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Bradykinin B_2 receptor-mediated chronotropic effect of bradykinin in isolated guinea pig atria

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Abstract

The present study was undertaken to characterize the direct chronotropic effect of bradykinin in isolated spontaneously beating atria of the guinea pig. Bradykinin caused concentration-dependent increases in the beating rate of atria. In contrast, the active metabolite of bradykinin and the typical bradykinin B_1 receptor agonist, Des-Arg^9-bradykinin, had no effect on the beating rate of atria. Inhibition of converting enzyme or neutral endopeptidase by captopril or SQ-28603, respectively, did not affect beating rate but potentiated bradykinin-induced increase in beating rate. The potent bradykinin B_2 receptor antagonist, HOE 140, antagonized bradykinin-induced chronotropic effect. In contrast, the bradykinin B_1 receptor antagonist, Lys-[Leu^8]Des-Arg^9-bradykinin, had no effect. The increase in beating rate caused by bradykinin was not affected by blockade of β_1-adrenoceptors, cyclooxygenase, or nitric oxide synthesis using atenolol, indomethacin and N^{ω}-nitro-L-arginine, respectively. Unlike bradykinin, angiotensin I and angiotensin II caused very small or no change in beating rate in the presence or absence of captopril and SQ-28603. These results indicate that bradykinin causes a direct positive chronotropic effect which is mediated by activation of bradykinin B_2 receptors independently of prostaglandins and β_1-adrenoceptors.

Keywords: Bradykinin; Bradykinin B_2 receptor; Sinus rate; Atrium, guinea pig

1. Introduction

The nonapeptide bradykinin has been widely studied for its potent vasodilator effects that are mediated by release of nitric oxide and prostacyclin via activation of bradykinin B_2 receptors (Sung et al., 1988). The effects of bradykinin can be modulated by inhibitors of angiotensin-converting enzyme which appears to be largely responsible for its proteolytic breakdown (Williams and Hollenberg, 1977; Swartz et al., 1979). Bradykinin has been shown to be released in ischemic heart and reduces reperfusion-ventricular arrhythmias (Kimura et al., 1973; Linz et al., 1990; Tito et al., 1991; Chahine et al., 1993). Its cardioprotective effect has been attributed to improvement in cardiac performance, increase in blood flow and/or modulation of noradrenaline release that could be mediated by activation of bradykinin B_1 receptors (Chahine et al., 1993). Previous reports have shown that intravenous or local administration of bradykinin to the epicardium of the canine heart causes an increase in heart rate (Nakamo, 1965; Staszewska-Barczak et al., 1976). Similarly bradykinin has been shown to stimulate the sino-atrial node in isolated guinea pig atria (Nakashima et al., 1982). In contrast, others have reported a decrease in heart rate in response to an intracoronary injection of bradykinin in intact or autonomically altered anesthetized dogs (Neto et al., 1974; Ribuot et al., 1993). The chronotropic effects of bradykinin, both in vivo and in vitro, remain controversial.

Because the isolated atrium is not under that influence of autonomic reflex or circulating hormones it offers direct quantitative measurement under equilibrium conditions of changes in sinus rate. This study was initiated to examine the direct chronotropic effects of bradykinin in isolated spontaneously beating guinea pig atria. The involvement of kinin receptors in the chronotropic effect was probed using potent and specific bradykinin B_1 and B_2 receptor antagonists, Lys-

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[Leu^8]des-Arg^9-bradykinin and HOE 140, respectively (Regoli and Barabé, 1988; Hock et al., 1991).

2. Materials and methods

2.1. Atrial preparation

Male Hartley guinea pigs (~ 300 g) were killed by exsanguination after asphyxia with carbon dioxide. The pericardium was carefully removed from the heart and the right atrium was dissected. A suture was tied to the upper and lower tip of the atrium. The spontaneously beating atrium was suspended between a fixed end and the distal end was connected to a force transducer for measurement of beating rate. Beating rates were determined by tachograph which integrated the beating rate to a linear scale on the recorder. The atria were placed in an organ bath filled with physiological salt solution containing (in mmol/l): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, disodium EDTA 0.026 and glucose 5.5. The solutions were kept at 37° C and were continuously gassed with 95% O_2-5% CO_2 to maintain the pH at 7.4. The resting tension was set at 1 g during a 1 h equilibration period. Cumulative concentration-response curves for the positive chronotropic effect of bradykinin were determined. The beating rate was assessed 1 min after the addition of each successive half-log concentration of agonist. Under these conditions there was no tachyphylaxis to bradykinin. At the end of the experiments, isoprenaline (10 μmol/l) was added to obtain maximal beating rate. Antagonists were added 30 min before addition of bradykinin in different atrial preparations.

2.2. Drugs

The pharmacological agents used were the following: angiotensin I, angiotensin II, atenolol, bradykinin, des-Arg^9-bradykinin, indomethacin, (−)-isoprenaline, Lys-[Leu^8]des-Arg^9-bradykinin, N^{ω}-nitro-L-arginine (Sigma Chemical, St. Louis, MO, USA), captopril and SQ-28603 (Bristol-Myers Squibb, Princeton, NJ, USA), HOE 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) (Peninsula Lab, Belmont, CA, USA). Unless otherwise specified, drugs were dissolved in distilled water. Indomethacin was prepared in 2% Na_2CO_3.

2.3. Data analysis

Changes in sinus rate are expressed as percent of maximum increase in beating rate caused by isoprenaline (10 μmol/l). The values are expressed as means ± S.E. Statistical evaluation of the data was made by use of repeated measures of analysis of variance or Student's *t*-test for paired comparisons of mean values. Values with P less than 0.05 were regarded as significant. In all experiments, n equals the number of guinea pigs from which the tissues were taken.

3. Results

3.1. Effects of bradykinin on beating rate

Bradykinin (0.1 nmol/l–10 μmol/l) caused a concentration-dependent positive chronotropic effect on the atria (EC_{50}: 281 ± 70 nmol/l, $n = 7$) (Fig. 1). In contrast, des-Arg^9-bradykinin (0.1 nmol/l–10 μmol/l), a bradykinin B_1 receptor agonist, had no effect on beating rate of the atria. Compared to isoprenaline, a β-adrenoceptor agonist, bradykinin was approximately 30-fold less potent in increasing the beating rate of the atria (isoprenaline EC_{50}: 7.1 ± 1.2 nmol/l, $n = 10$).

Atenolol (10 μmol/l), a β_1-adrenoceptor antagonist, had no effect on bradykinin-induced increase in beating rate (Fig. 2). By contrast, the same concentration of atenolol caused significant ($P < 0.05$ vs. control) rightward shift on isoprenaline-induced positive chronotropic effect (EC_{50}: 150 ± 46 nmol/l, $n = 8$).

3.2. Effects of bradykinin receptor antagonists

The potent bradykinin B_2 receptor antagonist, HOE 140 (0.1, 1 μmol/l) antagonized bradykinin-induced chronotropic effect (Fig. 1). In contrast, Lys-[Leu^8]des-Arg^9-bradykinin (0.1, 1 μmol/l), a bradykinin B_1 receptor antagonist, had no effect on the increase beating rate of atria (Fig. 1).

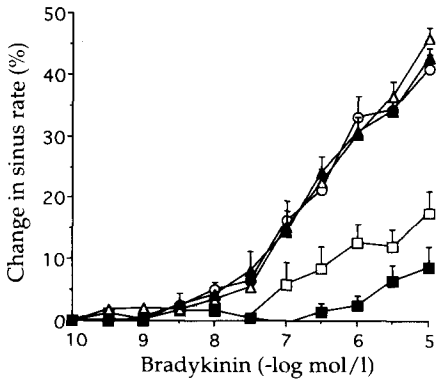

Fig. 1. Effects of bradykinin on beating rate: In control (○) spontaneously beating guinea pig atrium, bradykinin caused concentration-dependent positive chronotropic effect. HOE 140 in concentrations of 0.1 μmol/l (□) and 1 μmol/l (■) antagonized the increase in sinus rate resulting in a right-ward shift of bradykinin concentration-response curves. Lys-[Leu^8]Des-Arg^9-bradykinin (0.1 μmol/l (△) and 1 μmol/l ▲) had no effect on the chronotropic effects of bradykinin. Values are expressed as percent maximal response to isoprenaline (10 μmol/l), means ± S.E., $n = 7$.

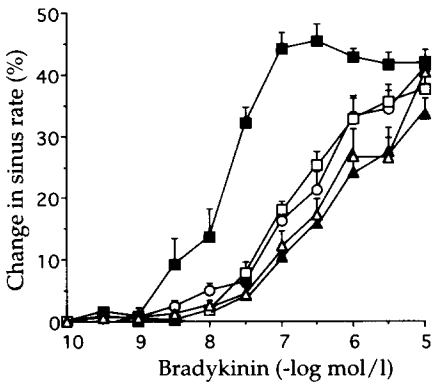

Fig. 2. Effects of inhibition of converting enzyme, β_1-adrenoceptor, cyclooxygenase and nitric oxide: Bradykinin-induced (control, ○) increase in beating rate was not affected by treating atria with atenolol (10 μmol/l, △), indomethacin (3 μmol/l, ▲) or N^{ω}-nitro-L-arginine (30 μmol/l, □). Captopril (1 μmol/l, ■) markedly augmented the bradykinin-induced increase in beating rate. $n = 6–7$.

3.3. Effects of captopril and SQ-28603

Inhibition of converting enzyme by captopril (1 μmol/l) did not affect basal beating rate but significantly ($P < 0.05$ vs. control) potentiated the bradykinin-induced increase in beating rate (EC_{50}: 16 ± 5.3 nmol/l, $n = 7$) (Fig. 2). Similarly, SQ-28603, a neutral endopeptidase inhibitor (1 μmol/l), enhanced bradykinin-induced chronotropic effects (EC_{50}: 100 ± 37 nmol/l, $n = 5$).

3.4. Role of prostanoids and nitric oxide

Treatment of guinea pig atria with indomethacin (3 μmol/l), a cyclooxygenase inhibitor, or N^{ω}-nitro-L-arginine (30 μmol/l), a nitric oxide synthesis inhibitor, had no significant effect on basal beating rate as well as on bradykinin-induced increase in beating rate (EC_{50}: 320 ± 238 and 140 ± 30 nmol/l, $n = 6$, 7, respectively) (Fig. 2).

3.5. Effects of angiotensin I and angiotensin II

Angiotensin I and angiotensin II (0.1 nmol/l–10 μmol/l) had very weak effect on the beating rate of atria (< 10% maximal isoprenaline) in the absence and presence of captopril or SQ-28603 (1 μmol/l) (data not shown).

4. Discussion

The present study demonstrates that bradykinin exhibits direct positive chronotropic effects in isolated atria. In contrast, in vivo studies have reported a decrease in heart rate in response to bradykinin injection in anesthetized dogs (Neto et al., 1974; Ribuot et al., 1993). The dissociation with in vivo studies could be at least in part due to an indirect vagally-mediated decrease in heart rate by a reflex mechanism. However, studies in anesthetized dogs in which baroreceptor-mediated responses were abolished by surgical bilateral vagotomy, bradykinin caused bradycardia (Ribuot et al., 1993). This could hypothetically be due to vasodilatory effects of bradykinin which could potentially decrease cardiac afterload and improve ventricular performance thereby overcoming its direct chronotropic effect. In the current study, it is unlikely that the tachycardia following bradykinin stimulation was due to indirect catecholamine release which in turn stimulates β_1-adrenoceptors because atenolol had no effect on the increase in beating rate caused by bradykinin. The concentration of atenolol was sufficient to displace the isoprenaline cumulative concentration-response curve more than 25-fold to the right. Similarly, others have shown that the β-adrenoceptor antagonist, propranolol, did not alter the chronotropic effect of bradykinin in guinea pig atrium (Nakashima et al., 1982). The augmentation of the positive chronotropic effect of bradykinin by captopril or SQ-28603 is consistent with the notion that inhibition of converting enzyme or neutral endopeptidase prevents degradation of bradykinin (Williams and Hollenberg, 1977; Swartz et al., 1979). Captopril had no direct intrinsic activity on basal beating rate of atria. An increase in prostacyclin and nitric oxide release have been described as potential mediators in cardioprotective and vasodilatory actions of bradykinin (Chahine et al., 1993; Van Gilst et al., 1987). In the isolated atria, the lack of effect of indomethacin and N^{ω}-nitro-L-arginine suggests that cyclooxygenase products and nitric oxide play no role in mediating the positive chronotropic effect of bradykinin. In contrast to bradykinin, angiotensin I and angiotensin II had very weak or no direct chronotropic effects in isolated atria. Others have also reported that angiotensin I and angiotensin II caused small increase in beating rate of isolated guinea pig atria (Nakashima et al., 1982).

It is now accepted that bradykinin acts mostly by interacting with the bradykinin B_1 and B_2 receptors (Regoli and Barabé, 1988). In an attempt to further characterize the receptors involved in mediating the chronotropic effect of bradykinin, we used selective bradykinin B_1 and B_2 receptor blockers (Hock et al., 1991). The inhibition by the selective bradykinin B_2 receptor antagonist, HOE 140, demonstrates that the positive chronotropic effect of bradykinin is likely mediated by stimulation of bradykinin B_2 receptors in the atrium. In contrast, the lack of effect of Lys-[Leu^8]Des-Arg^9-bradykinin indicates that bradykinin B_1 receptors are not involved in the chronotropic effects of bradykinin. This interpretation is further strengthened

by the fact that the most active and selective bradykinin B_1 receptor agonist, Des-Arg^9-bradykinin, did not cause a change in the beating rate suggesting non-involvement of bradykinin B_1 receptors in mediating the positive chronotropic effect in atria. Previous reports have shown that bradykinin activates peripheral nerve endings of capsaisin-sensitive primary sensory afferents in guinea pig atria which leads to the release of vasoactive neuropeptides such as substance P and calcitonin gene-related peptide (Geppetti, 1993). Because calcitonin gene-related peptide causes positive chronotropic effects in guinea pig atria, it may be possible that the effects of bradykinin could be mediated by release of vasoactive neuropeptides (Franco-Cereceda and Lundberg, 1985). Whether a concurrent release of neuropeptide and/or galanin from sympathetic nerve endings has an effect on the bradykinin-induced increase in beating rate remains to be examined.

The concentration of bradykinin to directly stimulate the sino-atrial node might be considered to be above plasma levels measured during physiological stimuli in animals. Therefore, an effect of bradykinin on heart rate under physiological conditions is unlikely but it is possible that high plasma concentrations may be reached during severe conditions such as hemorrhagic shock or myocardial ischemia (Kimura et al., 1973). In such abnormal cases, local generation of bradykinin at the sino-atrial node may potentially alter the heart rate considerably.

In summary, bradykinin exhibits an inherent positive chronotropic action on the atria, that is potentiated by converting enzyme inhibition. This chronotropic effect is mediated by bradykinin B_2 receptors independently of β-adrenoceptors, prostaglandins and nitric oxide. The small tachycardia may have physiological relevance only at high tissue levels of bradykinin that occur during severe pathological conditions.

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