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Differential presynaptic modulation of noradrenaline release in human atrial tissue in normoxia and anoxia

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1 Presynaptic modulation of noradrenaline release in human atrial tissue specimens was investigated under normoxic and anoxic conditions.

2 Noradrenaline release was induced by electrical stimulation and release during experimental intervention (S_2) was compared with release during a preceding control stimulation (S_1). The results were expressed as the geometric means and 95% confidence intervals of the S_2/S_1 ratio.

3 The α_2-adrenoceptor agonist, UK 14304 (0.1 μmol l^{-1}) significantly inhibited noradrenaline release, resulting in a S_2/S_1 ratio of 0.49 (0.40-0.59), and the α_2-adrenoceptor antagonist, yohimbine (1 μmol l^{-1}) increased noradrenaline release (S_2/S_1 1.83 [1.43-2.35]) during normoxia. Both compounds were ineffective during anoxia.

4 Adenosine (30 μmol l^{-1}) inhibited noradrenaline release with a S_2/S_1 ratio of 0.54 (0.42-0.66). The adenosine antagonist, 8-phenyltheophylline, alone had no effect during normoxia. During anoxia, neither adenosine nor 8-phenyltheophylline altered noradrenaline release.

5 The β_2-adrenoceptor agonist, terbutaline (1 μmol l^{-1}) increased (1.53 [1.14-2.01]) and the β-adrenoceptor antagonist, pindolol (1 μmol l^{-1}) suppressed noradrenaline release (0.62 [0.49-0.79]) under normoxic conditions. During anoxia, pindolol significantly inhibited noradrenaline release with a S_2/S_1 ratio of 0.66 (0.51-0.85), whereas terbutaline did not influence noradrenaline release.

6 Angiotensin II (0.1 μmol l^{-1}) enhanced noradrenaline release resulting in a S_2/S_1 ratio of 1.44 (1.34-1.54), while the angiotensin II antagonist, losartan (1 μmol l^{-1}) had no effect on noradrenaline release during normoxia. Conversely, angiotensin II did not increase noradrenaline release and losartan significantly inhibited noradrenaline release to a S_2/S_1 ratio of 0.60 (0.46-0.77) during anoxia.

7 In conclusion, human cardiac tissue possesses presynaptic inhibitory α_2-adrenoceptors and adenosine receptors, as well as facilitatory β_2-adrenoceptors and angiotensin II receptors regulating noradrenaline release under normoxic conditions. During anoxia the responses to α_2-adrenoceptors and adenosine receptor stimulation are lost, whereas facilitatory responses to β_2-adrenoceptors and angiotensin II receptor stimulation are maintained and these receptors appear to be maximally stimulated. This differential presynaptic modulation in anoxia may contribute to enhanced sympathetic activity in ischaemia.

Keywords: Anoxia; human cardiac tissue; noradrenaline release; presynaptic modulation; presynaptic receptors

Introduction

Substantial changes in the regulation of the cardiac sympathetic nervous system even after brief anoxic or ischaemic periods contribute to the generation of malignant arrhythmias and metabolic deterioration of the ischaemic myocardium (Corr & Gills, 1978; Rona, 1985; Schömig *et al.*, 1995). So far, investigations of sympathetic neurotransmission during ischaemia have concentrated mainly on the postsynaptic site (Mukherjee *et al.*, 1979; Butterfield & Chess-Williams, 1990). A few animal studies, however, suggest that presynaptic receptor-mediated inhibition of noradrenaline release may also be impaired during myocardial ischaemia (Dart *et al.*, 1984; Richardt *et al.*, 1987; Du *et al.*, 1990). Recently presynaptic receptor modulation of noradrenaline release has also been documented in human isolated atrial (Rump *et al.*, 1994) and ventricular myocardium (Matko *et al.*, 1994) under normoxic conditions. However, presynaptic modulation under ischaemic conditions is poorly understood, particularly in the human heart.

We studied presynaptic modulation of noradrenaline release in human atrial tissue specimens obtained from open heart surgery. Pharmacological and metabolic interventions are possible in this model and analogous conclusions on the intact human heart can be drawn on the basis of a previous comparative study (Seyfarth *et al.*, 1993). The key issue of the study was the direct comparison of presynaptic modulation under normoxic conditions and under pathophysiological relevant conditions such as anoxia.

In the present investigation only single concentrations of the various agonists and antagonists of presynaptic receptors were used, due to limited access to human atrial tissue specimen. We therefore always used supramaximal concentrations according to published animal studies (Bohmann *et al.*, 1993; Ordway *et al.*, 1993) or the previously published data of Rump *et al.* (1994). The effects of single concentrations of the agents on endogenous noradrenaline release under anoxic and normoxic incubation with otherwise identical protocols allows a direct comparison of presynaptic modulation under different metabolic conditions.

Methods

Tissue preparation and experimental protocol

Human atrial tissue was obtained during routine cardiac surgery from patients undergoing coronary artery bypass grafting or valve replacement, after informed consent had been obtained. Part of the right atrial appendage was excised during cannulation of the right atrium and immediately incubated in ice cold and gassed (95% O_2; 5% CO_2) Krebs-

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Henseleit solution with the following composition (mmol l^{-1}): NaCl 125; $NaHCO_3$ 16.9; NaH_2PO_4 0.2; KCl 4.0; $CaCl_2$ 1.85; $MgCl_2$ 1.0, glucose 11.0; and sodium EDTA 0.027. After transfer to the research laboratory, tissue was dissected into two to four parts of similar size weighing 50 to 100 mg. Pieces were placed between two paddle electrodes made of stainless steel wire netting and incubated in 3 ml Krebs-Henseleit solution in tubes at 37°C and constantly gassed with 95% O_2: 5% CO_2. This produced a pH between 7.35 and 7.45 and Po_2 of 400–500 mmHg in the buffer. Tissue was allowed to equilibrate for 45 min and thereafter incubation medium was changed by transferring the tissue immediately to the next tube with gassed Krebs-Henseleit buffer every 5 min. Desipramine (DMI, 0.1 μmol l^{-1}) was present in the buffer throughout the entire experiment to block the neuronal catecholamine carrier.

After 10 min of incubation with DMI, electrical field stimulation with 5 V, 4 Hz and a pulse width of 2 ms was carried out via the paddle electrodes for 5 min. Washout was allowed for a further 15 min before the second stimulation of 5 min was performed, followed by a second washout phase of 15 min until the end of the experiment. In every preparation, paired stimulations were carried out. Noradrenaline release during the first stimulation (S_1) was used as control in every experiment to compare the effect of metabolic intervention or drug effects on noradrenaline release during the second stimulation (S_2). Tissue was incubated after the control stimulation with agonist drugs for 10 min and with antagonist drugs for 15 min before S_2.

For experiments with anoxia the Krebs-Henseleit buffer was modified. The reducing agent, sodium dithionite 1 mmol l^{-1} was added, glucose was omitted and the solution was gassed with N_2 and CO_2. Thereafter $Po_2 < 1$ mmHg and pH of 7.40 was achieved. Anoxia started 5 min before each stimulation and was maintained throughout the 5 min stimulation period. Before each experiment, anoxia was confirmed by a blood gas detector (CIBA corning). After S_1 in anoxic buffer, tissue was allowed to recover for 15 min in normoxic incubation medium before anoxia was induced over a second period of 10 min, including S_2 covering the last 5 min. S_2 was also followed by a washout phase with normoxic incubation.

Noradrenaline determination

Noradrenaline concentration was measured in the incubation buffer after removal of the tissue. To prevent noradrenaline degradation, sodium EDTA (10 mmol l^{-1}) was added and the samples were immediately frozen at −60°C. Determination of endogenous noradrenaline was performed after a two-step extraction using high-performance liquid chromatography (h.p.l.c.) with a reversed phase C18 column and electrochemical detection as previously described (Schömig *et al.*, 1987). Recovery was 98%, the limit of detection was 0.1 nmol l^{-1}, and the coefficient of variation was 5.9%.

Agents used

Adenosine free base, angiotensin II acetate salt, desipramine hydrochloride, 8-phenyltheophylline, pindolol thiosulphate, terbutaline hemisulphate salt, tetrodoxin, and yohimbine hydrochloride were all obtained from Sigma Chemical Co, Deisenhofen, Germany. All Krebs salts and sodium EDTA (titriplex III) were obtained from Merck, Darmstadt, Germany. UK 14304 (5-bromo-6-(2-imidazolin-2yl amino) quinoxaline) was generously donated by Pfizer, Karlsruhe, Germany and losartan MK-954 from MSD, Darmstadt, Germany.

All drugs were dissolved in distilled water for stock solutions and further dilution was performed in Krebs-Henseleit buffer as required. Stock solution for 8-phenyltheophylline was made alkaline with a few drops of conc. NaOH, and stock solution for pindolol was acidified by adding a few drops of H_3PO_4. Stock solutions were either prepared freshly on the day of experiment or aliquots were kept at below −30°C.

Statistical analysis

Stimulation-induced noradrenaline release was calculated as cumulative overflow during the stimulation period and the following washout period of 15 min. The result of each atrial specimen was averaged from 2–4 pieces and considered as $n = 1$. Absolute noradrenaline release was expressed as arithmetric mean ± s.e.mean of $n = 6-8$ experiments in pmol g^{-1}. The first and second stimulation was compared and the ratio of noradrenaline release (S_2/S_1) was calculated. The results are presented as geometric means of the S_2/S_1 ratio with 95% confidence interval in $n = 6-8$ experiments. Statistical differences of the absolute noradrenaline overflow in paired stimulations (S_1 versus S_2) were analysed with Student's t test for paired data. A P value < 0.05 was considered statistically significant.

Results

Control experiments and effect of tetrodotoxin (TTX) (Figure 1)

Under normoxic conditions, stimulation-induced noradrenaline release from human atrial tissue amounted to 80.2 ± 8.9 and 78.3 ± 8.0 pmol g^{-1} during S_1 and S_2, respectively. The mean S_2/S_1 ratio was 0.98 (95% confidence interval: 0.85–1.12). During anoxia, stimulation-induced noradrenaline release increased significantly to 107.0 ± 4.0 (S_1) and

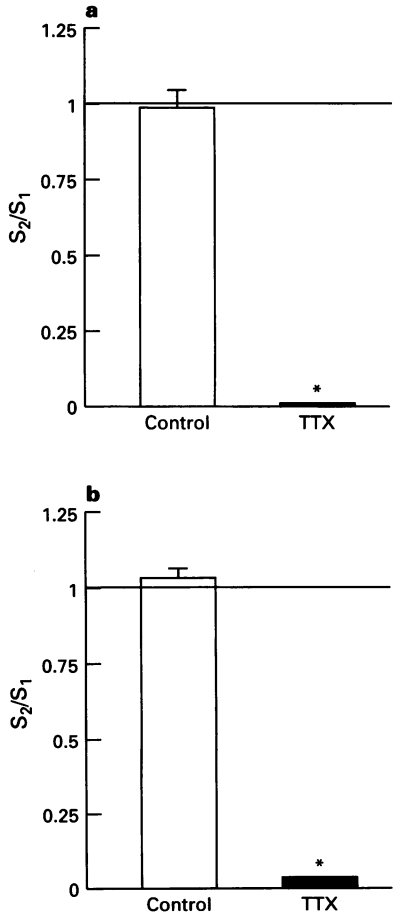

Figure 1 Effect of incubation with tetrodotoxin (TTX, 0.3 μmol l^{-1}) on noradrenaline overflow induced by electrical field stimulation of human incubated atrial tissue in normoxia (a) and anoxia (b). Paired stimulations were performed, with the first stimulation as individual control (S_1) and second stimulation during experimental intervention (S_2). Data are geometric means of S_2/S_1 ratios and 95% confidence intervals ($n = 6-8$) *$P < 0.05$.

109.8 ± 5.7 pmol g^{-1} (S_2) resulting in a mean S_2/S_1 ratio of 1.02 (0.93–1.13). Thus in neither normoxic nor anoxic control experiments, were significant differences of noradrenaline release found between S_1 and S_2 indicating reproducible conditions in the experimental settings.

Direct comparison of normoxia and anoxia revealed noradrenaline release of 80.2 ± 11.4 pmol g^{-1} in normoxia and 105.2 ± 10.3 pmol g^{-1} in anoxia with a resulting S_2/S_1 ratio of 1.32 (1.15–1.51) in paired experiments. There was a significant increase ($P<0.001$) of noradrenaline release in S_2 compared to S_1.

TTX incubation (0.3 μmol l^{-1}) during the second stimulation almost completely abolished the stimulation-induced noradrenaline overflow from human atrial tissue. In experiments with normoxia, the S_2/S_1 ratio was 0.01 (−0.02–0.04). Likewise, in anoxic experiments with TTX (0.3 μmol l^{-1}) noradrenaline release declined almost to zero and the resulting S_2/S_1 ratio was 0.03 (−0.00–0.06).

Presynaptic effects of α_2-adrenoceptor agonist and α_2-adrenoceptor antagonist (Figure 2)

Incubation of human atrial tissue with the selective α_2-adrenoceptor agonist, UK 14304 (0.1 μmol l^{-1}) reduced noradrenaline release under normoxic conditions. Consequently, the S_2/S_1 ratio declined to 0.49 (0.40–0.59). Incubation with the α_2-adrenoceptor antagonist yohimbine (1 μmol l^{-1}) led to a significant increase of stimulation-induced noradrenaline release (S_2/S_1: 1.83 [1.43–2.35]). The effect of UK 14304 was antagonized by simultaneous incubation with yohimbine, resulting in a S_2/S_1 ratio of 1.12 (0.82–1.55).

During anoxia, UK 14304 caused no significant change of noradrenaline release (S_2/S_1: 0.80 [0.58–1.05]). Likewise, in anoxic incubation, yohimbine had no significant effect on stimulation-induced noradrenaline release (S_2/S_1: 1.06 [0.87–1.28]). Anoxic incubation in the presence of both yohimbine and UK 14304 also had no significant effect on noradrenaline release (S_2/S_1: 0.84 [0.39–1.30]).

Presynaptic effects of adenosine and adenosine antagonists (Figure 3)

Adenosine (30 μmol l^{-1}) significantly inhibited noradrenaline release in normoxia, resulting in a S_2/S_1 ratio of 0.54 (0.42–0.66). Incubation with the adenosine receptor antagonist, 8-phenyltheophylline (8 PT) (1 μmol l^{-1}) alone, did not affect the release of noradrenaline (S_2/S_1: 0.95 [0.8–1.12]). Incubation with both 8-PT and adenosine antagonized the inhibitory action of adenosine (S_2/S_1: 0.88 [0.55–1.33]).

During anoxia, neither incubation with adenosine (30 μmol l^{-1}) (S_2/S_1: 0.90 [0.68–1.19]) nor 8-PT (10 μmol l^{-1}) led to a significant change in noradrenaline release (S_2/S_1: 0.87 [0.71–1.08]).

Presynaptic effects of β_2-adrenoceptor agonists and β_1/β_2-adrenoceptor antagonists (Figure 4)

The β_2-adrenoceptor agonist, terbutaline (1 μmol l^{-1}) caused a significant increase of stimulation-induced noradrenaline release (S_2/S_1: 1.53 [1.14–2.01]). Incubation with the non-selective β_1/β_2-adrenoceptor antagonist pindolol (1 μmol l^{-1}) significantly inhibited noradrenaline release, resulting in a $S_2/$

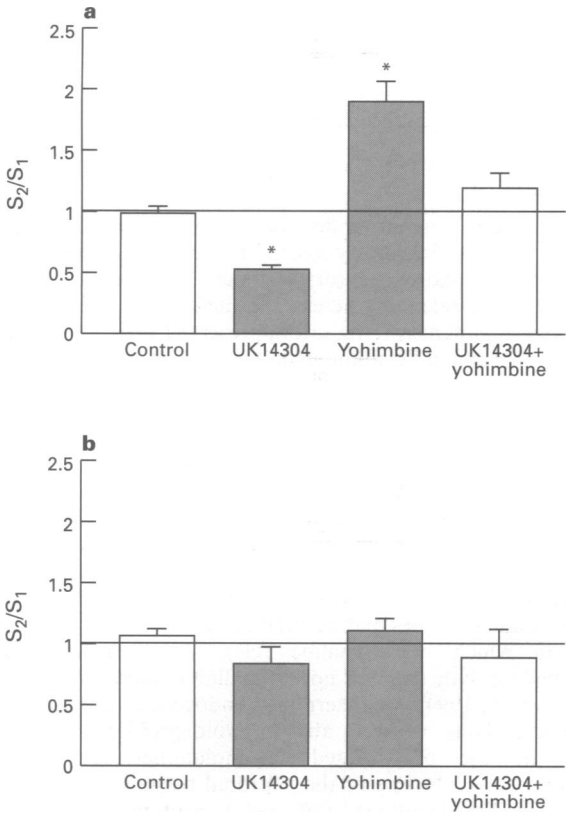

Figure 2 Effect of the α_2-adrenoceptor agonist, UK 14304 (0.1 μmol l^{-1}), the α_2-adrenoceptor antagonist, yohimbine (1 μmol l^{-1}), and combined incubation with both compounds on noradrenaline overflow induced by electrical field stimulation of human incubated atrial tissue in normoxia (a) and anoxia (b). Paired stimulations were performed, with the first stimulation as individual control (S_1) and second stimulation during experimental intervention (S_2). Data are geometric means of S_2/S_1 ratios and 95% confidence intervals ($n=6-8$) *$P<0.05$.

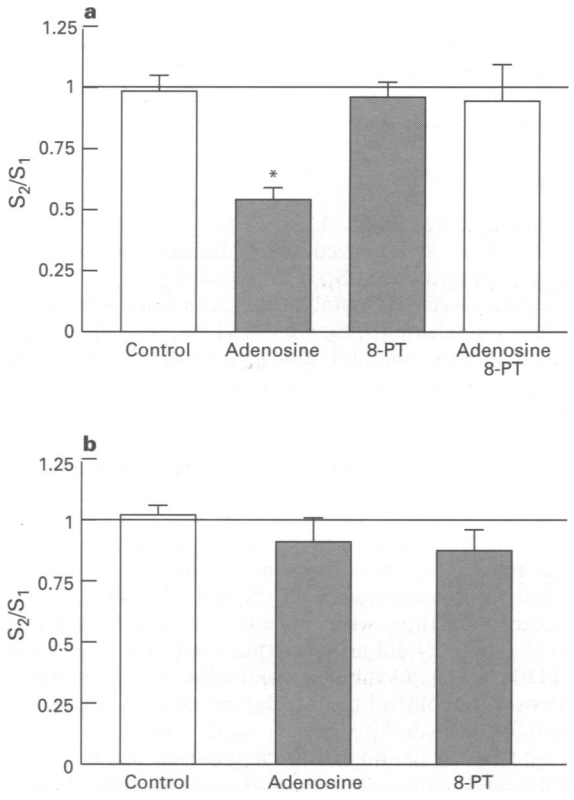

Figure 3 Effect of adenosine (30 μmol l^{-1}), the nonselective adenosine antagonist, 8-phenyltheophylline (8-PT) (1 μmol l^{-1}) and combined incubation with both compounds on noradrenaline overflow induced by electrical field stimulation of human incubated atrial tissue in normoxia (a) and anoxia (b). Paired stimulations were performed, with the first stimulation as individual control (S_1) and second stimulation during experimental intervention (S_2). Data are geometric means of S_2/S_1 ratios and 95% confidence intervals ($n=6-8$) *$P<0.05$.

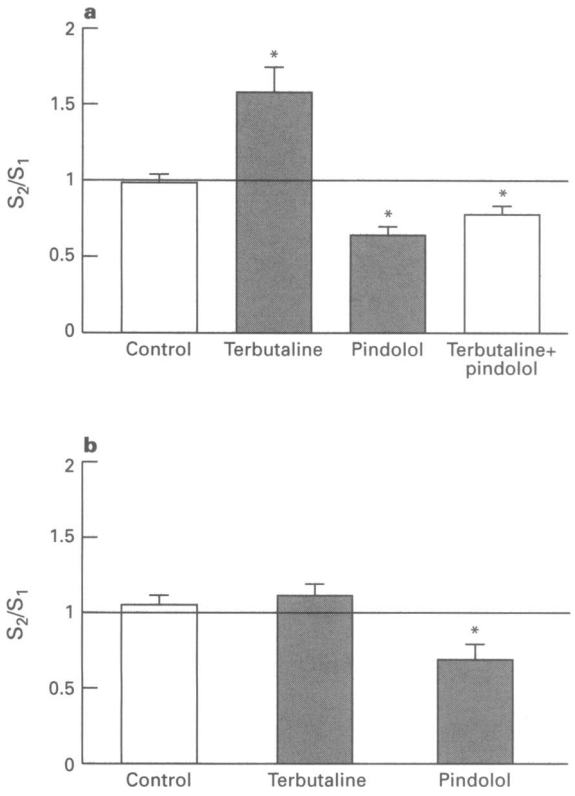

Figure 4 Effect of the β_2-adrenoceptor agonist, terbutaline (1 μmol l^{-1}), the β_1/β_2-adrenoceptor antagonist, pindolol (1 μmol l^{-1}), and combined incubation with both compounds on noradrenaline overflow induced by electrical field stimulation of human incubated atrial tissue in normoxia (a) and anoxia (b). Paired stimulations were performed, with the first stimulation as individual control (S_1) and second stimulation during experimental intervention (S_2). Data are geometric means of S_2/S_1 ratios and 95% confidence intervals ($n=6-8$) $^*P<0.05$.

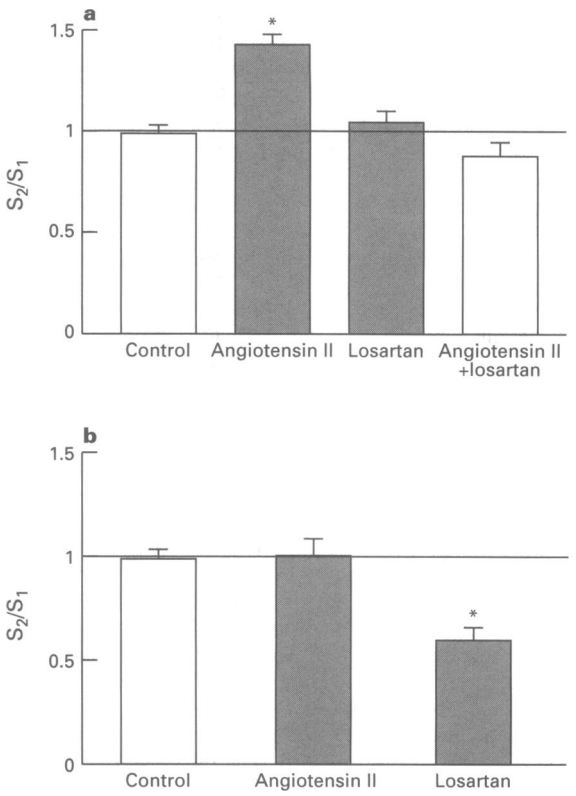

Figure 5 Effect of angiotensin II (0.1 μmol l^{-1}), the AT_1 antagonist, losartan (1 μmol l^{-1}), and combined incubation with both compounds on noradrenaline overflow induced by electrical field stimulation of human incubation atrial tissue in normoxia (a) and anoxia (b). Paired stimulations were performed, with the first stimulation as individual control (S_1) and second stimulation during experimental intervention (S_2). Data are geometric means of S_2/S_1 ratios and 95% confidence intervals ($n=6-8$) $^*P<0.05$.

S_1 ratio of 0.62 (0.49–0.79). The effect of terbutaline (1 μmol l^{-1}) was antagonized by simultaneous incubation with pindolol (1 μmol l^{-1}) (S_2/S_1: 0.75 [0.6–0.93]).

In anoxic buffer, terbutaline lost its facilitatory effect on noradrenaline release (S_2/S_1: 1.07 [0.92–1.24]). The β-adrenoceptor antagonist, pindolol, alone still produced a significant inhibition of noradrenaline release, as indicated in a S_2/S_1 ratio of 0.66 (0.51–0.85).

Presynaptic effects of angiotensin II and angiotensin II-antagonists (Figure 5)

Angiotensin II (0.1 μmol l^{-1}) caused a significant increase of stimulation-induced noradrenaline release under normoxic conditions, as documented by a S_2/S_1 ratio of 1.44 (1.34–1.54). Normoxic incubation with the selective AT_1 antagonist losartan (1 μmol l^{-1}) did not affect noradrenaline release (S_2/S_1: 1.04 [0.91–1.19]). Combined incubation with losartan and angiotensin II abolished the facilitatory effect of angiotensin II (S_2/S_1: 0.88 [0.67–1.08]).

In anoxia, incubation with angiotensin II did not significantly affect stimulation-induced noradrenaline release (S_2/S_1: 1.03 [0.85–1.25]). The AT_1 antagonist, losartan alone, however, significantly inhibited noradrenaline release in anoxia, resulting in an S_2/S_1 ratio of 0.60 (0.46–0.77).

Discussion

It was the unique finding of this investigation that modulation of exocytotic noradrenaline release by presynaptic receptors is fundamentally altered under anoxic conditions: In experimental anoxia, inhibitory receptors such as adenosine receptors and α_2-adrenoceptors lost their attenuating effect on noradrenaline release, whereas the facilitatory β_2-adrenoceptors and angiotensin II receptors preserved their potency and were even maximally stimulated.

Experimental model

As *in vivo* investigations on noradrenaline release in human hearts are difficult for obvious reasons, we used incubated human atrial tissue to study presynaptic modulation of exocytotic noradrenaline release. This *in vitro* model allows conclusions on the human heart which are not hampered by species or organ differences. In contrast to previously published studies of presynaptic modulation using incubated tissue, in which noradrenaline release was detected after prelabelling with tritiated noradrenaline (Matko *et al.*, 1994; Rump *et al.*, 1994), we determined endogenous noradrenaline by h.p.l.c. Thus, we were able to avoid problems associated with the use of tritiated catecholamines such as inhomogeneous labelling of the neuronal tissue with [^{3}H]-noradrenaline (Trendelenburg *et al.*, 1990) or interaction of tritium with the process of release itself (Trendelenburg *et al.*, 1983).

Control experiments performed as paired stimulations without pharmacological intervention proved the reproducibility of noradrenaline release induced by two subsequent stimulations under normoxic as well as under anoxic conditions. Thus, direct evaluation of drug effects was feasible within one preparation. The exocytotic nature of the stimulation-induced noradrenaline release could be documented, because inhibition of fast sodium channels by TTX completely antagonized

noradrenaline release both in normoxia and anoxia (for review: Caterall *et al.*, 1990). All experiments were carried out in the presence of DMI, which additionally excluded a non-exocytotic release of noradrenaline (Schömig, 1990). Notably, exocytotic noradrenaline release was significantly increased after brief anoxia in comparison to normoxia.

Inhibitory receptors

Under normoxic conditions, control of noradrenaline release by α_2-adrenoceptors was in a balanced state, i.e. stimulation of the receptor by endogenous agonists resulted in inhibition of noradrenaline release and antagonism of the receptor unmasked activation by the endogenous noradrenaline. These findings indicated the existence of an autoinhibitory loop modulating noradrenaline release in human atrial tissue via the α_2-adrenoceptor. Our observation was in agreement with a previous study on human papillary muscle, where autoinhibition of noradrenaline release via α_2-adrenoceptors was reported (Matko *et al.*, 1994). In the present study, α_2-adrenoceptor stimulation and antagonism did not affect noradrenaline release under anoxic conditions, although concentrations used of yohimbine and UK 14304 were beyond previously published K_d or EC_{50} values to achieve maximal response (Bohmann *et al.*, 1993; Ordway *et al.*, 1993). This observation indicated a loss of the inhibitory response to α_2-adrenoceptor stimulation during anoxia. A comparable impairment of presynaptic modulation via α_2-adrenoceptors during anoxia has been previously reported in rat isolated hearts (Dart *et al.*, 1984).

The experiments with adenosine documented the presence of inhibitory presynaptic adenosine receptors in normoxic human cardiac tissue. As endogenous adenosine is formed within the heart, the possibility of a transsynaptic modulation mechanism exists in human cardiac tissue. Adenosine receptor antagonism, however, did not reveal receptor activation by endogenous adenosine during normoxia. This finding was in agreement with previous experimental studies in isolated hearts, showing that adenosine formation is not biologically relevant, as long as only brief electrical stimulations are performed (Richardt *et al.*, 1987). Comparable to the finding with the α_2-adrenoceptor, the response to adenosine receptor stimulation was lost during anoxia, i.e. activation of the adenosine receptor by exogenous adenosine failed to inhibit stimulation-induced noradrenaline release. Although significant adenosine formation is known to occur after short periods of ischaemia (Schrader & Gerlach, 1976), functional activation of the receptor by endogenously formed adenosine could be excluded, as incubation with the adenosine antagonist, 8-PT, did not affect noradrenaline release during anoxia. The latter observation was in agreement with an investigation in rat isolated perfused hearts, where no modulation of stimulation-induced noradenaline release via presynaptic adenosine receptors was observed after an ischaemic period of 10 min, despite formation of substantial endogenous adenosine (Richardt *et al.*, 1987).

Facilitatory receptors

In the present study, the existence of presynaptic β_2-adrenoceptors was demonstrated at sympathetic nerve terminals in human atrial tissue. An increase in noradrenaline release by receptor stimulation with exogenous agonist, as well as inhibition of noradrenaline release by antagonism of the β_2-adrenoceptor could be documented. Modulation of noradrenaline release via activation of β_2-adrenoceptors with exogenous agonists was reported in a recent study in human atrial tissue, which has been prelabelled with tritiated noradrenaline (Rump *et al.*, 1994). In contrast to our study, auto-activation of β_2-adrenoceptors by endogenous noradren-aline was not found under their experimental conditions. This difference can be explained by the lower stimulation rate, which we used in our experiments, favouring the plasticity of presynaptic modulation (Stjärne *et al.*, 1992). Under anoxic conditions, an autoactivation of the β_2-adrenoceptor still occurred but no additional receptor stimulation was achieved by exogenous agonists. As supramaximal concentrations of terbutaline were used, endogenous activation of β_2-adrenoceptors appeared to be at a maximum in anoxic atrial tissue.

The presence of presynaptic angiotensin II receptors was indicated by increased noradrenaline release in the presence of exogenous angiotensin II. This stimulating effect could be antagonized by losartan, suggesting an involvement of the AT_1 receptor subclass. Although autoradiographic studies in the human heart revealed the presence of both the AT_1 and AT_2 receptor subtypes (Regitz-Zagrosek *et al.*, 1995), our observation indicating modulation of noradrenaline release via the AT_1 receptor subtype, in keeping with the previously published study by Rump and co-workers (1994). In the present study, there was no evidence for AT_1 receptor activation by endogenously formed angiotensin II during normoxic incubation, as demonstrated by unchanged noradrenaline release after antagonism of the AT_1 receptor. This is well explained by a negligible formation of angiotensin II in the heart during normoxic conditions (Noda *et al.*, 1993). In anoxia, however, the AT_1 receptor was maximally stimulated by endogenous angiotensin II, as documented by an attenuation of noradrenaline release by losartan alone and the lack of effect of exogenous angiotensin II in anoxia.

Mechanisms for differential presynaptic modulation in normoxia and anoxia

Our results suggest that inhibitory receptors such as α_2-adrenoceptors and adenosine are more prone to loss of function by anoxia than facilitatory receptors such as β_2-adrenoceptors and angiotensin II receptors. In a former study in the rat isolated heart, loss of presynaptic inhibition in ischaemia was explained with general impairment of the neuronal function due to progressive degradation of ATP after 10 min of ischaemia (Dart *et al.*, 1984). In the present study, however, selective impairment of inhibitory presynaptic receptors occurred, while exocytotic function was well preserved. The underlying cause for this observation could be the greater susceptibility of inhibitory guanine nucleotide binding proteins to acute myocardial ischaemia (Strasser & Marquetant, 1990; 1995; Niroomand *et al.*, 1992) compared to stimulatory guanine nucleotide binding proteins (Susanni *et al.*, 1989). The second fundamental change in presynaptic receptor regulation during anoxia was an enhanced activity of facilitatory receptors such as β_2-adrenoceptors and AT_1 angiotensin receptors. A reason for this finding could be a maximal activation of these receptors by endogenous transmitters during anoxia. In fact, a substantial increase of angiotensin II formation during ischaemia has been reported (Noda *et al.*, 1993). The increase of local angiotensin II concentrations at the receptor site may lead to maximal activation of the presynaptic AT_1 receptor by endogenous angiotensin II in anoxia.

Regarding presynaptic β_2-adrenoceptors, a moderate increase of noradrenaline release during anoxia as demonstrated in a previous study from our group (Seyfarth *et al.*, 1993), cannot completely explain the lacking effect of individual exogenous agonist administration at the β_2-adrenoceptors. Therefore, anoxia may also lead to an increased sensitivity of receptors or its postreceptor signal transduction. Moreover, the increased noradrenaline release during anoxia might not be solely explained by the observed differential presynaptic modulation. Multiple changes during anoxia, such as altered intracellular calcium concentration, functional status of the exocytotic machinery, noradrenaline metabolism and increased extra-neuronal uptake 2 could account for this. In fact, it was previously shown in isolated chromaffin cells that the exocytotic machinery develops an increased sensitivity to calcium in anoxia (Itoh *et al.*, 1994). Consequently, identical en-

dogenous noradrenaline concentrations would lead to an augmented response to receptor stimulation due to increased efficiency of subcellular pathways.

The lack of presynaptic modulation of inhibitory neurotransmitters released from other autonomic nerves, such as acetylcholine, could also contribute to enhanced noradrenaline release during anoxia. Acetylcholine plays a significant role in modulation of noradrenaline releases in human cardiac tissue in normoxia (Matko *et al.*, 1994). Du and colleagues (1990) previously described the failure of the inhibitory modulation of acetylcholine on noradrenaline release even after only 1 min of stop flow ischaemia in rat hearts. This is in accordance with our findings with an early loss of inhibitory presynaptic receptor function during anoxia.

The function of uptake carriers does not play a major role in altered presynaptic modulation of noradrenaline release during anoxia. In our study, the neuronal uptake 1 carrier was sufficiently blocked by desipramine, which was present in all experiments during the whole time course after the equilibration period. Extraneuronal uptake 2 is of minor relevance in man. This was previously shown in scintigraphic investigations in human subjects with a seletive tracer for uptake 2 (Carr *et al.*, 1979) and by a human volunteer study with tritiated noradrenaline with blockade of extraneuronal uptake 2 by cortisol (Esler *et al.*, 1981). Changes in the relative uptake 2 function during anoxia and ischaemia are not likely, as previous studies in rat isolated hearts revealed insignificant changes in exocytotic noradrenaline release during ischaemia during uptake 2 inhibition (Dart *et al.*, 1984).

Moreover, different activity of monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) during anoxia, could also contribute to altered catecholamine kinetics during anoxia. The strict oxygen-dependence of oxidative deamination of noradrenaline by MAO resulted in a decrease in DOPEG release and relative increase of noradrenaline release in anoxia (Schömig *et al.*, 1987). However, biochemical and physiological changes during early anoxia or ischaemia are extremely complex and this study is focusing on the alteration of presynaptic modulation of noradrenaline release during anoxia. Therefore, future experiments with a well characterized model with easier access than human tissue such as rat isolated hearts are required to clarify these issues.

Conclusion

In the present study, changes in sympathetic modulation in human atrial tissue in anoxia on the level of presynaptic receptors could be demonstrated. Inhibitory regulation via α_2-adrenoceptors and A_1 adenosine receptors in normoxia is changed to facilitatory regulation via β_2-adrenoceptors and AT_1 receptors in anoxia. The concomitantly increased exocytotic noradrenaline release during early anoxia could therefore by partly explained by this completely different presynaptic modulation. An increase in noradrenaline release together with increased sensitivity of excitatory postsynaptic receptors (Butterfield & Chess-Williams, 1990; Strasser & Marquetant, 1990; Strasser *et al.*, 1992; Niroomand *et al.*, 1992; 1995) could be a further reason for the deleterious effects of sympathetic activation in early ischaemia. As clinically effective pharmacological tools for the treatment of ischaemic heart disease, such as angiotensin- and β-adrenoceptor blocking agents, exert their effect in part by presynaptic modulation of noradrenaline release (Brodde, 1988; Pfeffer *et al.*, 1988), inhibition of noradrenaline release during anoxia may contribute to the beneficial effects of β-adrenoceptor antagonists and angiotensin inhibiting agents in the treatment of ischaemic heart disease.

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