EFFECTS OF TEMPERATURE CHANGE, OXYGEN DEPRIVATION AND CATIONS ON THE ATRIAL RESPONSES TO VAGAL STIMULATION

NOBORU TODA, MOTOHATSU FUJIWARA AND KIRO SHIMAMOTO

Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto

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It is generally believed that the cardiac fibers of the right vagus nerve terminate, in large part, near the sinoatrial node and some fibers distribute widely in the atria, and that most of the cardiac fibers of the left vagus supply the atrioventricular node and bundles. On the other hand, there are opposite conclusions regarding the existence of the vagus nerve endings in the ventricles. Electrical stimulation of the vagal fibers to the heart has usually been performed in the open chest animal or in the heart-lung preparation of the dog. Many investigators agree with the inhibitory nature of the vagal innervation on the heart, while there is some evidence for existence of the cardio-accelerator fibers in the vagus nerve (1-4). Middleton et al. (5) have concluded that some vagal fibers make connection with the chromaffin cells or the adrenergic neurons located in or near the heart by demonstrating an adrenaline-like substance in the perfusate of the cat's heart which is responding with the increase in rate and amplitude to vagal stimulation. McEwen (6) has observed an inhibitory effect of vagal stimulation in the rabbit's heart for many hours after the isolation. He, further, has demonstrated that the vagal stimulation restores the normal rhythmicity of contraction in the atria arrested by suspending in the bath for many hours, and that the same vagal stimulation inhibits the regular rhythm restored by the addition of acetylcholine. Using the same type of preparations, Burn and Rand (7) have observed the restarting effect of vagal stimulation on the heart which ceased to beat by cooling.

The marked acceleration of the repolarization phase in the atrial membrane potentials of the dog's and rat's hearts in situ following vagal stimulation has been shown by Hoffman and Suckling (8) and Biersteker et al. (9). Similar observation was made on the turtle's heart in situ by Churney et al. (10). Although the vagal effect on the membrane potentials of the isolated frog's heart was demonstrated by Hutter and Trautwein (11), the vagal effect on the atrial or ventricular membrane potentials of the isolated mammalian heart has not hitherto been presented. During the study of the effect of vagal stimulation on the transmembrane potentials (12), the authors have been confronted with many difficulties which should be previously removed in the iso-
lated atrial preparation with the vagal innervation. Accordingly, the present report concerns with the physiological studies on the effects of vagal stimulation on contraction rate and amplitude in the isolated rabbit's and guinea-pig's atria.

METHODS

Thirty rabbits weighing 1.8 to 2.5 kg and fifty guinea-pigs weighing 250 to 400 g were used.

Preparation of the vagally innervated atria of the rabbit or the guinea-pig: The preparation was made by the method of McEwen (6). Immediately after sacrifice of the animal by cutting the common carotid arteries the chest was opened by parasternal incision, and the heart with the bilateral vagi and sympathetic nerves, sternum, clavicles, ribs, esophagus, trachea, bronchi and cervical and upper thoracic vertebrae was isolated totally. The thoracic contents were immersed in the oxygenated nutrient solution at 30°C. After resection of the ventricles the cardiac branches of the vagi and atria were cleaned out from other tissues. The atria attached with the vagus nerves were suspended in the nutrient solution, the composition of which will be described below, and the atrial contraction magnified ten times via a spring lever was recorded on the smoked paper.

Stimulation of the vagus nerves: The square wave stimuli delivered from the Nihon-Koden Kogyo electronic stimulator (Type M5E-3) were applied, unless otherwise stated, to both vagi placed on the platinum electrodes above the surface of the nutrient solution. Care was taken to keep the nerve as wet as possible. The intensity of stimulation was usually submaximal and the time length of stimulation was 30 seconds.

Calculation of the changes in rate and amplitude of contraction before, during and after stimulation of the nerves: The rate of atrial contraction was counted for 30 seconds before, during and after stimulation and expressed in terms of rate/min. The experiment was never started before the rate of spontaneous contraction was constant and three successive vagal stimulation gave nearly equal responses. The interval of every stimulation was usually 5 minutes, being enough to obtain uniform and reproducible responses.

The negative chronotropic and inotropic responses to vagal stimulation were determined as percent reduction in rate and amplitude of the atrial contraction during stimulation of the vagi. For the comparison of the effects of various experimental treatments on the vagal responses, percent changes of the responses after treatments were calculated.

The nutrient solution: The composition of the nutrient solution was as follows: NaCl 9.00 g (158.3 mM), KCl 0.42 g (5.6 mM), CaCl₂ 0.12 g (0.82 mM), NaHCO₃ 0.30 g (3.5 mM) and glucose 1.00 g (5.6 mM) in 1,000 ml of distilled water. Compared with the composition of the nutrient solution employed usually for the isolated heart, the concentration of CaCl₂ was reduced to one-half.

Physiological experiments: The effects of temperature change on the vagal responses were determined 10 to 20 minutes after the modification of bath temperature. The effects of hypoxia on the vagal responses were repeatedly tested at intervals of 5 to
10 minutes after cessation of oxygen-bubbling into the nutrient solution. The effects of increase in concentration of cations such as calcium and potassium on the vagal responses were similarly tested after adding the required amount of the cation to the nutrient solution. In the study of the effect of low sodium, the concentration of sodium in the nutrient solution was decreased to one-half, one-fourth, one-sixth and one-eighth by replacing with the isotonic sucrose solution. The effect of magnesium was tested by adding MgCl₂ to the nutrient solution at multiples of the molar concentration of CaCl₂.

The concentration of the drugs or cations in the bath was expressed in terms of g/ml or mM/l.

RESULTS

1. Effects of stimulus frequency

Both vagi were stimulated submaximally at various frequencies from 1 to 800/sec for 30 seconds. The ordinates in Fig. 1-A and -B show the percent change in the vagal effect on the rate and amplitude of rabbit’s and guinea-pig’s atria, taking the effect at a frequency of 20/sec as 100%, and the abscissae show the logarithmic values of frequency. The vagal negative response of rabbit’s atria in rate and amplitude was facilitated with the increase in frequency from 1 to 20/sec. Further increase in frequency induced no more facilitation of the response, and the response-frequency curve reached a plateau. In the guinea-pig’s preparation, on the other hand, the facilitation of the vagal response at frequencies from 1 to 10/sec was marked in the rate, but was not so in amplitude. Further increase in frequency produced a plateau in the response-frequency curve, and at frequencies of 500 and 800/sec the vagal response in rate and amplitude decreased slightly. In the guinea-pig’s preparation the vagal responses in contractile rate at the frequency of 1/sec and 4/sec were 27% and 38%, respectively, of that at the frequency of 20/sec, while the corresponding values in amplitude were 59% and 82%, respectively. The results show that the maximal vagal response in rate and amplitude is obtained by stimulation at the frequency of 20/sec. Therefore, a 20/sec stimulus frequency was used for the further experiments, unless otherwise mentioned.
The change in the pulse width from 0.5 to 2.0 msec did not markedly affect the responses. However, the pulse width below 0.2 msec resulted in a marked reduction in the vagal response. Accordingly, a 1.0 msec pulse width was used throughout the further experiments.

2. Comparison of the right and the left vagal stimulation

The effects of stimulation of the right and the left vagus on the rate and amplitude of the atrial contraction were compared in the guinea-pig's atria. In 14 preparations which responded well to stimulation of both vagi with a decrease in rate, 9 preparations showed a stronger response to stimulation of the right vagus than that of the left vagus, and 4 preparations showed no detectable difference between the responses to stimulation of either nerve, and remaining one preparation showed a predominant response to stimulation of the left vagus. On the other hand, the decrease in amplitude by stimulation of the right vagus nerve was stronger than that by stimulation of the left nerve in 5 preparations and the reverse effects were observed in 3 preparations. In the remaining 6, no difference between the responses to the right and the left nerve could be seen. The differences between the stimulating effects of the right and the left vagus nerve on the rate and amplitude of the atrial contraction may be attributed to the differential distribution of the right and the left vagus to the atria.

3. Effects of temperature

The effects of warming or cooling on the responses of the atria to vagal stimulation and to acetylcholine were studied. The most marked response to vagal stimulation was obtained at the temperature of 29° to 30°C in rabbit's and guinea-pig's atria. Figs. 2-A and -B show the correlation between the temperature and the changes in rate and amplitude following vagal stimulation in rabbit's and guinea-pig's atria. In these figures, the vagal response at the temperature of 30°C was taken as 100%. The amplitude of spontaneous atrial contraction decreased linearly with the increase
in temperature from 21° to 35°C. However, a few preparations showed a slight increase in amplitude. On the other hand, the rate of spontaneous contraction showed a linear increase with the rise of the temperature from 21° to 35°C (straight lines).

The maximal vagal effects in contractile rate and amplitude were obtained in the temperature of 29° to 31°C, and 28° to 31°C, respectively. The cooling of the preparation below 28°C reduced the vagal effects in rate and amplitude of the atrial contraction. At the temperature of 21°C the vagal responses in rate and amplitude were reduced to 27% and 21% of that at 30°C, respectively. The rise of temperature above 32°C also reduced both responses (Fig. 3). The vagal response at a temperature of 37°C in

![Fig. 3. Effects of the rise of temperature on the vagal responses and the spontaneous contraction in the guinea-pig's preparation. Numbers on the top of each figure show the contraction rate/min before, during and after vagal stimulation. Both vagi were stimulated at the signals under each tracing.](image)

rate and amplitude was reduced to 30% and 54% of that at 30°C. Thus, the atrial responses to vagal stimulation were affected in the course of the rise or fall of temperature. The atrial response to vagal stimulation upon the re-adjustment of temperature to 30°C was weaker than the original response at 30°C, and 15 to 20 minutes were usually required until the original response was obtained. The effects of the temperature change on the atrial responses to vagal stimulation were almost the same in rabbit's and guinea-pig's preparations.

When acetylcholine (10⁻⁸ to 3×10⁻⁸) was applied to the guinea-pig's vagus-atria preparation at 30°C, the rate and amplitude of spontaneous contraction were reduced by 10 to 15% and 40 to 70% of controls, respectively. The effects of the same concentration of acetylcholine on the rate and amplitude of the guinea-pig's atria, which were immersed in the nutrient solution for 20 minutes at the temperature of 25°C and 35°C and reduced the response to vagal stimulation by 25 to 45%, were tested. As shown in Table 1, the negative chronotropic response to acetylcholine was considerably augmented by a fall of temperature to 25°C and was reduced by a rise of temperature
to 35°C. The negative inotropic response to acetylcholine was less affected by the changes of temperature. The figures in the table of the rate and amplitude in response to acetylcholine and vagal stimulation show the percent changes of the responses comparing those at 30°C.

### TABLE 1. Effects of temperature change on the responses to vagal stimulation and to added acetylcholine (10⁻⁸ to 3 x 10⁻⁸) in the guinea-pig's preparations. The figures in this table show the percent increase (plus) or decrease (minus) in responses, comparing with the responses at 30°C.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>No. of exp.</th>
<th>Response to vagal stim.</th>
<th>Response to ACh</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Rate</td>
<td>Amplitude</td>
</tr>
<tr>
<td>25°C</td>
<td>4</td>
<td>-30%</td>
<td>-27%</td>
</tr>
<tr>
<td>35°C</td>
<td>3</td>
<td>-41%</td>
<td>-20%</td>
</tr>
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s.e.: standard error of mean.

4. **Effects of oxygen deprivation**

The continuous cessation of oxygen-bubbling in the nutrient solution produced the progressive and marked decrease in amplitude of the spontaneous contraction and the slight but gradually developing decrease in the spontaneous rate. The vagal response in rate and amplitude of the atrial contraction, on the other hand, was gradually augmented 5 to 10 minutes after the cessation of oxygen supply and the peak effects were observed 20 to 40 minutes thereafter when the amplitude of the spontaneous contraction was markedly decreased (Fig. 4). Fig. 5 shows the percent increase of the vagal responses in rate and amplitude following the cessation of oxygen supply and the recovery from it due to a full re-oxygenation. Only one (case III) out of 5 preparations was not affected by the treatment. Remaining 4 preparations were markedly or moderately increased in the response by the cessation of oxygen supply, although the degree was subjected to significant individual variations. The decrease in rate and amplitude of the spontaneous contraction...
atrial contraction was easily reversed by a full re-oxygenation for 10 to 20 minutes, while the increased response to vagal stimulation recovered more slowly and 20 to 40 minutes were necessary for a full recovery (Fig. 4).

The negative chronotropic and inotropic effects of exogenously added acetylcholine on the atrial contraction were not significantly affected by the cessation of oxygen supply for 15 to 30 minutes.

5. Effects of calcium

The effects of excess calcium on the spontaneous atrial contraction and on the atrial responses to stimulation of the vagi were studied. A two-fold increase in the content of calcium gradually increased the amplitude of spontaneous contraction, and the maximum increase of 50 to 150% was attained 5 to 10 minutes later and lasted for 60 to 80 minutes or more. The rate of the spontaneous contraction did not show any significant change. Further increase in the concentration of calcium did not affect the rate, while it increased the amplitude.

However, the vagal responses in rate and amplitude were augmented 10 to 15 minutes after the increase in calcium concentration to 1.64 and 3.28 mM/1 (two- and four-fold increase). The increased responses lasted for 60 minutes or more. Fig. 6 shows the percent increase in the vagal responses of 7 preparations following the increases in calcium concentration. A two-fold increase in the content of calcium increased the vagal response by 30 to 260% in rate and 20 to 150% in amplitude. But a four-fold increase in calcium concentration did not produce further increase in the vagal response. The effect of the change in calcium concentration did not markedly differ between rabbit's and guinea-pig's atria.

The negative chronotropic and inotropic effects of added acetylcholine were not significantly affected by a two-fold or four-fold increase in calcium concentration.
6. Effects of magnesium

The effects of magnesium addition in molar concentrations of one and a half, two, three and four times of calcium in the nutrient solution were studied. The rate and amplitude of the spontaneous contraction were only slightly decreased by the addition of magnesium.

As shown in Fig. 7, the vagal responses in rate and amplitude of the atrial contraction were not definitely affected by the addition of magnesium in concentration of one and a half to four times of calcium. Among eight preparations tested, two showed a moderate reduction of the vagal response in rate, one a slight reduction in amplitude, and the remaining preparations showed no significant changes. The further addition of magnesium did not affect the vagal responses. The augmenting effects on the vagal responses of the increase in calcium concentration were not antagonized by the addition of magnesium. There was no difference of the effects of magnesium between rabbit's and guinea-pig's preparations.

7. Effects of potassium

A two-fold increase in concentration of potassium in the nutrient solution markedly decreased the rate and amplitude of the spontaneous atrial contraction. The decrease in amplitude manifested immediately after the addition of potassium, and the peak effect of about 40% depression was observed 10 to 20 minutes later. Thereafter, the depression slightly reduced in degree and about 20% depression was maintained from 30 minutes after addition. The atrial rate gradually decreased after the addition of potassium, and the decrease was about 15% at the time of the peak effect in amplitude and was restored gradually along with recovery of the amplitude. The rate of the spontaneous contraction at 30 minutes after addition of potassium approximated to the original rate, while the amplitude was not fully recovered (Fig. 8). The further
increase to a three-fold in potassium concentration abolished the spontaneous contraction within 5 to 15 minutes. However, the spontaneous contraction was easily restored by washing with the standard solution.

The vagal responses in rate and amplitude of the atrial contraction were augmented by a two-fold increase in potassium concentration. The augmenting effect of more than 100% was obtained 5 to 10 minutes after the addition of potassium, and successive stimulation of the vagi induced the marked and constant augmentation of the vagal response even during partial recovery in the spontaneous atrial contraction (Fig. 9). In some of preparations which exhibited the marked decrease in spontaneous rate and amplitude following the increase in concentration of potassium, marked positive chronotropic and inotropic effects (after-positivity) were observed immediately after the cessation of stimulation. A two- or four-fold increase in concentration of calcium antagonized the decrease in amplitude of the spontaneous contraction induced by excess potassium, but potassium and calcium cooperatively augmented the vagal response of the atria.

In the atria which ceased to beat or which were going to cease by an increase in concentration of potassium to 16.8 mM/l (three-fold increase), the vagal stimulation often restored the spontaneous beat (Fig. 10). However, the duration of the re-appearance of spontaneous beat was subjected to individual variations from several seconds to several minutes. When the spontaneous atrial contraction was abolished by an excess amount of potassium, the administration of acetylcholine in concentrations of $10^{-4}$ to $10^{-7}$ in guinea-pig's atria and of $10^{-9}$ to $5 \times 10^{-6}$ in rabbit's atria sometimes restarted the spontaneous beat transiently. However, such restarting effect of vagal stimulation or the administration of acetylcholine was obtained only within a short period after the atrial arrest and not after 20 minutes. The similar restarting effects were obtained by the addition of calcium in concentrations of 0.82 to 1.64 mM/l and of adrenaline in a concentration of $10^{-6}$. The restarting effect of calcium appeared gradually. The recovery in rate was incomplete and usually of 20 to 40 beats/min, while that in amplitude was considerably marked. On the other hand, the administration of adrenaline markedly restored both rate and amplitude, but the recovering effect lasted only for 5 to 10 minutes.
FIG. 10. Upper two figures show the vagal response before (the left figure) and after (the right) the first potassium addition, 5.6 mmol/L. The lower shows the restarting effect of vagal stimulation on the atria arrested by the second addition of potassium, 5.6 mmol/L. Numbers on the top of each figure show the contraction rate/min before, during and after vagal stimulation. Both vagi were stimulated at the signals.

FIG. 11. Effects of sodium reduction to one-fourth (upper figure) and to one-half (lower figure) of the standard concentration on the rate (solid line) and amplitude (broken line) of the spontaneous contraction. Ordinates: percent change in rate and amplitude. Abscissae: time after sodium reduction.

8. Effects of sodium

The replacement of a half of sodium in the nutrient solution with the isotonic sucrose solution significantly increased the amplitude of the atrial spontaneous contraction. The maximum increase in amplitude was observed 10 to 20 minutes after replacement and then declined gradually, but about 20% increase was still observed 40 minutes later (Fig. 11). The rate of the spontaneous contraction was not affected or slightly decreased by the replacement. The reduction of sodium to one-fourth in the nutrient solution produced a further increase in the amplitude and 80 to 90% increase was observed 20 to 30 minutes later. Thereafter, the increased amplitude declined slightly and the steady level of the amplitude was obtained. In contrast to the increase in amplitude, the rate of spontaneous contraction decreased gradually until about 40% decrease was observed 40 minutes after the reduction of sodium to one-fourth (Fig. 11). Sometimes, an extreme reduction in rate was followed by manifestation of arrhythmia or arrest of the spontaneous beat. The similar irregularities of the rate and amplitude and the abrupt arrest of the spontaneous beat were observed 5 to 22 minutes after the reduction of sodium to one-sixth or one-eighth. These effects of sodium replacement were variable in degree and time course. The re-adjustment of sodium-deficient solution to the original
composition restored promptly the normal rhythmic contraction.

The replacement of one-half of sodium with the isotonic sucrose produced an augmentation of the vagal response in the atrial rate in 4 out of 8 preparations but no effect in others. The vagal response in the amplitude was augmented by 43 and 67% in 2 of 9 preparations, insignificantly changed in 4 and reduced by 14 to 20% in remaining 3 following the replacement of one-half of sodium (Fig. 12). The reduction of sodium to one-fourth resulted in a marked dissociation of the effects on the vagal responses in rate and amplitude. The response in rate to vagal stimulation was augmented by 26 to 96% in 4 out of 6 atria and not affected in remaining 2, while the response in amplitude was reduced by 23 and 47% in 2 out of 6 atria and not affected in remaining 4. Therefore, it is likely that the replacement of sodium in the nutrient solution with the isotonic sucrose tends to augment the negative chronotropic effect of vagal stimulation but reduce or not affect the negative inotropic effect. The reduction of sodium to one-fourth often resulted in an appearance of the positive chronotropic and inotropic effects immediately after the cessation of vagal stimulation (after-positivity). In some cases the rate of the spontaneous contraction doubled to that before stimulation.

In the atria which ceased to beat by a reduction of sodium to one-fourth or one-sixth, vagal stimulation often restored the spontaneous contraction. The restarted contraction was maintained for 30 seconds to 10 minutes (Fig. 13). However, the atria arrested similarly by a reduction of sodium to one-eighth were never restarted by the vagal stimulation. The atria arrested by a replacement of sodium with the isotonic sucrose were not restarted by an addition of acetylcholine $10^{-8}$ to $10^{-5}$ but restarted by an addition of adrenaline $10^{-4}$. The pattern of the restart by adrenaline was closely similar to that by the vagal stimulation. The response of the rabbit's atria to the replacement of sodium did not markedly differ from that of the guinea-pig's atria.

9. Effects of barium

The effects of barium in concentrations of 1.5 to 3.0 mM/l on the spontaneous atrial contraction and the vagal responses of the atria were studied in rabbit's and guinea-pig's preparations. The addition of barium markedly increased the amplitude
of spontaneous contraction, while it decreased the rate. Whereas the similar effects on
the spontaneous contraction, associated with the augmentation of the vagal effect on
the rate, were obtained by the reduction of sodium to one-fourth, the effects of barium
were always associated with the reduction of the vagal effects in rate and amplitude.
The reduction of the atrial responses to vagal stimulation by addition of 1.5 mM/l of
barium is shown in Fig. 14. Before the addition of barium the negative chronotropic

FIG. 13. Restarting effects of vagal stimulation on the atria arrested
by sodium reduction to one-fourth in the guinea-pig's atria. Numbers on the top of each figure show the contraction rate/min
before, during and after vagal stimulation. Vagus nerves were
stimulated at the signals. The left of upper figures : 5 min
after sodium reduction. The right of upper figures : 15 min
after sodium reduction. The spontaneous contraction abruptly
stopped and was restarted by vagal stimulation. Lower
figure : 40 minutes after sodium reduction. Once the spontane-
ous contraction restarted, the vagal stimulation again produced
the negative chronotropic and inotropic responses.

FIG. 14. Effects of barium (1.5 mM/l) on the spontaneous contraction
and the vagal response in the guinea-pig's atria.

response to vagal stimulation was 55% (N=3), and corresponding value 20 to 30 minutes
after addition of barium in a concentration of 1.5 mM/l was 23% (N=3). Similarly,
the vagal response in the amplitude during stimulation was reduced from 92% (N=3)
to 33% (N=3) following the addition of barium in the same concentration.
DISCUSSION

The results obtained from the physiological studies of the rabbit's and guinea-pig's atria with the vagus nerves are summarized in Table 2. Though the vagus nerves-atria preparation of guinea-pig was easily isolated, the success of the preparation from rabbit was about 60%.

<table>
<thead>
<tr>
<th>Table 2. Summary of results obtained in the present experiments.</th>
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<tbody>
<tr>
<td>Response to vagal stim.</td>
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<tr>
<td>Rate</td>
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</tr>
<tr>
<td>Rise of temperature</td>
</tr>
<tr>
<td>Fall of temperature</td>
</tr>
<tr>
<td>Oxygen deprivation</td>
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<tr>
<td>Calcium addition</td>
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<tr>
<td>Magnesium addition</td>
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<tr>
<td>Potassium addition</td>
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<tr>
<td>Sodium reduction</td>
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<td>Barium addition</td>
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*: Data reported by Tibbs and Berman (42).

Intensity of electrical stimuli required for eliciting the negative chronotropic and inotropic effects to vagal stimulation strikingly differed according to the animal species and also individuals in the same species. The effective intensity of the stimuli usually ranged from 3.2 to 5.1 V for rabbit's preparations, while stimulation of the vagus nerves of guinea-pig's preparations at intensity of 0.5 to 2.0 V elicited the marked effects. Very weak stimuli such as 0.01 V occasionally produced significant vagal effects in guinea-pig's preparations. In accord with the differential sensitivity to vagal stimulation between the atria of both species, the negative response to added acetylcholine also differed between both species. Guinea-pig's atria responded well to concentrations of $10^{-8}$ to $3 \times 10^{-8}$ of acetylcholine, while rabbit's atria to a concentration of $10^{-4}$.

In the physiological experiments the spontaneous contraction and the atrial responses to vagal stimulation were tested under variable experimental conditions. A number of investigators obtained the negative chronotropic and inotropic responses to vagal stimulation of the isolated atrial preparation but the frequency of stimuli used varied considerably (7, 10, 20, 23 or 25/sec). Recently, Burn and Weetman (13) have reported that the responses of the vas deferens to hypogastric nerve stimulation of various frequencies differ not only quantitatively but also qualitatively. Detailed studies in the present experiments on the relationship between the stimulus frequency and the negative chronotropic and inotropic effects showed that the optimal frequency was 20/sec in rabbit's preparation and 10 to 20/sec in guinea-pig's preparation. The stimulus frequency giving a maximal response to the cat's heart in situ was 30/sec (14) and somewhat higher than the frequency required for the isolated rabbit's and guinea-pig's heart
in this experiment. The sub- or supramaximal stimulation of the vagus nerves at a frequency of 20/sec and a duration of 1 msec, and at intervals of 5 to 10 minutes elicited the reproducible response for 8 to 10 hours after setting-up the preparation.

Comparison of the atrial responses to stimulation of the right and the left vagus nerves showed that the chronotropic response was more dominant to the right than to the left nerve, while the inotropic response did scarcely differ between both nerves. If the negative chronotropic effect reflect the activity of the vagal innervation on the sinoatrial node, the results show clearly that innervation on the sinoatrial node of the right vagus predominates that of the left vagus. However, the fact that one-fourth of the preparations responded to stimulation of either nerve to almost same extent may indicate the cholinergic innervation of the sinoatrial node from the left as well as the right vagus, or alternatively the existence of rate-decreasing atrial mechanism independent of the sinoatrial node. On the other hand, if the negative inotropic effect derive from the activity of the cholinergic innervation on the contractile system in the atrial muscle, the extent of the innervation of the right and the left vagus on the atrial muscles should be almost equal since the response did scarcely differ between both sides.

Lowering of the temperature of the nutrient solution below 29°C gradually reduced the vagal responses in proportion to the temperature fall. On the other hand, the atrial response to added acetylcholine at 25°C was only slightly affected in amplitude and was rather augmented in rate. Cooling of the pelvic nerve-colon preparation of rabbits (15) and of the vagus nerve-stomach preparation of rats (16) firstly blocked the response to preganglionic nerve stimulation, then abolished the response to nicotine or DMPP and finally that to acetylcholine. Webb (17) and Innes et al. (18) showed that cooling did not affect the responses to added acetylcholine of the rabbit's atria and the guinea-pig's intestine. From these results, it is likely that the reduced response to vagal stimulation under cooling derives from blockade of the intracardiac cholinergic ganglia and, partly, of the postganglionic structures. It was reported that the amount of acetylcholine released in the perfusate of the superior cervical ganglia by preganglionic stimulation at a high frequency (10/sec) was reduced by cooling of the ganglia (19) but the acetylcholine release by a low frequency of stimulation (2/sec) was not affected (20). Therefore, blockade of the cardiac ganglia and the postganglionic structures under cooling may possibly derive from the diminution of the release of acetylcholine from the pre- and postganglionic terminals.

Rise of the temperature of the nutrient solution above 30°C also reduced the responses of the atria to vagal stimulation. The responses of the atria to added acetylcholine were also diminished by the elevation of the temperature to 35°C. Whittaker (21) has shown that the intragranular acetylcholine is totally released by incubation of the mitochondrial fraction of the brain homogenate in the isotonic sucrose solution for 90 minutes at 37°C. The amplitude of action potentials in the human vagus nerve was reported to be maximum at 56°C and reduced in proportion to the lowering of temperature (22). Although the conduction velocity of the rabbit vagus nerve and the
release of acetylcholine from the vagus nerve terminals may be increased by the rise of temperature, the decrease in sensitivity of the atria to acetylcholine may surpass them and result in the decrease in the vagal effects.

The responses of the atria to vagal stimulation were augmented by deprivation of oxygen supply but the responses to added acetylcholine were not affected. Washizu (23) has shown that the electrical excitability of the motoneuron in the anterior horn of the isolated toad spinal cord increases at the initial phase of oxygen deficiency. Brooks and Eccles (24) explained that the increased excitability of motoneuron derived from the depolarization of the synaptic membrane due to oxygen deficiency. Ruch and Fulton (25) describe that extreme anoxemic state results in increased outflow of the intracellular potassium from the heart and the corresponding changes of ECG, and that the similar ECG changes are produced by hyperkalemia. Trautwein et al. (26) have shown a reduction in the resting potential of isolated papillary muscle fibers and Purkinje fibers of the cat’s heart under oxygen deprivation. These facts lead us to the conclusion that deficiency of oxygen supply to the atria induces either increase in extracellular potassium or/and decrease in intracellular potassium and these changes in the potassium concentration result in the increased atrial response to vagal stimulation.

The calcium concentration in the standard nutrient solution used in the present studies was decreased to one-half of normal Ringer solution. The addition of calcium to the standard solution markedly increased the amplitude of the spontaneous beat but did not affect the rate. The similar effects of calcium on the spontaneous beat was shown by Webb (17). It is likely that the positive inotropic effect of calcium derives from the affection of E-C coupling by the increased uptake of calcium. A two-fold increase in calcium concentration markedly augmented the atrial responses to vagal stimulation. Further increase in calcium concentration to a four-fold did not further increase the effects of vagal stimulation, despite the manifestation of further increase in amplitude of the spontaneous contraction. It has been confirmed electrophysiologically that the addition of calcium does not affect the release of acetylcholine from the neuromyajal junction in resting state but this ion increases the release of acetylcholine following presynaptic stimulation. Hodgkin and Keynes (27) have shown that the influx of "CaCl₂ increases during stimulation of the squid giant axon. Citing the demonstration of Cleland and Slater (28) that calcium taken up by the heart muscle fiber destroys the structure of sarcosome, Hodgkin and Keynes have suggested that calcium taken up by the nerve fibers during stimulation destroys the synaptic vesicle located in the nerve terminals and thereby releases acetylcholine. The increased responses of the atria to vagal stimulation observed after the first addition of calcium may derive from the increased release of endogenous acetylcholine from the synaptic vesicles. Failure to increase the vagal response by further addition of calcium may be due to exhaustion of the easily available acetylcholine. The assumption is supported by the fact that calcium did not affect the responses of the atria to added acetylcholine.

The addition of magnesium has been reported to antagonize the action of calcium
on the neuromyal junction and to reduce the release of acetylcholine from the motor nerve endings (29, 30). Therefore, the dose of magnesium added to the nutrient solution was determined according to the concentration of calcium. The addition of magnesium in the concentration of 1.23 to 3.28 mM/l did not affect the atrial responses to vagal stimulation in many preparations, but reduced the responses in a few preparations. The depressant effect of magnesium was independent of the concentration employed. In addition, the application of magnesium did not affect the increase in the atrial responses to vagal stimulation following the addition of calcium.

The addition of potassium to the atrial preparation reduced the rate and amplitude of the spontaneous beat. The reduction in amplitude was long-lasting and recovered only partially about 30 minutes after the addition. The depressant effect of potassium may be due to the antagonistic action of potassium against calcium in the E-C coupling or alternatively, due to the depolarizing action of potassium with subsequent partial block of the conduction system. The depolarization of the cat’s and rabbit’s atrial fibers in the increased concentration of extracellular potassium was shown by Burgen and Terroux (31) and Hoffman (32). If it is possible that the decrease in the resting potential induces the conduction failure and thereby the decrease in the amplitude of spontaneous contraction, the partial recovery of the amplitude 30 minutes after the addition of potassium may result from a partial restoration of the resting potential due to intracellular uptake of added potassium. The addition of potassium in a higher concentration (three-fold increase) progressively depressed and finally abolished the spontaneous beat. Stimulation of the vagi or the addition of acetylcholine in the concentration of $10^{-8}$ to $10^{-7}$ in the guinea-pig’s atria and of $10^{-6}$ to $5 \times 10^{-6}$ in the rabbit’s atria sometimes restarted the spontaneous contraction. The restarting effect of vagal stimulation suggests that the vagi are more resistant than the atrial muscle to conduction failure resulting from the depolarization. Marshall and Vaughan Williams (33), Marshall (34) and Burn and Rand (35) have shown that higher concentrations of quinidine and eserine, or cooling of the preparation decreases the resting potential of the atrial muscle fibers below 60 mV and abolishes the spontaneous contraction by blocking the impulse conduction from the sinoatrial node to the atrial muscle, and that the addition of acetylcholine or vagal stimulation restarts the spontaneous contraction by recovering the impulse conduction with the increase in the resting potential. The results obtained in the present experiments that the stimulation of the vagus nerves or the addition of acetylcholine restarted the spontaneous rhythmicity of the atria arrested by potassium may be another example of the experiments of Burn et al.

The abolition of the spontaneous rhythm caused by the addition of potassium was easily and markedly antagonized by the addition of calcium and adrenaline. The antagonistic effect of calcium was clearly shown against the amplitude-decreasing effect of potassium. It has been reported (36) that the lowering of resting potential caused by excess extracellular potassium is diminished if extracellular calcium is elevated. However, it has not been proved whether the abolished rhythm caused by excess potassium
is recovered due to the increase in resting potential following the addition of calcium. On the other hand, the antagonistic effect of adrenaline against both rate- and amplitude-decreasing effects of potassium manifested abruptly and markedly but transiently. In the experiment using the toad's heart Hayashi and Azuma (37) showed that the decrease in resting potential caused by excess potassium could be recovered by the addition of adrenaline. However, such effect of adrenaline on the mammalian heart has not been reported elsewhere.

The addition of potassium augmented the atrial response to vagal stimulation. It has been postulated that the increase in concentration of extracellular potassium decreases the resting potential of the nerve fibers (38), and this depolarization may contribute to the increase in excitability of the nerve. Whittaker (21) showed that the increase in concentration of extracellular potassium did not affect the release of acetylcholine from the storage granules of brain homogenate. However, using the same experimental methods Takeno and Yanagiya (39) have demonstrated the increased release of acetylcholine in response to the increased concentration of potassium, and Kataoka (40) similarly observed the increase in serotonin release by potassium. Along with the electrophysiological aspects of potassium action, the increased release of acetylcholine by potassium may contribute to the augmentation of the atrial responses to vagal stimulation.

The decrease in concentration of sodium increased the amplitude of the spontaneous contraction, while it decreased the rate. Using the isolated frog's heart and the isolated rabbit's atria, Daly and Clark (41) and Tibbs and Berman (42) showed an increase in the amplitude of the spontaneous contraction following the reduction of sodium in the nutrient solution. The latter authors attempted to explain the effect with a mutual antagonism of calcium and sodium. In the present experiments the reduction of sodium to one-fourth of that in the standard solution markedly decreased the rate. The reduction in the concentration of sodium was shown to induce the reduction in the prepotential with subsequent decrease in the rate (43).

The reduction of sodium concentration augmented the vagal response in the rate and sometimes reduced the response in the amplitude. Tibbs and Berman (42) obtained the similar result following added acetylcholine on the atria suspended in the low sodium solution. While the potentiating effects of low sodium on the vagal negative chronotropic response can be interpreted by the increased release of endogenous acetylcholine and/or by the increased sensitivity of the atria to acetylcholine, the failure to augment the negative inotropic response may result from the increased contractility under low sodium. The reduction of sodium content in the nutrient solution was reported to depress the amplitude of the atrial transmembrane action potentials in cat's atrium (31). From the present results, however, it is unlikely that the reduction of the sodium content to one-half or sometimes to one-fourth strongly interferes with impulse conduction in the atrial fibers or release of acetylcholine from the postganglionic cholinergic nerve endings within the heart.

A marked after-positivity of the spontaneous beat was often observed immediately
after the interruption of vagal stimulation in the atria suspended in the sodium-reduced solution. The similar but more slight after-positivity was observed in one-third of the preparations suspended in the standard solution. The after-positivity was suggested to originate from the release of adrenaline-like substance somewhere in the heart (5). However, there is no evidence to support the assumption. The extreme reduction of sodium produced the arrest of the spontaneous rhythm. The decrease in the amplitude of the transmembrane action potential below critical level may be a cause of block of impulse propagation through the atrial muscle and subsequent cardiac arrest. When the spontaneous beat was abolished by the reduction of sodium content to one-fourth or one-sixth of the standard solution, vagal stimulation often restarted the atrial contraction. In contrast to the high potassium-arrested atria, the low sodium-arrested atria were never restarted by acetylcholine but restarted by adrenaline. The results described above strongly suggest that the after-positivity of the spontaneous beat observed immediately after the interruption of vagal stimulation and the restarting effect of vagal stimulation on the low sodium-arrested atria derive from the increased release of endogenous noradrenaline or from the increased sensitivity of the atria to noradrenaline released.

The addition of barium markedly increased the amplitude of spontaneous contraction, while it decreased the rate. The similar dissociation of the rate and the amplitude of the spontaneous beat was produced by cooling of the atria or low sodium in the nutrient solution. The addition of barium, moreover, reduced the atrial responses to vagal stimulation. It has been reported that barium markedly increases the release of acetylcholine in the superior cervical ganglia of cats and the releasing effect of barium is stronger than that of calcium (44). Bella et al. (16) have shown that the depression by cooling of the isolated stomach to vagal stimulation is restored by the addition of barium. The concentration of barium used in the present experiment was a quarter of that of the former authors and ten times of that of the latter authors. The difference of the barium concentration and the test preparation used might have led to discrepancy of the results.

SUMMARY

Effects of temperature change, oxygen deprivation and cations on the chronotropic and inotropic responses of the isolated rabbit’s and guinea-pig’s atria to stimulation of the vagus nerves and to added acetylcholine were studied in the present experiments.

1. The vagal stimulation at a frequency of 20/sec elicited maximum negative responses in rate and amplitude. The interval of five minutes between successive stimulations was enough to reproduce almost the same response.

2. The negative chronotropic response to stimulation of the right vagus was more marked than that to stimulation of the left vagus, while the negative inotropic response caused by stimulation of either nerve did not markedly differ.

3. The fall or rise of the temperature of the nutrient solution from 30°C reduced both negative chronotropic and inotropic responses of the atria to vagal stimulation.
The responses to added acetylcholine were reduced by the rise but not by the fall of the temperature.

4. The oxygen deprivation augmented both responses to vagal stimulation, while it did not affect the responses to added acetylcholine.

5. The addition of calcium increased the amplitude of the spontaneous contraction and augmented both responses to vagal stimulation. The addition of magnesium reduced the atrial responses to vagal stimulation only in some preparations. While the addition of potassium decreased the rate and amplitude of the spontaneous contraction, it augmented the atrial responses to vagal stimulation. The vagal stimulation or the addition of acetylcholine sometimes restarted the atria arrested by high concentration of potassium. The reduction of sodium resulted in the increase in amplitude accompanied with the decrease in rate of the spontaneous contraction. Low sodium augmented the negative chronotropic response but reduced the negative inotropic response to vagal stimulation. The vagal stimulation as well as the addition of adrenaline restarted the atria arrested by extreme reduction of sodium. The addition of barium reduced the atrial responses to vagal stimulation.

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