Naunyn-Schmiedeberg's Arch Pharmacol (1992) 346:482–485

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Positive inotropic response to 5-HT in human atrial but not in ventricular heart muscle

Ulrich Jahnel, Johanna Rupp, Rudolf Ertl, and Hermann Nawrath

Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Strasse 67, W-6500 Mainz, Federal Republic of Germany

Received June 2, 1992/Accepted July 17, 1992

Summary. The effects of 5-hydroxytryptamine (5-HT) on force of contraction (F_C), action potential (AP) and calcium current (I_{Ca}) were studied in human right atrial and left ventricular heart muscle. 5-HT exerted a concentration-dependent increase in F_C in multicellular atrial preparations; the EC_{50} was approximately 3×10^{-7} mol/l. Maximal increases in F_C (252±58% of control values; means ± SEM, $n=6$) were obtained at 5-HT 10^{-5} mol/l. At this concentration, I_{Ca} was increased four- to sevenfold in enzymatically isolated atrial myocytes. In contrast, ventricular preparations did not respond to 5-HT; F_C, AP and I_{Ca} remained unaffected. In the same preparations, F_C was increased by isoprenaline three- to fourfold. These results confirm the observation that 5-HT induces a positive inotropic effect in the human atrium, possibly mediated by activation of the adenylyl cyclase – cyclic AMP system. Our study demonstrates, however, the complete lack of functional 5-HT receptors, with respect to changes in F_C, in the human ventricle. Since the positive inotropic effect of 5-HT in the human heart is obviously restricted to the atrium, our findings question the concept of developing 5-HT receptor agonists for the treatment of heart failure.

Key words: Human heart – Force of contraction – 5-Hydroxytryptamine – Ca^{2+} current

Introduction

5-Hydroxytryptamine (5-HT) exerts complex effects on the cardiovascular system which have been ascribed to the activation of at least four subtypes of 5-HT receptors (for recent reviews see Saxena and Villalón 1990, 1991). 5-HT_4 receptors mediate the positive inotropic response to 5-HT in human isolated atrial preparations (Kaumann et al. 1990, 1991; Sanders and Kaumann 1992). A recent study in human atrial myocytes has shown that the inotropic effect of 5-HT is related to an increase in the calcium current (I_{Ca}) via an elevation of intracellular cyclic AMP levels and stimulation of cAMP-dependent protein kinase (Ouadid et al. 1992). It has been suggested that the development of 5-HT_4 agonists may offer new perspectives in the treatment of heart failure (Saxena and Villalón 1991; Ouadid et al. 1992). Essential to this goal would be a corresponding inotropic response to 5-HT in the ventricle. We have therefore investigated the effects of 5-HT on force of contraction (F_C), action potential (AP) and I_{Ca} in human isolated ventricular heart muscle. For comparison, atrial tissue was also studied.

Correspondence to: H. Nawrath at the above address

Methods

Preparations. Human papillary and atrial heart muscle preparations were obtained from patients undergoing open heart surgery. The patients (n = 9; 5 female, 4 male) ranged in age from 41 to 72 years. Atrial heart muscle samples were obtained from patients suffering from coronary heart disease without signs of heart failure. Left ventricular muscle strips were provided from patients suffering from combined mitral valve lesions (NYHA II–III). Most patients had been treated with antianginal drugs (organic nitrates, β-adrenoceptor blockers), diuretics (triamterene, spironolactone, hydrochlorothiazide) and cardiac glycosides (digitoxin) before surgery. None of the patients was known to have received any calcium entry blocking agent or 5-HT receptor antagonist. General anesthesia was performed with narcotic combinations (midazolam, enflurane and nitrous oxide), skeletal muscle relaxants (pancuronium, suxamethonium) and analgesics (fentanyl).

At surgery, approximately 1 cm^2 of atrial myocardium was removed from the right atrial appendage as a part of the cannulation procedure for cardiopulmonary bypass. Papillary muscle samples were excised before valve replacement. Immediately after excision, the tissue was immersed in cool (4 °C) preoxygenated Tyrode solution containing (mmol/l): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.6, EDTA 0.05, equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). The time between excision and the beginning of laboratory processes was 15–90 min.

Experiments in multicellular preparations. Ventricular heart muscle samples were transferred to a dissection chamber containing oxygenated warm Tyrode solution, pinned down on Sylgard, and cut into strips of about 3 mm in length and 1 mm in diameter. Both ends were ligated

with a fine silk suture. For the measurement of AP and F_C, the preparations were mounted horizontally in a 2 ml organ bath which was built into a perspex block that also contained a main reservoir of 100 ml Tyrode solution heated to 37 °C. Communication between both compartments was provided by connecting pores through which the fluids were driven by gas (95% O_2 and 5% CO_2). The preparations were fixed in the organ bath to keep the muscle length as constant as possible. One end of the preparation was positioned between two platinum electrodes and the other end connected to an inductive force displacement transducer via a stainless steel wire.

The muscle strips were electrically driven at 1 Hz by rectangular pulses of 0.1 ms duration at 10% above threshold intensity using a Grass stimulator (model S88) and isolation unit (SIU 5). F_C was recorded at the apex of the preload active tension curve by means of an inductive force displacement transducer whose output was fed to a Hellige carrier frequency preamplifier. The preparations were allowed to stabilize for at least 2 h. Transmembrane potential was detected intracellularly by using conventional microelectrodes filled with 3 mol/l KCl (resistances 10–20 MΩ). A voltage follower with input capacitance compensation was used to record transmembrane potential. Upstroke velocity (dV/dt) was obtained by analog differentiation. All signals were displayed on an oscilloscope (Nicolet 310) and stored digitally on videotape (VCR Panasonic NV-H75) in conjunction with an Instrutech VR 100 14-bit AD converter. For evaluation, data were plotted (Hewlett Packard, 7475A) or transcribed to an X-Y recorder (BBC Goerz Metrawatt SE 790).

Atrial heart muscle samples were cut into pieces of 5–7 mm in length and 3–5 mm in width and, for the measurement of F_C only, mounted vertically in 5 ml organ baths heated to 35 °C as described earlier (Nawrath et al. 1984). After a stabilisation period of about 1 h, the effects of 5-HT were investigated by exposure to cumulatively increasing concentrations after the establishment of a stable response (about 10 min). All test solutions contained, unless otherwise stated, atenolol (10 µmol/l) to exclude any effects of 5-HT on β-adrenoceptors.

Measurement of membrane currents in single myocytes. For isolation of atrial or ventricular myocytes, heart muscle samples (0.5–1 cm^2) were cut into small pieces. For removal of Ca^{2+}, the tissues were stirred in nominally Ca^{2+}-free, oxygenated solution (15 min) containing (mmol/l) NaCl 130.0, KCl 5.4, KH_2PO_4 1.2, $MgCl_2$ 1.2, HEPES 6.0, glucose 30; pH was adjusted to 7.2 by NaOH (37 °C). Single cells were obtained by enzymatic dissociation using collagenase (1 mg/ml) and protease (0.1 mg/ml) according to Escande et al. (1987). After a superfusion period of 45 min, the digestion procedure was continued for further 45–100 min in the absence of protease. After the appearance of rod-shaped striated cells, the muscle chunks were resuspended under gentle mechanical agitation and stored (4 °C) in high K^+, Ca^{2+} free solution (Kraftbrühe, KB) with the following composition (mmol/l): KOH 70, glutamic acid 50, KCl 40, KH_2PO_4 20, taurine 20, $MgCl_2$ 3, HEPES 10, EGTA 5, glucose 10; pH was adjusted to 7.4 by KOH.

For whole cell clamp experiments, a droplet of the cell suspension was transferred to a chamber mounted on the stage of an inverted microscope (Olympus IMT-2). After settling down on the glas bottom of the chamber, cells were finally exposed to test solution containing (mmol/l) NaCl 137.6; CsCl 5.4; $MgCl_2$ 0.5; $CaCl_2$ 1.8; HEPES 11.6; glucose 5.0; pH was adjusted to 7.4 by NaOH. The whole-cell configuration of the single-electrode patch-clamp technique was used for the measurement of membrane currents (Hamill et al. 1981).

I_{Ca} was measured in K^+-free test solution containing CsCl in order to block K^+ currents. The solution in the recording pipette (resistances 1–2 MΩ) contained (mmol/l) CsCl 125.0, $MgCl_2$ 6.0, $CaCl_2$ 0.15, Na_2ATP 5.0, HEPES 10.0, EGTA 5.0; pH was adjusted to 7.2 with CsOH. To inactivate the fast Na^+ current (I_{Na}), holding potential was set to −40 mV. I_{Ca} was measured in response to depolarizing voltage clamp steps of 100 ms in the range between −40 mV and +40 mV. Time interval between test pulses was 10 s. Transmembrane currents were measured with an L/M-EPC-7 amplifier (List-Medical, Darmstadt, FRG) which received rectangular command pulses from a PC equipped with a Labmaster interface (Scientific Solution, Solon, USA). Current signals were filtered at 3 kHz prior to digitization using an 8-pole Bessel filter (Rockland Systems Corp., Model 432, USA). Data acquisition and analysis were performed with pClamp software (Axon, Foster City, USA).

5-HT (10 µmol/l) was added to the superfusate which was kept at 35 °C using a Peltier-effect heating device. A stable response was generally achieved within 2–5 min.

Chemicals. The following drugs were used (abbreviations and sources in parentheses): collagenase A (Boehringer, Mannheim, FRG); HEPES (Serva, Heidelberg, FRG); isoprenaline sulphate dihydrate (Boehringer, Ingelheim, FRG); adenosine-5'- triphosphate (Na_2ATP), atenolol, glutamic acid, 5-hydroxytryptamine creatinine sulfate complex (5-HT), protease type XIV, taurine (Sigma, Munich, FRG). All other chemicals were obtained from Merck, Darmstadt (FRG).

Evaluation of results. Results are either shown as original records or expressed as means±SEM. Peak levels of phasic contractions (F_C) are given as % of control values. Action potential parameters were analyzed for maximal upstroke velocity (dV/dt_{max}), resting membrane potential, overshoot, amplitude and duration at 20% and 90% of repolarization. I_{Ca} was measured at peak levels of inwardly directed currents after onset of the pulses with reference to zero current.

Results

Influence of 5-HT of F_C

5-HT exerted a concentration-dependent positive inotropic effect in human right atrial heart muscle (Fig. 1; filled circles). The effects of cumulatively increasing concentrations developed within 2–5 min. F_C was increased up to about 250% of control values at 10 µmol/l 5-HT. The pD_2-value amounted to 6.61 (95% confidence interval: 6.49 – 6.73). In the same concentration range, 5-HT was ineffective in ventricular heart muscle (Fig. 1; filled squares). In 10 ventricular preparations obtained from 5 patients, F_C remained unchanged even at concentrations which elicited maximal changes in the atrium. In the same preparations, F_C was inreased to 355±15% by isoprenaline (1 µmol/l).

Influence of 5-HT on I_{Ca} in the atrium

5-HT (10 µmol/l) caused a pronounced increase in I_{Ca} in atrial myocytes. The original current traces of a represen-

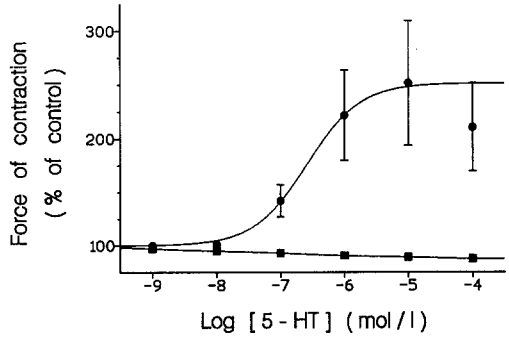

Fig. 1. Influence of 5-HT on force of contraction (F_C) in human right atrial and left ventricular heart muscle preparations. Cumulative concentration-response relationships. In the presence of atenolol (10 µmol/l), 5-HT elicited a concentration-dependent positive inotropic effect in the atrium (●; $n = 6$), whereas F_C remained unaffected in the ventricle (■; $n = 10$). Means±SEM. Standard error bars are partially within the size of the symbols

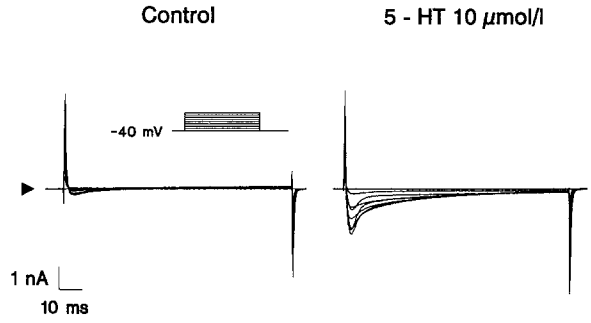

Fig. 2. Influence of 5-HT on calcium current (I_{Ca}) in a single human atrial myocyte. Original current traces in response to 9 depolarizing voltage clamp steps (10 mV increments; holding potential −40 mV; 100 ms) were superimposed under control conditions and in the presence of 5-HT (10 µmol/l). K^+ currents were suppressed by intra- and extracellularly applied Cs^+. Zero current is marked by ►. 2 minutes after the addition of 5-HT (10 µmol/l), a marked increase in I_{Ca} was observed

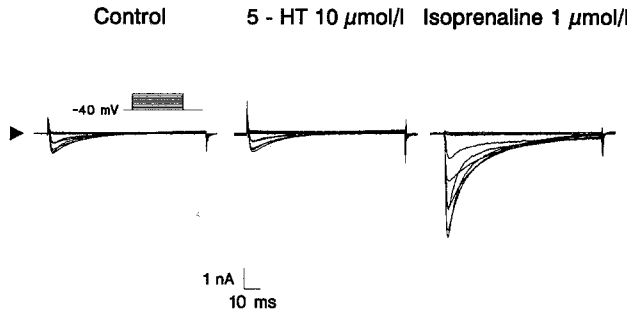

Fig. 4. Influence of 5-HT and isoprenaline on I_{Ca} in a single human ventricular myocyte. Original current traces in response to 9 depolarizing voltage clamp steps (10 mV increments; holding potential −40 mV; 100 ms) were superimposed under control conditions, in the presence of 5-HT (10 µmol/l) and in the presence of isoprenaline (1 µmol/l). K^+ currents were suppressed by intra- and extracellularly applied Cs^+. Zero current is marked by ►. I_{Ca} was recorded in the absence of atenolol. Superfusion of the myocyte with 5-HT (10 µmol/l) did not affect I_{Ca}; after washout of 5-HT, addition of isoprenaline (1 µmol/l) to the superfusate caused a pronounced increase in I_{Ca}

tative experiment are shown in Fig. 2. I_{Ca} was obtained in response to 100 ms depolarizing pulses from −40 mV (holding potential) up to +40 mV in 10 mV increments. Outward currents were suppressed by intra- and extracellularly applied Cs^+. 2 min after the addition of 5-HT, a sevenfold increase of peak I_{Ca} was observed. In three different experiments, I_{Ca} was increased four- to sevenfold by 5-HT. Similar increases of peak I_{Ca} were observed in the presence of isoprenaline (1 µmol/l) (data not shown).

Influence of 5-HT on AP and I_{Ca} in the ventricle

Figure 3 shows the lack of effect of 5-HT (100 µmol/l) on F_C, AP and dV/dt_{max} in human ventricular heart muscle. In this and 5 other preparations obtained from 5 patients, F_C and AP parameters remained completely unaffected by 5-HT. In the particular experiment in Fig. 4, I_{Ca} was recorded in the absence of atenolol (control conditions). Superfusion of the myocyte with 5-HT (10 µmol/l) did not affect the magnitude or time course of I_{Ca}. After washout of 5-HT, the addition of isoprenaline (1 µmol/l) caused a large increase in I_{Ca}.

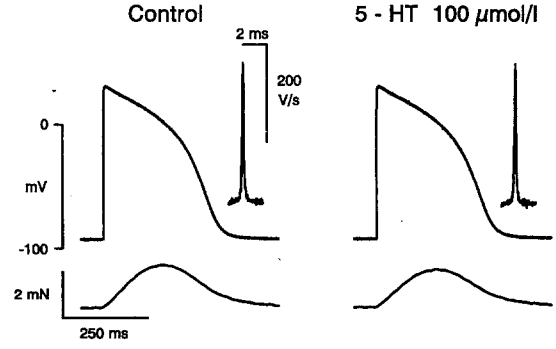

Fig. 3. Original recordings of action potential, upstroke velocity (dV/dt) and F_C obtained from a human papillary muscle preparation under control conditions and in the presence of 5-HT (100 µmol/l). Note the lack of a positive inotropic effect and the absence of changes in the action potential configuration in the presence of 5-HT

Discussion

5-HT causes a marked positive inotropic effect in human atrial heart muscle preparations. Kaumann et al. (1991) have shown that the receptors which are involved can be blocked by ICS 205-930 with a pK_B of 6.7, but not by antagonists at 5-HT_1, 5-HT_2 or 5-HT_3 receptors. These properties are similar to those of a new subtype of 5-HT receptor designated 5-HT_4 which is coupled positively to the adenylyl cyclase in mouse embryo colliculi neurons (Dumuis et al. 1988). The increase in F_C in the human atrium is also related to a cAMP-dependent stimulation of I_{Ca} thereby improving excitation-contraction coupling (Ouadid et al. 1992). In isolated atrial myocytes from rat, guinea-pig, rabbit or frog, neither a positive inotropic effect nor an increase in I_{Ca} was observed in response to 5-HT (Ouadid et al. 1992); thus, different responses to 5-HT among species are obvious. If effective, the action of 5-HT may involve the stimulation of various subtypes of receptors: a positive chronotropic effect is causally related to the stimulation of 5-HT_1 (cat; Saxena et al. 1985), 5-HT_2 (rat; Docherty 1988), 5-HT_3 (dog; Wilson et al. 1990) or 5-HT_4 receptors (pig; Kaumann 1990; Villalón et al. 1990).

The situation in the human heart may be summarized as follows: 5-HT causes an increase in the heart rate (LeMessurier et al. 1959) and a marked positive inotropic effect in the atrium (the latter mediated by 5-HT_4 receptors), whereas the ventricle seems to be completely unresponsive.

The reason for the lack of a direct positive inotropic effect of 5-HT in the ventricle is unclear. At least two different possibilities have to be discussed: first, the absence of the key 5-HT receptor, and, second, a missing link within the signal transduction pathway. Receptor-binding studies can partially resolve these questions. At present, however, a high-affinity radioligand for the 5-HT_4 receptor is not available (see Bockaert et al. 1992).

Due to the encouraging results in the human atrium to enhance the contractility via 5-HT receptors, a possible

therapeutic benefit of selective 5-HT_4 receptor agonists in the failing heart has been discussed (Saxena and Villalón 1991; Ouadid et al. 1992). However, in the light of a possible 5-HT_4-receptor-mediated tachycardia and the lack of direct effects of 5-HT on F_C in the human ventricle, the use of 5-HT agonists in the therapy of heart failure appears unlikely.

Acknowledgements. We would like to thank Prof. Satter (Abteilung für Herz-, Thorax- und Gefäßchirurgie der Johann Wolfgang Goethe-Universität Frankfurt, FRG) and Prof. Oelert (Abteilung für Herz-, Thorax- und Gefäßchirurgie der Johannes Gutenberg Universität Mainz, FRG) for supplying us with heart muscle preparations. This work was supported by grants from Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie and Naturwissenschaftlich-Medizinisches Forschungszentrum der Universität Mainz.

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