Functional studies on α_1 -adrenoceptor subtypes mediating inotropic effects in rat right ventricle

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1 We have studied the α_1 -adrenoceptor subtypes mediating inotropic effects of adrenaline in rat right ventricle and the Ca^{2+} sources used to elicit these effects. α_{1A} -Adrenoceptor-mediated contractile effects in rat vas deferens were studied for comparison in some cases.

2 Treatment with chloroethylclonidine did not affect the maximal β -adrenoceptor-mediated inotropic effects in rat right ventricle or the maximal α_{1A} -adrenoceptor-mediated contractile effects in rat vas deferens; it did not alter the potency of isoprenaline in the ventricle and reduced the potency of the α -adrenoceptor antagonists in vas deferens only slightly. Treatment of right ventricular strips with CdCl_2 markedly reduced resting tension and enhanced maximal inotropic effects of isoprenaline but did not affect its potency.

3 Inactivation of cardiac α_{1B} -adrenoceptors by treatment with chloroethylclonidine slightly enhanced the maximal inotropic effects of the full agonist, adrenaline and of several partial agonists.

4 Schild analysis of inhibition experiments with the α_{1A} -adrenoceptor-selective antagonists, 5-methylurapidil and ($\pm$)-tamsulosin, demonstrated that adrenaline causes its inotropic effects mainly via the α_{1B} -adrenoceptor subtype. Schild analysis of 5-methylurapidil inhibition experiments in chloroethylclonidine-treated ventricles indicated that only α_{1A} -adrenoceptors mediate the inotropic effects of adrenaline following inactivation of the α_{1B} -adrenoceptors.

5 In control ventricles the organic Ca^{2+} entry blocker, nitrendipine and treatment with the inorganic Ca^{2+} entry blocker, CdCl_2 did not reduce inotropic effects of adrenaline whereas ryanodine treatment inhibited them. In contrast, nitrendipine and CdCl_2 treatment had major inhibitory effects in chloroethylclonidine-treated but lacked inhibitory effects in phenoxybenzamine-treated ventricular strips.

6 We conclude that inotropic effects of adrenaline in rat heart are mediated mainly by α_{1B} -adrenoceptors via release of Ca^{2+} from an intracellular pool. Following inactivation of α_{1B} -adrenoceptors by chloroethylclonidine treatment, α_{1A} -adrenoceptors can fully compensate and mediate inotropic effects by promoting influx of extracellular Ca^{2+} at least partly via voltage-operated channels. Therefore, we speculate that α_{1B} -adrenoceptors exert a tonic inhibitory effect on α_{1A} -adrenoceptors.

Keywords: α_{1A} -Adrenoceptors; α_{1B} -adrenoceptors; rat heart; inotropy; Ca^{2+}

Introduction

Multiple α_1 -adrenoceptor subtypes can be distinguished by use of pharmacological and receptor cloning techniques but the physiological relevance of this heterogeneity is not fully understood (Lomasney *et al.*, 1991; Bylund, 1992; Michel *et al.*, 1992). In rat heart, α_{1A} - and α_{1B} -adrenoceptors have been detected by radioligand binding (Minneman *et al.*, 1988; Hanft & Gross, 1989; Hanft *et al.*, 1989; Wang *et al.*, 1991; Knowlton *et al.*, 1993; Michel *et al.*, 1994). Known functions of cardiac α_1 -adrenoceptors include inotropic and electrophysiological effects and (at least in cultured neonatal cardiomyocytes) the development of hypertrophy (Benfey, 1990; Endoh, 1991). Whereas the reduction in transient outward currents in rat ventricular cardiomyocytes is mediated by α_{1A} - and α_{1B} -adrenoceptors (Wang *et al.*, 1991), only α_{1A} -adrenoceptors appear to mediate the development of hypertrophy in cultured neonatal rat cardiomyocytes (Knowlton *et al.*, 1993). Therefore, the present study has investigated the α_1 -adrenoceptor subtypes mediating the inotropic effects in rat heart and the Ca^{2+} sources used to elicit them. We have previously carried out a pharmacological analysis of the α_1 -adrenoceptor subtypes present in rat heart and of the specificity and efficacy of the pharmacological tools used in the present study (preceding paper, Michel *et al.*, 1994).

Methods

Inotropic experiments in rat right ventricle and vas deferens

Male adult Wistar rats weighing 250–400 g were obtained from Lippische Versuchstierzucht (Extetal, Germany). α_1 -Adrenoceptor-mediated positive inotropic effects were determined in right ventricles of rat heart as previously described (Gross *et al.*, 1988; Hanft & Gross, 1989). Briefly, experiments were performed at 30°C in organ baths containing 20 ml modified carbogen-saturated Krebs-Henseleit solution of the following composition (mmol l⁻¹): NaCl 118, KCl 4.8, CaCl_2 2.5, MgSO_4 1.2, NaHCO_3 25, KH_2PO_4 1.2 and glucose 10. Additionally propranolol (10 $\mu\text{mol l}^{-1}$), oxaprotiline (0.3 $\mu\text{mol l}^{-1}$) and corticosterone (40 $\mu\text{mol l}^{-1}$) were added unless otherwise indicated, to block β -adrenoceptor-mediated responses and neuronal and extraneuronal uptake, respectively. The preparations were stimulated electrically at 1 Hz (3 ms, amplitude 20% above threshold voltage) under isometric conditions (preload 9.8 mN). Prior to each experiment endogenous catecholamine stores were depleted by treatment with tyramine (three times with 100 $\mu\text{mol l}^{-1}$). In some experiments, right ventricular strips were treated with vehicle, 100 $\mu\text{mol l}^{-1}$ of chloroethylclonidine, 100 $\mu\text{mol l}^{-1}$ CdCl_2 or both for 30 min followed by 60 min of washout.

Contractions of rat vas deferens were measured under the same conditions except that the contractions were recorded without electrical stimulation; the resting tension in vas deferens experiments was 4.9 mN.

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Chemicals

The compounds used in this study were obtained from the following sources: (–)-adrenaline bitartrate, (–)-phenylephrine hydrochloride, (–)-isoprenaline hydrochloride, oxymetazoline hydrochloride, (±)-propranolol hydrochloride, yohim-

bine hydrochloride, corticosterone, and CdCl_2 from Sigma (Munich, Germany), (+)-oxaprotiline hydrochloride from Ciba Geigy (Basel, Switzerland), nitrendipine, phenoxybenzamine hydrochloride, and chloroethylclonidine dihydrochloride from Research Biochemicals (Natick, MA, U.S.A.), (±)-tamsulosin hydrochloride (formerly known as (±)-YM 12617 or (±)-YM 617) from Yamanouchi Pharmaceutical Co. (Tokyo, Japan), 5-methyl-urapidil from Byk Gulden (Konstanz, Germany), indanidine (formerly known as Sgd 101/75) from Siegfried (Zofingen, Switzerland), cirazoline hydrochloride from Synthelabo (Paris, France), and (–)-amidephrine mesylate from Bristol Myers (Evansville, IN, U.S.A.).

Results

Effects of chloroethylclonidine and CdCl_2 treatment on β -adrenoceptor-mediated positive inotropic effects in right ventricle

Treatment with chloroethylclonidine, CdCl_2 or both did not affect the potency of isoprenaline in eliciting inotropic effects in rat right ventricle (Figure 1); propranolol was omitted from the organ bath in these experiments. Thus, the pD_2 values for isoprenaline were similar under control conditions (8.27 ± 0.18 ; $n = 8$), in chloroethylclonidine-treated strips (8.28 ± 0.16 ; $n = 8$), in CdCl_2 -treated strips (8.13 ± 0.17 ; $n = 7$), and in strips treated with the combination of chloroethylclonidine plus CdCl_2 (8.19 ± 0.15 ; $n = 8$). The maximal inotropic effects of isoprenaline were also not significantly affected by chloroethylclonidine treatment (9.8 ± 1.3 mN in treated

Figure 1 Effects of treatment with chloroethylclonidine and/or CdCl_2 on isoprenaline-induced positive inotropic effects. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (○), $100 \mu\text{mol l}^{-1}$ chloroethylclonidine (■), $100 \mu\text{mol l}^{-1}$ CdCl_2 (□) or $100 \mu\text{mol l}^{-1}$ CdCl_2 or their combination (Δ). Following 60 min of washout, cumulative concentration-response curves for isoprenaline were constructed. Data are mean $\pm$ s.e. mean of 7–8 experiments.

Figure 2 Effects of treatment with chloroethylclonidine on α_1 -adrenoceptor-mediated positive inotropic effects. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (○) or $100 \mu\text{mol l}^{-1}$ chloroethylclonidine (■). Following 60 min of washout, cumulative concentration-response curves for the indicated agonists were constructed. Data are mean $\pm$ s.e. mean of (a) 49 (control adrenaline), 37–42 (chloroethylclonidine-treated adrenaline), (b) 6 (amidephrine), (c) 8 (phenylephrine) and (d) 9 experiments (cirazoline).

Table 1 Effect of chloroethylclonidine-treatment on α -adrenoceptor agonist-stimulated positive inotropic effects in rat vas deferens

	Control		Chloroethylclonidine-treated	
	Developed tension (mN)	pD_2	Developed tension (mN)	pD_2
Amidephrine	13.8 ± 1.4	5.35 ± 0.08	13.9 ± 1.8	5.14 ± 0.06
Cirazoline	13.6 ± 1.0	6.57 ± 0.03	13.8 ± 1.0	$6.32 \pm 0.04^{***}$
Phenylephrine	21.1 ± 1.5	5.53 ± 0.03	21.4 ± 1.9	$5.22 \pm 0.03^{***}$

Contractile effects were determined in rat vas deferens following a 30 min treatment with $100 \mu\text{mol l}^{-1}$ chloroethylclonidine and subsequent 60 min washout or under time-matched control conditions. Data are mean $\pm$ s.e.mean of 6 (amidephrine), 8 (cirazoline), and 8 (phenylephrine) experiments. $^{***}P < 0.001$ vs. control.

Table 2 Effect of chloroethylclonidine-treatment on α -adrenoceptor agonist-stimulated positive inotropic effects in rat right ventricle

	Control		Chloroethylclonidine-treated	
	Developed tension (mN)	pD_2	Developed tension (mN)	pD_2
Adrenaline	10.6 ± 0.4	6.88 ± 0.17	$12.5 \pm 0.4^{***}$	$6.10 \pm 0.17^{***}$
Amidephrine	$8.3 \pm 1.0^\dagger$	5.47 ± 0.06	$9.3 \pm 0.9^\dagger$	$5.17 \pm 0.03^{***}$
Cirazoline	$6.6 \pm 0.7^{\dagger\dagger}$	6.23 ± 0.02	$8.1 \pm 0.9^{\dagger\dagger}$	$5.97 \pm 0.07^{**}$
Phenylephrine	$8.6 \pm 0.7^\dagger$	5.85 ± 0.02	$10.5 \pm 0.6^\dagger$	$5.03 \pm 0.07^{***}$

Positive inotropic effects were determined in rat right ventricular strips following a 30 min treatment with $100 \mu\text{mol l}^{-1}$ chloroethylclonidine and subsequent 60 min washout or under time-matched control conditions. Data are mean $\pm$ s.e.mean of 49 (adrenaline control), 42 (adrenaline-treated), 6 (amidephrine), 9 (cirazoline), and 8 (phenylephrine) experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$, vs. control; $^\dagger P < 0.05$ and $^{\dagger\dagger}P < 0.001$, vs. adrenaline. A graphic representation of these data is given in Figure 1.

Figure 3 Effects of chloroethylclonidine treatment on α_1 -adrenoceptor subtypes mediating the positive inotropic effects of adrenaline. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (b, open symbols) or $100 \mu\text{mol l}^{-1}$ chloroethylclonidine (a, filled symbols). Following 60 min of washout, cumulative concentration-response curves for adrenaline were constructed in the absence ($\bullet$, $\circ$) and presence of 100 nmol l^{-1} 5-methyl-urapidil ($\blacksquare$, $\square$) or prazosin ($\blacktriangle$, $\triangle$). Data are mean $\pm$ s.e.mean of 11–14 and 6–19 experiments under control conditions (b) and after chloroethylclonidine treatment (a) respectively.

vs. $8.1 \pm 0.8 \text{ mN}$ in control strips; Figure 1), but treatment with CdCl_2 almost doubled the inotropic effects of isoprenaline to $16.1 \pm 2.3 \text{ mN}$ in CdCl_2 -treated and 17.2 ± 2.2 in CdCl_2 plus chloroethylclonidine-treated strips. The enhanced inotropic effects of isoprenaline were mainly due to reduced basal force of contraction since basal force was similar in control ($27.4 \pm 1.3 \text{ mN}$) and chloroethylclonidine-treated strips ($23.3 \pm 0.6 \text{ mN}$) but significantly reduced in CdCl_2 -treated ($13.2 \pm 1.2 \text{ mN}$, $P < 0.001$ vs. control) or CdCl_2 plus chloroethylclonidine-treated strips ($15.8 \pm 1.7 \text{ mN}$, $P < 0.001$ vs. chloroethylclonidine-treated strips).

Effects of chloroethylclonidine treatment on α_{1A} -adrenoceptor-mediated contractile effects in vas deferens

In rat vas deferens, α_1 -adrenoceptor agonists elicited contractile effects with the order of potency: cirazoline $>$ phenylephrine $\geq$ amidephrine; maximal contractile effects for amidephrine and cirazoline were approximately 35% smaller than those for phenylephrine (Table 1). Chloroethylclonidine treatment did not alter maximal contractile effects of the partial agonists amidephrine and cirazoline or the more efficacious agonist phenylephrine (Table 1). The potency of the agonists was slightly reduced in chloroethylclonidine-treated strips but this reached statistical significance only for cirazoline and phenylephrine (Table 1).

Effects of chloroethylclonidine treatment on α_1 -adrenoceptor-mediated positive inotropic effects in right ventricle

α -Adrenoceptor agonists increased force of contraction with a rank order of potency of adrenaline $>$ cirazoline $>$ phenylephrine $>$ amidephrine (Figure 2, Table 2). Relative to adrenaline (increase in force of contraction $10.6 \pm 2.6 \text{ mN}$, $n = 49$) the other compounds were only partial agonists with efficacies of 62% (cirazoline, $n = 9$), 81% (phenylephrine, $n = 8$) and 78% (amidephrine, $n = 6$); indanidine was a very weak partial agonist ($\sim 7\%$ of the adrenaline effect at

Figure 4 Effects of chloroethylclonidine treatment on α_1 -adrenoceptor subtypes mediating the positive inotropic effects of adrenaline. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (left panels) or 100 $\mu\text{mol l}^{-1}$ chloroethylclonidine (right panels) with subsequent 60 min of washout. The panels show a Schild analysis of data similar to and including those shown in Figure 3. Each data point represents the shift of the dose-ratio (DR) obtained in the presence of the indicated antagonist concentration in a single experiment; DR was determined as the ratio of the concentration of adrenaline required for half-maximal stimulatory effects in the presence and absence of the antagonist concentration.

300 $\mu\text{mol l}^{-1}$), and oxymetazoline did not cause consistent positive inotropic effects but rather caused negative inotropic effects in concentrations exceeding 100 $\mu\text{mol l}^{-1}$ which were not affected by addition of 1 $\mu\text{mol l}^{-1}$ prazosin, i.e. apparently unrelated to α_1 -adrenoceptors.

Inactivation of α_{1B} -adrenoceptors by treatment with chloroethylclonidine did not alter basal force of contraction (see above) and reduced the potency of the α -adrenoceptor agonists by factors of less than 10 (Figure 2, Table 2). The maximal inotropic effects of adrenaline were significantly enhanced following chloroethylclonidine treatment by approximately 20% ($n = 42$, $P < 0.0001$, Figure 2, Table 2). Maximal inotropic effects of the partial α -adrenoceptor agonists were increased to a similar extent but this did not reach statistically significant levels with the given number of experiments (Figure 2, Table 2).

Effects of chloroethylclonidine treatment on the subtype mediating positive inotropic effects of adrenaline in right ventricle

Prazosin (1–100 nmol l^{-1}), 5-methyl-urapidil (10–1000 nmol l^{-1}), and ($\pm$)-tamsulosin (30–300 nmol l^{-1}) concentration-dependently shifted the adrenaline concentration-response

curve to the right towards higher concentrations without significantly affecting the maximal inotropic effect (Figures 3 and 4). Whereas 100 nmol l^{-1} 5-methyl-urapidil caused a much smaller right shift in control than in chloroethylclonidine-treated ventricular strips, 100 nmol l^{-1} prazosin shifted the adrenaline concentration-response curve to a similar extent under both conditions (Figure 3); thus, the α_{1A} -selective 5-methyl-urapidil caused a much smaller shift than non-selective prazosin under both conditions, whereas both drugs caused similar shifts following chloroethylclonidine treatment. We quantitatively analyzed these antagonistic effects with Schild plots (Arunlakshana & Schild, 1959). The regression line of the Schild plots for prazosin had a slope which did not significantly differ from unity in control (0.89 ± 0.05) and chloroethylclonidine-treated strips (0.99 ± 0.10) and yielded pA_2 values of 9.01 and 8.36, respectively (Figure 4). The α_2 -adrenoceptor selective antagonist, yohimbine (50 nmol l^{-1}) failed to shift the adrenaline concentration-response curve in chloroethylclonidine-treated strips. Schild analysis of the 5-methyl-urapidil shifts revealed a slope which was less than unity in control strips (0.61 ± 0.08 ; Figure 4). Therefore a pA_2 value could not be calculated; pK_b values calculated for the individual 5-methyl-urapidil concentrations ranged from 8.12 to 7.25. Following chloroethylclonidine treatment the slope of the Schild regression for 5-

methyl-urapidil was steepened and no longer significantly differed from unity (0.88 ± 0.09); the pA_2 value calculated from data obtained with chloroethylclonidine-treated strips was 8.24 (Figure 4). Schild analysis of antagonism by ($\pm$)-tamsulosin yielded a slope which was somewhat steeper than unity (1.52 ± 0.14) and the x-axis intercept was 8.09 (Figure 4); in contrast ($\pm$)-tamsulosin yielded a pA_2 value of 9.32 for the α_{1A} -adrenoceptor-mediated contraction of rat vas deferens (slope 1.16 ± 0.08).

Effects of chloroethylclonidine treatment on Ca^{2+} sources used by adrenaline for its positive inotropic effects in right ventricle

The organic Ca^{2+} entry blocker, nitrendipine (100 nmol l^{-1}) did not significantly affect resting tension in control ($22.4 \pm 1.7 \text{ mN}$ in the absence and $20.6 \pm 0.9 \text{ mN}$ in the presence, $n = 8$ and 7, respectively) or chloroethylclonidine-treated rat right ventricle ($19.5 \pm 1.9 \text{ mN}$ in the absence and $17.8 \pm 1.4 \text{ mN}$ in the presence, $n = 8$ and 12, respectively). Addition of nitrendipine slightly but significantly enhanced the inotropic effects of adrenaline in control strips (pD_2 value from 6.74 ± 0.05 to 7.00 ± 0.05 , $n = 8$ and 9, respectively, $P = 0.0023$) but did not alter the maximal response to adrenaline under control conditions (10.0 ± 0.7 and $10.5 \pm 0.5 \text{ mN}$ in the absence and presence of nitrendipine, respectively; Figure 5). In chloroethylclonidine-treated strips, however, nitrendipine did not enhance the inotropic response to adrenaline (pD_2 value 6.10 ± 0.07 in the absence and 6.11 ± 0.03 the presence of nitrendipine, $n = 8$ and 12, respectively) but significantly

Figure 5 Effects of chloroethylclonidine-treatment and nitrendipine on α_1 -adrenoceptor-mediated positive inotropic effects. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (a) or $100 \mu\text{mol l}^{-1}$ chloroethylclonidine (b) with subsequent 60 min of washout. Concentration-response curves for adrenaline were constructed in the absence (open symbols) and presence of 100 nmol l^{-1} nitrendipine (solid symbols). Data are mean $\pm$ s.e. mean of 8–12 experiments.

reduced the maximal inotropic response to adrenaline from 14.0 ± 0.8 to 11.1 ± 0.8 ($P = 0.0218$; Figure 5).

Treatment with the inorganic Ca^{2+} entry blocker, CdCl_2 ($100 \mu\text{mol l}^{-1}$ for 30 min followed by 60 min washout) had little effect on the sensitivity towards the inotropic effects of adrenaline (pD_2 value 6.95 in control and 6.72 in treated strips with $n = 22$ and 18, respectively; Figure 6) and simul-

Figure 6 Effects of chloroethylclonidine- and CdCl_2 -treatment on α_1 -adrenoceptor-mediated positive inotropic effects. Tissue strips were treated for 30 min at 30°C in the organ bath with vehicle (○), $100 \mu\text{mol l}^{-1}$ chloroethylclonidine (□), $100 \mu\text{mol l}^{-1}$ CdCl_2 (●) or the combination of both (■) with subsequent 60 min of washout. Concentration-response curves for adrenaline were constructed in control and chloroethylclonidine- and CdCl_2 -treated strips but could not be obtained in strips treated concomitantly with both. Data are mean of 18–22 experiments; error bars were omitted for clarity.

Figure 7 Effects of ryanodine on α_1 -adrenoceptor-mediated contractile effects in heart and vas deferens. Concentration-response curves for adrenaline were constructed in the absence (○) and presence of 300 nmol l^{-1} ryanodine (■) in right atrial (b) and vas deferens strips (a). Data are mean $\pm$ s.e. mean of 8 experiments.

Figure 8 Effects of nitrendipine on α_1 -adrenoceptor-mediated inotropic effects in cardiac strips pretreated with phenoxybenzamine. Right ventricular strips were treated with 100 nmol l^{-1} phenoxybenzamine for 5 (a) and 10 min (b) followed by 60 min washout. Adrenaline concentration-response curves were constructed in the absence (○) and presence of 100 nmol l^{-1} nitrendipine (■). Data are mean $\pm$ s.e.mean of 3 experiments.

taneously slightly enhanced the maximal responses towards adrenaline. In contrast, treatment with CdCl_2 reduced the inotropic response to $10 \mu\text{mol l}^{-1}$ adrenaline by almost 90% in chloroethylclonidine-treated strips (Figure 6), and the reduction in force of contraction was so pronounced that concentration-response curves could not be analyzed.

Addition of 300 nmol l^{-1} ryanodine to the organ bath 1 h prior to adrenaline reduced basal force of contraction from 23.5 ± 0.8 to $5.3 \pm 0.5 \text{ mN}$ ($n = 8$ each, $P < 0.0001$). Ryanodine also reduced the (presumably α_{1B} -adrenoceptor-mediated) maximal inotropic responses of adrenaline by $\sim 50\%$ but the sensitivity of the remaining responses of adrenaline (pD_2 value 6.90 ± 0.09) was similar to that in paired control strips (pD_2 value 6.85 ± 0.03 ; Figure 7). In contrast, ryanodine did not alter the α_{1A} -adrenoceptor-mediated contractile response towards adrenaline in vas deferens with regard to maximal effects or sensitivity (Figure 7).

Non-selective alkylation of α_{1A} - and α_{1B} -adrenoceptors in rat right ventricle by phenoxybenzamine (100 nmol l^{-1} for 5 or 10 min) reduced the maximal inotropic effects of adrenaline (Figure 8) and also slightly decreased its potency (pD_2 values 6.57 ± 0.06 and 6.22 ± 0.15 , respectively, $n = 3$ each). In phenoxybenzamine-treated strips, however, addition of 100 nmol l^{-1} nitrendipine did not cause any further reduction of the inotropic effects of adrenaline (Figure 8).

Discussion

Previous studies have investigated the role of cardiac α_1 -adrenoceptor subtypes in signalling and growth regulation;

α_1 -adrenoceptor stimulation reduces a voltage-activated transient outward current in adult cardiomyocytes; while either α_{1A} - or α_{1B} -adrenoceptor inhibition was sufficient to block the late component of this current, only combined α_{1A} - and α_{1B} -adrenoceptor inhibition could antagonize its peak component (Wang *et al.*, 1991). The α_1 -adrenoceptor-mediated formation of inositol phosphates and induction of a hypertrophic phenotype in rat cultured neonatal cardiomyocytes involves only the α_{1A} -adrenoceptor subtype (Del Balzo *et al.*, 1990; Knowlton *et al.*, 1993). The present study has investigated the role of α_1 -adrenoceptor subtypes in the enhancement of contractility.

The specificity of the tools, 5-methyl-urapidil, ($\pm$)-tamsulosin, oxaprotiline, nitrendipine and of the treatments with chloroethylclonidine and/or CdCl_2 has been evaluated in our previous studies (Hanft & Gross, 1989; Hanft *et al.*, 1989; Michel *et al.*, 1994). Moreover, our conditions of chloroethylclonidine-treatment do not non-specifically interfere with contractility of cardiac or vas deferens smooth muscle and do not inhibit inotropic effects mediated by β -adrenoceptor stimulation or contractile effects in vas deferens which are mediated by α_{1A} -adrenoceptors (Han *et al.*, 1987; Hanft & Gross, 1989). Thus, functional effects of our chloroethylclonidine treatments are unlikely to result from anything but α_{1B} -adrenoceptor inactivation.

Competitively binding drugs and the irreversible antagonist, chloroethylclonidine, detect similar proportions of α_{1A} - and α_{1B} -adrenoceptors in rat heart in radioligand binding studies (Minneman *et al.*, 1988; Hanft & Gross, 1989; Hanft *et al.*, 1989; Han & Minneman, 1991). However, the two approaches did not yield compatible results with regard to the α_1 -adrenoceptor subtype mediating positive inotropic effects in the present study. Based on Schild analysis of the antagonism by the α_{1A} -adrenoceptor-selective WB 4101 and 5-methyl-urapidil, we have previously suggested that adrenaline causes inotropic effects in rat right ventricle via α_{1B} -adrenoceptors (Hanft & Gross, 1989). Our present results with the α_{1A} -adrenoceptor-selective ($\pm$)-tamsulosin (Hanft *et al.*, 1989; Michel *et al.*, 1993) support this conclusion. The use of a wider range of 5-methyl-urapidil concentrations than in our previous study (Hanft & Gross, 1989) reduces the slope of the Schild regression to values of less than unity indicating the possible involvement of more than one site and thus not allowing the calculation of pA_2 values. However, pK_b values calculated for the antagonism by the individual 5-methyl-urapidil concentrations were all much closer to its α_{1B} - than its α_{1A} -adrenoceptor affinity. Thus, our previous (Hanft & Gross, 1989) and present data with competitive antagonists suggest that adrenaline causes its positive inotropic effects in rat heart mainly if not exclusively via α_{1B} -adrenoceptors when β -adrenoceptors are blocked. However, a minor contribution of α_{1A} -adrenoceptors, which is not recognized by WB 4101 or ($\pm$)-tamsulosin, cannot be ruled out.

On the other hand, effective inactivation of α_{1B} -adrenoceptors by chloroethylclonidine treatment (Michel *et al.*, 1994) yielded apparently opposite results. Thus, chloroethylclonidine treatment did not reduce but rather enhanced the maximal inotropic effects of the full agonist adrenaline or the partial agonists amidephrine, cirazoline or phenylephrine (for which by definition no receptor reserve exists). Chloroethylclonidine treatment steepened the slope of the Schild-regression for the antagonistic effects of 5-methyl-urapidil and resulted in a pA_2 value which is closer to its α_{1A} -adrenoceptor affinity. Rat heart contains much fewer α_{1A} - than α_{1B} -adrenoceptors (Minneman *et al.*, 1988; Hanft & Gross, 1989; Han & Minneman, 1991; Michel *et al.*, 1994) and appears to lack α_{1C} -adrenoceptors in radioligand binding (Minneman *et al.*, 1988; Hanft & Gross, 1989; Han & Minneman, 1991) and Northern blot analysis (Lomasney *et al.*, 1991). Thus, either α_{1A} -adrenoceptors couple much more efficiently to inotropy than α_{1B} -adrenoceptors, or α_{1B} -adrenoceptors exert a tonic inhibitory effect on α_{1A} -adrenoceptors under control conditions which makes the contribution of the latter hard to detect. Although

we lack definitive proof of either hypothesis, we favour the latter for the following reasons. Firstly, lack of reduction of maximal effects by α_{1B} -adrenoceptor inactivation is compatible with the idea of more efficient α_{1A} -adrenoceptor coupling only if this subtype completely dominates the inotropic response. The low antagonistic potencies of the α_{1A} -selective antagonists, WB 4101, ($\pm$)-tamsulosin and 5-methyl-urapidil rule out this possibility. Secondly, enhanced inotropic effects of adrenaline following chloroethylclonidine treatment are completely implausible unless a tonic inhibitory effect of α_{1B} -adrenoceptors is postulated. In another study neither WB 4101 nor chloroethylclonidine had affected basal contractility of adult rat cardiomyocytes, but WB 4101 inhibited and chloroethylclonidine enhanced the contractile effects of the α_1 -adrenoceptor agonist phenylephrine (Gambassi *et al.*, 1991). Therefore, we speculate that α_{1A} - and α_{1B} -adrenoceptors cross-talk in rat heart. As a result a fully-fledged α_{1A} -adrenoceptor-mediated inotropic response becomes detectable only following α_{1B} -adrenoceptor inactivation. Since the cellular localization of the various adrenoceptor subtypes within the myocardium is unclear, a more detailed understanding of this interaction will probably require the use of a cell line co-expressing the subtypes. On the other hand such α_{1A}/α_{1B} -adrenoceptor cross-talk may not occur in all species since chloroethylclonidine treatment almost completely abolishes the α_1 -adrenoceptor-mediated inotropic effects in rabbit myocardium (Takanashi *et al.*, 1991).

Elevations of intracellular Ca^{2+} are crucial for the development of inotropy (Langer, 1992; Stern & Lakatta, 1992), but Ca^{2+} sensitization of the myofilaments may also play an important role in α_1 -adrenoceptor-mediated inotropy (Puceat *et al.*, 1992). Since α_{1A} - and α_{1B} -adrenoceptors increase intracellular Ca^{2+} by different mechanisms in extra-cardiac tissues (Minneman, 1988; Wilson & Minneman, 1990), we investigated the sources of Ca^{2+} used by adrenaline in control (mainly α_{1B} -adrenoceptors) and chloroethylclonidine-treated right ventricular strips (mainly α_{1A} -adrenoceptors) to elicit positive inotropic effects; in some cases α_{1A} -adrenoceptor-mediated contractions of rat vas deferens (Han *et al.*, 1987; Hanft & Gross, 1989) were used as an additional model system. In control strips nitrendipine and $CdCl_2$ treatment did not inhibit the inotropic effects of adrenaline but rather slightly enhanced its potency and increased its maximal effects, respectively. Thus, influx of extracellular Ca^{2+} does not appear to be required for the α_{1B} -adrenoceptor-mediated positive inotropic effects. Similarly, α_{1B} -adrenoceptor-mediated vasoconstriction elicited by full agonist in rat aorta (Beckerlingh *et al.*, 1984) and generation of inositol phosphates in rat hepatocytes (Wilson & Minneman, 1990) are insensitive to blockade of Ca^{2+} influx. In contrast, ryanodine which modulates Ca^{2+} release from the sarcoplasmic reticulum (Stern & Lakatta, 1992) decreased maximal inotropic effects of adrenaline without altering its potency, indicating the importance of Ca^{2+} release from the sarcoplasmic reticulum. This Ca^{2+} release is unlikely to involve inositol phosphate formation since (a) inositol phosphate formation is too slow to participate in control of the heart beat (Stern & Lakatta, 1992), (b) (at least in neonatal rat cardiomyocytes) inositol phosphate formation occurs via α_{1A} - but not via α_{1B} -adrenoceptors (Del Balzo *et al.*, 1990; Knowlton *et al.*, 1993), and (c) because the ryanodine receptor is distinct from the inositol phosphate receptor (Otsu *et al.*, 1990). Thus, cardiac α_{1B} -adrenoceptors are similar to those in other tissues since they rely on mobilization of Ca^{2+} from intracellular pools rather than on influx of extracellular Ca^{2+} to elicit inotropic effects but are distinct since they are unlikely to do so via generation of inositol phosphates.

A different picture was obtained in the α_{1A} -adrenoceptor models, i.e. in chloroethylclonidine-treated heart and in vas deferens. In chloroethylclonidine-treated heart, $CdCl_2$ treat-

ment and nitrendipine reduced the maximal inotropic effects of adrenaline by 88% and 21%, respectively. The weaker inhibition by nitrendipine compared to $CdCl_2$ treatment could indicate incomplete blockade of voltage-operated Ca^{2+} channels or the concomitant involvement of such channels and other pathways of transmembrane Ca^{2+} fluxes such as the Na^+/Ca^{2+} exchange which can operate bidirectionally (Hilton, 1985). We cannot completely answer this question since higher concentrations of nitrendipine interfered with α_1 -adrenoceptor binding but favour the latter possibility since 100 nmol l⁻¹ nitrendipine is considerable in excess of its affinity at voltage-operated Ca^{2+} channels (Goa & Sorkin, 1987). In vas deferens, ryanodine failed to alter the α_{1A} -adrenoceptor-mediated contractile response to adrenaline.

In some model systems of α_{1B} -adrenoceptors such as rat aorta, full agonists can rely on mobilization of intracellular Ca^{2+} only whereas partial agonists require influx of extracellular Ca^{2+} (Beckerlingh *et al.*, 1984); following partial inactivation of α_{1B} -adrenoceptors by treatment with the alkylating agent, phenoxybenzamine, full agonists may also require influx of extracellular Ca^{2+} to elicit vasoconstriction (Ruffolo *et al.*, 1991). In the present study treatment with phenoxybenzamine failed to cause nitrendipine-sensitivity in rat right ventricle although it reduced the potency of adrenaline to a similar extent to chloroethylclonidine treatment and, in contrast to chloroethylclonidine, even reduced the maximal inotropic response. Thus, α_{1B} -adrenoceptor-mediated inotropic effects do not require influx of extracellular Ca^{2+} but may involve Ca^{2+} release from the sarcoplasmic reticulum via a ryanodine receptor as well as Ca^{2+} sensitization of the myofilaments (Puceat *et al.*, 1992). In contrast α_{1A} -adrenoceptor-mediated contractile effects in rat heart and vas deferens require influx of extracellular Ca^{2+} which occurs at least partly via a voltage-operated Ca^{2+} channel (see also, Han *et al.*, 1987); Ca^{2+} mobilization from ryanodine-sensitive stores may not be involved.

In summary, we have demonstrated that adrenaline (in the presence of propranolol) causes positive inotropic effects via α_{1B} -adrenoceptors independently of influx of extracellular Ca^{2+} in control myocardium. Following inactivation of α_{1B} -adrenoceptors by chloroethylclonidine treatment, α_{1A} -adrenoceptors more than compensate by mediating even greater positive inotropic effects by stimulation of the influx of extracellular Ca^{2+} . Together with previously published data (Del Balzo *et al.*, 1990; Anyukhovsky & Rosen, 1991; Wang *et al.*, 1991; Knowlton *et al.*, 1993) we conclude that α_{1A} - and α_{1B} -adrenoceptors may be coexpressed in rat myocardium to activate distinct physiological responses.

The apparent cross-talk between α_{1A} - and α_{1B} -adrenoceptors may be important under pathophysiological conditions. Thus, in hearts from spontaneously hypertensive rats, α_1 -adrenoceptor-mediated inotropic effects appear to be enhanced although the number of α_1 -adrenoceptors and their G-protein G_q are unchanged (Brodde & Michel, 1992). Moreover, preliminary data from our laboratory indicate that the ratio of α_{1A}/α_{1B} -adrenoceptors is similar in normotensive WKY ($28 \pm 3/72 \pm 3$) and spontaneously hypertensive rats ($26 \pm 2/74 \pm 2$, $n = 6$ each, unpublished observations). Since β -adrenoceptor-mediated inotropy is reduced rather than enhanced in hypertensive hearts (Brodde & Michel, 1992) and, thus, alterations of the contractile apparatus cannot explain enhanced inotropy, the intriguing possibility exists that enhanced α_1 -adrenoceptor function may be related to an altered cross-talk between α_{1A} - and α_{1B} -adrenoceptors.

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