

## NEURAL REGULATION OF THE HEART MUSCLE IN AN ISOPOD CRUSTACEAN: ACCELERATION AND PERIPHERAL INHIBITION

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### SUMMARY

1. In the neurogenic heart of the isopod crustacean *Porcellio dilatatus*, repetitive electrical stimulation of the cardiac nerves elicited either cardio-acceleratory or cardio-inhibitory effects depending on the stimulation parameters.

2. Acceleratory effects were accompanied by a decrease of membrane potential and by changes in the contour of the spontaneous electrical responses: increase in the speed of the rising phase and enhancement of the plateau phase.

3. Inhibitory stimulation acted on rhythmicity and/or contour of spontaneous responses. At stimulation pulse frequencies beyond 25/s a hyperpolarization appeared after the cessation of the inhibitory train.

4. Inhibitory stimulation elicited IJPs in the myocardium. Their reversal potential was found to be close to the value of the resting membrane potential. During inhibitory stimulation, the membrane resistance of the heart muscle was frequently decreased.

5. The effects of changing the external chloride content, and of adding GABA and picrotoxin support the hypothesis that the inhibitory impulses increased the myocardium permeability to  $\text{Cl}^-$ .

6. On the basis of these findings it is assumed that cardio-inhibitory fibres act on both cardiac ganglion and myocardium.

7. Comparisons are established between the wood-louse's heart and the skeletal or heart muscle of some arthropods. The functional significance of peripheral inhibition is further discussed in relation to the nature of the spontaneous electrical responses and to contraction.

### INTRODUCTION

The main investigations of the extrinsic nervous regulation of the neurogenic heart of Crustacea have been undertaken in Decapoda and Stomatopoda. Some work has been concerned with the effects of cardio-regulatory impulses on the heartbeat (Wiersma & Novitski, 1942; Smith, 1947; Maynard, 1953; Florey, 1960) while a number of extensive studies have dealt with the action of the regulatory nerve stimulation on the cardiac ganglion responsible for the automaticity (Terzuolo & Bullock, 1958; Watanabe, Obara & Akiyama, 1968, 1969).

It has generally been assumed that the cardio-regulatory nerves acted on the cardiac ganglion rather than on the heart muscle. However, Hagiwara (1961) and Maynard (1961) put forward the hypothesis that, in addition to their effects on the ganglion, the regulatory impulses might act on the myocardial membrane.

Peripheral inhibition has been proved to be a particularity of the skeletal muscle of a variety of Arthropoda (Insecta: Usherwood & Grundfest, 1965; Crustacea: Boistel & Fatt, 1958; Dudel & Kuffler, 1961; Atwood, 1968; Arachnida: Brenner, 1972; Merostomata: Parnas *et al.* 1968). To our knowledge, peripheral inhibition has not been shown in the heart of Decapoda. On the contrary, Hallet (1971) pointed out that, in the lobster heart, the stimulation of the cardiac regulator fibres (either acceleratory or inhibitory) did not induce direct modification of the myocardial membrane activity, the effect of the stimulation being explained by indirect action through the cardiac ganglion. On the other hand, in the heart of the terrestrial isopod crustacean, *Porcellio dilatatus* (wood-louse), previous results suggest the existence of peripheral inhibition (Holley, 1968).

The aim of the present investigations was to study the cardio-regulatory mechanisms in the heart of the wood-louse and particularly the functional significance of peripheral inhibition in the control of electrogenesis and contraction. Preliminary reports of this work have been published already (Delaleu & Holley, 1973; Delaleu, 1974).

## METHODS

### *Preparation*

Detailed studies using light and electron microscopy have been undertaken in the heart of *Porcellio* (Delaleu, 1970, 1974). The heart of the wood-louse is neurogenic, and a cardiac ganglion containing neurones lies at the dorsal inner limit of the myocardium wall. The ganglion is connected to a pair of fine cardiac nerves, originating from the central nervous system and from the stomatogastric system and running along the 'aorta'.

The methods of dissection and the composition of the bathing medium have been described elsewhere (Holley & Delaleu, 1972). In these experiments, the heart tube was completely isolated from the posterior part of the exoskeleton but the 'aorta', which prolongs the heart, remained *in situ*, attached to the tergites of the first four thoracic segments. This prevented the cardiac nerves being damaged.

### *Stimulation and electrical recording*

The stimulation of the cardiac nerves was achieved by means of two Ag-AgCl wires, 100  $\mu\text{m}$  in diameter, isolated by polyethylene tubes, except at the tips. The stimulating electrodes were placed on the rostral part of the aorta, which helped locate the nearly invisible nerves. They were connected to a stimulator through an isolation unit. The stimulations were trains of 1 ms square pulses at various frequencies. The electrical activity of the heart was recorded with conventional glass microelectrodes, filled either with 3 M-KCl or 0.6 M  $\text{K}_2\text{SO}_4$ , impaled in the anterior part of the heart. The distance between the foci of stimulation and recording was approximately 2 mm. Measurements of membrane resistance or electrical polariza-

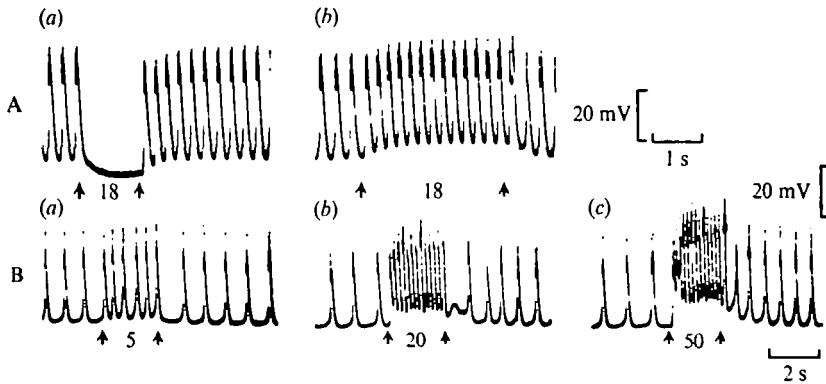


Fig. 1. Cardio-inhibition and cardio-acceleration in *Porcellio*. A. Inhibition (a) and acceleration (b) of the rhythmic electrical activity recorded from the same heart when the frequency of impulses in the train is kept constant (18/s) but when their intensity is varied. (a) 4 V; (b) 3.5 V. B. Cardio-acceleratory effects (other heart). (a) Train at 5/s; (b) at 20/s; (c) at 50/s. In this figure and in the following the numbers indicate the stimulation frequency (pulses/s). The distance between the arrows indicates the duration of the train.

tions of the myocardium were performed by inserting a second polarizing micro-electrode close to the recording electrode. The mechanogram was recorded by means of an RCA 5734 transducer.

## RESULTS

The traces in Fig. 1 A (a, b) clearly indicate that when a constant frequency of stimulation pulses (18/s) was applied to the same heart, variations of the intensity of stimulation elicited either cardio-inhibitory effects (a, 4 V) or cardio-acceleratory effects (b, 3.5 V). These results strongly suggest the duality of the regulatory fibres contained in the cardiac nerves. Generally, the threshold for acceleration was lower than that for inhibition. Attempts to separate these two effects were not often successful, and inhibitory effects were more easily recorded.

### *Cardio-acceleration*

Stimulation at frequencies not higher than 5/s shortened the interval between two spontaneous electrical responses and depolarized the myocardial membrane. These effects became more pronounced when frequency was increased to 50/s. Marked changes were observed in preparations whose spontaneous rhythmicity was slow, as illustrated by the traces in Fig. 1 B which show that stimulation from 5 to 50/s caused the frequency of the spontaneous activity to increase three and a half times. The acceleratory effects were accompanied by a decline in membrane potential, but this decline did not exceed 10 mV during maximal acceleration (1 B, c). During cardio-acceleratory effects, the time course of the spontaneous responses was altered: the plateau was enhanced and the rate of rise was increased, but the amplitude of the total upstroke showed little variation (sometimes slightly decreased). The increase in rhythm was hardly affected when the myocardial membrane was experimentally either depolarized or hyperpolarized before and during the application of the train of impulses. A decrease in heart membrane resistance could be observed during or immediately following an acceleratory stimulation. At the end of the excitatory

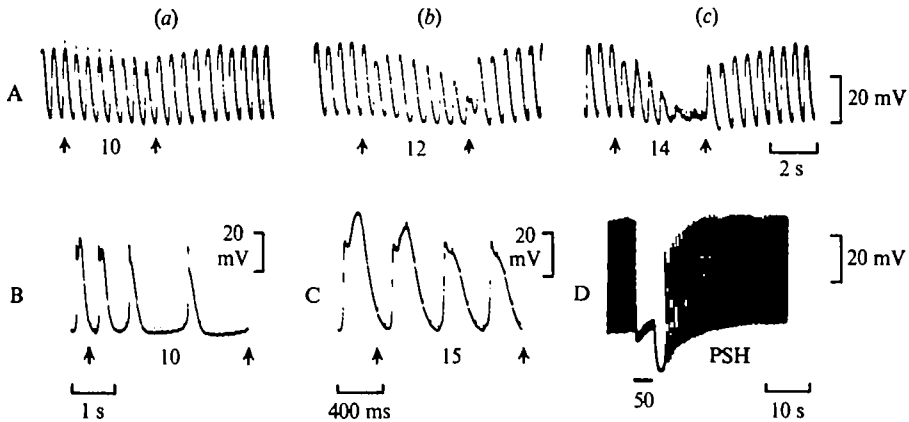


Fig. 2. The cardio-inhibitory effects. A. Changes in spontaneous activity when frequency is progressively increased. (a) 10/s; (b) 12/s; (c) 14/s. B and C. Effect of inhibitory trains on two hearts whose spontaneous electrical responses displayed humped plateaus. D. One example of a post-stimulus hyperpolarization (PSH) recorded when applying a 5 s duration 50/s train (horizontal bar).

stimulation, marked after-effects were not generally observed; membrane potential returned to its resting value and the original rhythm rapidly recovered.

### Cardio-inhibition

#### General aspects of inhibition

According to the frequencies used, the inhibitory stimulation altered the rhythm, the time course of the spontaneous responses, and the resting potential. At low frequencies (less than 12/s) these parameters were not identically affected in all the hearts tested. In some instances the contour and the amplitude of the responses were modified before there was any marked change in rate of beating as exemplified by traces (a), (b), (c) on Fig. 2A. It should be noted that myocardial membrane hyperpolarized during the stimulation. In other instances (Fig. 2B), the changes in rhythm preceded any reduction of the initial upstroke, and electrical stimulation did not cause any change in resting membrane potential. This trace and trace C in the same figure show that the time course of the plateau could be affected by stimulating at frequencies that did not noticeably reduce the amplitude of the upstroke. This point is worth emphasizing as it suggests the heterogeneity of the components (upstroke and plateau) of the electrical response.

The cessation of the activity occurred at frequencies from 12 to 20/s depending on the hearts studied but occurred at constant frequency for a given heart. At moderate frequencies, up to 15/s, the after-effects were slight but nevertheless they were more important than those observed during cardio-acceleration. For example, after the application of the stimulation the plateau was transiently enhanced and prolonged.

At frequencies higher than 25/s the post-stimulation effects became more pronounced. In particular, following the end of the stimulating pulse trains, a long-lasting hyperpolarization was observed (Fig. 2D). This post-stimulation hyperpolarization (PSH) varied in amplitude and duration with the frequency and duration of the stimulating volley. For instance, trains of pulses at 50/s, lasting 5 s, resulted in

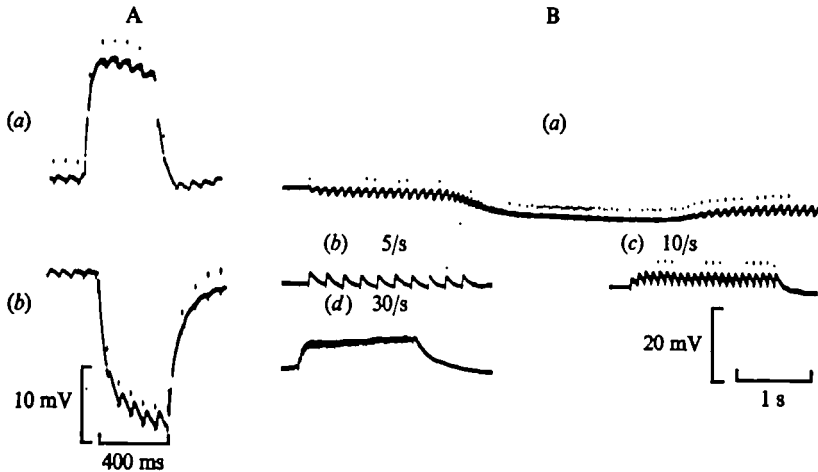


Fig. 3. Synaptic potentials recorded in the myocardium. A. The inhibitory potentials (IJPs), depolarizing at normal resting potential, are reversed by the application of outward currents (a) and increased in size when the myocardial membrane was experimentally hyperpolarized (b). Intensity of current:  $\pm 7 \times 10^{-8}$  A; train at 15/s. In this experiment  $E_{IJP} = -58$  mV. B. IJPs in presence of tetraethylammonium chloride (10 mM/l). (a) Effect of progressive increments in frequency (from 10/s up to 30/s) on hyperpolarizing potentials. (b), (c) and (d) Summation of depolarizing potentials (frequency, respectively 5, 10 and 30/s).

a PSH of 10 mV and lasting 25 s (means of 40 experiments). This PSH exhibited unusual properties, namely a paradoxical increase in membrane resistance. Detailed study showed that this PSH was not a simple development of the inhibitory processes brought into play during stimulation, but was a phenomenon justifying separate investigation. Accordingly, the data reported in this paper mainly deal with the inhibitory processes induced by stimulation not exceeding 25/s, i.e. frequencies at which the special phenomenon represented by the PSH is thought to be absent or minimal.

#### *Inhibitory junctional potentials*

Intracellular records from myocardial fibres during the cardiac nerve stimulation revealed the presence of inhibitory junctional potentials (IJPs) which could be either depolarizing or, more rarely, hyperpolarizing. In some hearts no detectable IJPs were recorded unless the membrane potential was varied experimentally under conditioning current injection. As illustrated in Fig. 3 A, this method was used to determine the reversal potential of the IJPs. In most cases this reversal potential was found to be roughly equal to that of the resting potential or more positive by a few millivolts.

Whereas the amplitude of IJPs did not exceed 2 mV in normal saline, nerve stimulation applied in presence of tetraethylammonium chloride (TEA, 10 mM/l) elicited synaptic potentials whose amplitude could reach 4–5 mV. Contrasting to the situation in normal saline, TEA allowed us to record hyperpolarizing IJPs more frequently than depolarizing IJPs. This can be partly attributed to the depolarizing effect of TEA in the wood-louse's myocardium (Delaleu *et al.* 1972). In Fig. 3 B, trace (a) shows the hyperpolarizing IJPs summated during a progressive increase in

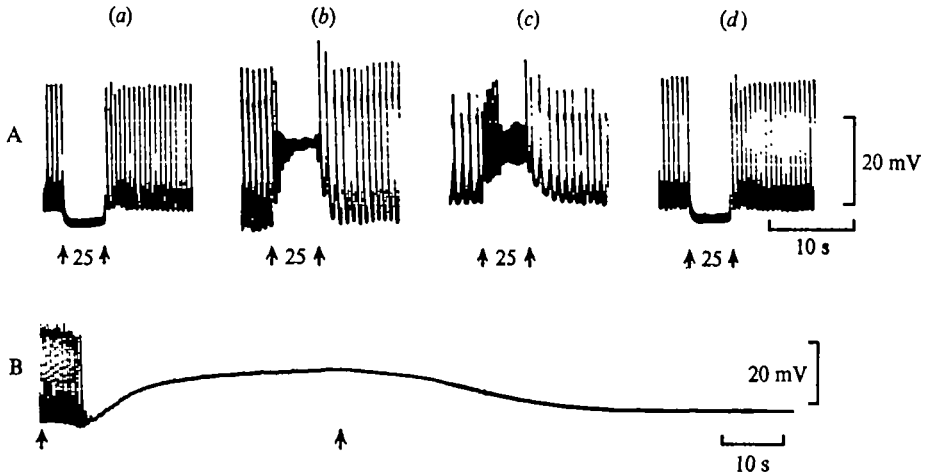


Fig. 4. The cardio-inhibitory effects in presence of  $\text{Cl}^-$ -deficient solutions and/or GABA. A. Shift of the level of the summated IJPs in presence of a  $\text{Cl}^-$ -deficient (methylsulphate) saline (b) after 1 min, (c) after 2 min. The normal saline controls are in (a) and (d). Note the 'paradoxical' acceleration in (c). Trains at 25/s. Resting membrane potential:  $-60$  mV. B. Inhibition of the spontaneous activity and membrane potential changes, successively in presence of a  $\text{Cl}^-$ -deficient solution containing GABA (between the arrows) and in the normal saline containing GABA ( $0.1$  mM/l).

stimulation frequency in TEA medium. At the beginning of the stimulation at 10/s, the synaptic potentials exhibited some degree of facilitation. Conversely, tracings (b), (c), (d), represent depolarizing IJPs recorded in another heart also perfused with a TEA solution. Under optical examination, the myocardium was seen to contract under the summated IJPs. The ability of the inhibitory stimulations to clamp the membrane potential close to its resting value was further illustrated under particular conditions. As previously reported (cf. the above reference), in presence of TEA the myocardium membrane potential could be suddenly blocked close to electrical zero. It was observed that stimulation of the cardiac nerves at 50/s caused the myocardial membrane to repolarize in an all-or-none manner.

#### *Effect of varying the external chloride concentration and action of GABA*

Since the heart of *Porcellio* was shown to have common features with crustacean skeletal muscle, we suspected  $\text{Cl}^-$  ions to be implicated in the IJPs. To test this possibility, the external  $\text{Cl}^-$  content ( $[\text{Cl}^-]_o$ ) was varied. Gamma-aminobutyric acid (GABA), the transmitter that increases the chloride permeability at the crustacean inhibitory neuromuscular junction, was added in the bathing medium. In  $\text{Cl}^-$ -deficient solutions, the cardiac nerves were stimulated at 25/s, and we observed the level of the membrane potential, determined by the summated IJPs.

When the inhibitory stimulation was applied 60 s after the introduction of the modified saline (90% of the external  $\text{Cl}^-$  was replaced by the large methylsulphate anion), the level of the summated IJPs shifted by 15–20 mV in the positive direction (Fig. 4A, b). This was observed about fifty times in twelve hearts. If the same experiment was performed one minute later, the same inhibitory stimulation did not cause the heart to stop immediately, but a marked acceleration of the heartbeats could be

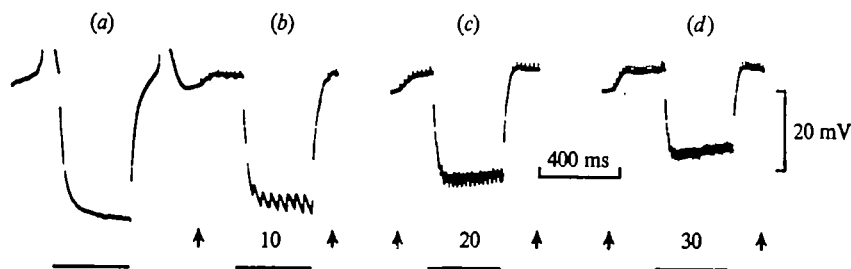


Fig. 5. Membrane resistance changes during inhibitory stimulation. (a) Control. (b), (c) and (d) Effect of inhibitory trains on the electrotonic potential resulting from the application of an intracellular current pulse ( $-8 \times 10^{-8}$  A) to the myocardium. The horizontal bars indicate the pulse duration.

observed (c). Higher frequencies were then required to obtain an arrest. When the normal saline was replaced the previous stimulation again induced cessation of the spontaneous activity (d).

The traces in Fig. 4 B show that when a  $\text{Cl}^-$ -deficient solution containing GABA (0.1 mM/l) was introduced (first arrow) the electrical activity stopped and the myocardial membrane depolarized by 15 mV. If the normal saline containing GABA was replaced (second arrow) the membrane potential returned close to its normal resting value. In one preparation, the introduction of the  $\text{Cl}^-$ -deficient solution containing GABA resulted in a temporary acceleration of the heartbeat. Thus GABA mimicked the effects of the inhibitory stimulation in both normal and  $\text{Cl}^-$ -deficient salines.

#### *Changes in membrane resistance during inhibitory stimulation*

The inhibitory stimulation decreased the membrane resistance ( $R_M$ ) (15 experiments). The traces in Fig. 5 represent the variations of the hyperpolarizing effects of a similar current pulse applied during stimulation of cardiac nerves at different rates. The reduction in size of the electrotonic potentials indicates a decrease in membrane resistance caused by stimulation. This decrease in  $R_M$  was a function of the stimulation frequency and, in this experiment,  $R_M$  was halved by stimulation at 30/s. It should be noted that inhibitory stimulations did not always result in such a diminution of  $R_M$ . Actually, in some experiments no variation in  $R_M$  was recorded although the stimulation was efficient, at least on the pacemaker neurones, as judged by the cessation of the spontaneous activity. Furthermore, in a few instances a slight increase in  $R_M$  even appeared. The lack of consistency of the results concerning the changes in  $R_M$ , especially the paradoxical increase in  $R_M$ , reflected the dual effect of the stimulation.

#### *Inhibitory stimulation and contraction*

In so far as mechanical activity is controlled by the myocardial membrane polarization (Holley & Delaleu, 1972), the inhibitory stimulation that modifies the electrical responses and the resting potential can be expected to act on the contraction and the heart tonus. This could be verified: stimulation of the cardiac nerves at rates that did not cause an arrest of the heart, but simply reduced the amplitude of the

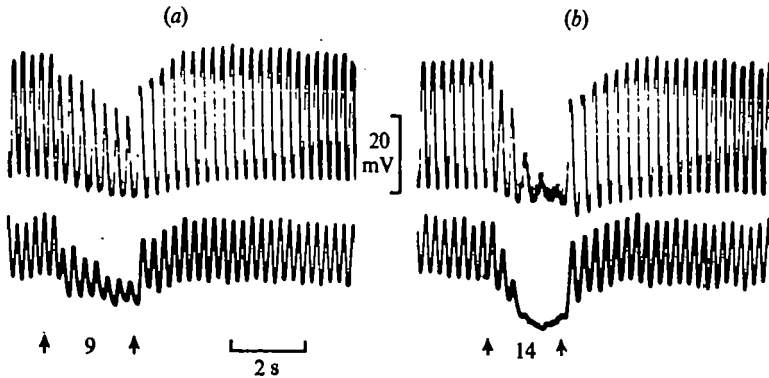


Fig. 6. Cardio-inhibitory effects and contraction. Effect of inhibitory stimulation on electrical activity (upper traces) and on mechanogram (lower traces). Frequency of stimulation (a), 9/s; (b), 14/s.

spontaneous electrical responses, markedly affected the mechanogram. The contractions were reduced as soon as the plateau of the responses was altered even though the initial upstroke was not yet affected. The diastolic tonus of the heart was also modified by the stimulation as illustrated in Fig. 6. In this case the stimulation that hyperpolarized the heart membrane induced a marked reduction of the diastolic tonus. It can be seen that a hyperpolarization of less than 5 mV (b) during stimulation at 14/s caused a decrease in mechanical tension equal to the amplitude of the normal contractions. This confirms the presence of a large diastolic tonus in normal conditions.

#### DISCUSSION

Stimulation of the cardiac nerves led to either acceleration or, more frequently, inhibition of the heartbeat, which agrees with previous mechanogram examination (Holley & Delaleu, 1967; Holley, 1967). It is probable that each cardiac nerve contains two categories of fibres. Selective stimulation of only one type of fibre was attempted by finely adjusting the stimulation parameters, together with changes in placement of the stimulating electrodes, and apparently was sometimes achieved, for example when clear acceleratory effects were recorded. Under these conditions a weak increase in the intensity of stimulation (but not in frequency) frequently caused a complete inversion of the effects, suggesting that the fibres were scarce, and that the inversion was due to recruitment of an inhibitory fibre. Histological studies revealed the scarceness of the fibres.

#### *Cardio-acceleration*

The increase in the rhythm and rate of rise of the spontaneous electrical responses might at first be attributed to an acceleration of the periodical activity of the cardiac ganglion cells along with an increase in the spike discharge frequency within each burst. Well-known studies on the crustacean heart support this hypothesis (see review by Maynard, 1960).

The question arises as to whether the cardio-acceleratory fibres also acted on the myocardial membrane. In *Porcellio*, acceleratory effects decreased the membrane



potential and caused a sustained depolarization that looked like that observed by Watanabe *et al.* (1969) in pacemaker neurones of *Squilla* during stimulation of the  $\beta$  nerves. However, in our opinion, this change in membrane polarization of the wood-lice heart does not represent proof for a direct peripheral effect, for this finding may be explained as being the consequence of an acceleration causing a 'tetanization', hence preventing full repolarization before the appearance of a new depolarizing response. Several attempts to attribute a change in membrane resistance to the functioning of 'acceleratory' junctions on the myocardium were unsuccessful. Changes in membrane resistance were explainable as a consequence of the increase in rhythm of the electrical responses, which partly consist of excitatory junctional potentials. The direct effect of the cardio-acceleratory stimulation on the myocardium could have been demonstrated by the presence of junctional potentials, but we failed to identify such potentials.

#### Cardio-inhibition

In addition to the action of inhibitory stimulation on the cardiac ganglion, there is convincing evidence for direct inhibitory action on the myocardial membrane, as indicated by the presence of IJPs and the changes in membrane resistance during stimulation. Therefore the physiology of the heart muscle of *Porcellio* presents features in common with the crustacean skeletal muscle in which this mode of post-synaptic inhibition has been frequently observed (Boistel & Fatt, 1968; Atwood, 1968). On the other hand, the heart of this isopod differs from that of the lobster in which Hallet (1971) failed to record synaptic potentials when stimulating the cardio-inhibitory nerves.

The value of the reversal potential for the IJPs, close to that of the membrane potential, supports the comparison of the heart of *Porcellio* with the skeletal muscle of Crustacea studied by Fatt & Katz (1953), and Hoyle & Wiersma (1958). The shift in the level of the summated IJPs in  $\text{Cl}^-$ -deficient solutions strongly suggests that during inhibition there was an increase in chloride conductance, the level of membrane polarization during the stimulation presumably tending to follow the shift in the equilibrium potential for  $\text{Cl}^-$  ( $E_{\text{Cl}^-}$ ). In other words the effect of cardio-inhibitory impulses would be to clamp the membrane potential close to  $E_{\text{Cl}^-}$ .

In some preparations the efficiency of inhibitory stimulation decreased in methylsulphate solutions. In addition, an acceleration of the spontaneous responses was noted under inhibitory stimulation. This paradoxical phenomenon may be attributed to an inversion, in low  $[\text{Cl}^-]_o$ , of the effects of inhibitory transmission at the level of the pacemaker cells of the cardiac ganglion. These results argue that the chloride conductance was normally involved in 'central' ganglion inhibition as in peripheral inhibition. The observed acceleration probably resulted from the depolarizing effect of the stimulation that increased chloride permeability when  $E_{\text{Cl}^-}$  had shifted in the positive direction (decrease in  $[\text{Cl}^-]_o$ ). There have been similar findings with molluscan neurones during iontophoretic application of acetylcholine in chloride-deficient solutions (Kerkut & Thomas, 1963).

Several hypotheses may be proposed to account for the increase in IJP amplitude in TEA solutions. Since this substance increases the membrane resistance (Delaleu *et al.* 1972), it is conceivable that this effect could induce larger junctional potentials. Another tentative explanation would be that TEA increased the amount of

transmitter released through a lengthening of the presynaptic action potentials as observed by Kusano *et al.* (1967).

GABA mimicked the effects of the stimulation of the inhibitory fibres since it abolished the spontaneous activity; without doubt it acted on the cardiac ganglion. GABA also acted on the myocardial membrane, as indicated by the shift in membrane potential recorded in GABA-methylsulphate solution. This change in membrane potential was somewhat comparable with that observed when the inhibitory fibres were stimulated in methylsulphate saline. This is consistent with an increased  $\text{Cl}^-$  conductance caused by GABA. The decrease in  $R_M$  in the presence of GABA reported in a previous paper (Holley & Delaleu, 1972) strengthens this interpretation.

The acceleration sometimes observed in GABA-methylsulphate solutions could be explained by assuming that the  $\text{Cl}^-$  conductance mediated the action of GABA at the level of the pacemaker cells. The action of the inhibitory fibres at this level was shown to depend on the same mediation. While awaiting further analysis of the inhibitory process in *Porcellio*, it should be emphasized that picrotoxin, known as an antagonist of GABA, antagonized the inhibitory effect of the cardiac nerve stimulation. A close relationship is indicated between the properties of GABA and those of the natural inhibitory transmitter; both these substances could act on the same receptor of the postsynaptic membrane or on receptors coupled with a common ionophore.

The changes in  $R_M$  recorded during the inhibitory stimulation suggest an action of the inhibitory fibres on the myocardium, and confirm the hypothesis of peripheral inhibition put forward by Holley (1968). Our results provide further evidence that the heart of the wood-lice shares some physiological properties with the skeletal muscle of some arthropods (see reviews by Atwood, 1968 and Pearson, 1973). The increase in  $R_M$  that was sometimes recorded in *Porcellio*, but not in the skeletal muscle, may be explained by stimulation of the cardio-regulatory nerves having two effects, A and B, with opposite action on  $R_M$ . The A effect would be the synaptic processes leading to an increase in chloride conductance, as analysed in the present paper. The B effect appears essentially as an increase in membrane resistance resulting in a post-stimulation hyperpolarization (PSH) at rather high rates of stimulation. This hypothesis will be presented and discussed in a subsequent paper. Since the A and B effects lead to opposite changes in membrane resistance it is conceivable that their combination in various proportions determines various values of the global membrane resistance. It must be further stated that picrotoxin depressed the increase in chloride conductance (A effect), thus revealing the decrease in conductance characterizing the B effect, at the very beginning of the cardiac nerve stimulation.

Whereas the myocardium of the wood-lice shares some properties of the skeletal muscle of decapods it seems to differ from their heart muscle, since peripheral inhibition could not be demonstrated in the lobster (Hallet, 1971). The significance of these important differences in cardiac regulation mechanisms in the two orders can be understood if we refer to the difference in nature of the processes regulating contraction, i.e. the spontaneous electrical activity. The normal spontaneous response of the heart of *Homarus* has been interpreted as a temporal summation of JPs resulting from the synaptic action of the cardiac ganglion on passive membrane conductances (see review by Anderson, 1973). On the other hand, the responses in *Porcellio* show appreciable differences, since the plateau was thought to bring into play active

membrane conductances. If the contractile activity depends directly on the amplitude and duration of the depolarization in both species (Hallet, 1971; Holley & Delaleu, 1972; Delaleu *et al.* 1972), the regulation of the contraction may be specifically achieved through a control of the plateau. In the lobster, this control can be performed by the action of the extrinsic regulatory fibres on the unique cardiac ganglion, since the postsynaptic electrical responses closely depend on the characteristics (number of spikes, frequency, duration) of the ganglion bursts. In the wood-louse the problem is different: the plateau appears to depend strongly on the myocardial membrane properties and it is then conceivable that the plateau and the related contraction are regulated at the peripheral level. As a matter of fact it could be observed in *Porcellio* that inhibitory stimulation had a specific depressive action on the sustained depolarization and consequently on the contraction at low frequencies that were not out of a possible physiological range.

In conclusion, inhibitory regulation operates at two different levels in the heart of *Porcellio*: the frequency of beating is regulated by the effect of inhibitory impulses on the cardiac ganglion, while the tonus and the amplitude of the contraction are chiefly modulated at a peripheral level by impulses reaching the myocardial membrane.

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