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CARDIAC GANGLION OF *LIMULUS*: INTRACELLULAR ACTIVITY IN THE UNIPOLAR CELLS

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INTRODUCTION

The spontaneous rhythmic beating of the heart of the horseshoe crab, *Limulus polyphemus*, has its origin in a cardiac ganglion located on the external dorsal surface of the heart (Carlson, 1904; 1909). The heart in *Limulus* is a long tubular structure divided into nine unequal segments by eight pairs of ostia. The cardiac ganglion is a cord-like structure extending almost the entire length of the heart.

The microscopic anatomy of the cardiac ganglion has been recently described (Bursey & Pax, 1970). The ganglion consists of two parts, a fibre-tract portion and a cellular portion. The fibre tract extends the entire length of the ganglion, but the cellular portion reaches only as far forward as the anterior of the third heart segment. Within the cellular portion of the ganglion four different cell types can be recognized: large pigmented unipolar cells, large pigmented bipolar cells, pigmented multipolar cells and non-pigmented multipolar cells.

A number of investigators have made an effort to determine the site within the cardiac ganglion where electrical activity originates, that is, the site of the pacemaker. Carlson (1905) and Edwards (1920), noting that activity generally begins first in the middle third of the heart, concluded that the pacemaker for the heart is located in this region. Garrey (1930, 1932) and Bullock, Burr & Nims (1943), however, demonstrated that the site at which activity first appears within the ganglion at each heartbeat can vary. A number of procedures, notably local heating or cooling, may shift the pacemaker as far anterior as the anterior end of the third segment or as far posterior as the eighth segment.

On the basis of sectioning experiments and subsequent histological studies Heinbecker (1936) concluded that the large unipolar cells are probably the pacemakers in the ganglion. We report here the results of experiments we have performed in an effort to define more clearly the role of these large unipolar cells in the production of the spontaneous rhythm seen in the cardiac ganglion of *Limulus*.

MATERIALS AND METHODS

The animals used in these experiments were obtained from the Gulf Specimen Co., Panacea, Florida, and were maintained until use in an artificial sea-water aquarium at 13–16 °C. Both males and females with carapace widths from 20 to 30 cm were used. The length of the heart in such animals measured between 11 and 17 cm. All recordings were made from cardiac ganglia *in situ* on isolated spontaneously beating hearts.

Procedures used for isolating the hearts have been described previously (Pax & Sanborn, 1967). In our experiments, however, a dark-coloured glass rod (O.D. 7 mm) was inserted into the lumen of the heart through a transverse cut at the first pair of ostia. This dark glass rod makes it easier to distinguish details within the cardiac ganglion, particularly the positions of the large unipolar cells. Isolated hearts were bathed in an artificial sea water (Instant Ocean, Aquarium Systems, Inc.) and all experiments were carried out at room temperature (22–26 °C).

Glass microelectrodes filled with 3 M-KCl or 0·5 M-K_2SO_4 with resistances between 5 and 50 MΩ were used for recording of intracellular electrical activity. The electrodes were connected through Ag–AgCl wires to negative capacitance pre-amplifiers (Argonaut Model LRA 043 or W-P-Instruments Model M-4). A large Ag–AgCl wire served as an indifferent electrode.

For intracellular current injection both KCl-filled and K_2SO_4-filled electrodes with resistances between 5 and 40 MΩ were used. In these experiments the built-in bridge circuit of the M-4 Electrometer which permits simultaneous current injection and potential recording from a single electrode was used. Extracellular stimulation was accomplished by means of suction electrodes applied to either the ganglion or small side branches from the ganglion.

With a magnification of 42 × the large unipolar cells are readily visible in the isolated heart. A number of features distinguish them from others in the ganglion (Bursey & Pax, 1970). They are of large size (100 × 140 μm), are spherical in shape, have an asymmetrical pigment distribution and often protrude from the ganglion. The only other cells within the ganglion of the same general size are the large pigmented bipolar cells. These, however, have a symmetrical pigment distribution and do not protrude from the ganglion. Records were taken only from cells which could be clearly identified as large pigmented unipolar cells.

RESULTS

(A) *Spontaneous activity*

Intracellular records were obtained from a total of 214 unipolar cells from 66 different isolated spontaneously beating hearts. The mean resting potential recorded was −43 mV (range −18 to −64 mV). Correlated with each contraction of the heart there occurs in these cells a burst of activity which consists of a sustained depolarization of several seconds on which is superimposed a series of small spike-like potentials of 2–5 mV. Figs. 1 and 2 give several examples of the kind of activity recorded from unipolar cells. In the cells we examined the maximum depolarization reached during activity averaged 23 mV (range 5–48 mV) and was directly correlated with the size of the resting potential ($r = 0{\cdot}65$). In no case did maximum depolarization pass zero, the membrane remaining polarized by at least 5 mV in all cases.

The sustained depolarization that one records from these cells during each heart contraction follows a rather complex time course and, though this time course is fairly constant from beat to beat in any particular cell, it varies from cell to cell. In general, one sees a rapid initial depolarization to some peak value which is maintained for varying periods of time. There then often follows a fairly rapid partial repolarization followed by either a plateau and then a slow repolarization to the resting level, or a simple slow repolarization to the resting level without an intervening plateau (Fig. 2).

In most instances one can readily distinguish two phases in the activity. There is an early phase during which the small, spike-like potentials are irregular in both amplitude and frequency. This is followed by a second phase during which the spikes are much more regular in amplitude and frequency. The transition from the first phase to the second is usually obvious and is often marked by a short period with no spike activity or by a fairly rapid partial repolarization of the cell.

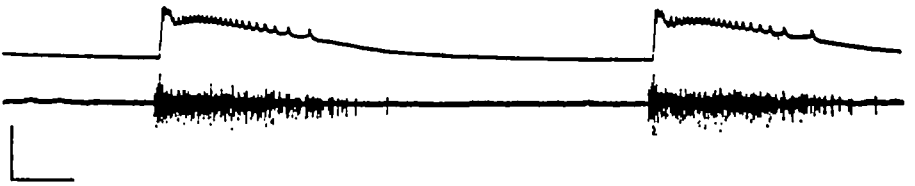

Fig. 1. Simultaneous activity recorded intracellularly from a unipolar ganglion cell in segment 7 (top trace) and extracellularly from the whole ganglion from segment 3 (bottom trace). The onset of a spontaneous burst of the ganglion approximately coincides with the initial depolarization from the unipolar cell. The ganglion continues to fire throughout the period of spike activity in the cell. Voltage scale, 30 mV, top trace; 100 μV, bottom trace. Time scale, 400 ms.

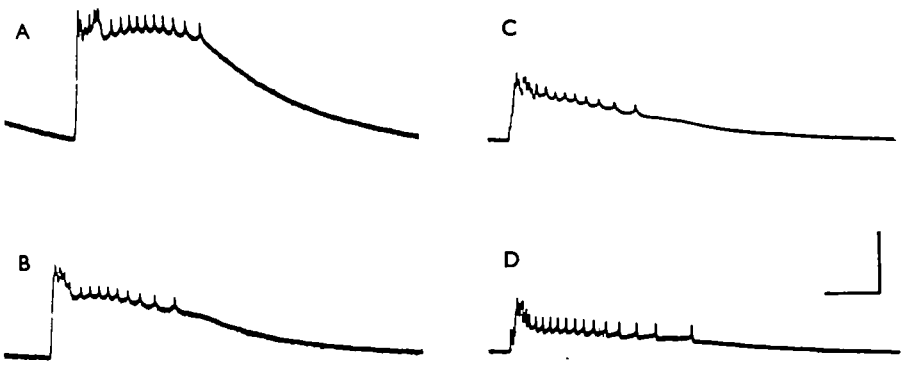

Fig. 2. Spontaneous intracellular electrical activity recorded from the somata of four unipolar cells. Note the differences in the rise times of the initial depolarizations. Resting membrane potentials were 54, 45, 32 and 22 mV for A, B, C and D respectively. Voltage scale, 20 mV; time scale, 400 ms.

For purposes of discussion, the time from the beginning of the depolarization of the cell to the point where the spike-like potentials become regular we will designate the initial phase of the activity. The time from the end of the initial phase to the time when the membrane returns to the resting level we will designate the recovery phase.

(B) *Phases of activity*

Initial phase

The initial phase—the time from the beginning of the depolarization to the beginning of the recovery phase—may last from 23 to 450 ms. The irregular, spike-like potentials one sees during this phase may not begin until maximum depolarization is reached, or a number of these spikes may be evident on the rising phase of the depolarization. Of the 214 cells we examined 33% had no spikes on the rising phase (Fig. 2A), while 67% had from one to six spikes (Fig. 2C, D). In most instances these spikes are discernible only by a slight notch on the rising phase of the depolarization (Fig. 2C), but in some cases (38 cells out of 214) the spikes are larger and easily discernible as distinct spikes (Fig. 2D).

The time to reach peak depolarization and the presence or absence of spikes are correlated. Thus for 15 cells for which quantitative data were obtained in which there were no spikes on the initial depolarization the rise time averaged 17 ms; for 15 cells in which barely discernible spikes were present the rise time averaged 39 ms; and for 15 cells in which large spikes were evident the rise time averaged 113 ms. There were no significant differences in the peak depolarizations seen in these different groups of cells. Table 1 summarizes the data we have obtained from these cells.

Table 1. *A summary of the electrical properties of unipolar cardiac ganglion cells*

	N	Range	$\bar{X}$	S.D.
Resting membrane potential (mV)	112	18–64	43	5·7
Peak depolarization (mV)	112	5–48	23	5·2
Duration of initial phase (ms)	152	23–450	170	65·6
Duration of recovery phase (s)	152	0·13–6·28	2·66	0·96
Total number of spikes on recovery phase	152	1–31	11	5·4
Duration of spike activity on recovery phase (s)	152	0·063–2·98	0·92	0·43
Average frequency of spikes on recovery phase (spikes/s)	152	2·8–169·4	15·4	8·9

Recovery phase

As mentioned above, several features mark the end of the initial phase and the beginning of the recovery phase. Chief among these are the greater regularity in the superimposed spikes and the partial repolarization of the membrane. The partial repolarization may be barely discernible (Fig. 2A), or may be as much as 50% of the maximum depolarization (Fig. 2D).

The spikes superimposed on the recovery phase initially occur at a fairly high frequency but decline in frequency over time. Generally, the interval between the first two spikes is only about $\frac{1}{8}$ the interval between the last two spikes. In only rare instances was the average spike frequency on the recovery phase greater than 30/s. During the time that the spikes are occurring on the recovery phase the membrane potential may be maintained at some more or less steady potential (Figs. 2A, B) or it may be slowly declining with time (Fig. 2C). In either case, following the end of the last spike on the recovery phase, the potential slowly declines to the resting level. The time required for the membrane potential to reach resting level following the end of the last spike ranged from 0·41 to 1·96 s.

There is no obvious correlation between the frequency of spikes on the recovery phase and the level of membrane depolarization during this time. There is also no evident correlation between the level of membrane depolarization and the time the last spike occurs and, in fact, in some instances the membrane is nearly completely repolarized at the time the last spike on the recovery phase is seen (Fig. 2D). Table 1 summarizes some of the characteristics of the recovery phase in these cells.

(C) *Intracellular current injection*

In order to gain further information about the membrane properties of the unipolar cell soma and about the potentials recorded from these cells, intracellular depolarizing and hyperpolarizing currents were applied to a number of cells. This was accomplished

through a bridge circuit which permitted simultaneous current injection and potential recording from the same electrode. Fig. 3 shows several examples of the results obtained from the application of such currents.

Hyperpolarizing currents applied during the recovery phase when spikes are occurring cause the number and frequency of the spikes to decrease (Fig. 3 A). If the intensity of the applied hyperpolarizing current is of sufficient strength, the spikes can be completely eliminated. In our experiments, the current required to decrease the number of spikes by one ranged from 0·6 to $1{\cdot}9 \times 10^{-9}$ A, and the current required to eliminate the spikes completely ranged from 1·0 to $4{\cdot}4 \times 10^{-9}$ A.

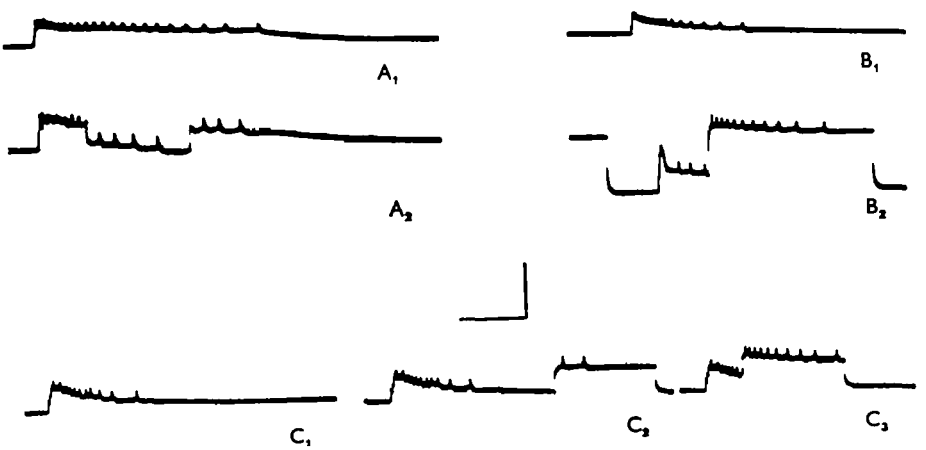

Fig. 3. Effects of intracellularly applied current on the spontaneous electrical activity recorded from unipolar cells. A_1, B_1 and C_1 represent normal activity. In A_2 a hyperpolarizing current of $1{\cdot}0 \times 10^{-9}$ A was applied during the spike activity on the recovery phase. A decrease in the number of spikes is evident. In B_2 a hyperpolarizing current of $4{\cdot}4 \times 10^{-9}$ A was applied just prior to a spontaneous burst. A resultant increase in amplitude of the initial phase occurs. In C_2 and C_3 a depolarizing current of $1{\cdot}0 \times 10^{-9}$ A is applied during the recovery phase. In both instances several spikes were induced by the current. Voltage scale, 35 mV; time scale, 500 ms.

Hyperpolarizing pulses applied at the beginning of an intracellular burst of activity result in an increase in the amplitude of the initial depolarization (Fig. 3 B). In such cases the number of spikes during the rising phase of the initial depolarization does not change, but the amplitude of these spikes is larger. The total duration of the initial phase is not affected.

Depolarizing currents applied during the quiet period between heartbeats, if of sufficient strength, induce small spikes similar in shape and size to those seen during the recovery phase. The number of spikes induced is dependent on the current intensity, but the size of the spikes is independent of current strength (Fig. 3 C_2). In our experiments the minimum current intensity needed to elicit a single spike ranged from 0·6 to $2{\cdot}5 \times 10^{-9}$ A, with an average of $1{\cdot}1 \times 10^{-9}$ A.

Depolarizing currents applied during the period of spike activity of the recovery phase cause the appearance of extra spikes during this phase (Fig. 3 C_3). In some instances as many as ten extra spikes could be induced. The number of extra spikes is dependent on the strength of the depolarizing current (generally, a current of greater than $0{\cdot}8 \times 10^{-9}$ A was required) and the time at which the current is applied. The

minimum intensity of current needed to induce such potentials during the spike activity of the recovery phase is less than that required during the quiet period—generally 20–30% less.

Depolarizing pulses applied during the initial phase result in a decrease in the amplitude of the initial depolarization, but there is no effect on the duration of the initial phase. If shortly before the onset of a spontaneous burst a depolarizing current is applied, no change in the interburst interval appears. In no case were we able to drive the cells into bursts of activity by the application of current, nor at any time could we elicit somal action potentials.

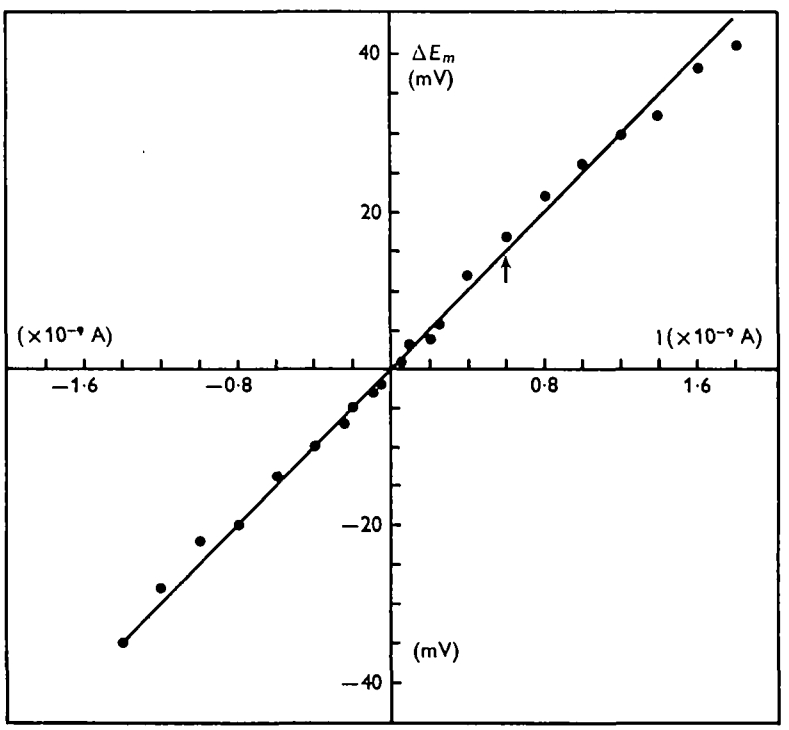

Fig. 4. Current–voltage relationship of the soma membrane of a unipolar cell. Note that the relationship is linear in both the hyperpolarizing and depolarizing regions. Arrow indicates the current intensity at which spikes were induced in the cell. The line was calculated by the method of least squares.

(D) *Specific membrane resistance, time constant and specific membrane capacitance*

In order to determine the total membrane resistance (R_{tot}), a number of depolarizing and hyperpolarizing currents were injected into the somata of the unipolar cells and the changes in membrane potential were recorded. In order to minimize electrode polarization the current polarity was reversed for each stimulation. Fig. 4 gives one example of the results obtained from such experiments. In this case R_{tot} had a value of 15·1 MΩ. Measured from 21 cells from six different animals, R_{tot} had a mean value of 28·1 MΩ (range 8·3–56·1 MΩ).

If it is assumed that the unipolar cells of the cardiac ganglion of *Limulus* are spherical in shape and have a diameter of 120 μm (Bursey & Pax, 1970), the surface area of the

membrane of the soma is approximately $4{\cdot}5 \times 10^{-4}$ cm^2. The specific membrane resistance for these cells is then 28·1 MΩ $\times 10^{-4}$ cm^2 or 12700 Ω.cm^2 (range 3750–26000 Ω.cm^2).

The specific membrane resistance of the unipolar cells of *Limulus* is much larger than that reported for many vertebrate cells, but is near that observed in some invertebrate nerve cells and in the photoreceptor cells of *Limulus* (for review see Coombs, Curtis & Eccles, 1959; Fuortes, 1958; Millecchia & Mauro, 1969). The high resistance we observe may be due to the thick capsule of connective tissue surrounding the unipolar cells (Bursey & Pax, 1970). Kusano (1966) determined that the resistance of the myelin sheath layer of 10 μm thickness which surrounds the giant nerve fibres of a *kuruma* shrimp is $2{\cdot}3 \times 10^5$ Ω. The capsules of the unipolar cells in *Limulus* are often thicker than 10 μm, and if the resistance of these is approximately one order of magnitude larger than that of the myelin sheath of the *kuruma* shrimp, the capsule resistance could be significant in measurements from the unipolar ganglion cells. Several attempts to measure the resistance of the capsule surrounding the unipolar cells in *Limulus* were made but proved unsuccessful.

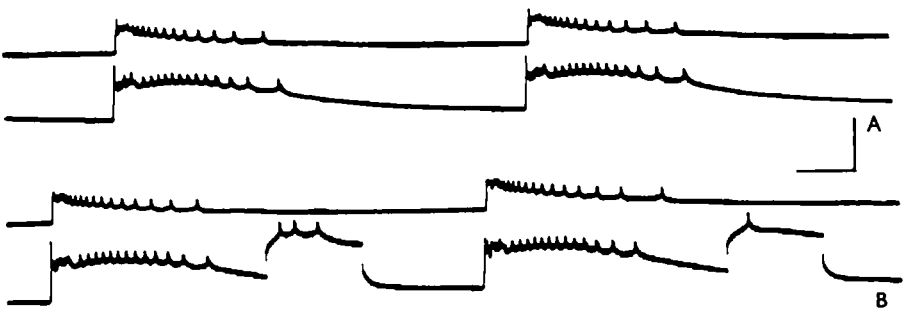

Fig. 5. Simultaneous recordings from a pair of unipolar cells in which current is applied intracellularly to one cell of the pair. In A is shown the normal spontaneous intracellular electrical activity. In B a current of $1{\cdot}0 \times 10^{-9}$ A was applied to the cell in the bottom trace. The current was sufficient to induce spikes in the stimulated cell, but no evident change in potential is seen in the cell in the top trace. Voltage scale, 30 mV; time scale, 500 msec.

The time constant was measured in these experiments from nine cells from four different animals and averaged 19·6 ms (range 4–40 ms). With these values of specific membrane resistance and membrane time constant the calculated specific membrane capacitance has a mean value of 1·54 μF/cm^2 (range 0·15–7·47 μF/cm^2).

(E) *Simultaneous recordings*

In order to examine the relationship between unipolar cells simultaneous records were made from pairs of unipolar cells. A total of 43 such pairs from 11 different hearts were examined. Fig. 5A shows one example of such recordings.

The activity of the two cells in such paired recordings is never strictly synchronized, there being a difference in the time at which activity begins in each of the cells at each heartbeat. The magnitude of the difference is highly correlated (r = 0·86) with the distance between the cells of a pair, so that cells near each other show a smaller time difference than do cells relatively far apart. For example, in one heart a pair of cells 8 mm apart had a time difference of 12 ms, while in the same heart a pair of cells 15 mm

apart had a time difference of 28 ms. On the average, the time difference increased by 3·0 ms for every millimetre increase in distance between the cells. The maximum time difference seen in any case was 56 ms and this was for two cells situated 17 mm apart.

The pattern of activity seen in each of the cells of a pair is not identical. Generally there is a difference in the total duration of activity, and no one-to-one relationship between spikes on the recovery phase can be discerned. Usually the total number of spikes on the recovery phase is different in each of the cells.

The above-mentioned recordings indicate that there is probably no 'tight coupling' between the unipolar cells, but in order to check this more closely simultaneous records were also made from pairs of unipolar cells while depolarizing or hyperpolarizing currents were passed into one of the cells of a pair. In these experiments a total of 17 pairs of cells from six different hearts were examined. Fig. 5B shows one example of such experiments. Neither depolarizing nor hyperpolarizing currents had any effect upon the non-stimulated cell. Even in cell pairs less than 100 μm apart, no evidence of electronic spread of injected currents into the non-stimulated cell could be seen. In cases where depolarizing currents elicited spikes in one cell of a pair, there were no extra spikes in the non-stimulated cell (Fig. 5B).

(F) *External stimulation*

The responses of the unipolar cells to external stimulation were also examined in a number of cases, a total of 48 cells from 18 different hearts being examined. In these experiments the external stimulation consisted of single or low-frequency pulses ($<$ 1/s; 5–10 V; 0·2–2·0 ms) applied through a suction electrode either to the cardiac ganglion anterior to the third pair of ostia or to side branches from the ganglion. The responses one sees with such stimulation are dependent upon a number of factors, such as the particular cell from which one is recording, the site of stimulation and the timing of the stimulus with respect to the normal rhythmic activity in the cell.

The most pronounced response is one in which a single stimulus applied to a side branch or the anterior end of the ganglion produced within a particular cell a complex burst of activity nearly identical to a normal spontaneous burst. The latency of this response averaged 125 ms (range 38–285 ms). This occurred only when the stimulus was given late in the normal interburst interval and not in every cell examined. Only 63% of the cells examined showed such a response, and not every site of stimulation produced the response in a particular cell.

In those cases where a complex burst was produced by a single stimulus the evoked response replaced the normal spontaneous burst; and, with stimulation frequencies slightly greater than the normal rhythm in the cell, the cell could be driven as a function of the frequency of stimulation. Figs. 6A and B give two examples of such driven responses. The maximum increase in burst frequency ever obtained was 25 beats/min, but usually averaged about 10 beats/min.

If the frequency of stimulation exceeded the limits that the cell could follow, then the burst frequency reverted to its normal frequency. In such cases the timings of the stimuli were such that each succeeding stimulus occurred earlier in the interburst interval. In some instances the stimuli became completely ineffective when they arrived earlier in the interburst interval. In other instances a partial burst of activity would

occur and this would become simpler as the stimuli were delivered earlier in the interburst interval. Eventually either they would consist of only one or a few simple potentials or they would completely disappear.

In many of the cells examined single stimuli did not produce complex responses capable of re-setting the normal spontaneous rhythm. Instead, single stimuli produced only partial bursts of the type described above and these partial bursts had no measurable effect on the on-going spontaneous bursts. The complexity of these bursts

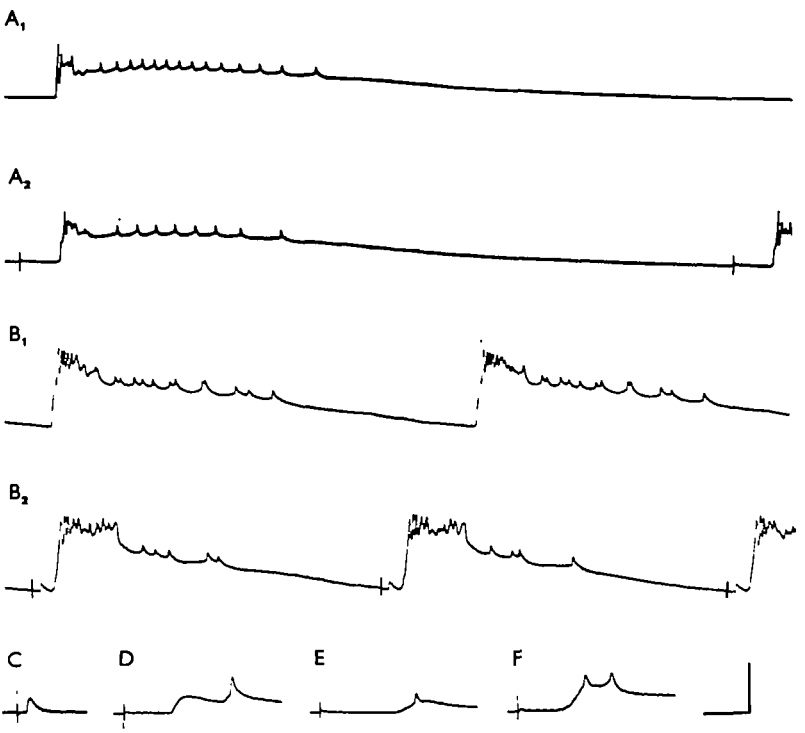

Fig. 6. Effects of extracellular stimulation upon the electrical activity of unipolar cells. A_1 and B_1 represent normal activity from two different cells. A_2 and B_2 are driven responses of these cells. B_2 shows a small pre-potential preceding the initial depolarization. C–F show a variety of non-burst responses from different cells elicited by single stimuli to the ganglion. Voltage scale, 40 mV, A, C, D, E and F; 20 mV for B. Time scale, 200 ms, A, B and C; 100 ms, D, E and F.

ranged all the way from bursts approaching in complexity those of the normal spontaneous bursts to merely one or a few simple depolarizations (Figs. 6D–F). At their simplest they appeared to be simple, slowly rising potentials on which there were sometimes superimposed one or a few small spikes. In some instances the complexity of the burst elicited was directly related to the timing of the stimulus, that is, more complex responses occurred late in the interburst interval, but in others only simple responses could be obtained regardless of the timing of the stimulus. The latency for these responses averaged 148 ms—nearly the same as for the driven responses described above.

In a few instances, in cells which could be driven by external stimuli, a small rapidly rising potential occurred prior to the beginning of the complex burst (Fig. $6B_2$).

Such small potentials were also sometimes seen as the only response to stimuli given to cells which could not be driven (Fig. 6C). These small, fast-rising potentials were generally easily distinguishable. Their latency was much less than that of the other responses described above—the average latency being only 17 ms (range 1–32 ms). Their rise time and decay time was also much less than the above-described responses. For these responses the time for 50% recovery averaged 27 ms (range 8–81 ms). By contrast, the 50% recovery time averaged 195 ms (range 87–277 ms) for the simple slowly rising potentials without spikes.

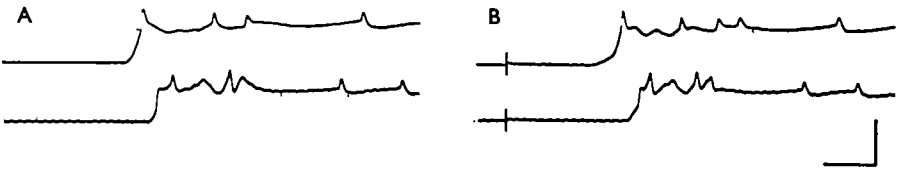

Fig. 7. Simultaneous recordings from two unipolar cells showing the results of an external stimulus applied to the ganglion at segment 3. A is the normal spontaneous activity and B shows the driven response. Upper trace shows activity from a cell located in segment 6; lower trace, from a cell located in segment 7. In both instances the anterior cell showed signs of activity before the posterior cell. Voltage scale, 30 mV; time scale, 100 ms.

To better determine the effect of stimulation on different unipolar cells, pairs of cells were simultaneously penetrated (a total of ten pairs of cells from four hearts) and their responses to extracellular stimulation were observed. In all cases if a driven response was evoked in one of the cells it was also present in the other. It was not possible to cause this type of response in only one cell of the pair (Fig. 7). Frequency limits and voltage thresholds for this driven response were identical in both cells of the pair.

Partial responses which did not re-set the rhythm were also observed in these paired-cell preparations. In some cases both cells responded to a stimulus with some type of complex activity pattern. However, it was also possible to evoke such a response in only one of the pair, the stimulus apparently having no effect on the other cell.

DISCUSSION

Spontaneous activity

The spontaneous activity recorded from the unipolar cells does not appear to have a simple single origin. From the experiments we have presented here it appears likely that this activity has at least two main components—one pre-synaptically evoked and another endogenous to the cell. Since from the intracellular current-injection experiments the soma of the unipolar cell appears electrically inexcitable, the activity recorded in the cell soma probably represents only attenuated electronically spreading activity.

One component of the activity which we feel is endogenous to the cell is the spike activity during the recovery phase. These spikes, small because of the electrical inexcitability of the cell soma, probably represent active responses confined to the processes of the cell. They appear to be endogenous to the cell because they can be eliminated by hyperpolarizing pulses or elicited by depolarizing pulses.

The sustained depolarization, both during the initial phase and the recovery phase during a spontaneous burst of activity in the unipolar cell, appears to be the result of pre-synaptic inputs to these cells. Depolarizing currents caused its amplitude to decrease, while hyperpolarizing currents caused its amplitude to increase. There was no blocking of activity to hyperpolarization, nor could intracellular bursts of activity be initiated by application of depolarizing currents. The spikes on the initial phase also appear to be pre-synaptically evoked. Their numbers were unaffected by injection currents, but their amplitudes were altered by such treatment.

Interconnexions between cells

In the cardiac ganglion of the lobster the interactions among neurones occur through synapses and electrical connexions (Hagiwara, Watanabe & Saito, 1959; Watanabe, 1958). In *Squilla*, on the other hand, transmission occurs solely by electrical connexions (Watanabe & Takeda, 1963).

There appear to be no connexions between the unipolar ganglion cells of *Limulus*. Neither spikes induced by depolarizing currents nor hyperpolarizing nor depolarizing currents which alter the activity in one cell of a pair had any measurable effect on the activity in the other cell of a pair. From these data it appears likely that the synchrony in the activity seen in these cells must be brought about by some common input to these cells rather than by some sort of direct interaction between them.

Function of the unipolar cells

The data we have presented here suggest, contrary to the conclusion drawn by Heinbecker (1933; 1936), that the unipolar cells in *Limulus* are follower cells and not pacemaker cells. Several indirect lines of evidence lead to this conclusion.

As mentioned above, at least part of the activity seen in these cells seems to derive from a pre-synaptic input—a situation one would expect in a follower cell and not in a pacemaker cell. Some of the results of external stimulation also support the idea that these cells are follower or motor cells. The small, short-latency, rapidly rising potentials sometimes seen either alone or prior to the production of a complex burst could be direct antidromic spikes in motor axons. Their similarity to the spikes on the recovery phase and their comparatively short latency support this possibility. A failure to observe these antidromic spikes in a majority of the cases of external stimulation is probably due to the failure to stimulate that side branch from the ganglion through which a particular cell sends its motor axon. Because of physical difficulties it was usually impossible to manœuvre the suction electrode over that side branch nearest the cell from which one was recording at a particular time.

The intracellular activity recorded from unipolar cells of the cardiac ganglion of *Limulus* is also similar in a number of respects to the electrical activity of the simple follower (Type A) cells in the crustacean cardiac ganglion (Bullock & Terzuolo, 1957; Hagiwara & Bullock, 1957; Watanabe, 1958). In *Limulus*, as in the Crustacea, the activity consists of a fast-rising initial depolarization followed by a slow repolarization, and superimposed on the depolarization there is a series of small spikes. As in these follower cells of the Crustacea, no pacemaker potentials have ever been seen in the *Limulus* unipolar cells.

In the *Limulus* cardiac ganglion, however, there are a number of features which make

it difficult to assign a definite function to these cells. All regions of the ganglion from segments five to eight are potential pacemaker regions, and under particular conditions the pacemaker region may be shifted from place to place (Bullock *et al.* 1943; Garrey, 1930, 1932). Thus, even though the cells from which we recorded do not show evidences of a pacemaker function at the time we were recording from them, they may under other circumstances have this function.

Another difficulty in trying to assign a function to these cells is that the cell soma itself appears to be electrically inexcitable, and the activity recorded is restricted to the axons of these cells. Pacemaker potentials, if present in these cells, might be unrecordable by our methods because they may be occurring at sites distant from the point of recording.

A further difficulty is pointed out by the results of external stimulation. In the majority of cases such stimulation produced in the unipolar cells a complex response which appeared to be produced by some sort of synaptic input to these cells. If these unipolar cells are the follower or motor cells of the ganglion, this production of complex, synaptically evoked responses indicates that there is a slightly greater complexity of structure than a simple pacemaker–follower cell interaction.

The results of the external stimulation could be explained if there were some sort of feedback system from the unipolar cells to the pacemakers which is activated by external stimulation. It is also possible that afferent fibres of some sort are carried in the side branches from the ganglion and that these, activated by external stimulation, exert effects on the unipolar cells directly or indirectly.

Obviously the experiments we have described here do not unequivocally demonstrate the function of the unipolar cells in the cardiac ganglion of *Limulus*. This will only become possible once the physiology of some of the other cell types within the ganglion has been examined and some of the interrelationships between the various cell types become known.

SUMMARY

1. The electrical activity of unipolar cells of the cardiac ganglion of *Limulus* has been examined with microelectrodes to determine the electrical properties and the functional relationship between unipolar cells.

2. Resting membrane potential in these cell averages −43 mV.

3. Correlated with each contraction of the heart there occurs a burst of activity which consists of a sustained depolarization of several seconds on which is superimposed a series of small 2–5 mV spikes.

4. Activity which occurs during the initial phase appears to be the result of presynaptic inputs to these cells. Spike activity during the recovery phase is probably endogenous to the cell from which we are recording.

5. The specific membrane resistance for the soma membrane averages 12 700 $\Omega \cdot cm^2$. The time constant has a value of 19·6 ms. The specific membrane capacitance measures 1·54 $\mu F/cm^2$.

6. External stimulation produces a number of different responses in the unipolar cells which are dependent upon a number of factors. These responses can be classified into at least three types: (*a*) a driven response similar to a spontaneous burst; (*b*) slowly rising potentials which may be antidromic spikes.

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