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Modulatory effect of bradykinin on noradrenaline release in isolated atria from normal and B_2 knockout transgenic mice

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Abstract

The modulatory effect of bradykinin on electrically-induced noradrenaline release was assessed in isolated atria from normal and B_2 knockout transgenic mice preincubated with [^{3}H]noradrenaline. Concentrations of 1, 3 and 10 nM of bradykinin did not significantly alter the outflow of radioactivity whereas higher concentrations of bradykinin (30 and 100 nM) enhanced it. The facilitatory effect of 30 nM bradykinin was inhibited by a selective bradykinin B_2 receptor antagonist, Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin, 30 nM), and by a protein kinase C inhibitor, bisindolylmaleimide (1 μM). The co-administration of bradykinin (1 to 100 nM) with either [Leu^8]des-Arg^9-bradykinin (100 nM), AcLys[DβNal^7,Ile^8]des-Arg^9-bradykinin (30 nM) (bradykinin B_1 receptor antagonists) or diclofenac (1 μM) (a cyclooxygenase inhibitor), shifted the facilitatory effect of bradykinin to lower concentrations. The facilitatory effect of bradykinin also was enhanced by enalaprilat (1 μM) and mergetpa (1 μM), inhibitors of angiotensin-converting enzyme (kininase II) and kininase I, respectively. In contrast, selective bradykinin B_1 receptor agonists, des-Arg^9-bradykinin (1 to 100 nM) and Sar[D-Phe^8]des-Arg^9-bradykinin (1 to 100 nM), did not significantly affect the stimulation-induced outflow of radioactivity. Neither bradykinin (100 nM) nor des-Arg^9-bradykinin (100 nM) had any modulatory effect in B_2 knockout transgenic mice. These findings suggest that the facilitatory effect of bradykinin on noradrenaline release in the mouse atria is mediated exclusively by presynaptic bradykinin B_2 receptors which are linked to protein kinase C. The greater release of noradrenaline with bradykinin under inhibition of prostaglandins production and kininases I and II activity might be of importance in pharmacotherapies. © 1998 Elsevier Science B.V.

Keywords: Noradrenaline release; Bradykinin; Kinin receptor; Prostaglandin; Atrium mouse; Protein kinase C; Kininase; B_2 knockout transgenic mouse

1. Introduction

The nonapeptide bradykinin has been shown to influence the cardiovascular system. For instance, bradykinin causes vasodilatation, enhances vascular permeability, interacts with cardiovascular regulatory hormones such as angiotensin II and exerts a cardioprotective action on ischemic myocardium by preventing the development of lethal arrhythmias (Bhoola et al., 1992; Linz et al., 1995). Bradykinin can also alter heart rate and cardiac contractility by modulating cardiac sympathetic neurotransmission (Burch et al., 1990; Bhoola et al., 1992).

Experimental animal models have shown that bradykinin modulates sympathetic postganglionic neurotransmission either by enhancing or inhibiting noradrenaline release from sympathetic nerve terminals. Bradykinin potentiates the release of noradrenaline induced by electrical field stimulation in the pithed spontaneously hypertensive rat (Dominiak et al., 1992), in the rat and mouse vas deferens (Llona et al., 1991), in the rat hypothalamus (Tsuda et al., 1993), atrium (Chulak et al., 1995), ventricle (Vaz-da-Silva et al., 1996) and heart (Minshall et al., 1994). In contrast, bradykinin inhibits noradrenaline release by enhancing prostaglandins biosynthesis in the rabbit isolated pulmonary artery and heart (Starke et al., 1977).

Most of the biological actions of bradykinin are mediated by the activation of constitutive bradykinin B_2 receptor (Regoli and Barabé, 1980). Bradykinin B_1 receptor which is highly sensitive to the active bradykinin metabolite, des-Arg^9-bradykinin, is induced following tissue injury and after the administration of endotoxins or specific interleukins (Marceau et al., 1983; Deblois et al., 1988; Marceau, 1995). In addition, constitutive and functional bradykinin B_1 receptor has been reported in the dog vasculature (Rhaleb et al., 1989; Nakhostine et al., 1993), as

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well as in the mouse vas deferens (Maas et al., 1995) and stomach (Nsa Allogho et al., 1995). It seems that the bradykinin B_1 receptor distribution in the mouse is tissue specific since it has not been found in arterial and venous mesenteric bed (Berthiaume et al., 1997). In this species, the relative contribution of bradykinin B_1 and B_2 receptors in cardiac function and notably in the modulation of atrium sympathetic neurotransmission remains to be addressed.

The aims of the present study were: (1) to evaluate the effect of bradykinin on the release of radioactive noradrenaline induced by electrical stimulation in mouse isolated atria; (2) to characterize the subtype of bradykinin receptors involved with the use of selective agonists and antagonists for bradykinin B_1 and B_2 receptors; (3) to compare the response to bradykinin in B_2 knockout transgenic mice (Borkowski et al., 1995); (4) to determine whether bradykinin B_1 receptor is upregulated in B_2 knockout transgenic mice; (5) to assess the contribution of protein kinase C and endogenous prostaglandins in the response to bradykinin; and (6) to evaluate the contribution of kininases I and II in the degradation of bradykinin in mouse atria.

2. Materials and methods

2.1. Animal

Most of the experiments were conducted in female CD1 mice (20–24 g, $n = 252$; Charles River, St-Constant, Québec, Canada). Additional experiments were performed on atria of transgenic B_2 knockout J129sv × C57Bl/6 mice (23–28 g, $n = 16$; Merck, Rahway, NJ, USA) which were obtained from Dr. Pedro D'Orléans-Juste at Université de Sherbrooke (Sherbrooke, Canada) during the course of his experiments on the arterial and venous mesenteric bed. Wild-type female C57Bl/6 mice (23–28 g, $n = 19$; Charles River, St-Constant, Québec, Canada) were used as control group for the B_2 knockout trangenic mice.

All research procedures and the care of the animals were in accordance with the principles and guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of our university.

2.2. Experimental protocol

On the day of the experiments, the animals were decapitated and the hearts quickly removed. The atria were dissected from the ventricles and incubated in a Krebs–Henseleit solution containing [^{3}H]noradrenaline (4 μCi/ml, 0.2 μM), gassed with 5% CO_2 and 95% O_2 for 20 min at 37°C. Thereafter, the atria were transferred into 0.3 ml perfusion chambers (one pair of atria per chamber). The rate of superfusion of the system (Brandel SF6 Suprafusion system, Brandel, Gaithersburg, USA) was 0.4 ml/min and the temperature of the system was kept constant at 37°C. The atria were washed with Krebs–Henseleit solution for a period of 75 min during which a priming stimulation (5 Hz frequency for 60 s at intensity of 50 mA, 2 ms pulses) was given at 40 min. After the washing period, the effluent was collected for 12 periods of 5 min each (total of 60 min) to estimate the radioactivity. During this collection procedure, the atria were stimulated twice (5 Hz frequency for 60 s at intensity of 50 mA, 2 ms pulses) at 10 min (S_1) and 45 min (S_2). The drugs were added to the perfusion system 20 min before S_2 and remained throughout the experimental protocol to determine their effect on the stimulation-induced outflow of radioactivity.

At the end of the experiment, the atria were dissolved in NCS-II (Amersham, Mississauga, Canada). The radioactivity in both tissues and samples was determined by liquid scintillation counting (Beckman model LS3801, Beckman, Irvine, USA). Corrections for counting efficiency (69%) were made by automatic external standardization. The resting radioactive outflow was taken during the 5-min period prior to the first stimulation (R_1) or the second stimulation (R_2). Stimulation-induced outflow of radioactivity was calculated by subtracting the resting radioactive outflow from the total radioactivity content of the 5-min stimulation period. The stimulation-induced outflow of radioactivity measured during the second period of stimulation (S_2) was expressed as the percentage of that obtained during the first period of stimulation (S_1). The values were standardized for the total amount of radioactivity measured during the course of the experiment (12 periods of 5 min each) and defined as fractional release (FR) according to the formula: $FR_1 = [(S_1 - R_1)/(\text{total release of radioactivity during the experiment})] \times 100$; $FR_2 = [(S_2 - R_2)/(\text{total release of radioactivity during the experiment})] \times 100$. The effect of the drugs is determined by the expression FR_2/FR_1.

2.3. Drugs and materials

The Krebs–Henseleit solution contained (in mM): NaCl: 118; KCl: 4.7; $CaCl_2$: 2.5; $MgSO_4$: 0.45; glucose: 11.1; EDTA: 0.067; KH_2PO_4: 1.03; $NaHCO_3$: 25 and ascorbic acid: 0.14. Following the [^{3}H]noradrenaline incubation period, bovine serum albumin 0.1% was added to the Krebs–Henseleit solution for the remainder of the experimental protocol. L-[ring-2,5,6-^{3}H]noradrenaline was from Du Pont (Mississauga, Canada) and mergetpa (DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid) from Calbiochem (San Diego, CA). Bradykinin and diclofenac were obtained from Sigma (St. Louis, USA) and Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) from Hoechst (Frankfurt, Germany). [Leu^8]des-Arg^9-bradykinin and des-Arg^9-bradykinin were obtained from Hukabel Scientific (Montréal, Canada) and bisindolylmaleimide from Boehringer Mannheim (Laval, Canada). Sar[D-Phe^8]des-Arg^9-bradykinin and AcLys[DβNal^7,Ile^8]des-Arg^9-

bradykinin were a generous gift of Dr. Domenico Regoli from Université de Sherbrooke (Sherbrooke, Canada) and enalaprilat was obtained from Merck Frosst (Pointe-Claire-Dorval, Canada).

2.4. Statistical analysis of data

Results were given as mean ± S.E.M of (n) pairs of atria. Statistical differences were evaluated using a one-way analysis of variance followed by a Bonferroni test for multiple comparisons. Only probability values (P) less than 0.05 were considered to be statistically significant.

3. Results

In control experiments, the stimulation-induced outflow of radioactivity for both stimulations (S_1 and S_2) was about the same and thus FR_2/FR_1 was 93.6 ± 2.5% (n = 6) for the CD1 mice, 105.0 ± 10.1% (n = 6) for the wild-type C57Bl/6 mice and 105.9 ± 10.1% (n = 5) for the B_2 knockout mice, indicating the stability of the preparation. The fractional resting outflow of radioactivity (R_1) and the stimulation-induced outflow of radioactivity during S_1 (FR_1), in the absence of treatment, were, respectively, smaller and larger in CD1 mice compared to the wild-type C57Bl/6 mice and B_2 knockout mice (Table 1). The total tissue radioactivity was significantly greater for the wild-type C57Bl/6 mice compared to the CD1 mice or the B_2 knockout mice (Table 1). The resting outflow ratio (R_2/R_1) was not changed by any treatment used in that study (data not shown).

3.1. Effect of bradykinin on noradrenaline release

Bradykinin did not affect the stimulation-induced outflow of radioactivity from isolated mouse atria at 1 nM and 3 nM (data not shown) or 10 nM (Fig. 1). However, at higher concentrations (30 and 100 nM), bradykinin significantly enhanced the stimulation-induced outflow of radioactivity from the tissue (Fig. 1 shows the response of bradykinin 30 nM). In transgenic B_2 knockout mice, a concentration of 100 nM of bradykinin did not alter the outflow of radioactivity while a significant increase was seen in wild-type mice (Table 2).

Table 1
Control experimental conditions for the atria isolated from CD1, wild-type (C57Bl/6) or B_2 knockout transgenic mice

Animal	R_1(%)	FR_1(%)	TTR (dpm × 10^5)	n
CD1	0.4 ± 0.0[a]	1.0 ± 0.0[a]	1.1 ± 0.0	252
Wild-type	0.5 ± 0.0	0.5 ± 0.1	1.7 ± 0.2[b]	19
B_2 Knockout	0.5 ± 0.0	0.6 ± 0.1	1.2 ± 0.2	16

The fractional resting outflow of radioactivity (R_1) which was measured during the collecting period preceding S_1 and the stimulation-induced outflow of radioactivity (FR_1) in the absence of treatment, both expressed as a percentage of the total release of radioactivity, and the total tissue radioactivity (TTR) are shown. Data represent the means ± S.E.M. of (n) pairs of atria.
[a] $P < 0.05$ vs. wild-type mice and B_2 knockout mice.
[b] $P < 0.05$ vs. CD1 mice and B_2 knockout mice.

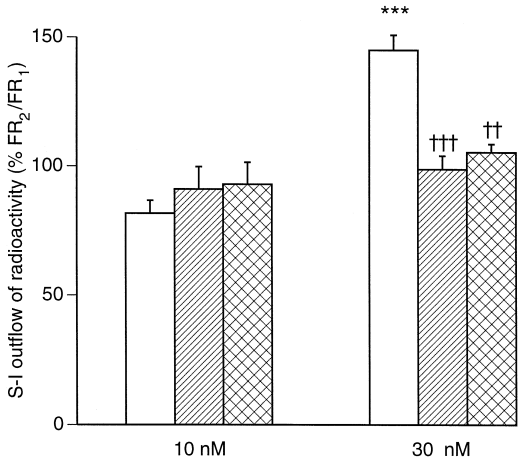

Fig. 1. Effects of bradykinin (10 and 30 nM) without (□) or with Hoe 140 (30 nM, (square with diagonal lines)) or bisindolylmaleimide (1 μM, (square with intersecting diagonal lines)) on the stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]noradrenaline. Values are means ± S.E.M of five to 11 experiments per group. *** $P < 0.001$ vs. respective treatment alone (see Table 3). †† $P < 0.01$ and ††† $P < 0.001$ vs. bradykinin 30 nM alone.

3.2. Influence of a bradykinin B_2 receptor antagonist and a protein kinase C inhibitor on the facilitatory action of bradykinin

The bradykinin B_2 receptor antagonist, Hoe 140 (30 nM), which had no significant effect by itself (Table 3), abolished the facilitatory effect of 30 nM bradykinin (Fig. 1), suggesting a major role of those receptors upon the modulatory action of bradykinin. The compound bisindolylmaleimide, a protein kinase C inhibitor, was used in order to investigate the implication of this intracellular signalling pathway in the facilitatory action of bradykinin. Bisindolylmaleimide (1 μM) had no significant effect on the outflow of radioactivity per its own (Table 3). However, the facilitatory effect of 30 nM of bradykinin was significantly blocked in the presence of this inhibitor (Fig. 1).

3.3. Influence of bradykinin B_1 receptor antagonists on the action of bradykinin

The bradykinin B_1 receptor antagonist [Leu^8]des-Arg^9-bradykinin (100 nM) by itself did not significantly modify the stimulation-induced outflow of radioactivity (Table 3). However, in its presence, the effect of bradykinin was significantly enhanced. Indeed, a significant difference of the stimulation-induced outflow of radioactivity was ob-

Table 2
Fractional release of [^{3}H]noradrenaline (FR_2/FR_1 %) in isolated atria from wild-type (C57Bl/6) or B_2 knockout transgenic mice under different treatments

Animal	Treatment	Fractional release of [^{3}H]noradrenaline FR_2/FR_1 (%)	*n*
Wild-type	control	106.2 ± 10.8	6
	bradykinin (100 nM)	147.3 ± 14.1[a]	7
	des-Arg^9-bradykinin (100 nM)	110.9 ± 4.9	6
B_2 knockout	control	98.0 ± 10.1	5
	bradykinin (100 nM)	100.9 ± 8.1	7
	des-Arg^9-bradykinin (100 nM)	82.9 ± 11.9	4

Data represent the means ± S.E.M. of (*n*) pairs of atria.
[a] $P < 0.05$ vs. control group of wild-type mice.

served with bradykinin 10 nM under bradykinin B_1 receptor blockade, whereas on its own, bradykinin 10 nM had no effect (Fig. 2). This facilitatory effect was completely inhibited by the bradykinin B_2 receptor antagonist, Hoe 140 (30 nM, Fig. 2). The release of noradrenaline induced by higher bradykinin concentrations (30, 100 nM), was not modified by 100 nM of [Leu^8]des-Arg^9-bradykinin (Fig. 2 shows the response of bradykinin at 30 nM). Similar results were obtained with the bradykinin B_1 receptor antagonist, AcLys[DβNal^7,Ile^8]des-Arg^9-bradykinin (30 nM). This bradykinin B_1 receptor antagonist which is devoid of agonist activity at the mouse bradykinin B_1 receptor contrary to [Leu^8]des-Arg^9-bradykinin (Nsa Allogho et al., 1995) was without effect on its own (Table 3) but potentiated significantly the effect of 10 nM of bradykinin ($FR_2/FR_1 = 120.2 \pm 8.5\%$, $n = 11$, $P < 0.01$).

3.4. Lack of effect of bradykinin B_1 receptor agonists on noradrenaline release

The active metabolite of bradykinin and selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, did not significantly affect the stimulation-induced outflow of radioactivity at concentrations up to 100 nM (Fig. 2). Moreover, the metabolically stable bradykinin B_1 receptor agonist, Sar[D-Phe^8]des-Arg^9-bradykinin (Drapeau et al., 1991), was also found to be inactive when applied at 100 nM (Fig. 2). In addition, des-Arg^9-bradykinin (100 nM) did not influence the outflow of radioactivity in the transgenic B_2 knockout mouse (Table 2).

3.5. Influence of diclofenac on the action of bradykinin

Prostaglandins are mediators of various indirect effects of bradykinin (see Hall, 1992). To examine whether or not prostaglandins are involved in the modulatory action of bradykinin, the cyclooxygenase inhibitor, diclofenac, was used. Diclofenac (1 μM) had no significant effect on the outflow of radioactivity on its own (Table 3). However, in its presence, a significant increase of noradrenaline release occurred with 3 and 10 nM of bradykinin (Fig. 3). Therefore, the release of noradrenaline induced by the three concentrations of bradykinin (3, 10 and 30 nM) was similar in the presence of diclofenac (Fig. 3).

3.6. Effect of an angiotensin-converting enzyme and kininase I inhibitor on the action of bradykinin

Angiotensin-converting enzyme and kininase I are the two main enzymes involved in the degradation of bradykinin (Erdös, 1990); their inhibition may consequently lead to bradykinin accumulation. Thus, the angiotensin-converting enzyme inhibitor enalaprilat and the kininase I inhibitor mergetpa were used in our preparation. By themselves, enalaprilat (1 μM) and mergetpa (1 μM) given individually or together did not alter the

Table 3
Fractional release of [^{3}H]noradrenaline (FR_2/FR_1 %) under different treatments

Treatment	Fractional release of [^{3}H]noradrenaline FR_2/FR_1 (%)	*n*
Control	93.6 ± 2.5	6
Hoe 140 (30 nM)	88.7 ± 6.1	5
Bisindolylmaleimide (1 μM)	103.7 ± 6.5	10
[Leu^8]des-Arg^9-bradykinin (100 nM)	90.0 ± 4.1	5
AcLys[DβNal^7,Ile^8]des-Arg^9-bradykinin (30 nM)	86.7 ± 5.5	9
Diclofenac (1 μM)	100.8 ± 9.0	5
Enalaprilat (1 μM)	95.8 ± 5.0	6
Mergetpa (1 μM)	82.1 ± 3.0	6
Enalaprilat + mergetpa (1 μM + 1 μM)	107.4 ± 7.8	6

Data represent the means ± S.E.M. of (*n*) pairs of atria. No statistical significance was found between treatments and control values.

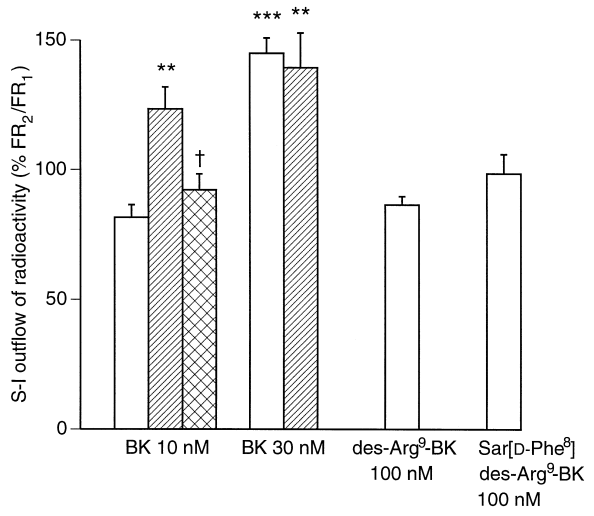

Fig. 2. Effects of bradykinin (BK: 10, 30 nM), des-Arg^9-bradykinin (100 nM) or Sar[D-Phe^8]des-Arg^9-bradykinin (100 nM) on the stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]noradrenaline. Bradykinin was also shown with [Leu^8]des-Arg^9-bradykinin (100 nM, (square with diagonal lines)) or [Leu^8]des-Arg^9-bradykinin (100 nM) plus Hoe 140 (30 nM) (square with intersecting diagonal lines). Values are means ± S.E.M of five to 11 experiments per group. ∗∗ $P < 0.01$ and ∗∗∗ $P < 0.001$ vs. respective treatment alone (see Table 3). † $P < 0.05$ vs. BK (10 nM) in the presence of [Leu^8]-des-Arg^9-bradykinin (100 nM).

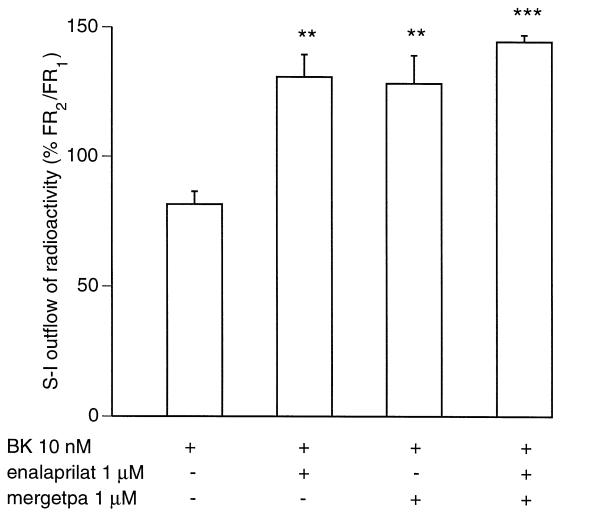

Fig. 4. Effects of bradykinin (BK, 10 nM) in the absence or presence of enalaprilat (1 μM) and/or mergetpa (1 μM) on the stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]noradrenaline. Values are means ± S.E.M of five to 11 experiments per group. ∗∗ $P < 0.01$ and ∗∗∗ $P < 0.001$ vs. respective treatment alone (see Table 3).

stimulation-induced outflow of radioactivity (Table 3). In the presence of enalaprilat or mergetpa, bradykinin 10 nM increased significantly the stimulation-induced outflow of radioactivity which was not the case in the absence of these inhibitors (Fig. 4). The somewhat greater noradrenaline release induced by 10 nM of bradykinin in the presence of both agents was not significantly different from that measured by bradykinin in the presence of each inhibitor given separately. The facilitatory effect of bradykinin (10 nM), when co-administered with enalaprilat, was completely abolished by the addition of Hoe 140 ($FR_2/FR_1 = 98.3 \pm 6.3\%$, $n = 5$, $P = 0.01$) but was unaffected by the bradykinin B_1 receptor antagonist ([Leu^8]des-Arg^9-bradykinin, 100 nM, $FR_2/FR_1 = 140.9 \pm 8.2\%$, $n = 5$). On its own, the co-administration of Hoe 140 and enalaprilat did not alter the stimulation-induced outflow of radioactivity ($FR_2/FR_1 = 96.0 \pm 9.6\%$, $n = 5$). Finally, the administration of des-Arg^9-bradykinin (100 nM), a bradykinin B_1 receptor agonist, did not affect significantly the stimulation-induced outflow of radioactivity in the presence of the angiotensin-converting enzyme and/or kininase I inhibitor (data not shown).

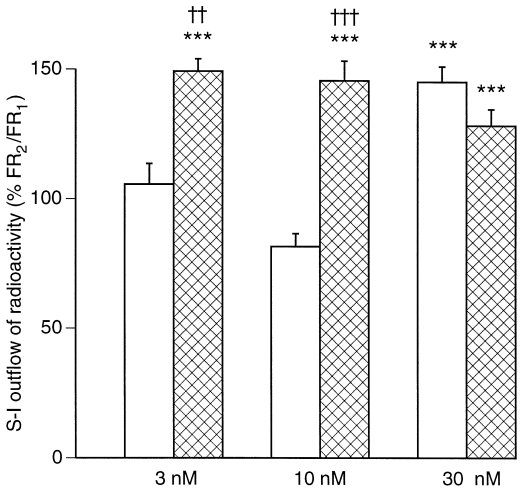

Fig. 3. Effects of bradykinin (3, 10, 30 nM) in the absence (□) or presence (square with intersecting diagonal lines) of diclofenac (1 μM) on the stimulation-induced (S-I) outflow of radioactivity from mouse atria incubated with [^{3}H]noradrenaline. Values are means ± S.E.M of five to 11 experiments per group. ∗∗∗ $P < 0.001$ vs. respective treatment alone (see Table 3). †† $P < 0.01$ vs. BK (3 nM) alone and ††† $P < 0.001$ vs. BK (10 nM) alone.

4. Discussion

The present study revealed a modulatory effect of bradykinin on postganglionic sympathetic fibers in the mouse atria. The electrical stimulation-induced outflow of radioactivity from mouse atria pre-incubated with [^{3}H]noradrenaline was used as an index of noradrenaline release from sympathetic fibers (Johnston et al., 1987). The inhibition of the facilitatory action of bradykinin (starting at 30 nM) by the selective antagonist, Hoe 140 (Hock et al., 1991; Lembeck et al., 1991), strongly suggests that bradykinin exerts a facilitatory effect through bradykinin B_2 receptor in the mouse atria. This is further supported by the lack of effect of bradykinin in atria isolated from B_2 knockout transgenic mice. The involvement of bradykinin

B_2 receptor in the bradykinin facilitatory effect is consistent with earlier studies in rat atria (Chulak et al., 1995), rat heart (Minshall et al., 1994), rat ventricle (Vaz-da-Silva et al., 1996) and rat vas deferens (Llona et al., 1991). The rabbit heart seems to be the only preparation in which bradykinin has been shown to inhibit the stimulation-induced outflow of noradrenaline (Starke et al., 1977).

The presence of bradykinin B_1 receptor antagonists allowed lower concentration of bradykinin (10 nM) to increase significantly the outflow of noradrenaline. However, the lack of direct inhibitory effects of selective bradykinin B_1 receptor agonists (des-Arg^9-bradykinin and Sar-[D-Phe^8]des-Arg^9-bradykinin) on noradrenaline release in atria from normal (CD1), wild-type (C57Bl/6) and transgenic B_2 knockout mice argues against a direct function of bradykinin B_1 receptors in the mouse atria. Furthermore, the low affinity of bradykinin for B_1 receptors makes a direct effect of bradykinin on this receptor unlikely at 10 nM. The bradykinin B_1 receptor, which is usually induced in pathological states (Marceau, 1995), was found in some peripheral tissues of the normal mouse notably in the vas deferens and stomach (Maas et al., 1995; Nsa Allogho et al., 1995, 1997) but was absent in the arterial and venous mesenteric bed (Berthiaume et al., 1997). Although the expression of bradykinin B_1 receptor in the mouse may be different from that of other species, this receptor does not seem to be directly involved in the modulation of noradrenaline release in the atrium. It is noteworthy that bradykinin B_1 receptor does not appear to be upregulated in atria of transgenic B_2 knockout mice as assessed by the lack of effect of des-Arg^9-bradykinin. This finding is consistent with the lack of bradykinin B_1 receptor mediated response in the arterial and venous perfused mesenteric circuits of both normal and transgenic B_2 knockout mice (Berthiaume et al., 1997). The enhancing effects of the bradykinin B_1 receptor antagonists on bradykinin (10 nM) remain unexplained at this time and do not seem to be due to a direct agonist activity since both antagonists were devoid of direct action when given alone. Moreover, AcLys[DβNal^7,Ile^8]des-Arg^9-bradykinin was reported to be the most suitable antagonist developed so far at mouse bradykinin B_1 receptor (Nsa Allogho et al., 1995).

The use of bisindolylmaleimide, a protein kinase C inhibitor (Toullec et al., 1991), significantly reduced the facilitatory effect of bradykinin, suggesting a contribution of this intracellular pathway following bradykinin B_2 receptor activation in the mouse atria. The protein kinase C intracellular signalling pathway has been shown to be involved in the action of prejunctional facilitatory receptors on sympathetic nerve ending (Majewski et al., 1990). Although the bradykinin B_2 receptor has been linked to the activation of various second messenger systems, the phospholipase C/protein kinase C pathway seems to be the most frequent (Bhoola et al., 1992; Hall, 1992). Similarly, we have previously shown that the facilitatory effect of bradykinin in the rat atria also was inhibited by bisindolylmaleimide (Chulak et al., 1995).

The biological action of bradykinin has been shown to be mediated indirectly through prostaglandins in different tissues (Hall, 1992). A cyclooxygenase inhibitor, diclofenac, was used to evaluate the putative involvement of prostaglandins in the modulation of noradrenaline release by bradykinin in our preparation. The results suggest a local bradykinin-induced prostaglandins synthesis that inhibits the B_2-mediated facilitatory effect of bradykinin on noradrenaline release since diclofenac unmasked a facilitatory effect with lower concentrations of bradykinin. Thus, the postsynaptic production of inhibitory prostaglandins induced by bradykinin reduces the presynaptic action of bradykinin, especially at low peptide concentration, in the mouse atria. Likewise, Starke et al. (1977) have previously observed an inhibition of noradrenaline release mediated by bradykinin via the production of prostaglandins in the rabbit heart.

One of the main metabolic pathway of bradykinin operates through angiotensin-converting enzyme which produces inactive metabolites (Erdös, 1990). It is well known that angiotensin-converting enzyme also is involved in the conversion of angiotensin I to angiotensin II (Erdös, 1990). Thus, the use of angiotensin-converting enzyme inhibitors can influence sympathetic neurotransmission either by blocking angiotensin II formation and/or by preventing bradykinin degradation (Minshall et al., 1994; Schwieler et al., 1993; Schwieler et al., 1994). In this study, we found that the angiotensin-converting enzyme inhibitor, enalaprilat, unmasked a facilitatory effect of bradykinin at the concentration of 10 nM which was blocked by a bradykinin B_2 receptor antagonist. Thus, this effect of enalaprilat is likely due to an increase in the effective concentration of bradykinin and suggests the presence of angiotensin-converting enzyme in the mouse atria.

Another relevant enzyme involved in the metabolism of kinins is kininase I which hydrolysis bradykinin into its active metabolite des-Arg^9-bradykinin (Marceau, 1995). The blockade of this pathway with mergetpa enhanced the facilitatory effect of bradykinin most likely by preventing the conversion of bradykinin into des-Arg^9-bradykinin, thereby increasing the effective concentration of bradykinin. Hence, the observation that bradykinin mediates a facilitatory action on noradrenaline release at lower concentrations in the presence of enalaprilat or mergetpa strongly suggests an implication of both angiotensin-converting enzyme and kininase I in the metabolism of bradykinin in the mouse atria. However, blockade of both catabolic routes was not additive, suggesting either that they represent alternative fully effective pathways or that the facilitatory effect of bradykinin is maximal and cannot be augmented further. This latter explanation is congruent with the maximum effect observed with 30 and 100 nM of bradykinin, which is similar to that obtained with 10 nM bradykinin in the presence of both inhibitors.

In conclusion, the present study suggests that the facilitatory effect of bradykinin on noradrenaline release is mediated by a presynaptic bradykinin B_2 receptor in the isolated mouse atria which is linked to protein kinase C. Bradykinin B_1 receptors do not seem to be directly implicated in the stimulation or inhibition of noradrenaline release, although bradykinin B_1 receptor blockade enhances the ability of bradykinin to act through bradykinin B_2 receptors. Amplification of the effect of bradykinin on noradrenaline release can also be produced by a cyclooxygenase inhibitor, a angiotensin-converting enzyme inhibitor or a blocker of kininase I. It remains to be determined if administration of non-steroid anti-inflammatory drugs or angiotensin-converting enzyme inhibitors can alter cardiac function in physiological and pathological situations by enhancing the presynaptic facilitatory effect of endogenous bradykinin on noradrenaline release.

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