THE EFFECT OF EXTRACELLULAR STIMULATION ON THE UNIPOLAR CELLS OF THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS

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by

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ABSTRACT

THE EFFECT OF EXTRACELLULAR STIMULATION ON THE UNIPOLAR CELLS OF THE CARDIAC GANGLION OF *LIMULUS POLYPHEMUS*

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Jane L. Becker

The intracellular response of the unipolar cell in the cardiac ganglion of limulus to extracellular ganglion and side branch stimulation was examined to gain a better understanding of the functional role of these cells within the pacemaker ganglion.

Three types of responses were produced in the unipolar cells: (a) driven responses similar in appearance to a spontaneous burst, (b) slow non-driven responses of varying complexity and (c) rapid non-driven responses simple in form.

The driven response observed in any unipolar cell or cell pair is a reflection of the driving of the entire ganglion's rhythm.

Timing of the stimulation with regard to the normal burst frequency can determine if the response seen will be driven or slow. The delay times of both responses are alike, 120 msec. for the driven and 139 msec. for the slow. The stimulus pathway for both of these seems to be the same and probably goes through the pacemaker cells.

The rapid non-driven response is distinct and separate from both the driven and slow activity. The rapid response is not a reflection of a ganglion-wide activity. It has a short delay time (18 msec.) and is not affected by stimulus timing during the burst. It may be an antidromic spike.

All unipolar cells of a heart seem to share a common input and probably do not normally function in a pacemaker capacity.

ACKNOWLEDGMENTS

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INTRODUCTION

The heartbeat of Limulus polyphemus was shown by Carlson (1904, 1905) to be neurogenic. Until recently, all efforts to determine a location for the pacemaker within the cardiac ganglion concerned external electrical recordings. However, studies using intracellular electrodes are necessary for a complete understanding of the functional interactions among ganglion cells and of the physiology of the heart.

ANATOMY OF THE HEART AND CARDIAC GANGLION

The tubular heart of limulus is located just beneath the carapace and immediately dorsal to the gut (Patten and Redenbaugh, 1899). In the adult animal, the heart reaches a length of 10 - 17 cm. Eight pairs of ostia, located dorso-laterally, divide the heart into nine unequal segments.

The cardiac ganglion extends along the external mid-dorsal surface for almost the whole length of the heart. Between ostial pairs 3 and 6 the ganglion is widest, tapering anteriorly and posteriorly. Bursey (1969) has shown the ganglion to consist of a ventral cellular portion containing most of the ganglion cell somata and a smaller dorsal fiber tract containing mostly nerve fibers. Numerous branches, not constant in number or position from heart to heart, are given off from the ganglion in the area of each pair of ostia. The largest of these bundles of nerve fibers is found at the fifth ostia. At each of the remaining ostia, there are one or two branches larger than the rest. Some of the branches given off penetrate deep into the cardiac muscle. Other branches remain on the surface of the heart and form somewhat definite tracts known as the lateral cardiac nerves at the lateral edges of the heart. The lateral nerves do not contain ganglion cell bodies (Bursey, 1969).

Bursey (1969) has demonstrated the presence of six cell types within the cardiac ganglion. The large unipolar cells (120μ dia.) have their pigment granules concentrated in one large group and are encapsulated. They occur in all segments of the heart posterior to ostia 3, but are concentrated in segments 5 and 6. An average of 124 large unipolar cells are located in the ganglion. The spindle-shaped pigmented large bipolar cells (120μ x 90μ) are also heavily encapsulated, but have their pigment granules concentrated at both poles. They too occur throughout the segments posterior to ostia 3, while averaging only 55 cells per ganglion. Approximately 21 pigmented multipolar cells (60 - 80μ dia.) are located throughout the ganglion. They possess no distinct capsule and concentrate their pigment granules near the processes. The 40μ long pigmented bipolar cells are to be found in all segments of the heart. Like the large bipolar cells, they too are spindle-shaped and encapsulated. An average of 31 small bipolar cells are found in the ganglion. The non-pigmented multipolar cells average 20 - 30μ in diameter and are usually found in close association with the pigmented cells. Isolated groups of these cells are also found anterior to ostia 3. Large numbers of small non-pigmented cells 7 - 10μ in diameter are found to occur within the capsules of the large unipolar cells and around the non-pigmented multipolar cells.

ORIGIN OF THE HEARTBEAT

The neurogenic origin of the heartbeat was first demonstrated by Carlson (1904, 1905). He found that the integrity of the cardiac ganglion, rather than that of the myocardium, was necessary for the normal rhythmic synchronous contraction of all parts of the heart. Lesioning of the cardiac ganglion and its associated lateral nerves terminated the coordination of cardiac muscle contraction on either side of the cut. Sectioning of the cardiac muscle without disturbance of the ganglion had no effect on the heartbeat. Removal of the cardiac ganglion resulted in the cessation of contraction in the muscle.

Several investigators (Carlson, 1904, 1905; Rijlant, 1931; Edwards, 1920) have shown the automatism of segments five through seven to be greater than that of segments anterior or posterior to this area. This middle third of the heart also beats slightly earlier than the other portions and seems the normal pacemaker location within the ganglion. By various means of manipulation, including local heating or stretching, the primary pacemaker area can be shifted to other segments (Garrey, 1930). Thus, it appears that cells in segments three through eight have the potential for becoming pacemaker centers.

Heinbecker (1933, 1936) suggested on the basis of several observations that large cells are the pacemakers within the ganglion. Firstly, these cells are concentrated in the middle third of the heart where the cardiac rhythm seems normally to originate. The observed repetitive type of activity of the small ganglion cells indicated to Heinbecker that these cells are directly responsible for impulses causing the cardiac muscle to contract and do not have a pacemaker function. The fact that external stimulation of what Heinbecker calls the large cells could induce activity in the smaller cells also favored a pacemaker function for the large cells.

Intracellular recordings from the ganglion cells was logically the next step in a comprehensive study of the limulus heart. Bursey and Pax's (1970) study of the microanatomy of the cardiac ganglion made it possible to limit intracellular studies to one morphological and, thus presumably, to one physiological type of cell. Of the cell types recognized, the unipolar cell seems to me the best target for intracellular studies. There are several reasons for this choice. These large cells (120μ dia.), found in segments four through nine and concentrated in five and six, have a distinguishing pigment arrangement and are recognizable under the dissecting microscope. The fact that they are the only cells that protrude dorsally and laterally from the ganglion not only aids in their identification, but also makes them more easily penetrable.

The normal spontaneous burst pattern of the unipolar cells has recently been studied by Palese (1970) with the use of intracellular microelectrode techniques. A rapid depolarization to some peak value initiates the burst. There may be many to no small spike-like potentials visible on the rising phase of this initial depolarization. A rather rapid repolarization then occurs and is quickly followed by either a continued slow repolarization or a slight slow depolarization. Upon this slow depolarization or repolarization one sees superimposed several spike-like potentials. Following the convention of Palese _et_ _al_. (1970), the time from the start of the initial depolarization to the point where the spike-like potentials become regular in frequency will be designated the initial phase of burst activity. The time from the end of the initial phase to the time when the membrane returns to resting level will be termed the recovery phase. The division between the initial and recovery phases is also marked by the rapid repolarization of the membrane. The recovery phase itself consists of an active portion, during which the spike-like potentials are present, and a quiet portion lacking spikes. The interburst interval is measured from the beginning of one initial depolarization of the cell to the beginning of the next initial depolarization.

Palese (1970) demonstrated that the injection of current into the soma of one unipolar cell does not bring about any changes in other unipolar cells within the same heart. This suggests that there are neither electrical nor synaptic connections among unipolar cells. He could change the amplitude but not the number of spikes on the rising phase of burst activity. This indicates that these spikes are presynaptically evoked. Since the spike-like potentials superimposed on the recovery phase can be eliminated by the injection of a hyperpolarizing current or elicited by a depolarizing current during this phase, it is thought that this spike activity is endogenous to the cell being stimulated intracellularly. Palese also suggests that the large unipolar cell probably does not normally function in a pacemaker capacity, since at least part of the burst activity seen in the unipolar cell is presynaptically elicited by a ganglion cell type or types other than the unipolar.

If the unipolar cells do not play the role of pacemakers, it is possible that they are motor in function. One would then expect processes of these cells to be sent out to the myocardium. The large, presumably motor cells of the lobster cardiac ganglion have recently been studied from the standpoint of their branching within the ganglion. Hartline (1967) has traced the axonal processes of these cells by stimulating various nerves leaving the ganglion while recording along the length of the ganglion. By noting the change of the antidromic response to a monophasic conformation as it approached the inexcitable cell soma, he was able to single out a particular cell's process from the complex external record. By using the intracellular unipolar recording in place of the external ganglion record, this technique could be put to use in the study of the limulus ganglion.

It is the purpose of this study to investigate the output of the unipolar cell, to attempt through the use of external stimulation to determine if processes of unipolar cells leave the ganglion for the muscle and to gain a better understanding of the functional role of these cells within the cardiac ganglion of limulus.

METHODS AND MATERIALS

CARE AND MAINTENANCE OF ANIMALS

Mature male and female specimens of the horseshoe crab, *Limulus polyphemus* (carapace width 20 - 25 cm.), were used in all experiments. They were shipped periodically by air express from the Gulf Specimen Co., Panacea, Florida. The animals were maintained until use at 13 - 16°C. in a Dayno Co. Model 703 artificial sea water aquarium.

ISOLATION OF THE HEART

All experiments were performed on an isolated heart-ganglion preparation. Two cuts made through the dorsal exoskeleton just lateral to the heart were joined by anterior and posterior transverse cuts. The resulting rectangular piece of carapace overlying the heart was then lifted and dissected free of the underlying tissue. Removal of the internal extensor muscles of the opisthosoma, which cover the heart in the cephalothorax, fully exposed the heart.

A transverse cut through the cardiac muscle at the first pair of ostia allowed insertion of a glass rod (O.D. 7 mm.) into the entire length of the heart lumen. The rod was lifted and the tissue anchoring the heart in the pericardium was cut free from the preparation.

With the rod still in the lumen, the heart was placed in a paraffin chamber filled with artificial sea water ("Instant Ocean," Aquarium Systems, Inc.). Removal of the strands of connective tissue overlying the cardiac ganglion was accomplished under the dissecting microscope. The heart-ganglion preparation was rinsed several times with sea water prior to experimentation. Throughout the course of the experiment, the sea water in the chamber was changed approximately every 60 minutes. All experiments were run at room temperature (22 - 26°C.).

INTRACELLULAR RECORDING

Only unipolar ganglion cells were penetrated for intracellular study. These cells are distinguishable from the other cell types present in the cardiac ganglion (Bursey and Pax, 1970). Furthermore, their obvious protrusion from the ganglion and their large size made them easily penetrable for study with microelectrode recording techniques.

Microelectrodes were drawn out from Kimax glass capillary tubing (O.D. 0.8 - 1.2 mm.) with a Narishige microelectrode puller. The electrodes were filled under vacuum with methanol, then by diffusion displacement first with distilled water and finally with 3$\underline{M}$ KCl. Only electrodes of 10 - 30 Mohms resistance were used for the penetration of cells.

The microelectrodes were placed on a chlorided silver wire clamped to a Narishige micromanipulator, by which the electrodes were positioned. A large chlorided silver wire placed in the paraffin chamber served as a reference electrode. The microelectrode-chlorided wire unit was coupled to a preamplifier (Argonaut Model LRA 043 or WP Instruments Model M-4). The output of the preamplifier was fed into a Tektronix 502A Dual Beam Oscilloscope. Electrical activity was recorded from the oscilloscope screen by means of a Polaroid Oscilloscope C-27 or Grass C4-K Kymograph camera.

EXTRACELLULAR STIMULATION

In order to study the effects of external stimulation on the unipolar cells, a glass suction electrode was used. Kimax glass tubing (O.D. 4 mm.) was drawn out to an internal tip diameter of 400μ at one end. A length of plastic tubing pulled over the other end led back to a 35 cc. plastic syringe, the source of suction pressure. A 4 cm. length of #26 gauge silver wire soldered to an insulated copper wire was inserted through the plastic tubing around the opening made for the wire. The suction electrode was maneuvered with a Narishige micromanipulator.

The tip of this stimulating electrode was lowered to the point where it just touched the ganglion. Suction was applied until a firm seal, as visually determined through the dissecting microscope, was made between the tip of the electrode and the nervous tissue. Sites of stimulation were the ganglionic trunk in the mid-portion of segment three and areas approximately 2 mm. out from the trunk on side branches leaving the ganglion for the muscle. Side branches located at ostial pairs 4 through 8 and from both sides of the ganglion were used. Since the number and arrangement of side branches at any given ostial level varies from heart to heart, it is not possible to compare specific side branches in different animals. Side branches at the level of a particular ostium may be compared, their location being approximately identical in all hearts. For example, the results of stimulating any ostia 5 branches of different hearts could be compared.

DATA AND OBSERVATIONS

RESPONSES TO EXTRACELLULAR STIMULATION

The responses of unipolar cells to external stimulation were recorded intracellularly, and quantitative data were obtained for a total of 48 cells from 18 different hearts. External stimulation consisted of single or low frequency pulses (<1/sec., 5 - 10 V., 0.2 - 2.0 msec.) applied either directly to the ganglion in the middle of segment three or to side branches leaving the ganglion at the level of the various ostia.

Three types of responses could be produced in the unipolar cells by extracellular stimulation. It was sometimes possible with a single stimulus to cause a complex burst of activity nearly identical to the normal spontaneous burst of the cell being penetrated. This response could be made to follow stimuli given at a frequency slightly greater than the normal burst frequency of the cell and would replace the normal bursts. Such responses were termed driven. In many cases, single stimuli did not reset the normal spontaneous rhythm, but caused only partial bursts of activity. At times, these non-driven responses were simple, slow-rising potentials on which were superimposed one or a few small spikes. In contrast to these slow non-driven responses, small rapidly-rising potentials with no superimposed spikes were seen in some cells. Such responses were termed rapid non-driven responses. A total of 80 responses, including these three types -- driven, slow non-driven and rapid non-driven -- were obtained.

The type of intracellular response seen due to external stimulation depended upon several factors, such as the particular cell from which one was recording, the site of stimulation along the ganglion and the timing

of the stimulus with respect to the normal rhythmic activity in the cell. It was possible to evoke more than one type of response in any given cell, depending upon the site and timing of stimulation. Also, any given stimulus site was capable of eliciting differing responses in different unipolar cells.

DRIVEN RESPONSES

Of all the responses obtained as a result of external stimulation, 61% were of the driven type (Figure 1). This driven response was seen in 73% of the cells examined. However, it was not possible to stimulate all ganglionic side branches in turn while recording intracellularly from any given cell, due to difficulties in maneuvering the stimulating electrode. Therefore, it is possible that for cells not showing the driven response, a branch capable of driving the cell was not stimulated. In any given heart, a stimulus site that caused the driven response in one unipolar cell caused it in all the unipolar cells examined.

The driven response occurred only if the stimulus was given during the latter part of the quiet period. As mentioned above, the resulting driven burst in any given cell resembled the normal unstimulated burst pattern of that cell, but the burst frequency of the cell was increased to that of the stimulus being given.

In cells where complete records of several normal pre-stimulus, driven and post-stimulus bursts were taken, it was found that any changes in burst characteristics due to stimulation were usually slight and readily reversible with the cessation of stimulation (Figure 1B). Obviously, the interburst interval was shortened, as this change is implied in the concept of the driven response, where the burst is set to follow a stimulus frequency slightly higher than that of the cell. This decrease was taken up in the recovery phase of the burst, the initial phase remaining unchanged or increasing (average increase 44 msec.). There was usually a decrease in the number of spikes seen in the active portion of the recovery phase and an associated decrease in the length of the active portion. There was sometimes a few millivolts decrease from the normal amplitude and an increase in the rise time (average increase 20 msec.) of the initial depolarization. In some cases, the character of the rising phase of the initial depolarization was changed (Figure $1A_3$), with additional spikes appearing. The few changed characteristics returned to pre-stimulus conditions and the normal firing rate of the cell was resumed within two to four beats following the removal of the stimulating pulses.

The latency of the driven response following the stimulus averaged 120 msec. (range 40 - 192 msec.). This delay time seemed correlated to neither the cell position nor the stimulus site alone. However, when examining results from one stimulus site at a time, it was noted that for some sites the delay time increased with the distance between the cell and the stimulating electrode. Delay times and the distance of the cell from the point of stimulation were highly correlated (correlation coefficient, $r = 0.9696$) for stimulation applied directly to the ganglion in the middle of segment three. Side branches leaving the ganglion at the level of ostia 5 also showed this correlation ($r = 0.5664$), while branches leaving from ostia 4 and 6 did not. Data were insufficient for branches at other ostia.

The driven burst replaced the normally occurring activity and was pushed above the spontaneous rhythm in the cell as a function of the

Figure 1 The driven responses seen in two unipolar cells as a result of extracellular stimulation.

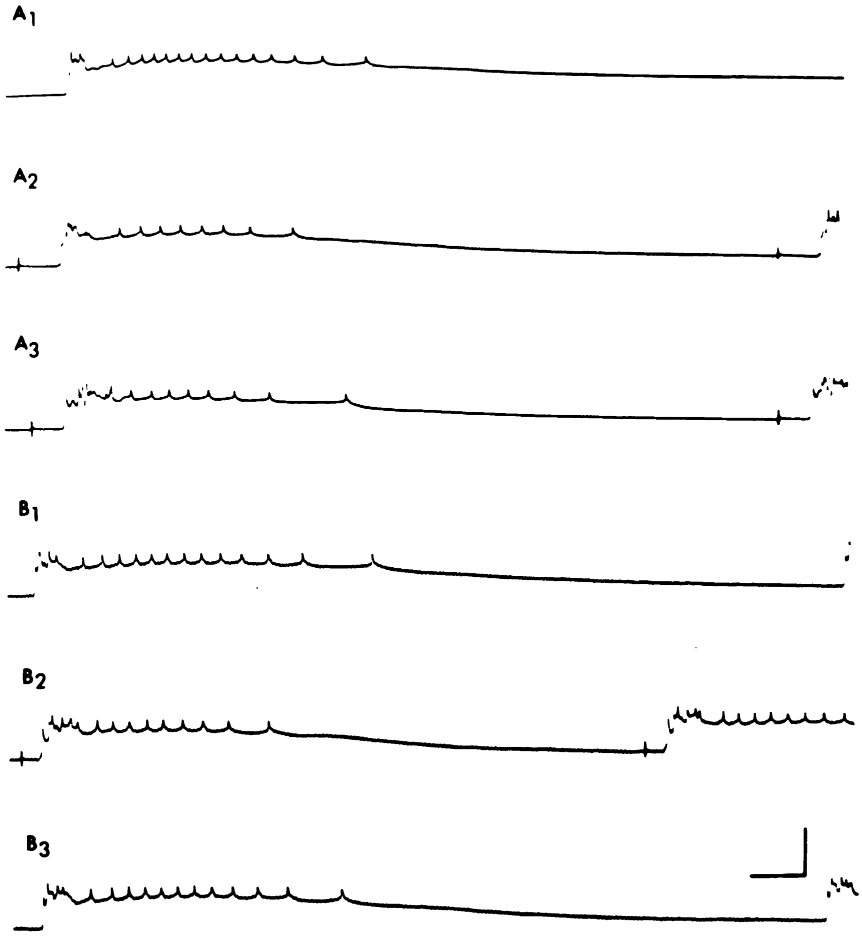
Figure 1A The normal and driven responses in a unipolar cell located in the middle of segment nine.

- A_1 The normal unstimulated burst activity.
- A_2 Stimulation of a side branch at ostia 5. Note that the burst patterns of normal and stimulated cases are similar. The biggest differences occur in burst length and number of spikes on the recovery phase (Stimulus -- 5V-2 msec.-3 beats/sec.).
- A_3 Stimulation on a different side branch (at ostia 6). Here the driven burst pattern differs considerably from the normal (Stimulus -- 5V-2 msec.-0.3 beats/sec.).

Figure 1B A comparison of pre-stimulus, driven and post-stimulus bursts in a unipolar cell in the posterior portion of segment 7. Stimulation is applied at a ganglion branch at ostia 5.

- B_1 The normal pre-stimulus burst pattern.
- B_2 The driven response. Note that the burst frequency is increased, the number of recovery phase spikes is decreased and both the active and quiet portions of the recovery phase are decreased in length (5V-2 msec.-0.37 beats/sec.).
- B_3 The burst pattern of the cell three beats after the cessation of stimulation. The burst has almost returned to the pre-stimulus pattern characteristics.

Voltage scale - 40 mV; Time scale - 200 msec.


A1
A2
A3
B1
B2
B3

frequency of stimulation. In order to see to what extent the cells could be pushed, the stimulus frequency was increased by small increments until consecutive stimuli were not able to cause the driven response. Thus, a frequency limit for the cell could be found. The maximum increase in burst frequency ever obtained was 23 beats/min. in a cell changing from 32 to 54 beats/min. Bullock et al. (1942) could increase the limulus heart frequency six-fold by stimulating the ganglion with a d-c input. A frequency value half way between the normal beat frequency and the driven frequency limit was set for each cell, and the stimulus intensity decreased until a threshold was reached for maintaining the driven response. It was not possible to maintain the driven burst at its frequency limit long enough for threshold determination. Below this voltage threshold, the stimulus was not sufficient to hold the burst to the stimulus frequency, and the cell reverted to its normal rhythm. The average threshold at this half-limit frequency was 4.67 V. (range 0.27 - 36.0 V.). There seemed to be no correlation among cell position, stimulus position, normal frequency, frequency limit, threshold and change in frequency due to stimulation. However, it was noted that a stimulus site causing the driven response in all the cells observed in any given heart tended to produce similar frequency limits and thresholds among cells, while greater differences in these measurements were seen for different stimulus sites recorded for one cell. Data obtained on the driven responses of paired unipolar cells indicates some possible reasons for the lack of relationships between driven frequencies and thresholds.

For some cells, stimulation of a particular stimulus site produced only the driven response. That is, if the stimulus was applied too early in the burst to elicit a driven response, no response at all was recorded, regardless of the stimulus intensity, and no change in the interburst interval occurred. In other cells, such a change in stimulus timing produced less complex responses on the background of the normal spontaneous burst. For some cells it was not possible to cause a fully driven response, but it was possible to partially drive them. That is, every other stimulus would cause a full, nearly normal looking burst, while the alternate stimuli produced a less than complete burst. The stimulus site was not related to whether a cell would show only driven or both driven and less complex responses. All the cells of any one heart reacted in only one of these two ways, indicating this to be a heart-wide phenomenon. Hearts responding with only the driven response were found to have significantly faster normal spontaneous rhythms (2.5 - 5% probability, Mann-Whitney U Test) than those which could respond with both driven and less complex responses during the burst.

SLOW NON-DRIVEN RESPONSES

The slow non-driven responses varied from activity almost as complex as the normal burst, having spike-like depolarizations superimposed upon a slow depolarization, to simple slow potentials lacking spikes (Figure 2A). The average peak amplitude was 15 mV, ranging from a low of 3.4 mV in the case of a spikeless slow potential to a high of 36.9 mV in a complex slow response. The slow response accounted for 30% of all responses obtained. Such responses were seen in 42% of the cells examined, both in those that were and those that were not capable of being driven.

The complexity of the response varied with the timing of the stimulus with respect to the normal burst rhythms. This could readily be seen when a stimulus frequency slightly more or less than the normal burst firing frequency was used. Then with each normal beat of the cell, the stimulus would occur at a different point in the burst pattern and produce responses of differing complexity. If the stimulus hit during the active portion of the recovery phase, a response was not detectable. Normal variability in the spontaneous occurrence of the spikes on this phase prevented the identification of any stimulus-evoked changes in spike timing that might have been present. However, the spike number was not altered. Stimulation applied during the early part of the quiet period while the cell was still greatly depolarized caused rather complex responses. As the unipolar cell moved closer toward the resting state, however, it became more difficult to elicit even a simple response. When caused by stimuli applied after this point of apparent unexcitability, responses became increasingly complex. The most complex responses were due to stimulation applied later in the interburst interval. However, sometimes the slow responses remained unchanged in complexity as a single spikeless slow potential regardless of the stimulus timing but increased in amplitude the later the stimulus was given in the burst. It cannot be said at this point whether the complexity of the response follows the excitability level of the impaled cell or of any cells having inputs to the unipolar cell or to a combination of these two possibilities. The relationship between stimulus timing and the complexity of the slow non-driven response is illustrated in figure 2B.

In those slow responses where spike-like depolarizations were superimposed upon the slow depolarization, these spikes closely resembled those seen in the active portion of the normal spontaneous burst for that cell. Thus, they may be produced in the same way, the stimulating pulse may follow the same pathway to the unipolar cell that is followed in spontaneous burst generation.

Figure 2 Slow and rapid non-driven responses.

Figure 2A Four examples of slow non-driven responses.

Figure 2B The effect of stimulus timing on slow response complexity. The cell is located in the middle of segment 6 and the stimulus was applied to the ganglion in mid-segment 3 (7V-1 msec.-single).

Figure 2C Examples of the rapid non-driven response.

C_1 In paired cells penetrated simultaneously, the rapid response is seen twice in one of the cells. For the stimulus given later in the interburst interval, a slow response can be seen in both cells. (Top trace - cell in mid-segment 6; bottom trace - cell in posterior segment 7; stimulus applied at ostia 6 branch, 10V-2 msec.-single).

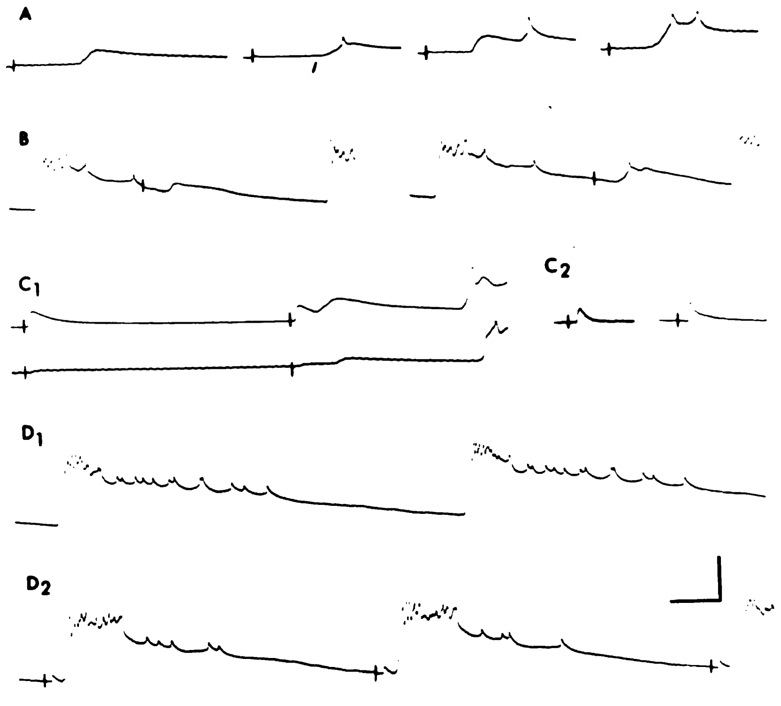
C_2 Two additional examples showing the short time course of the rapid response.

Figure 2D The rapid response in conjunction with the driven response.

D_1 The normal burst pattern.

D_2 The rapid response as a prepotential to the driven burst. (Cell in posterior segment 5; stimulus applied to ganglion mid-segment 3, 5V-1 msec.-0.7 beats/sec.).

Voltage scale - 20 mV (B, D), 40 mV (A, C); Time scale - 100 msec. (A, C), 200 msec. (B, D).


A
B
C1
C2
D1
D2

The delay time for the slow non-driven response approximated that for the driven response, averaging 139 msec. (range 70 - 426 msec.) versus the average driven delay of 120 msec. The only correlation between delay time and cell position was found when stimulation was applied anteriorly on the ganglion at segment three ($r = 0.8246$). Very little correlation was found ($r = 0.1440$) when a comparison of distance between the cell and stimulating electrode and the delay time was made for all slow responses due to side branch stimulation. Stimulation of branches at ostia 5 alone produced even less of a correlation ($r = 0.09$), in contrast to the results obtained from the driven responses.

When the slow response was evoked during a burst, the burst length was either increased, unchanged or decreased compared to that of the normal unstimulated burst. An increase in burst length was slightly more common, but the results in general were very inconsistent.

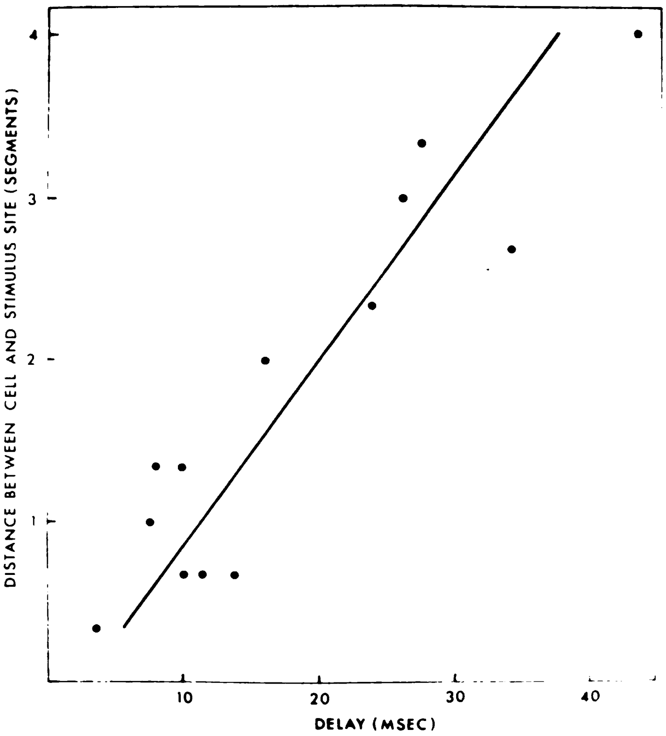
RAPID NON-DRIVEN RESPONSES

The rapid non-driven response was seen alone (7% of the total responses) or in conjunction with the driven response (12% of all driven responses) and in two cases with the slow response. The timing of the stimulus in relation to the cell burst frequency did not affect the complexity of the response. This response, then, may follow a different pathway than does the slow response. In cells which showed the rapid response along with the driven response, the small rapid depolarization preceded the driven burst by the same time interval in each beat. Whether occurring alone or in conjunction with other response types, the rapid response was characteristically a small (average depolarization 9.8 mV), fast rising and declining potential similar in appearance to those seen on the normal recovery phase (Figure 2C, D). The average latency of 18 msec. (range 3.5 - 43 msec.) was much shorter than those seen in either the driven (120 msec.) or slow non-driven (139 msec.) responses. The time needed for 50% recovery was measured for the rapid response and the slow spikeless response. For the rapid response, 50% recovery took 27 msec. (range 8 - 81 msec.), while that for the slow response averaged 195 msec. (range 87 - 277 msec.). These two responses, then, may be produced by different means. The rapid and slow responses may be compared within the same cell in figure $2C_1$.

When delay times for the fast response were compared to the distance separating the cell and the stimulating electrode for all stimulus sites used, a strong correlation ($r = 0.9230$) resulted (Figure 3). Responses in cells further from the stimulus site had a greater latency. A wide range of conduction times have been computed for the limulus cardiac ganglion -- 15 - 75 cm./sec. (Carlson, 1906a; Heinbecker, 1933; Rijlant, 1931). If the values obtained for the delay time are divided by the distance of the unipolar cell from the stimulating electrode, the values calculated are in the same area as for straight conduction through the ganglion. This may imply a direct route for the stimulus pulse from the stimulating electrode to the unipolar cell showing the rapid response.

This rapid response was most clearly distinguishable from the slow response by its smaller latency, much shorter rise and decay times and less complex form. It seemed separate from the driven response as well. In any given heart, a particular stimulus site would cause a driven response in all the unipolar cells examined in the heart. A site that caused the rapid response, however, did so in only one of the cells observed. For any given unipolar cell showing the rapid response, only

Figure 3 Relationship between delay time and the distance between the cell and the stimulus site for the rapid non-driven response. The line was calculated by the method of linear regression. The correlation coefficient for the relationship is r = 0.9230.


DISTANCE BETWEEN CELL AND STIMULUS SITE (SEGMENTS)
4
3
2
1
10
20
30
40
DELAY (MSEC)

one of the sites stimulated produced the response. This implies that the rapid response is a phenomenon of the individual unipolar cell. It must be remembered, however, that not all stimulus sites could possibly be stimulated for each cell investigated. Also, not all unipolar cells could be examined to see if a particular stimulus site could evoke the rapid response in more than one of them. These are limitations imposed by the experimental set-up, especially the delicate placement of the intracellular electrode.

SIMULTANEOUS RECORDINGS

To better understand the effect of stimulation on different unipolar cells, pairs of these cells were simultaneously penetrated (a total of 10 pairs from 4 hearts) and their responses to extracellular stimulation observed. As determined by Palese (1970), the normal spontaneous bursts in any two unipolar cells of the same ganglion are not identical. The more anterior of the pair usually begins its burst activity before the more posterior cell. There is a difference in the duration of initial and recovery phases, in the duration of active and quiet portions of the recovery phase and in the number and timing of spikes during the active portion as well. The three types of responses to extracellular stimulation were examined in the paired preparations.

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 response in only one cell of the pair (Figure 4A). Even in the cases where the driven response was only partial, with only alternate bursts being complete, the same type of response was seen in both cells. Frequency limits and voltage thresholds for the driven response were found to be identical for both cells of any pair, when measured as in the individual cells previously. Thus, the variation seen in these two measurements among individual cells was probably due to nothing more than variability in the state of excitability of the cell or cells affecting the unipolar cells. These data suggest a common input for the driven response in all the unipolar cells of a heart.

It was necessary to determine if the driven response seen in any two unipolar cells reflected a change in burst frequency throughout the heart, as the paired cell data seemed to suggest. Some investigators (Garrey, 1930, 1932 a, b; Samojloff, 1930) showed that the cardiac muscle could be accelerated due to direct stimulation of the ganglion at a point between the third and posterior segments. Driving of the entire heart was accomplished by Bullock _et_ _al_. (1942) with the application of a direct current input to the ganglion. They noticed the return within one beat to the normal beat frequency upon terminating the stimulation. Since they were observing the externally recorded ganglionic bursts, the subtle changes seen in the unipolar cell burst patterns which took 2 - 4 beats to reverse to the normal would not have been detected. The entire ganglion, then, would be expected to be affected just as the unipolar cells are.

In order to verify this, a unipolar cell was penetrated, the anterior portion of the ganglion freed so that it could be lifted onto platinum hook electrodes for recording and a stimulus applied to a side branch by means of a suction electrode. When a side branch was found to elicit a driven response in a cell, the cell's response was monitored simultaneously with the external recording of the anterior ganglion activity. The normal and driven responses for one such cell-ganglion recording are seen in

Figure 4 Responses to extracellular stimulation in a simultaneously penetrated unipolar cell pair (Top trace - cell in mid-segment 6; bottom trace - cell in posterior segment 7).

Figure 4A The driven response occurs in both cells in all cases. The inter-cell latency remains the same in pre-stimulus (A_1) and driven (A_2) bursts. (Stimulus applied at ostia 4 branch, 10V-2 msec.-0.32 beats/sec.).

Figure 4B The slow non-driven response is seen to be more complex in the top cell, which is closer to the stimulating electrode. (Stimulus applied at ostia 4 branch, 10V-2 msec.-single). Initial phases of spontaneous bursts immediately preceding and following the evoked slow response are shown so that the almost identical inter-cell latencies in these cases may be compared with the longer latency between the two slow responses. Due to limitations of space, two portions of the interburst interval are removed (2180 and 930 msec. respectively).

Figure 4C The rapid non-driven response was only seen in one of the two cells of any pair. Initial phases of the spontaneous bursts immediately preceding and following the evoked rapid response are presented to show that no change in inter-cell latency occurs in these bursts due to the rapid response (stimulus applied at ostia 6 branch, 10V-2 msec.-single).

Voltage scale - 40 mV; Time scale - 100 msec.

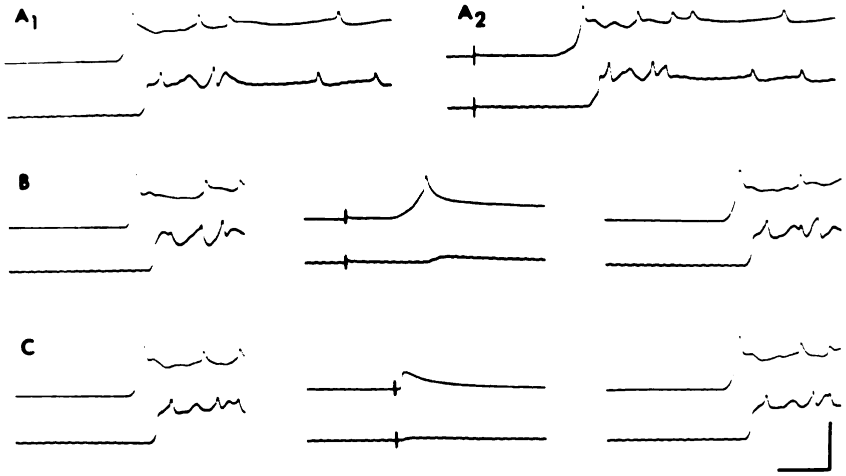

A1
A2
B
C

figure 5. The time relationship between the start of the initial depolarization of the unipolar cell and the beginning of burst activity in the ganglion remains constant under normal and driven conditions. A great deal of variability in the length of the externally recorded ganglionic burst can be seen. Therefore, a comparison of the normal and driven ganglionic bursts does not result in any clearly defined and consistent differences. However, it does appear that the stimulating pulse is affecting the cell or cells responsible for initiating the ganglionic burst, since the external record shows the entire ganglion to be reset by the stimulus.

The particular example given in figure 5A shows the unipolar cell burst leading that of the ganglion. This might seem to indicate that the unipolar cell is initiating the burst and is the pacemaker for the ganglion. However, this relationship may be due to two factors -- conduction time among the ganglion cells and location of the pacemaker. It is probable that primarily motor output is being recorded from the anterior ganglion, since no cell bodies are located there. Such output would generally be expected to lag behind burst activity recorded in cell somata if conduction time were the only factor to be considered.

The distance of the cell from the pacemaker area probably determines in part whether the cell or ganglionic burst will lead. This possibility was examined in intracellular and ganglionic burst comparisons for a total of 17 unipolar cells from 5 different hearts. It has been demonstrated (Carlson, 1904, 1905; Rijlant, 1931; Edwards, 1920) that the pacemaker is normally located in the middle third of the ganglion. For those cells in segments 7 and 8, presumably further from the pacemaker area than was the site of external stimulation, the cells always followed the ganglionic burst. The average ganglion lead in these cases was 53 msec. (range 24 - 100 msec.). Within any heart, however, there are variations in this cell-ganglion latency for cells located in approximately the same area. That is, the latency was not linearly related to the distance of these cells from the middle of the heart. Such variability might be explained by different conduction times between the pacemaker area and the various cells. For cells in the middle third (segments 4 - 6) of the heart, there was little consistency as to whether the cell or the anterior ganglion led. Some cells led the ganglion (maximum cell lead 28 msec.), some followed the ganglion (maximum ganglion lead 36 msec.) and some cells burst simultaneously with the anterior ganglion (Figure 5). Conduction time is probably the cause of variation among cells in this middle third of the heart, since the cells all lie within the general area of the normal pacemaker. Information concerning impulse transmission rates among cell types must be gained before the above data may be fully understood.

In the paired cell preparations, the time difference between the start of intracellular burst activity in the cells, which we may term the inter-cell latency, was compared between the normal spontaneous bursts and the driven bursts. In all cases, the inter-cell latency was increased in the driven condition. The normal inter-cell latency averaged 56.8 msec. That for the driven pair averaged 77.6 msec., almost a 40% increase over the normal. The pre-stimulus latency returned with the cessation of stimulation (Figure 4A).

Slow non-driven responses were also seen in the paired cells. In some cases, only one cell of the pair, the one closer to the stimulating electrode, responded with slow activity. Where both cells responded, the inter-cell latency for the slow response was always greater (by almost 50%)

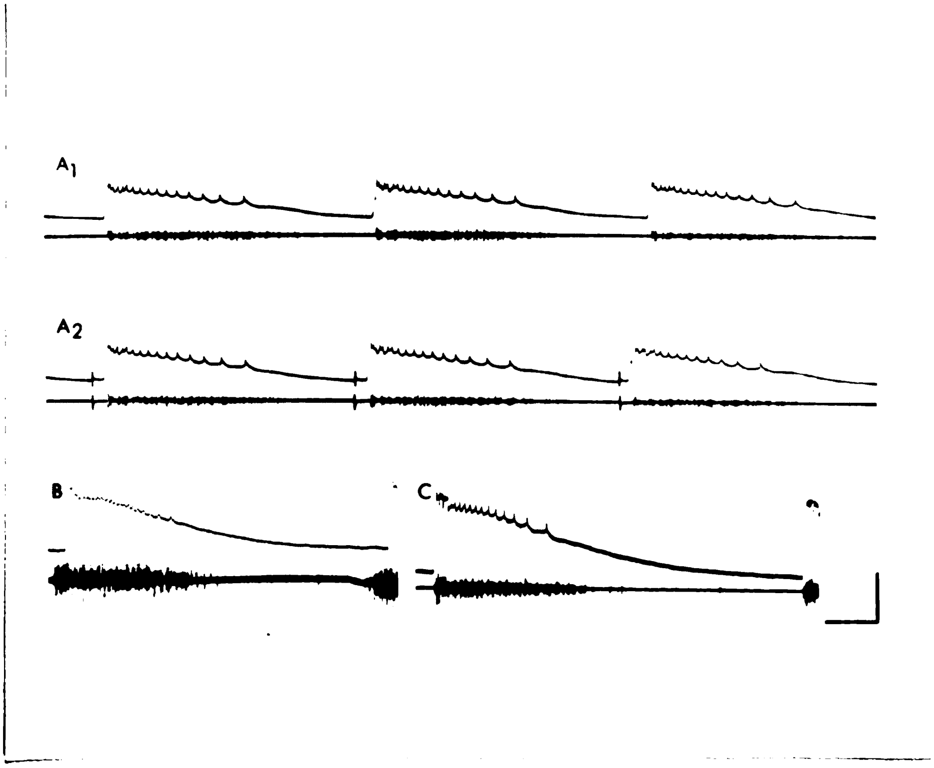
Figure 5 The time relationship between intracellular unipolar and external ganglionic recordings (External record taken from ganglion at level of ostia 2).

Figure 5A A cell located in the posterior part of segment 4 is seen to burst before the ganglion both spontaneously (A_1) and when driven (A_2) by stimulation applied to an ostia 5 branch (10V-2 msec.-0.45 beats/sec.). Note that under spontaneous or driven conditions the external record shows variation in length. Also, the latency between the intracellular and external records remains the same under both conditions, showing the whole ganglion to be driven.

Figure 5B The anterior ganglion burst leads the normal burst of a unipolar cell from segment 8.

Figure 5C No latency is seen between the external ganglion recording and the spontaneous intracellular recording from a cell located in segment 6.

Voltage scale - 20 mV (B, C), 40 mV (A); External recording - 125µV (A, B), 250µV (C); Time scale - 400 msec. (A, C & D).


A1
A2
B
C

than that seen between pre-stimulus bursts of the cells. The inter-cell latency for the spontaneous bursts following the slow response was not greatly changed from the normal, being either slightly greater or lesser than in the pre-stimulus condition. In such pairs, it was also noted that the slow response in the cell closer to the stimulating electrode was always more complex in nature than that of the more posterior cell (Figure 4B).

The rapid non-driven response was also seen. However, for any given stimulus site, only one cell of the pair showed it, always the one closer to the stimulus. This rapid response produced no change in the inter-cell latency of the following spontaneous beat (Figure 4C).

In a few cases, it was possible to maneuver the stimulating electrode to the middle of segment nine for direct stimulation of the ganglion. Few somata of any cell type are located in this area (Bursey, 1969). The driven response was again seen to occur in both cells of the pair, but caused the more posterior cell to fire first. With termination of the stimulating pulses, the situation reverted to the normal, with the more anterior cell bursting first (Figure 6A). When posterior ganglionic stimulation caused a slow response in each cell, the posterior cell again led, and the response of that cell was the more complex of the two. In the spontaneous burst following this slow response, however, the normal anterior lead was re-established (Figure 6B). These data suggest that the pacemaker center may be shifted, if only temporarily, by the application of stimulating pulses to the ganglion.

Figure 6 Effects of posterior (Segment 9) ganglionic stimulation on two unipolar cells viewed simultaneously (Top trace - cell in posterior segment 6; bottom trace - cell in posterior segment 7).

Figure 6A A driven response evoked in both cells.

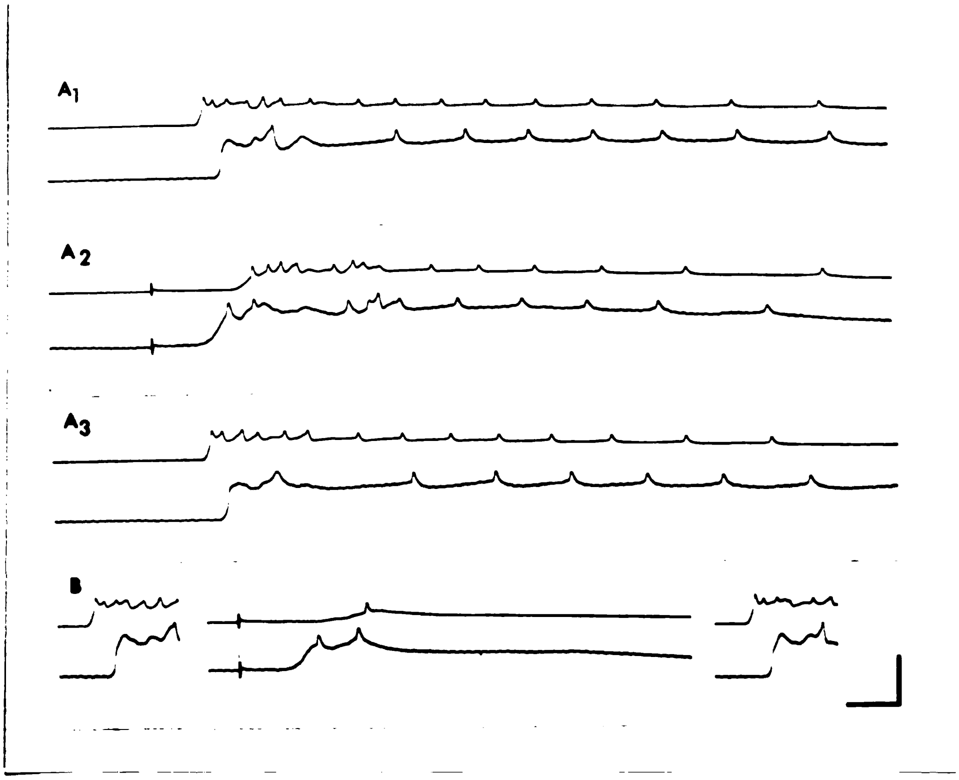
A_1 The normal unstimulated burst; more anterior cell leads.

A_2 The driven burst; more posterior cell, closer to stimulating electrode, now leads (stimulus - 10V-2 msec.-0.3 beats/sec.).

A_3 The spontaneous burst immediately following the cessation of stimulation. Note the return to the normal anterior lead and inter-cell latency.

Figure 6B Slow non-driven response evoked in both cells. Note that the cell closer to the site of stimulation exhibits the more complex slow activity and the shorter delay time. Initial phases of the spontaneous bursts immediately preceding and following the evoked responses demonstrate that the normal anterior lead and inter-cell latency are unchanged by the production of the slow responses.

Voltage scale - 40 mV; Time scale - 100 msec.


A1
A2
A3
B

STIMULUS POSITION EFFECTS

One factor that remained to be examined was the site of extracellular stimulation. Direct stimulation of the ganglion anteriorly always produced a response of some type in any cell. Stimulation of ganglionic branches was not as effective, only a certain percentage of the stimulus attempts resulting in a response. The highest percentage of successful stimulus attempts was seen for ostia 5 branch stimulation (58%). Ostia 6 branches (38%) were the next most productive stimulus sites, followed by branches at ostia 4 (33%), 7 (31%) and 8 (13%). It was difficult to free for stimulation the few small branches present at ostia 2 and 3 from the abundant connective tissue covering them.

Ostia 5 branches seemed equally effective in eliciting responses from cells in segments 5 through 9. Stimulation of ostia 4 branches was most effective in segment 5 cells, ostia 6 branch stimulation gave the greatest number of responses in cells located in segment 7 and, likewise, ostia 7 branches most effectively worked on segment 8 cells. However, responses for all these ostia were seen in cells located throughout the heart, and the arrangement of stimulating and recording equipment favored stimulation of side branches anterior to the cells being monitored. The few responses seen for ostia 8 branch stimulation were not concentrated in any one segment.

The types of responses seen were not necessarily related to the stimulus site. Both driven and non-driven responses could be evoked in cells throughout the heart by stimulation anteriorly on the ganglion or on ostia 4, 5 and 6 branches. In the case of the rapid non-driven response, ostia 6 stimulation caused rapid activity in segments 6 and 7 cells, while stimulation of branches at ostia 5 caused the response in cells of segments 5, 6, 7 and 8. Since relatively few rapid responses were observed, the segmental effects of ostia 5 and 6 branches may not be representative.

DISCUSSION

STIMULUS-EVOKED ACTIVITY

Stimulation causing the driven response does not seem to initiate new activity within the unipolar cells, but appears rather to reset the normal rhythm of the entire ganglion and, therefore, the rhythm of the pacemaker cell or cells. This is clearly shown in the paired cell preparations as well as in the comparison between intracellular unipolar and extracellular ganglionic recordings. The fact that certain side branches in a given heart will cause driven responses in all unipolar cells of that heart and that in paired cell preparations the frequency and threshold limits are identical for the two cells indicates a common input for all unipolar cells in a given heart. These data, plus the information that the unipolar cells are neither ephaptically nor synaptically connected (Palese, 1970) suggest that the unipolar cells are regulated and their bursts synchronized by another cell type within the ganglion which must be acting either as the pacemaker or as an interneuron between pacemaker and unipolar cells.

It appears that both the driven and slow activities must be caused by stimulation of the same pathway, since both may be evoked by the same stimulus site. The timing of the stimulus pulse determines which of the two responses will be seen. The pathway must include stimulation of the pacemaker cells, for the cardiac rhythm can be reset. The fact that stimulation of some hearts produced only driven, while stimulation of other hearts produced both driven and slow non-driven responses, may possibly be explained in terms of the refractory period of the pacemaker cells. Hearts that only could be driven had faster normal rhythms than those that could respond with either driven or slow activity. It may be that for the rapidly beating hearts, the refractory period of the pacemaker cells controlling the rhythm extends almost to the start of the next burst. In the more slowly beating hearts, however, the pacemaker cells may be ready to fire much sooner before the next spontaneous burst occurs.

The possibility of such a sustained refractory period seemed doubtful. However, Carlson (1907) demonstrated a refractoriness associated with the whole limulus heart. He stimulated the ganglion while recording the cardiac rhythm mechanically from the muscle. Stimuli applied to the ganglion at the beginning of systole in the muscle had no effect on the rhythm. If applied a fraction of a second before the muscle systole, it still had no effect, indicating that the ganglion itself was refractory. The muscle was shown to be refractory as well, by stimulating a portion of it where the ganglion had been removed. The ganglion refractory period remains in effect until toward the end of diastole when the next beat could be forced to occur earlier than usual. The shortening of the beat seen in this case is, in effect, the driven response seen here in the intracellular recordings as well as in the ganglionic recordings. The refractory period thus demonstrated is not absolute. With a sufficient increase in shock intensity, Carlson was able to get some response during this time. Therefore, the lengthy refractory period seen in the cardiac ganglion has to be defined in terms of greatly diminished excitability rather than in terms of inexcitability. Carlson (1906b) showed this refractory period in other invertebrates as well. He also indicated that automatism, such as the cardiac rhythm, need not be the result of refractoriness, although in several animals he found the more automatic parts of the heart to have shorter refractory periods.

The refractory period of the unipolar cells as well as that of the pacemakers may affect the variation in slow non-driven responses. Perhaps those evoked later in the interburst interval were more complex in form since they were further from the refractory period of the unipolar cell and/or pacemaker cells. Also, it could be that, with time, more pacemaker cells leave the refractory state allowing for a more intense input to the unipolar cell. Perhaps the side branches whose stimulation did not bring about the driven but only the slow response contained an insufficient number of fibers which affected the pacemaker cells.

The rapid non-driven activity seems to be a distinct response, while the driven and slow non-driven activities seen in the ganglion appear to be just differently timed manifestations of the same response. The latency, form and time course of the rapid response are quite different from the other two types of activity. This indicates that the rapid response follows a different pathway to the unipolar cell. Also, this response did not seem to be ganglion-wide in scope since it was never seen in both cells of a simultaneously penetrated pair. It would seem that the rapid response might be the result of direct stimulation of a branch of the unipolar cell axon, for the response latency seen in any cell is quite short and linearly related, regardless of the site of stimulation, to the distance between the cell and the stimulating electrode.

Palese (1970) has indicated that the small spikes intracellularly recorded from the unipolar cell soma during the recovery phase are probably spikes originating in the axon of the cell and spreading electrotonically back to the soma. He bases this upon the fact that he can elicit or remove these spikes by intrasomal injection of current of different polarities. The resemblance of the rapid responses to these spikes leads me to believe that they are also the result of electrotonic spread into the inexcitable soma, probably due to antidromic spikes caused by external stimulation. An antidromic spike is also suggested by the short latency plus the rather constant amplitude regardless of the stimulus timing. The small number of these responses obtained may be due to the limited possibility of matching any given unipolar cell among the 125 or so present in the ganglion with a side branch containing a process from the cell. It is also possible that some antidromic spikes might be blocked at points of bifurcation in the unipolar cell process. Such blockage has been seen in aplysia giant cells by Tauc and Hughes (1963).

The possibility of shifting the pacemaker center of the heart was seen in the posterior stimulation of the ganglion in the paired cell preparations. In the spontaneous bursts, as well as in anterior ganglionic or side branch evoked responses, the more anterior cell of the pair always led the more posterior one in firing. The reverse was seen in driven responses caused by posterior stimulation of the ganglion. Thus, the pacemaker center seems to have been moved closer to the more posterior cell of the pair. All the pacemaker cells must necessarily be interconnected since both unipolar cell firing and muscle contraction throughout the heart are synchronized. It may be that the accelerated rhythm of the pacemaker cell or cells being directly stimulated feeds back to control the normal pacemaker area. The rapid return to the pre-stimulus relationship between anterior and posterior cell burst timing shows this shift of pacemaker location to be transient and readily reversible. Bullock *et al*. (1943) found they could change the position of the acting pacemaker by the application of direct current at different points along the ganglion. They explained the change in terms of a polarizing field effect. Certain cells would be decelerated while others would be accelerated by the same imposed field. The accelerated cells would then take on the pacemaker role. The shorter pulse duration used in the present study may not have such a long lasting effect.

Shifts in the pacemaker region were not obvious with anterior ganglionic or side branch stimulation. However, it was seen that the inter-cell latency between paired cells increased in the driven response over that in the spontaneous unstimulated bursts. This change in the inter-cell latency may reflect a shift in the pacemaker position. Garrey (1930 a, b, 1932) has shown that direct ganglionic stimulation as well as local stretching caused a shift of pacemaker to the site of stimulation. If the side branch stimulation does indeed affect the pacemaker cells, as it seems to, it should be capable of shifting the pacemaker to some extent at least.

It also appeared in paired preparations that the site of stimulation was an important factor in the complexity of the slow response. Such responses in the cell closer to the stimulating electrode were always more complex. This may be due to a local condition, there being perhaps an increased number of inputs to the pacemaker cells closer to the site of stimulation.

PATHWAYS OF EXTRACELLULAR STIMULATION

Some important questions brought up by this study have not been answered, among them -- what fibers does the suction electrode actually stimulate and what pathways does the stimulus pulse follow to produce the response visible in the unipolar cells. The data collected suggests several possible answers to these questions.

Firstly, direct stimulation of the ganglion trunk most likely stimulates more than one type of fiber running in the fiber tract. It is reasonable to expect that the pacemaker cells themselves could be stimulated directly. This would explain the ease with which the driven response was obtained with ganglion trunk stimulation. It is probable that some unipolar cell processes might reach as far forward as segment three. Although this was not apparent in the cells observed, few unipolar cells anterior to segment five were investigated. In one case only was the rapid response, presumably an antidromic one, seen with anterior ganglionic stimulation and that for a cell located in segment five. Surely it is likely that other cell types within the ganglion were stimulated as well. However, a study of each of the additional cell types would be necessary before it could be said with certainty what the effect of stimulation of these would be upon the unipolar cell burst pattern. Also, ganglionic stimulation at the level of segment three is always more anterior than the pacemaker area of the ganglion. This explains the linear relation between distance separating the cell and stimulating electrode and delay time. More posterior cells are further from the pacemaker center and would be expected to have greater driven response latencies than more anterior cells.

Fewer numbers of fibers are being stimulated in the side branch than was the case with the ganglion trunk. Bursey (1969) found that the number of fibers located in the area of ganglionic stimulation averaged 80 - 100, while the largest side branch, located at ostia 5, contained 60 - 100. Most other branches along the length of the heart average about 20 - 60 fibers. The relative success of ostia 5 branch stimulation may be due to the large number and size of branches at that location. It may also be that fewer types of cells were directly stimulated at these side branches as all cell types would not necessarily send processes out to the cardiac muscle. It is believed, as a result of the rapid, apparently antidromic response seen, that the unipolar cells do send processes out through the side branches. Thus, there seems to be an antidromic pathway within the side branches leading directly to the unipolar cells.

It seems evident that processes of cells affecting the pacemaker cells of the heart must also be present within the side branches. There are several possible schemes that could explain the stimulation of the pacemaker. The scheme which seems least feasible is one in which a process of the unipolar cell with a feed back to the pacemaker cell is stimulated within a side branch. If this were the case, one would expect that stimulation of the unipolar cell would cause a change in the cardiac rhythm as was seen in the spiny lobster cardiac ganglion (Watanabe & Bullock, 1960). In these experiments, intracellular stimuli of long duration (200 msec.) were injected into the large ganglion cells which serve as followers in that ganglion. Hyperpolarizing current caused a slowing of the pacemaker rhythm of the heart, while depolarizing current speeded the heart. These effects were not seen in the limulus ganglion, where Palese (1970) performed a similar series of current injections into the unipolar cells with no effect seen on the cardiac rhythm. If such current moving orthodromically cannot reset the pacemaker rhythm, it seems less likely that a single, short duration antidromic pulse could do so. Information for other cell types is not sufficient to eliminate this feedback pathway for all cells of the ganglion. It seems likely that the resetting of the cardiac rhythm is not accomplished through the unipolar cells.

Another possibility would be for the pacemaker cells to send processes out through the side branches, allowing these cells to act, in part, as motor outputs to the muscle. Such an arrangement might cause asynchrony within the heart. Possibly this side branch pacemaker could cause muscle contraction before motor cells innervated by the pacemaker could affect the muscle. Information on transmission delays between cell types would be needed before this pathway could be proven correct or incorrect.

A more tenable argument can be made for the existence within the ganglion side branches of sensory fibers feeding back to the pacemakers information on cardiac muscle condition. The heart has already been shown to be sensitive to stretch (Garrey, 1930, 1932) and such a local sensory pathway might be involved in the speed-up of the heartbeat in response to stretching. Since the unipolar cells seem to be eliminated as sensory elements, it may be that the other cell types within the ganglion or perhaps cells whose somata are located within the myocardium may be acting in a sensory fashion. It has been theorized (Bullock et al., 1954; Maynard, 1955) that pacemaker cells might act as stretch receptors in the decapod cardiac ganglion by sending out processes to the myocardium. However, no experiments have actually been reported for the crustaceans to test this possibility. If any of the other nerve cells within or without the ganglion of limulus act as local sensory elements, it appears that they affect the pacemaker cells, since both the driven and slow responses appear to be mediated through the pacemaker.

If indeed sensory elements do feed information on local changes in myocardium condition (i.e. stretching) back to the pacemakers, it is likely that such pathways are not generally in use. An excess input, above the usual amount, that might reach the pacemakers via side branch sensory pathways might help explain the slight changes from the normal burst characteristics, frequency limits and threshold differences and changes in the inter-cell latency seen in the driven burst. If several inputs were to lead to different pacemaker cells within the local area of the side branch being stimulated, the timing of the pacemaker cells' firing could change slightly resulting in a change in unipolar cell burst properties. Mechanically applied stretch in the area of a side branch capable of driving the heart would demonstrate if changes similar to those caused by electrical stimulation could be mimicked. However, maintaining the microelectrode within the unipolar cell while stretching the musculature was not possible with the present heart preparation.

FUNCTION OF THE UNIPOLAR CELL

There are probably stronger arguments to be made for what the function of the unipolar cell is not than there are for what its function is. It does not seem, under normal conditions, to play the role of pacemaker within the cardiac ganglion. There are no connections necessary for pacemaker synchronization shown between unipolar cells, intracellular stimulation of unipolar cells does not modify in any way the cardiac rhythm (Palese, 1970) and unipolar cells receive modifying inputs from at least one other cell type. All of these factors appear to rule out a pacemaker function for the unipolar cell. Since unipolar cells do not affect the rhythm of the heart, they are probably not acting as sensory elements either.

The probability of unipolar cell processes reaching via the ganglion side branches to the myocardium hints at the possibility of a motor function for this cell type. Simultaneous monitoring of unipolar cells and muscle cells during unipolar cell stimulation must be done to test this possibility. A role as an interneuron cannot be ruled out for the unipolar cells either. They might connect pacemaker cells with ganglion cells acting as motor units.

It must be obvious at this point that neither the role of the unipolar cell nor the pathways producing the driven, slow and rapid responses can be learned until more is known of the physiological properties and anatomical arrangement of the processes of all the cell types within the ganglion. Recently, the intrasomal injection of procion dyes has enabled researchers to trace the processes of individual nerve cells (Stretton and Kravitz, 1968). Such treatment of the various cell types within the cardiac ganglion of limulus would demonstrate which cells had outputs to the cardiac muscle and the extent of area covered by the processes of each cell. In this way, vital anatomical data could be gained for each cell being studied physiologically. Physiological studies of each cell type similar to those already done on the unipolar cell are necessary as well. Several cell types must also be penetrated simultaneously and their interactions observed. The fact that none of the other cell types, with the possible exception of the large bipolar cell, is as easily recognizable or accessible as the unipolar cell may hinder this aspect of cardiac ganglion research. Nevertheless, such studies will be needed for a complete understanding of functional interactions within the cardiac ganglion of limulus.

SUMMARY

1. The intracellular response of the unipolar cell in the cardiac ganglion of limulus to extracellular ganglion and side branch stimulation was examined to gain a better understanding of the functional role of these cells within the pacemaker ganglion.

2. Three types of responses were produced in the unipolar cells: (a) driven responses similar in appearance to a spontaneous burst, (b) slow non-driven responses of varying complexity and (c) rapid non-driven responses simple in form.

3. The driven response observed in any unipolar cell or cell pair is a reflection of the driving of the entire ganglion's rhythm.

4. Timing of the stimulation with regard to the normal burst frequency can determine if the response seen will be driven or slow. The delay times of both responses are alike, 120 msec. for the driven and 139 msec. for the slow. The stimulus pathway for both of these seems to be the same and probably goes through the pacemaker cells.

5. The rapid non-driven response is distinct and separate from both the driven and slow activity. The rapid response is not a reflection of a ganglion-wide activity. It has a short delay time (18 msec.) and is not affected by stimulus timing during the burst. It may be an antidromic spike.

6. All unipolar cells of a heart seem to share a common input and probably do not normally function in a pacemaker capacity.

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