Studies on the Nervous Regulation of the Heart Beat in Decapod Crustacea

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ABSTRACT The effect of electrical stimulation of cardioaccelerator and cardioinhibitor nerves on the mechanically recorded heart beat of crayfish was studied. Similar experiments were performed with the lobster, *Homarus americanus*.

Quantitative relationships between uni- and bilateral accelerator and/or inhibitor nerve stimulation and the resulting change in frequency and amplitude of the heart beat were established.

With increasing frequency of stimulation the accelerator nerves show a relative decrease in their action, while that of the inhibitor nerves increases. It appears that left and right regulator nerves have synaptic contacts at the same areas of the postsynaptic cells within the heart ganglion. Similar results are obtained whether all impulses arrive over one, or over the other, or over both accelerator (or inhibitor) nerves; the resulting acceleration or inhibition depends strictly on the number of accelerator, or inhibitor impulses arriving at the ganglion.

The ganglion cells can adapt to the inhibitor action. This is shown to be a postsynaptic phenomenon. Adaptation to accelerator stimulation is virtually absent.

Characteristic after-effects of the accelerator and inhibitor action were observed and quantitatively evaluated.

The interpretation of the results is based on the assumption of chemical transmitter substances. It is concluded that the accelerating transmitter decays slowly while the inhibitory transmitter is inactivated relatively rapidly.

INTRODUCTION

The heart of decapod crustacea has been of considerable interest to physiologists because of the neurogenic origin of its beating and because of its peculiar pharmacology which differs so strikingly from that of the vertebrate heart. It is now well established that the decapod heart contains a ganglion, consisting

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of very few nerve cells, which controls the rhythmic contractions of the heart muscle. It is equally well known that the heart beat is regulated by the central nervous system through accelerator and inhibitor nerve fibers, and one generally assumes that these cardioregulator nerves act on the heart ganglion rather than on the heart muscle. The pertinent literature has recently been reviewed by Krijgsman (10).

To the general physiologist as well as to the neurophysiologist, the heart ganglia of crustacea offer a particular challenge; they consist of a definite, small number of neurons, each of which can be identified, and all can be assumed to interact in much the same way that we can assume neurons of any central nervous system interact. The potentiality of preparations of isolated crustacean heart ganglia for the study of mechanisms of nervous integration was first recognized by Welsh and Maynard (18), and subsequently utilized by Maynard (12–14), Bullock, Cohen, and Maynard (2), Hagiwara and Bullock (8), Bullock and Terzuolo (3), and Terzuolo and Bullock (16). With the exception of the first, all these investigations used hearts of the spiny lobster, *Panulirus interruptus*. The results indicate that four of the nine ganglion cells stimulate the other five by rhythmically occurring bursts of action potentials. The five large cells, called followers or motor neurons, activate the heart muscle. The motor cells also influence the four "pacemaker" cells by way of collaterals. These four small cells have long dendritic processes extending into the heart muscle. The cardioregulator nerves which enter the heart from left and right, consist on each side of two accelerator fibers and one inhibitor fiber (see Maynard (13)). Terzuolo and Bullock (16) conclude that certain of the follower neurons of the ganglion are directly affected by the accelerator fibers while the others are only indirectly influenced by the increased activity of the pace-maker cells resulting from stimulation of the accelerator neurons. The inhibitor fibers apparently have endings on all ganglion cells, but their stimulation leads to depolarization of cells 1 and 2 of the follower neurons, while it causes hyperpolarization and consequent inhibition of burst formation in the other cells. For a detailed account of the electrophysiological behavior of the heart ganglion cells the reader is referred to the original papers.

In spite of these extensive studies on isolated heart ganglia and on the activities and responses of individual ganglion cells, very little is known about the integrated response to the action of regulatory nerves of the heart ganglion and the resultant effects on the heart beat. The best data and discussions available in the literature are those given by Wiersma and Novitski (19) with regard to the crayfish heart and of Maynard (11) concerning *Palinurus*.

There are, however, only incomplete records of the relationship between frequency of stimulation of regulator nerves and extent of the resulting acceleration or deceleration of the heart beat. Quantitative relationships between

the effects of bilateral and of unilateral stimulation of accelerators and/or inhibitors have not yet been established, and information concerning the interaction of accelerator and inhibitor fibers of ipsilateral and contralateral cardioaccelerators and cardioinhibitors is lacking.

The present paper is an attempt to establish the quantitative and qualitative aspects of the nervous regulation of the heart beat. Particular consideration was given to the following questions: (1) How does the response vary with different frequencies of stimulation of the regulator fibers? (2) Does the action of accelerators and inhibitors result in facilitation? (3) How is the rate of facilitation related to the frequency of stimulation? (4) Does the heart ganglion show adaptation to continued action of regulator nerves? (5) What are the after-effects of stimulation of regulator nerves? (6) If it occurs, does the adaptation process alter the after-effects? (7) How do ipsilateral and contralateral accelerators and/or inhibitors interact and can adaptation and after-effects sum in the case of alternating stimulation of inhibitor and accelerator fibers? (8) Can the results be interpreted as indicative of chemical transmission of nerve impulses from regulator fibers to ganglion cells?

Alexandrowicz (1) showed that the organization of the heart ganglion of the crayfish (*Astacus fluviatilis*) is very similar to that of the lobsters (*Homarus* and *Palinurus*), the major difference being the number of ganglion cells: in the crayfish there are (probably) eight small and eight large cells. In view of the fact that the previous study of Wiersma and Novitski (19) was done on the crayfish heart it seemed profitable to use again a crayfish as experimental animal. Occasional experiments on hearts of lobsters (*Homarus americanus*) have shown that the observations on crayfish hearts apply very well also to the hearts of other decapods.

The experiments which will be described in detail below consisted of electrical stimulation of cardioregulator fibers and mechanical recording from the perfused, intact heart. The interpretation of the recordings obtained was based on the following assumptions (see also reference 19): (1) Each heart beat is caused by a brief burst of nerve impulses sent out from the ganglion. (2) The amplitude of the beat is proportional to the frequency of impulses. (3) The duration of the beat is proportional to the duration of the burst.

Methods

The experimental animals used were: *Pacifastacus leniusculus* (Dana), *Orconectes virilis* Hagen, and *Homarus americanus* L. The phenomena to be described were observed in all three species of decapods, but the majority of experiments were performed using *Pacifastacus leniusculus*. Unless otherwise stated, the results described are those obtained with this species.

Pacifastacus were obtained from the Columbia River near Portland, Oregon, *Orconectes* from Oshkosh, Wisconsin. The crayfish used were almost exclusively males

having a body length of 4 to 5 inches. They were kept in a concrete tank with shallow, running fresh water. *Homarus* were received by air freight from the Atlantic coast of Maine. They were all males with a body length of 10 to 12 inches.

Crayfish and lobsters were prepared in the following way: The cephalothorax was opened along the cervical groove and eviscerated. The abdomen was completely removed and the thoracic flexor muscles of the abdomen were cut out. The ventral nerve cord was destroyed by pushing a red hot needle into the "nerve canal." The pericardium was left intact but was pierced by a glass cannula frontolateral of the heart. The prepared cephalothorax was mounted in an upright position. By way of the cannula the pericardium was perfused at a rate of 2 ml./minute. The perfusion fluid was pumped out by the heart through the arteries which were cut just outside the pericardium. The perfusion fluid dripped from the inner dorsal surface of the carapace and could be collected if desired. For crayfish a perfusion medium patterned

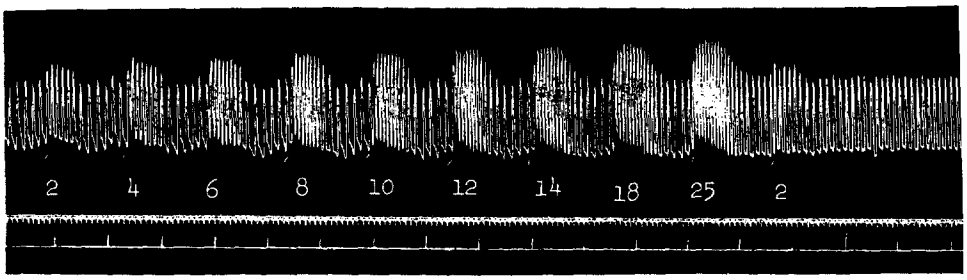


FIGURE 1. *Pacifastacus leniusculus*. Perfused heart. Record of heartbeats during serial stimulation of left cardioaccelerator nerve. Upper time base, seconds. The figures indicate the number of stimuli per second applied to the nerve.

after Van Harreveld's (17) solution was devised which had the following composition in mM: NaCl 190.0, KCl 5.4, $CaCl_2$ 13.5, $MgCl_2$ 2.6, $NaHPO_4$ 5.0, and Na_2PO_4 5.0. The pH was adjusted to 6.6 using drops of 0.1 N HCl or NaOH. The crayfish saline medium described by Elliott and Florey (4) was found to have rather adverse effects on the heart. This is due to the content of tris-buffer. The *Homarus* hearts were perfused with filtered sea water.

All experiments were carried out at a room temperature of 25°C. The perfusion media were in equilibrium with this temperature. The flow rates were kept constant, using Mariotte bottles.

Micromanipulators were used to guide pairs of fine platinum electrodes and to gently press them against the exposed cardioregulator nerves a few millimeters from their point of origin from the nerve cord. The position of these nerves in the crayfish has been adequately described by Wiersma and Novitski (19). It is rather similar in the lobster. While the inhibitor nerves have a superficial course the accelerators have to be exposed by cutting away those layers of the thoracic flexor muscles beneath which they lie.

Electrical stimuli in the form of square waves were provided by two stimulators,

American Electronics Laboratories, Model 104-A. The instruments were usually set to produce trains of 5 sec. duration at 10 sec. intervals, of pulses 1 msec. long of a potential of 1 to 5 volts. The frequency of the stimuli was varied as desired. This mode of stimulation will be referred to as *serial stimulation.*

The heart beats were recorded with a balanced, light heart lever on a smoked drum of a slow motion kymograph. For this purpose a small heart clamp was affixed to the frontomedial portion of the pericardium or to the ventral wall of the heart itself. Signal magnets were used to indicate the duration of stimulation. Due to certain imperfections of the recording the signal marks were replaced by white lines or timer traces during the preparation of the illustrations for this paper.

The results reported in this paper are derived from over 200 experiments carried out through a period of 2 years. No consistent seasonal variations were noted.

RESULTS

Cardioacceleration

Stimulation of the cardioaccelerator nerve of one side only with a single shock shortens the interval between the preceding beat and the one immediately following the stimulus. Repetitive stimulation with frequencies of 0.5/sec. or 1/sec. thus causes an irregular heart beat, the total number of beats per unit time being larger than before or after the stimulation sequence. Stimulation with frequencies from 2/sec. upward causes a smooth acceleration and an increase in amplitude of the heart beat. The inotropic effect is very pronounced in *Pacifastacus* but in *Orconectes* it is often small and inconspicuous. The chronotropic effects of accelerator stimulation are very similar in both species.

With increasing frequency of stimulation there is a graded increase of the (chronotropic and inotropic) response which reaches a maximum with stimulation of about 30 to 40 pulses/sec. No further acceleration results with higher frequencies and the increase in amplitude may be less than that produced by stimulation frequencies below 30/sec. Examples of the effects of serial stimulation of the accelerator nerve are shown in Fig. 1.

Particularly in *Pacifastacus* hearts the inotropic action of the accelerator nerves shows definite facilitation. Maximum amplitudes are reached several seconds after onset of stimulation. The higher the frequency the later is the maximum achieved. The longest facilitation times have been recorded during stimulation with 25 to 35 pulses/sec. and amount to 10 to 15 seconds. Maximum acceleration is usually reached within the first 3 seconds of accelerator action; this means that the second diastole after the onset of stimulation is commonly the shortest.

During prolonged accelerator action there is very little adaptation. In fact, if adaptation occurs at all it takes place during the first 3 to 5 heart beats, but even then it is minimal. With the exception of the first few beats which may or

may not be of a higher frequency, the acceleration remains constant even through stimulation periods as long as 1 or 2 minutes.

Only in "aged" preparations or in cases in which supramaximal frequencies of stimulation are used, does a slight decline of the response occur during prolonged excitation.

The relationship between frequency of accelerator stimulation and the frequency of the accelerated heart beat has been determined in a series of experiments in which the nerves were stimulated for 10 or 15 second periods at intervals of at least 1 minute. The results obtained from *Pacifastacus* and from *Orconectes* hearts are quantitatively similar. A representative and typical series of measurements is represented graphically in Fig. 2.

If the accelerator nerve is stimulated with frequencies greater than about

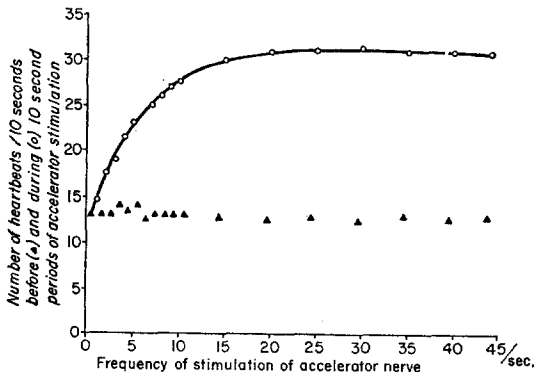


FIGURE 2. *Orconectes virilis*. Perfused heart. Relationship between heart rate and frequency of stimulation of right cardioaccelerator nerve.

5/sec. the chronotropic and inotropic effects always outlast the period of stimulation. The duration of this after-effect clearly depends on the frequency of the preceding stimulation. This can be particularly well studied in slowly beating hearts. The duration of the after-effect is defined as the number of seconds between the end of accelerator stimulation and the time at which the heart beat has returned to a frequency equal to that exhibited just prior to the stimulation. It is interesting to note that the duration of the after-effect is independent of the duration of the preceding stimulation, provided this lasted for a minimum of about 10 seconds. The relationship between duration of stimulation and duration of after-effect is indicated in Table I.

The relationship between frequency of preceding stimulation and the duration of the after-effect is typically represented by Fig. 3. The same graph also relates the duration of the after-effect to the direct response of the heart to accelerator stimulation. The two curves shown in Fig. 3 appear to be very similar. The fact that the maximum of the after-effect curve is to the right of the

curve which represents number of heart beats is no coincidence. Maximum after-effects were usually found after stimulation with about 40 pulses/sec. The after-effects of stimulation frequencies higher than 40 to 50/sec. are shorter and variable.

When the accelerator nerves of both sides are stimulated, their effects sum. Thus, if both are stimulated at a frequency of 5/sec. the resulting acceleration and increase in amplitude equal that produced by stimulation of one accelerator with 10 pulses/sec. The time course of facilitation is equal for both modes of stimulation. This behavior has been ascertained in *Pacifastacus* as well as *Orconectes* over the whole range of acceleratory responses (see also Fig. 4). When stimulation is applied to one accelerator for 10 seconds and 5 seconds

TABLE I

Duration	Stimulation	Duration of after-effect		
		Heart 1	Heart 2	Heart 3
sec.	*pulses/sec.*	*sec.*	*sec.*	*sec.*
2	5	5	7	10
5	5	15	18	20
10	5	20	25	25
15	5	20	27	25
30	5	18	25	27
60	5	20	27	25
2	10	5	15	6
5	10	37	43	40
10	10	38	47	43
15	10	41	47	45
30	10	40	47	42
60	10	42	46	45

after its onset the other is stimulated also, a stepwise acceleration and amplitude change occur, as shown in Fig. 4*a*. The same result is obtained if during a 10 second period of stimulation of one accelerator the frequency is suddenly raised (Fig. 4*b*). It appears that the heart ganglion responds simply to the number of accelerator pulses per unit time, regardless of the path on which they arrive. This is borne out also by the fact that the after-effect of accelerator stimulation is about equal whether all pulses arrived from one side or whether some arrived from the left and the others from the right side (Table II).

Cardioinhibition

Stimulation of the cardioinhibitor nerve of one side leads to slowing or complete diastolic arrest of the heart beat, depending on the frequency of stimulation. Serial stimulation in a large number of preparations has shown that complete stoppage is achieved with frequencies between 25 and 40/sec. and

higher. The critical frequency is remarkably constant in each individual case and little variation was found even during prolonged experiments, lasting over several hours. As with accelerator action, the effect on frequency and on amplitude varies independently. The frequency changes are always more pronounced. Records of the effects of serial stimulation of one inhibitor nerve are shown in Fig. 5. The relationship between frequency of stimulation and the induced decrease in the frequency of the heart beat is graphically represented in Fig. 6.

Facilitation of the inhibitor action is quite obvious (see Fig. 5), but it can also be seen that it proceeds rapidly. Facilitation periods were never found to exceed 2 seconds, and the maximum effect is established within the period of 1

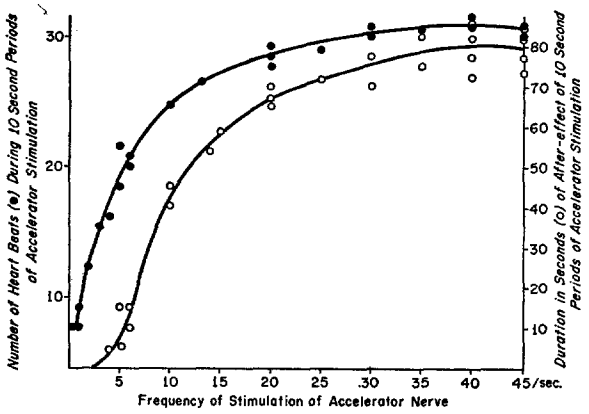


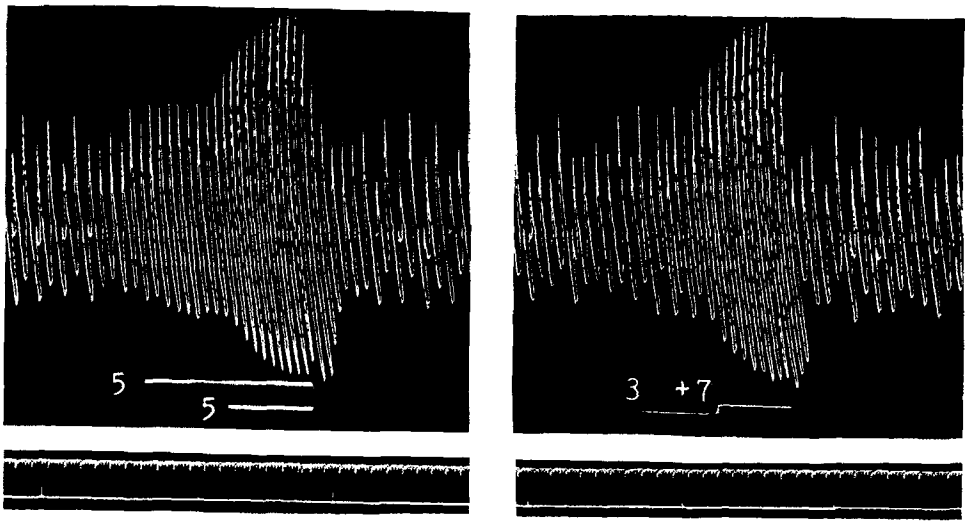
FIGURE 3. *Pacifastacus leniusculus*. Perfused heart. Relationship between frequency of stimulation of right cardioaccelerator nerve and heart rate and accelerator after-effect. Note that detectable after-effects begin only at frequencies above 5/sec., while marked acceleration results already with stimulation frequencies between 1 and 5/sec.

to 3 heart beats. Facilitation proceeds more rapidly the higher the frequency of stimulation. The exact relationships have, however, not been worked out.

With a large number of preparations the records show a conspicuous drop in the baseline concomitant with the establishment of inhibition of the heart beat. A typical case is shown in Fig. 7. It is striking that the maximum of this apparent relaxation is reached with the same stimulation frequencies which give maximal inhibition of the heart beat.

In their paper on "The mechanism of the nervous regulation of the crayfish heart," Wiersma and Novitski (19) state that "immediately after stopping stimulation of the inhibitor, the heart invariably beats once. However, the pause between this beat and the following one is usually longer than normal, and for the first few beats this interval between beats may even lengthen somewhat before gradually returning to normal."

The same type of behavior was observed in this study in many preparations



(*a*) (*b*)

FIGURE 4. *Pacifastacus leniusculus*. Perfused heart. Record of heart beats. (*a*) During stimulation of left accelerator nerve with 5 pulses/sec. the right accelerator is stimulated with 5 pulses/sec. Note the increment in the resulting acceleration which is equal in magnitude to that produced by stimulation of one of the accelerators with 10 pulses/sec. (*b*) Change in stimulation frequency applied to left accelerator nerve from 3 to 7/sec. Note increment in response. Lower and upper straight lines indicate stimulation of left and right accelerator nerve respectively. Lower time trace, 10 sec.

TABLE II

Frequency of stimulation of accelerator nerve		Duration of accelerator after-effect, as measured in		
Left	Right	Heart 1	Heart 2	Heart 3
pulses/sec.	*pulses/sec.*	*sec.*	*sec.*	*sec.*
5	—	7	0	0
—	5	8	0	2
5	5	15	5	10
10	—	17	10	12
—	10	14	5	10
10	10	24	18	25
20	—	29	23	27
—	20	22	19	26
20	20	30	42	35
40	—	28	35	30
—	40	33	46	32

of *Pacifastacus* and *Orconectes* hearts (an example is shown in Fig. 8). The after-effects of inhibition show, however, great variation. In many preparations the state of complete diastolic arrest continued for several seconds after the end of stimulation of the cardioinhibitor nerve (as shown in Fig. 9), while in other

preparations the heart seemed to resume its normal action the instant the inhibitor stimulation was ended. It can, however, be stated that in all cases

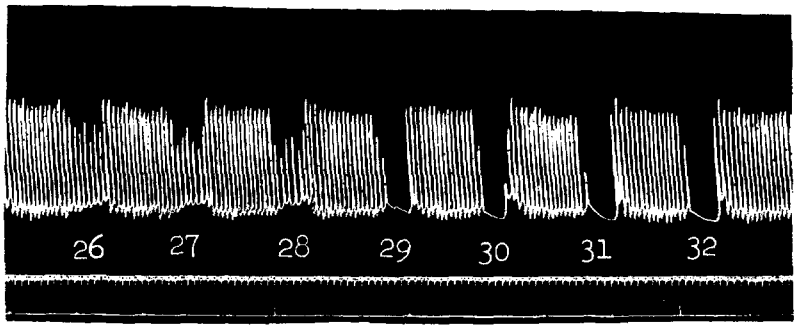


FIGURE 5. *Pacifastacus leniusculus.* Perfused heart. Record of heart beat during serial stimulation of left cardioinhibitor nerve. Figures indicate number of stimuli applied per second. Upper time trace, seconds.

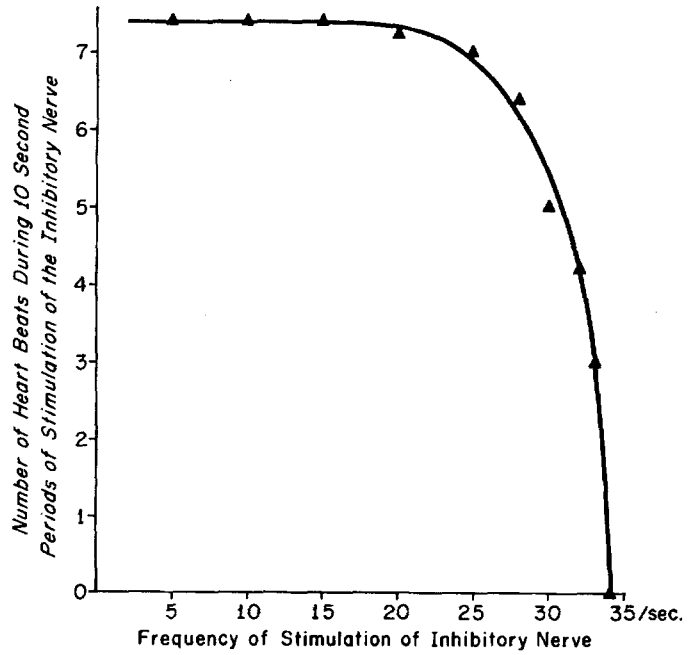


FIGURE 6. *Pacifastacus leniusculus.* Relationship between heart rate and frequency of stimulation of left cardioinhibitor nerve as measured during 10 second periods of stimulation.

studied the amplitude of the heart beats immediately following the state of complete inhibition (whether this coincided with the end of stimulation or occurred seconds later) was equal (if not larger) to that of the heart beats preceding the onset of inhibitor stimulation. Whenever inhibitor after-effects

occur, their duration is proportional to the frequency of the discontinued stimulation of the inhibitor nerve, but never exceeds a maximum of 10 seconds.

If inhibitor stimulation was prolonged, an adaptation to the inhibitor influence could be noted; even if the stimulation frequency was high enough to

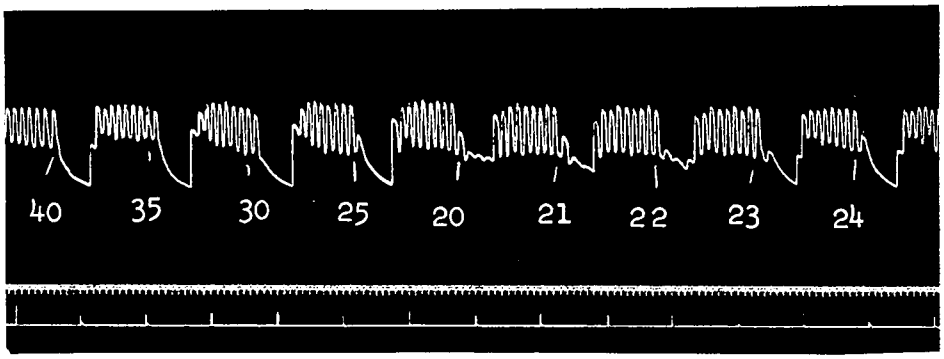


FIGURE 7. *Pacifastacus leniusculus.* Perfused heart. Record of heart beat during serial stimulation of right cardioinhibitor nerve. The figures indicate the number of stimuli per second applied to the nerve. Upper time trace, seconds. Note the drop in baseline which coincides with the occurrence of complete diastolic arrest.

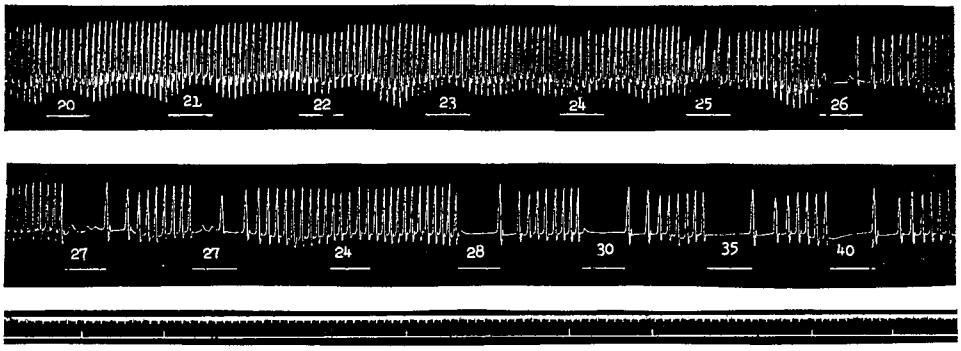


FIGURE 8. *Pacifastacus leniusculus.* Perfused heart. Record of heart beat during serial stimulation of left cardioinhibitor nerve. The figures indicate the number of stimuli per second applied to the nerve. Lower time trace, 10 sec. Note that with higher frequencies the end of stimulation is immediately followed by a single heart beat. Notice how the inhibitory after-effects increase with increasing frequency of inhibitor stimulation.

induce diastolic arrest of the heart, heart beats, usually of normal or near normal amplitude, appeared with gradually increasing frequency. The time interval between onset of diastolic arrest and the appearance of the first beat during inhibitor stimulation clearly depends on the frequency of stimulation of the inhibitor nerve. An example of such behavior is shown in Fig. 10. If prolonged stimulation of the inhibitor nerve was stopped there occurred in many prep-

arations a rather pronounced "rebound" phenomenon: the heart beat showed sudden acceleration above the normal rate and a gradual decline to its original frequency of beating. A similar postinhibitory excitation has been described by Maynard (13) and by Terzuolo and Bullock (16). An example is shown in Fig. 11. In many cases this postinhibitory excitation did not occur and at the end of inhibitor stimulation the heart beat resumed more or less immediately its original frequency.

The effect of simultaneous stimulation of both inhibitor nerves was equal to the sum of the effects of stimulation of each; if both nerves were stimulated with a frequency of 15/sec., the result equaled that of stimulation of either nerve with 30 pulses/sec. It was astonishing to find that stimulation frequencies as low as 5/sec. (which, if applied to only one of the nerves would have no detectable effect), would sum with submaximal frequencies applied to the

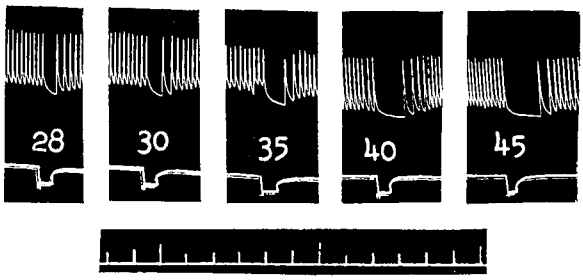


FIGURE 9. *Orconectes virilis*. Perfused heart. Record of heart beat during stimulation of left cardioinhibitor nerve with different frequencies of stimuli. The figures indicate the number of pulses per second applied to the nerve. Time base, 10 sec. Note the very long lasting after-effects and the absence of a beat following cessation of inhibitor stimulation.

other nerve to give increased inhibition. If a total number of 30 pulses/sec. was needed to cause complete diastolic arrest through stimulation of one of the two inhibitor nerves, the same full inhibition could be obtained if one of the inhibitors was stimulated with only 25 pulses/sec. and the other, simultaneously, with 5/sec. This is shown in Fig. 12.

It should be mentioned here that both inhibitors were found to be equally affective and that the threshold frequency for complete inhibition of the heart beat was exactly the same, regardless of whether the one or the other was stimulated. It was, in fact, possible to detect slight differences in the calibration of the two stimulators used, by stimulating both inhibitors of one heart alternately through the two stimulators and by exchanging the leads (see Fig. 12).

A series of experiments was conducted in an attempt to discover whether the synaptic endings of both inhibitors are on the same or on different cells of the heart ganglion. The argument used was the following: if the left inhibitor innervates only part of the ganglion cells, the adaptation to continued inhibi-

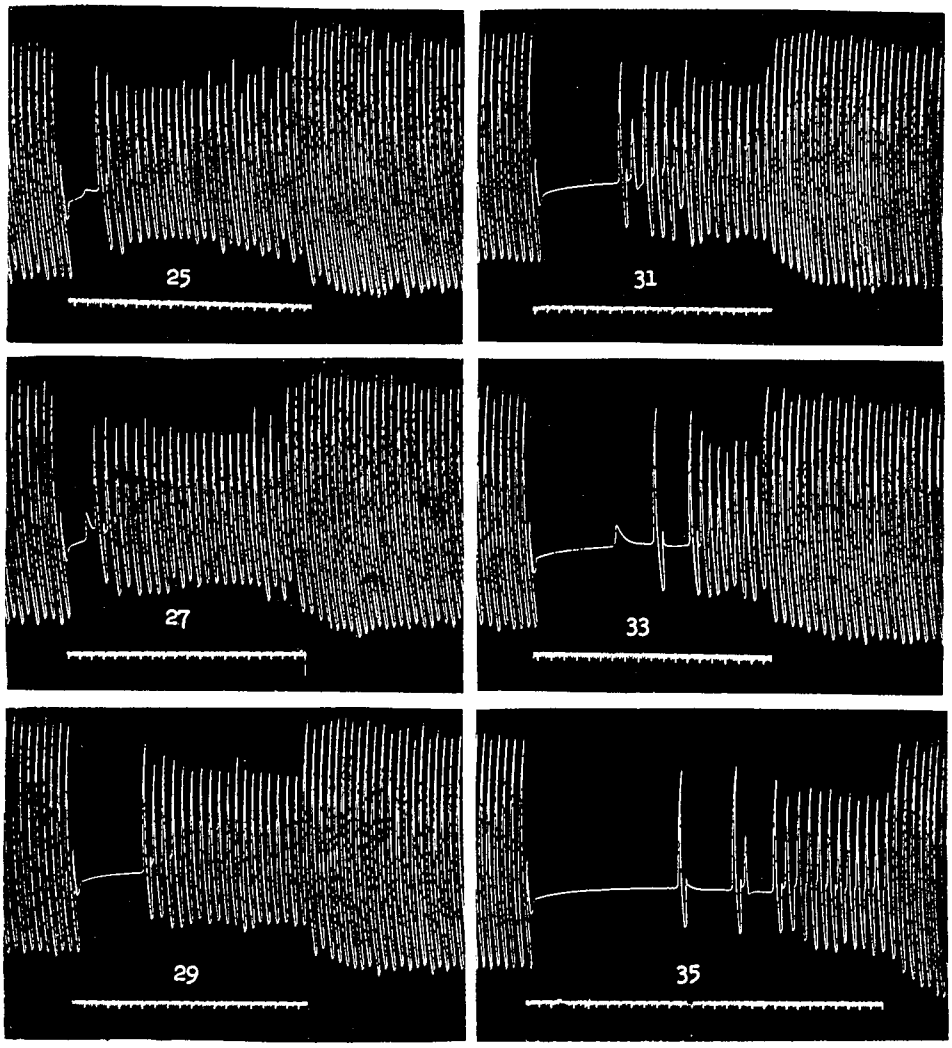


FIGURE 10. *Pacifastacus leniusculus*. Perfused heart. Records of heart beats during stimulation of left cardioinhibitor nerve. The length of the time bases (which indicate one-fifth and 1 sec.) shows the duration of applied stimulation. Note the recurrence of heart beats during continued inhibitor stimulation and the relationship between duration of inhibition and frequency of stimulation.

tion should affect only those cells and complete inhibition should once more result if stimulation were switched to the other inhibitor nerve, which would now inhibit the other set of (unadapted) nerve cells.

Frequencies of stimulation were selected, which were slightly above those required to give complete diastolic arrest. Stimulation was continued until adaptation was well under way and a regular beat was resumed. In an instant

stimulation of the one inhibitory nerve was stopped and stimulation of the other with identical frequency was begun. The records (see Fig. 13) showed that the process of adaptation continued as if no change had taken place. This behavior has been found in all preparations studied. It can, therefore, be concluded that both inhibitors make synaptic contact with the same cells. In addition these results demonstrate clearly that the adaptation phenomenon cannot be the result of fatigue of the inhibitor neurons or fatigue in the synaptic region as the stimulation is switched to an unfatigued system. It seems likely then,

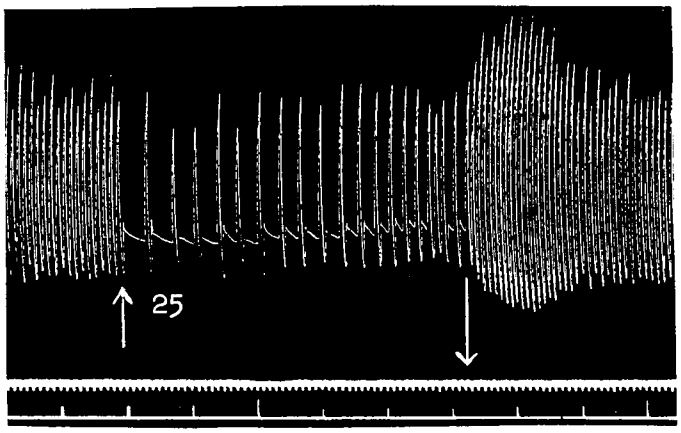


FIGURE 11. *Pacifastacus leniusculus*. Perfused heart. Record of heart beat during stimulation of left cardioinhibitor nerve, with 25 pulses/sec. Upper time trace, seconds. Note adaptation and accelerator rebound at end of inhibitor stimulation.

that the adaptation to inhibition is a reaction of the postsynaptic structure, the ganglion cells. The phenomenon of adaptation in the crayfish heart thus becomes comparable to that found with crustacean stretch receptor neurons (Florey (5, 6)) and many other receptors (see Hensel (9)).

Simultaneous and Alternating Stimulation of Cardioinhibitors and Cardioaccelerators

If during stimulation of one accelerator nerve the ipsilateral or the contralateral inhibitor nerve was stimulated, the extent of the resulting inhibition was equal to that obtained without the simultaneous action of the accelerator. It was astonishing to find that even supramaximal accelerator stimulation did not shift the threshold for complete diastolic arrest. If this was 30 pulses/sec. applied to the cardioinhibitor, it remained at 30/sec. even during stimulation of one or the other accelerator nerve with 50 or 100 pulses/sec.

If during stimulation of the inhibitor nerve the accelerator (ipsilateral or contralateral) was stimulated, the inhibition remained unaltered (Fig. 14).

Occasional exceptions were encountered when during threshold inhibition supramaximal frequencies were applied to the ipsilateral accelerator nerve. In such cases a few very small beats would result during accelerator action. A similar pattern has been described by Smith (15) for *Cancer irroratus.*

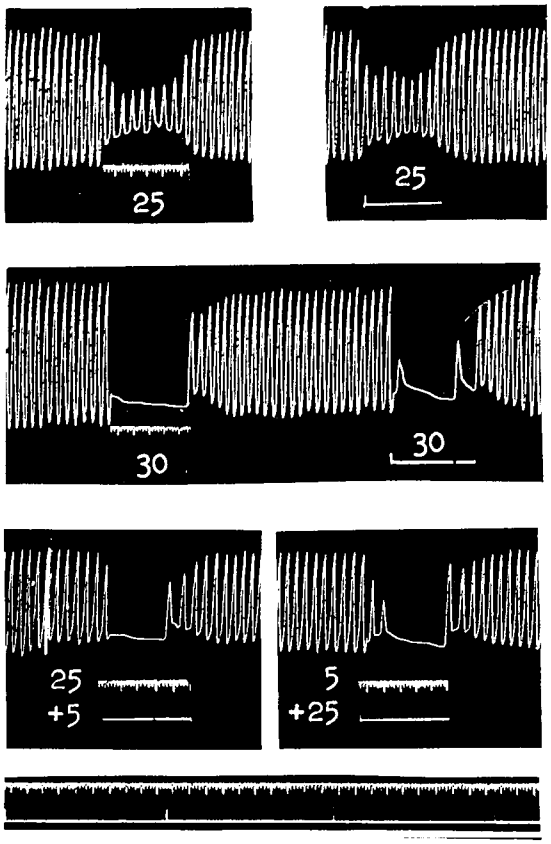


FIGURE 12. *Pacifastacus leniusculus.* Perfused heart. Record of heart beat during stimulation of left and/or right cardioinhibitor nerve. The short time traces below each record indicate duration of stimulation of left (upper) and right (lower trace) inhibitor nerve. The figures indicate the number of pulses applied to the nerve(s). Upper time trace, one-fifth and 1 sec. Note the slight difference in response to action of left and right inhibitor nerve. This is due to differences in calibration of the stimulators used.

In three out of twenty preparations, a different pattern of response was found: in these cases the inhibitor nerves did not dominate, but during simultaneous action of accelerator and inhibitor nerve the frequency of the heart beat was altered according to the resultant effects of both (Fig. 15). This seems to be the situation found by Wiersma and Novitski (19) in *Procambarus clarkii.* In the present study this pattern was the exception and occurred only in late phases of some experiments on *Pacifastacus.*

If accelerator and inhibitor stimulation were stopped simultaneously, there occurred an excitatory after-effect, the time course of which was similar to the acceleratory after-effect following stimulation of the accelerator nerve alone. In those preparations in which prolonged inhibitor stimulation was followed by postinhibitory excitation, this rebound summed with the postexcitatory acceleration when both inhibitor and accelerator had been stimulated simultaneously.

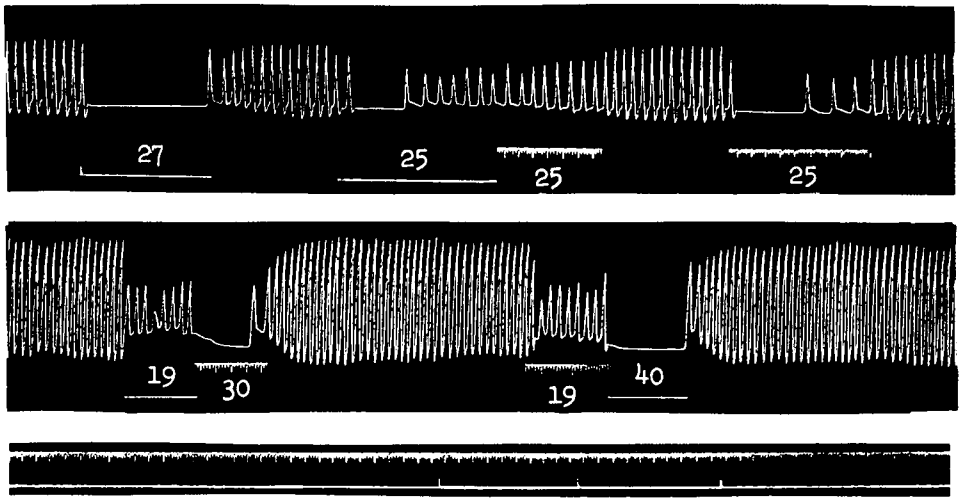


FIGURE 13. *Pacifastacus leniusculus*. Perfused heart. Record of heart beat during stimulation of left or right cardioinhibitor nerve. The upper and lower time traces below each record indicate duration of stimulation of left and right inhibitor nerve. The figures indicate the numbers of pulses applied to each nerve. Lower time trace, 10 sec. Note that no inhibition occurs in response to inhibitory stimulation of one inhibitor nerve if the ganglion had adapted to stimulation of the other. Inhibition does, however, occur if the second nerve is stimulated with higher frequencies.

If an inhibitor nerve was stimulated during an acceleratory after-effect, the resulting inhibition interrupted the prolonged acceleration, but did not alter its time course; a few seconds after the end of the inhibitor action, the heart beat would show the same residual acceleration it would have shown had there been no inhibitory interruption.

DISCUSSION

The difference between the effectiveness of accelerator and inhibitor neurons on the heart ganglion is very conspicuous. With linearly increasing frequency of stimulation the rate of increase of accelerator action decreases to zero while

the rate of increase of inhibitor action rises toward infinity. This situation is illustrated in Figs. 16 and 17, in which the data of Figs. 2 and 6 are replotted.

In addition there is a striking difference in the after-effects; these are of long duration in the case of the accelerators, but are absent or short lived in the case of inhibition. On the whole, the results confirm and extend those of Maynard (11) obtained with *Panulirus argus.*

There is also a pronounced difference with regard to the time course of facilitation and of adaptation. Facilitation of chronotropic action is brief in both cases but that of the inotropic action is conspicuous in acceleration and very brief in inhibition. Adaptation is very pronounced during inhibition, but almost absent during acceleration.

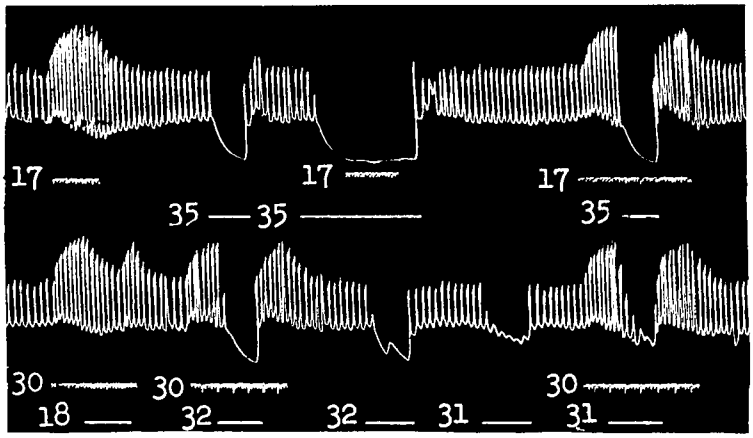


FIGURE 14. *Pacifastacus leniusculus.* Perfused heart. Record of heart beat during stimulation of left cardioinhibitor, and/or right cardioaccelerator nerve. The figures indicate the number of pulses applied to each nerve. Upper and lower time traces indicate duration (in one-fifth and 1 sec.) of stimulation of accelerator and inhibitor nerve. Note the dominance of the inhibitor action over the accelerator action.

In my opinion the results can best be explained if one assumes that both accelerator and inhibitor neurons act on the ganglion cells by release of excitatory and inhibitory transmitter substances. The release of an excitatory transmitter by accelerator neurons is indicated by the persistence of the action after cessation of stimulation and by the fact that the duration of the after-effect is proportional to the frequency of the preceding stimulation. This does, of course, imply a rather slow inactivation of the transmitter. The slow inactivation would explain the "smooth" acceleration produced by stimulation with frequencies as low as 2/sec.

Two explanations may be offered for the fact that the rate of increase of acceleration decreases with linearly increasing frequency of stimulation: (1) The amount of transmitter released per unit time may reach a level sufficient to saturate the receptor molecules on the postsynaptic membranes. (2) The

amount of transmitter released per nerve impulse declines with increasing frequency of stimulation. The second explanation seems to be more likely because it is supported by the fact that the rate of increase of the acceleratory

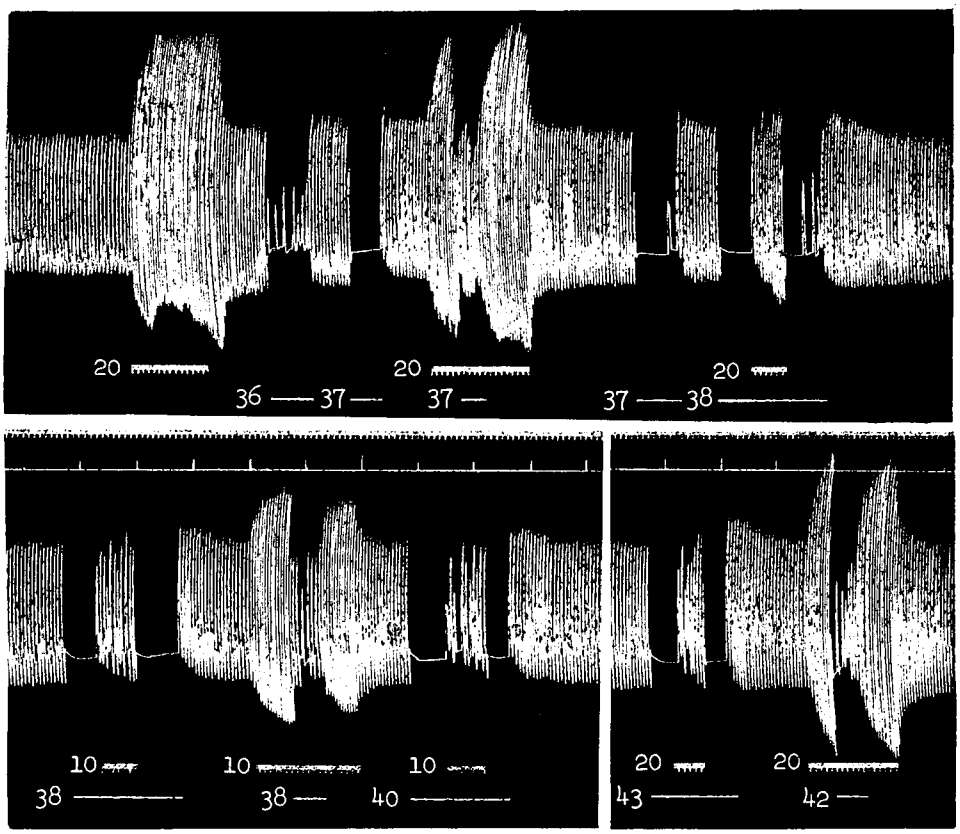


FIGURE 15. *Pacifastacus leniusculus*. Perfused heart. Record of heart beats during stimulation of left cardioinhibitor and/or right cardioaccelerator nerve. The figures indicate the number of pulses applied to each nerve. Upper and lower time traces indicate duration of stimulation of accelerator and inhibitor nerves respectively. Time marks on upper trace, 1 sec. Note that action of accelerator nerve definitely diminishes the action of inhibitor nerve.

after-effects decreases with linearly increasing frequencies of the preceding stimulations at about the same rate as does the rate of increase of the acceleration during stimulation (see Fig. 3 on page 1068). If the amount of transmitter released per impulse remained constant one would expect an increasing accumulation of transmitter and an increasing duration of the after-effect, even beyond the range of frequencies of stimulation which cause just maximum acceleration.

That the inhibitor neurons release a transmitter is indicated by those experiments in which the diastolic arrest of the heart continued for several seconds after the end of inhibitor stimulation. Direct evidence for the inhibitor transmitter will be presented in a subsequent publication (a preliminary account has been given by Florey (7)). The results described in this paper imply a rapid inactivation of the inhibitor transmitter, since in most cases after-effects are absent or very brief. With the assumption of a small time constant of the transmitter action, the characteristic increase in efficiency of the inhibitor

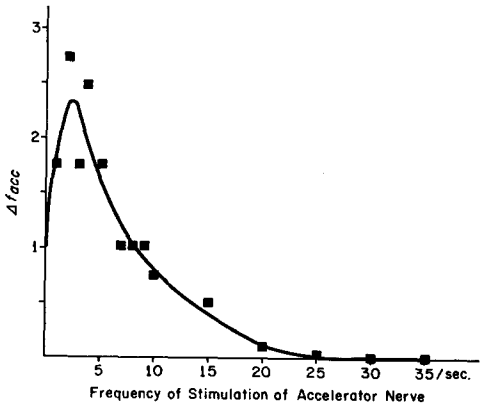


FIG. 16

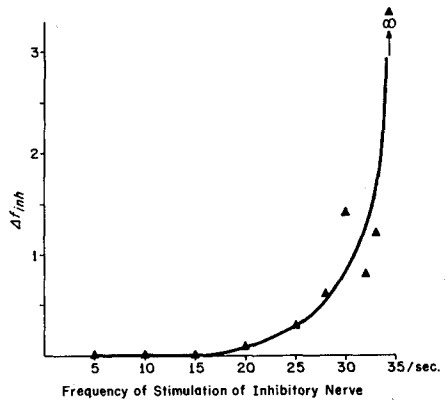


FIG. 17

FIGURE 16. *Orconectes virilis*. Perfused heart. Relationship between rate of change of acceleration of heart beat and frequency of stimulation of the left cardioaccelerator nerve. The abscissa indicates the number of stimuli per second applied to the accelerator nerve during 10 sec. periods of stimulation. The ordinate indicates the incremental change in the number of heart beats for each additional stimulus/second.

FIGURE 17. *Pacifastacus leniusculus*. Perfused heart. Relationship between rate of change of inhibition of heart rate and frequency of stimulation of left cardioinhibitor nerve. The abscissa indicates the stimuli per second applied to the inhibitor nerve during 10 second periods of stimulation. The ordinate indicates the decremental change in the number of heart beats for each additional stimulus per second.

nerve with increasing frequency of stimulation can be readily explained: With longer intervals between pulses the transmitter action decays before the arrival of a new pulse. As the time between pulses decreases, there will be a moment when pulses will arrive while some transmitter still persists from the previous pulse. From then on the build-up of transmitter will be exponential with linearly increasing pulse frequencies. The situation is diagrammatically represented in Fig. 18.

The absence of noticeable adaptation or of an increase of the response to continued accelerator stimulation may be explained if one assumes that the accumulation of excitatory transmitter is balanced by an adaptation so that the resultant frequency of the heart beat remains constant. Both the time

course of the accumulation of transmitter for any given frequency of stimulation, and the time course of adaptation can be expected to be exponential functions of time, asymptotically approaching a steady state. If the two curves are symmetrical, there will be no change of heart frequency once the initial acceleration is achieved.

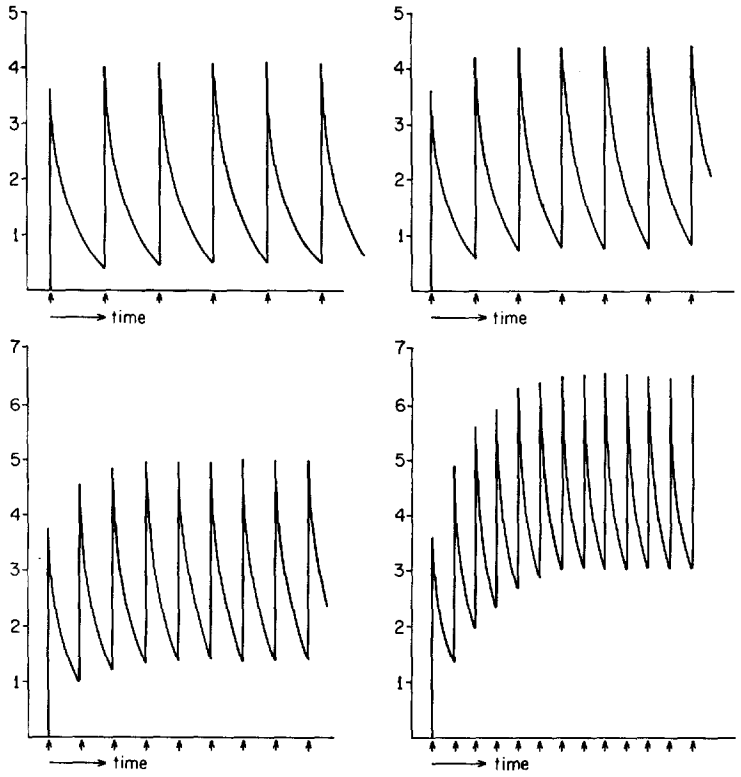


FIGURE 18. Hypothetical changes in the amount of synaptic transmitter substance in relation to the frequency of presynaptic impulses. The curves are calculated assuming that the inactivation of the transmitter follows an equation for a first order reaction and that the interval between impulses decreases below the half-life time of the transmitter substance. The units given are arbitrary. Note the accumulation of transmitter substance as the interval becomes shorter than the time of decay of the substance released with each impulse. The impulse intervals are related as 5:4:3:2.

If the time constant of the accumulation of transmitter were smaller than that of the adaptation process, adaptation would become noticeable. This may very well be the explanation for the remarkable adaptation during inhibitor stimulation.

The dominance of the inhibitor action over that of the accelerators is surprising. It may be due to a strategic location of the inhibitor synapses, but it is also possible that the inhibitor transmitter acts as a blocking agent for the excitatory transmitter.

The neurochemical regulation of the heart beat will be the subject of a later publication.

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