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Enhancement of noradrenaline release by angiotensin II and bradykinin in mouse atria: evidence for cross-talk between $G_{q/11}$ protein- and $G_{i/o}$ protein-coupled receptors

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1 The interaction between α_2-autoreceptors and receptors for angiotensin (AT_1) and bradykinin (B_2) was studied in mouse isolated atria. The preparations were labelled with [^{3}H]-noradrenaline and then superfused with desipramine-containing medium and stimulated electrically.

2 Angiotensin II (10^{-11}–10^{-7} M), angiotensin III (10^{-10}–10^{-6} M) and bradykinin (10^{-11}–10^{-7} M) enhanced the evoked overflow of tritium when preparations were stimulated with conditions that led to marked α_2-autoinhibition (120 pulses at 3 Hz), but not when stimulated with conditions that led to little α_2-autoinhibition (20 pulses at 50 Hz).

3 Blockade of α-adrenoceptors by phentolamine (1 or 10 μM) reduced or abolished the effect of angiotensin II and bradykinin on the overflow response to 120 pulses at 3 Hz.

4 Addition of the δ-opioid agonist [D-Ser^2]-leucine enkephalin-Thr (DSLET, 0.1 μM), or of neuropeptide Y (0.1 μM), together with phentolamine, restored the effect of angiotensin II and bradykinin.

5 The β-adrenoceptor agonist terbutaline (10^{-9}–10^{-4} M) enhanced the evoked overflow of tritium irrespective of the degree of autoinhibition.

6 The experiments show that (i) a marked prejunctional facilitatory effect of angiotensin and bradykinin in mouse isolated atria requires prejunctional α_2-autoinhibition; (ii) in the absence of α_2-autoinhibition, activation of other prejunctional $G_{i/o}$ protein-coupled reeptors, namely opioid and neuropeptide Y receptors, restores a marked effect of angiotensin II and bradykinin; and (iii) the facilitatory effect of terbutaline is not dependent upon the degree of α_2-autoinhibition. The findings indicate that the major part of the release-enhancing effect elicited through prejunctional $G_{q/11}$ protein-coupled receptors is due to disruption of an ongoing, α_2-autoreceptor-triggered $G_{i/o}$ protein mediated inhibition.

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Abbreviations: DSLET, [D-Ser^2]-leucine enkephalin-Thr; Hoe 140, D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin; NPY, neuropeptide Y; Phent, phentolamine; PSS, physiological salt solution

Introduction

As in many other tissues, angiotensin II increases the average release of noradrenaline per sympathetic nerve action potential in the heart (Starke *et al*., 1969; for review see Fuder & Muscholl, 1995). Bradykinin also modulates cardiac sympathetic neurotransmission, but its effects are less uniform. Bradykinin increases the release of noradrenaline in mouse (Chulak *et al*., 1998), rat (Chulak *et al*., 1995; Rump *et al*., 1997a) and human atria (Rump *et al*., 1997a), rat ventricle (Vaz-da-Silva *et al*., 1996) and synaptosomes of the guinea-pig heart (Seyedi *et al*., 1997). However, bradykinin inhibits noradrenaline release in the rabbit heart, perhaps because in that tissue, stimulation of prostaglandin synthesis by bradykinin and subsequent inhibition of noradrenaline release by prostaglandins prevails (Starke *et al*., 1977; see also Chulak *et al*., 1998).

It was early observed that angiotensin II lost its effect in rabbit hearts pretreated with phenoxybenzamine, which by itself increased the release of noradrenaline by blocking presynaptic α_2-autoreceptors; the explanation at that time was that release was already maximal after phenoxybenzamine alone (Starke & Schümann, 1972). Brasch *et al*. (1995) found a similar phenomenon in guinea-pig atria: angiotensin II failed to increase the stimulation-induced release of noradrenaline when it had already been increased by the α_2-adrenoceptor antagonist idazoxan. However, angiotensin also failed to increase release when a special nerve stimulation protocol was used which, for unknown reasons and without any use of drugs, prevented the development of α_2-autoinhibition. The authors concluded that ongoing α_2-autoinhibition is a prerequisite for the effect of angiotensin, perhaps because 'the intracellular signal transduction pathways of α_2-adrenoceptors and-AT_1-receptors are linked at some stage, perhaps *via* a common G-protein or by a protein kinase' (Brasch *et al*., 1995). In contrast to these studies, angiotensin became more, rather than less effective after blockade of α_2-adrenoceptors in the rabbit pulmonary artery (Costa & Majewski, 1988).

Cross-talk between prejunctional receptors is not new, but the majority of studies have focused on interactions between receptors coupled to $G_{i/o}$ proteins, such as α_2-autoreceptors and opioid receptors (for review see Hertting *et al*., 1990; Schlicker & Göthert, 1998). Presynaptic angiotensin receptors couple to $G_{q/11}$ proteins (Musgrave *et al*., 1991; Chulak *et al*., 1995). It was of interest, therefore, to investigate further the

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interaction between presynaptic α_2-autoreceptors and angiotensin receptors, especially in view of the discrepancy mentioned above. The present study was undertaken in mouse atria where the prejunctional angiotensin receptor has been characterized as AT_1 (Cox *et al*., 1999). The interaction between α_2-autoreceptors and bradykinin B_2-receptors was also of interest because prejunctional B_2-receptors also couple to $G_{q/11}$ (Chulak *et al*., 1995).

Methods

Preparation and protocols

Adult male NMRI mice were killed by exsanguination. The heart was placed in physiological salt solution (PSS), which had been bubbled with 95% CO_2 and 5% O_2, and stored on ice. The wall of each pair of atria was cut into six to eight pieces. Pieces of atria (12 to 14) were incubated in 2 ml of PSS containing [^{3}H]-noradrenaline (0.1 μM) for 30 min at 37°C. Twelve preparations were then superfused in parallel in twelve superfusion chambers with PSS at a flow rate of 1.2 ml min^{-1}. The preparations were subjected to electrical field stimulation by square wave pulses (p) of 1 ms width and 80 mA current strength, yielding a voltage drop of 45 V cm^{-1} between the electrodes of each chamber. A 'priming' stimulation period (180 p at 3 Hz) was applied at $t=30$ min ($t=0$ min being the beginning of superfusion). At $t=54$ min, the preparations were subjected to either six (S_1 to S_6) or three (S_1 to S_3) periods of electrical field stimulation (either 20 p at 50 Hz, or 36, 60 or 120 p at 3 Hz), delivered 18 min apart. Consecutive 2-min samples of the superfusate were collected from $t=50$ min onwards. At the end of the experiment the tissue was dissolved in 0.5 ml of Soluene (Packard, Frankfurt am Main, Germany) and tritium was determined in the superfusate samples and preparations.

Concentration-response curves to the peptides and to terbutaline were determined in experiments with six stimulation periods; increasing concentrations were introduced after S_1, 12 min before S_2-S_6). The effect of single concentrations of drugs was usually determined by introducing them after S_2, 12 min before S_3, and keeping them in for the remainder of the experiment (with three or six stimulation periods). Other details are explained in the Results section.

Drugs and radiochemicals

Unless stated otherwise, the PSS used for superfusion had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, ethylenediaminetetraacetic acid disodium salt 0.03 and desipramine 0.001. The PSS for incubation with [^{3}H]-noradrenaline contained no desipramine and 0.2 mM $CaCl_2$ (see Limberger *et al*., 1992).

The following drugs were used: angiotensin II (human), angiotensin III (human), bradykinin, [D-Ser^2]-leucine enkephalin-Thr (DSLET), desipramine hydrochloride, rauwolscine hydrochloride, (±)-terbutaline hemisulphate (Sigma, Deisenhofen, Germany); D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140; gift from Hoechst, Frankfurt am Main, Germany); neuropeptide Y (NPY; human; Bachem, Heidelberg, Germany); losartan (gift from Merck, Darmstadt, Germany); phentolamine hydrochloride (gift from Ciba-Geigy, Basel, Switzerland); (−)-[2,5,6-^{3}H]-noradrenaline (New England Nuclear, Dreieich, Germany, specific activity 46.8–62.3 Ci mmol^{-1}).

Analysis of data

The outflow of tritium was calculated as a fraction of the tritium content of the tissue at the onset of the respective collection period, per min. The overflow of tritium evoked by electrical field stimulation was calculated as the total tritium outflow during the collection period in which stimulation was applied and during the next collection period thereafter, minus the estimated basal outflow. Basal outflow was assumed to decline linearly from the collection period before stimulation to the second collection period after stimulation. The evoked overflow was expressed as a percentage of the tritium content of the tissue at the time of stimulation. Overflow ratios (S_n/S_1) were then determined for each period of stimulation. Percentage changes of S_n/S_1 ratios caused by a drug added

Table 1 Electrically evoked overflow of tritium from mouse atria (stimulation period S_1)

	Stimulation conditions	*Calcium concentration* (mM)	*Drugs present before S_1 (in addition to desipramine)*	*Evoked tritium overflow (S_1; % of tissue tritium)*	n
1	20 p at 50 Hz	2.5	–	0.46±0.03	58
2	36 p at 3 Hz	2.5	–	0.40±0.08	13
3	36 p at 3 Hz	2.5	Phent 1	0.98±0.07[a]	8
4	60 p at 3 Hz	2.5	–	0.44±0.04	11
5	60 p at 3 Hz	2.5	Phent 1	1.58±0.07[b]	8
6	120 p at 3 Hz	2.5	–	0.98±0.04	162
7	120 p at 3 Hz	2.5	Phent 1	3.40±0.24[c]	25
8	120 p at 3 Hz	2.5	Phent 10	3.70±0.19[c]	8
9	120 p at 3 Hz	2.5	Phent 1 + Losartan 0.1	3.23±0.24	10
10	120 p at 3 Hz	2.5	Phent 1 + Hoe 140 0.01	3.34±0.20	10
11	120 p at 3 Hz	2.5	Phent 1 + DSLET 0.1	0.79±0.10[d]	27
12	120 p at 3 Hz	2.5	Phent 1 + NPY 0.1	0.63±0.21[d]	22
13	120 p at 3 Hz	1.3	–	0.47±0.02	24
14	120 p at 3 Hz	1.3	Phent 1	1.96±0.07[e]	12
15	120 p at 3 Hz	0.65	–	0.24±0.01	16
16	120 p at 3 Hz	0.65	Phent 1	1.33±0.07[f]	11

Mouse atrial preparations preincubated with [^{3}H]-noradrenaline were superfused with PSS containing 1 μM desipramine, different calcium concentrations and additional drugs as indicated (Phent, phentolamine; μM concentrations). Values are means±s.e.mean of the overflow of tritium evoked by the first stimulation period, S_1, from *n* preparations; all experiments of this study pooled. Significant differences ($P<0.05$): [a]from line 2, [b]from line 4, [c]from line 6, [d]from line 7, [e]from line 13, [f]from line 15.

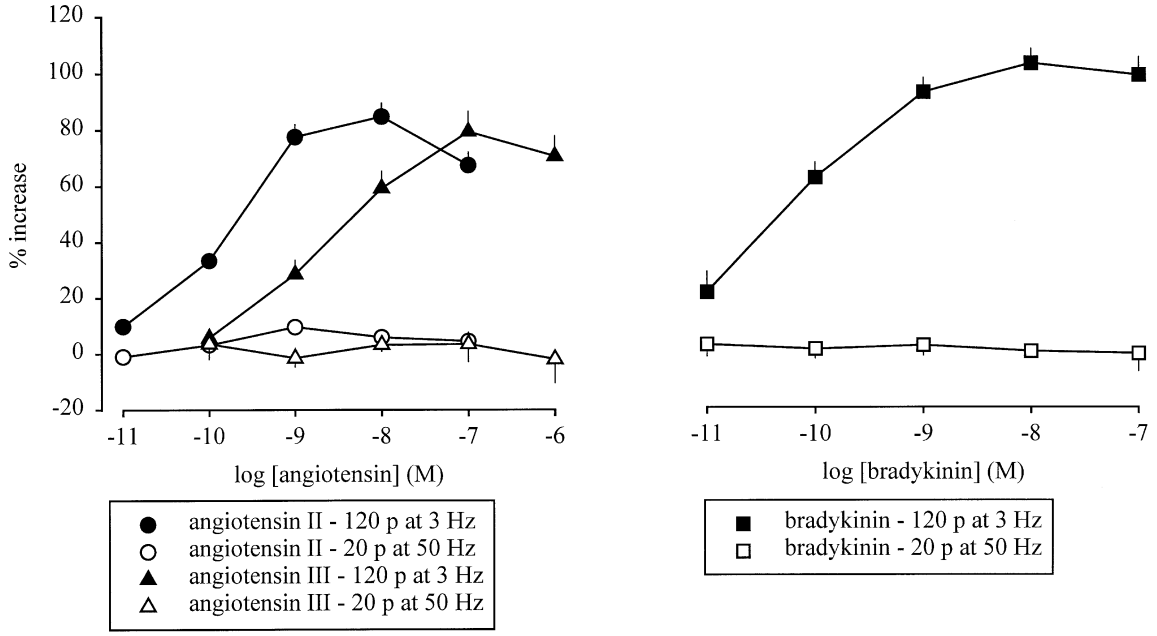

Figure 1 Effect of angiotensin II, angiotensin III (left panel) and bradykinin (right panel) on the overflow of tritium, evoked by stimulation trains leading either to marked or to little autoinhibition. The preparations were stimulated for 6 periods (S_1-S_6) by either 120 p at 3 Hz (marked autoinhibiton) or 20 p at 50 Hz (little autoinhibition). Angiotensin II, angiotensin III and bradykinin were introduced into the PSS in increasing concentrations, 12 min before S_2-S_6. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of the angiotensins and bradykinin. The vertical lines represent the s.e.mean from 4–30 preparations. Most of the experiments with angiotensin II and angiotensin III, 120 p at 3 Hz, are from Cox *et al.* (1999).

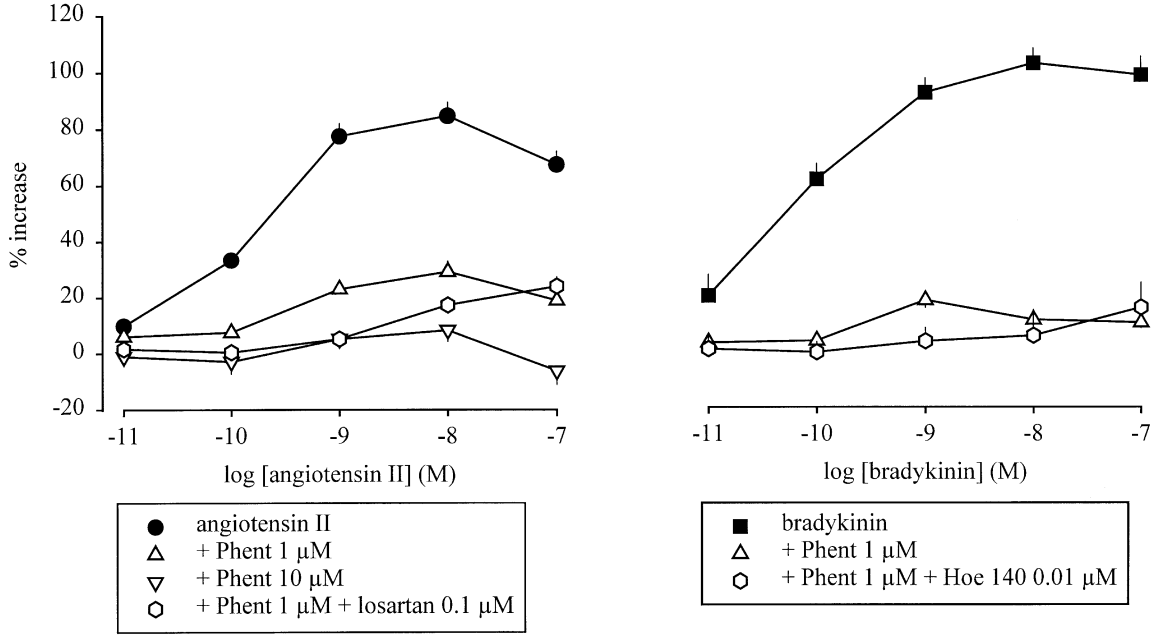

Figure 2 Effect of angiotensin II (left panel) and bradykinin (right panel) on the overflow of tritium, evoked by stimulation trains leading to marked autoinhibition, in the absence and presence of phentolamine. The preparations were stimulated for 6 periods (S_1-S_6) by 120 p at 3 Hz. Angiotensin II and bradykinin were introduced into the PSS either alone or in the presence of phentolamine (Phent, 1 or 10 μM), which was present throughout superfusion. In some experiments, losartan (0.1 μM) or Hoe 140 (0.01 μM) was also present throughout superfusion. Angiotensin II and bradykinin were introduced in increasing concentrations, 12 min before S_2-S_6. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of angiotensin II and bradykinin. The vertical lines represent the s.e.mean from 4–30 preparations. The curves showing the effect in the absence of phentolamine are from Figure 1.

after S_1 were calculated for each preparation, taking as reference value the average corresponding S_n/S_1 ratio in otherwise identical control experiments, in the absence of the drug. Effects of drugs added after S_1 on basal tritium outflow were calculated in the same manner, based on samples collected immediately before stimulation.

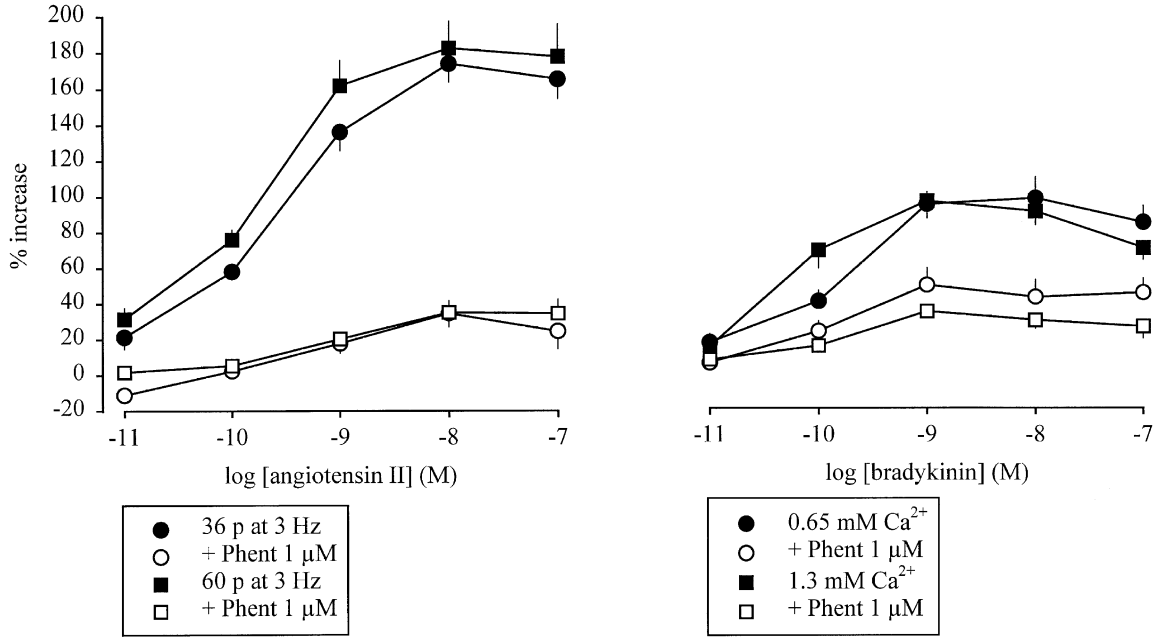

Figure 3 Effect of angiotensin II (left panel) and bradykinin (right panel) on the overflow of tritium, evoked by stimulation trains leading to marked autoinhibition, in the absence and presence of phentolamine: influence of a reduction of S_1 overflow values. The preparations were stimulated for six periods (S_1-S_6). Angiotensin II and bradykinin were introduced into the PSS either alone or in the presence of phentolamine (Phent, 1 μM), which was present throughout superfusion. Angiotensin II and bradykinin were introduced into the PSS in increasing concentrations, 12 min before S_2-S_6. Left panel: preparations were stimulated with either 36 or 60 p (instead of the usual 120 p) at 3 Hz. Right panel: preparations were stimulated with 120 p at 3 Hz; the PSS contained either 0.65 or 1.3 mM (instead of the usual 2.5 mM) Ca^{2+}. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of angiotensin II and bradykinin. The vertical lines represent the s.e.mean from 4–14 preparations.

Data are expressed as means ± standard errors of the mean (s.e.mean); n denotes the number of preparations. The statistical significance of differences between groups was determined by Mann-Whitney test with Bonferroni correction. In all cases probability levels less than 0.05 ($P<0.05$) were taken to indicate significant differences.

Results

The basal efflux of tritium was similar to that previously reported (Cox *et al*., 1999). Electrical field stimulation with either 120 p at 3 Hz or 20 p at 50 Hz greatly increased tritium outflow. The electrically evoked overflow of tritium elicited by the first periods of stimulation, expressed as per cent of tissue tritium, is shown in Table 1.

Effect of angiotensin II, angiotensin III and bradykinin on trains leading to marked autoinhibition: 120 p at 3 Hz

Phentolamine, when introduced 12 min before the third of six periods of stimulation by 120 p at 3 Hz (S_3), increased the evoked overflow of tritium by 358 ± 50% (S_3; similar at S_4-S_6; $n=6$), indicating marked autoinhibition of transmitter release.

Under these stimulation conditions, in the absence of phentolamine, angiotensin II ($10^{-11}-10^{-7}$ M), angiotensin III ($10^{-10}-10^{-6}$ M) and bradykinin ($10^{-11}-10^{-7}$ M) increased the evoked overflow of tritium in a concentration-dependent manner (Figure 1). The maximal enhancement produced by the angiotensins and bradykinin was about 80 and 100%, respectively. The EC_{50} values, determined as the concentrations causing half-maximal enhancement, i.e. 40% in the case of the angiotensins and 50% in the case of bradykinin, amounted to 0.17 nM for angiotensin II, 2.2 nM for angiotensin III and 0.04 nM for bradykinin (interpolated from the nearest points of the concentration-response curve, see also Cox *et al*., 1999). Addition before the third of three stimulation periods of either angiotensin II (0.1 μM) alone, bradykinin (0.1 μM) alone, or the two combined yielded similar enhancement of the overflow at S_3, indicating that the effects of the peptides were not additive (angiotensin II 83 ± 10% increase, $n=4$; bradykinin 92 ± 12% increase, $n=5$; combination 117 ± 10% increase, $n=7$).

The facilitatory effect of the angiotensins and bradykinin was blocked respectively by the AT_1-receptor antagonist losartan (0.1 μM) and the B_2-receptor antagonist Hoe 140 (0.01 μM; data not shown, but see Cox *et al*. (1999) for angiotensin and Chulak *et al*. (1998) for bradykinin).

None of the drugs altered basal tritium efflux (data not shown).

Effect of angiotensin II, angiotensin III and bradykinin on trains leading to little autoinhibition: 20 p at 50 Hz

Stimulation with single, brief trains of 20 p at 50 Hz produced very little autoinhibition: phentolamine, when introduced 12 min before the third of six periods of stimulation, enhanced the overflow of tritium significantly, but only by 31 ± 7% (S_3; similar at S_4-S_6; $n=4$). When the preparations were stimulated under these conditions, neither angiotensin II, angiotensin III nor bradykinin altered the evoked overflow of tritium (Figure 1). Again none of the drugs tested altered basal tritium efflux (data not shown).

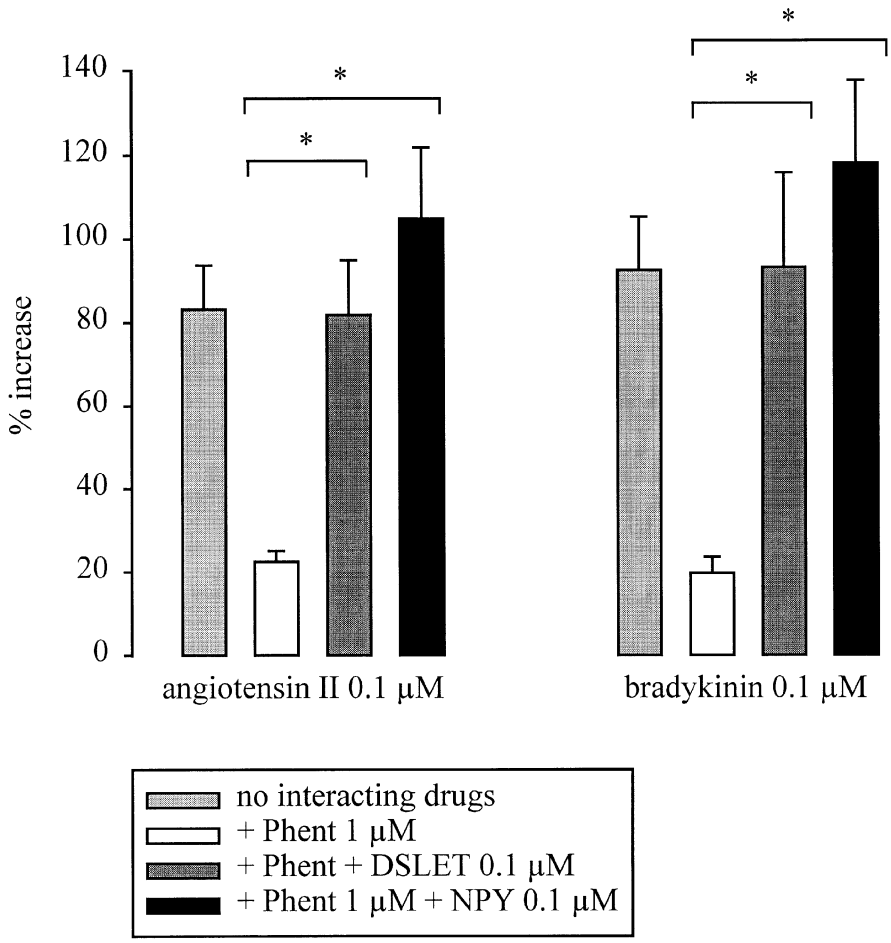

Figure 4 Effect of angiotensin II and bradykinin on the overflow of tritium, evoked by stimulation trains leading to marked autoinhibition, in the absence and presence of phentolamine: interaction with DSLET and NPY. The preparations were stimulated for three periods (S_1-S_3) by 120 p at 3 Hz. Angiotensin II and bradykinin were introduced into the PSS either alone, or in the presence of phentolamine (Phent, 1 μM), or in the presence of Phent plus DSLET (0.1 μM) or NPY (0.1 μM). Phent, when given alone, was present from the beginning of superfusion. The combinations of Phent plus DSLET or NPY were present from $t=30$ min. Angiotensin II and bradykinin were introduced 12 min before S_3. The columns represent the mean percentage increase, calculated from S_3/S_1 values and corrected for changes observed in control experiments in the absence of angiotensin II or bradykinin. The vertical lines represent the s.e.mean from 5–15 preparations. * Indicates a significant difference ($P<0.05$). All increases were significant ($P<0.05$) as compared to control experiments without angiotensin II and bradykinin (not shown).

Effect of angiotensin II and bradykinin with α_2-adrenoceptor blockade

Another approach to investigating the influence of prejunctional α_2-adrenoceptors on the facilitatory effect of angiotensin II and bradykinin was to block α_2-adrenoceptors during stimulation with normally autoinhibition-rich trains such as 120 p at 3 Hz. Phentolamine (1 or 10 μM), when present throughout superfusion, increased the overflow (S_1) elicited by stimulation with 120 p at 3 Hz from, on average, 0.98 to 3.40% and 3.70%, respectively (Table 1), in accord with its effect when added before S_3 (see above). Phentolamine (1 μM) markedly reduced the facilitatory effect of both angiotensin II and bradykinin on the overflow response to 120 p at 3 Hz (Figure 2). A 10 fold higher concentration of phentolamine abolished the effect of angiotensin II (Figure 2). Most of the small effects of angiotensin and bradykinin remaining in the presence of phentolamine (1 μM) were abolished by losartan (0.1 μM) and Hoe 140 (0.01 μM), respectively (Figure 2). To eliminate a possible non-selective effect of phentolamine, the selective α_2-adrenoceptor antagonist rauwolscine was also used. Like phentolamine, rauwolscine (1 μM), when present throughout superfusion, increased the overflow at S_1 ($2.49\pm0.38\%$, $n=10$). The facilitatory effect of bradykinin was reduced by rauwolscine (1 μM; data not shown, $n=6$). None of the receptor antagonists when introduced alone, or in combination with each other, had any effect on basal tritium efflux (data not shown).

Effect of angiotensin II and bradykinin with reduced pulse number and reduced calcium concentration

The overflow of tritium from preparations stimulated with 120 p at 3 Hz was markedly enhanced by the α-adrenoceptor antagonists (see above). The possibility was considered that the increase in overflow *per se* rather than the blockade of α_2-adrenoceptors might minimise the effect of angiotensin II and bradykinin, or that the release of noradrenaline might be maximally enhanced by the α_2-adrenoceptor antagonists, as previously suggested (Starke & Schümann, 1972), and thus no further increase by angiotensin II or bradykinin be possible. Two approaches were employed in order to reduce the evoked overflow, in the presence of phentolamine, to levels obtained with 120 p at 3 Hz in the absence of phentolamine. Firstly, the number of electrical pulses was reduced from 120 to 60 and 36. Even when S_1 overflow values were reduced from 3.40%

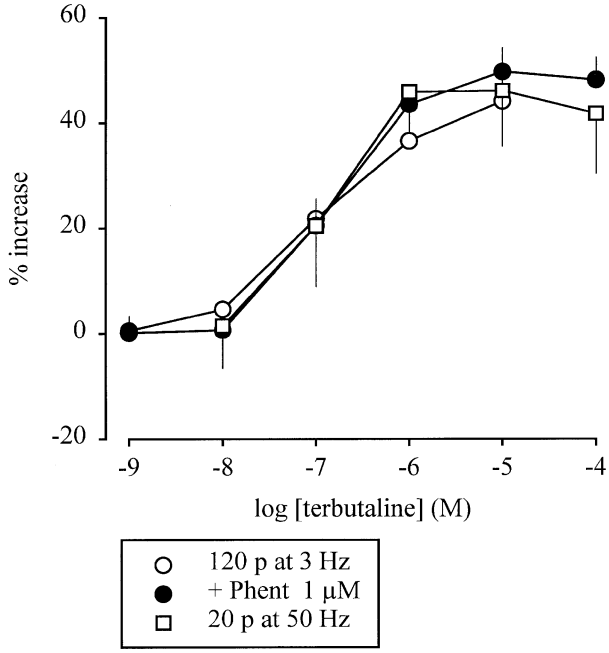

Figure 5 Effect of terbutaline on the evoked overflow of tritium. The preparations were stimulated for six periods (S_1-S_6) by either 120 p at 3 Hz or 20 p at 50 Hz. Terbutaline was introduced into the PSS either alone or in the presence of phentolamine (Phent, 1 μM), which was present throughout superfusion. Terbutaline was introduced in increasing concentrations, 12 min before S_2-S_6. The symbols represent the mean percentage increase, calculated from S_n/S_1 values and corrected for changes observed in control experiments in the absence of terbutaline. The vertical lines represent the s.e.mean from 4–10 preparations.

(120 p, phentolamine 1 μM) to 1.58 and 0.98% (60 and 36 p, respectively, phentolamine 1 μM; Table 1), the facilitatory effect of angiotensin II was still greatly attenuated by phentolamine (Figure 3). In the absence of phentolamine, angiotensin II enhanced tritium overflow even to a greater extent, by about 180% maximally (Figure 3), than in experiments with 120 p at 3 Hz (80%; Figure 1).

The second procedure to reduce S_1 overflow was to keep the number of pulses constant (120 p at 3 Hz) but to decrease the extracellular calcium concentration to 1.3 or 0.65 mM. This approach was used for experiments with bradykinin. Even when S_1 overflow values were reduced from 3.40% (2.5 mM calcium, phentolamine 1 μM) to 1.96 and 1.33% (1.3 and 0.65 mM calcium respectively, phentolamine 1 μM; Table 1), the facilitatory effect of bradykinin was still greatly attenuated by phentolamine (Figure 3). In the absence of phentolamine, bradykinin enhanced tritium overflow to the same extent, about 100% (Figure 3), as it did at 2.5 mM calcium (Figure 1).

It should be noted that marked α_2-autoinhibition prevailed in trains of 60 and 36 p, as well as in trains of 120 p applied in low calcium media: phentolamine (1 μM) in each case greatly increased the evoked overflow of tritium (Table 1).

Effect of angiotensin II and bradykinin in the presence of phentolamine plus DSLET or NPY

The possibility was considered that the $G_{q/11}$-coupled receptors for angiotensin and bradykinin require the activation of a $G_{i/o}$-coupled receptor such as the α_2-autoreceptor in order to facilitate noradrenaline release. To investigate this possibility, prejunctional non-α_2, $G_{i/o}$-coupled receptors were activated under conditions where the α_2-autoreceptor was blocked: namely the δ-opioid and the Y_2 NPY receptor. The experiments consisted of three periods of stimulation with 120 p at 3 Hz. The δ-opioid receptor agonist DSLET and the Y_2 NPY receptor agonist NPY (both 0.1 μM), when added together with phentolamine (1 μM) from $t=30$ min of superfusion onwards, reduced the evoked overflow of tritium as compared to phentolamine alone (S_1; Table 1). In the presence of phentolamine alone, the effect of angiotensin II and bradykinin was greatly attenuated (Figure 4). Addition of either DSLET or NPY together with phentolamine restored the effect of angiotensin II and bradykinin (Figure 4).

The combination of DSLET and phentolamine slightly increased basal tritium efflux. The combination of NPY and phentolamine was without significant effect on basal tritium efflux (data not shown).

Effect of terbutaline

The results thus far suggested an interaction between $G_{i/o}$- and $G_{q/11}$-coupled receptors. To investigate whether this was a specific phenomenon, we investigated the interaction between α_2-autoreceptors and a G_S-coupled release-enhancing receptor, namely the prejunctional β_2-adrenoceptor. The β_2-adrenoceptor agonist terbutaline enhanced tritium overflow in the same, concentration-dependent manner, irrespective of whether pulse trains leading to marked (120 p at 3 Hz) or little (20 p at 50 Hz) autoinhibition were used or whether, in the former case, the autoinhibition was blocked by phentolamine (1 μM) (Figure 5). The maximal increase by terbutaline was about 50% in each case (Figure 5). Terbutaline did not alter basal tritium efflux (data not shown).

Discussion

Under appropriate conditions, angiotensin II, angiotensin III and bradykinin all greatly enhanced, by up to 80–180%, the electrically evoked overflow of tritium, or, in other words (see Starke, 1977), the action potential-evoked release of [^{3}H]-noradrenaline. These effects have previously been documented in mouse atria (Musgrave & Majewski, 1989; Rajanayagam *et al*., 1989; Chulak *et al*., 1998; Cox *et al*., 1999). They were blocked respectively by losartan and Hoe 140, which supports the general consensus that they were mediated *via* AT_1- and B_2-receptors, respectively (Chulak *et al*., 1998; Cox *et al*., 1999).

The 'appropriate conditions' for a pronounced effect of the peptides were: stimulation by trains of 120, 60 or 36 p at 3 Hz in 2.5 mM calcium medium and stimulation by 120 p at 3 Hz in 1.3 or 0.65 mM calcium medium. In contrast, the facilitatory effects of the angiotensins and bradykinin were much smaller or even absent when atria were stimulated by trains of 20 p at 50 Hz, or by trains of 120, 60 or 36 p at 3 Hz in 2.5, 1.3 or 0.65 mM calcium medium in the presence of phentolamine or rauwolscine. This difference was not due to a difference in the pre-angiotensin or pre-bradykinin release (S_1), or to an upper limit of release when release was very high (120 p at 3 Hz in the presence of phentolamine or rauwolscine). In fact, the pre-angiotensin and pre-bradykinin release values overlapped in the two groups: the 'appropriate conditions' produced release values (S_1) between 0.24% (120 p at 3 Hz, 0.65 mM calcium) and 0.98% (120 p at 3 Hz, 2.5 mM calcium), whereas the conditions permitting at best a minor effect of the peptides produced values between 0.46% (20 p at 50 Hz) and 3.7% (120 p at 3 Hz, phentolamine 10 μM; Table 1). Rather, what distinguished conditions that permitted a marked effect of

angiotensin or bradykinin from those that did not, was the operation of α_2-autoinhibition: the angiotensins and bradykinin were very effective when α_2-autoinhibition was pronounced, as shown by the large release-enhancing effects of phentolamine (or rauwolscine); the effects of the peptides were weak when either pulse trains were too brief for autoinhibition (20 p at 50 Hz), or autoinhibition was interrupted by α_2-antagonists.

Our findings in mouse atria confirm and extend previous observations with angiotensin II in the rabbit heart and in guinea-pig atria (Starke & Schümann, 1972; Brasch *et al*., 1995). They support the view of the latter authors that it is the degree of α_2-autoinhibition that determines the effectiveness of angiotensin II. Bradykinin, it seems, requires the same conditions for its prejunctional effect.

Interestingly, some electrophysiological observations may also be explained by dependence of the effect of angiotensin on α_2-autoinhibition. In three guinea-pig tissues, the uterine artery and vas deferens (Bell, 1972; Ziogas & Cunnane, 1991) and the mesenteric artery (Onaka *et al*., 1997), angiotensin II did not increase the amplitude of the first excitatory junction potential (e.j.p.) in a train of pulses but increased the amplitudes of subsequent e.j.p.s. Transmitter release (in this case cotransmitter ATP release) by the first pulse, after a period of rest, is free of α_2-autoinhibition; α_2-autoinhibition develops from the second pulse onwards (Illes & Starke, 1983; Brock *et al*., 1990). In the pulmonary artery of the rabbit, phentolamine actually increased the release-facilitating effect of angiotensin II (Costa & Majewski, 1988), the opposite of what one would expect from the results discussed so far; the reason for the difference is not known.

Our experiments provide some evidence for the view that the interaction between prejunctional α_2-autoreceptors, prejunctional AT_1-receptors and prejunctional B_2-receptors takes place at the level of the cellular transduction pathways (see Brasch *et al*., 1995; see also Limberger *et al*., 1988; Hertting *et al*., 1990; Schlicker & Göthert, 1998). In support of this idea, angiotensin II and bradykinin shared both the dependence on α_2-autoinhibition and a common transduction step, as shown by the lack of additivity of their releasing-enhancing effects. The common transduction step might for example be activation of one and the same $G_{q/11}$ protein. Also in support of an interaction during signal transduction, α_2-autoinhibition did not modify the facilitation of noradrenaline release by terbutaline, which acts through prejunctional β_2-adrenoceptors and hence, the G_S pathway. We suspected that the two $G_{q/11}$-coupled receptors might require an ongoing $G_{i/o}$-mediated inhibition for a marked effect. For this reason, we activated two $G_{i/o}$-coupled receptors, namely the δ-opioid (Werling *et al*., 1989) and the Y_2 NPY (Rump *et al*., 1997b) receptor, while the α_2-autoreceptors were blocked by phentolamine. In fact, the δ-opioid agonist DSLET as well as NPY restored the effects of angiotensin II and bradykinin after they had been minimised by phentolamine.

If indeed $G_{q/11}$-mediated enhancement of noradrenaline release requires, or is at least much amplified by, an ongoing $G_{i/o}$-mediated inhibition, what might be the mechanism? A likely possibility is that protein kinase C (PKC), activated by the $G_{q/11}$ pathway, interferes with the ongoing $G_{i/o}$ pathway. The prejunctional $G_{i/o}$ pathway is thought to involve *inter alia* the inhibition of N-type calcium channels (see Starke *et al*., 1989; Lipscombe *et al*., 1989), and PKC has been reported to attenuate the $G_{i/o}$-mediated inhibition, although not of prejunctional, at least of somato-dendritic N-type calcium channels (see Hamid *et al*., 1999). PKC might disrupt the $G_{i/o}$ pathway by phosphorylating either the $G_{i/o}$-coupled receptor (for example the α_{2A}-adrenoceptor; Liang *et al*., 1998), $G_{i/o}$ itself (Katada *et al*., 1985), or the N-type calcium channel (see Hamid *et al*., 1999). PKC activation increases transmitter release in atrial preparations (Musgrave *et al*., 1991; Brasch, 1993; Chulak *et al*., 1995). Interestingly, a PKC activating phorbol ester did not increase noradrenaline release in guinea-pig atria after α_2-adrenoceptor blockade on the one hand, and under autoinhibition-free stimulation conditions on the other hand (Brasch, 1993), like angiotensin II and bradykinin in our experiments. Taken together, these findings and those of our study are compatible with the idea that prejunctional AT_1- and B_2-receptors, once activated, (i) stimulate $G_{q/11}$ and thereby PKC; that PKC then (ii) phosphorylates and inactivates a protein involved in the $G_{i/o}$-mediated prejunctional α_2-autoinhibition; and that (iii) ongoing autoinhibition is thus disrupted and transmitter release disinhibited.

In conclusion, apart from confirming the requirement of α_2-autoinhibition for a marked facilitatory effect of angiotensin II, we extend these observations to angiotensin III and also provide evidence for the same requirement in the case of another $G_{q/11}$-coupled receptor, the bradykinin B_2-receptor. When α_2-autoinhibition is disrupted, activation of other prejunctional $G_{i/o}$-coupled receptors, namely opioid and NPY receptors, restores a marked effect of angiotensin II and bradykinin. No such interaction is obtained between α_2-autoinhibition and the G_S-coupled prejunctional β_2-adrenoceptor. Thus, we provide further evidence in support of a special type of prejunctional receptor cross-talk in mouse atria. The mechanism of this cross-talk and whether it occurs in other tissues and species is of particular interest.

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