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SHORT COMMUNICATION

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Presynaptic imidazoline receptors and α_2-adrenoceptors in the human heart: discrimination by clonidine and moxonidine

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Abstract The involvement of presynaptic α_2-autoreceptors and imidazoline receptors in the modulation of noradrenaline release was investigated in strips from human atrial appendages preincubated with [^{3}H]noradrenaline and superfused with medium containing desipramine and corticosterone. Electrical impulses were applied transmurally at 2 Hz. The imidazoline derivatives moxonidine and clonidine reduced the evoked tritium overflow in a concentration-dependent manner. Moxonidine was 18-fold more potent than clonidine. The concentration-response curve for moxonidine, but not for clonidine was shifted to the right by the α_2-adrenoceptor antagonist rauwolscine. The apparent pA_2 value of rauwolscine against moxonidine was 8.41. An inhibitory effect was also observed for the imidazoline derivative BDF 6143 (4-chloro-2-(2-imidazolin-2-ylamino)-isoindoline), a mixed α_2-adrenoceptor antagonist/imidazoline receptor agonist; BDF 6143 was about 2-fold more potent than clonidine. Rauwolscine (1 μM) did not substantially shift the concentration-response curve of BDF 6143.

It is concluded that noradrenaline release in the human atrium is inhibited not only via presynaptic α_2-autoreceptors but also via presynaptic non-I_1, non-I_2 imidazoline receptors. The failure of rauwolscine to antagonize the inhibitory effect of clonidine suggests that clonidine preferentially stimulates the presynaptic imidazoline receptors; the α_2-adrenoceptor component of its action is probably suppressed by an inhibitory interaction between the two receptors. In contrast, moxonidine acts via presynaptic α_2-autoreceptors, leaving the presynaptic imidazoline receptor unaffected.

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Introduction

Inhibition of the release of noradrenaline from sympathetic neurones is a typical response to the imidazoline derivatives clonidine and moxonidine (Starke 1987; Schlicker et al. 1990). Originally, this effect was thought to be mediated exclusively by presynaptic α_2-adrenoceptors (for review, see Starke 1977, 1987). Meanwhile, presynaptic imidazoline receptors mediating an inhibition of noradrenaline release have been identified in the rabbit pulmonary artery, aorta and heart (Göthert and Molderings 1991; Molderings et al. 1991; Fuder and Schwarz 1993) as well as in the human pulmonary artery (Göthert et al. 1995). These receptors do not belong to the imidazoline I_1 or I_2 receptor classes (Molderings and Göthert 1995).

The following pharmacological features of presynaptic imidazoline receptors were found in the rabbit aorta and pulmonary artery. The imidazoline derivative cirazoline exhibited no effect at α_2-autoreceptors, but behaved as a selective presynaptic imidazoline receptor agonist. BDF 6143 proved to be a mixed imidazoline receptor agonist/α_2-adrenoceptor antagonist. Rauwolscine was a highly potent antagonist at the α_2-autoreceptors, but an at best weak antagonist at imidazoline receptors. Clonidine acted as an agonist at both receptors. The preferential I_1 receptor agonist moxonidine (Ernsberger et al. 1992; Haxhiu et al. 1992) did not activate the presynaptic non-I_1, non-I_2 imidazoline receptor but was an agonist at α_2-adrenoceptors. Evidence was also obtained that the presynaptic α_2-autoreceptor and the presynaptic imidazoline receptor interacted with each other: when the imidazoline receptor was activated, the effect of an α_2-adrenoceptor agonist was attenuated (Göthert et al. 1995). Such an interaction was suggested to be the reason for the relatively low potency of clonidine in inhibiting noradrenaline release in the rabbit pulmonary artery and aorta and for the only very weak po-

tency of rauwolscine in antagonizing this effect (Molderings et al. 1991, Molderings and Göthert 1995): preferential stimulation of the imidazoline receptor by the mixed imidazoline/α_2-receptor agonist clonidine suppressed its simultaneous activating effect on the α_2 autoreceptor (Göthert et al. 1995).

The first aim of the present study was to examine whether inhibitory imidazoline receptors also occur at the sympathetic axon terminals of human atrial appendages where α_2-autoreceptors were recently identified by Rump et al. (1995a,b). Furthermore we investigated whether the effects of moxonidine and clonidine in this tissue resembled those in the rabbit pulmonary artery.

Methods

Segments of macroscopically normal human right atrial appendages were obtained from normotensive 35 to 70 year old male or female patients undergoing open heart surgery. The atrial appendages were routinely removed for cannulation of the right atria. The patients were not treated with adrenoceptor agonists or antagonists or with drugs influencing the storage or release of noradrenaline. After premedication with pethidine and promethazine, the patients were anaesthetized (both induction and maintenance) with flunitrazepam and fentanyl. During maintenance of anaesthesia, they were ventilated with mixtures of oxygen and air. Pancuronium was administered for neuromuscular blockade. The study was approved by the local ethics committee.

The segments were cut into strips of about 3×15 mm. These strips were incubated for 60 min in 1.5 ml physiological salt solution (37°C) containing (-)-(ring-2,5,6-^{3}H)-noradrenaline 0.2 μM (specific activity 57.3 Ci/mmol). Subsequently, they were mounted vertically in an organ bath (tension adjusted to 2.0 g) between two parallel platinum electrodes and superfused with [^{3}H]noradrenaline-free physiological salt solution of 37°C at a rate of 2 ml/min. The composition of the solution was (mM): NaCl 118, NaH_2PO_4 1.2, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, glucose 11.0, ascorbic acid 0.3, Na_2EDTA 0.03 (aerated with 95% O_2 and 5% CO_2). Throughout superfusion this solution contained desipramine 0.6 μM and corticosterone 40 μM for blockade of the neuronal and extraneuronal uptake of noradrenaline, respectively.

The superfusate was collected in 4-min fractions. Five (in a few experiments three) 3-min periods of transmural electrical stimulation (2 Hz; rectangular pulses of 200 mA and 0.3 ms) were applied to each strip after 94 (S_1), 126 (S_2), 158 (S_3), 190 (S_4) and 222 (S_5) min of superfusion. At the end of superfusion the strips were solubilized with Soluene (Packard), and the radioactivity in the superfusate samples and blood vessels was determined by liquid scintillation counting.

The agonists under investigation were applied at concentrations increasing by a factor of 10 from 12 min before until 20 min after the onset of S_3, S_4 and S_5, respectively. Rauwolscine was present in the superfusion fluid from 14 min before S_1 until the end of the experiments. Separate control experiments (no agonist) were carried out for each agonist (clonidine, moxonidine and BDF 6143) in the absence as well as in the presence of rauwolscine.

Tritium efflux was calculated as the fraction of tritium present in the strip at the onset of the respective collection period. Basal tritium efflux was expressed as the ratio of the fractional rate during the collection period immediately before S_3, S_4 or S_5 (i.e., t_3, t_4, t_5) over that immediately before S_2 (t_2). Stimulation-evoked tritium overflow was calculated by subtraction of the basal efflux from the total efflux during the 16 min subsequent to the onset of stimulation; basal efflux was assumed to decrease linearly from the collection period before to that 16–20 min after onset of stimulation. Evoked tritium overflow was calculated as a percentage of tissue tritium at the onset of stimulation, and the ratios of the overflow evoked by S_3, S_4 or S_5 over that evoked by S_2 were determined.

Results are given as means ± SEM of data obtained in n tissue strips. Student's *t*-test was used for comparison of the mean values. As an estimate of agonist potency, $pIC_{30\%}$ values (negative logarithm of the concentration producing 30% inhibition of evoked tritium overflow) were determined by interpolation from the nearest points of the concentration-response curves. Apparent pA_2 values were determined according to formula (4) of Furchgott (1972).

Drugs used were (–)-[ring-2, 5, 6-^{3}H]-noradrenaline (spec. activity 57.3 Ci/mmol; New England Nuclear, Dreieich, Germany); desipramine hydrochloride (Ciba-Geigy, Wehr, Germany); corticosterone (Sigma, München, Germany); BDF 6143 (4-chloro-2-(2-imidazolin-2-ylamino)-isoindoline)fumarate, moxonidine (Beiersdorf, Hamburg, FRG); clonidine hydrochloride (Boehringer, Ingelheim, FRG); rauwolscine hydrochloride (Roth, Karlsruhe, FRG). Drugs were dissolved in saline or water with the following exception: corticosterone was dissolved in 1,2-propandiol and the stock solution was further diluted with saline.

Results

Basal tritium efflux

When neither rauwolscine nor another test drug was administered at S_1-S_5, basal tritium efflux during t_2 from segments of atrial appendages preincubated with [^{3}H]noradrenaline was 2.7 ± 0.4 nCi/min (n = 7), corresponding to a fractional rate of efflux of 0.0007 ± 0.0001 min^{-1}. Basal efflux decreased with time, as reflected by t_n/t_2 ratios, which declined from t_3/t_2 (0.86 ± 0.02) to t_5/t_2 (0.77 ± 0.02). In the presence of rauwolscine 1 μM (from 14 min before S_1 until the end of the experiment), basal tritium efflux did not significantly differ from that in the absence of the drug. Clonidine, moxonidine and BDF 6143 did not modify basal tritium efflux.

Electrically evoked tritium overflow in control experiments

When neither rauwolscine nor another test drug was administered at S_1–S_5, the tritium overflow evoked by S_2 amounted to 17.2 ± 3.9 nCi (corresponding to 1.01 ± 0.24% of tissue tritium; n = 7) and remained approximately constant throughout the experiments, as reflected by S_3/S_2 to S_5/S_2 values close to unity (between 0.89 ± 0.04 and 1.04 ± 0.02). Rauwolscine 1 μM (present from 14 min before S_1 until the end of the experiments; n = 6) increased the electrically evoked tritium overflow (by 68 ± 41% at S_2) but, possibly due to the considerable scatter, the difference was not significant. In the presence of rauwolscine, tritium overflow gradually decreased from stimulation period to stimulation period (S_3/S_2: 0.91 ± 0.09; S_5/S_2: 0.72 ± 0.07).

Effects of clonidine, moxonidine and BDF 6143 on electrically evoked tritium overflow

In the absence of rauwolscine, clonidine caused a concentration-dependent inhibition of the electrically evoked

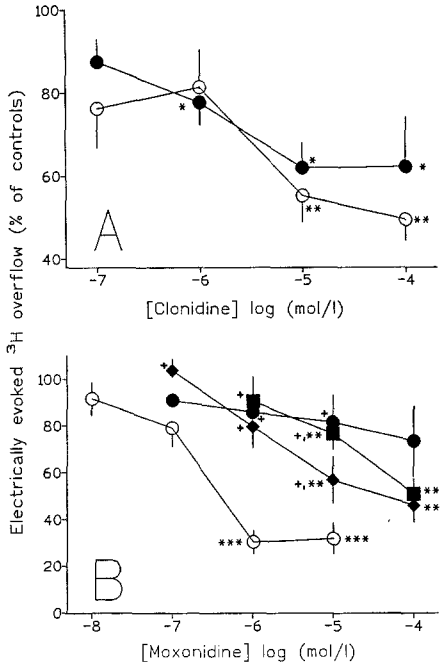

Fig. 1 Effect of clonidine (**A**) and moxonidine (**B**) on electrically evoked tritium overflow from segments of human atrial appendages and interaction with rauwolscine. Experiments without rauwolscine (○); experiments in the presence of rauwolscine 0.1 μM (◆), 0.3 μM (■) or 1 μM (●) from 14 min before S_1 until the end of the experiments. Five 3-min periods of transmural electrical stimulation (2 Hz; S_1-S_5) were applied after 94, 126, 158, 190 and 222 min of superfusion. Clonidine or moxonidine was added at increasing concentrations from 12 min before until 20 min after onset of S_3, S_4 or S_5. Ordinate, S_3/S_2, S_4/S_2 and S_5/S_2 overflow ratios, expressed as percentage of ratios in respective control experiments without clonidine and moxonidine administration. Means ± SEM from 5–11 tissue strips. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (compared with the corresponding controls); + $P<0.05$ (compared with the effect of the same moxonidine concentration in the absence of rauwolscine; the effects of clonidine in the absence and presence of rauwolscine were not significantly different)

overflow of tritium (Fig. 1A; $pIC_{30\%}$ value: 5.57; 100 μM clonidine produced an inhibition by about 50%). Rauwolscine 1 μmol/l failed to influence the concentration-response curve for clonidine (Fig. 1A; $pIC_{30\%}$ value of clonidine in the presence of rauwolscine 1 μM: 5.50).

Moxonidine also reduced the electrically evoked tritium overflow in the absence of rauwolscine (Fig. 1B; $pIC_{30\%}$: 6.82). The concentration-response curve for moxonidine was shifted to the right by rauwolscine in a concentration-dependent manner (Fig. 1B). The mean apparent pA_2 value for rauwolscine determined at the level of $IC_{30\%}$ of moxonidine was 8.41 (individual pA_2 values at rauwolscine 0.1 μM and 0.3 μM were 8.22 and 8.60, respectively). Rauwolscine 1 μM abolished the moxonidine-induced inhibition of tritium overflow.

In the absence of rauwolscine, an inhibitory effect was also obtained with BDF 6143 (Fig. 2; $pIC_{30\%}$ 5.74). Rauwolscine 1 μM tended to shift the concentration-response curve for BDF 6143 to the left (Fig. 2; $pIC_{30\%}$ 6.20).

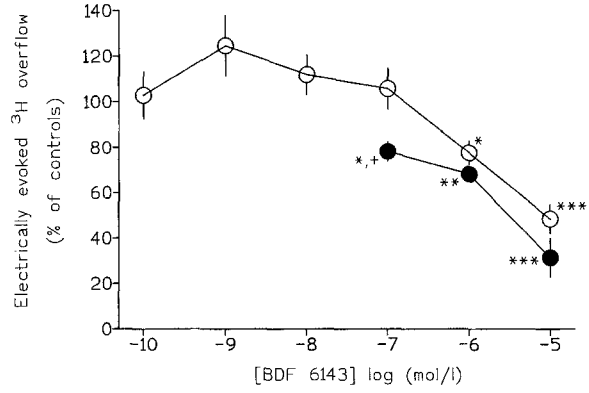

Fig. 2 Effect of BDF 6143 on electrically evoked tritium overflow from segments of human atrial appendages and interaction with rauwolscine. Experiments without rauwolscine (○); experiments in the presence of rauwolscine 1 μM (●) from 3 min before S_1 until the end of the experiments. For details see legend to Fig. 1. Ordinate, S_3/S_2, S_4/S_2 and S_5/S_2 overflow ratios, expressed as percentage of ratios in respective control experiments without BDF 6143 administration. Means ± SEM from 5–8 tissue strips. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (compared with the corresponding controls); + $P<0.05$ (compared with the effect of the same BDF 6143 concentration in the absence of rauwolscine)

Discussion

The purpose of the study was to investigate whether inhibitory presynaptic imidazoline receptors are present on the sympathetic axon terminals of human cardiac tissue in addition to α_2-autoreceptors and whether moxonidine and clonidine are able to discriminate between these presynaptic receptors. Under the present conditions (blockade of neuronal and extraneuronal uptake) the electrically evoked overflow of tritium was considered to be an estimate of the action-potential-induced release of noradrenaline from the sympathetic neurones.

Two findings indicate the occurrence of presynaptic imidazoline receptors.

Firstly, after blockade of α_2-adrenoceptors by a high concentration of rauwolscine, the mixed α_2-adrenoceptor antagonist/imidazoline receptor agonist BDF 6143 inhibited noradrenaline release (as in the rabbit pulmonary artery and aorta: Docherty et al. 1982; Göthert and Molderings 1991). The finding that BDF 6143 tended to be less potent in the absence of rauwolscine can be explained by its α_2-adrenoceptor blocking property: in the absence of rauwolscine, α_2-autoreceptor blockade by BDF 6143 tends to increase the release of noradrenaline and thus attenuates the imidazoline receptor-mediated inhibition; no such attenuation occurs when noradrenaline release is already disinhibited by preapplication of rauwolscine.

Secondly, clonidine, a preferential agonist at presynaptic non-I_1, non-I_2 imidazoline receptors (as compared to its potency at presynaptic α_2-adrenoceptors in the rabbit pulmonary artery and aorta; Molderings et al. 1991; Molderings and Göthert 1995), inhibited the release of noradrenaline in a manner resistant to antagonism by 1 μM rauwolscine (in rabbit pulmonary artery and aorta, rauwolscine was a weak antagonist against clonidine: apparent pA_2

6.77 and 6.55, respectively; Molderings and Göthert 1995). In contrast, at this high concentration rauwolscine abolished the inhibitory effect of moxonidine, an α_2-adrenoceptor agonist devoid of an agonist effect at the presynaptic non-I_1, non-I_2 imidazoline receptors in rabbit vascular tissue (Molderings et al. 1991; Göthert et al. 1995). At 0.1 and 0.3 μM rauwolscine, the concentration-response curve of moxonidine was shifted to the right, yielding a mean apparent pA_2 value of rauwolscine of 8.4 consistent with the values of 8.6 and 9.1 that Rump et al. (1995a,b) determined for human atrial α_2-adrenoceptors against the agonist UK14304. The results with moxonidine and clonidine are in agreement with the view that these drugs exhibit similar properties at the presynaptic α_2-adrenoceptors and imidazoline receptors in human cardiac tissue on the one hand and in the rabbit vasculature on the other: moxonidine is a selective α_2-autoreceptor agonist, whereas clonidine preferentially activates presynaptic imidazoline receptors.

As in rabbit blood vessels, a plausible explanation of the preference of clonidine for presynaptic imidazoline (over α_2) receptors is an interaction between the two, in that imidazoline receptor activation suppresses the stimulation of α_2-autoreceptors (Göthert et al. 1995). This inhibitory interaction may also be one reason for the 18 times lower potency of clonidine than moxonidine in inhibiting noradrenaline release although in radioligand binding studies clonidine exhibited higher affinity for α_2-adrenoceptors than moxonidine (Uhlén et al. 1995; Munk et al. 1996). However, additional unknown mechanisms may contribute to the low potency of clonidine and its apparent inability to activate the α_2-autoreceptors.

Taken together, the present findings provide evidence for the existence of inhibitory presynaptic imidazoline receptors, in addition to the α_2-autoreceptors, on the postganglionic sympathetic nerve terminals in human atrial appendages. The human receptors agree with rabbit vascular presynaptic imidazoline receptors in that they are activated by BDF 6143 and clonidine but not by moxonidine. Both can be classified as non-I_1, non-I_2 (Göthert et al. 1995; Molderings and Göthert 1995).

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