ELSEVIER

European Journal of Pharmacology 309 (1996) 51–58

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Inhibition by bertosamil of cardiac responses to pinacidil or Bay k 8644 in isolated dog atria and ventricles

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Received 26 March 1996; revised 22 April 1996; accepted 23 April 1996

Abstract

We investigated the effects of a novel bradycardic agent, bertosamil (3-isobutyl-7-isopropyl-9,9-pentamethylene-3,7-diazabicyclo[3.3.1]nonane sesquihydrogenfumarate), on the sinus rate and atrial contractile force and the left ventricular contractile force in isolated, blood-perfused dog hearts and the blocking effects of bertosamil on the chronotropic and inotropic responses to pinacidil and Bay k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-piridine-5-carboxylate). Bertosamil (0.1–100 nmol) caused transient positive, followed by continuous negative, chronotropic responses and positive inotropic responses in atria, and increased the left ventricular contractile force. Neither propranolol nor atropine affected the cardiac responses to bertosamil. Bertosamil (3–100 nmol) dose dependently attenuated the negative chronotropic and inotropic responses to pinacidil but not to acetylcholine. Bertosamil at a high dose attenuated the positive cardiac responses to Bay k 8644, norepinephrine and isoproterenol. These results suggest that bertosamil inhibits negative cardiac responses mediated by an ATP-sensitive K^+ channel but not an acetylcholine muscarinic receptor and, at a high dose, attenuates the L-type Ca^{2+} channel-mediated positive cardiac responses in isolated dog hearts.

Keywords: Bertosamil; K^+ channel, ATP-sensitive; Ca^{2+} channel, L-type; Chronotropism; Inotropism; Heart, dog

1. Introduction

Reduction of heart rate as a mechanism to decrease myocardial oxygen consumption in patients with ischemic heart disease is one of the major effects of β-adrenoceptor antagonists and certain Ca^{2+} channel blockers. However, these agents have negative inotropic and hypotensive effects concomitantly, which would aggravate heart failure, and excessive hypotension would impair coronary circulation. Some specific bradycardic agents (referred to as sinus node inhibitors) have been developed to reduce heart rate selectively without influencing contractility. In our recent studies of bradycardic agents, zatebradine decreased sinus rate with an increase in atrial contractile force, but at high doses decreased ventricular contractile force in isolated, perfused dog heart preparations (Furukawa et al., 1993). Other bradycardic agents, such as alinidine, falipamil, and E 4080, reduced atrial contractile force in the isolated, perfused right atrium (Ogiwara et al., 1987, 1988; Sawaki et al., 1993).

Bertosamil (3-isobutyl-7-isopropyl-9,9-pentamethylene-3,7-diazabicyclo[3.3.1]nonane sesquihydrogenfumarate) has been developed as a bradycardic, antiarrhythmic and antiischemic agent. However, little information is available on the properties of this compound. Bertosamil (0.5–5.0 μmol/kg) caused elevation of the blood pressure, reduced heart rate, diminished the double product (heart rate × blood pressure) and possessed ventricular and atrial antiarrhythmic activity in anesthetized cats (Papp et al., 1992). In electrophysiological studies, bertosamil decreased the maximum rate of depolarization (V_{max}), lengthened the action potential duration, and prolonged the effective refractory period markedly in atrial and ventricular myocardial cells of the rabbit (Krassói and Papp, 1992). These results indicate that bertosamil possesses both class I and III antiarrhythmic features. Bertosamil inhibited the currents of the cloned K^+ channels, K_v 1.2 (a delayed rectifier type, IK_v 1.2) and K_v 1.4 (a transient outward type, IK_v 1.4) expressed in *Xenopus* oocytes (Yamagishi et al., 1995). However, there is no available report of the

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PII S0014-2999(96)00322-6

effects of bertosamil on the sinus rate and myocardial contractility, and the mechanisms of the effects of bertosamil on the heart.

In the present study, therefore, we tried to investigate the effects of bertosamil on the rate, atrial force and ventricular force in the isolated, blood-perfused dog heart preparations. We also investigated the effects of bertosamil on the cardiac responses to autonomic interventions and ion channel agonists, pinacidil and Bay k 8644.

2. Materials and methods

2.1. Preparations

An isolated right atrial or left ventricular preparation was perfused with heparinized arterial blood from an anesthetized support dog. The details of these preparations have been described in previous papers (Chiba et al., 1975; Chiba, 1976). Support dogs, weighing 10–30 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and ventilated artificially through a cuffed tracheal tube with room air by using a Harvard respirator (Harvard Apparatus Co., Millis, MA, USA, model 607). Sodium heparin (500 United States Pharmacopoeia (USP) units/kg i.v.) was administered to each dog at the beginning of the perfusion of the isolated atrial or ventricular preparation and 200 USP U/kg was given each hour thereafter.

Isolated right atrial or left ventricular preparations were obtained from other mongrel dogs weighing from 8 to 19 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After sodium heparin (200 USP U/kg, i.v.) was administered, the right atrium or left ventricle was excised and immersed in cold Ringer's solution of the following composition (mM): NaCl, 154.0; KCl, 5.6; $CaCl_2$, 2.2 and $NaHCO_3$, 3.6. The sinus node artery of the isolated right atrium or the anterior descending branch of the left coronary artery of the isolated left ventricle was cannulated and each preparation was perfused with heparinized blood from the carotid artery of the anesthetized support dog by the aid of a peristaltic pump (Harvard Apparatus, model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mm Hg. The rate of blood flow to the atrial or ventricular preparation was 3–10 ml/min. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through an external jugular vein.

The preparation was anchored to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (Nihon Kohden, Tokyo, Japan, AP-620G) by a silk thread. The cardiac tissue was usually stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden, RTA-1200). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation in order to record the atrial electrogram or to drive the left ventricle electrically. The left ventricular preparation was electrically paced at a frequency of 2 Hz with a 1-ms pulse dulation and supramaximal voltages (4 V). The atrial rate was derived from the electrogram with a cardiotachometer (Nihon Kohden, AT-600G). The femoral arterial blood pressure and heart rate derived from lead II of the ECG of the support dog and the rate of blood flow to a preparation were monitored simultaneously.

2.2. Experimental protocols

In the first series, we examined the effects of bertosamil on the sinoatrial nodal pacemaker activity and atrial myocardial contractility in the isolated right atrium and the left ventricular myocardial contractility in the isolated left ventricle. Bertosamil at a dose of 0.1–100 nmol was injected into the sinus node artery of the isolated atrium ($n = 9$) or the anterior descending branch of the left coronary artery of the isolated ventricle ($n = 5$), and we then determined its chronotropic and inotropic effects. To investigate the effects of bertosamil on the autonomic nervous system, we studied the effects of atropine (10 nmol, $n = 6$) and propranolol (10 nmol, $n = 5$) on the responses to bertosamil (30 nmol) in the isolated atrium.

In the second series, to investigate the effects of bertosamil on the ATP-sensitive K^+ channel (K^+_{ATP})-mediated cardiac responses, we studied the effects of bertosamil (3–100 nmol) on the negative chronotropic and inotropic responses to K^+_{ATP} channel openers, pinacidil (0.01–3 μmol, $n = 5$) and cromakalim (0.01–0.3 μmol, $n = 3$), in the isolated atrial preparation and on the negative inotropic response to pinacidil ($n = 3$) in the isolated left ventricle. The negative cardiac responses to cumulative administration of pinacidil were observed before and 2 min after the application of bertosamil. Each dose of bertosamil was injected at intervals of more than 30 min. We also studied the effects of bertosamil (30 nmol) on the negative chronotropic and inotropic responses to acetylcholine (0.3, 1 and 3 nmol) in the isolated atrium.

In the third series, to investigate the effects of bertosamil on the L-type Ca^{2+} channel-mediated cardiac responses, we studied the effects of bertosamil (1–100 nmol) on the positive chronotropic and inotropic responses to an L-type Ca^{2+} channel agonist, Bay k 8644 (10 nmol, $n = 6$) and to β-adrenoceptor agonists, norepinephrine (0.3 nmol, $n = 6$) and isoproterenol (3 or 10 pmol, $n = 5$) in the isolated atrial preparation.

2.3. Drugs

The drugs used in the present experiments were bertosamil {3-isobutyl-7-isopropyl-9,9-pentamethylene-3,7-diazabicyclo[3.3.1]nonane sesquihydrogenfumarate}

(generously donated by Solvay Pharma Deutschland, Germany), acetylcholine chloride (Daiichi Seiyaku, Tokyo, Japan), atropine sulfate (Wako Pure Chemicals, Tokyo, Japan), norepinephrine hydrochloride (Sankyo, Tokyo, Japan), propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), *l*-isoproterenol hydrochloride (Nikken Kagaku, Tokyo, Japan), pinacidil (Shionogi, Osaka, Japan), and methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-piridine-5-carboxylate (Bay k 8644, generously donated by Dr F. Seuter, Bayer, Wuppertal-Elberfeld, Germany). Pinacidil was dissolved in 0.1 N HCl to make a stock solution of 100 mM and then diluted with saline to obtain low concentrations. Bay k 8644 was dissolved in ethanol and other drugs were dissolved in saline before the start of the experiments. The amount of drug solution injected was 1–30 μl.

2.4. Statistical analysis

The data are shown as the maximal change in response to each drug and are expressed as means ± S.E.M. The data were analyzed with an analysis of variance and Bonferroni's method for multiple comparisons of data. Student's *t*-test for paired or unpaired data was used for comparisons between the two groups. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of bertosamil on sinoatrial nodal pacemaker activity, atrial contractility and ventricular contractility

When bertosamil (0.1–100 nmol) was injected into the sinus node artery of an isolated right atrium, bertosamil

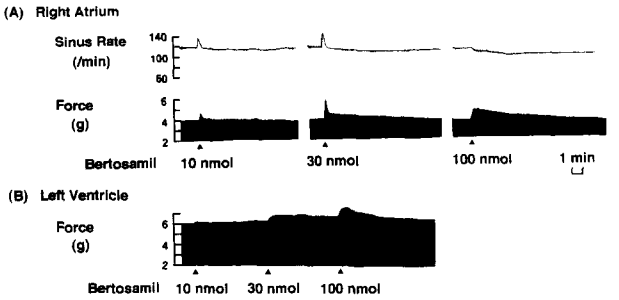

Fig. 1. Chronotropic and inotropic effects of bertosamil at doses of 10, 30 and 100 nmol in isolated, blood-perfused right atrium of the dog (A) and inotropic effects of bertosamil at doses of 10, 30 and 100 nmol in isolated, blood-perfused left ventricle (B).

decreased sinus rate and increased atrial contractile force (Fig. 1A and Fig. 2A). Bertosamil decreased sinus rate with a transient positive chronotropic effect in the early phase, but in 2 of 9 preparations, no positive chronotropic effect was observed. The duration of the negative chronotropic effect was much longer than that of the positive inotropic effect. Bertosamil (0.1–100 nmol) increased the left ventricular contractile force in the isolated, blood-perfused left ventricle (Fig. 1B and Fig. 2B).

We tested whether the cardiac responses to bertosamil were mediated by activation of the autonomic nervous system. Propranolol (10 nmol) did not change significantly the positive followed by negative chronotropic and positive inotropic responses to bertosamil (30 nmol) while the positive cardiac responses to norepinephrine (0.1 nmol) were suppressed significantly ($P < 0.05$) in 5 isolated atria (Fig. 3A).

While atropine (10 nmol) blocked the negative chronotropic and inotropic responses to acetylcholine (1 nmol), it did not significantly affect the negative

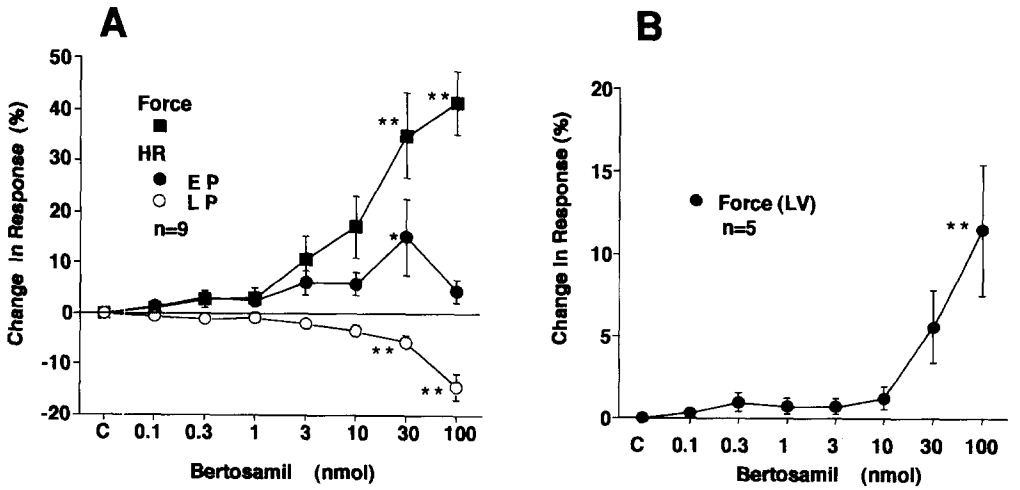

Fig. 2. Positive inotropic (■) and positive (early phase, E.P.) (○) followed by negative (late phase, L.P.) (●) chronotropic responses to bertosamil at doses of 0.1–100 nmol in 9 isolated, blood-perfused canine atria (A) and positive inotropic responses (●) to bertosamil in 5 isolated, blood-perfused canine left ventricles (B). Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 9 isolated atria were 111 ± 2.3 (mean ± S.E.) beats/min and 2.5 ± 0.4 g, respectively. The basal left ventricular contractile force in 5 isolated ventricles was 2.6 ± 0.6 g. * $P < 0.01$ vs. control.

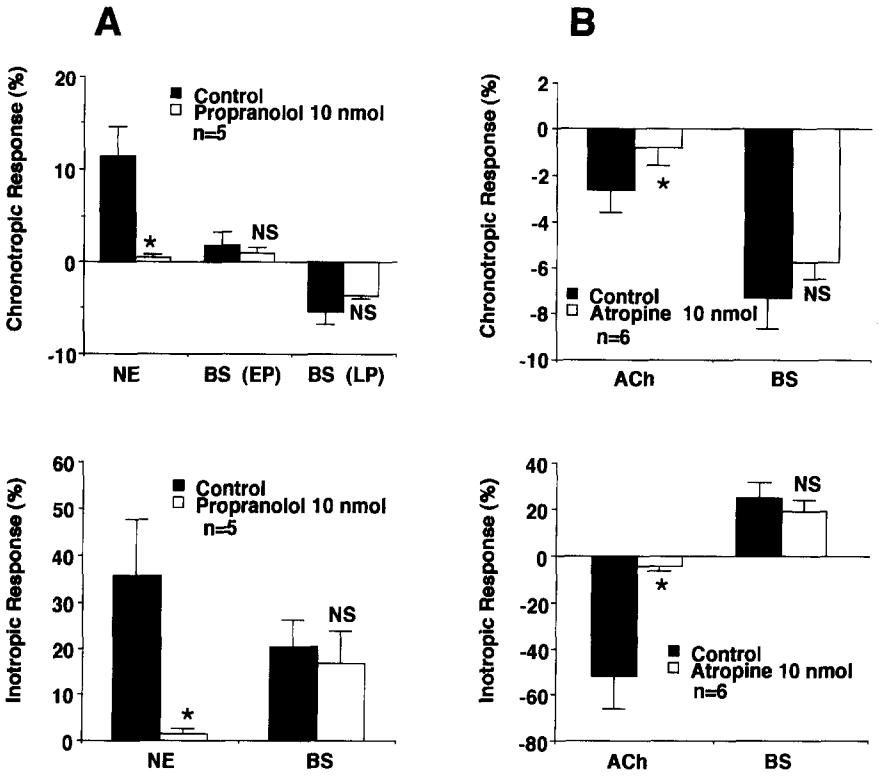

Fig. 3. Effects of 10 nmol of propranolol on the chronotropic and inotropic responses to bertosamil (BS, 30 nmol) and the responses to norepinephrine (NE, 0.1 nmol) in 5 isolated, blood-perfused dog atria (A). The basal sinus rate and contractile force in 5 isolated atria were 101 ± 5.2 beats/min and 2.8 ± 0.5 g. Effects of 10 nmol of atropine on the chronotropic and inotropic responses to bertosamil (BS, 30 nmol) and the responses to ACh (1 nmol) in 6 isolated, blood-perfused dog atria (B). The basal sinus rate and atrial contractile force in 6 isolated atria were 97 ± 10.4 beats/min and 3 ± 0.2 g. Vertical bars show S.E.M. * $P < 0.05$; NS, not significant vs. control.

chronotropic and positive inotropic responses to bertosamil (30 nmol) in 6 isolated atria (Fig. 3B).

3.2. Effects of bertosamil on the K^+ channel-mediated negative cardiac responses to pinacidil or acetylcholine

An ATP-sensitive K^+ channel opener, pinacidil (0.03–3 μmol), when injected cumulatively, induced negative chronotropic and inotropic responses in a dose-related manner and at a dose of 1 μmol decreased sinus rate and atrial contractile force by 15 ± 4.8% and 90 ± 4.4%, respectively, in 5 isolated atria. Fig. 4 shows the inhibition by bertosamil (100 nmol) of the negative chronotropic and inotropic effects of pinacidil in a dose of 0.03–3 μmol in an isolated, blood-perfused dog atrium. Bertosamil (3–100 nmol) dose dependently inhibited the negative chronotropic and inotropic responses to pinacidil ($P < 0.05$, Fig. 5). The ID_{50} values of bertosamil for the negative chronotropic and inotropic effects of pinacidil at a dose of 0.3 μmol were 48.2 ± 10.4 and 37.0 ± 5.6 nmol, respectively. In the 3 isolated left ventricular preparations, bertosamil (3–100 nmol) also inhibited the negative inotropic responses to pinacidil (0.03–3 μmol) injected cumulatively (Fig. 5C).

We also studied the effects of bertosamil (10 and 100 nmol) on the negative responses to cromakalim, another ATP-sensitive K^+ channel opener, in 3 isolated atria. Bertosamil (100 nmol) attenuated the negative chronotropic and inotropic responses to 0.3 μmol of cromakalim by 6.4 ± 4.5% and 56.5 ± 2.3% ($P < 0.05$), respectively, from the control response (100%).

To investigate whether bertosamil has an antimuscarinic property, we studied the effects of bertosamil (30 nmol) on the negative chronotropic and inotropic responses to acetylcholine. Two minutes after the injection of bertosamil, the negative chronotropic and inotropic responses to acetylcholine (0.3–3 nmol) were not affected significantly by bertosamil (Fig. 6).

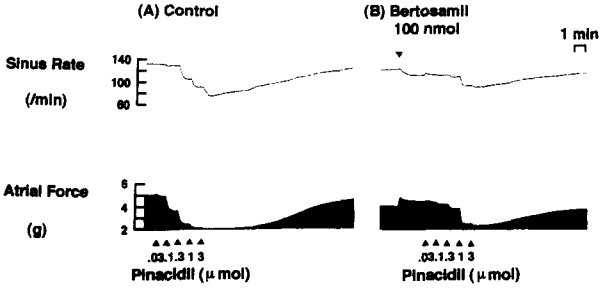

Fig. 4. Representative tracings of the effects of bertosamil on the negative chronotropic and inotropic responses to pinacidil injected cumulatively into the sinus node artery in an isolated, blood-perfused canine atrium. Pinacidil-induced cardiac responses were determined before (control) (A) or 2 min after the treatment with bertosamil (100 nmol) (B).

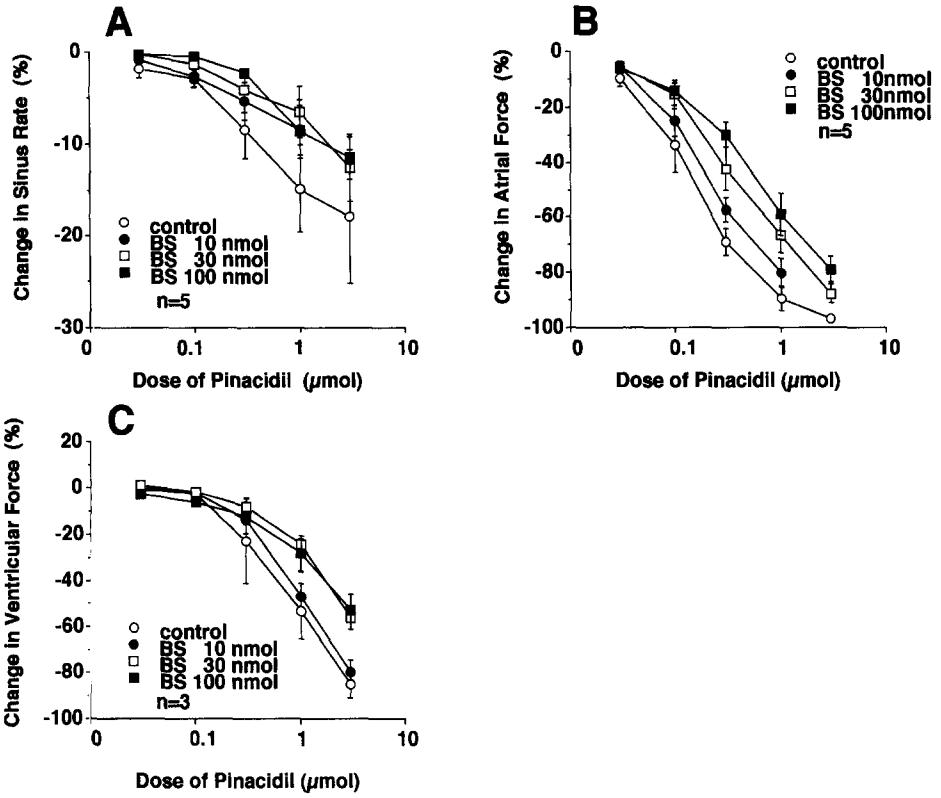

Fig. 5. Effects of bertosamil on the negative chronotropic (A) and inotropic (B) responses to pinacidil in 5 isolated, blood-perfused canine atria, and on the negative inotropic response to pinacidil in 3 isolated, blood-perfused canine ventricles (C). Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 5 isolated atria were 90 ± 3.5 beats/min and 2.3 ± 0.1 g. The basal contractile force in 3 left ventricles was 3.0 ± 1.0 g.

3.3. Effects of bertosamil on the positive cardiac responses to Bay k 8644 and norepinephrine

Bay k 8644 (10 nmol), an L-type Ca^{2+} channel agonist, increased sinus rate and atrial contractile force by 21 ± 6.1 beats/min (22 ± 6.5%) and 2.2 ± 0.5 g (85 ± 20.7%), respectively, in 6 isolated atria. Pretreatment with bertosamil (1–100 nmol) attenuated the positive chronotropic and inotropic responses to Bay k 8644 dose dependently ($P < 0.01$, Fig. 7).

Bertosamil (100 nmol) significantly attenuated the positive chronotropic response ($P < 0.01$) but not the inotropic response to norepinephrine at a dose of 0.3 nmol (Fig. 7). However, bertosamil did not affect the positive responses to 1 nmol of norepinephrine significantly (data not shown). Norepinephrine at 0.3 and 1 nmol increased sinus rate and

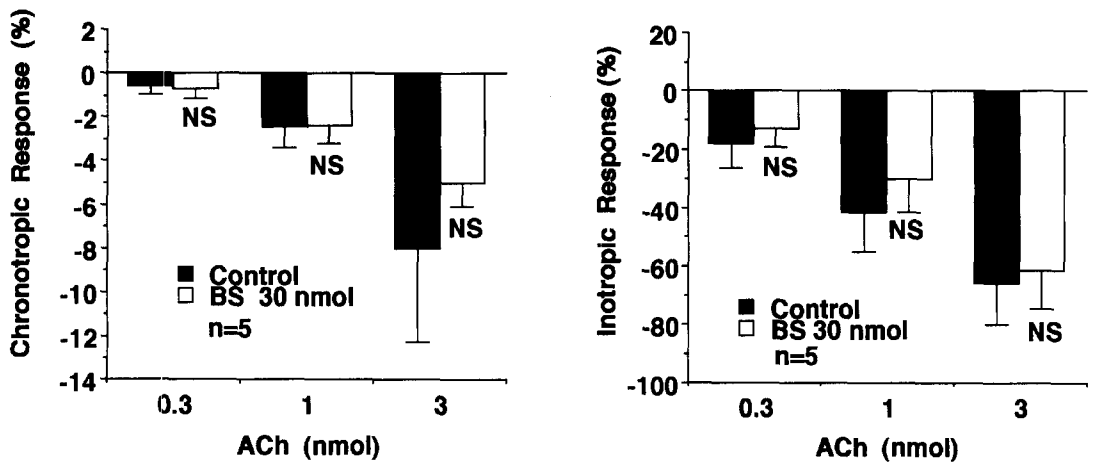

Fig. 6. Effects of bertosamil (30 nmol) on the negative chronotropic (left panel) and inotropic (right panel) responses to acetylcholine (ACh, 0.3–3 nmol) in 5 isolated, blood-perfused dog atria. Vertical bars show S.E.M. NS, not significant vs. control. The basal sinus rate and atrial contractile force in 5 isolated atria were 134 ± 12.0 beats/min and 1.7 ± 0.5 g.

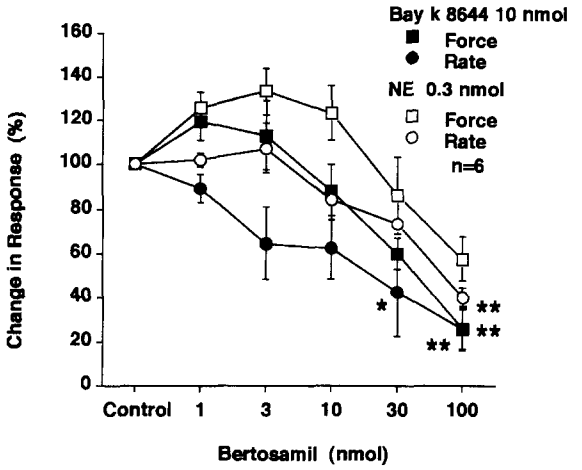

Fig. 7. Effects of bertosamil (1–100 nmol) on the positive chronotropic (●) and inotropic (■) responses to Bay k 8644 (10 nmol) and on the positive chronotropic (○) and inotropic (□) responses to norepinephrine (NE, 0.3 nmol) in the isolated, blood-perfused dog atrium. Data from 6 experiments are shown as the percentage changes from the control values in response to each drug. Vertical bars show S.E.M. The basal sinus rate and atrial contractile force in 6 isolated atria were 94 ± 12.6 beats/min and 2.6 ± 0.1 g. * $P < 0.05$; ** $P < 0.01$ vs. control.

atrial contractile force by 19 ± 4.7 beats/min (20 ± 5.0%) and 1.5 ± 0.2 g (59 ± 7.6%) and 53 ± 12.4 beats/min (54 ± 12.9%) and 5.2 ± 1.3 g (202 ± 78.6%), respectively, from the respective control values. Bertosamil (100 nmol) significantly ($P < 0.05$) attenuated the positive chronotropic and inotropic responses to isoproterenol to 48 ± 11.3% and 48 ± 11.2%, respectively, from the control (100%) in 5 isolated atria. Isoproterenol (3 or 10 pmol) increased sinus rate and atrial contractile force by 26 ± 4.7 beats/min (24 ± 5.0%) and 1.8 ± 0.3 g (105 ± 28.4%), respectively, from the basal control values.

4. Discussion

In the present study, we demonstrated that bertosamil caused a decrease in sinus rate with a transient increase in sinus rate, and an increase in atrial contractile force and an increase in left ventricular contractile force in the isolated, blood-perfused atrial and ventricular preparations of the dog. The cardiac responses to bertosamil were influenced by neither atropine nor propranolol (Fig. 3). Therefore, we suggest that bertosamil induced negative chronotropic and positive inotropic responses not due to direct activation of muscarinic receptors or β-adrenoceptors in isolated, blood-perfused dog heart preparations. It was reported briefly that bertosamil attenuates the maximum rate of depolarization and prolongs the action potential duration and effective refractory period in rabbit atrial and ventricular myocytes (Krassói and Papp, 1992). Bertosamil also inhibits the currents of the cloned K^+ channels, K_v 1.2 (a delayed rectifier type) and K_v 1.4 (a transient outward type) in *Xenopus* oocytes (Yamagishi et al., 1995). The Class I antiarrhythmic agents, quinidine and disopyramide, decrease sinus rate and contractile force in the isolated dog atrium (Chiba et al., 1979; Watanabe and Chiba, 1982), a transient outward current inhibitor, 4-aminopyridine, causes a positive inotropic effect with a small positive chronotropic effect in isolated dog atrium (Furukawa et al., 1985) and E4031, a delayed rectifier K^+ current inhibitor, causes negative chronotropic responses without changes in contractile force in isolated guinea pig hearts (Wettwer et al., 1991). Thus, it is conceivable that the chronotropic and inotropic effects of bertosamil are induced by the inhibition of the several ionic currents by bertosamil in the isolated, dog heart preparations.

In addition to the direct cardiac effects of bertosamil, we found that bertosamil inhibited the negative chronotropic and inotropic responses to ATP-sensitive K^+ channel activation induced by pinacidil and cromakalim in the isolated perfused dog atrial and ventricular preparations and that the ID_{50} for the cardiac responses to pinacidil at a dose of 0.3 μmol was 50 nmol (Fig. 5). However, bertosamil did not affect the negative cardiac responses to acetylcholine. Therefore, we suggest that bertosamil has an inhibitory property on the ATP-sensitive K^+ channel in addition to the antiarrhythmic properties, i.e., inhibition of the maximum rate of depolarization and prolongation of the atrial and ventricular effective refractory period (Krassói and Papp, 1992), in the isolated dog heart. In the isolated, perfused atrium of the dog, an ATP-sensitive K^+ channel blocker, glibenclamide, blocks the negative chronotropic and inotropic responses to pinacidil with an ID_{50} of 0.6 μmol for the negative cardiac responses to 0.3 μmol of pinacidil (Murakami et al., 1992). Thus, bertosamil is more potent as an ATP-sensitive K^+ channel blocker than glibenclamide in the dog heart. It has been reported that class I, III and IV antiarrhythmic drugs antagonize the ATP-sensitive K^+ channel-mediated responses in isolated rabbit hearts (Friedrichs et al., 1993), isolated rat myocardial cells (Haworth et al., 1989) and *Xenopus* oocytes (Sakura et al., 1993). However, the inhibition by quinidine, verapamil or amiodarone of the ATP-sensitive K^+ channel-mediated responses is much less effective than that by glibenclamide in the rat heart cell (Haworth et al., 1989). On the other hand, a class III antiarrhythmic agent, MS-551, attenuates the ATP-sensitive K^+ channel-related ventricular fibrillation, suggesting a role of the antiarrhythmic effects of ATP-sensitive K^+ channel blockers in the heart (Friedrichs et al., 1994).

Glibenclamide at a high concentration inhibits the cardiac responses to acetylcholine in isolated rat ventricular myocytes (Kirsch et al., 1990) and in isolated perfused dog atria (Murakami et al., 1992). Some antiarrhythmics such as quinidine, disopyramide and MS-551 have antimuscarinic effects on the heart (Mirro et al., 1980; Iisalo and Aaltonen, 1983; Nowrath et al., 1984; Mori et al., 1995). Thus, although antimuscarinic effects of antiarrhythmic

agents and an ATP-sensitive K^+ channel blocker have been reported, bertosamil in the doses used did not inhibit the negative cardiac responses to acetylcholine in the isolated dog atrium (Fig. 6).

Bertosamil also attenuated the positive chronotropic and inotropic responses to Bay k 8644 and norepinephrine in the isolated, perfused dog atrium (Fig. 7), suggesting that bertosamil inhibits L-type Ca^{2+} channel activation or intracellular Ca^{2+} handling following L-type Ca^{2+} channel activation in the dog heart. The positive chronotropic and inotropic responses to Bay k 8644 are blocked by nicardipine and verapamil in the isolated, perfused dog atrium (Furukawa et al., 1988). However, it is unlikely that bertosamil inhibits the hyperpolarization-activated inward current in the dog heart because bertosamil inhibited both positive chronotropic and inotropic responses to norepinephrine and isoproterenol in the isolated dog atrium. A hyperpolarization-activated inward current inhibitor, zatebradine, selectively inhibits the positive chronotropic but not the positive inotropic responses to norepinephrine and isoproterenol but inhibits neither positive chronotropic nor positive inotropic responses to Bay k 8644 (Sawaki et al., 1995). Together with a brief previous report (Krassói and Papp, 1992) we, therefore, suggest that bertosamil inhibits the ATP-sensitive K^+ channel and L-type Ca^{2+} channel in addition to its class I and III antiarrhythmic properties in the heart. A class III antiarrhythmic agent, MS-551, inhibits K^+ channels and at higher doses attenuates the Ca^{2+} current but not V_{max} in rabbit ventricular cells (Nakaya et al., 1993). Although another antiarrhythmic agent, amiodarone, has the properties of class I, II, III, and IV antiarrhythmic agents, the precise mechanisms of the antiarrhythmic effects of amiodarone are still not known (Singh and Sarma, 1994). Thus, bertosamil may act as an antiarrhythmic agent with properties of class I, III and IV agents, but the potential antiarrhythmic activity of bertosamil should be examined further in animal arrhythmia models.

Acknowledgements

We thank Solvay Pharma Deutschland GMBH (Hannover, Germany) for the generous supply of bertosamil.

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