β2-Adrenoceptor-mediated positive inotropic effect of adrenaline in human ventricular myocardium

Quantitative discrepancies with binding and adenylate cyclase stimulation

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Summary. Experiments were designed to unravel the relative contribution of β1- and β2-adrenoceptors to the positive inotropic effects of adrenaline and noradrenaline in isolated tissues of left ventricular myocardium of man. We also analyzed relationships between the fractions of human left ventricular β1- and β2-adrenoceptors, estimated from binding assays, and stimulation of adenylate cyclase and contractile force by adrenaline and noradrenaline. 1) Selective blockade of β2-adrenoceptors by erythro-(±)-(α-methyl-indan-4-yloxy)-3-isopropylaminobutan-2-ol (ICI 118,551) attenuated the increase of contractile force caused by adrenaline but not by noradrenaline, suggesting some involvement of β2-adrenoceptors. Selective blockade of β2-adrenoceptors without affecting β1-adrenoceptors still enabled both adrenaline and noradrenaline to cause maximum possible increases of contractile force through β1-adrenoceptors. 2) A direct involvement of β2-adrenoceptors became manifest by selectively antagonizing β1-adrenoceptors by 1-2[(3-carbamoyl-4-hydroxy)phenoxoxy]ethylaminol-3-[4(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxoxy]-2-propanol (CGP 20712 A) without affecting β2-adrenoceptor. β2-adrenoceptors can mediate half of the maximum increase of contractile force elicited by low concentrations of adrenaline and also contribute to the increase of contractile force caused by high concentrations of noradrenaline. 3) β-adrenoceptors were labelled in membrane particles with 3H-(−)-bupranolol in the absence (β1 & β2) and presence of 500 nmol/l CGP 20712 A (β2). 71% of the β-adrenoceptors were β1 and 29% β2. Binding inhibition experiments with CGP 20712 A and ICI 118,551 yielded 74% β1 and 26% β2. 4) With the help of ICI 118,551 and CGP 20712 A it was found that, in membrane particles, 33 - 36% and 64 - 67% of maximum stimulation of the adenylate cyclase by adrenaline and noradrenaline were fit and 29% f12. Binding inhibition experiments with radiolabelled fit- and fl2-adrenoceptors in human left ventricle consistent with a partial involvement of both sinoatrial fit- and fl2-adrenoceptors. Data in other species are consistent with an involvement of both sinoatrial β1- and β2-adrenoceptors in the chronotropic effects of adrenaline, noradrenaline and other agonists (Lemoine et al. 1985a - guinea pig; Kaumann 1986 - rat; McCaffey et al. 1986 - man).

A mixed population of β1- and β2-adrenoceptors has been verified with radioligand binding in various heart regions of guinea pig and cat (Hedberg et al. 1980; Kaumann and Lemoine 1985; Lemoine et al. 1985a). In cat ventricle and left atria the minor β2-population appears to mediate a minor part of the positive inotropic effects to either adrenaline (Kaumann et al. 1985) and the β2-selective agonist procaterol (Kaumann et al. 1983).

β1- and β2-adrenoceptors, labelled in human atria (Brodde et al. 1983), both appear to participate in the positive inotropic effects of non-physiological agonists (Zerkowski et al. 1986) and physiological catecholamines (Gille et al. 1985; Kaumann and Lobbig 1986).

Although binding studies have also revealed the coexistence of β1- and β2-adrenoceptors in human ventricular myocardium (Robberecht et al. 1983; Stiles et al. 1983; Heitz et al. 1983) little is known about their function. Recent reports suggest a role of β2-adrenoceptors in the positive inotropic effects of adrenaline (Kaumann et al. 1985 - left ventricle) and zinterol (Bristow and Ginsburg 1986 - right ventricle) in man.

We now present evidence obtained from isolated tissues of human left ventricle consistent with a partial involvement of β2-adrenoceptors in the positive inotropic effects of adrenaline and noradrenaline. We also present some quantitative comparisons between the fractions of radiolabelled β1- and β2-adrenoceptors in human left ventricle and their relative contribution to adenylate cyclase stimulation and positive inotropic effects of adrenaline and noradrenaline.

Methods

Isolated tissues. Left ventricular tissues were excised from patients undergoing open heart surgery. Thirteen patients...
had mitral lesions and 6 patients had hypertrophic obstructive cardiomyopathy (HOCM). Fentanyl was used for the induction of anaesthesia, enflurane (ethrane) as anaesthetic and pancuronium as muscle relaxant. The patients did not receive β-adrenoceptor blocking agents or sympathicomimetics for at least one week before surgery. The tissues were obtained, transported, dissected and set up as described before (Kaumann et al. 1982; Kaumann and Lobnig 1986). Thin strips (width < 0.8 mm) were dissected in the direction of the preformed trabecular fibres without causing visible damage to the endocardium and mounted in an apparatus with a 50 ml organ bath (Blinks 1965) containing a solution at 37°C with (mmol/l), 140 Na⁺, 5 K⁺, 2.25 Ca²⁺, 0.5 Mg²⁺, 98.5 Cl⁻, 34 HCO₃⁻, 1 HPO₄²⁻, 0.5 SO₄²⁻, 5 fumarate, 5 pyruvate, 5 glutamate, 10 glucose, 0.04 EDTA equilibrated with 95% O₂ and 5% CO₂; the water was deionized and double distilled. The tissues were paced at 5 s intervals by square-wave pulses of barely threshold intensity. The tissues were pretreated for 2 h with 5 μmol/l (-)-isoprenaline followed by washout, a procedure that causes irreversible blockade of both α-adrenoceptors (Kaumann 1970) and neuronal capture of catecholamines in human heart (Gille et al. 1985).

Two or three cumulative concentration-effect curves for (-)-noradrenaline or (-)-adrenaline were determined in the absence and presence of a blocking agent selective for β₁ and β₂-adrenoceptors. The positive inotropic effects of the catecholamines were expressed as percentage of the response to noradrenaline (Gille et al. 1985) and neuronal capture of catecholamines in human heart (Gille et al. 1985).

Results

Inotropic effects mediated through β₁- and β₂-adrenoceptors

In agreement with previous observations (Kaumann et al. 1982; Gille et al. 1985), adrenaline and noradrenaline increased the contractile force of ventricular strips of human heart with similar potency (Figs. 1–3). To disclose a possible participation of β₂-adrenoceptors in the responses to adrenaline and noradrenaline we used the β₂-selective antagonist ICI 118,551, known to exhibit an up to 300-fold higher affinity for β₂- than for β₁-adrenoceptors in isolated tissues (Bilski et al. 1983; Lemoine et al. 1985a). ICI 118,551 50 nmol/l antagonized adrenaline-induced increases in the contractile force of ventricular strips. The blockade was surmounted by higher adrenaline concentrations. Fast speed tracings of contractions enhanced by adrenaline revealed similar time courses of contractions in the absence and presence of 50 nmol/l ICI 118,551 (Fig. 1a). The blockade of the responses to adrenaline was greater than expected from the affinity of ICI 118,551 for β₁-adrenoceptors (Fig. 1b), suggesting some involvement of β₂-adrenoceptors in the increases of contractile force by adrenaline. The responses to noradrenaline were only slightly antagonized by ICI 118,551, to an extent expected from its affinity for β₂-adrenoceptors (Fig. 1c).

In order to unmask β₂-adrenoceptors which cause responses to adrenaline and noradrenaline, we blocked β₁-adrenoceptors with CGP 20712 A, which is at least 10,000 times more selective for β₁- than for β₂-adrenoceptors (Dooley and Bittiger 1984; Lemoine et al. 1985c). CGP 20712 A 300 nmol/l revealed effects of low concentrations of adrenaline which were resistant to blockade; only the effects of higher concentrations of adrenaline were antagonized by CGP 20712 A (Figs. 2a and 3a). The CGP 20712 A-resistant component of the concentration-effect curve for (-)-adrenaline comprised approximately one half of the maximum response to adrenaline (Figs. 2a and 3a). Saturation of β₁-adrenoceptors by 300 nmol/l CGP 20712 A antagonized the responses to both low and high concentrations of noradrenaline (Figs. 2b and 3b). However, the shift of the concentration-effect curve for noradrenaline by 300 nmol/l CGP 20712 A was smaller than expected (Fig. 2b, dashed lines) from the affinity of CGP 20712 A for β₁-adrenoceptors.

Is the CGP 20712 A-resistant component of the adrenaline responses due to stimulation of β₂-adrenoceptors? The answer is yes, because saturation of β₂-adrenoceptors by 50 nmol/l ICI 118,551 abolished the CGP 20712 A-resistant responses to adrenaline (Fig. 3a). ICI 118,551 50 nmol/l also caused additional blockade of the effects of high concentrations of noradrenaline in the presence of 300 nmol/l CGP 20712 A (Fig. 3b, suggesting also an involvement of β₂-adrenoceptors.

Proportion of β₁- and β₂-adrenoceptors

Saturation binding revealed a component that was not inhibited by 500 nmol/l CGP 20712 A, amounting to 29% ± 6% of the total saturable binding (Fig. 4). Since 500 nmol/l is equivalent to 300 times Kᵦ₅ of CGP 20712 A.