Acceleratory Synapses on Pacemaker Neurons in the Heart Ganglion of a Stomatopod, *Squilla oratoria*

AKIRA WATANABE, SHOSAKU OBARA, and TOYOHIRO AKIYAMA

From the Department of Physiology, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan. Dr. Akiyama’s present address is the Department of Neurology, College of Physicians and Surgeons, Columbia University, New York 10032

ABSTRACT The pacemaker neurons of the heart ganglion are innervated from the CNS through two pairs of acceleratory nerves. The effect of acceleratory nerve stimulation was examined with intracellular electrodes from the pacemaker cells. The major effects on the pacemaker potential were an increase in the rate of rise of the spontaneous depolarization and in the duration of the plateau. The aftereffect of stimulation could last for minutes. No clear excitatory postsynaptic potential (EPSP) was observed, however. On high frequency stimulation, a small depolarizing response (the initial response) was sometimes observed, but the major postsynaptic event was the following slow depolarization, or the enhancement of the pacemaker potential (the late response). With hyperpolarization the initial response did not significantly change its amplitude, but the late response disappeared, showing that the latter has the property of the local response. The membrane conductance did not increase with acceleratory stimulation. The injection of depolarizing current increased the rate of rise of the spontaneous depolarization, but only slightly in comparison with acceleratory stimulation, and did not increase the burst duration. It is concluded that the acceleratory effect is not mediated by the EPSP but is due to a direct action of the transmitter on the pacemaker membrane.

INTRODUCTION

Pacemaker neurons in the *Squilla* heart ganglion are innervated by the CNS with inhibitory and acceleratory nerve fibers (Alexandrowicz, 1934). The effect of inhibitory nerve stimulation on the pacemaker activity has been described in a previous paper (Watanabe, Obara, and Akiyama, 1968). In the present paper, the effect of acceleratory nerve stimulation will be described. It will be shown that there are several important asymmetries between inhibitory and acceleratory effects. The inhibitory postsynaptic poten-
tial is clearly observable, but the excitatory postsynaptic potential is quite obscure even when the acceleratory effect is conspicuous. The potential change associated with the acceleratory effect can be eliminated by applying hyperpolarizing current. These phenomena seem to indicate that the electrically excitable membrane is deeply involved in the acceleratory effects produced in the postsynaptic neurons.

METHODS

The anatomy of the preparation is described in detail in previous papers (Watanabe, Obara, Akiyama, and Yumoto, 1967; Watanabe, Obara, and Akiyama, 1968). Only a summary will be presented here. The *Squilla* heart is a long tube made of striated muscle fibers. On its dorsal surface, the ganglionic trunk runs longitudinally. About 15 somata appear on the ganglionic trunk with variable distances of several millimeters. They are numbered from the rostral side and are called Gc. 1 [i.e. ganglion cell 1], Gc. 2, etc. The rostral cells are pacemakers for the spontaneous burst discharge in the ganglion. The pacemaker cells are innervated from the CNS through three pairs of regulator nerves, which are called $\alpha$, $\beta$, and $\gamma$, respectively, from the rostral side. The $\alpha$-nerve is inhibitory, while the $\beta$- and $\gamma$-nerve are acceleratory to the heart beat.

In this study one of the acceleratory nerves was stimulated with a metal electrode or a suction electrode, and the postsynaptic potential change was recorded from the soma with the glass capillary intracellular electrode. Results were obtained from Gc. 3-6, whereas Gc. 1 and Gc. 2 were not penetrated because of their small size and poor visibility. The experimental setup was the same as that of the previous paper (Watanabe et al., 1968).

Neurons in the pacemaker region are electrotonically connected (Watanabe, Obara, Akiyama, and Yumoto, 1967) and therefore potential changes observed in one soma are influenced by those in neighboring somata. Whenever necessary, recording was made from two neighboring cells simultaneously, and polarizing current was applied to one of the cells through a third intracellular electrode. As seen in Fig. 4 or Fig. 8, in most cases the complication due to the electrotonic coupling was small, and many conclusions could be drawn from observations made from a single cell.

Unlike the $\alpha$-nerve, which contains only a single axon, both $\beta$- and $\gamma$-nerve contain several axons. The effect of stimulation sometimes showed a stepwise difference in the grade of the acceleratory effect according to stimulus intensity. Usually we used the intensity which was strong enough to produce the largest effect.

As in the previous paper (Watanabe et al., 1968), normal saline with the following composition was used in most experiments for the external medium (mm): NaCl, 450; KCl, 15; CaCl$_2$, 10; MgCl$_2$, 20.

RESULTS

The Effect of Acceleratory Nerve Stimulation on the Spontaneous Discharge

Fig. 1 presents a series of experiments in which the $\beta$-nerve was stimulated repetitively when the cell was spontaneously active. Even when the stimulus
frequency was very low, the acceleratory effect was clearly recognized. In Fig. 1 F, the stimulus frequency was 2.5/sec, and only about three shocks were applied during one interburst period. In spite of it, the first interburst period during stimulation (1.24 sec) was significantly shorter than that of the control (ranging from 1.45 to 1.72 sec). Another indication of the acceleratory effect was the rate of depolarization during the interburst period. Before and after the period of stimulation, the rate of depolarization (measured at its linear part) was about 1.4 mv/sec. During stimulation, it was increased to about 2.4 mv/sec.

At higher frequencies of stimulation, the effect was enhanced. Increase in burst frequency or in rate of depolarization became more prominent (Fig. 1 D and E). Another important effect was the prolongation of the plateau phase (Fig. 1 B and C). When the stimulus frequency was lower, the level for the peak of afterhyperpolarization was almost constant, and the pacemaker depolarization was still almost linear (Fig. 1 D-F). With higher stimulus frequency, the peak of the afterhyperpolarization also moved up (Fig. 1 B and C). The pacemaker depolarization lost its linear time course, and the rate of depolarization increased rapidly, and therefore the time course of the membrane potential between two bursts took the form of a catenary. In Fig. 1 C the period of the plateau became almost the same as the period of the pause, and in Fig. 1 B, the membrane stayed in the state of plateau in most periods of stimulation. When the acceleratory effect attained its extreme, the membrane potential was fixed to the state of plateau during the whole period.
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of stimulation, and the spike frequency could paradoxically be decreased, probably because of cathodal depression (Fig. 1 A).

The number of spikes was usually increased during a burst, as seen in Fig. 1 C, but sometimes the change was not significant. Even a decrease would sometimes be encountered. It is known that the spike number per burst is a complicated function of the burst frequency, and stimulation of the accelerator nerve does not necessarily increase this parameter (Brown, 1964).

The spike amplitude was usually reduced during acceleration especially when the acceleratory effect was prominent (Fig. 1 A and B; Fig. 2). This is probably due to refractoriness of the preceding spikes, and to cathodal depression because the base line is depolarized. Amplitudes of antidromic spikes were also decreased during acceleratory nerve stimulation, but the decrease was only modest (10–20 %) and not as drastic as in the case of inhibitory nerve stimulation (Watanabe et al., 1968), even when the spike frequency was high.

EFFECTS OF OUTSIDE DIVALENT CATIONS When the outside medium was the normal saline, most preparations showed clear acceleration in response to stimulation of the β- or the γ-nerve. The acceleration was only very rarely observed in seawater, and even then the effect was very weak and transient. In Mg saline (in which the divalent cations in the normal saline are all re-
placed with Mg), the acceleratory effect was never observed. These results are in parallel with the effect of inhibition in response to α-nerve stimulation. In the case of acceleration, the lack of definite EPSP's (see below) makes a quantitative estimation of the acceleratory effect difficult, but it seems safe to conclude that, without some amount of Ca ions, the acceleratory transmitter is not released from the presynaptic terminal, as is the case in almost all chemical synapses so far examined (see, e.g., del Castillo and Engbaek, 1954; Takeuchi and Takeuchi, 1962).

The outside concentration of divalent cations also influenced the pacemaker activity. With an increase in divalent cation concentration, the burst frequency was usually decreased. Sometimes increase in resting potential and membrane resistance was also noticed. These effects may be called the "stabilizing action" of the divalent cations on the membrane. Ca ions showed a stronger stabilizing action than Mg ions. With 80 mM Ca and 20 mM Mg, the spontaneous activity was abolished (see Fig. 6 D-G), although excitability in response to stimulation was preserved, and postsynaptic potential was enhanced because of high Ca concentration. Seawater contains about 50 mM Mg and about 10 mM Ca, and fresh preparations in seawater showed a spontaneous periodic discharge at a frequency of about one-fourth per second. In normal saline most preparations showed a burst frequency of 1 1/2 per sec.

**Differences between β- and γ-nerves**  Acceleratory effects were constantly observed when stimulation was applied to either the β-nerve or to the γ-nerve. No consistent difference was recognized in these effects, although, probably, the effect on the γ-nerve was weaker.

One consistent finding was that the slow potential was never elicited in Gc. 6 when the stimuli were applied to the β-nerves. When the recording was made simultaneously from Gc. 5 and Gc. 6, and the β-nerve was tetanically stimulated, a slow depolarization started at once in Gc. 5, developed gradually, and eventually evoked a burst discharge without creating a sharp inflection point between slow depolarization and the burst. In Gc. 6, the pacemaker depolarization was hardly enhanced, although at the later stage a small increase in depolarization rate was noticed. This is to be regarded as the electrotonic spread from Gc. 5, since the amount of increased depolarization was less than one-fifth of that in Gc. 5. When the burst of spikes started in Gc. 5, it appeared also in Gc. 6, with a sharp inflection point between slow depolarization and burst. Thus the effect of β-nerve stimulation on Gc. 6 was mostly confined to an earlier appearance of the burst discharge of spikes, whereas the time course of pacemaker depolarization was barely affected. The γ-nerve produced a typical enhancement of the pacemaker potential in both Gc. 5 and Gc. 6. Probably the β-nerve does not innervate Gc. 6. Gc. 3-5 produced the slow potential in response to stimulation of either β- or γ-nerves.
AFTEREFFECTS OF ACCELERATION When an acceleratory nerve was stimulated tetanically at a high frequency, the acceleratory effect persisted for a considerable length of time. In Fig. 1 A, the burst frequency was about 0.56/sec before β-nerve stimulation. After the period of stimulation, the burst frequency was at first 1.1/sec, and then decreased only very gradually towards the value before stimulation. Actually, in the records shown as Fig. 1 B-F, the aftereffect of the preceding acceleration had certain influences on the burst frequency before the next period of stimulation, although they were recorded at intervals of at least 1 min.

Fig. 2 shows the time course of the aftereffect. The actual record of the membrane potential is illustrated in Fig. 2 A, and two parameters of the spontaneous activity are plotted in Fig. 2 B. It will be seen that the two parameters of the burst behave differently. The number of spikes in a burst subsides in a short time after stimulation. This corresponds to the fact that the prolongation of the plateau does not last for a long time after stimulation. On the other hand, the frequency of the burst does not return quickly to the original value. In the case of Fig. 2, it returned to the original value only after about 2 min.

The long-lasting aftereffect constitutes one of the major asymmetries between acceleration and inhibition observed in this ganglion. A similar asymmetry is also observed in the lobster heart ganglion (Maynard, 1961). Usually the inhibitory effect terminates at once when the IPSP subsides with a time constant of a fraction of a second (Watanabe et al., 1968). Sometimes the postinhibitory polarization keeps the membrane potential to a hyperpolarized level, but the effect lasts at most only for several seconds. The lack of symmetry between acceleration and inhibition will be corroborated further in later sections of this paper.

EFFECTS OF ACCELERATORY NERVE STIMULATION ON THE POTENTIAL CHANGE OF THE POSTSYNAPTIC MEMBRANE For examining the effect of acceleratory impulses on the postsynaptic membrane in more detail, the sweep of the oscilloscope was synchronized with the spontaneous burst, and stimuli were applied to the acceleratory nerves for a period with a definite phase relation to the spontaneous pacemaker activity.

As has been shown in Fig. 1 F, the acceleratory effect was apparent even when a small number of stimuli was applied in an interburst period. In Fig. 3 A, only a single shock was given to the β-nerve at the initial part of the interburst period. The rate of depolarization was increased from 2.8 mv/sec to 3.5 mv/sec and the burst interval shortened by about 0.1 sec from the control. With two or three shocks, the effect was far more apparent (Fig. 3 B and C). The records show that a major effect of acceleratory nerve stimulation on the postsynaptic membrane potential is to increase the rate of spontaneous
depolarization in the pacemaker cell. A decrease in apparent firing level for spikes in accelerator stimulation (Fig. 3 B and C) is probably due to invasion of conducted spikes from other neurons where the effect of acceleration was larger.

In spite of the discrete increase in rate of depolarization, no rapid potential change was observed in response to each stimulus. Several records are presented in Fig. 3 D-F to confirm this point, using higher amplification for the potential recording. No record reveals, however, brief depolarizing responses, which might be expected if the acceleratory effect were mediated by some excitatory postsynaptic potential (EPSP).

With high frequency stimulation, however, a small potential change was observed with a brief latency, at least in some preparations (Fig. 5). The response was a depolarizing process which grew rather slowly and saturated after about 100 msec. The plateau phase might persist for a while until the rate of depolarization was accelerated to form the next burst.

The over-all response to brief tetanic acceleratory stimulation is thus composed of two phases. The initial response is produced with a brief latency, but its amplitude is not more than 1.5 mv, and may not be detected in many preparations. The late response is the major observable postsynaptic potential change. This is usually recognized as an increased rate of the pacemaker depolarization. It may be defined as the difference between two potential changes, one with stimulation and another without stimulation, before the burst discharge takes place. By adjusting the period and phase of the tetanic
stimulation (see Figs. 4 and 8), the time course of the late response can sometimes be traced up to its end, without being obscured by the following burst.

**THE LATE RESPONSE** Since the late response is the major postsynaptic event after acceleratory nerve stimulation, properties of this response will first be described. An important test to determine the nature of a postsynaptic potential is to apply polarizing current through the membrane and then to examine its effect on the postsynaptic potential. Fig. 4 shows one experiment to test the effect of hyperpolarizing current on the late response. Tetanic stimulation at a frequency of 100/sec was applied for a brief period to the γ-nerve, and recording was made simultaneously from Gc. 5 and Gc. 6.

![Figure 4](image)

**Figure 4.** The effect of hyperpolarization on the late response. Upper beam, Gc. 5, cell 161. Lower beam, Gc. 6, cell 162. A third electrode was inserted into Gc. 5 to pass polarizing current. The γ-nerve was stimulated at a frequency of 100/sec for a brief period. A, without polarization. A1, the late response was subthreshold to evoke a burst. A2, the period of tetanic stimulation was slightly later, and the late response evoked a burst. B, with polarization. B1, four sweeps were superimposed; each sweep shows potential change with or without γ-nerve stimulation, or with or without hyperpolarizing current. B2, eight sweeps were superimposed. Potential changes without polarization and with three different intensities of polarization are shown with and without γ-nerve stimulation. K2SO4 electrode. The distance between the two somata was 5.4 mm.

Fig. 4 A1 shows that the late response failed to evoke a burst, whereas Fig. 4 A2 shows that the same stimulation produced a late response which in turn evoked a burst discharge. Undoubtedly the late response is a major cause in producing acceleration of the spontaneous burst.

Fig. 4 B shows experiments in which hyperpolarizing current was applied with a third intracellular electrode inserted into Gc. 5. In Fig. 4 B1, four sweeps were superimposed, each of them showing the potential change with or without γ-nerve stimulation, or with or without the hyperpolarizing pulse. It will be seen that in Gc. 5 the hyperpolarizing pulse of this intensity almost eliminated the late response. In Gc. 6, the membrane was also slightly hyperpolarized, because of the electrotonic coupling among the pacemaker cells.
(Watanabe, Obara, and Akiyama, 1967). The late response was reduced in size, but was not abolished. In Fig. 4 B4, the intensity of the hyperpolarizing pulse was changed in four steps. With hyperpolarization of moderate intensity, the amplitude of the late response was reduced, and with stronger hyperpolarization it disappeared almost completely.

The above results indicate that the property of the late response is different from that of postsynaptic potentials observed in other synapses, where the depolarizing response usually becomes larger in amplitude when the membrane is hyperpolarized. To explain this, one may assume that the membrane resistance increases during the late response. This explanation is, however, not likely here, because if it were true we should find a reversal of the response when the membrane is strongly hyperpolarized. In fact no such reversal has been observed; the late response simply disappeared with strong hyperpolarization (see also Fig. 7). Another explanation is to assume that the emf of the membrane changes according to the level of the membrane potential. This is the assumption we have adopted in this paper. Thus the late response is an enhanced pacemaker potential. It is known that the pacemaker potential is suppressed by hyperpolarization (Watanabe, Obara, and Akiyama, 1967). With acceleratory stimulation, the rate of depolarization is greatly increased, but still the hyperpolarization can suppress the whole process.

In Fig. 4 B4, the amount of hyperpolarization in Gc. 6 was only about 1.6 mv, and it was enough to reduce the late response to about one-fifth that of the control. The large effect of hyperpolarization indicates that the late response possesses the property of self-regenerative potential change. Since depolarization at the initial stage determines the next amount of depolarization, a slight difference in the initial step can cause a large difference in the later time course.

THE INITIAL RESPONSE

The behavior of the late postsynaptic response is not quite similar to that observed in other postsynaptic membranes. Usually the primary reaction of the postsynaptic membrane is either an EPSP or an IPSP, with characteristics such that their emf and conductance do not depend on the potential level of the postsynaptic membrane (see del Castillo and Katz, 1956; Grundfest, 1959). It is therefore important to know whether the late response is the primary effect of the transmitter substance on the postsynaptic membrane.

As mentioned before, at least some of the cells showed a small response with a brief latency. We call this the initial response. Since its size was often very small, it was easily overlooked. However, because of the characteristics of the larger late response, it is necessary to examine the properties of the initial response more carefully.

An example of the initial response is shown in Fig. 5. The latency of the
The initial response was usually less than 10 msec. With repetitive stimulation it grew gradually, but attained a saturation point after about 100 msec, and usually the depolarization became continuous with the late response or with the next burst discharge. The amplitude was around 1 mv, and to some extent it varied according to the phase of stimulation. The amplitude of the initial response was only 0.3 mv in Fig. 5 A, but was about 0.6 mv in Fig. 5 B and C. This is probably due to the increase in membrane resistance at the later period of the pacemaker depolarization (see Watanabe, Obara, and Akiyama, 1967).

![Figure 5](image_url)

**Figure 5.** The initial response to the γ-nerve stimulation at a frequency of 100/sec. Gc. 4, cell 142. Ten sweeps were superimposed, five with stimulation and five without stimulation. The period of stimulation is indicated by a bar under each record. K₂SO₄ electrode.

With hyperpolarization, the initial response did not show any significant increase in amplitude. It remained constant, or decreased slightly, with increased hyperpolarization. Fig. 6 A-C shows one example, in which the initial response was more distinctly recognized than usual. Without hyperpolarization it elicited a burst (Fig. 6 A), but with hyperpolarization of about 5 mv the burst was eliminated (Fig. 6 B). The initial response was then about 1 mv. With hyperpolarization of about 12 mv the initial response was reduced to about 0.7 mv (Fig. 6 C). The size of the initial response was plotted against the membrane potential, and a gradual decrease in the size was recognized with increased hyperpolarization, but with hyperpolarization of more than 10 mv, the size remained constant. Even with hyperpolarization of about 25 mv, no detectable increase in size was recognized. If we assume that the rever-
sal potential of the EPSP is near the outside level, we can expect a 50% increase in size with this amount of hyperpolarization, since the resting potential of the membrane was about 50 mv. No such increase was actually observed.

These findings do not support the idea that the initial response is the EPSP. In spite of this, we can assume that we are picking up the usual EPSP from a point too far from its focus to detect any increase in size with hyperpolarization. The decrease in size with hyperpolarization might be explained by superposition of the late response which must be taking place at the same time.

![Figure 6. The effect of hyperpolarization on the initial response. A-G, an experiment with normal saline. Upper beam, Gc. 5, cell 153. Lower beam, polarizing current through a second electrode in the same cell. The ß-nerve was stimulated repetitively at a frequency of 100/sec for a period indicated below the lower beam. KCl electrode. D-G, another experiment with high Ca saline (NaCl, 435; KCl, 15; CaCl₂, 80; MgCl₂, 20, in mm). Gc. 5, cell 237. No spontaneous discharge. Two sweeps are superimposed, one without stimulation and another with ß-nerve stimulation at a frequency of 100/sec for a period indicated by a bar in each record. D, without polarization. E-G, hyperpolarizing current was applied through a second electrode in the same cell. The dotted line shows the resting level. KCl electrode.]

When the Ca concentration in the outside medium was higher, a considerable increase in size of the initial response was observed. With hyperpolarization, a slight increase in size of the initial response was sometimes observed under such conditions. One example is shown in Fig. 6 D-G. The effect was, however, not always observable in different preparations.

In conclusion, we could not get definite evidence for the existence of an EPSP in this preparation, when the recording was made from the soma at any rate, while the acceleratory effect itself was quite evidently observed at the soma as the enhancement of the pacemaker depolarization. It is also to be noted that the IPSP can very easily be recorded at the soma in response to ß-nerve stimulation (Watanabe et al., 1968).
During the search for the EPSP, we have encountered many small responses which could deceptively be identified as the primary response to the accelerator stimulation. Apart from the stimulus artefact and the externally recorded axon spikes, the appearance of the small prepotential (see Watanabe, Obara, Akiyama, and Yumoto, 1967, p. 831) sometimes posed a confusing problem. The small prepotential can be identified from its larger amplitude and shorter duration, although its postsynaptic origin can only be proved by its disappearance on application of hyperpolarizing current. We consider the small prepotential to be a spike of dendrites of the penetrated neuron, as has been discussed in a previous paper (Watanabe, Obara, Akiyama, and Yumoto, 1967).

**Figure 7.** The current-voltage relationship and its change with the acceleratory nerve stimulation. Two KCl electrodes were inserted into Gc. 5, cell 238, and polarizing current pulses of about 0.8 sec were applied. The lowest (most negative) membrane potential during current application was plotted against intensity of the polarizing current. Open circles, control. Filled circles, with stimulation of the $\beta$-nerve at a frequency of 50/sec.

**Resistance Changes of the Postsynaptic Membrane During Acceleration** Fig. 7 compares the I-V relationship of the soma with and without acceleratory stimulation. Since the membrane potential of the pacemaker neuron fluctuates, especially during acceleration, some convention has to be adopted to prepare the I-V curve. A good correlation was obtained when the lowest (most negative) membrane potential was plotted against the polarizing current.

Without polarizing current, the lowest level of the membrane potential moved up by about 10 mv with acceleratory stimulation. On application of hyperpolarizing current, this "acceleratory depolarization" was reduced in size. The stronger the hyperpolarizing current, the less the amount of the acceleratory depolarization, until finally the amount was almost fixed at a constant value. In the example of Fig. 7, the acceleratory depolarization had
a constant value of 2–3 mv when the hyperpolarization was larger than about 30 mv. In other materials, the acceleratory depolarization was often reduced to an insignificant value with strong hyperpolarization.

The I-V relationship within the range of moderate hyperpolarization showed a lower slope conductance during acceleration. When the intensity of the hyperpolarizing current was further increased, however, the slope conductance became larger and almost the same as that without acceleration.

The above characteristics of the I-V relationship can be explained if one assumes that the conductance of the postsynaptic membrane decreases during acceleration. According to this, however, one would expect that the two I-V curves would cross at some hyperpolarized level, and then with further hyperpolarization the membrane potential would move in the hyperpolarizing direction, in response to acceleratory stimulation. Since such phenomena were actually never observed, we do not think that the above assumption is appropriate. Instead we would like to assume that the decreased slope conductance during acceleration is due to a change in membrane emf which depends on the level of the membrane potential. At a less hyperpolarized level the acceleration of the pacemaker potential increases and accordingly the over-all level of the membrane potential is moved more effectively in the depolarizing direction. When hyperpolarizing current is strong enough to suppress the pacemaker potential completely, the slope of the I-V curve during acceleration is almost the same as that of the control because the pacemaker potential does not contribute to the membrane potential.

Even with hyperpolarization of more than 30 mv, a small depolarization sometimes remained during acceleration. This may be due to the initial response or to electrotonic spread from the neighboring neurons, where the suppression of the acceleratory depolarization must be less effective.

It is concluded that with acceleratory stimulation no conductance increase is observable at the soma. Decrease in slope conductance can be regarded as due to enhancement of the pacemaker activity, since it depends on the postsynaptic membrane level.

With application of the depolarizing current the membrane conductance was markedly increased, and stimulation of the acceleratory nerve made only a small contribution to the lowest value of the fluctuating membrane potential.

**COMPARISON BETWEEN CURRENT INJECTION AND ACCELERATORY STIMULATION**

As far as observations made at the cell soma, if there is any EPSP, it is quite small, and the major postsynaptic event is represented by the late response, or the increase in rate of the spontaneous depolarization. A straightforward inference from the above findings is that the acceleratory transmitter acts directly on the membrane which is producing the pacemaker potential. An alternative hypothesis is that the EPSP, however small, still triggers the
late response electrically. We therefore applied some electric current to the soma, for the purpose of testing whether it can produce a potential change similar to the late response produced by acceleratory nerve stimulation.

Fig. 8 shows one experiment. A tetanic train of stimuli to the β-nerve produced the late response in Gc. 4 and Gc. 5, but in Fig. 8 A1 it was just subthreshold for triggering the burst discharge of spikes. Fig. 8 A2 shows that the same stimulation elicited a larger late response which in turn triggered the burst discharge. The initial response was recognizable in neither Gc. 4 nor in Gc. 5 and one may conclude that it was less than 0.5 mv.

Fig. 8 shows a comparison between acceleratory nerve stimulation and injection of depolarizing current. Upper beam, Gc. 4, cell 164. Lower beam, Gc. 5, cell 165. K₂SO₄ electrodes. A, without polarization. The β-nerve was stimulated at a frequency of about 60/sec. A₁, the subthreshold late response. A₂, the late response with an evoked burst. B, without β-nerve stimulation. The polarizing current was injected into Gc. 4 with a third electrode. B₁, the subthreshold electrotonic potential. B₂, the electrotonic potential with an evoked burst.

In Fig. 8 B₁, a polarizing current pulse was injected into Gc. 4 through an intracellular current electrode. The duration of the pulse was the same as the period of repetitive stimulation. An electrotonic potential of about 7 mv was produced in Gc. 4 and its electrotonic spread created a depolarization of about 1.3 mv in Gc. 5. After the electrotonic potential, we did not observe any potential change comparable to the late response shown in Fig. 8 A, nor did the rate of spontaneous depolarization change significantly. We may conclude, therefore, that current injection into the soma does not produce the effect of tetanic stimulation of the acceleratory nerve, with a period of stimulation comparable to the duration of the injected current pulse.

Fig. 8 B₂ shows that when the injected current was superthreshold a burst discharge was elicited. No slow depolarization could be recognized after the burst, but definite conclusions may not be drawn from this because of complications with the spike production.
In Fig. 9 current pulses of longer duration were applied during the interburst period. Without polarizing current, the rate of depolarization was about 0.27 mv/sec (Fig. 9 A). The rate was much reduced by applying strong hyperpolarization (Fig. 9 B). With a moderate strength of depolarizing current, the rate was increased to about 0.46 mv/sec (Fig. 9 C), but further increase in current strength did not increase the rate; instead, even some decrease could be noticed (Fig. 9 D). Further increase produced a burst discharge, but afterwards the rate of depolarization was again less conspicuous (Fig. 9 E). The effect was totally different with acceleratory stimulation. Fig. 9 F shows that tetanic stimulation with a frequency of about 20/sec for a brief period (eight shocks) produced a remarkable increase in the rate of rise of the slow depolarization. (The rate of rise, measured at the time of the last shock, was about 8.4 mv/sec. On application of hyperpolarization of about 15 mv (not shown), the rate of rise at the time of the last shock was reduced to a value of about 2.6 mv/sec, showing that at least the major part of the depolarization is not due to the EPSP.) The elicited burst manifested a plateau which was far longer than that of the spontaneous burst. The burst elicited by current injection did not show a comparable plateau formation.

The experiments suggest that the effect of acceleration cannot be reproduced completely by the injection of depolarizing current. Therefore, it is quite unlikely that the acceleratory effect is produced by depolarizing synaptic current. Indeed, the injected current modifies the time course of the slow potential, but the effect of injected current is usually far weaker than the effect of acceleratory stimulation, irrespective of the intensity of the current.
It can therefore be inferred that the primary response of the postsynaptic membrane to the transmitter is the enhancement of the pacemaker potential. If we assume that the primary response is the production of EPSP, and that the enhancement of the pacemaker potential is secondarily produced by the local current created by the EPSP, we cannot understand why the injected current does not reproduce the effect of acceleratory stimulation.

INTERACTION BETWEEN ACCELERATION AND INHIBITION The effect of inhibition in the course of the acceleratory process has been examined by stimulating the α-nerve during the period of acceleratory nerve stimulation. As has been described for decapod hearts (Wiersma and Novitski, 1942; Florey, 1960), the inhibitory effect dominated and the burst activity was completely stopped with α-nerve stimulation when both α- and β-nerves were stimulated at the same frequency. Fig. 10 shows one example. With β-nerve stimulation at the frequency of 100/sec, a long-lasting plateau was produced in Gc. 4 (Fig. 10 A). When a period of α-nerve stimulation at the same frequency was interposed, the plateau was almost completely abolished (Fig. 10 B). After the period of α-nerve stimulation, the plateau formation was at once resumed with a rebound burst discharge of spikes. In comparison with the effect of α-nerve stimulation only (Fig. 10 C), the combined effect (Fig. 10 B) showed a higher rate of membrane depolarization during the period of inhibition, indicating that the acceleratory process was still going on. When both nerves were stimulated simultaneously for a longer period, discharges of spikes started sporadically with irregular intervals. One such spike is seen in Fig. 10 B near the end of the period of inhibition. This is presumably due to some
accommodation process of the postsynaptic membrane to the inhibitory transmitter.

The effect of acceleratory stimulation persisted for a period far longer than that of inhibitory stimulation (see Fig. 2). Therefore, when both nerves were stimulated simultaneously, the burst discharge was inhibited during stimulation, but was accelerated after stimulation (see Wiersma and Novitski, 1942). The aftereffect of acceleratory nerve stimulation persisted even when the inhibitory nerve was stimulated subsequently to suppress development of the pacemaker potential. An experiment is presented in Fig. 11. In this particular experiment, KCl-filled intracellular electrodes were employed, and therefore the IPSP appeared as a distinct depolarizing response. The period of acceleratory nerve stimulation was only 30 msec, but a conspicuous increase in the rate of rise of the spontaneous depolarization was elicited, and the burst interval was shortened by about 1.7 sec (Fig. 11 A3) from that in the control (Fig. 11 A1). When the α-nerve was also stimulated at the same time, acceleration was suppressed during the period of inhibition, but after the
period of inhibition the burst discharge took place at once (Fig. 11 A1). The high rate of depolarization before the burst indicates that the acceleratory effect was never eliminated, but only temporarily suppressed by the inhibitory process. The postinhibitory facilitation (see Watanabe et al., 1968) does not explain the acceleration because stimulation of the α-nerve only did not shorten the burst interval significantly (Fig. 11 A2). The acceleratory effect was observed even after the period of inhibition which lasted for about 1.5 sec (Fig. 11 A4). It is interesting to note that after the period of inhibition the following burst started after approximately the same period (Fig. 11 B1-B3). The decrease in apparent critical voltage in B2 and B3 suggests that in this cell the inhibitory process lasted longer than in some other cells so that the spikes in the next burst started from the other cells.

The time course of the membrane potential during inhibition did not change with stimulation of the acceleratory nerve (see Fig. 11 B3). The acceleratory effect persisted, but was not expressed as a significant change in the membrane potential. This is due either to a very slow diffusion process of the acceleratory transmitter from the receptor site on the postsynaptic neuron, or to the fact that the transmitter has a long-lasting influence on the postsynaptic membrane. Whatever the reason, it is unlikely that the initial response plays a definite role in the observed acceleration, since its depolarizing action must have been completely eliminated by the long-lasting IPSP (see Fig. 6 A-C for the possible duration of the initial response).

DISCUSSION

It has been found that with stimulation of the acceleratory nerve the pacemaker activity of the Squilla heart ganglion is greatly enhanced. The rate of spontaneous depolarization during the interburst period is increased even with a brief period of tetanic stimulation, causing the shortening of the burst interval. In addition, the plateau of the burst phase is prolonged. In spite of the strong acceleratory effects, we failed to identify the typical EPSP, with a definite emf and a conductance increase which are independent of the postsynaptic membrane potential, although the small initial response probably is the summated EPSP with its focus far from the soma. Furthermore, current injection into the soma does not reproduce the effect of acceleratory stimulation. Application of depolarizing current achieves increase in rate of depolarization to only a small extent, and the plateau formation is not enhanced.

It seems necessary, therefore, to assume that the enhancement of the pacemaker activity is due to a direct action of the transmitter on the pacemaker membrane. It seems inadequate to suppose that the enhancement is indirectly achieved by local current, with its sink at the focus of the EPSP. The acceleratory effect in this ganglion is thus more similar to acceleratory effects observed
in mammalian hearts (Otsuka, 1958) or to the effect of catecholamines on mammalian smooth muscle (Born and Bübring, 1956; Axelsson et al., 1961). In these preparations, too, the EPSP or IPSP is not supposed to constitute an essential link between the release of transmitter and the final acceleratory or inhibitory effect. In all these cases the postsynaptic elements are spontaneously active, and extrinsic nerves only regulate, rather than operate, the effectors.

In the lobster heart ganglion, the response to acceleratory nerve stimulation is different from that reported here; mostly the acceleration is not associated with any slow potential change, and only on some occasions are responses purely of the EPSP type (Terzuolo and Bullock, 1958). In that material the recording was made exclusively from follower cells, and one might suppose that the response would be more similar to the present material if one could record the potential change from the pacemaker cell, because the general pattern of response is quite similar in the two preparations as far as the observations made extracellularly are concerned (Wiersma and Novitski, 1942; Maynard, 1953; Florey, 1960).

In mammalian heart muscle (Trautwein and Schmidt, 1960) as well as in smooth muscle (Burnstock, 1958), it has been shown that the effect of transmitter can be modified and even reversed with the application of metabolic inhibitors. In intestinal smooth muscle phosphorylase activity is increased with adrenaline (Axelsson, Bueding, and Bübring, 1961). These findings suggest that the change in membrane potential is mediated by a change in the rate of the intracellular metabolism. A similar effect has been demonstrated also in nerve cells (Nakajima and Takahashi, 1966). We therefore examined the effects of several procedures which might have influence on the metabolic rate of the cell in the Sea urchin heart ganglion. It was found that application of iodoacetic acid (1 mM) or ouabain (0.1 mM) did not prevent the acceleratory effects produced by β- or γ-nerve stimulation. Application of cold saline (around 5°C) probably reduced the acceleratory effect, but did not eliminate it. Replacement of Na with Li caused depolarization of the membrane, but at least in some preparations the acceleratory effect could be observed. At present, therefore, the metabolic rate does not seem to be closely linked with production of the acceleratory effect.

A full interpretation of the action of the acceleratory transmitter on the postsynaptic membrane is, however, beyond the scope of the present paper, and we are only trying to establish that in this material the acceleratory effect cannot be accounted for solely as a result of EPSP production. A direct action of the transmitter on the pacemaker membrane has to be assumed, although EPSP contribution cannot be excluded completely. It is possible that similar mechanisms of synaptic acceleration are at work at some places in the CNS, especially when the postsynaptic neuron manifests intrinsic rhythmical activity.
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