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ELECTROPHYSIOLOGY OF THE HEART OF AN ISOPOD CRUSTACEAN: *PORCELLIO DILATATUS*

I. GENERAL PROPERTIES

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INTRODUCTION

The crustacean skeletal muscle has undergone a great deal of experimental investigation (Atwood, 1967; Fatt & Katz, 1953; Hagiwara, Naka & Chichibu, 1964; Wiersma, 1961). There are many reasons for this interest. For instance, the large size of the fibres facilitates biochemical and electrophysiological examination. Moreover, crustacean neuromuscular systems provide simplified models for studies of the integration of diverse synaptic inputs.

For the same reason the cardiac ganglia of some crustacea have been studied in detail by several workers (Matsui, 1955; Maynard, 1953, 1958; Watanabe *et al.* 1967; Welsh & Maynard, 1951). In these studies emphasis has been given to the electrical events in pacemaker and follower neurones, and to the interactions between them. However, few studies have been made on electrophysiological properties of the heart fibres. Some early papers described the whole electrical activity in the heart of several Decapoda with special reference to the problem of the tetanic or non-tetanic nature of the contraction (Dubuisson & Monnier, 1931; Arvanitaki, Cardot & Tai-Lee, 1934). Later, microelectrode technique was used to record intracellular activity in various species of Decapoda. However, these studies only describe spontaneous electrical events and do not pay attention to the ionic mechanisms and membrane properties which are involved. Brown (1964) gave some interesting data on *Squilla* heart as a neuromuscular system and raised important questions concerning the relationship between membrane potential and contraction. Recently, van der Kloot (1970), Lassalle & Guilbaut (1970) dealt with the study of ionic mechanisms and membrane properties which are responsible for the activity. Anderson & Cooke (1971) and Hallet (1971) investigated the relationship between the ganglion activity and the heart-muscle activation.

The present study is concerned with some of the main physiological properties of the heart of a terrestrial isopod crustacean, the wood-louse *Porcellio dilatatus* (Brandt). No electrophysiological studies had been undertaken previously. It offers a convenient preparation for studies dealing with membrane ionic permeabilities, for test fluids have a very rapid access to the single, thin, muscular layer. An attempt to analyse electrophysiological characteristics of the myocardium has been made by applying

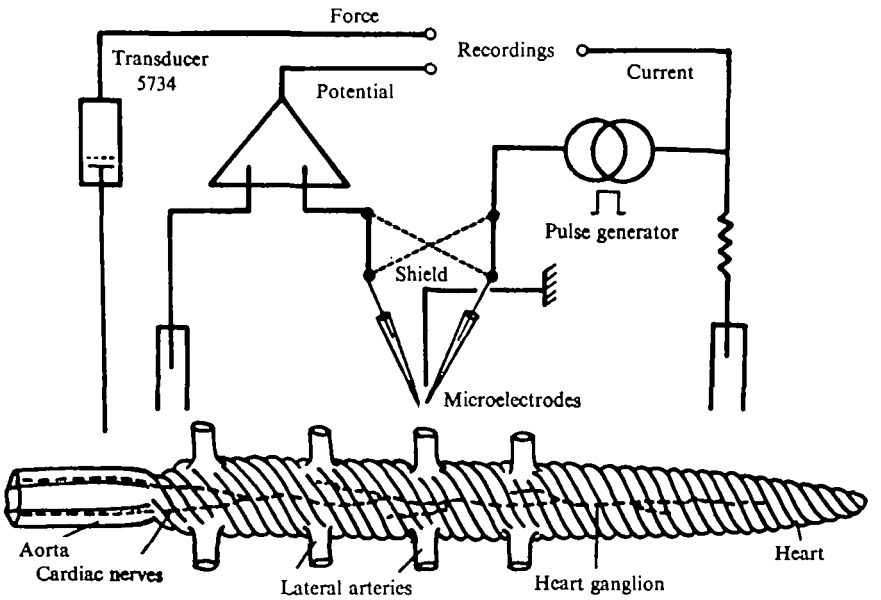

Fig. 1. Diagram of the experimental arrangement and of the dorsal view of *Porcellio* heart. Explanation in the text.

intracellular current pulses. Additional data have been obtained on the action of chemicals considered as possible transmitter substances in Crustacea. Finally, some particularities of the relationship between contraction and membrane potential have been investigated. The purpose of this paper is not to present an exhaustive basic analysis of each property or mechanism. We have attempted (i) to enlarge the inventory of the properties of cardiac muscle in Arthropoda and (ii) to bring to light some points of interest for further comparative studies on crustacean skeletal and heart muscles.

Anatomy and histology

The *Porcellio* heart is shaped like a tube and is about 5–6 mm long and 300–400 μm in diameter in the largest specimens. The wall is pierced by ostia. Eleven arteries with valves arise from the heart. Three are joined and run forward, the others are paired lateral arteries situated in the anterior half of the heart (Fig. 1).

The wall is composed of a single layer of muscle fibres, 10–20 μm thick, which seem to be arranged in a network. Cross-walls delimiting distinct fibres are rarely distinguished. In this layer, fibres (or branches) run in a right-handed helix. In each fibre not very dense myofibrils are collected in a central core surrounded by sarcoplasm.

The muscular wall is covered by a very thin sheath of connective tissue. On the inner side of the muscle layer there is a plexus of strands of connective tissue sometimes including cells with highly refringent inclusions.

As pointed out by Alexandrowicz (1952), there are, in the isopod *Ligia*, three nerve element systems connected with the heart: (i) a local nervous system or cardiac ganglion, (ii) nerves connecting the local nervous system with the central nervous system, and (iii) nerves of the arterial valves. In *Porcellio* the three systems have been

identified by using methylene blue *in situ* preparations or sectioning techniques. A nerve trunk runs along the mid-line of the dorsal wall of the heart, on the inner side of the wall. Six cell bodies lie in the trunk (Tanita, 1939). The cardiac ganglion is connected by a pair of nerves running alongside the aorta, the cardiac nerves, which originate in the nerve cord and probably in the visceral nervous system (Delaleu, 1970). What is more, the arterial valves are densely innervated.

MATERIALS AND METHODS

Dissection

The preparations were obtained from male or female specimens, 10–15 mm in length. The animals were decapitated, the legs were removed and the body pinned, ventral side up, in the experimental chamber filled with physiological saline. Two lateral incisions were made extending from the thoracic region to the caudal tip of the abdomen. The sectioned portion of the exoskeleton, the nerve cord and the visceral material were carefully removed and the heart was thus exposed. Such a semi-isolated preparation was used directly in several experiments designed to determine what effects the isolation process had on cardiac physiology, and for studying the regulatory action of the cardiac nerves running along the intact aorta.

Quasi-normal mechanical conditions being thus maintained, the heart survived well for as long as 12 h in a suitable medium. However, the presence of the intact pericardial septum adhering to the ventral wall of the heart was a serious obstacle to the penetration of the microelectrodes.

In most experiments the heart was completely isolated from the carapace. Arteries and other lateral attachments were first cut, and the aorta was carefully pulled off from the dorsal tegument and tied up to the arm of a mechano-electrical transducer. The transducer was then moved by means of a micromanipulator in order to lift up and turn back the heart under slight stretch. This procedure favours the section of the numerous small links by which the dorsal heart wall adheres to the tegument and permits an oscillographic control of the degree of stretch.

In spite of these precautions, the complete isolation was a traumatic operation. Numerous hearts collapsed and stopped beating after the cutting of the lateral attachments, suggesting that the stretch maintained by lateral structures exerts some retro-action on the spontaneous firing of the ganglionic pacemaker. However, most of them usually recovered their activity during the subsequent phases of the dissection.

In an intact specimen cardiac rate depended on age (or size), temperature and other internal or external conditions. The mean rate of beating in an animal 10 mm in length was 250/min at 20 °C. The heart rate did not usually decrease in semi-isolated preparations, but in isolated hearts rates as high as 150/min were not often recorded.

Physiological supporting medium

A modified Ringer solution, according to amounts of ionic species determined in haemolymph (Holley & Regondaud, 1963), was used as normal saline. Its composition, expressed in mM/l, was the following: Na^+, 306·6; K^+, 6; Ca^{2+}, 13·5; Cl^-, 323·7; CO_3H^-, 2·4; pH was 7·6.

Experiments were carried out at room temperature with temperature variation less than 2 °C during the course of one experiment.

Recording apparatus (*Fig.* 1)

Conventional KCl-filled glass microelectrodes of tip diameter approximately 0·5 μm (resistance: 5–15 MΩ) were used for recording from heart fibres. They were connected to the input of an electrometer amplifier (Medistor) by means of an Ag–AgCl wire. The reference electrode consisted of an Ag–AgCl wire embedded in Agar–Ringer. The output of the amplifier was displayed on a Tektronix 502A oscilloscope. Permanent records were obtained by photographing the screen of the oscilloscope with an Alvar 'cathograph' camera.

In some experiments the stimulating device described by Weidmann (1951) was used for applying intracellular current pulses through a second microelectrode.

The contractions of the isolated heart were recorded in the following way. The aorta was tied to the extremity of a fine needle welded to the mobile arm of a RCA 5734 transducer. The posterior end of the cardiac tube was pinned down in the experimental chamber by means of the suspensory ligaments. The movement of the transducer by micro-manipulation enabled us to adjust the mechanical tension imposed on the heart. The contractile fibres form a spiral about the main axis; the mechanical tension recorded during the myocardial contraction only corresponded to the longitudinal component of the total tension. The effect of the transverse component gave a rhythmic torsion to the heart.

RESULTS

Spontaneous electrical activity

Once inserted in an isolated heart, microelectrodes could often record the membrane potential for several minutes. The maximum diastolic potential ranged from −50 to −70 mV. During spontaneous activity depolarization was rarely more than 40 mV, the upstroke never exceeding the zero potential level. The intracellular electrogram appeared similar in contour and time course, but not in amplitude, to those described for insect hearts (McCann, 1963) or vertebrate myocardium (Coraboeuf & Weidmann, 1949). After an S-shaped depolarization with a slow rate of rise (from 1 to 2 V/sec) it generally included a prolonged repolarization resulting in a 'plateau' (Fig. 2A: *a*, *b*). It is important to note that the 'plateau' is smooth (Fig. 2B) and does not present additional peaks or wavelets, unlike the cardiac intracellular recordings of *Limulus* or *Squilla* (McCann, 1962; Irisawa *et al.* 1962). The amplitude and the shape of this plateau varied from one preparation to another and from one point to another in the same preparation, and spontaneous alterations even occurred in a series of responses recorded at the same point. However, it was not possible to demonstrate a consistent physiological differentiation of various areas of the heart.

As shown in Fig. 2C, the repolarization was altered by the degree of mechanical tension imposed on the isolated heart; if the passive tension on the heart was increased by 5 mg, the initial phase of repolarization including plateau was accelerated. In this respect it may be supposed that the spontaneous alterations of the plateau

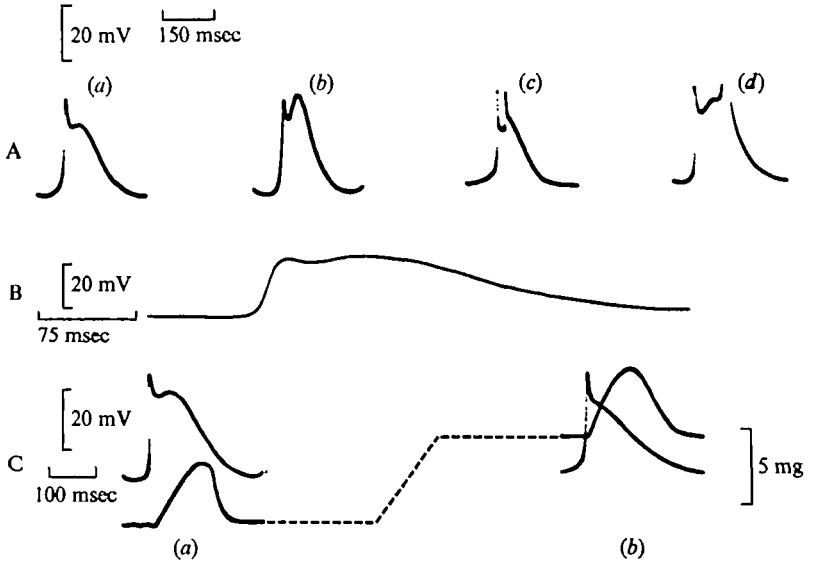

Fig. 2. (A) Various types of intracellular electrical responses recorded in the myocardium. (*a*, *b*) Most common responses. (*c*, *d*) Two modifications occurring during the repolarization phase. (B) Electrical response recorded at a rapid sweep speed. (C) Effect of stretching the heart on the contour of the responses (mechanogram tracings connected by dotted line). (*a*) Recordings under usual conditions. (*b*) Recording after stretching.

phase reflected fluctuations of the pressure exerted by the microelectrode on the heart wall.

Depolarization did not start abruptly, except in hearts with a very slow beat. It was preceded by a slow phase which frequently looked like a pacemaker potential.

Two modifications of this general picture of the intracellular electrogram were sometimes observed: firstly, the early repolarization of the initial upstroke was followed by a secondary depolarization that did not exceed the level of the first one (Fig. 2A: *c*); and secondly, exceptionally, a spike was also observed which originated from the end of the plateau phase, so that the whole amplitude of the response, which showed an overshoot, was then about 65 mV (Fig. 2A: *d*). This phenomenon looked like the action potentials obtained when tetraethylammonium chloride or caffeine were added to the bathing medium (second paper).

The whole *Porcellio* heart contracted synchronously. It exhibited neither the phenomenon of 'reversal-beat' nor peristaltic waving. When two microelectrodes were inserted 1·2 mm apart, the rising phases of the two responses were separated by no more than 2 to 3 msec. Accurate measurements were difficult owing to the fact that there was no abrupt variation or break in the electrogram, but an apparent condition velocity of 50 cm/sec was evaluated.

The effect of intracellular current pulses

On the spontaneous activity of the isolated heart

This activity was recorded during application of constant current pulses of opposite polarities (magnitude: from 4×10^{-8} A to 4×10^{-7} A; duration: 1 sec) delivered by a polarizing microelectrode 50–200 μm from the recording microelectrode. The varia-

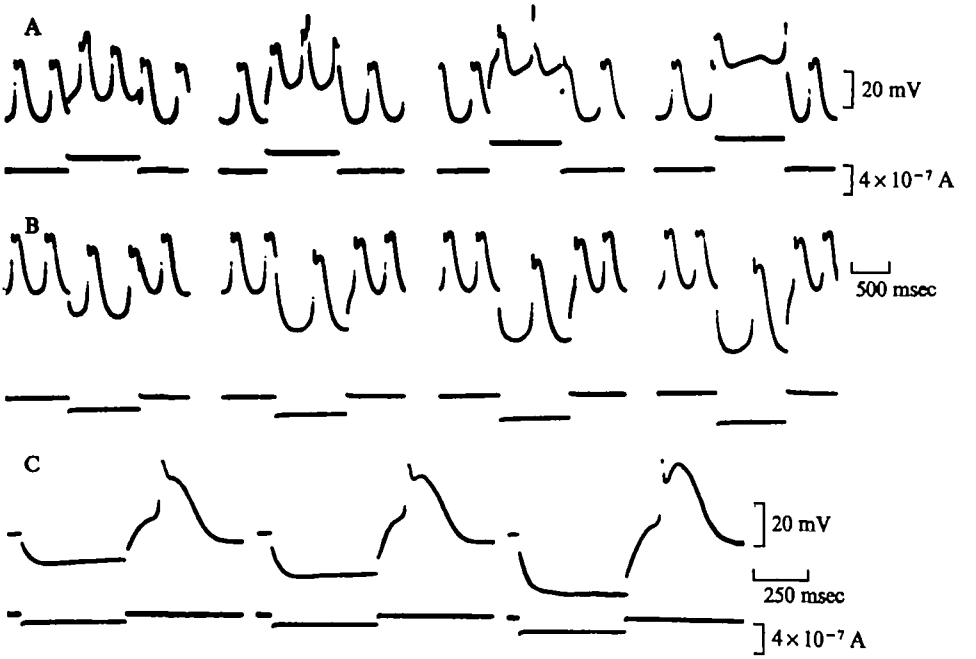

Fig. 3. Effect of intracellular currents (lower traces) on the spontaneous electrical responses (upper traces). Progressive increments (A) of depolarizing current, (B) of hyperpolarizing current, (C) of hyperpolarizing current preceding the electrical response.

tions imposed on the potential were propagated decrementially with an apparent space constant λ of about 1 mm. Fig. 3B shows a decrease in rhythm of heart beats during hyperpolarizing currents. In some preparations the spontaneous activity ceased in response to current pulses of about 4×10^{-7} A, which produced a 40 mV hyperpolarization of the myocardial membrane. The amplitude of the spontaneous responses also increased during the application of the current pulses. For example, in the experiment illustrated by Fig. 3B the magnitude of the normal response was 32 mV and a current pulse of 4×10^{-7} A, which hyperpolarized the membrane by 35 mV, increased the response to 48 mV. During hyperpolarizing pulses the shape of the response was thus modified: the initial rate of rise was reduced and the repolarization was marked by a relative lowering of the plateau.

Fig. 3A illustrates the effect of depolarizing pulses. For currents of about 4×10^{-8} A there was an increase in frequency of the spontaneous heart beats, but when the intensity of current reached 4×10^{-7} A the frequency was lowered and cessation of activity might occur. However, even during a complete inhibition, some small oscillations of the membrane potential remained. The magnitude of the responses was also modified by depolarizing currents. Two types of responses could be then recorded: either (i) responses resembling normal ones, but whose amplitude decreased with increasing intensity of the polarizing current, or (ii) responses with an enhanced plateau phase. This phenomenon becoming more marked, a spike could be then triggered.

In Fig. 4 are plotted the data of such an experiment. Over the range of intensitie

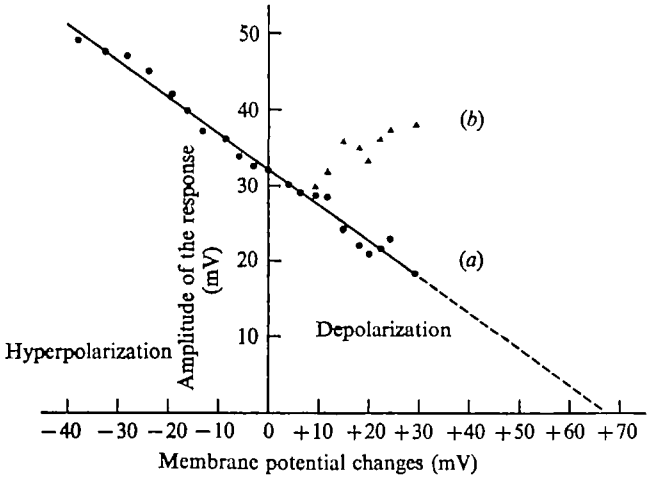

Fig. 4. Relationship between the total amplitude of the responses and the variations of the voltage across the membrane produced by polarizing currents (duration 500 msec). Two classes (*a*, *b*) of membrane responses during applied depolarizing currents. The intensity of current varied from $\pm 4 \times 10^{-8}$ A to $\pm 4 \times 10^{-7}$ A. Detailed explanation is in text.

tested there is a direct relationship between the amplitude of the electrical response and the membrane polarization, the slope of the curve (*a*) being 5 mV for a variation of 10 mV of the diastolic potential. The separated points (*b*) show a different behaviour for the second class of responses; their height increases with depolarization, but the relationship between the membrane depolarization and the response magnitude does not seem to be linear.

During other experiments several steps of hyperpolarizing current, of 500 msec duration, were applied to the heart immediately before a spontaneous response. It can be seen in Fig. 3C that the stronger the previous hyperpolarization the more enhanced the plateau.

On the myocardial membrane without spontaneous activity

The spontaneous rhythmicity was abolished by destroying the cardiac ganglion in the posterior part of the heart, and intracellular current pulses (duration: 500 msec) of different intensities (from 3×10^{-8} to 4×10^{-7} A) and of opposite polarities were then applied to the intact areas of heart.

Fig. 5 shows two types of responses (A and B) commonly recorded by a microelectrode 100–150 μm from the polarizing microelectrode. Graphs A and B of Fig. 6 represent corresponding I/V curves. The values of the potential were measured 500 msec after the beginning of the current step, for A and (*a*) of B, and at the maximum of the response for (*b*) of B. In example A, during hyperpolarizing pulses, the I/V ratio measured at 500 msec is constant; from the slope of the curve the polarization resistance was found to be about $10^5\ \Omega$. In the hyperpolarization traces in 5B, and to a lesser degree in 5A, the conductance increases with time (delayed rectification of hyperpolarization). However, this delayed rectification tends to decrease, then falls to zero for currents of approximately -4×10^{-7} A. The junction of curves (*a*) and (*b*) in Fig. 6B represents such changes. The cessation of the hyperpolarizing

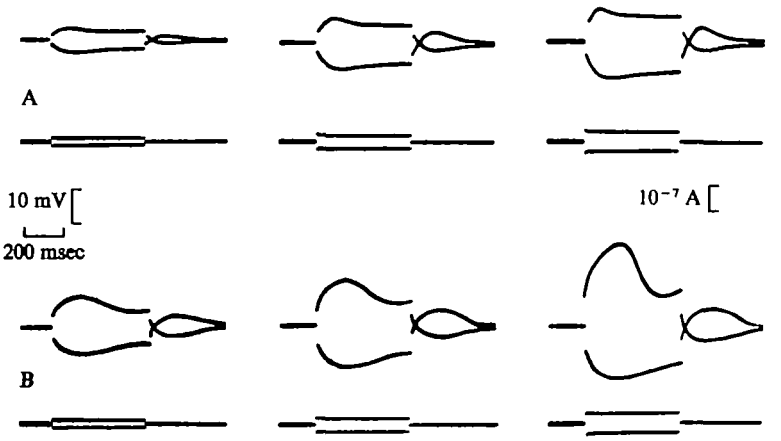

Fig. 5. Two examples of the effects of intracellular currents (lower traces) on the myocardial membrane potential at rest (upper traces). (A) the membrane displays a 'normal' rectification associated with a weak delayed rectification. (B) (other preparation): presence of a graded active response of the membrane during the application of depolarizing pulses. Distance between internal stimulating and recording electrodes, about 100 μm.

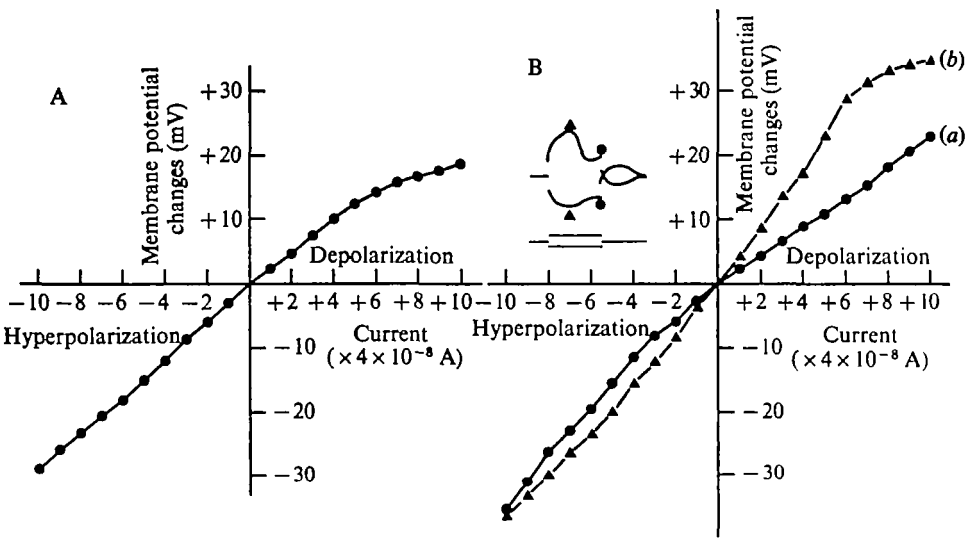

Fig. 6. Relationship between the applied currents (duration: 500 msec) and the membrane potential (myocardium at rest). (A) Measurements from an experiment some data of which are shown in Fig. 5A. (B) Curve (*a*), time course of the membrane potential measured 500 msec after the beginning of the stimulation. In (*b*) the measurements are made at maximum amplitude of the graded response (see inset diagram); some traces corresponding to this experiment are shown in Fig. 5B.

current was followed by a transient depolarization. Depolarizing pulses evoked rather unlike responses in the two examples described, but we never recorded true action potentials. In Fig. 5A the depolarization traces displayed a 'normal' rectification and a weak delayed rectification appeared. At the end of these anodic pulses there was a transient phase of hyperpolarization. Examples 5B and 6B (*a*) show a weak 'normal' rectification when the potential was measured 500 msec after

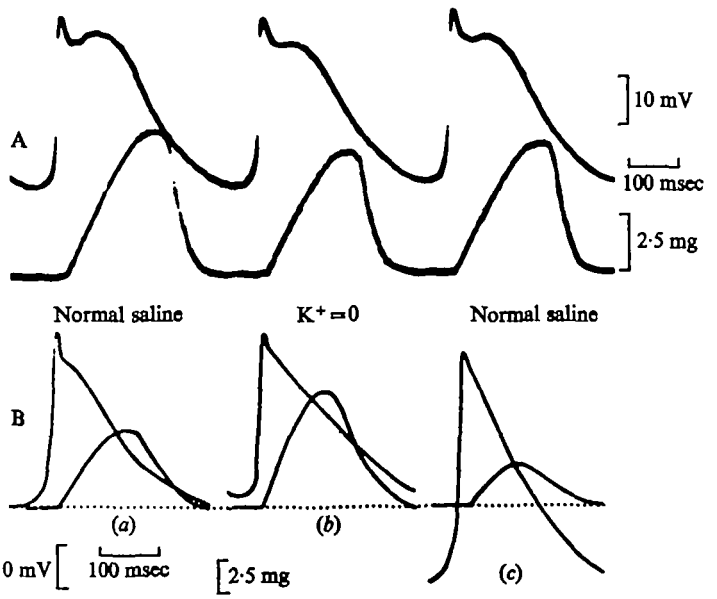

Fig. 7. (A) Simultaneous recording of electrical responses (upper traces) and corresponding contractions (lower traces) showing that the magnitude of the mechanogram fluctuates in conjunction with the level of the electrical plateau. (B) Drawings showing the effect of a K^+-free solution: (*a*) electrical response and mechanogram under normal conditions $[K^+]_o$ = 6 m-equiv/l; (*b*) K^+-free medium (after 8 min); (*c*) return to normal concentration of K^+ (we have not taken into consideration the slow variations of the mechanical tension; more details in text).

the beginning of the applied pulses. But this measurement does not concern a stable state for medium and strong values of polarizing current. If the measurements are made during maximum response (curve *b* in Fig. 6B) the I/V relationship shows an 'anomalous' rectification. However, it is likely that the recordings made during depolarizing pulses are the resultant of several components, one of which might be an active, graded response of the membrane.

Relationship between membrane potential and contraction

During spontaneous activity

The simultaneous recording of the intracellular activity and of the overall mechanogram (Fig. 7A) shows that the mechanical activity begins about 50 msec after the early depolarization, approximately corresponding with the top of the upstroke. The mechanogram value reached its maximum when the repolarization phase was already more than 60% completed. It is important to point out the correlation between the value obtained from the mechanogram and that of the plateau of the electrical response. Indeed, it appeared that during sequences of spontaneous activity, electrical responses differed from one to another, especially as to their plateaus, the other parameters being constant. At the same time the amplitude of the mechanogram fluctuated similarly from one cycle to another; the more elevated plateau corresponded to the greater mechanogram value.

Fig. 7B shows the same type of simultaneous records made in a K^+-free solution.

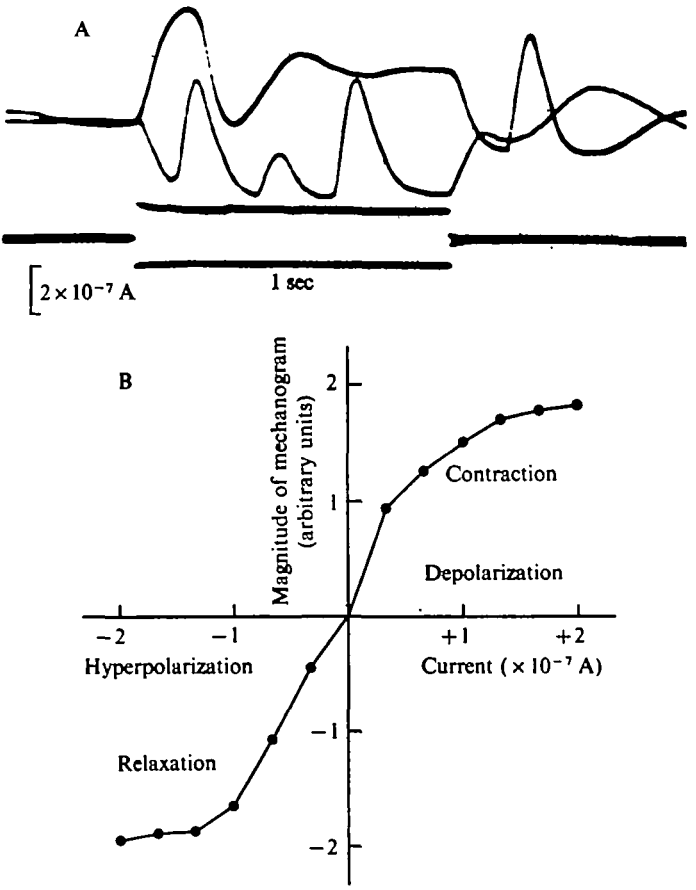

Fig. 8. (A) Contractile effect (upper traces) of transmembrane currents (lower traces); an upward deflexion of the contraction tracings corresponds to an active increase of tension, a downward deflexion indicates a relaxation of the heart; spontaneous oscillation is superimposed; the variation of the mechanical tension changed direction with the current polarity. (B) Evolution of the magnitude of the mechanogram as a function of the intensity of the polarizing current (measurements made 100 msec after the beginning of current).

Owing to possible shifting of the transducer base-line we did not take into consideration the slow variations of the mechanical tension. Only the difference of tension between systole and diastole were taken into account. After prolonged action of this K^+-free solution (8 min) the membrane was slightly depolarized, and at the same time the amplitude of the systolic contraction increased (*b*). As soon as the normal saline was re-introduced (*c*), the diastolic membrane potential increased strongly (18 mV) and then the amplitude of the electrical response increased by 34% in relation to the amplitude in normal solution. However, the top of the large responses remained lower than the top of a normal response. So, in spite of the large amplitude of the electrical response, the mechanogram indicated a tension lower than the value recorded under normal conditions or during the application of a K^+-free solution.

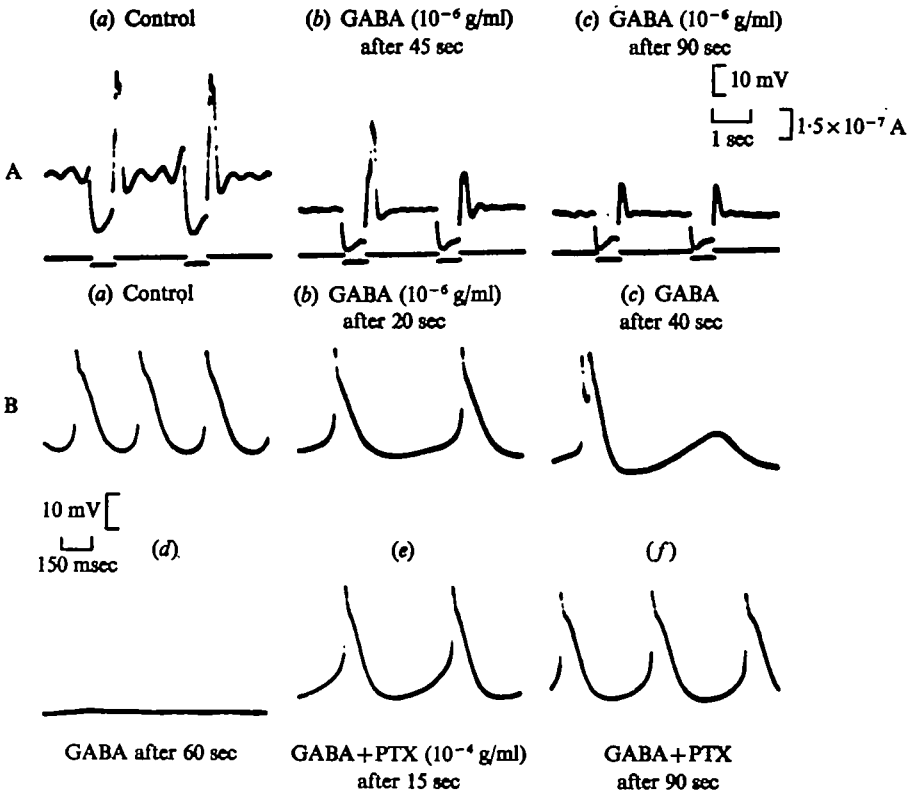

Fig. 9. Effect of γ-aminobutryic acid (GABA) (10^{-6} g/ml). (A) On the membrane resistance: (*a*) control; (*b*) and (*c*) respectively 45 sec and 90 sec after GABA was introduced. (B) On electrical spontaneous responses: (*a*) control; (*b*) in GABA after 20 sec; (*c*) after 40 sec and (*d*) after 60 sec; (*e*) 15 sec after the introduction of picrotoxin (PTX) (10^{-4} g/ml) in GABA-containing saline; (*f*) after 90 sec.

During application of currents

The mechanical activity was recorded during the application of transmembrane current. Unfortunately, the conditions of these preliminary experiments did not allow the simultaneous recording of the membrane potential variation.

A depolarizing pulse of 10^{-7} A led to a local reduction of the heart diameter *in situ*. For stronger currents (4×10^{-7} A) the contraction of the myocardium was greater and spread to about one-third of its length, which corresponded approximately to the value of space constant λ. Also, it is worth noting that when a hyperpolarizing pulse was applied the cardiac tube underwent an important expansion which lasted as long as the stimulation. In both cases, after the cessation of current pulses, the heart recovered its usual diameter.

The same currents were applied to isolated preparations at rest (Fig. 8A). Depolarizing pulses produced a torsion of the heart and an increase in mechanical tension. Hyperpolarizing pulses produced a reversed torsion and a reduction in mechanical tension. The figure also shows an oscillation, which indicates a parallel oscillation of the potential. In particular, there appeared, during the application of hyperpolarizing current, three contractions which certainly originated from electrical

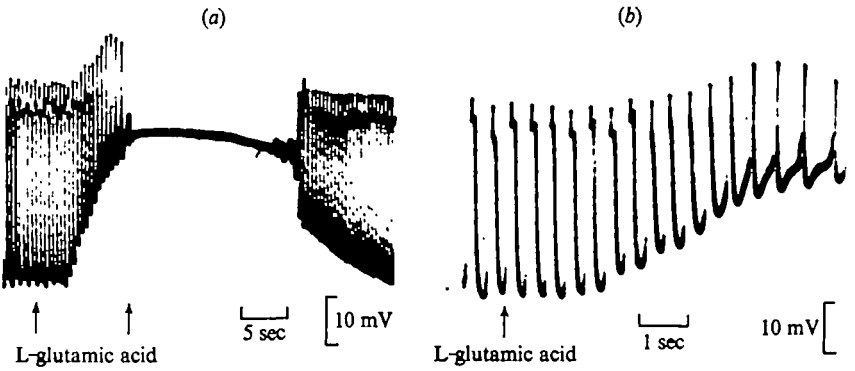

Fig. 10. Effect of L-glutamic acid (10^{-4} g/ml) recorded at two different sweep speeds (*a*, *b*). Arrows point out the moment when this substance was introduced, then withdrawn.

responses produced by a mechanical activation of the cardiac ganglion. The experimental points from such experiments are plotted in Fig. 8B. The curve shows the gradual changing of the amplitude of the mechanogram measured 100 msec after the beginning of the current pulses. The curve is S-shaped, the middle part suggests a quasi-linear relationship between the amount of contraction and the intensity of the applied current.

Effects of γ-aminobutyric acid and of L-*glutamic acid*

Fig. 9A shows that the addition of gamma-aminobutyric acid (GABA) at 10^{-6} g/ml to the normal saline appreciably decreased (40%) the resting membrane resistance. On preparations beating spontaneously, 10^{-7} g/ml of GABA reduced the frequency of the intracellular responses. The recordings from (*a*) and (*f*) on Fig. 9B show the effect of this substance used at 10^{-6} g/ml. The frequency of the heart was decreased by about 50%, the plateau phase declined and the rising phase was slowed. After some abortive or double-peaked responses (*c*), total cessation of activity occurred after 60 sec (*d*). In addition, it is interesting to note that GABA increased the membrane potential by about 8 mV. If picrotoxin (10^{-4} g/ml), known as an antagonist of GABA, was then introduced into the bathing solution, electrical responses reappeared. Within a few seconds they resembled normal responses (*e*), although their frequency was somewhat slowed down. When picrotoxin and GABA were applied simultaneously on the heart, only a decrease in the cardiac rhythmicity occurred. The withdrawal of picrotoxin was not found to restore rapidly the inhibitory properties of GABA, for after 15 min some responses still remained.

The application of 10^{-5} g/ml of L-glutamic acid produced a slight diminution of the membrane voltage (about 5 mV), but at 10^{-4} g/ml its effect was very marked and rapid (see Fig. 10*a*, *b* recorded at two different sweep speeds); as soon as it was added to the normal saline a strong depolarization (which reached about 30 mV after 30 sec) appeared. First, the response amplitude decreased by an amount equivalent to that for depolarization. Then the maximum response exceeded that usually recorded by 7–8 mV. The amplitude thus obtained remained constant for a few seconds before decreasing again until activity ceased. The frequency was decreased

only when diastolic polarization was decreased by 15 mV. The response path obtained showed pronounced modifications, the plateau phase being lowered and the rate of fall increased. Reversibility proved to be both rapid and total. The withdrawal of L-glutamic acid enabled nearly normal responses to be recorded 15 sec later.

DISCUSSION

Nature of the electrical response

A possible way to understand the nature of the response is to compare the myocardial activity of *Porcellio dilatatus* with that of other arthropods which have already been studied by many investigators.

The heart of the horse-shoe crab *Limulus* is considered a typical example of neurogenic automatism. Its intracellular electrogram includes an abrupt and weak depolarization without overshoot, followed by a slow phase of repolarization characterized by numerous and irregular small peaks. This normal neurogenic response has been interpreted as a temporal summation of junctional potentials resulting from the synaptic action of the cardiac ganglion (Robb & Recht, 1964; Abbott, Lang & Parnas, 1969; Rulon, Hermsmeyer & Sperelakis, 1971).

The electrical activity of the heart of the stomatopod *Squilla*, investigated by Irisawa *et al.* (1962) and by Brown (1964), presents numerous analogies with that of *Limulus*, and an identical interpretation was proposed by Brown.

Electrogenesis of *Porcellio* heart-beat shows some appreciable differences. Indeed, though the respective amplitudes of the resting potential and response are approximately similar, two important differences must be emphasized: firstly, the plateau phase is smooth and uninterrupted; and secondly, a pre-potential precedes the rapid upstroke.

The intracellular electrogram does not show a better similarity with those of decapods, where the sustained plateau of the response 'has a somewhat jagged appearance' (*Procambarus*: van der Kloot, 1970). The myocardial responses of decapods are generally greater than that recorded in the *Porcellio* heart. As for the presence of a slow pre-potential preceding the rapid upstroke, it seems that only Laplaud *et al.* (1961) mentioned it in *Carcinus*.

On the other hand, the usual response of *Porcellio* appears similar in contour to that described for the insect heart (McCann, 1965), whose automatism is nevertheless considered as myogenic. Indeed, the two types of activity have an unstable diastolic polarization and a rapid phase of depolarization followed by a smooth plateau. Thus, the simple comparison of the transmembrane activity of the heart of *Porcellio* with several types of activity recorded in arthropods does not lead to an understanding of its nature. If we assume that this heart is neurogenic (presence of a cardiac ganglion), the phenomenon may be considered *prima facie* as either (i) a synaptic potential, a summation of several synaptic potentials, an action potential, or (ii) a composite phenomenon including an active response of the membrane with a post-synaptic activity.

The experiments consisting in shifting the membrane potential during activity or during periods of rest give information which can be used for choosing between these proposals. During spontaneous activity the imposed current alters the rhythm,

time course and amplitude of the response. With regard to the rhythm variations they seem to support *prima facie* the hypothesis of a myogenic origin of the heart beat, since the depolarization of the myocardial membrane leads to an increase in the spontaneous rhythmicity whereas hyperpolarization decreases it. In this connexion we have always noted the important effect of passive or active mechanical tension on the frequency of the heart-beat, so we do not think that the observations mentioned above support the view that the heart-beat is myogenic. We prefer to consider that some of the effects of polarizing pulses are due to a retro-action of the mechanical consequences of the potential variations on the rhythmic discharges of the cardiac ganglion (see further explanation concerning the relation between electrical activity and mechanical tension).

With regard to the variations of the response magnitude, the relation (apparently linear) which links it to the value of the membrane potential is consistent with the post-synaptic or junctional potential hypothesis. However, it was not possible to prove the existence of a 'reversal potential'. Indeed, the microelectrode did not allow us to deliver current intense enough to depolarize the membrane more than 40 mV; moreover the strong depolarization altered the electrical activity (cf. part *b* of the curve, Fig. 4) or more frequently stopped it. It occurred during strong or medium depolarizations and seemed to depend on feed back from the mechanical tension to the activity of the cardiac ganglion. By extrapolating the measurements one may expect that the response would become zero for a depolarization of 65 mV. This value is approximately that of the resting potential; consequently one may suppose that the phenomenon has its reversal potential near zero.

Let us consider now the hypothesis that the electrical response is a true action potential. Compared to the myocardial action potential of vertebrates, it differs principally in its magnitude and its rate of rise. The action potentials of the ventricular tissue range from 100 mV to 120 mV with an overshoot (Coraboeuf, 1960). In the tissues characterized by spontaneous activity this magnitude is less important (West, 1955*a*, *b*). As far as the maximal rate of rise is concerned, it varies greatly according to the cardiac regions investigated. In the spontaneously active tissues, such as the pacemaker, both amplitude and rate of rise are reduced (West, 1955*a*, *b*).

Hence it is with the pacemaker tissue of vertebrates that the myocardium of *Porcellio* shows the greatest similarities (at least in respect to the contour). However, the experiments involving electrical transmembrane stimulation have shown that under normal conditions the myocardial membrane does not display the properties required for a complete regenerative activity. Provisionally, one may consider that the initial upstroke of the electrical response corresponds principally to a synaptic event. But the repolarization phase does not exhibit the exponential decay typical of synaptic potentials. Hence we might infer that this phase results from the temporal summation of several junctional potentials, but they would have to be perfectly summed since the plateau phase is smooth, unlike the electrogram of *Limulus* and *Squilla*. There is, however, another possible interpretation of the plateau and repolarization termination phases which can be obtained by making use of the data from electrical stimulation experiments. As pointed out, the membrane exhibits some degree of rectification and occasionally a weak graded response during applied depolarizing pulses. Consequently the plateau might be considered as the membrane

response to the synaptic depolarization; this depolarization would slowly activate a system of conductances, the nature of which cannot be yet defined here. Then again, the contour of the repolarization and especially the plateau phase (particularly its magnitude with respect to that of the upstroke) presents a large variability which could depend on fluctuations of the conductance ratio during this phase. Here it should be noted that the plateau can be altered by imposing a shift on the resting potential during or just before the response (Fig. 3). It could be supposed that the transformation of the responses by depolarizing pulses would result from a depolarization larger than that reached by synaptic excitation. As for the effect of the hyperpolarizing pulses preceding the response, it is possible to interpret them, according to the model proposed by Hodgkin & Huxley (1952), as being the consequence of the 'disinactivation' of a conductance system.

Furthermore, it is possible that fluctuations of humoral activity could alter the plateau phase, acting through the conductance system. Indeed, under similar experimental conditions one can record individual variations for the time course of both spontaneous and imposed responses. As mentioned above, there is another factor which has an effect upon the repolarization profile: the amount of tension applied to the heart (cf. Fig. 2C). At present we have no explanation for this phenomenon.

Under normal conditions a relative increase in permeability for entering ions would not be sufficient to elicit regenerative activity. However, all-or-none activity is exceptionally recorded (Fig. 2A, *d*). Besides, some pharmacological substances, such as caffeine, procaine and TEA, easily induce this kind of activity. Therefore it seems that the physiological properties of the myocardial membrane of *Porcellio* differ quantitatively rather than qualitatively from those of numerous electrically excitable membranes which generate action potentials.

The apparent conduction velocity, measured during spontaneous activity, has too high a value to be explained as a true propagation of a response with such a low rate of rise. Thus it is likely that impulses arising in the ganglion would activate the different areas of the heart almost simultaneously. The short delays observed would originate mainly in the nervous system.

Moreover, the relatively high value of the space constant enables us to define indirectly the heart structure. Since this value is near those found for single muscle fibres (Fatt & Katz, 1953), it indicates that the microelectrodes were both inserted, for each measurement, in an electrically homogeneous region. Therefore it seems that the muscular network, as observed with optical microscopy, does not consist of a plurality of isolated cellular units, but rather of fibres which, although separated, have interconnexions of low electrical resistance.

Relationships between membrane potential and contraction

The examination of the excitation/contraction relationship during spontaneous activity shows the behaviour of the *Porcellio* heart as being quite different from that of the 'slow' skeletal-muscle fibre of the crustacean, which contracts without an appreciable change in membrane potential (Hoyle & Wiersma, 1958). On the contrary, in every case an electrical event always precedes the contraction. However, the study of the mechanogram during weak fluctuation of the plateau phase indicates that the electrical response cannot be considered as just a signal triggering, in an all-or-none

manner, a mechanical process that is a function of parameters which are independent of electrogenesis. On the contrary, as in numerous muscular tissues, the time course and the amplitude of the electrical events have an effect on the profile and degree of contraction. The experimental data provided by intracellular stimulation support these results. Progressive increments of depolarizing pulses induce contractions whose amplitude and rate of rise are simultaneously increased (despite not having directly verified the membrane polarization during these experiments, we postulate that the relationship current/tension described previously is maintained).

We are not able to assume that there is a simple proportionality between the variation in voltage and the variation in mechanical tension. The experiments carried out in a K^+-free saline suggest that the predominant factor is the polarization level from which the depolarization has been established, or the level at which the depolarization ends. It would seem that the mechanical 'efficiency' of the variation of the membrane voltage depends on the absolute level at which this variation takes place. In this respect our results may be compared with those of Orkand (1962) obtained from experiments on crayfish muscle fibres depolarized by different hyperpotassic solutions. As a matter of fact, in his preparation, the degree of mechanical tension is not dependent on the height of the variation of polarization but on the absolute value of the membrane potential. Brown (1964) also showed that in *Squilla* heart it is the absolute level of the membrane potential and not the height of the junction potential which determines the amount of local tension in the fibre. In view of these data one can understand why, in the strongly hyperpolarized myocardium of *Porcellio* (action of K^+-free solution), a weak contraction was recorded even when there was a large electrical response. With regard to the generally admitted concept of a threshold for contraction the results we have obtained do not allow us to verify its validity for the myocardium of *Porcellio*. This notion of threshold implies that the fibre develops an active tension only when its membrane polarization has decreased to a given critical value. Now, in our preparation, the application of hyperpolarizing currents shows (by reduction of tension) the presence of an active permanent tension, present during apparently normal conditions. It would imply firstly, that the membrane polarization is always above the hypothetical threshold, and secondly, that the myocardium is able to develop extremely prolonged tonic contractions. In keeping with the first point, Atwood, Hoyle & Smith (1965) established that, in crab muscle, a category of fibres have a contraction threshold very near that of the resting potential. Reuben *et al.* (1967) contest the validity of the notion of contraction threshold for crayfish muscle fibres. Finally, Brown (1964) has been able to show that hyperpolarizing currents cause a local expansion, when applied to the myocardium of *Squilla*. Recently Vassort, Rougier & Favelier (1971) pointed out that strips of frog heart, studied by voltage-clamp technique, contracted slowly during depolarizing pulses too weak to trigger the slow inward current; a symmetrical decrease of tension could be produced by hyperpolarizing pulses. Rather similar results were sometimes obtained by Leoty (1971) with the same preparation.

With respect to the second point, we can mention numerous observations made on the semi-isolated heart of *Porcellio*. The diastolic diameter of the cardiac tube often varied *in situ* during activity. Some of the variations occurred slowly, others, on the contrary, were very abrupt. A slight mechanical stimulation of the wall (e.g. by

the tip of microelectrode) frequently produced an intense contraction which lasted several minutes. We noted that these mechanical effects were concomitant with the variations of the membrane potential.

If the level of the electrical polarization determines the amount of mechanical tension, then each alteration of the plateau phase of an electrical response will induce a change in the systolic tension. Now, it was shown (Holley, 1967) that the plateau of the electrical response of the heart of *Porcellio* was modified by stimulation of the cardio-regulator nerves. It is, then, conceivable that under physiological conditions the extrinsic cardio-regulator system takes advantage of the relationship between the electrical polarization and contraction to adjust finely the cardiac flow.

Effect of GABA and L-*glutamic acid*

GABA inhibits the functioning of the heart at relatively weak concentrations and picrotoxin antagonizes this effect. A similarity of behaviour between this preparation and the skeletal muscle of decapods can thus be seen. Part of the effect of GABA concerns the cardiac ganglion, taking into consideration the frequency variation of the heart-beats. Moreover, the modification of the membrane resistance, the hyperpolarization and the variations of contour observed lead to the conclusion that there could be a direct action on the membrane. In this connexion the physiological properties of the heart of *Porcellio* seem to differ from those of the lobster heart since Hallet (1971) reported that GABA had no clear influence on the heart muscle cells of *Homarus*. Further experiments will be necessary to determine whether the substance acts specifically on neuromuscular areas of the membrane; this can be postulated in analogy to its well-known action on decapod skeletal muscle (Takeuchi & Takeuchi, 1965, 1966).

As for glutamate, it is likely that an important part of its action directly concerns the myocardial membrane, as shown by the strong depolarization observed. We have not enough data to explain the mechanisms of this depolarization. However, it is conceivable that, as for the skeletal muscle of decapods, L-glutamic acid acts at the level of the excitatory neuromuscular junction. In the crayfish Takeuchi & Takeuchi (1964) have shown that the equilibrium potential for the activity of glutamate was near zero, which would mean a non-specific increase in permeability. In the lobster heart glutamate caused the heart cell membrane to become depolarized (Hallet, 1971). Thus the hearts of decapods and isopods behave similarly with respect to the action of glutamate and differently with regard to the effect of GABA. Additional investigations are needed to determine whether these data are relevant to any physiological differences in the nerve control of the heart activity.

SUMMARY

1. The electrical properties (recorded with intracellular microelectrodes) and the mechanical properties of the myocardium of the wood-louse *Porcellio dilatatus* (Brandt), a terrestrial isopod crustacean, have been investigated.

2. The diastolic membrane potential varied from -50 to -70 mV. Several types of spontaneous electrical responses have been recorded. Their amplitude was usually between 30 and 45 mV and their duration varied from 150 to 400 msec. Except for very rare cases, overshoot did not occur.

3. The phase of depolarization began slowly; it became faster but the maximum rate of rise never exceeded 1–2 V/sec. The rising phase, devoid of steps, was followed by a partial repolarization leading to a more or less sustained smooth plateau. Superimposed changes of potential occurred very rarely. A jagged appearance of the plateau, as seen in numerous neurogenic hearts and interpreted as junction potentials, could not be observed.

4. During spontaneous activity intracellularly applied currents modified the frequency, the time course and the amplitude of the responses. The junctional nature of the rising phase is suggested.

5. At rest the myocardial membrane displayed a 'normal' rectifying property and a weak delayed rectification; in addition, some preparations showed an active graded response of the membrane. A complete regenerative activity was never triggered.

6. A tentative explanation of the electrical response is proposed: the rising phase could chiefly correspond to a junctional potential, the plateau might be a response of the membrane to the synaptic depolarization.

7. The value of the membrane space constant (about 1 mm) suggests that the small muscle fibres, as observed under optical microscope, are interconnected electrically. The impulses delivered by the heart ganglion would activate the whole myocardium almost simultaneously, resulting in a high apparent conduction velocity and a good synchronization from one end to the other.

8. The degree of tension or of relaxation of the fibres closely depended on the value of the membrane voltage. The magnitude of the contraction depended on the absolute level of the potential and the length of time during which the depolarization was maintained. The functional importance of the plateau phase is considered. Intracellular depolarizing pulses led to a contraction of the heart; conversely, hyperpolarizing pulses led to its relaxation.

9. GABA (10^{-6} g/ml) inhibited the functioning of the heart and reduced the membrane resistance. Picrotoxin (10^{-4} g/ml) acted as an antagonist to GABA. L-Glutamic acid (10^{-4} g/ml) strongly depolarized the membrane, leading to the cessation of the activity.

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