Hyperpolarization of the Quiescent Heart and Inhibition of the Heart Beat by Vagal Impulses

Youko Satow

Department of Physiology (Prof. T. Wakabayashi),
Tokyo Medical College, Tokyo

Hyperpolarization by the vagus stimulation of the quiescent sinus of bullfrog’s heart was recorded. The slope and magnitude of hyperpolarization varied with the pattern of vagal impulses. The hyperpolarization became larger with increase in the number of shocks, but after the magnitude of hyperpolarization attained its maximum the potential fell slowly toward the initial level during the stimulation. The optimal interval for the slope and magnitude of hyperpolarization was about 50 to 100 msec. Outside these intervals, the slope and magnitude of hyperpolarization were slow and small.

Secondly, the electrogram was recorded from the tortoise’s heart and the relation between the pattern of vagus stimulation and the quiescent period after the stimulation was investigated, and similar results were obtained. Of course, the phenomenon of escape occurred on continued vagus stimulation.

The results were explained on the assumption of accumulation of the transmitter, its diffusion or destruction and the failure of nervous impulses, but the reduction of potential as well as the vagus escape in the later period of stimulation were interpreted as suggesting a development of the desensitization to the transmitter. Moreover, delayed response, change of firing potential, shift of pacemaker and rebound excitation were described.

A change in the membrane potential of the heart muscle by vagus stimulation has been reported by a number of authors. In 1887 Gaskell showed that the demarcation current of the tortoise auricle was increased by vagus stimulation. Recently del Castillo and Katz and Hutter and Trautwein studied the hyperpolarized potentials brought about by vagus stimulation in the pacemaker region of the frog’s heart. They suggested that the inhibitory transmitter, i.e., acetylcholine released by vagus stimulation changed the permeability of the membrane to specific ions responsible for the changes of potential level. However, the effect of variation in the pattern of vagal impulses — variation in the stimulation interval and total number of stimuli — on the membrane potential has not been studied. In the present report the effect of the pattern of vagus stimulation on the hyperpolarized potentials of the sinus fibers is described, and the relation between the hyperpolarization and the output of transmitter is discussed.

Received for publication, September 4, 1967.
This work was partly supported by a grant from the Ministry of Education.
thermore, the relation between the length of the inhibitory period of the heart beat remaining after the end of vagus stimulation and the pattern of vagus stimulation is presented.

**METHODS**

Almost all the experiments using intracellular electrodes were done on excised bullfrog's sinus preparations (*Rana catesbiana*), and a few on toad’s sinus (*Bufo vulgaris formosus*). Some experiments were also done on the tortoise's heart (*Clemmys japonica*) in situ using extracellular electrodes. For the intracellular recording the sinoauricular region of the excised bullfrog's heart was carefully cut open in normal Ringer solution and pinned with its dorsal wall underneath on a cork board. It was suitably stretched so as to assure a steady impalement of the electrode. Then, the bathing solution was exchanged with hypertonic and Cl-deficient Ringer solution (the osmolarity was increased to three times the normal by adding sucrose (Hodgkin and Horowicz), and NaCl in the normal Ringer was replaced by an equimolar amount of sodium propionate). The recording was made after arresting the heart beat by immersing the preparation in hypertonic Ringer solution for thirty to sixty minutes. Empirically it was found to be not so difficult to impale the electrode into the cell in this Cl-deficient Ringer solution.

The electrodes filled with 0.6 M Na₂SO₄ (80–100 MΩ) or with 3 M KCl (15–30 MΩ) were used. The electrode was made movable by connecting with a silver wire of about 60 μ in diameter in order to make the recordings free from mechanical artifact. A conventional electrophysiological apparatus, including a counter of the number of stimulating pulses (Nihon Kohden Co.), was used. A central part of the vagus nerve which was electrically isolated carefully from the ganglion jugulare was placed in a fluid electrode for stimulation. Supramaximal rectangular pulses of 1 msec duration were adopted for stimulation.

For investigating the relation between the pattern of vagus stimulation and the period of the standstill of the heart beat, the tortoise's heart was used. The heart was exposed after taking away the ventral plastron. The auricle and ventricle in situ were hung up by serre-fines, through which the electrogram was recorded with a pen writer. For stimulation the right vagus was placed in a fluid electrode.

**RESULTS**

1) **Intracellular potentials in the beating heart**

An example of the recording from the sinus preparation, which was not yet quiescent at an early stage of immersing in the hypertonic solution, is shown in Fig. 1. (Only in this case the bathing medium was made hypertonic by adding sucrose to normal Ringer.) A repetitive vagus stimulation of ten shocks with a pulse interval of 58 msec caused a hyperpolarization in the sinus fiber and inhibited
Fig. 1. Hyperpolarization by vagus stimulation. All records of subsequent figures were obtained with an intracellular electrode, unless otherwise stated. Bullfrog's heart. Repetitive stimulation of ten shocks with 58 msec interval and indicated by break in the upper line, was given to the right vagus. A marked hyperpolarization was transiently evoked and followed by a period of quiescence (upper record); the same stimulation was conducted again (lower record, direct continuation of the upper). At the end of the record the potential became unstable. Time mark is 0.5 c.p.s. and arrows indicate zero level; these apply to all the figures in the present paper. Cal.: 20 mV. 3 times hypertonic sucrose Ringer. 3 M KCl electrode. Temperature: 23.8°C (room). Oct. 12, 1966.

In some cases (Fig. 2 A) the prepotential was very small. It seems that the intracellular electrode had not been impaled just into the pacemaker cell, or that the rising phase of prepotential was slow in the cell impaled and its discharge was evoked by the conduction from the pacemaker cell. Fig. 2 A shows that the inhibition evoked by repetitive stimulation of 20 shocks brought about a marked hyperpolarization and a delayed spike potential which was preceded by a slow and large prepotential. After the appearance of two discharges a long quiescent period ensued (about 13 sec): then, somewhat small action potentials with no prepotential appeared. Fig. 2 B shows an example of a slowly beating heart, in which 50 shocks with 115 msec pulse interval evoked a long inhibition. On the contrary, in Fig. 2 B, the prepotential which had observed before the stimulation disappeared at the
first discharge after the inhibition. In Fig. 2 C and D, a hyperpolarization appeared even in the fiber which usually showed no prepotential.

2) The number of stimuli and the hyperpolarization

A number of experiments were done on the quiescent heart in Cl-deficient hypertonic sucrose Ringer solution, and the hyperpolarization elicited by vagus stimulation with varying number of stimuli was recorded. In a few preparations of the bullfrog's heart, a single vagus stimulation could evoke a small hyperpolarization. In most cases, however, the summation of suitably spaced stimuli was needed to produce hyperpolarization. Fig. 3 shows the hyperpolarization by 5, 10, 50, 100, 200 and 300 shocks of 58 msec pulse interval. The hyperpolarization became larger with the increasing number of shock, but after its magnitude attained a
Fig. 3. Effect of varying the number of left vagus stimulation on bullfrog’s sinus, made quiescent in Cl-deficient hypertonic sucrose Ringer solution. With the increase in the number of stimuli, hyperpolarization became gradually increased, but with the stimuli of more than 50 shocks the magnitude did not increase. Stimulation interval: 58 msec. A; 5, B; 10, C; 50, D; 100, E; 200, and F; 300 shocks. All records were made from the same point of the cell. Cal.: 10 mV. 0.6 M Na$_2$SO$_4$ electrode. Temperature: 23°C (room). Oct. 18, 1966.

maximum, the hyperpolarization decreased again toward the initial membrane potential level in spite of still continued stimulation (C,D,E and F). In other words the magnitude of hyperpolarization increased exponentially in general to attain a final level, and a further continuation of stimuli only contributed to the prolongation of hyperpolarization. With further continuation of repetitive stimulation, the magnitude of hyperpolarization could not be maintained and decayed during the continued stimulation. In Fig. 4, the hyperpolarized potentials by different numbers of shocks were superposed. The effect of pulse interval will be shown below. Fig. 7 A indicates the relationship between the number of
Fig. 4. Superposed representation of the hyperpolarization. The records in Fig. 3; A, B, C, D and E were traced and superposed. Interval 58 msec. The arrow shows the initial time of stimulation. The latency is about 500 msec. A, B, C, D and E indicate the end of each stimulation of 5, 10, 50, 100 and 200 shocks of stimuli, respectively. Cal.: 5 mV, 1 sec.

Fig. 5. Effect of changing the interval of right vagus stimulation of bullfrog's sinus. All records from the same point of the cell. The period of stimulation was 5.8 sec. A, 11.5; B, 23; C, 58; D, 115; E, 240; and F, 610 msec interval. Optimal interval was 58 msec. 0.6 M Na$_2$SO$_4$ electrode. Cal.: 20 mV. Temperature: 23.6°C (room). Oct. 28, 1966.
stimuli and the maximal magnitude of hyperpolarization in a case of 58 msec interval.

3) **Effect of the pulse interval with constant stimulation period**

Fig. 5 shows the effect of changing pulse interval upon the maximal magnitude of hyperpolarization, keeping the stimulating period constant at 5.8 sec. Both the gradient of initial hyperpolarization and its magnitude attained their maximum at 58 msec interval. The relationship between the pulse interval and the maximum magnitude of hyperpolarization was shown in Fig. 7 B.

4) **Effect of the pulse interval with fixed number of shock**

Fig. 6 shows the effect of forty stimuli of varying intervals (A; 11.5, B; 23, C; 58, D; 115, E; 240 and F; 610 msec) on hyperpolarization: from 11.5 to 115 msec

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Fig. 6. Effect of changing interval of the left vagus stimulation in the quiescent bullfrog's sinus. Forty stimuli. A, 11.5; B, 23; C, 58; D, 115; E, 240; and F, 610 msec interval. All records from the same point of the cell. Both the initial gradient of hyperpolarization and its magnitude were maximum with 58 msec interval. In D and E spontaneous heart heat occurred. E is the continuation of the record D without interruption. 0.6 M Na₂SO₄ electrode. Cal.: 10 mV. Temperature: 23°C (room). Oct. 18, 1966.
intervals the magnitude became larger. In D, the initial gradient of hyperpolarization was reduced and the potential began to decrease during continued stimulation, while in E and F, the gradient and the magnitude were smaller, but the hyperpolarization continued to increase during the whole period of stimulation. The relationship between the maximum magnitude of hyperpolarization and the interval of pulse was shown in Fig. 7 C.

![Fig. 7](image)

**Fig. 7.** A: The relationship between the number of stimuli of 58 msec interval (abscissae; logarithm of stimulus number, N) and the magnitude of maximal hyperpolarization measured from diastolic potential level to the trough of hyperpolarization (ordinates; in mV) obtained from the records of Fig. 3. B: The relationship between the magnitude of maximal hyperpolarization in mV and the interval of stimuli in msec, the same as shown in Fig. 5. Abscissae: interval of stimulation (msec) in logarithm. Ordinates: maximum magnitude of hyperpolarization (mV). C: The relationship between maximal magnitude of hyperpolarization (ordinates; in mV) and the interval of stimulation (abscissae; in msec) the same as in Fig. 6.

5) **Effect of long stimulation**

Examples of the stimulation period of 11 to 28 sec are shown in Fig. 8. In A (58 msec interval) the hyperpolarization developed steeply and the potential began to return toward the normal level while the stimulation continued. In B (610 msec interval) the potential attained a plateau and was maintained for the

period of continued stimulation. In C (1.1 sec interval) the small hyperpolarization gradually increased during the whole period of stimulation, and this tendency further continued for a short period after cessation of the stimulation as was above described.

6) Delayed response

As is shown in Figs. 5 and 6, the latent period of the evoked hyperpolarization was about 500 msec. It is worth while noticing that in the case of a small number of stimuli (Fig. 3 A), the hyperpolarization started after a latent period and grew to the maximum in about 1 sec. Figs. 6F and 8B and C show the cases in which the course of hyperpolarization continued after the end of stimulation for such a short period as was comparable with the latent period.

7) So-called rebound excitation

In some cases, the spontaneous discharge of the sinus often occurred in the quiescent heart after the hyperpolarization caused by vagus stimulation (58 msec interval, 30 shocks) had recovered to a nearly original level (Fig. 9 A). In Fig. 9B and C, spontaneous discharge occurred once or twice immediately after the hyperpolarization. In D left, stimuli were given during continuing spontaneous discharge. The stimulation resulted in quiescence of the heart and an evoked hyperpolarization was followed by one action potential. The continuous
Fig. 9. An example of so-called rebound excitation. Bullfrog's sinus. When the hyperpolarization evoked by the vagus stimulation almost subsided, a few spontaneous discharges appeared. A: Very slowly beating state. 58 msec interval and 30 shocks. Temperature; 24.9°C (room). Oct. 14, 1966. B: Quiescent state. 23 msec and 100 shocks. Temperature; 24.9°C (room). Oct. 1, 1966. C: Quiescent state. 58 msec and 100 shocks. Temperature; 22.5°C (room). Oct. 21, 1966. D: At first beating. The right and the left records from the same point of the cell. The hyperpolarization in the right appears larger than in the left, but the maximal magnitude of hyperpolarization measured from zero level are nearly equal in both records. 58 msec and 50 shocks. 0.6 M Na₂SO₄ electrode. Cal.: 20 mV. Temperature: 23°C (room). Oct. 18, 1966.

record is shown on the right of D; the similar response in a quiescent heart. In comparing them, it is noticed that the magnitude of hyperpolarization measured from the zero level of the potential was nearly equal. While the magnitude of evoked hyperpolarization measured from the diastolic level in the beating heart was smaller than the magnitude of hyperpolarization in the quiescent one, namely the quiescent fiber which had a lower membrane potential was hyperpolarized to the same level.

8) Inhibition of the heart beat and the inhibition period after vagus stimulation of various patterns

The experiments mentioned in this section were carried out on the tortoise's heart. With vagus stimulation of various patterns, the heart ceased to beat as reported above. The remaining inhibition period (T_{eff}) in the present paper was defined as the time from the end of stimulation to the first discharge of the heart. In Fig. 10, T_{eff} were plotted against the interval of stimuli. The curves indicate the results obtained with various stimulation periods. It shows that, within a wide
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Fig. 10. The relation between the period of remaining inhibition (T\text{eff} in sec, ordinates) and the interval of stimulation of the right vagus (msec on logarithmic scale, abscissae). Tortoise's heart, extracellular recording. Period of stimulation: $\ast$, 5; $\circ$, 10; $\bullet$, 20; and $+$, 50 seconds. The plot of 250 msec on the axis shows the escape during the vagus stimulation. The plateau in each curve shows the range of constant T\text{eff}. Temperature: 20.2°C (room). Mar. 17, 1965.

range of intervals, maximal T\text{eff} was about 17 sec in the 10 and 20 sec stimulation period and about 12 sec in 5 and 50 sec stimulation period. It will be easily expected that T\text{eff} increased with prolongation of stimulation period. It will be discussed later why T\text{eff} after 50 sec stimulation was much shorter than that after 20 sec stimulation. With an extraordinarily long stimulation, escape firing occurred during the stimulation as will be described in the next section. The relations between T\text{eff}, the number and interval of stimuli are shown in Fig. 11. The optimal number and the optimal interval of stimuli to obtain a maximum T\text{eff} were thus found. It was experienced, though not shown in the figure, that the quiescent state of the heart continued for more than thirty minutes with a continuous stimulation of 100 msec interval.

9) Vagus escape

When vagus stimulation was continued too long, the heart began to discharge spontaneously (vagus escape) despite of the continuation of vagal stimulation. There were two types of escape. One of them was complete escape, which was induced by the stimulation of short interval: the heart began to beat with a rhythm similar to the normal (Fig. 12, upper). The other was incomplete escape: this was induced by the stimulation of a relatively long interval. In the latter the rhythm of the escaping beat was very slow and somewhat irregular as compared with the normal one (Fig. 12, lower). With further continuation of stimulation, the occurrence of the escape tended to become more and more regular, but its period was still much longer than the normal (not indicated in the figure).
DISCUSSION

Accumulation of transmitter, exhaustibility and desensitization. In the present experiments, the hyperpolarization and the inhibition of the heart beat by vagus stimulation were investigated. It is not certain whether the magnitude of hyperpolarization or the duration of inhibition ($T_{eff}$) is related linearly with the amount of transmitter released, even if the sensitivity to the transmitter is taken to be unaltered. Tentatively, the results will be explained by the assumption that
Fig. 12. Tachogram showing the vagus escape. Tortoise's heart. Stimulation period: 100 sec. Upper: complete escape; 20 msec interval stimulation. Lower: incomplete escape; 500 msec interval stimulation. The rhythm of complete escape was normal, and that of incomplete escape was slow and irregular. Temperature: 21°C (room). Feb. 12, 1965.

the hyperpolarization or the period of remaining inhibition runs parallel with the accumulation of the transmitter; that is to say, they increase or decrease with the amount of accumulated transmitter so far as the sensitivity to transmitter is unchanged. Moreover it is simply assumed that by repetitive stimulation the transmitter accumulates near the nerve terminals, but at the same time it decreases during the stimulation by destruction and/or diffusion depending on the grade of its accumulation. Thus, the amount of transmitter will be expected to increase exponentially to a certain saturation level. As regards the membrane potential, it is probable that it changes non-linearly with the accumulation of transmitter and practically saturated by its property. Therefore the saturation of hyperpolarization, as shown in Figs. 4, 5, 6 and 8, will be possible by two different factors: the dissipation of transmitter and the membrane property.

By comparing A with B in Fig. 5, it is seen that the gradient of hyperpolarization at the end of stimulation of 5.8 sec with 23 msec interval (B) is almost
horizontal, while the gradient at the half point of stimulation with 11.5 msec interval (A) is definitely increasing. The number of stimuli was the same in both cases. Similarly the gradient of hyperpolarization at the end of 5.8 sec stimulation with 58 msec interval (Fig. 5 C) is decreasing, while that in the case of stimulation of 23 msec interval (B) is increasing at the half point of stimulation (2.9 sec) at which the number of stimuli was the same as in (C). Similar relations are found in other recordings. If the reduction of hyperpolarization is brought about by the exhaustion of transmitter due to rapid repetition of stimuli, it will be expected to be less pronounced in the case of long interval stimulation. But by comparing the gradients of hyperpolarization evoked by the same number of stimuli, the reduction was found rather stronger in cases of long interval stimuli. Therefore some factors other than the exhaustion of transmitter may play a part; some kind of desensitization to the transmitter probably appears during continued stimulation. Similar results are found by comparing the figure of hyperpolarization evoked by the constant number (40) of stimulation in Fig. 6 except A, where the stimulation interval is too short. Accumulation of transmitter may be expected to occur sooner with short interval stimuli since the time for dissipation is short. But the magnitude of hyperpolarization with the interval of 11.5 msec (Fig. 6 A) was far smaller. This apparent paradox can easily be interpreted by inevitable failure of nervous conduction due to the refractoriness of the stimulated nerve. The shorter the interval, the more failure will occur especially during the latter stage of continued stimulation. Thus, there was an optimal interval of stimuli for evoking the hyperpolarization as is shown in Fig. 7 B and C.

The period of remaining inhibition (T_{eff}) is mainly related to the amount of accumulated transmitter at the end of stimulation. The optimal interval for the maximal T_{eff} is indicated in Figs. 10 and 11. Fig. 10 shows that T_{eff} was saturated by the stimulation with equal intervals for 10 and 20 sec at a higher level than by the stimulation for 5 sec; but T_{eff} was lower with a long stimulation for 50 sec. Moreover with longer continued stimulation, the vagus escape appeared. As demonstrated in Fig. 11, by the stimuli of interval from 10 to 100 msec, T_{eff} curves ascended together with prolonged stimulation period, although the same was not necessarily true for other experiments. However, the curves of shorter interval always reached earlier their maximum. Probable explanation for this is as follows: because of failure of nervous conduction in the case of shorter interval stimulation, as stated above, the number of the effective stimuli is expected fewer than that of the given stimuli to the nerve, especially at the later stage of continued stimulation. With longer continued stimulation, the vagus escape appeared which is indicated by plotting on the axis T_{eff}=0.

The curves for 200 msec and 500 msec in Fig. 11 are quite different from others. The curves of T_{eff} for 200 msec are already descending at 10 sec in spite of fewer number of stimuli as compared with that of 100 msec. Since it is probable that the exhaustion of transmitter, if it occurs, will be more remarkable with
increasing number of stimuli with short interval stimulation than with long interval stimulation. The contradictory result is seen in the lower figure. It may easily be supposed that a smaller amount of transmitter will accumulate and smaller magnitude of hyperpolarization is produced by longer interval stimulation. As a result rapid dissipation of transmitter is expected to occur and makes $T_{\text{eff}}$ shorter. However, why the incomplete vagus escape (Fig. 12, lower) appeared during a continued stimulation cannot be explained only on the basis of the amount of transmitter. It must be assumed that such an opposing factor as is observed in the case of hyperpolarization may be growing desensitization to the transmitter in the course of stimulation.

The inhibition of the heart beat is originated from the pacemaker, whereas the hyperpolarization occurs not only at the pacemaker but also in other regions. However, from the present results, it seems very likely to assume that the both events might be attributed to the release of the transmitter.

**Shift of pacemaker.** When the recorded region began to fire spontaneously after hyperpolarization by the vagus stimulation, the firing level was not always constant. Hutter and Trautwein explained this phenomenon by assuming a shift of the pacemaker or alternation of excitability of the pacemaker. They showed only a record which suggested that the region of the pacemaker became more distant from the recording electrode; i.e., the critical level of the recurring discharge occurred already at a more hyperpolarized level than before. Such results were also obtained in the present experiments. Moreover, in rare instances, the discharge did not appear after vagus inhibition until a more depolarized level was attained than before (Fig. 2A). It suggests an approach of the pacemaker to the electrode. These phenomena of firing at a hyperpolarized or depolarized level present evidence of a shift of the pacemaker by vagus stimulation.

**Recovery of the discharge.** The form of discharge which appears after the inhibition by vagus stimulation is at first extraordinarily low and sharp (Figs. 1 and 2) as was already observed by Hutter and Trautwein. This may be attributed to incomplete recovery of the membrane activity, but further studies from the viewpoint of ionic hypothesis are still required.

**Prepotential and hyperpolarization.** Even when the pacemaker potential could not be recorded because the electrode was probably impaled too far from the pacemaker cell, hyperpolarization by vagus stimulation could be recorded in some cases (Fig. 2 C and D). The nerve ending which evoked the hyperpolarization might probably be located near the electrode.

Moreover, in some cases the prepotential appeared preceding the discharge, after the hyperpolarization produced by vagus stimulation had decayed, although the prepotential was very small before the stimulation (Fig. 2 A). In Fig. 2 B, the form of prepotential after vagus stimulation was different from that before stimulation. From these it cannot be generally predicted that the hyperpolarization by
vagus stimulation does not appear when the prepotential cannot be observed before the stimulation.

**So-called rebound phenomena.** Bennett\(^5\) reported that during the quiescent state of the smooth muscle of the guinea-pig taenia coli one or several discharges occurred spontaneously after the hyperpolarization produced by the inhibitory nerve stimulation (rebound excitation). Moreover, by the application of hyperpolarizing current, similar excitatory responses were elicited in the heart of chick embryo (Sperelakis and Lehmkuhl\(^6\)). The records of Fig. 9 in the present report are quite similar to their result. The resemblance between the records should be pointed out in this connection. Its explanation awaits further studies.

**Acknowledgment**

I am greatly indebted to Professor T. Wakabayashi for his advice and interest throughout the work. I am also grateful to Drs. T. Tosaka, S. Iwasaki and S. Yamagishi for their suggestions. Thanks are also due to Dr. S. Nakajima for his revision of the manuscript.

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