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European Journal of Pharmacology 334 (1997) 191–200

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Different sympathetic–parasympathetic interactions on sinus rate and atrioventricular conduction in dog hearts

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Received 10 April 1997; revised 14 July 1997; accepted 18 July 1997

Abstract

We investigated the sympathetic–parasympathetic interactions involved in SA nodal pacemaker activity and AV conductivity in the anesthetized dog heart. Stimulation of the intracardiac parasympathetic nerves to the SA nodal region (SAPS) and stimulation of the intracardiac parasympathetic nerves to the AV nodal region (AVPS) induced negative chronotropic and dromotropic responses, respectively. Cardiac sympathetic stimulation, aminophylline, 3-isobutyl-1-methylxanthine (IBMX, a relatively pure nonselective phosphodiesterase inhibitor) and methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-piridine-5-carboxylate (Bay k 8644, a Ca^{2+} channel agonist) increased sinus rate and decreased AV conduction time. Sympathetic stimulation augmented the negative chronotropic response to SAPS but not the negative dromotropic response to AVPS, IBMX augmented both responses, Bay k 8644 augmented the chronotropic response and attenuated the dromotropic response, and aminophylline did not affect the chronotropic response to SAPS and inhibited the dromotropic response to AVPS. Additionally, when Bay k 8644 directly given via the AV node artery decreased AV conduction time, it attenuated the negative dromotropic response to AVPS and carbachol injected into the AV node artery. These results suggest that the differential sympathetic–parasympathetic interactions on sinus rate and AV conduction are at least partly induced by an interaction between changes in slow inward Ca^{2+} current or intracellular Ca^{2+} and the cardiac effects of acetylcholine in the heart in situ. © 1997 Elsevier Science B.V.

Keywords: Autonomic nervous system; Sympathetic-parasympathetic interaction; Sinus rate; Atrioventricular conduction; Aminophylline

1. Introduction

Parasympathetic nerve stimulation decreases heart rate more in the presence of tonic sympathetic nerve stimulation than in its absence (Samaan, 1935; Levy and Zieske, 1969; Warner and Russel, 1969). On the other hand, the sympathetic–parasympathetic interactions on atrioventricular (AV) conductivity were not consistently present in previous studies. In some studies in anesthetized dogs the increase in AV conduction time in response to parasympathetic nerve stimulation was not influenced by sympathetic nerve stimulation (Levy and Zieske, 1969; Wallick et al., 1982). In other studies, however, parasympathetic nerve stimulation during sympathetic stimulation produced either a greater increase in AV conduction time in anesthetized dogs (Takahashi and Zipes, 1983) or a smaller increase in AV conduction time in anesthetized puppies (Urthaler et al., 1986). Thus, the sympathetic–parasympathetic interactions on the SA nodal pacemaker activity are different from those on the AV conductivity.

The autonomic interactions occur at prejunctional and postjunctional levels of the autonomic neuroeffector junction in the heart (Levy, 1971, 1989; Watanabe, 1984). Prejunctionally, acetylcholine released from parasympathetic nerve endings inhibits the release of norepinephrine from neighboring sympathetic nerve endings (Löffelholz and Muscholl, 1969; Levy and Blattberg, 1976). The postjunctional interactions occur at the level of the cardiac effector cells and are caused by a cyclic AMP-dependent mechanism, i.e., a reduction of increased intracellular concentrations of cyclic AMP, or a cyclic AMP-independent mechanism (Levy, 1971; Watanabe, 1984; Endoh et al., 1985). The reduction of the increased cyclic AMP levels is

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mediated at a site in the cyclic AMP cascade proximal to the catalytic unit of adenylyl cyclase (LaRaia and Sonnenblick, 1971; Endoh et al., 1985; Hescheler et al., 1986; DiFrancesco and Tromba, 1988). Cholinomimetics may also inhibit cyclic AMP-dependent effects at sites in the cyclic AMP cascade distal to the catalytic unit of adenylyl cyclase (Lindemann and Watanabe, 1985; MacLeod, 1985; Furukawa et al., 1989).

In preliminary studies, we observed that aminophylline attenuated the negative dromotropic response but not chronotropic response. Aminophylline is the widely used methylxanthine derivative in the treatment of cardiovascular and respiratory diseases and has three basic cellular actions, namely, to increase the accumulation of cyclic AMP by inhibition of phosphodiesterase, to translocate intracellular Ca^{2+}, and to block adenosine receptors (Rall, 1990). Thus, we hypothesized that the differential sympathetic–parasympathetic interactions on the SA nodal pacemaker activity and AV conductivity were mediated at sites distal to the catalytic unit of adenylyl cyclase in the heart. To test this hypothesis, we studied the effects of cardiac sympathetic nerve stimulation, aminophylline, 3-isobutyl-1-methylxanthine (IBMX, another methylxanthine derivative with relatively pure phosphodiesterase inhibitory activity) and Bay k 8644 (a Ca^{2+} channel agonist) (Schramm and Towart, 1985), on the negative chronotropic response to stimulation of the intracardiac parasympathetic nerves to the SA nodal region (SAPS) and on the negative dromotropic response to stimulation of the intracardiac parasympathetic nerves to the AV nodal region (AVPS) in anesthetized, open-chest dogs. Then, we also discussed the effects of aminophylline with complex actions on the negative chronotropic and dromotropic responses to parasympathetic nerve stimulation.

2. Materials and methods

2.1. Preparations

The animal experiments were approved by the Shinshu University School of Medicine animal experimentation committee and animals were obtained through the Animal Laboratory for Research of Shinshu University School of Medicine.

Thirty-one mongrel dogs that weighed 10–25 kg were used. Each dog was anesthetized by sodium pentobarbital (30 mg/kg, i.v.). A tracheal cannula was inserted and intermittent positive-pressure ventilation was started. The chest was opened at the fifth intercostal space. Each cervical vagus nerve was crushed with a tight ligature and the ansae subclaviae on both sides were ligated tightly at their junctions with the stellate ganglia. These maneuvers remove virtually almost all tonic neural activity to the heart (Levy et al., 1966).

Bipolar silver electrodes were placed on the epicardial surfaces of the right atrial appendage and right ventricle to record atrial and ventricular electrograms. Heart rate (atrial rate) and AV conduction time (AV interval) were measured and displayed on a heat-writing rectigraph (Nihon Kohden, Tokyo, WT685T). The left femoral artery was cannulated for monitoring systemic arterial pressure. The left femoral vein was also cannulated for drug injection and for physiological saline infusion to adjust spontaneous fluid losses.

Two bipolar silver electrodes, 1.5 mm interelectrode distance, were used to stimulate the regional intracardiac parasympathetic nerves (Fig. 1; Furukawa et al., 1990). One bipolar electrode was placed on the fatty tissue overlying the right side of the atrial junction with the right pulmonary veins, and it was used to stimulate the intracardiac parasympathetic nerves to the SA nodal region (SAPS). The second bipolar electrode was placed on the fatty tissue at the junction of the inferior vena cava and left atrium. It was used to stimulate the intracardiac parasympathetic nerves to the AV nodal region (AVPS). We used steady stimulation (Nihon Kohden SEN7103) with 5 mA pulse amplitude, less than 0.05 ms pulse duration, and a frequency of 5 to 30 Hz for 20 or 30 s. This stimulation intensity was subthreshold for activation of pacemaker cells or cardiac muscle cells. Each stimulation was separated by intervals of at least 2 min to allow sufficient recovery time. Treatment with atropine (0.1 or 0.2 mg/kg i.v.) abolished the responses to stimulation.

To study the effects of Bay k 8644 administered via the AV node artery on the negative dromotropic responses to AVPS and carbachol, the distal branch of the left circumflex coronary artery was cannulated and the AV node artery was perfused with heparinized arterial blood from the femoral artery by means of a peristaltic pump (Harvard Apparatus, Miles, MA, model 1210) (Fig. 1). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mm Hg. Sodium heparin, 500 USP u/kg i.v., was given at the beginning of the perfusion and 200 USP u/kg were added at 1 h intervals. Details of the experimental procedure have been reported previously (Chiba and Hashimoto, 1970).

2.2. Protocol

We carried out two series of experiments. In the first series, we studied the effects of nerve stimulation ($n = 5$), aminophylline ($n = 6$), IBMX ($n = 6$) and Bay k 8644 ($n = 5$) on the negative chronotropic response to SAPS and on the negative dromotropic response to AVPS. Before each intervention, we adjusted the level of SAPS to induce approximately 20 (low) and 40 (high) beats/min decrease in heart rate; these represented the control responses. The low levels of SAPS were achieved with frequencies of 5 or 10 Hz and the high levels with

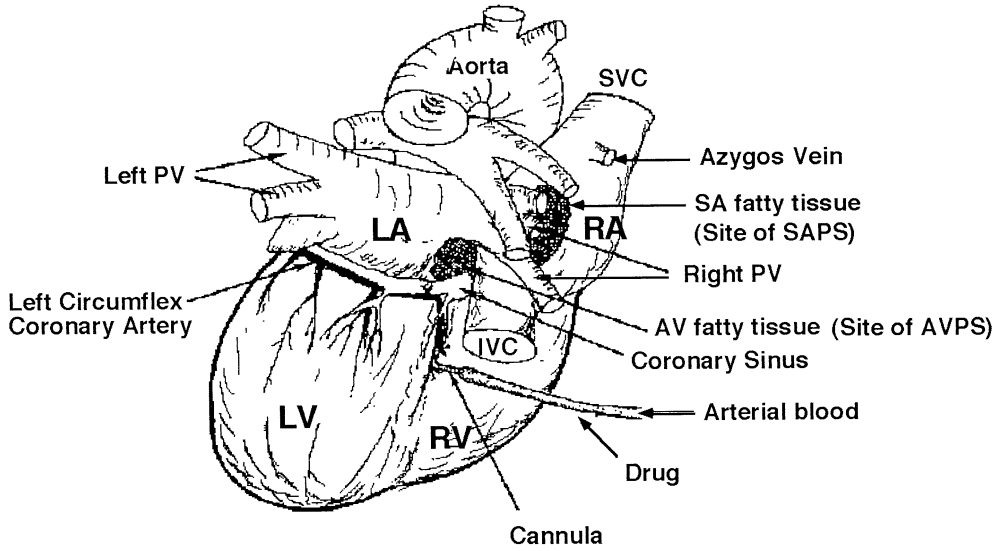

Fig. 1. Dorsal view of the dog heart showing loci (shaded area) for intracardiac parasympathetic nerve stimulation to the SA nodal region (SA fatty tissue) and to the AV nodal region (AV fatty tissue), and the cannula to the distal branch of the left circumflex coronary artery for selective perfusion of the AV nodal region. AV, atrioventricular; AVPS, stimulation of the intracardiac parasympathetic nerves to the AV nodal region; SA, sinoatrial; SAPS, stimulation of the intracardiac parasympathetic nerves to the SA nodal region; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; PA, pulmonary artery; PV, pulmonary vein; IVC, inferior vena cava; SVC, superior vena cava.

frequencies of 10 or 30 Hz. Similarly, the levels of AVPS were adjusted to induce approximately 20 and 40 ms increases in AV conduction time as control responses. The low levels of AVPS were achieved with frequencies of 5 or 10 Hz, and the high levels with frequencies of 10 or 30 Hz.

Effects of sympathetic stimulation on the negative chronotropic response to SAPS and on the negative dromotropic response to AVPS were studied in 5 anesthetized dogs. Two bipolar iridium electrodes were placed on the cardiac sides of the right and left stellate ganglia and connected to an electrical stimulator (Nihon Kohden MSE-3). We used a steady stimulation of 10 V, 1 ms pulse duration and with 'low' frequencies of 0.5–1 Hz and 'high' frequencies of 1–4 Hz. Before and during sympathetic stimulation, we determined the chronotropic response to SAPS and the dromotropic response to AVPS, both at the low and high frequency levels. The order of SAPS or AVPS was randomized in each experiment.

The effects of aminophylline on the chronotropic response to SAPS and on the dromotropic response to AVPS were investigated in 6 animals. Aminophylline was infused at rates of 0.37, 1.2 and 3.7 mg/kg/min i.v. for 5 min or longer before determination of the responses to SAPS and to AVPS. Before and during aminophylline infusion, we determined the chronotropic response to SAPS and the dromotropic response to AVPS, both at the low and high frequency levels. The plasma concentrations of theophylline were measured by the standard fluorescence polarization immunoassay with TDX theophylline (Dainabot, Tokyo) before and 8 to 10 min after the beginning of aminophylline infusion. Because aminophylline decreased the systemic arterial blood pressure, to investigate the influence of reduction of the blood pressure on the responses to nerve stimulation, we studied the effects of isosorbide dinitrate at doses of 0.02–0.1 mg/kg i.v. on the negative chronotropic response to SAPS and the negative dromotropic response to AVPS in 4 anesthetized dogs. The effects of another methylxanthine derivative, IBMX on the chronotropic response to SAPS and on the dromotropic response to AVPS were also investigated in 6 anesthetized dogs. IBMX was given at doses of 0.025, 0.05 and 0.12 mg/kg i.v.

The effects of Bay k 8644 on the responses to SAPS and AVPS were studied in 5 dogs after treatment with 1 mg/kg i.v. of propranolol. Before and after the administration of Bay k 8644 (10 and 30 μg/kg i.v.), we carried out SAPS and AVPS. We adjusted the levels of SAPS and AVPS to elicit approximately a 40 beats/min decrease and a 40 ms increase, respectively, in the chronotropic and dromotropic responses in the IBMX, isosorbide dinitrate and Bay k 8644 treatment groups.

In the second series, we investigated whether the effects of Bay k 8644 on the negative dromotropic response to AVPS are induced at the postjunctional site in the heart. To achieve this aim, we studied the effects of Bay k 8644 (8.4 and 28 nmol) injected via the AV node artery on the response to AVPS and to carbachol (0.1–0.3 nmol) injected to the AV node artery in 5 experiments. We adjusted the level of AVPS and the dose of carbachol to induce approximately 40 ms increases in AV conduction time. At the start of each experiment, we repeated the SAPS, AVPS or the carbachol injection to confirm the stability of the chronotropic or dromotropic response.

2.3. Drugs

The chemicals used in the present study were aminophylline (theophylline ethylenediamine) and isosorbide dinitrate (Eisai, Osaka), methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-piridine-5-carboxylate (Bay k 8644, generously donated by Bayer AG, Wuppertal–Elberfelt), carbamylcholine chloride (carbachol) and 3-isobutyl-1-methylxanthine (IBMX) (Aldrich, Milwaukee, WI), propranolol hydrochloride (Sigma, St. Louis, MO), and atropine sulfate (Wako Pure Chemicals, Osaka). Bay k 8644 was dissolved in ethanol and other drugs were dissolved in saline.

2.4. Statistical analysis

All data are shown as mean ± S.E.M. We analyzed the effects of sympathetic nerve stimulation or aminophylline on the responses to SAPS or to AVPS by two-way analysis of variance with randomized block design. Other results were analyzed by one-way analysis of variance. Two levels of stimulation frequencies for SAPS or AVPS and two levels of sympathetic stimulation frequencies or doses of each substance were considered to be fixed factors. When the F statistic was significant, we compared the data between two values by Bonferroni t-test (Wallenstein et al., 1980). P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Responses to SAPS, AVPS and carbachol injection

SAPS decreased the heart rate and AVPS increased the AV conduction time (AV interval) without changing the heart rate in an autonomically decentralized, open-chest anesthetized dog (Fig. 2A). The response to each stimulation increased when the frequency of stimulation was raised. When carbachol (0.01–0.3 nmol) was directly injected into the AV node artery, the AV conduction time increased dose-dependently, but it did not affect the heart rate or arterial blood pressure (Fig. 2B). The negative chronotropic response to SAPS and the dromotropic response to AVPS before the interventions of sympathetic stimulation, aminophylline, IBMX, Bay k 8644 or isosorbide dinitrate are shown in Table 1.

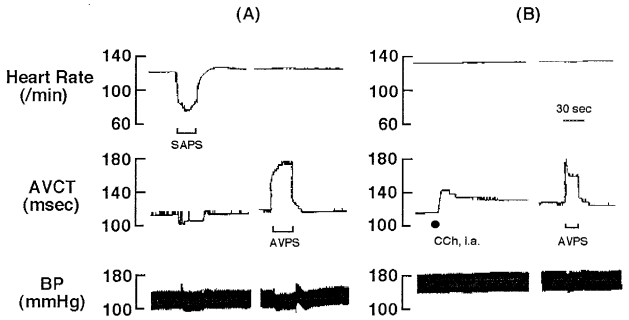

Fig. 2. A negative chronotropic response to stimulation of the discrete parasympathetic nerves to the SA nodal region (SAPS) and a negative dromotropic response to stimulation of the discrete parasympathetic nerves to the AV nodal region (AVPS) in an autonomically decentralized, anesthetized open-chest dog (A), and negative dromotropic responses to AVPS and carbachol (0.1 nmol) injection into the AV node artery in an autonomically decentralized, anesthetized open-chest dog after heparin treatment (B). AVCT, atrioventricular conduction time; BP, arterial blood pressure. Parasympathetic nerves to the SA nodal region were stimulated by 5 mA, 30 Hz and 0.03 ms and parasympathetic nerves to the AV nodal region were stimulated by 5 mA, 30 Hz and 0.03 ms (A) or 0.05 ms (B).

3.2. Effects of sympathetic stimulation, aminophylline, IBMX and Bay k 8644 on the responses to parasympathetic stimulation

Stimulation of cardiac sympathetic nerve fibers increased sinus rate and mean arterial blood pressure, and decreased AV conduction time in autonomically decentral-

Table 1
Decreases in heart rate in responses to stimulation of the discrete intracardiac parasympathetic nerves to the SA nodal region (SAPS) and increases in AV conduction time (AVCT) in response to stimulation of the discrete intracardiac parasympathetic nerves to the AV nodal region (AVPS) before each intervention (sympathetic stimulation, aminophylline, IBMX, Bay k 8644 or isosorbide dinitrate (ISDN)) in 5 experimental groups

Group	Decreases in heart rate induced by SAPS (beats/min)	Increases in AVCT induced by AVPS (ms)
Sympathetic stimulation ($n = 5$)		
Low level	20 ± 4.0	10 ± 4.0
High level	41 ± 3.1	52 ± 11.3
Aminophylline ($n = 6$)		
Low level	19 ± 4.1	16 ± 2.7
High level	49 ± 5.6	47 ± 6.5
IBMX ($n = 6$)	40 ± 2.0	40 ± 3.8
Bay k 8644 ($n = 5$)	36 ± 3.0	45 ± 1.2
ISDN ($n = 4$)	35 ± 3.7	46 ± 8.3

Data are shown as mean ± S.E.M.

Table 2
Direct effects of sympathetic stimulation, aminophylline infusion, IBMX, Bay k 8644 and isosorbide dinitrate (ISDN) on the heart rate, AV conduction time (AVCT) and mean arterial blood pressure (MABP) in the autonomically decentralized, anesthetized, open-chest dogs

Group	Heart rate (beats/min)	AVCT (ms)	MABP (mm Hg)
Sympathetic stimulation ($n = 5$)			
Control	110 ± 3.2	128 ± 4.6	109 ± 4.4
Low level	140 ± 3.3 [b]	114 ± 3.6 [a]	114 ± 5.6 [b]
High level	169 ± 7.1 [b]	110 ± 4.1 [b]	119 ± 4.3 [b]
Aminophylline ($n = 6$)			
Control	121 ± 8.6	127 ± 3.4	124 ± 6.4
0.37 mg/kg/min	130 ± 10.6	122 ± 3.2 [a]	125 ± 7.9
1.2 mg/kg/min	144 ± 12.7 [b]	115 ± 3.8 [b]	109 ± 10.3 [a]
3.7 mg/kg/min	166 ± 14.4 [b]	105 ± 4.8 [b]	63 ± 9.5 [b]
IBMX ($n = 6$)			
Control	115 ± 6.5	152 ± 8.4	113 ± 8.5
0.025 mg/kg	126 ± 3.5 [a]	137 ± 10.5	115 ± 12.6
0.05 mg/kg	152 ± 5.6 [b]	128 ± 6.9 [b]	113 ± 10.2
0.12 mg/kg	172 ± 9.3 [b]	120 ± 5.8 [b]	101 ± 12.8
Bay k 8644 ($n = 5$)			
Control	102 ± 5.1	134 ± 5.8	105 ± 7.7
0.01 mg/kg	108 ± 5.9	130 ± 6.1	152 ± 9.2 [b]
0.03 mg/kg (n = 4)	118 ± 8.3 [b]	118 ± 9.0 [b]	174 ± 16.2 [b]
ISDN ($n = 4$)			
Control	101 ± 13.2	105 ± 10.0	93 ± 13.6
0.02–0.03 mg/kg	103 ± 14.4	104 ± 9.8	79 ± 11.7 [a]
0.05 mg/kg	104 ± 13.3	105 ± 9.5	69 ± 12.5 [b]
0.09–1.2 mg/kg	104 + 13.4	104 + 9.5	66 + 13.5 [b]

Data are shown as mean ± S.E.M.
[a] $P < 0.05$, [b] $P < 0.01$ vs. control.

ized, anesthetized dogs (Table 2). The positive chronotropic and dromotropic effects increased as we raised the frequency of stimulation. SAPS and AVPS induced negative chronotropic and dromotropic responses, respectively, in a frequency-dependent manner ($P < 0.001$, Fig. 3). During sympathetic stimulation the negative chronotropic response to concomitant SAPS was significantly ($P < 0.005$) increased and the augmentation of chronotropic response varied directly with the level of sympathetic stimulation (Fig. 3, upper panel). On the other hand, during sympathetic stimulation the negative dromotropic response to concomitant AVPS was not affected significantly (Fig. 3, lower panel).

Infusion of aminophylline at rates of 0.37, 1.2 and 3.7 mg/kg/min i.v. induced positive chronotropic and dromotropic effects, and the responses varied directly with the dose. Aminophylline infused at rates of 1.2 and 3.7 mg/kg/min deceased mean arterial blood pressure (Table 2). The plasma concentration of theophylline 8 to 10 min after beginning of aminophylline infusion increased with the rate of infusion. The plasma concentrations of theophylline were 10.5 ± 1.9, 37.3 ± 3.7 and 105.5 ± 11.9 μg/ml during infusion of aminophylline at rates of 0.3, 1 and 3 mg/kg/min, respectively. During the aminophylline infusion, the negative chronotropic response to SAPS did not change significantly (Fig. 4, upper panel). During the aminophylline infusion at the rate of 3.7 mg/kg/min high frequency SAPS induced an AV junctional rhythm in 3 of 6 experiments. On the other hand, the negative dromotropic response to AVPS was attenuated in a dose-dependent manner ($P < 0.001$) by the aminophylline infusion (Fig. 4, lower panel). We found that aminophylline affected the negative chronotropic and dromotropic responses to parasympathetic stimulation similarly in propranolol treated and non-treated groups. Therefore, although 3 of 6 experiments were carried out after treatment with propranolol (1 mg/kg i.v.), we combined the data obtained in experiments with and without propranolol treatment.

To elucidate the effects of the reduction of the arterial blood pressure on the chronotropic response to SAPS and the dromotropic response to AVPS, we studied the effects of isosorbide dinitrate on the responses to parasympathetic nerve stimulations in 4 anesthetized dogs. When isosorbide dinitrate in doses of 0.02–0.1 mg/kg i.v. dose-dependently ($P < 0.001$) decreased mean arterial blood pressure (Table 2), the negative chronotropic response to SAPS and the negative dromotropic response to AVPS were not significantly changed.

IBMX in doses of 0.025, 0.05 and 0.12 mg/kg i.v. induced the positive chronotropic and dromotropic responses, and the responses varied directly with the dose (Table 2). After IBMX treatment, the negative chronotropic response to SAPS was augmented in a dose-dependent

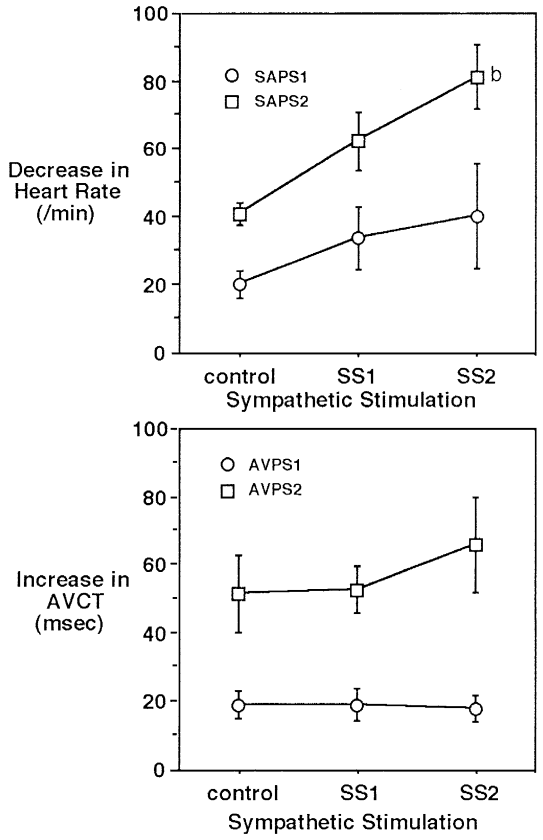

Fig. 3. Effects of sympathetic nerve stimulation on the negative chronotropic response to stimulation of the parasympathetic nerves to the SA nodal region (SAPS, upper panel) and the negative dromotropic response to stimulation of the parasympathetic nerves to the AV nodal region (AVPS, lower panel) in 5 anesthetized dogs. Vertical bars show S.E.M. SS1, low level of sympathetic nerve stimulation; SS2, high level of sympathetic nerve stimulation; SAPS1, low level of SAPS; SAPS2, high level of SAPS; AVPS1, low level of AVPS; AVPS2, high level of AVPS; AVCT, atrioventricular conduction time. [b] $P < 0.01$ vs. control.

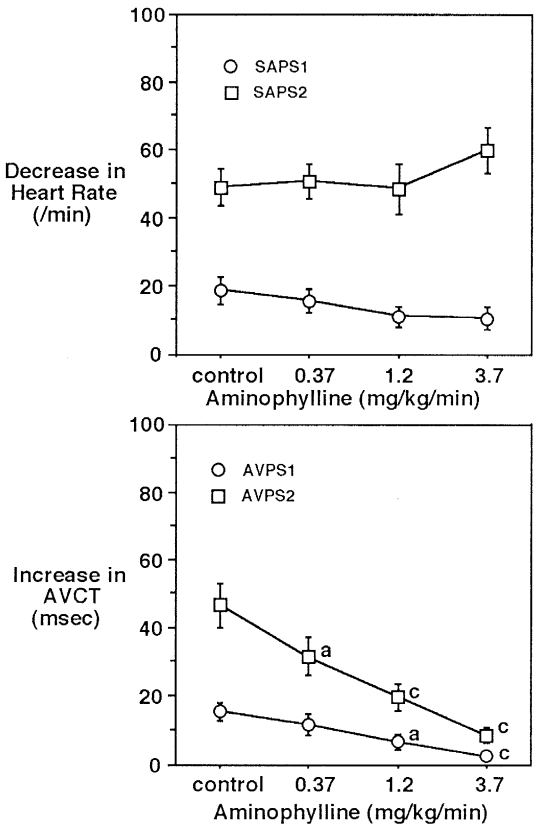

Fig. 4. Effects of aminophylline (0.37, 1.2 and 3.7 mg/kg/min) on the negative chronotropic response to stimulation of the parasympathetic nerves to the SA nodal region (SAPS, upper panel) and the negative dromotropic response to stimulation of the parasympathetic nerves to the AV nodal region (AVPS, lower panel) in 6 open-chest anesthetized dogs. Vertical bars show S.E.M. SAPS1, low level of SAPS; SAPS2, high level of SAPS; AVPS1, low level of AVPS; AVPS2, high level of AVPS; AVCT, atrioventricular conduction time. [a] $P < 0.05$, [c] $P < 0.001$ vs. control.

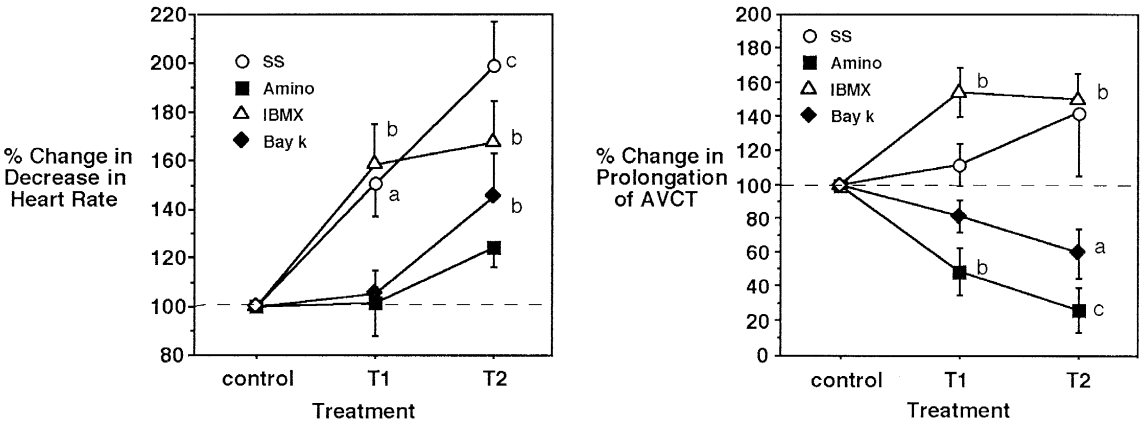

Fig. 5. Effects of sympathetic nerve stimulation, aminophylline (1.2 and 3.7 mg/kg/min), IBMX (50 and 120 μg/kg) and Bay k 8644 (10 and 30 μg/kg) on the percentage changes in decrease in heart rate in response to stimulation of the parasympathetic nerves to the SA nodal region and on the percentage changes in prolongation of atrioventricular conduction time (AVCT) in response to stimulation of the parasympathetic nerves to the AV nodal region. Vertical bars show S.E.M. Treatment T1 and T2 show the low and high levels of sympathetic stimulation and low and high doses of substances, respectively. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. control.

manner ($P < 0.01$, Fig. 5 left panel) and the negative dromotropic response to AVPS was also potentiated ($P < 0.01$, Fig. 5 right panel).

Bay k 8644 in doses of 0.01 and 0.03 mg/kg i.v. elicited increases in sinus rate and mean arterial blood pressure, and elicited decreases in AV conduction time in 5 dogs after propranolol treatment (Table 2). The negative chronotropic response to SAPS was potentiated ($P < 0.05$, Fig. 5 left panel) by Bay k 8644, but the negative dromotropic response to AVPS was attenuated significantly ($P < 0.01$, Fig. 5 right panel).

To compare the effects of interventions on the negative chronotropic response to SAPS and the negative dromotropic response to AVPS, we summarized in Fig. 5 the percentage changes in response to parasympathetic stimulations at a high level during sympathetic stimulation and aminophylline (1.2 and 3.7 mg/kg/min) or after IBMX (0.05 and 0.12 mg/kg) and Bay k 8644 (0.01 and 0.03 mg/kg). Sympathetic stimulation, IBMX and Bay k 8644 dose-dependently ($P < 0.01$) potentiated the percentage decreases in sinus rate in response to SAPS, but aminophylline did not. On the other hand, aminophylline and Bay k 8644 attenuated the percentage increases in AV conduction time in response to AVPS in a dose-dependent manner ($P < 0.01$), while IBMX augmented the percentage increases in AV conduction time. Sympathetic nerve stimulation did not affect the percentage increases in AV conduction time induced by AVPS.

3.3. Effects of Bay k 8644 on the negative dromotropic responses to AVPS and carbachol injection

Intravenous injection of Bay k 8644 attenuated the negative dromotropic response to AVPS when Bay k 8644 increased mean arterial blood pressure (Table 2). Thus, we studied the direct effects of Bay k 8644 on the negative dromotropic response to AVPS and to carbachol injected into the AV node artery. When Bay k 8644 was injected directly into the AV node artery, it decreased AV conduction time in a dose-dependent manner, but it did not affect the sinus rate and arterial blood pressure (Fig. 2B). When Bay k 8644 at a dose of 8.4 nmol decreased the AV conduction time by 25 ± 7.9 ms in 5 anesthetized dogs, the negative dromotropic response to AVPS and carbachol was significantly ($P < 0.01$) attenuated (Fig. 6). In 3 experiments, Bay k 8644 at a dose of 28 nmol elicited a greater attenuation of the negative dromotropic response to AVPS and carbachol.

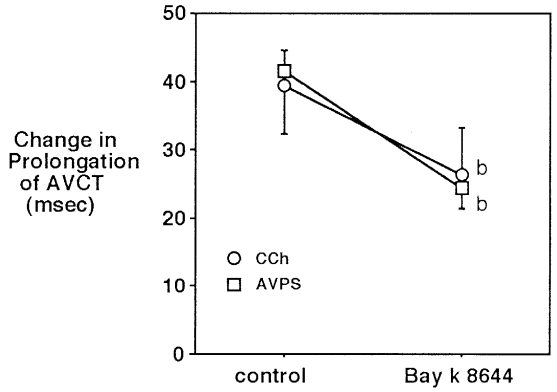

Fig. 6. Effects of Bay k 8644 (8.4 nmol) on the negative dromotropic responses to stimulation of the parasympathetic nerves to the AV nodal region (AVPS) and carbachol (0.1–0.3 nmol) injection to the AV node artery in 5 autonomically decentralized, anesthetized dogs after treatment with heparin. Vertical bars show S.E.M. [a] $P < 0.05$ vs. control.

4. Discussion

In the present study, we demonstrated that a nonselective phosphodiesterase inhibitor, IBMX augmented the negative dromotropic as well as chronotropic responses to parasympathetic nerve stimulation in the anesthetized dog. However, Bay k 8644 attenuated the negative dromotropic response to parasympathetic stimulation and to carbachol injected directly into the AV node artery, while it augmented the negative chronotropic response to parasympathetic stimulation. We therefore suggest that the disparate sympathetic–parasympathetic interactions in the regulation of SA nodal automaticity and AV conductivity are at least partly due to a cyclic AMP-independent mechanism, and complex interactions exist. That is, the dromotropic response to concomitant stimulation of sympathetic and parasympathetic nerves is the sum of the attenuation by parasympathetic stimulation of the cyclic AMP-dependent positive dromotropic response and the attenuation by sympathetic stimulation of the cyclic AMP-independent negative dromotropic response. We also demonstrated that aminophylline attenuated the negative dromotropic but not the negative chronotropic response to parasympathetic nerve stimulation in anesthetized dogs.

4.1. Different sympathetic–parasympathetic interactions on sinus rate and AV conduction

In the autonomically decentralized, open-chest dog, SAPS concomitant with sympathetic nerve stimulation decreased heart rate (sinus rate) more than did SAPS alone, but the prolongation of the AV conduction time (AV interval) induced by AVPS was not significantly affected by sympathetic nerve stimulation (Fig. 3). These results confirm previous reports on the autonomic interactions of the SA node (Samaan, 1935; Levy and Zieske, 1969; Urthaler et al., 1986) and the AV conductivity (Levy and Zieske, 1969; Wallick et al., 1982), that is, parasympathetic effects predominate on sinus rate but not on AV conduction.

In the present study, both negative chronotropic and dromotropic responses to parasympathetic nerve stimulation were augmented dose-dependently after administration

of IBMX, a nonselective phosphodiesterase inhibitor (Chasin and Harris, 1976; Weishaar et al., 1985; Katano and Endoh, 1993). These results suggest that the cyclic AMP-dependent mechanism is effective on AV conduction as well as on sinus rate in the dog heart. Very recently, Habuchi et al. (1996) reported that I_{Ca} increased by IBMX was attenuated by acetylcholine in the AV nodal cell of the rabbit, but the maximal response to IBMX or by 8-bromo-cyclic AMP was not depressed by acetylcholine, although they did not study the effects of acetylcholine on the increased I_{Ca} quantitatively. The used doses of IBMX, the concentration of acetylcholine at the neuroeffector junction and/or AV interval determined in anesthetized dogs in the present study might be different from their experimental conditions. It has been also reported that cyclic AMP increased I_{Ca} and I_K differentially in guinea pig ventricular myocytes (Harada and Iijima, 1994).

The intravenous injection of Bay k 8644, a Ca^{2+} channel agonist, augmented the negative chronotropic response to SAPS but attenuated the negative dromotropic response to AVPS (Fig. 5), whereas Bay k 8644 increased systemic arterial blood pressure. Similarly, Bay k 8644 injected into the AV node artery attenuated the negative dromotropic response to AVPS and to injection of carbachol into the AV node artery (Fig. 6). These results suggest that Bay k 8644 attenuates the negative dromotropic response to AVPS by acting at some postjunctional sites in the heart. Bay k 8644 increases intracellular Ca^{2+} by activating the slow inward current (Schramm et al., 1983; Endoh et al., 1986). On the other hand, acetylcholine does not reduce I_{Ca} induced by dialyzing the cell with cyclic AMP or with the catalytic subunits of cyclic AMP-dependent protein kinase in guinea pig ventricular myocytes (Hescheler et al., 1986), although I_{Ca} was depressed by acetylcholine and its depressive effect might be due to a reduction of cyclic AMP in the rabbit SA nodal myocytes (DiFrancesco and Tromba, 1988). The activated inhibitory G protein (G_i) obtained from human erythrocytes had no effects on I_{Ca} activity but the activated stimulatory G protein (G_s) activates I_{Ca} in guinea pig ventricular myocytes (Yatani et al., 1987). Therefore, Bay k 8644 probably attenuates the negative dromotropic responses to carbachol and parasympathetic nerve stimulation 'indirectly'. That is, the increase in I_{Ca} induced by Bay k 8644 antagonizes the effects mediated by the acetylcholine activated K^+ channels, namely, shortening of the action potential duration and hyperpolarization of the cell membrane.

Yatani et al. (1990) reported that the hyperpolarization activated current (I_f) is regulated by both G_s and G_i, but G_i is more potent than G_s in SA nodal pacemaker cells. G_s can regulate Ca^{2+} channels directly (Yatani et al., 1987). Thus, the interaction between G_i and G_s on I_f may be responsible for the augmentation by Bay k 8644 of the negative chronotropic response to parasympathetic nerve stimulation in the anesthetized dog heart. We, therefore, suggest that the postjunctional sympathetic–parasympathetic interactions are modified by cyclic AMP-independent mechanisms as well as by the cyclic AMP-dependent mechanisms as reported previously (Levy, 1971, 1989; Endoh et al., 1985; Lindemann and Watanabe, 1985; DiFrancesco and Tromba, 1988). The cyclic AMP-independent mechanisms are the indirect interactions between the effects mediated by Ca^{2+} channels and acetylcholine-activated K^+ channels and the regulation of I_f in each cardiac tissue. We also suggest that the differential autonomic interactions involved in SA nodal pacemaker activity and AV conductivity reflect the balance among these cyclic AMP-independent and -dependent mechanisms. However, we also need further studies of the role of phosphatidylinositol transduction and cyclic GMP on the autonomic interactions.

4.2. Effects of aminophylline on the negative chronotropic and dromotropic responses to parasympathetic stimulation

Aminophylline (theophylline ethylenediamine) induced positive chronotropic and dromotropic effects in a dose-dependent manner and attenuated the negative dromotropic response to AVPS. However, it did not attenuate the negative chronotropic response to SAPS (Fig. 4, Table 2). On the other hand, IBMX augmented both the negative dromotropic and chronotropic responses to parasympathetic stimulation (Fig. 5). Aminophylline and other methylxanthines have three basic cellular actions: (a) translocation of intracellular Ca^{2+}, (b) accumulation of cyclic AMP induced by phosphodiesterase inhibition, and (c) blockade of adenosine receptors (Rall, 1990). When we infused aminophylline at doses of 1.2 and 3.7 mg/kg/min i.v., plasma concentrations of theophylline were 37 and 106 μg/ml, respectively. These concentrations correspond to the concentrations of theophylline (2×10^{-4}–2×10^{-3} M) that inhibit phosphodiesterase in isolated mammalian cardiac tissues (Rall and West, 1963; Endoh, 1980). Thus, if aminophylline acted only as a phosphodiesterase inhibitor in the present study, the negative chronotropic response to SAPS would have been augmented by aminophylline as IBMX did, as previously reported (Endoh, 1979, 1980; MacLeod, 1985; Furukawa et al., 1989). Therefore, we suggest that in the present study, aminophylline acted on the chronotropic and dromotropic responses to parasympathetic stimulation (a) by inhibiting phosphodiesterase and (b) by translocating intracellular Ca^{2+} in the heart (Johnson and Inesi, 1969; Marcus et al., 1972; Donges et al., 1977; Rall, 1990). Actually, theophylline (0.1–0.3 mM) has a biphasic effect, early and late phases, on the intracellular Ca^{2+} transient in the canine ventricular muscle cells and theophylline in its late phase increases I_{Ca} cyclic AMP-independently (Endoh, 1994), although the biphasic effect of IBMX has not been observed yet. If aminophylline blocked adenosine receptors and thereby attenuated the negative dromotropic response to AVPS, it would also attenuate the negative chronotropic response to SAPS.

Adenosine receptors control SA nodal pacemaker activity and AV conductivity. Thus, the adenosine receptor blocking effect is probably not the main mechanism by which aminophylline attenuates the negative dromotropic response to parasympathetic stimulation in our experiments. Although aminophylline may increase plasma catecholamine concentrations in man (Vestal et al., 1983), aminophylline attenuated the negative dromotropic response to AVPS in dogs with or without propranolol treatment (Fig. 4). Therefore, aminophylline probably attenuated the negative dromotropic response to parasympathetic nerve stimulation by increases in I_{Ca} and translocating intracellular Ca^{2+}. This vagolytic effect may explain how high doses of aminophylline tend to inhibit the negative chronotropic effects of low frequency parasympathetic stimulation, as previously reported (Hadhazy, 1971, 1972; Urthaler and James, 1976). This effect and the results from Bay k 8644-induced attenuation of the dromotropic response to AVPS suggest that changes in intracellular Ca^{2+} as well as receptor transduction are responsible for the different sympathetic–parasympathetic interactions that characterize pacemaker activity and AV conductivity in the heart.

Acknowledgements

We thank Dr. Matthew N. Levy for editorial advice and Miss H. Tsubota for skilled technical assistance. This work was supported in part by Scientific Research Grant-in-Aid 4670112 and 08670105 from the Ministry of Education, Science and Culture, Japan.

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