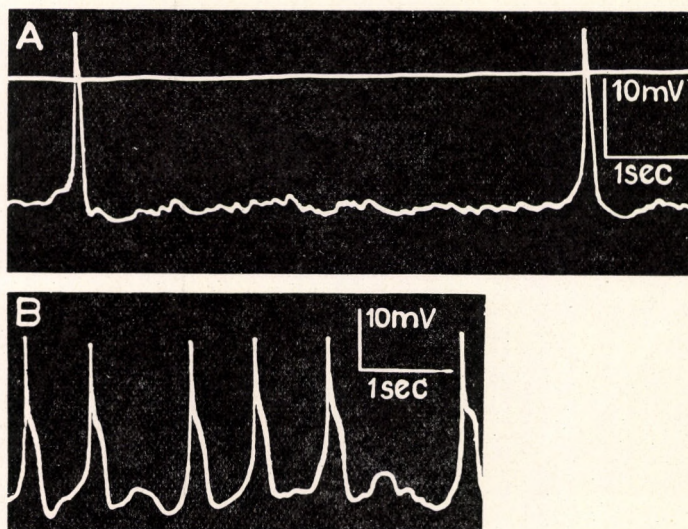


Fig. 6. Action potentials registered from *Tettigonia* (A) and *Ephippigera* (B) hearts

TABLE II
Variations of the resting and the action potentials in different segments of
Locusta heart

segment N°	resting potential (mV \pm S.D.)	action potential (mV \pm S.D.)	overshoot (mV)
1	23.8 \pm 4.35	30.3 \pm 8.60	6.5
2	30.4 \pm 6.16	32.5 \pm 9.65	2.1
3	27.3 \pm 7.87	33.7 \pm 8.36	6.4
4	25.0 \pm 4.58	30.0 \pm 5.29	5.0
5	29.1 \pm 5.74	35.7 \pm 5.91	6.6
6	25.1 \pm 6.08	32.1 \pm 8.06	7.0
7	23.7 \pm 3.74	30.0 \pm 4.58	6.3
8	23.0 \pm 4.47	33.6 \pm 4.12	10.6
9	28.0 \pm 6.92	36.6 \pm 5.74	8.6

In different segments no significant differences were observed in the action potential. The value of the resting potential was comparatively low at the 7th and 8th segments and as a consequence the overshoot showed some increase towards the caudal region.

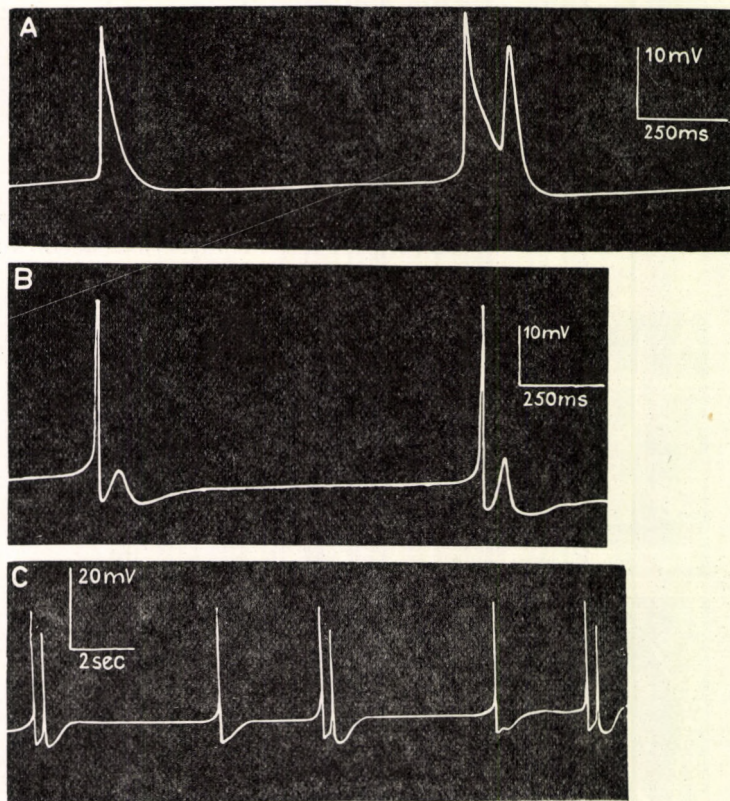


Fig. 7. Doubled action potentials from *Locusta* heart
A. — The first step in the dividing of the potential
B. and C. — further steps of the dividing of the potential

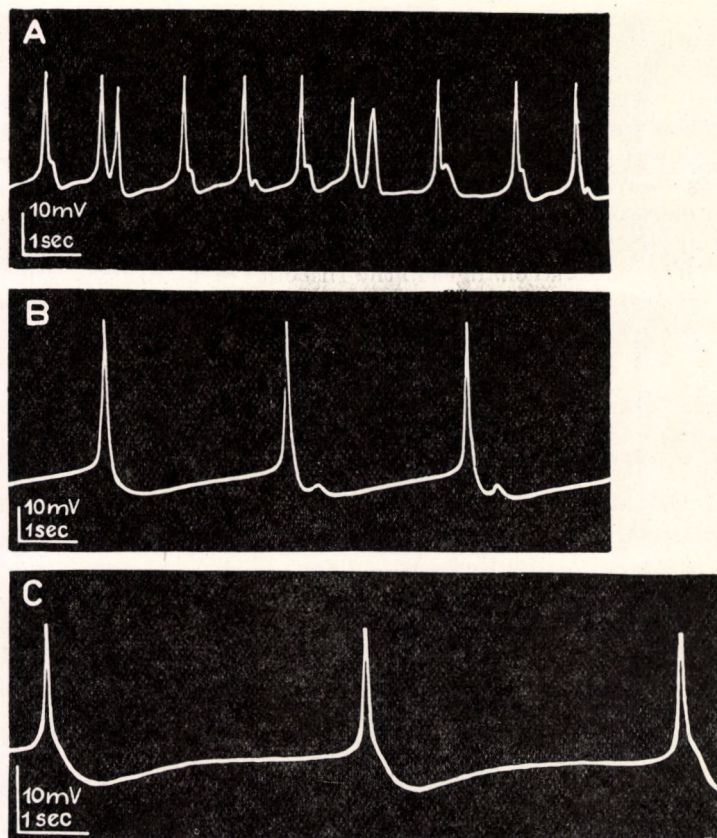


Fig. 8. Type of action potentials registered from the *Mantis* heart on the border of the 4th and 5th segments in different time

Further investigations showed that from time to time changes in the value of the resting and action potentials were caused by the "reversal". This is a physiological phenomenon when the direction of the impulse conduction changes from apical-caudal to the caudal-apical and it is common in all hearts of tubular structures (McCANN, 1970). This phenomenon was also observed by us many times. The reversal registered from *Locusta* heart is demonstrated in Fig. 7.

The reversal starts with the dividing of the action potential into two parts involving only the upper part or spreading down to the whole spike (Fig. 7A). Further on a separated second spike arises. This was of lower amplitude and generated immediately after the first one (Fig. 7B). The next step of the reversal characterized by doubled spikes in every second place while between those only a single spike is observable (Fig. 7C). The single spikes following the doubled ones are generated with longer intervals than the doubled spikes following the singles (Fig. 7C). In the last step the additional second spike is postponed in comparison with the first one and after having finished the reversal the heart produced the spikes again with constant intervals. The demonstrated case was registered from the heart chamber

of the third segment, in *Locusta*. Doubled spikes could be observed from all segments both in case of potentials without initial depolarization as well as of pacemaker type potentials.

In *Mantis* heart the reversal and the dividing of the spikes proved to be the same as in *Locusta* heart. In Fig. 8 the dividings of the spike can be seen registered on the border of the 4th and 5th segments. Between A and B registrations there is 30 minutes difference without any changes in the position of the electrodes. In the same area no sign of spike dividing was found in other experiments (Fig. 8C).

Thus the functional changes in the Insecta hearts influence the magnitude and shape of action potentials in a higher degree than it is known in other groups of animals.

Discussion

From the heart muscle fibres of the investigated Insecta species no higher than 40 mV of resting potentials were registered. Higher resting potential values were reported in other Arthropods: 52 mV in *Hyalophora cecropia* (McCANN, 1963), 45 mV in *Geolycosa missouriensis* (SCHERMAN and PAX, 1969), 45 mV in *Sarcophaga bullata* (BRUEN and BALLARD, 1970). On the vertebrate heart the resting potential exceeded even these values, corresponding to 96.4 mV in the rabbit ventricle (ROBB, 1965). Variable resting potential values were reported for Mollusca hearts, 32.1 mV in *Oyster* (KURIYAMA et al. 1960), 45.2 mV in *Mytilus* (IRISAWA et al. 1967), 50.6 mV in *Helix* heart (KISS and S.-RÓZSA, 1971).

The action potentials registered in our experiments never exceeded 50 mV. This value is lower than the action potential registered from *Hyalophora cecropiac* (McCANN, 1963) corresponding to 59.5 mV. In our experiments the action potential of the hearts in all species showed an overshoot in contrast to the action potentials of *Musca domestica* (FOURCROY, 1969), *Sarcophaga bullata* (BRUEN and BALLARD, 1970) and different Molluscan hearts (KURIYAMA et al. 1960; IRISAWA et al. 1967; KISS and S.-RÓZSA, 1971) where the action potential never exceeded the resting potential. On the vertebrate hearts overshoot was obtained only from the muscle fibres of the ventricle and auricle (15–20 mV), however, the pacemaker cells failed to show any overshoot (ROBB, 1965). Further differences from literary data (McCANN, 1963; 1965; BRUEN and BALLARD, 1970) were found, in our experiments the duration of the action potentials was shorter than that of earlier reports.

In different species some differences can be found in the shape of the action potentials, too. In the heart of *Hyalophora cecropia*, McCANN (1965) demonstrated two types of action potentials which could be registered from the whole heart. According to our results only the pacemaker type of action potentials can be registered from *Locusta* heart in a normal heart function while the potentials with delayed repolarization in most cases are followed after reversal. Nevertheless on *Mantis* and *Ephippigera* hearts similarly to *Hyalophora cecropia* the plateau type potentials can be registered all the time. It means that in the type of the potentials considerable differences may exist between the different Insects.

In the investigated Insecta species both latent and dominant pacemaker potentials were observed. In the case of the dominant pacemaker the diastolic depolarization was slow and turned smoothly to the ascending phase of the next action potential (*Fig. 1A, Fig. 4A*). It can be suggested that the dominant pacemaker potential has its origin in the heart muscle cells, however in the case of the latent pacemaker potentials the possibility that the impulses have nervous origin must be taken into consideration too. The first suggestion supports the view of myogenic origin of the heart rhythm. This assumption is further supported by the fact that junction potentials which in the innervated heart are superimposed on the basic line between the spikes (MILLER, 1969) were registered only in a negligible number of the experiments and in spite of this the heart was able to work for a long time. The present experiments confirm the supposition that all the cells of Insecta heart are capable of producing an impulse (MILLER, 1969; McCANN, 1970) and there are not anatomically separated pacemaker, conductive and contractile systems. On the other hand, from the absence of the anatomical specialization follows that the cells of Insecta hearts are multifunctional: they can play not only the role of a pacemaker, but they are able also for conduction or contraction. These functions change during activity and as a result reversion may arise. After reversal, when the spikes become doubled, the cells usually lost their dominant pacemaker quality (*Fig. 7*) and would produce latent pacemaker potentials or potentials without initial depolarization (*Fig. 3*). It is not yet clear how frequently one cell may change its function. To answer this question much longer registration is needed than was done in our experiments.

The comparison of the resting and action potentials in different segments showed that the values recorded from two caudal segments differ from that of the other ones. However, it is difficult to tell whether it is in correlation with the generation of the potentials or not. To explain the differences further experiments are demanded. As to the type of the action potentials we did not find favoured segments, so the earlier supposition according to which the plateau type of the action potentials would occur more frequently in the first segment was not proved. The dominant pacemaker was localized by TENNEY (1953) in the first, while by IRISAWA et al. (1956) in the seventh segment. However, on the basis of our results the permanent change of the place of the dominant pacemaker (McCANN, 1970) seems to be more acceptable and not the favoured role of one or another segment in the generation of the activity. Consequently, the dominant pacemaker can be localized only functionally and not anatomically. The change of the site of the dominant pacemaker is in close connection with the alteration of the resting potential as the place having lower resting potential converts into dominant pacemaker independently of its anatomical localization.

Summary

Electrophysiological characteristics of the heart of *Locusta migratoria migratorioides*, *Mantis religiosa*, *Phaneroptera nana*, *Ephippigera ephippiger*, *Tettigonia viridissima* and *Gryllotalpa vulgaris* were studied by microelectrodes. It was found that the value of the resting potentials varies between 12–40 mV in the different species. The magnitude of the action potentials was 10–50 mV.

In all the investigated species both the minimal and the maximal values were registered. The duration of the action potentials was between 30–170 msec. The action potential showed overshoot in each species.

The heart chambers located in the different segments did not show different types of potential and there are no constant values of resting and action potentials for the same segment.

The pacemaker type of potential was found to be the prevailing form. The pacemaker potentials may originate from latent or dominant pacemakers. In *Locusta* heart plateau type potential was observed only after the so-called reversal phenomenon. Action potentials with typical delayed repolarization and well developed plateau were registered only from the heart of *Mantis* and *Ephippigera*.

The form of the potentials depends on the functional state of the heart muscle fibres, and the cells proved to be multifunctional. Reversal reflects the changes of the function of the single cells and in this course the latent pacemaker can turn into dominant one and vice versa, meaning that the type of the potential would vary not only from one cell to another but from time to time also in the same cell.

The results seem to prove the supposition about the myogenic origin of the heart rhythm in the investigated Insecta species.

REFERENCES

- BRUEN J., R. C. BALLARD (1970): An intracellular study of the myocardial cells of the flesh fly, *Sarcophaga bullata*. — *Comp. Biochem. Physiol.* **32**, 227–236.
- FOURCROY S. J. (1967): Microelectrode studies of the house fly heart, *Musca domestica* L. — (*Master's thesis, San Jose State College*).
- GEROULD J. H. (1933): Orders of insects with heart beat reversals. — *Biol. Bull.* **64**, 424–431.
- IRISAWA H., A. F. IRISAWA, T. KADOTANI (1956): Findings on the electrograms of the cicada's heart (*Cryptotympana japonensis* KATO). — *Jap. J. Physiol.* **6**, 150–161.
- IRISAWA H., N. SHIGETO, M. OTANI (1967): Effect of Na⁺ and Ca⁺⁺ on the *Mytilus* (Bivalve) heart muscle. — *Comp. Biochem. Physiol.* **23**, 199–212.
- ISHIKAWA S. (1959): Membrane potentials of dorsal vessel of the silkworm, *Bombyx mori*, by intracellular method. — *J. Sericult. Sci. Japan* **28**, 295–297.
- KISS T., K. S.-RÓZSA (1971): Studies on the effects of ions in the spontaneous activity of the heart in Gastropoda. — *Acta Physiol. Acad. Sci. Hung.* (in press).
- KURIYAMA H. A., M. GOTO, T. MAENO, Y. ABE, S. OZAKI (1960): Comparative studies on transmembrane potentials and electrical characteristics of cardiac muscle. — Ingakushoin, Hongo Tokyo, 1960. Reprinted from "The Electrical Activity of Single Cells", pp. 243–266.
- LUDWIG D., K. TRACEY, M. L. BURNS (1957): Rations of ions required to maintain heart beat of the American cockroach, *Periplaneta americana*. — *Ann. Ent. Soc. Amer.* **50**, 244–246.
- MCCANN F. V. (1963): Electrophysiology of an insect heart. — *J. Gen. Physiol.* **46**, 803–821.
- MCCANN F. V. (1965): Unique properties of the moth myocardium. — *Ann. N. Y. Acad. Sci.* **127**, 84–99.
- MCCANN F. V., V. C. L. WIRA (1967): Transmembrane ionic gradients in Lepidoptera as related to cardiac electrical activity. — *Comp. Biochem. Physiol.* **22**, 611–615.
- MCCANN F. V. (1970): Physiology of insect hearts. — *Ann. Rev. Entomol.* **15**, 173–200.
- MILLER T. (1969): Initiation of activity in the cockroach heart. — In *Comparative Physiology of the Heart: Current Trends*. — *Experientia Suppl.* **15**, (MCCANN F. V., Ed.: Birkhauser, Basel. 206–218).
- MILLER T., R. L. METCALF (1968): Site of action of pharmacologically active compounds on the heart of *Periplaneta americana*. — *J. Insect Physiol.* **14**, 383–394.

- ROBB J. S. (1965): Comparative basic cardiology. — *Grune and Stratton, New York* 602 pp.
- S.-RÓZSA K., I. V.-SZŐKE (1970): Investigations on the chemical sensitivity of insect heart. — *Annal. Biol. Tihany* **37**, 99—109.
- SNEFF R. E. (1967): The electrophysiology of the adult heart of *Periplaneta americana*: evidence for a neurogenic heart. — *Dissertation Abstr. Sect. B.* **27/7**, 2499 B—2500 B.
- SHERMAN R. G., A. PAX (1969): Electrical activity in single muscle cells of a spider heart. — *Comp. Biochem. Physiol.* **28**, 487—489.
- TENNEY S. M. (1963): Observations of the lepidopteran heart with special reference to reversal of the beat. — *Physiol. Comp. Oecologia* **3**, 286—306.
- VÉRÓ M. (1970): Negatív kapacitású, nagybemenő impedanciájú erősítő FET tranzisztorral, mikroelektrodás vizsgálatokhoz. — *Magyar Élettani Társaság XXXVI Vándorgyűlése, Szeged*, p. 141.
- ZWICKY K. T., S. M. HODSON (1965): Occurrence of myogenic hearts in Arthropods. — *Nature* **207**, 778—779.

SZÍVIZOMSEJTEK NYUGALMI ÉS AKCIÓS POTENCIÁLJAINAK JELLEMZŐI NÉHÁNY ROVARFAJBAN

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Összefoglalás

Vizsgálták *Locusta migratoria migratorioides*, *Mantis religiosa*, *Phaneroptera nana*, *Ephippigera ephippiger*, *Tettigonia viridissima* és *Gryllotalpa vulgaris* szívének elektrofiziológiai jellemzőit intracelluláris elvezetésben. Megállapították, hogy a nyugalmi potenciál a különböző fajokon 12—40 mV között változik. Az akciós potenciál nagysága 10—50 mV. Valamennyi fajon regisztrálható a különböző sejtekből mind az alsó, mind a felső érték. Az akciós potenciálok időtartama 30—167 msec között változik. Valamennyi szív akciós potenciáljára overshoot jelenléte jellemző.

Az egyes szegmentumok szívkamrái nem mutatnak jellegzetes potenciálformát és állandó nyugalmi és akciós potenciál értékeket.

Potenciálforma tekintetében a pacemaker típusú potenciál a leggyakoribb típus. A pacemaker potenciálok származhatnak valódi vagy latens pacemaker működéséből. A *Locusta* szív nem pacemaker, plátós potenciált tartósan csak az ún. megfordulási effektus után generál. Késleltetett repolarizációval és kifejezett plátóval bíró akciós potenciál csak a *Mantis* és *Ephippigera* szíveken volt megfigyelhető.

A potenciálforma a szívizomsejtek funkcionális állapotának függvénye, s e sejtek multifunkcionálisak. A megfordulás jelensége a rostok funkcióváltozását tükrözi, s ennek kapcsán latens pacemaker valódi pacemakerre alakulhat és fordítva. A potenciálforma tehát nemcsak egyik sejtről a másokra változhat, hanem ugyanazon sejtben is az idő függvényében.

Az eredmények a szívritmus myogén eredetére vonatkozó elképzeléseket látszanak igazolni.