Comparison of the positive inotropic effects of serotonin, histamine, angiotensin II, endothelin and isoprenaline in the isolated human right atrium

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Summary. The receptor systems through which serotonin (5-HT), histamine, angiotensin II and endothelin increase the force of contraction were studied in isolated right atria from patients without apparent heart failure.

All agonists increased the atrial force of contraction in a concentration-dependent manner; maximal effects, however, were significantly less than those evoked by isoprenaline or Ca2+. 5-HT and histamine, but not angiotensin II and endothelin, activated adenylate cyclase, whereas endothelin and angiotensin II stimulated inositol phosphate generation. Experiments with subtype-selective antagonists revealed that histamine effects were mediated by H2-receptors (sensitive to ranitidine), 5-HT-effects by 5-HT4-receptors (sensitive to SDZ 205-557) and angiotensin II effects by AT1-receptors (sensitive to losartan).

We conclude that in human right atria the force of contraction can be increased by cyclic AMP-dependent (histamine, 5-HT) and -independent (angiotensin II, endothelin) pathways. Compared to β-adrenoceptors, however, all other receptor systems increase the force of contraction only submaximally indicating that the β-adrenoceptor pathway is the most important physiological mechanism to regulate force of contraction and/or heart rate in the human heart.

Key words: Serotonin – Histamine – Angiotensin II – Endothelin – Cardiac adenylate cyclase – Cardiac inositol phosphates – Human heart

Introduction

Catecholamines acting through β1- and β2-adrenoceptors mediate positive inotropic and chronotropic effects in vitro and in vivo in the human heart (Jones et al. 1989; McDevitt 1989; Bristow et al. 1990; Brodde 1991). Recent evidence, however, suggests that other receptor systems that can increase contractility and/or heart rate may also exist in the human heart. Among these are α1-adrenoceptors (Böhm et al. 1988; Bristow et al. 1988; Kohl et al. 1989; Steinfeld et al. 1992) as well as receptors for histamine (Bristow et al. 1982; Baumgaertner et al. 1983), serotonin (5-HT) (Kaumann et al. 1990, 1991; Sanders and Kaumann 1992), angiotensin II (Schomisch Moravec et al. 1990), and endothelin (Schomisch Moravec et al. 1989). The aim of this study was to compare the positive inotropic efficacy of several of these receptor systems with the β-adrenoceptor system, and to investigate their mechanism of action. For this purpose we assessed, on isolated electrically driven right atria from patients without apparent heart failure undergoing coronary artery bypass grafting, i) the positive inotropic effects of 5-HT, histamine, angiotensin II and endothelin in relation to that of isoprenaline; ii) the receptor subtype involved in the positive inotropic effects of 5-HT, histamine and angiotensin II, and iii) the effects of the agonists on right atrial adenylate cyclase activity and inositol phosphate generation.

Materials and methods

Patients. Right atrial appendages were obtained from 86 patients (26 female, 60 male; mean age: 55.3±3.1 [range: 33–75] years) without apparent heart failure undergoing coronary artery bypass grafting. None of the patients had been treated with sympathomimetic drugs or β-adrenoceptor antagonists for at least three weeks before operation. However, patients had received nitrates (n = 78), calcium antagonists (75), diuretics (14) and occasionally digitalis glycosides (3) and angiotensin-converting enzyme (ACE) inhibitors (4), alone or in combination. We avoided angiotension II experiments in atria from patients who had been treated with ACE-inhibitors.

Premedication consisted of 2 mg flunitrazepam given orally on the evening before and on the morning of surgery. The operation was carried out under modified neurolept anaesthesia with flunitrazepam and fentanyl; controlled ventilation was performed with a 1:1 mixture of oxygen and N2O with addition of isoflurane up to 1.0% (v/v). Pancuronium was used as muscle relaxant. In all patients right atrial appendages were removed during installation of the cardiopulmonary bypass. Immediately after excision all specimens were placed in sealed...

Preparation of tissues and organ-bath experiments. Preparation of atrial tissue usually began within 5–20 minutes of surgical removal in oxygenated Krebs-Henseleit solution at room temperature. The right atrial appendages were dissected to yield trabecular strips (4–5 mm in length and 1 mm or less in diameter) without endocardial damage and with fibres running parallel to the length.

The preparations were mounted in 25 ml organ bath containing Krebs-Henseleit solution of the following composition (mmol/l): NaCl 119; KCl 4.8; CaCl2 2.5; MgSO4 1.2; KH2PO4 1.2; NaHCO3 24.9; glucose 10; EDTA 0.1; ascorbic acid 0.057; equilibrated with carbogen at 37 °C. Myocardial strips were electrically stimulated by square wave pulses about 20% above threshold (3–8 V; mean: 4 V) at a frequency of stimulation of 1 Hz (Stimulator II, Hugo Sachs Elektronik, March-Hugstetten, FRG). The developed tension of the preparation (maintained under a resting tension of 4.9 mm) was recorded via a strain gauge on a Hellige recorder (Hellige, Freiburg, FRG). Preparations were allowed to equilibrate for at least 1 h in Krebs-Henseleit solution that contained 300 mmol/l of the highly selective β1-adrenoceptor antagonist CGP 20712 A (Dooley et al. 1986) in order to block positive inotropic effects of endogenous noradrenaline that may be released and, in the human right atrium, acts solely via β1-adrenoceptor stimulation (for references see Brodde 1991). Thereafter cumulative concentration-response curves for 5-HT, histamine, angiotensin II and endothelin were determined as detailed elsewhere (Motomura et al. 1990). For experiments with the 5-HT1-receptor antagonist SDZ 205-557, the 5-HT1-receptor antagonist IC50 930, the H1-histamine receptor antagonist diphenhydramine, the H2-histamine receptor antagonist ranitidine and the angiotensin II-AT1-receptor antagonist losartan (formerly known as DuP 753), preparations were equilibrated for at least 1 h with the antagonists. Each muscle preparation was used for one concentration-response curve only to exclude desensitization phenomena.

When the concentration-response curves to the agonists had reached a plateau, a high concentration of isoprenaline (10–100 μmol/l) was added to determine the maximum of force of contraction. After washout, a concentration-response curve for calcium (1.8–12.6 mmol/l) was determined at the end of each experiment.

Adenylate cyclase activity. Adenylate cyclase activity was assessed by the method of Salomon et al. (1974), as recently described (Brown et al. 1992). Briefly, membranes (20–40 μg of protein) were incubated for 10 min at 37°C in a final volume of 100 μl containing 40 mmol/l HEPES buffer pH 7.4, 5 mmol/l MgCl2, 1 mmol/l EDTA, 10 mmol/l GTP, 500 μmol/l [α-32P]-ATP, 100 μmol/l cyclic AMP and an ATP regenerating system (5 mmol/l phosphate and 50 units/ml creatine phosphokinase) with or without the agonists. The reaction was stopped by addition of 0.8 ml of 50 mmol/l Tris-HCl buffer pH 7.4 containing 40 mmol/l ATP and 1.4 mmol/l cyclic AMP. [3H]-Cyclic AMP (5000–10 000 cpm) was added to monitor the recovery of [3H]-cyclic AMP (Brown et al. 1992).

The protein content of the samples was determined by the method of Bradford (1976) using bovine immunoglobulin G as a standard.

Inositol phosphate determination. Right atrial tissue was chopped into 250×250 μm slices with a McIlwain tissue chopper (Bachofner, Reutlingen, FRG). The slices were resuspended in Krebs-Henseleit buffer of the following composition (mmol/l): NaCl 108; KCl 4.7; CaCl2 1.3; MgSO4 1.2; KH2PO4 1.2; NaHCO3 24.9; glucose 11; EDTA 0.001. The buffer was supplemented with 10 mmol/l LiCl to block inositol phosphate degradation, 20 μmol/l cocaline, 2 μmol/l adenosine deaminase to remove adenosine possibly liberated during tissue chopping, and 10 μmol/l propranolol. The slices were placed into fresh buffer twice during a 30 min incubation at 37°C. The incubation was continued for another 60 min following the addition of 100 μCi of [3H]-myoinositol/5 ml of suspension containing 200–300 mg of slices. Then, 300 μl aliquotes of the suspension (corresponding to 12–18 mg slice wet weight) were pipetted into flat bottom polystyrene tubes under gentle swirling and agonists and antagonists were added to yield a final volume of 330 μl. After 45 min in the absence or presence of agonists and/or antagonists, the incubation was stopped by addition of 330 μl icecold methanol and 660 μl chloroform. The mixture was vigorously vortexed twice and thereafter the phases were separated by centrifugation at 12 000 g for 10 min at 4°C. Aliquots (450 μl) of the upper phase were placed on Dowex AG 1-X8 columns (200 mg/column). Free inositol was eluted twice, each time with 5 ml H2O followed by 5 ml of 60 mmol/l ammonium formate. Total inositol phosphates were eluted by addition of 1 ml of 1 mol/l ammonium formate dissolved in 100 mmol/l formic acid. Eight ml of scintillator (Quickszint, Zinsser, Maidenhead, Berks, UK) were added to each sample. Following vigorous shaking the samples were counted in a scintillation counter (Beckman LS 9000, Beckman Instruments, Fullerton, Calif., USA) at 42% efficiency.

Statistical evaluation. The experimental data are given as means±SEM of n experiments. The significance of differences between two groups was estimated by unpaired two-tailed Student's t-test. A P-value smaller than 0.05 was considered to be significant. PD2-values were determined as described by Van Rossum (1963), PA2-values as described by Arunlakshana and Schild (1959). Kp-values for inhibition of 5-HT- or histamine-stimulated adenylate cyclase activity by IC50 205-930 and SDZ 205-557 and ranitidine, respectively, were determined according to the equation of Cheng and Prusoff (1973): Kp = IC50 (SI/EC50 + 1) where IC50 = concentration of antagonists necessary to inhibit 5-HT- or histamine-induced stimulation by 50%; [S] = concentration of 5-HT (100 μmol/l) or histamine (100 μmol/l) in the assay and EC50 = concentration of 5-HT and histamine, respectively, necessary to produce 50% of maximal stimulation of adenylate cyclase.

Drugs used. (-)-Isoprenaline sulfate, histamine dihydrochloride, human angiotensin II acetate were purchased from SIGMA (Munich, FRG), human endothelin I from Saxon Biochemicals (Hanover, FRG), 5-hydroxytryptamine creatinine sulfate from SERVA (Heidelberg, FRG), and ranitidine hydrochloride from Research Biochemicals (Köln, FRG). Diphenhydramine hydrochloride was a gift from Heumann (Nürnberg, FRG), ICS 205-930 (([3α,τropanyl]-1H-indole-3-carboxylic acid ester hydrochloride) and SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)-ethyl ester hydrochloride) from Sandoz (Basel, Switzerland), losartan (DuP 753) from DuPont Merck (Wilmington, DE, USA), and CGP 20712A (1-[3-(3-carbamoyl-4-hy- droxylphenoxyl)ethylamino]-3-[4-[(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxyl]2-propanol methanesulfonate) from Ciba-Geigy (Basel, Switzerland). [α-32P]-ATP (specific activity 30 Ci/mmol), [3H]-cyclic AMP (specific activity 44.5 Ci/mmol) and [3H]-myo-inositol (specific activity 80–120 Ci/mmol) were obtained from New England Nuclear (Dreieich, FRG).

Results

Positive inotropic effects

Serotonin. 5-HT (3×10−9−10−4 mol/l) caused concentration-dependent increases in force of contraction (Fig. 1); the PD2-value amounted to 6.53±0.11 (n = 23). When saturating concentrations of 5-HT had induced maximal increases in force of contraction, addition of 10–100 μmol/l isoprenaline to the organ bath produced further increases up to a maximum that was not significantly different from that evoked by saturating concentrations of Ca2+. Thus, 5-HT caused maximal increases in force of contraction that were only 56.9±5.1% (n = 23, P<0.01 vs. isoprenaline = 100%) of those brought about by isoprenaline or Ca2+ (Fig. 1). The 5-HT1-receptor antagonist SDZ 205-557 (3×10−8 to 3×10−7 mol/l) (Buchheit et al. 1991, 1992) and the 5-HT3-receptor antagonist ICS 205-930 (3×10−7 to 7×10−8 mol/l) (Richardson et al. 1985) shifted the con-