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Possible Involvement of Cyclic Adenosine Monophosphate–independent Mechanism in the Positive Chronotropic Effect of Norepinephrine in the Isolated Guinea Pig Right Atrium

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Background: **Although both positive chronotropic and inotropic effects of β-adrenergic stimulation are thought to be mediated by cyclic adenosine 3'5'-monophosphate, phosphodiesterase III inhibitors such as amrinone and milrinone potentiate the positive inotropic effect of catecholamines with minimum influence on the heart rate in clinical setting. The aim of the current study was to compare the positive chronotropic effect of norepinephrine with that of forskolin to elucidate whether cyclic adenosine monophosphate is relevant to the chronotropic effect of norepinephrine.**

Methods: **Concentration–response curves for the positive chronotropic effects of norepinephrine and forskolin on the spontaneously beating right atria of guinea pigs were determined in the absence and presence of phosphodiesterase inhibitors or ion channel inhibitors. In some experiments, the left atria driven electrically were used to determine the positive inotropic effect of norepinephrine.**

Results: **Norepinephrine and forskolin increased the beating rate in a concentration-dependent manner. The positive chronotropic effect of forskolin was potentiated by amrinone and 3-isobutyl-1-methylxanthine, whereas the positive chronotropic effect of norepinephrine was not potentiated by the phosphodiesterase inhibitors. In contrast, the positive inotropic effect of norepinephrine was potentiated by amrinone. The hyperpolarization-activated inward current inhibitor cesium chloride and L-type voltage-dependent Ca^{2+} current inhibitor verapamil suppressed the chronotropic effect of norepinephrine, whereas these inhibitors did not affect the chronotropic effect of forskolin.**

Conclusion: **Norepinephrine increases the spontaneously beating rate by a different mechanism from that of forskolin, suggesting that cyclic adenosine monophosphate is causally unrelated to the positive chronotropic effect of norepinephrine in the guinea pig heart.**

THERAPEUTIC approaches with phosphodiesterase III inhibitors, such as amorinone and milrinone, to patients with low cardiac output syndrome have been established in perioperative circulatory management.[1,2] These agents produce an increase in cardiac contractility and simultaneous vasodilation by inhibiting degradation of cyclic adenosine monophosphate (AMP) in the cardiomyocytes and vascular smooth muscle cells.[3,4] Interestingly, either administration of phosphodiesterase III inhibitor alone or combined treatment with catecholamines such as dobutamine increases the cardiac contractility effectively with a minimal increase in heart rate in clinical settings.[1,5–8] Inasmuch as an increase in cyclic AMP is thought to be involved in the β-adrenoceptor–mediated positive chronotropic effect as well as the positive inotropic effect,[9] it appears strange that the phosphodiesterase III inhibitors modulate the β-adrenoceptor–mediated inotropic and chronotropic effects differently.

Recently, cyclic AMP–independent effects of β-adrenoceptor agonists have been reported in several tissues, such as tracheal smooth muscles, coronary smooth muscles, and cardiac myocytes.[10–12] The reports suggest that the direct interaction of stimulatory guanosine 5'-triphosphate–binding protein (Gs) with ion channels is relevant to the cyclic AMP–independent mechanism of the β-adrenergic stimulation. β-Adrenoceptor agonists produce the positive chronotropic effect by changing several ionic currents, such as the L-type voltage-dependent Ca^{2+} current ($I_{Ca(L)}$) and the hyperpolarization-activated inward current (I_f), which contribute to the action potential configuration of the sinoatrial node pacemaker cell.[13] Therefore, it may be possible that β-adrenoceptor agonists modulate those ion channels in a membrane-delimited manner without corresponding change of the second messenger cyclic AMP.

The aim of the current study was to clarify whether an increase in intracellular cyclic AMP was involved in the β-adrenoceptor–mediated positive chronotropic effect. For this purpose, we compared pharmacologically the positive chronotropic effect of norepinephrine, an endogenous sympathetic neurotransmitter, with that of forskolin, a direct activator of adenylate cyclase, using phosphodiesterase inhibitors, the voltage-dependent Ca^{2+} channel inhibitor verapamil, and the I_f channel inhibitor cesium chloride (CsCl) in the spontaneously beating guinea pig right atria. If the positive chronotropic effect of norepinephrine is mediated by an activation of adenylate cyclase, it would be potentiated by phosphodiesterase inhibitors. In addition, verapamil and CsCl should modulate the chronotropic responses to norepinephrine and forskolin in a similar manner.

Materials and Methods

All experiments were performed in conformity with the Guiding Principles for Research Involving Animals

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Male guinea pigs weighing 250–350 g were used. After the midline thoracotomy was performed during anesthesia with diethyl ether, the heart was rapidly excised and placed in a dissection dish filled with oxygenated Krebs-Henseleit solution of the following composition: 119.0 mM NaCl, 2.5 mM $CaCl_2$, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, and 10.0 mM glucose.

Chronotropic Response of the Right Atrium to Norepinephrine and Forskolin

The spontaneously beating right atrium was carefully dissected and mounted vertically in a 10-ml double-walled glass chamber filled with Krebs-Henseleit solution, gassed continuously with 95% O_2 and 5% CO_2 (pH 7.4), and maintained at 36°C. The lower end of the right atrium was fixed on a hook, and the upper end was connected by a silk thread to an isometric force-displacement transducer (model UL-10GR, Minebea, Nagano, Japan) through a preamplifier (model AS2103, Sanei-Sokki, Tokyo, Japan). Diastolic tension was adjusted to 0.5 g. Spontaneously beating rate was counted on a chart recording of its developed tension. The preparation was equilibrated for at least 60 min before the experiment began.

The concentration–response curves for the positive chronotropic effect of norepinephrine and forskolin were determined in a cumulative manner by increasing their concentrations in steps of 0.5 molar log unit. The difference between the maximum beating rate produced by the agents and the basal beating rate was taken as 100%, and the percent changes in the beating rate produced by various concentrations of the agents were plotted. When examining the influence of an α_1-adrenoceptor antagonist, a β-adrenoceptor antagonist, phosphodiesterase inhibitors, or ion channel inhibitors on the chronotropic effects of the agents, the concentration–response curves were constructed 30 min after the application of each test compound. Because the phosphodiesterase inhibitors and ion channel inhibitors changed the spontaneously beating rate, the beating rate in the presence of each compound was taken as the basal beating rate. The concentrations of norepinephrine or forskolin producing half-maximal response (EC_{50}) in the absence and presence of various test compounds were obtained from log-probit plots of individual response as a function of concentration. The following equation was used to obtain pK_B value:

$$pK_B = \log([A]/[A]_0 - 1) - \log[B]$$

where [A] is the EC_{50} of an agonist in the presence of an antagonist, $[A]_0$ is the EC_{50} of an agonist in the absence of an antagonist, and [B] is a concentration of an antagonist.

To make effects of the test compounds on the chronotropic effects of norepinephrine and forskolin comprehensible, the concentration–response curves depicted on the basis of absolute values of the beating rate were inset into the corresponding figures.

Inotropic Response of the Left Atrium to Norepinephrine

The left atrium was dissected and mounted vertically in the glass chamber as previously described, except that the bath was kept at a temperature of 30°C. The resting tension applied to the preparation was adjusted to 1 g. The atrium was paced electrically by rectangular pulses of 0.5 Hz in frequency, 3 ms in duration, and 1.2 times the threshold voltage, delivered by a pair of spiral platinum electrodes connected to an electric stimulator (SEN-7203, Nihon-Kohden, Tokyo, Japan) through an isolation unit (SS-201J, Nihon-Kohden). The preparation was equilibrated for at least 60 min before the experiment began.

The concentration–response curve for the inotropic effect of norepinephrine was determined in a cumulative manner by increasing its concentration in steps of 0.5 log molar unit. The difference between the maximum contractile force produced by norepinephrine and the basal contractile force was taken as 100%. When examining the influence of amrinone on the inotropic effect of norepinephine, the concentration–response curves were constructed 30 min after the application of amrinone. Because amrinone increased the contractile force, that in the presence of amrinone was taken as the basal contractile force.

Drugs

The following compounds were used: norepinephrine, atenolol, prazosin, amrinone, (±) verapamil, forskolin, 3-isobutyl-1-methylxanthine (IBMX), and CsCl (Sigma Chemical Co., St. Louis, MO). Atenolol, forskolin, and amrinone were dissolved in dimethyl sulfoxide, and further dilution was made with distilled water. All other chemicals were dissolved in distilled water.

Statistics

Values are given as mean ± SD. Comparisons of values obtained from the concentration–response curves were made by one-way analysis of variance followed by Bonferroni *t* test. $P < 0.05$ was considered significant. The EC_{50} values were obtained directly from the concentration–response curves depicted using the Kaleida Graph software program (Synergy Software, Reading, PA).

Results

Positive Chronotropic Effects of Norepinephrine and Forskolin

Norepinephrine increased the spontaneously beating rate of the guinea pig right atria in a concentration-

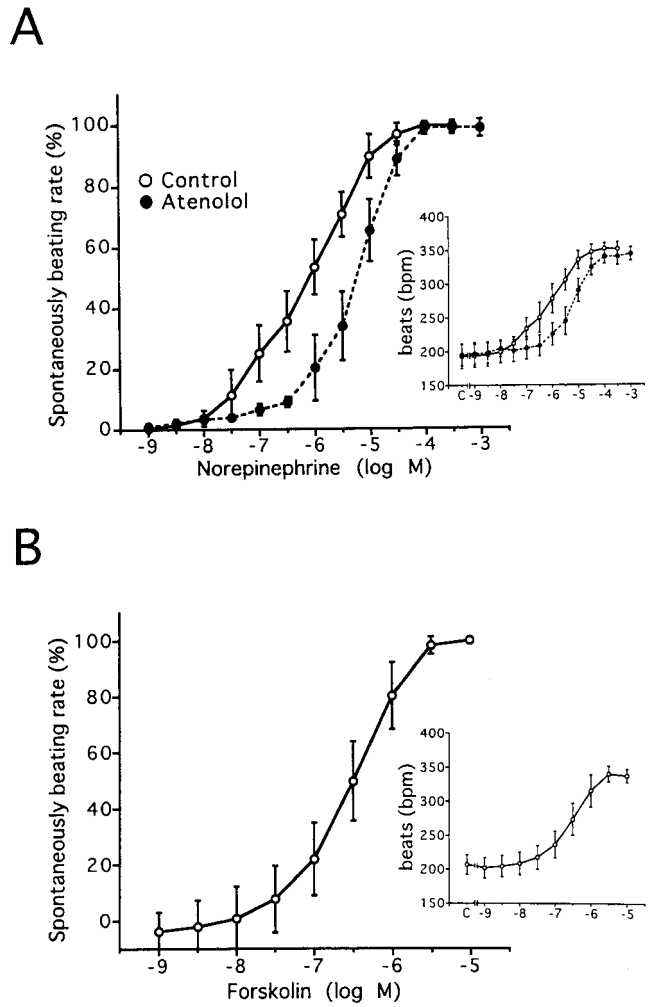

Fig. 1. Concentration–response curves for the positive chronotropic effects of norepinephrine in the absence (open circle; n = 6) and presence (closed circle; n = 6) of atenolol (1 μM) (*A*) and forskolin (n = 8) (*B*) in the spontaneously beating guinea pig right atrium. Points are mean ± SD. The increase in the beating rate (beats/min [bpm]) produced maximally by norepinephrine or forskolin was taken as 100%. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the beating rate. C = control.

dependent manner (fig. 1A). The agent at a concentration of 100 μM maximally increased the beating rate by 159 ± 15 beats/min from the basal rate of 193 ± 18 beats/min (n = 6). Atenorol at a concentration of 1 μM shifted rightward the concentration–response curve in a parallel manner without affecting the maximum response of the atria to norepinephrine. The EC_{50} values of norepinephrine in the absence and presence of atenolol were 0.88 ± 0.55 μM and 5.93 ± 2.64 μM, respectively (n = 6). The pK_B value of atenolol at a concentration of 1 μM was 6.76 (170 nM). Norepinephrine also increased the beating rate by 150 ± 17 beats/min (n = 6) with the EC_{50} value of 0.61 ± 0.20 μM in the presence of 0.3 μM prazosin. These values were not significantly different from those in the absence of prazosin. The direct adenylate cyclase activator forskolin also increased the beating rate in a concentration-dependent manner, with an EC_{50} value of 0.32 ± 0.16 μM (n = 8; fig. 1B). The maximum increase in the beating rate of 137 ± 11 beats/min produced by forskolin at a concentration of 10 μM was not significantly different from that produced by norepinephrine.

Influences of Phosphodiesterase Inhibitors on the Positive Chronotropic Effects of Forskolin and Norepinephrine

The normalized concentration–response curves for the positive chronotropic effect of forskolin in the presence of amrinone are shown in figure 2A. Amrinone at concentrations of 10 and 100 μM increased the spontaneously beating rate by 13 ± 4 beats/min (n = 12) and 37 ± 6 beats/min (n = 10), respectively. Amrinone accelerated the rate of an increase in the beating rate produced by forskolin, resulting in the leftward shift of the normalized concentration–response curves. The maximum increases in the beating rate produced by forskolin plus 10 μM (146 ± 15 beats/min; n = 6) and 100 μM (155 ± 8 beats/min; n = 4) amrinone from the predrug beating rate were not significantly different from that produced by forskolin alone. In contrast to forskolin, the rate of an increase in the beating rate produced by norepinephrine was not accelerated by amrinone even at a concentration of 100 μM (fig. 2B). Norepinephrine plus amrinone increased maximally the beating rate from the predrug basal rate by 151 ± 15 beats/min (n = 6), which was not significantly different from that produced by norepinephrine alone. Figure 3 also shows the conflicting influences of the nonselective phosphodiesterase inhibitor IBMX on the positive chronotropic effects of forskolin and norepinephrine. IBMX at a concentration of 3 μM accelerated the rate of an increase in the beating rate produced by forskolin, resulting in the leftward shift of the normalized concentration–response curve. On the other hand, IBMX did not affect the concentration–response curve for the positive chronotropic effect of norepinephrine. IBMX at a concentration of 3 μM itself increased the spontaneously beating rate by 21 ± 6 beats/min (n = 9). Because norepinephrine activates both α_1-, and β-adrenoceptors, activation of α_1-adrenoceptor might interfere the potentiating effect of the phosphodiesterase inhibitors. However, in the presence of 0.3 μM prazosin, the normalized concentration–response curve for the positive chronotropic effect of norepinephrine in the amrinone-treated atria was superimposable on that in the amrinone-untreated atria (n = 6 for each group, data not shown).

Influences of CsCl and Verapamil on the Positive Chronotropic Effects of Forskolin and Norepinephrine

The influences of CsCl, an I_f inhibitor, and verapamil, an $I_{Ca(L)}$ inhibitor, on the positive chronotropic effects of

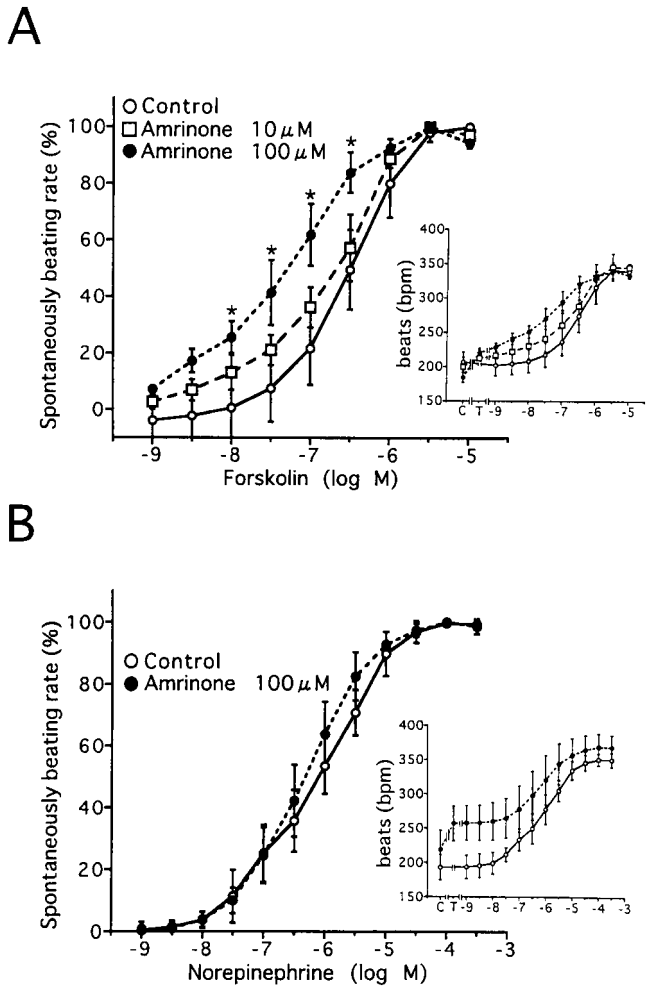

Fig. 2. The concentration–response curves for forskolin (*A*) and norepinephrine (*B*) in the absence (open circle; n = 8 for forskolin and n = 6 for norepinephrine) and presence of amrinone 10 μM (open square; n = 6 for forskolin) or 100 μM (closed circle; n = 4 for forskolin and n = 6 for norepinephrine) in the spontaneously beating guinea pig right atrium. Points are mean ± SD. The increase in the beating rate (beats/min [bpm]) produced maximally by forskolin or norepinephrine was taken as 100%. When the preparation was treated with amrinone, the beating rate in the presence of amrinone was taken as the basal beating rate. *Significantly different from corresponding control ($P < 0.05$) by analysis of variance followed by Bonferroni *t* test. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the beating rate. C = control; T = treatment.

forskolin and norepinephrine are shown in figures 4 and 5. CsCl at a concentration of 2 mM itself decreased the spontaneously beating rate by 56 ± 11 beats/min (n = 12), whereas verapamil at a concentration of 0.2 μM resulted in a decrease of 27 ± 19 beats/min (n = 9). In the presence of CsCl, forskolin and norepinephrine increased the beating rate by 87 ± 11 beats/min (n = 6) and 82 ± 9 beats/min (n = 6), respectively. However, the influences of CsCl on the normalized concentration–response curves of norepinephrine and forskolin were conflicting. CsCl tended to potentiate the positive chronotropic effect of forskolin at lower concentrations and suppress that of the agent at higher concentrations, resulting in the cross of the two concentration–response curves at a forskolin concentration of around 0.5 μM. However, as a whole, CsCl did not affect significantly the normalized concentration–response curve for forskolin (fig. 4A). On the other hand, it inhibited significantly the normalized concentration–response curve for norepinephrine (fig. 4B). As in the case of CsCl, although the concentration–response curve for forskolin in the presence of verapamil crossed the control response curve at the similar point, verapamil did not affect the positive chronotropic effect of forskolin as a whole (fig. 5A). In

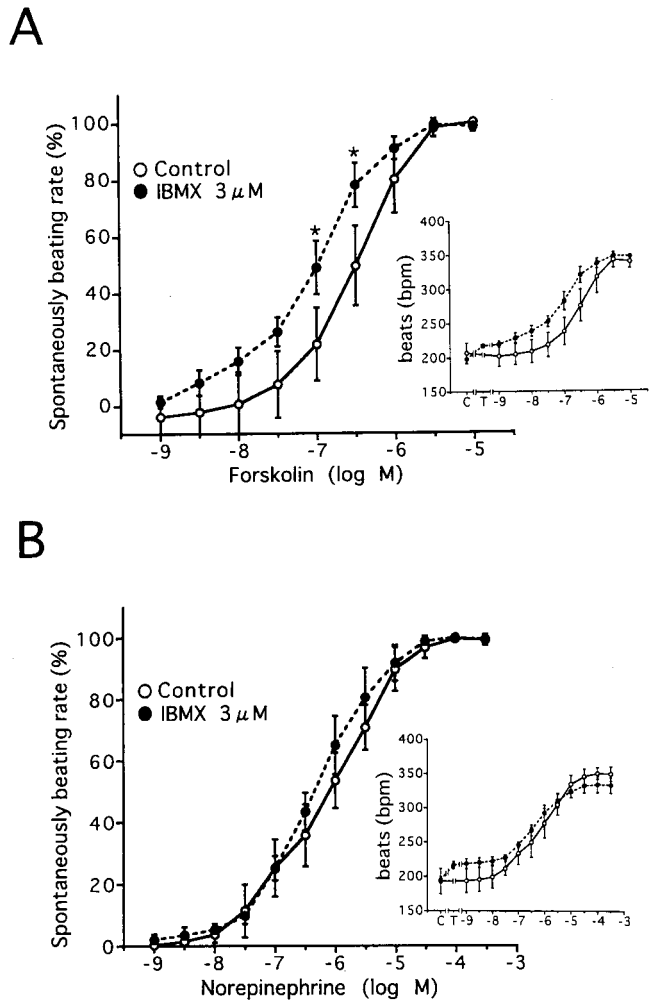

Fig. 3. The concentration–response curves for forskolin (*A*) and norepinephrine (*B*) in the absence (open circle; n = 8 for forskolin and n = 6 for norepinephrine) and presence of 3 μM 3-isobutyl-1-methylxanthine (IBMX; closed circle; n = 4 for forskolin and n = 5 for norepinephrine) in the spontaneously beating guinea pig right atrium. Points are mean ± SD. The increase in the beating rate (beats/min [bpm]) produced maximally by forskolin or norepinephrine was taken as 100%. When the preparation was treated with IBMX, the beating rate in the presence of IBMX was taken as the basal beating rate. *Significantly different from corresponding control ($P < 0.05$) by analysis of variance followed by Bonferroni *t* test. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the beating rate. C = control; T = treatment.

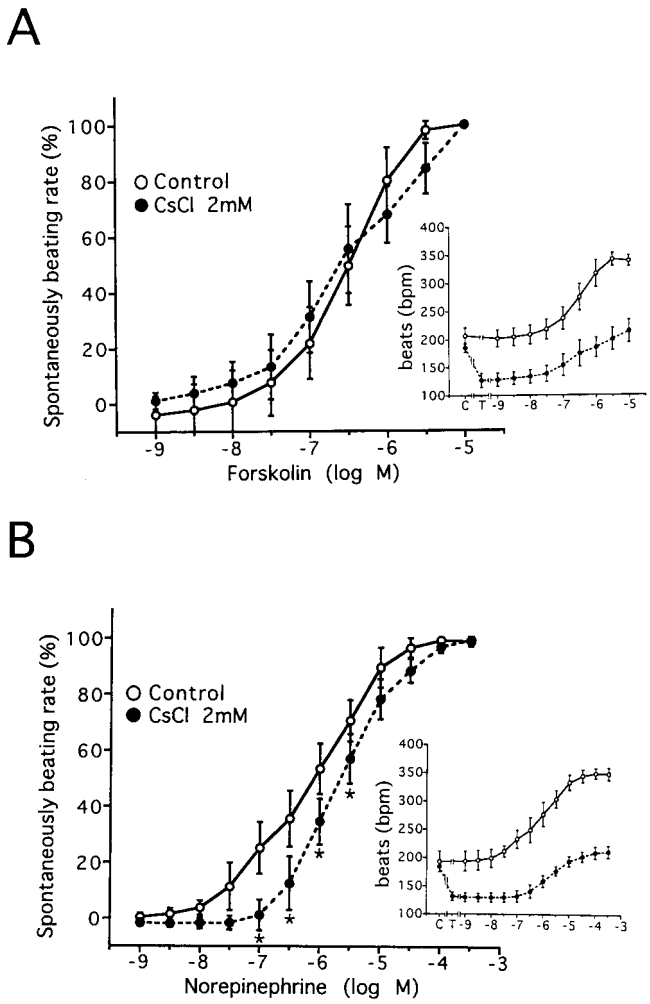

Fig. 4. The concentration–response curves for forskolin (*A*) and norepinephrine (*B*) in the absence (open circle; n = 8 for forskolin and n = 6 for norepinephrine) and presence of 2 mM CsCl (closed circle; n = 6 for forskolin and n = 6 for norepinephrine) in the spontaneously beating guinea pig right atrium. Points are mean ± SD. The increase in the beating rate (beats/min [bpm]) produced maximally by forskolin or norepinephrine was taken as 100%. When the preparation was treated with CsCl, the beating rate in the presence of CsCl was taken as the basal beating rate. *Significantly different from corresponding control ($P < 0.05$) by analysis of variance followed by Bonferroni *t* test. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the beating rate. C = control; T = treatment.

contrast, verapamil inhibited significantly the positive chronotropic effect of norepinephrine (fig. 5B). In the presence of verapamil, forskolin and norepinephrine increased the beating rate by 149 ± 10 beats/min (n = 4) and 124 ± 16 beats/min (n = 5), respectively.

Influence of Amrinone on the Positive Inotropic Effect of Norepinephrine

Norepinephrine increased the contractile force of the electrically driven left atria in a concentration-dependent manner. In contrast to the positive chronotropic effect of norepinephrine, amrinone at a concentration of 100 μM accelerated the increase in the force of contraction produced by norepinephrine, as shown in figure 6. Amrinone itself increased the contractile force by approximately 23%.

Discussion

In the current study we demonstrated that β-adrenoceptor–mediated positive chronotropic effect was not potentiated by the phosphodiesterase inhibitors amrinone and IBMX, whereas forskolin-produced positive chronotropic effect was potentiated by both agents. The I_f inhibitor CsCl and the $I_{Ca(L)}$ inhibitor verapamil decel-

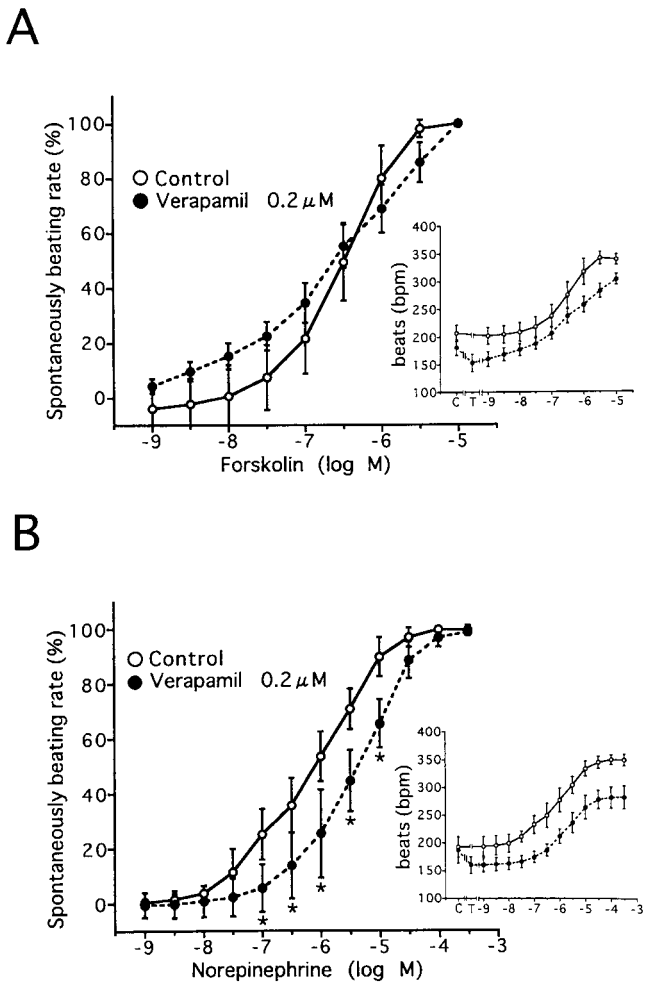

Fig. 5. The concentration–response curves for forskolin (*A*) and norepinephrine (*B*) in the absence (open circle; n = 8 for forskolin and n = 6 for norepinephrine) and presence of 0.2 μM verapamil (closed circle; n = 4 for forskolin and n = 5 for norepinephrine) in the spontaneously beating guinea pig right atrium. Points are mean ± SD. The increase in the beating rate (beats/min [bpm]) produced maximally by forskolin or norepinephrine was taken as 100%. When the preparation was treated with verapamil, the beating rate in the presence of verapamil was taken as the basal beating rate. *Significantly different from corresponding control ($P < 0.05$) by analysis of variance followed by Bonferroni *t* test. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the beating rate. C = control; T = treatment.

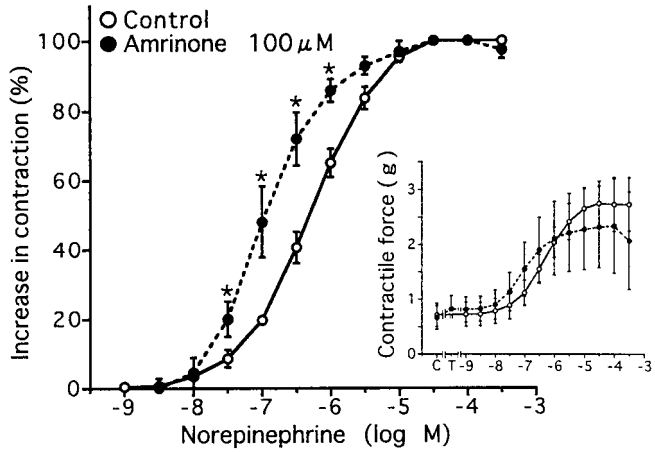

Fig. 6. The concentration–response curves for the positive inotropic effects of norepinephrine in the absence (open circle; n = 4) and presence of 100 μM amrinone (closed circle; n = 5) in the electrically driven guinea pig left atrium. Points are mean ± SD. The increase in the contractile force produced maximally by norepinephrine was taken as 100%. When the preparation was treated with amrinone, the contractile force in the presence of amrinone was taken as the basal contractile force. *Significantly different from corresponding control ($P < 0.05$) by analysis of variance followed by Bonferroni *t* test. (*Inset*) Concentration–response curves depicted on the basis of changes in absolute values of the contractile force. C = control; T = treatment.

erated the rate of an increase in the beating rate produced by norepinephrine. On the other hand, neither CsCl nor verapamil affected significantly the normalized concentration–response curve for the positive chronotropic effect of forskolin. These results suggest that the underlying mechanism of the positive chronotropic effect of the β-adrenergic activation is different from that of the adenylate cyclase activator forskolin.

Cardiac sympathetic activation produces both positive inotropic and chronotropic effects *via* β-adrenoceptors in the cardiac myocytes and the sinoatrial node cells. The adenylate cyclase–cyclic AMP system has been thought to have a pivotal role in the β-adrenoceptor–mediated positive chronotropic effect as well as the positive inotropic effect.[9,14–16] Supporting this notion, forskolin produces the positive chronotropic effect, as shown in the current study. Intracellular application of cyclic AMP also produces the positive chronotropic effect.[17,18]

Inasmuch as cyclic AMP is degenerated by phosphodiesterase, phosphodiesterase inhibitors could theoretically not only mimic but also potentiate the cyclic AMP–mediated responses. In fact, it has been demonstrated that the positive inotropic effect of isoprenaline on human and rabbit ventricular cells, and the antimigratory effect of forskolin on rat aortic vascular smooth muscle cells, are potentiated by phosphodiesterase inhibitors.[19–21] If the positive chronotropic effect of β-adrenergic stimulation would also be mediated by cyclic AMP, the effect should be potentiated by the phosphodiesterase inhibition. However, the phosphodiesterase III inhibitor amrinone did not potentiate the positive chronotropic effect of norepinephrine, although amrinone not only increased the beating rate by itself but also potentiated the positive chronotropic effect of forskolin. Because there are several types of phosphodiesterase,[22] cyclic AMP increased by β-adrenergic stimulation in the guinea pig sinoatrial node cell might not be degenerated by phosphodiesterase III. To elucidate this possibility, we also examined the influences of the nonspecific phosphodiesterase inhibitor IBMX on the positive chronotropic effects of norepinephrine and forskolin. Consistent with the result obtained from amrinone, IBMX potentiated the effect of forskolin but not the effect of norepinephrine. In addition, amrinone could potentiate the positive inotropic effect of norepinephrine in the guinea pig left atria. Shahid and Rodger[20] also reported that both amrinone and IBMX potentiated the positive inotropic effect of isoprenaline in the rabbit papillary muscles but not the positive chronotropic effect of the agent in the rabbit right atria.

There are at least three possible ways to interpret the current results. First, the positive chronotropic effect of norepinephrine is independent of β-adrenoceptors. Second, the cyclic AMP increased by the β-adrenergic activation is compartmentalized in the pacemaker cell, as proposed by Jurevicius and Fischmeister,[23] and the phosphodiesterase inhibitors used are hard to access this compartment. Third, cyclic AMP generation is causally unrelated to the positive chronotropic effect of norepinephrine. As shown in figure 1, the β_1-adrenoceptor selective antagonist atenolol shifted rightward the concentration–response curve for the positive chronotropic effect of norepinephrine. The pK_B value of 6.8 for atenolol at a concentration of 1 μM indicated the involvement of β_1-adrenoceptors in the positive chronotropic effect of norepinephrine in the current experiment.[24] Recent reports have demonstrated the involvement of α_1-adrenoceptor in regulating heart rate.[25,26] However, prazosin did not modify the positive chronotropic effect of norepinephrine in the current study, indicating that α_1-adrenoceptor plays a minor role in controlling the heart rate during the current experimental condition. Amrinone did not potentiate the positive chronotropic effect of norepinephrine even in the presence of prazosin. Therefore, the α_1-adrenoceptors activated by norepinephrine are also unrelated to the inability of amrinone for potentiating the positive chronotropic effect of norepinephrine.

The sinus rate is determined by the spontaneously firing rate of the action potential of the pacemaker cell in the sinoatrial node. β-Adrenoceptor agonists as well as an increase in intracellular cyclic AMP are thought to increase the spontaneously firing rate by means of activation of the I_f channels and the $I_{Ca(L)}$ channels.[13,17,27] Therefore, we examined influences of the I_f channel

inhibitor CsCl[28] and the Ca^{2+} channel inhibitor verapamil on the positive chronotropic effects of norepinephrine and forskolin. CsCl and verapamil shifted the normalized concentration–response curve for norepinephrine to the rightward direction. On the other hand, neither CsCl nor verapamil affected significantly the normalized concentration–response curve for forskolin. Even if cyclic AMP that were increased by norepinephrine would be compartmentalized, as far as cyclic AMP were relevant to the positive chronotropic effect of norepinephrine, both CsCl and verapamil should modulate the positive chronotropic effects of norepinephrine and forskolin in a similar manner. However, this is not the case in the current results. Taken together, the current results suggest that cyclic AMP is causally unrelated to the underlying mechanism of the positive chronotropic effect of norepinephrine in the guinea pig heart. It should be mentioned, however, that a decisive conclusion cannot be drawn until direct evidence is obtained, such as no increase in cyclic AMP in the pacemaker cell after treatment with norepinephrine or norepinephrine-produced increase in the ion channel activities in the membrane-delimited model.

Yatani *et al.*[29,30] reported that the β-adrenoceptor–coupled guanosine 5'-triphosphate binding protein Gs could activate both $I_{Ca(L)}$ and I_f channels directly, although cyclic AMP–dependent activation of both channels had already been demonstrated.[16,31] They also showed the time course of the isoproterenol-produced increase in I_{Ca} in isolated guinea pig cardiomyocytes.[32] Isoproterenol increased it in a biphasic manner, with a fast time constant of 150 ms and a slow time constant of 36 s. Forskolin and IBMX increased the I_{Ca} slowly, with time constants of 56 and 150 s, respectively. Therefore, they concluded that the fast response of the myocyte to isoproterenol was caused by the direct modulation of Gs on $I_{Ca(L)}$ channels, and the slow response was mediated by the second messenger cyclic AMP.[32] Isoproterenol also activated I_f rapidly, with time constant of 800 ms in cell-free, inside-out patch configuration.[30] These direct modulations of β-adrenoceptor–activated Gs on $I_{Ca(L)}$ and I_f channels are considered convenient for the sympathetic nervous system for beat-to-beat regulation of heart rate.[33,34] The current study might demonstrate these direct interactions of the β-adrenoceptor–Gs ion channels, which have been shown using patch clamp experiments, in the functional experiments.

However, it should be noted that there are some limitations to extrapolate the current data to the clinical setting. We used the atrial tissues and counted the number of the muscle contraction per minute instead of counting the number of the action potential in the pacemaker cells. In addition, we used the isolated tissues. Although isolation of a tissue or a cell makes complicated problem simple, much caution should be used in extrapolating the data to a whole body, which is regulated in a complicated manner by the nervous system and hormonal control. We also have to consider the species difference. The basic heart rate of the guinea pig is much faster than that of humans, suggesting the existence of a delicate difference as to the intracellular regulation on the firing of the action potential in the pacemaker cells between humans and the guinea pig.

Use of the phosphodiesterase III inhibitors amrinone and milrinone is an important therapeutic approach, especially combined with catecholamines, in patients with low cardiac output syndrome.[2] The combination of the phosphodiesterase III inhibitors with catecholamines increases cardiac contractility synergistically, whereas the increase in the heart rate is minimal.[1,8] These features seem in accord with the ideal inotropic management for perioperative low cardiac output,[1] because an increase in heart rate jeopardizes the balance of myocardial oxygen demand and supply.[35] The current study might explain, at least partially, the reason for the deferential effects of the phosphodiesterase III inhibitors on the contractility and heart rate observed in the clinical setting. Inasmuch as the heart is innervated by the sympathetic nervous system, the cardiac β-adrenoceptors are activated to a various extent by endogenous norepinephrine. Therefore, the phosphodiesterase III inhibitors could potentiate synergistically the positive inotropic effect of endogenous and exogenous catecholamines, minimally affecting those chronotropic effects.

In conclusion, amrinone and IBMX potentiate the positive chronotropic effect of forskolin. However, the agents did not potentiate the chronotropic effect of norepinephrine. Both CsCl and verapamil shifted rightward the normalized concentration–response curve for the positive chronotropic effect of norepinephrine without affecting the concentration–response curve for that of forskolin. These results strongly suggest that cyclic AMP is irrelevant to the positive chronotropic effect of β-adrenoceptor activation in the guinea pig heart.

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