Receptor Subtypes Involved in the α_1 -Adrenoceptor Mediated Increase in $^{86}\text{Rb}^+$ Efflux from the Rat Heart

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ABSTRACT—The aim of this study was to determine the involvement of the different α_1 -adrenoceptor subtypes in the α_1 -adrenoceptor mediated increase in $^{86}\text{Rb}^+$ efflux from rat hearts. Isolated hearts were perfused in the presence of a β -adrenoceptor antagonist (1 μM timolol). After loading with $^{86}\text{Rb}^+$, the efflux was measured during α_1 -adrenoceptor stimulation by phenylephrine (30 μM). Phenylephrine increased the $^{86}\text{Rb}^+$ efflux by about 30%. Pretreatment with the preferentially α_{1B} -adrenoceptor inhibitor chloroethylclonidine (CEC), reduced the response to phenylephrine by about 50%. The preferential α_{1D} -adrenoceptor inhibitor BMY 7378 inhibited the response to phenylephrine by 35%, with a $\text{pK}_I = 8.4$ (95% C.I. 8.2–8.6). The response was sensitive to the preferential α_{1A} -adrenoceptor inhibitors (+)niguldipine, 5-methylurapidil (5-MU) and WB-4101 at relatively high concentrations, and 5-MU inhibited the response with a $\text{pK}_I = 7.7$ (95% C.I. 7.2–8.0) in CEC pretreated hearts. In conclusion, the phenylephrine stimulated increase in $^{86}\text{Rb}^+$ efflux in the rat heart is not specifically linked to only one of the α_1 -adrenoceptor subtypes, but involves the α_{1B} - and the α_{1D} -adrenoceptor subtypes, and probably the α_{1A} -adrenoceptor subtype as well.

Keywords: α_1 -Adrenoceptor subtype, $^{86}\text{Rb}^+$ efflux, Rat heart, Chloroethylclonidine, BMY 7378

α_1 -Adrenoceptors mediate many physiological effects of catecholamines in the mammalian heart (1, 2). Stimulation of myocardial α_1 -adrenoceptors elicits a positive inotropic response (1, 3), induces cardiac myocyte hypertrophy and gene transcription (4) and stimulates ionic transport (5, 6). Now three α_1 -adrenoceptor subtypes, showing high sensitivity to prazosin, have been identified by pharmacological methods in contrast to the original subdivision into α_{1A} - and α_{1B} -adrenoceptor subtypes (7). Thus the current classification includes three α_1 -adrenoceptors named α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor subtypes (8). In addition, three α_1 -adrenoceptor subtypes have been cloned (9–12). We have previously reported that phenylephrine during β -adrenoceptor blockade causes a concentration-dependent increase in $^{86}\text{Rb}^+$ efflux in the rat heart (13). The response showed a high sensitivity to prazosin ($K_I = 6.1 \times 10^{-11}$ M), implying that it is mediated through α_1 -adrenoceptors. In order to extend the characterization of the involved α_1 -adrenoceptors with respect to the subtypes, the effect of phenylephrine stimulation on $^{86}\text{Rb}^+$ efflux in the presence of subtype preferential antagonists was studied.

MATERIALS AND METHODS

Heart perfusion

Hearts from male Wistar rats, fed ad libitum, with body weight of 200–300 g, were isolated under ether anesthesia. The aorta was cannulated, and the hearts were spontaneously beating and perfused in a non-recirculating system. The flow was kept constant (10 ml/min) by a Bifok FIA 08 roller pump or a HiLoad Pump P-50 reciprocating piston pump (both pumps from Pharmacia Biotech, Uppsala, Sweden). The basic perfusion medium contained: 119.3 mM NaCl, 3.0 mM KCl, 2.4 mM KH_2PO_4 , 1.2 mM MgSO_4 , 2.0 mM CaCl_2 , 24.9 mM NaHCO_3 , 10.0 mM glucose, and 2.2 mM mannitol and was equilibrated with 95% O_2 / 5% CO_2 at 31 °C (pH 7.4). When used, $^{86}\text{Rb}^+$ was added to the perfusion medium to yield approximately $1\text{--}2 \times 10^5$ cpm/ml.

Experimental design

All hearts were perfused with a basic perfusion medium containing a β -adrenoceptor antagonist (timolol, 1 μM) throughout the experiment to suppress the β -adrenoceptor component of phenylephrine stimulation (14). After a

perfusion period of 10 min, the hearts were loaded with the K^+ analogue $^{86}\text{Rb}^+$ for 30 min, followed by a 25-min control period and a 30-min period of agonist stimulation. Perfusate fractions of 3 ml (collection period, 18 sec) were collected consecutively with an LKB 7000 Ultro Rac fraction collector (Pharmacia Biotech) during the washout period. At the end of the experiment, the hearts were freeze-clamped with an aluminium clamp precooled in liquid nitrogen and pulverized in a cooled percussion mortar. The hearts weighed 1.0–1.45 g (wet weight). The tissue powder was stored in plastic vials at -80°C until $^{86}\text{Rb}^+$ tissue radioactivity was determined. When used, one of the preferential α_{1A} -adrenoceptor antagonists, WB-4101, (+)niguldipine and 5-methylurapidil (5-MU), or the preferential α_{1D} -adrenoceptor antagonist BMY 7378 was added to the perfusion buffer at the start of the washout period. 5-MU was preferred among the α_{1A} -adrenoceptor antagonists when a concentration-inhibition curve was constructed because it preferentially binds to α_{1A} -adrenoceptors (15), with a higher degree of selectivity than WB-4101 (16–18), and avoids the problems related to the high lipophilicity of (+)niguldipine (19, 20).

When used, the irreversible preferential α_{1B} -adrenoceptor antagonist chloroethylclonidine (CEC) was preincubated for 30 min (the same 30 min period in which the hearts were loaded with $^{86}\text{Rb}^+$) and was then washed out for 25 min before the hearts were exposed to the α_1 -adrenoceptor agonist phenylephrine ($30\text{ }\mu\text{M}$). Corresponding and separate groups were exposed to phenylephrine in the presence or absence of CEC, (+)niguldipine, 5-MU or BMY 7378, respectively. In addition, groups with the combination of CEC and either (+)niguldipine, 5-MU or WB 4101 were included. The maximal response to α_1 -adrenoceptor stimulation varied to some degree between the experimental series. Thus the effect of each subtype receptor antagonists were compared with separate groups of hearts exposed to phenylephrine alone.

Determination of $^{86}\text{Rb}^+$ -radioactivity

Radioactivity in the collected perfusate fractions, and in the heart tissue powder was measured with a Packard, model 1900 TR Tri-Carb liquid-scintillation spectrometer (Packard Instrument Company, Downers Grove, IL, USA). The count rates were corrected for radioactivity decay. The radioactivity of the perfusate fractions was determined as Cerenkov radiation directly in the aqueous medium. The heart tissue powder (150 mg) was homogenized in 7.5% trichloroacetic acid at a dilution of 1:10 wt./vol. The radioactivity in 0.1 ml supernatant was determined as Cerenkov radiation after addition of 2.9 ml basic perfusion medium. The contents of the heart

radioactivity at time points corresponding to the sampling time of each perfusate fraction were calculated. To the $^{86}\text{Rb}^+$ contents in the hearts at the end of perfusion, the contents in each previous perfusate fraction were added sequentially and cumulatively.

Calculations of $^{86}\text{Rb}^+$ washout kinetics and efflux rate

The rate of $^{86}\text{Rb}^+$ efflux was calculated as radioactivity appearing in the perfusate per min (cpm/min) during washout. The efflux rate index ("fractional efflux" (in min^{-1})) could be expressed as the ratio between perfusate $^{86}\text{Rb}^+$ radioactivity and $^{86}\text{Rb}^+$ contents of the heart at the corresponding time points. A curve fit computer program was used to perform a regression analysis that described the course of the efflux rate index in the control period (25 min) before agonist stimulation. The equation of a hyperbola was used in the computer program ($y=(c/x-b)+a$, (13)). When the regression line constructed from the data points in the first 15 min period was extrapolated beyond this period, it correlated well with the actual observed course of the efflux rate index in control experiments ($r^2=0.86$, Fig. 1A). Thus the regression lines were calculated individually from the control periods before intervention and were extrapolated into the stimulation period (Fig. 1A). In this way, each heart was used as its own control. The response was expressed as percentage of the extrapolated control index during the corresponding part of the washout period (Fig. 1B). The maximal response in each individual experiment was determined as the maximal increase in percent irrespective of time within the 30-min observation period. The effect of the antagonists was measured as the difference between the maximal response in the presence or absence of antagonists (mean values), respectively.

Construction of concentration-inhibition curves and statistics

The relative response to each concentration of antagonist was plotted as the mean value of the different experiments. The concentration giving 50% reduction (IC_{50}) of phenylephrine-evoked $^{86}\text{Rb}^+$ efflux was estimated by a curve-fitting computer program (Graph Pad PrismTM; Graph Pad Software Inc., San Diego, CA, USA). The estimated IC_{50} value was used together with the EC_{50} value for phenylephrine stimulated $^{86}\text{Rb}^+$ efflux ($0.45\text{ }\mu\text{M}$) obtained from previous work (13) to calculate the K_i (inhibition constant) value according to the Cheng and Prusoff equation (21). Values are given as means with S.E.M., except for the pK_i values ($-\log K_i$) which are given with 95% confidence interval (C.I.). Parametric statistical analysis was used (Student's *t*-test); $P<0.05$ was considered to reflect statistically significant differences.

Drugs

Phenylephrine hydrochloride, timolol maleate, manitol and WB 4101 [2-(2,6-dimethoxyphenoxyethyl)amino-methyl-1,4 benzodioxane] were purchased from Sigma Chemical, St. Louis, MO, USA) and BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) and chloroethylclonidine dihydrochloride, from Research Biochemicals International (Natick, MA, USA). (+)Niguldipine and 5-methylurapidil were the kind gifts of Dr. Sanders at Byk Gulden Lomberg (Konstanz, Germany), made available through Pharmacia AS (Oslo, Norway). Stock solutions were prepared in double-distilled water and maintained at -20°C . Further dilutions were made fresh daily and kept cool ($0-4^\circ\text{C}$). Rubidium-86 (specific activity in the range of 37–403 MBq/mg Rb-86) was purchased from Du Pont (Brussel, Belgium) or from Amersham International (Buckinghamshire, UK).

RESULTS

Effect of α_1 -adrenoceptor stimulation

The basal $^{86}\text{Rb}^+$ efflux rate index decreased with time during the washout period (Fig. 1A). After the 25-min washout (time of agonist intervention), the basal efflux rate index was $3.0 \pm 0.05 \times 10^{-2} \text{ min}^{-1}$ ($n=32$). Within 5 min after phenylephrine ($30 \mu\text{M}$) stimulation, the $^{86}\text{Rb}^+$ efflux rate index increased and reached a plateau level after $19 \pm 0.9 \text{ min}$ (Fig. 1A). The new plateau was $0.8 \pm 0.04 \times 10^{-2} \text{ min}^{-1}$ higher than the basal index at the corresponding time point ($P < 0.0001$, $n=32$). The maximal increase in $^{86}\text{Rb}^+$ efflux after α_1 -adrenoceptor stimulation by phenylephrine was $28 \pm 1.3\%$ (Fig. 2A, $P < 0.0001$, $n=32$).

Influence of the preferentially α_{1B} -adrenoceptor subtype antagonist CEC

CEC pretreatment did not affect the basal $^{86}\text{Rb}^+$ efflux curve, but the α_1 -adrenoceptor mediated increase in $^{86}\text{Rb}^+$ efflux was reduced (Fig. 2A). Pretreatment with a maximal concentration of CEC ($10 \mu\text{M}$, 30 min) reduced the phenylephrine mediated increase in $^{86}\text{Rb}^+$ efflux to $51 \pm 5.0\%$ of the maximal response (Fig. 3). Time to reach the maximal response was not changed in the CEC pretreated hearts ($21 \pm 1.2 \text{ min}$ vs $23 \pm 1.7 \text{ min}$ with or without CEC, respectively).

Influence of preferentially α_{1A} -adrenoceptor subtype antagonists in CEC pretreated hearts

In order to increase the experimental selectivity, the effect of three different preferentially α_{1A} -adrenoceptor antagonists was tested against the CEC insensitive part of the response to phenylephrine. 5-MU inhibited the

Fig. 1. α_1 -Adrenoceptor stimulated increase in $^{86}\text{Rb}^+$ efflux rate index from the rat heart. A: $^{86}\text{Rb}^+$ efflux rate index in the presence of $1 \mu\text{M}$ timolol. Data from one control and one stimulated experiment are shown. Open squares: Values from a single control experiment. Solid line: Regression line given by the equation $y = (1.22/x + 377.7) + 0.015$. The regression line is constructed from the data points during the 15-min period starting after the 10-min wash-out. The line is extrapolated into the next 30-min observation time for comparison with the actually observed control data ($r^2 = 0.86$, see text for details concerning the curve fit calculations). Open circles: Efflux rate index of a single heart exposed to α_1 -adrenoceptor stimulation (arrow). Broken line: Regression line calculated from the data points in the 15-min control period before agonist intervention and extrapolated into the 30-min intervention period in order to describe the extended course of the rate index in the control situation. Ordinate: $^{86}\text{Rb}^+$ efflux rate index (min^{-1}); Cpm ($^{86}\text{Rb}^+$) per fusate $\times \text{min}^{-1}$ /Cpm ($^{86}\text{Rb}^+$) heart. B: Increase in $^{86}\text{Rb}^+$ efflux rate index in percent of basal rate. The results from the phenylephrine stimulation experiment in panel A (open circles) is expressed as the increase in percent of the calculated control curve. Broken line: The basal values are taken as 100%. Arrow: Addition of phenylephrine. Ordinate: $^{86}\text{Rb}^+$ efflux rate index, in percent of the basal values. Abscissae: Time in min after start of $^{86}\text{Rb}^+$ washout.

response concentration-dependently (Fig. 4) with a $-\log IC_{50}$ value of 5.6 ± 0.14 , corresponding to a calculated $pK_i = 7.7$ ($-\log K_i$, 95% C.I. 7.2–8.0). The combination of 5-MU and CEC were tolerated well by the spontaneously beating heart, and high concentrations (10 μM) of both antagonists almost eliminated the α_1 -adrenoceptor mediated increase in $^{86}Rb^+$ efflux (Figs. 2B and 3). Also WB 4101 and (+)niguldipine inhibited the response in a concentration-dependent manner (Fig. 3). Both antagonists gave a statistically significant reduction compared to

	μM							
PHE	30	+	+	+	+	+	+	+
CEC	10	-	+	+	+	+	+	+
MU	10	-	-	+	-	-	-	-
NIG	0.1	-	-	-	+	-	-	-
NIG	1	-	-	-	-	+	-	-
WB	0.1	-	-	-	-	-	+	-
WB	1	-	-	-	-	-	-	+

Fig. 3. Effect of chloroethylclonidine (CEC) pretreatment and three different preferential α_{1A} -adrenoceptor subtype antagonists on the α_1 -adrenoceptor mediated increase in $^{86}Rb^+$ efflux rate index (in the presence of 1 μM timolol). Groups of hearts were subjected to stimulation by phenylephrine (PHE) alone or after CEC pretreatment without or with either 5-methylurapidil (MU), niguldipine (NIG) or WB 4101 (WB). The response was expressed as increase of $^{86}Rb^+$ efflux rate index in percent of the maximal response to PHE stimulation in the absence of receptor antagonists. Values are given as mean values $\pm$ S.E.M. $^{**}P < 0.001$, vs PHE alone; $^{§§}P < 0.001$, vs PHE + CEC.

Fig. 2.

Fig. 2. Effect of the preferential α_{1B} -adrenoceptor subtype antagonist chloroethylclonidine (CEC pretreatment, 10 μM , 30 min, 31°C), the preferential α_{1A} -adrenoceptor subtype antagonist 5-methylurapidil (5-MU) and the combination of 5-MU and CEC pretreatment on the phenylephrine (PHE) stimulated increase in $^{86}Rb^+$ efflux rate index (in the presence of 1 μM timolol). Each data point represent the mean value of 5–26 separate hearts. All groups were stimulated by PHE (arrow). A: Open circles: Efflux rate index in PHE-stimulated hearts in the absence of α_1 -receptor antagonists ($n=7$). Closed circles: Efflux rate index in PHE-stimulated hearts ($n=11$) pretreated by CEC. Broken line: Extrapolated control values expressed as 100%. B: Open circles: Efflux rate index in PHE-stimulated hearts in the absence of receptor antagonists ($n=26$). Closed circles: Efflux rate index in CEC-pretreated hearts (closed circles from panel A included for comparison). Open triangles: Efflux rate index in non-CEC-pretreated hearts in the presence of 5-MU (10 μM , $n=8$). Open squares: Efflux rate index in CEC-pretreated hearts in the presence of 5-MU (10 μM , $n=5$). Ordinate: Increase in $^{86}Rb^+$ efflux rate index in percent of basal efflux rate. Abscissa: Time in min after addition of agonist.

Fig. 4. Concentration-response relationship for the effect of 5-MU on the phenylephrine-stimulated $^{86}\text{Rb}^+$ efflux rate index in CEC pretreated hearts (in the presence of $1\ \mu\text{M}$ timolol). Each heart was subjected to one concentration of antagonist only. Closed circles: Response in the presence of CEC and increasing concentrations of 5-MU (mean values $\pm$ S.E.M.). Line: Regression line constructed from a computer program. The concentration response curves were constructed from 23 separate hearts. Ordinate: Increase in $^{86}\text{Rb}^+$ efflux rate index in percent of the maximal response. Abscissa: Log molar concentration of 5-MU.

CEC alone ($P \leq 0.001$, Fig. 3). The occurrence of heart arrhythmias prevented the use of higher concentrations of (+)niguldipine.

Influence of preferentially α_{1A} -adrenoceptor subtype antagonists in the absence of CEC pretreatment

5-MU also inhibited the response to phenylephrine in the absence of CEC pretreatment (Fig. 2B), reducing the response to $27 \pm 5.1\%$ of the maximal response at $10\ \mu\text{M}$ (Fig. 5). Time to reach the maximal response was also reduced from 18 ± 1.7 with phenylephrine alone ($n=8$) to 10 ± 1.7 min in the presence of 5-MU ($n=8$, $P=0.006$, Fig. 2B). The phenylephrine mediated increase in $^{86}\text{Rb}^+$ efflux was reduced to $71 \pm 7.8\%$ and $57 \pm 6.5\%$ of the maximal response by the presence of 0.1 and $1\ \mu\text{M}$ (+)niguldipine, respectively (Fig. 5). Again, the occurrence of heart arrhythmias prevented the use of higher concentrations of (+)niguldipine.

Influence of the preferential α_{1D} -adrenoceptor subtype antagonist BMY 7378

To investigate the possible involvement of the α_{1D} -adrenoceptor subtype, phenylephrine stimulation was performed in the presence of BMY 7378 (Fig. 6). The effect of BMY 7378 was studied without CEC pretreatment of the hearts because the α_{1D} -adrenoceptor subtype shows a relatively high sensitivity to CEC inactivation

	μM					
PHE	30	+	+	+	+	+
MU	10	-	+	-	-	-
NIG	0.1	-	-	+	-	-
NIG	1	-	-	-	+	-
BMY	1	-	-	-	-	+

Fig. 5. Effect of α_{1A} - or α_{1D} -adrenoceptor subtype selective antagonists on the phenylephrine stimulated increase in $^{86}\text{Rb}^+$ efflux rate index in non-CEC pretreated hearts (in the presence of $1\ \mu\text{M}$ timolol). Groups of hearts were subjected to stimulation by phenylephrine (PHE) alone or in the presence of either 5-methylurapidil (MU), niguldipine (NIG) or BMY 7378 (BMY). The response is expressed as increase in the $^{86}\text{Rb}^+$ efflux rate index in percent of the maximal response to PHE stimulation in the absence of receptor antagonists. Values are given as mean values $\pm$ S.E.M. * $P < 0.01$, ** $P < 0.001$, vs PHE alone.

(22, 23). The α_1 -adrenoceptor mediated increase in $^{86}\text{Rb}^+$ efflux rate index was inhibited by BMY 7378 with a $-\log \text{IC}_{50}$ value of 6.6 ± 0.2 , corresponding to a calculated $\text{pK}_1 = 8.4$ (95% C.I. 8.2–8.6, Fig. 7). The response was reduced to $69 \pm 5.6\%$ of the maximal response (Fig. 5) by $1\ \mu\text{M}$ BMY 7378, without changing the time to reach the maximal response (19 ± 3.1 min, $n=5$). A higher concentration of BMY 7378 ($10\ \mu\text{M}$) gave no further statistically significant inhibition of the response ($66 \pm 6.8\%$ of maximal response, $n=11$).

DISCUSSION

We have studied the phenylephrine stimulated increase in $^{86}\text{Rb}^+$ efflux in the rat heart in the presence of subtype preferential antagonists against the α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor subtypes. The increased efflux was partially inhibited by antagonists against all three subtypes. The

Fig. 6. Effect of BMY 7378 on the phenylephrine stimulated increase in $^{86}\text{Rb}^+$ efflux rate index (in the presence of $1\ \mu\text{M}$ timolol). Each data point represents mean values of 5–9 separate hearts. Open circles: Efflux rate index in phenylephrine-stimulated hearts in the absence of α_1 -receptor antagonist ($n=9$). Closed circles: Efflux rate index in the presence of BMY 7378 ($1\ \mu\text{M}$, $n=5$). Ordinate: Increase in $^{86}\text{Rb}^+$ efflux rate index in percent of basal efflux rate. Abscissa: Time in min after addition of agonist (arrow).

Fig. 7. Concentration-response relationship for BMY 7378 and phenylephrine stimulated $^{86}\text{Rb}^+$ efflux rate (in the presence of $1\ \mu\text{M}$ timolol). Each heart was subjected to one concentration of antagonist only. Closed squares: Response in the presence of increasing concentrations of BMY 7378 (mean values $\pm$ S.E.M.). Line: Regression line constructed from a computer program. The concentration response curve was constructed from 46 separate hearts. Ordinate: Increase in $^{86}\text{Rb}^+$ efflux rate index in percent of the maximal response to phenylephrine stimulation in the absence of receptor antagonists. Abscissa: Log molar concentration of BMY 7378.

average maximal increase in $^{86}\text{Rb}^+$ efflux after phenylephrine stimulation was about 30%, which is in agreement with previously reported experiments from our laboratory (5) and with recently reported studies showing similar increases (in percent of the control) in both $^{86}\text{Rb}^+$ and $^{42}\text{K}^+$ efflux in the rat heart after α_1 -adrenoceptor stimulation (13).

Evidence for involvement of the α_{1B} -adrenoceptor subtype

The 50% reduction of the α_1 -adrenoceptor-stimulated increase in $^{86}\text{Rb}^+$ efflux after pretreatment with CEC in the present study shows the involvement of the α_{1B} -adrenoceptor subtype. This was supported by the effects of 5-MU without or with CEC pretreatment, showing a part of the response (about 30%) which was 5-MU insensitive, but was eliminated by CEC pretreatment. The 5-MU insensitive part of the response must be mediated mainly by the α_{1B} -adrenoceptor subtype since 5-MU has higher affinity for the α_{1D} -subtype than for the α_{1B} -adrenoceptor subtype (8). The alkylating substance CEC irreversibly and preferentially inactivates α_{1B} -adrenoceptors (24, 25), and the degree of inactivation depends on concentration, incubation time and temperature (26). The experimental conditions ($10\ \mu\text{M}$, CEC, 30 min, 31°C) were chosen in order to obtain inactivation of the major part of the α_{1B} -adrenoceptor population (27) with a minimum effect on the α_{1A} -adrenoceptors. CEC inactivates the α_{1D} -adrenoceptor subtype with an affinity that is intermediate between the affinity for α_{1B} - and α_{1A} -adrenoceptors (23), and about 50% of the α_{1D} -adrenoceptor population can be inactivated by CEC treatment (12, 28). A 25-min washout of CEC before phenylephrine stimulation prevented the possibility of remaining CEC acting as a reversible antagonist at the other α_1 -adrenoceptors (18, 26). As pretreatment with $10\ \mu\text{M}$ CEC in the rat heart reduced the maximal number of high affinity sites for prazosin by 86% (29), the present CEC treatment probably inactivated a major part of the α_{1B} -adrenoceptors. The results thus indicate that the α_{1B} -subtype apparently contributes to the α_1 -adrenoceptor stimulated $^{86}\text{Rb}^+$ efflux.

Evidence for involvement of the α_{1D} -adrenoceptor subtype

We found that BMY 7378 inhibited the phenylephrine evoked response in a concentration-dependent manner with a calculated pK_i value=8.4, corresponding to the value reported for the rat α_{1D} -adrenoceptor ($\text{pK}_i=8.2$, ref. 30). Thus most of the inhibitory effect of BMY 7378 is probably due to inhibition of the α_{1D} -adrenoceptors. As CEC is able to inactivate a part of the α_{1D} -adrenoceptor population (see above), the effect of BMY 7378 was thus

studied without CEC pretreatment of the hearts. BMY 7378 was recently found to be a selective (approximately 100-fold compared to the α_{1A} - and the α_{1B} -subtype) antagonist against the α_{1D} -adrenoceptor subtype (30–32). Most binding studies have shown that the rat heart contains α_{1A} - and α_{1B} -adrenoceptors in a 20 : 80% ratio (16, 33). Han and Minneman (20), however, found in the CEC pretreated rat heart a significant proportion of low affinity sites for 5-MU which could at least partly represent the α_{1D} -adrenoceptor subtype. Recent studies have found 5-MU to have affinity for the α_{1D} -adrenoceptor that is intermediate between the α_{1A} - and α_{1B} -adrenoceptor subtypes (12, 34). Thus the α_{1D} -subtype may escape detection when only CEC and 5-MU are used as antagonists. Very recently, BMY 7378 was shown to inhibit the prazosin binding and the inotropic response and phospholipase C activation by phenylephrine in rat myocardium (28). The contribution of α_{1D} -adrenoceptors to the inotropic response seems, however, to be small (35). The present study indicates that the α_{1D} -adrenoceptor subtype apparently contributes to the phenylephrine stimulated $^{86}\text{Rb}^+$ efflux in the rat heart.

Is the α_{1A} -adrenoceptor subtype involved?

The phenylephrine evoked response was extensively reduced by three different preferentially α_{1A} -adrenoceptor subtype antagonists. This could indicate the involvement of the α_{1A} -subtype in the response to phenylephrine. The reduction, however, appeared at concentrations that may influence also the α_{1B} - and the α_{1D} -adrenoceptor subtypes. The estimated pK_i value = 7.7 for 5-MU in CEC treated hearts was lower than the values usually found for the α_{1A} -subtype (mean 8.8, range 7.7–9.3; ref. 26). This indicates that 5-MU may have acted on the α_{1B} - (mean 7.1, range 6.0–7.8) or α_{1D} -adrenoceptor (intermediate affinity) subtypes (26), and makes a contribution from the α_{1A} -adrenoceptors somewhat uncertain.

There are, however, additional arguments in favor of an involvement of the α_{1A} -subtype in addition to the α_{1B} - and the α_{1D} -adrenoceptor subtypes. The relative reduction (in percent) of the response to phenylephrine by 5-MU was higher in CEC treated hearts than in untreated ones. 5-MU influenced the time course of the response (Fig. 2B) to phenylephrine differently compared to CEC and BMY 7378. While CEC treatment or the presence of BMY 7378 reduced the response to phenylephrine without affecting the time to the maximal response, 5-MU reduced the time to the maximal response significantly. A possible explanation for these differences may be that the different α_1 -adrenoceptor subtypes are coupled to different $^{86}\text{Rb}^+$ translocation mechanisms. The order of potency of (+)niguldipine and 5-MU with respect to the inhibitory effect (in CEC pretreated hearts) may also

suggest a contribution from the α_{1A} -adrenoceptors to the phenylephrine stimulated $^{86}\text{Rb}^+$ efflux. Thus the results as a whole can apparently best be accounted for by assuming that the α_{1A} -adrenoceptor subtype is also involved in the stimulated $^{86}\text{Rb}^+$ efflux.

Concluding remarks

The results of this study revealed that the increased $^{86}\text{Rb}^+$ efflux involves the α_{1D} - and the α_{1B} -adrenoceptors. Also the α_{1A} -adrenoceptor subtype seems to be involved in the response to phenylephrine, although these results are less clear. It is, however, difficult to quantify the contribution of the different receptor subtypes, because of the lack of complete selectivity of the antagonists available. The cross inhibition of the antagonists is obvious from the finding that the sum of inhibition (at maximal concentrations of antagonists) exceeded 100%. It is likely that several mechanisms are involved in the phenylephrine-induced increase of total $^{86}\text{Rb}^+$ efflux. This may be related to a contribution from all three α_1 -adrenoceptor subtypes, as they may at least partly be coupled to different translocation mechanisms. In conclusion, the demonstrated inhibition obtained by subtype selective antagonists indicates that the phenylephrine stimulated increase in $^{86}\text{Rb}^+$ efflux in the rat heart is not specifically linked to only one of the α_1 -adrenoceptor subtypes, but involves the α_{1B} - and the α_{1D} -adrenoceptor subtypes, and probably the α_{1A} -adrenoceptor subtype as well.

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REFERENCES

- 1 Terzic A, Puceat M, Vassort G and Vogel S: Cardiac α_1 -receptors: An Overview. *Pharmacol Rev* **42**, 147–175 (1993)
- 2 Hieble JP, Bondinell WE and Ruffolo RJ: Alpha- and beta-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. *J Med Chem* **38**, 3415–3444 (1995)
- 3 Osnes JB, Christiansen HB, Horgmo GI, Schiander IG and Skomedal T: Alpha-adrenergic stimulation contributes to the inotropic effect of noradrenaline. *Biomed Biochim Acta* **46**, 8–9 (1987)
- 4 Simpson PC, Kariya K, Karns LR, Long CS and Karliner JS: Adrenergic hormones and control of cardiac myocyte growth. *Mol Cell Biochem* **104**, 35–43 (1991)
- 5 Hanem S, Skomedal T and Osnes J-B: Increased $^{86}\text{Rb}^+$ efflux from perfused rat hearts exposed to α_1 -adrenergic stimulation. *J Cardiovasc Pharmacol* **22**, 350–355 (1993)
- 6 Hanem S, Enger M, Skomedal T and Osnes J-B: α_1 -Adrenoceptor stimulation changes heart K-balance. *Pharmacol Toxicol* **76**, Suppl II, 22 (1995)

- 7 Morrow AL and Creese I: Characterization of alpha 1-adrenergic receptor subtypes in rat brain: a reevaluation of [³H]WB4104 and [³H]prazosin binding. *Mol Pharmacol* **29**, 321–330 (1986)
- 8 Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP and Ruffolo RR Jr: International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: Consensus update. *Pharmacol Rev* **47**, 267–270 (1995)
- 9 Cotecchia S, Schwinn DA, Randall RR, Lefkowitz RJ, Caron MG and Kobilka BK: Molecular cloning and expression of the cDNA for the hamster alpha 1-adrenergic receptor. *Proc Natl Acad Sci USA* **85**, 7159–7163 (1988)
- 10 Schwinn DA, Lomasney JW, Lorenz W, Szklut PJ, Fremeau RT Jr, Yang-Feng TL, Caron MG, Lefkowitz RJ and Cotecchia S: Molecular cloning and expression of the cDNA for a novel alpha 1-adrenergic receptor subtype. *J Biol Chem* **265**, 8183–8189 (1990)
- 11 Lomasney JW, Cotecchia S, Lorenz W, Leung W-Y, Schwinn DA, Yang-Feng TL, Brownstein M, Lefkowitz RJ and Caron MG: Molecular cloning and expression of the cDNA for the α_{1A} -adrenergic receptor, the gene for which is located on human chromosome 5. *J Biol Chem* **266**, 6365–6369 (1991)
- 12 Perez DM, Piascik MT and Graham RM: Solution-phase library screening for the identification of rare clones: isolation of an alpha 1D-adrenergic receptor cDNA. *Mol Pharmacol* **40**, 876–883 (1991)
- 13 Andersen G, Enger M, Skomedal T and Osnes J-B: Concentration-response relationship of α_1 -adrenoceptor stimulated increase of ⁸⁶Rb⁺ efflux in rat heart. *Pharmacol Toxicol* **79**, 169–176 (1996)
- 14 Skomedal T, Schiander IG and Osnes J-B: Both alpha and beta adrenoceptor-mediated components contribute to final inotropic response to norepinephrine in rat heart. *J Pharmacol Exp Ther* **247**, 1204–1210 (1988)
- 15 Michel MC and Insel PA: Comparison of cloned and pharmacologically defined rat tissue alpha 1-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* **350**, 136–142 (1994)
- 16 Hanft G and Gross G: Subclassification of alpha 1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. *Br J Pharmacol* **97**, 691–700 (1989)
- 17 Gross G, Hanft G and Rugevics C: 5-Methyl-urapidil discriminates between subtypes of the alpha 1-adrenoceptor. *Eur J Pharmacol* **151**, 333–335 (1988)
- 18 Lazou A, Fuller SJ, Bogoyevitch MA, Orfali KA and Sugden PH: Characterization of stimulation of phosphoinositide hydrolysis by alpha 1-adrenergic agonists in adult rat hearts. *Am J Physiol* **267**, H970–H978 (1994)
- 19 Boer R, Grassegger A, Schudt C and Glossmann H: (+)-Niguldipine binds with very high affinity to Ca²⁺ channels and to a subtype of alpha 1-adrenoceptors. *Eur J Pharmacol* **172**, 131–145 (1989)
- 20 Han CD and Minneman KP: Interaction of subtype-selective antagonists with alpha 1-adrenergic receptor binding sites in rat tissues. *Mol Pharmacol* **40**, 531–538 (1991)
- 21 Cheng Y-C and Prusoff WH: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC₅₀) of an enzymatic reaction. *Biochem Pharmacol* **22**, 3099–3108 (1973)
- 22 Perez DM, Piascik MT, Malik N, Gaivin R and Graham RM: Cloning, expression, and tissue distribution of the rat homologue of the bovine alpha 1C-adrenergic receptor provide evidence for its classification as the alpha 1A subtype. *Mol Pharmacol* **46**, 823–831 (1994)
- 23 Schwinn DA, Johnston GI, Page SO, Mosley MJ, Wilson KH, Worman NP, Campbell S, Fidock MD, Furness LM, Parry-Smith DJ, Peter B and Bailey DS: Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. *J Pharmacol Exp Ther* **272**, 134–142 (1995)
- 24 Han C, Abel PW and Minneman KP: Heterogeneity of alpha 1-adrenergic receptors revealed by chloroethylclonidine. *Mol Pharmacol* **32**, 505–510 (1987)
- 25 Minneman KP, Han C and Abel PW: Comparison of alpha 1-adrenergic receptor subtypes distinguished by chloroethylclonidine and WB 4101. *Mol Pharmacol* **33**, 509–514 (1988)
- 26 Michel MC, Kenny B and Schwinn DA: Classification of alpha 1-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* **352**, 1–10 (1995)
- 27 Horie K, Hirasawa A and Tsujimoto G: The pharmacological profile of cloned and stably expressed alpha 1b-adrenoceptor in CHO cells. *Eur J Pharmacol* **268**, 399–407 (1994)
- 28 Deng XF, Chemtob S and Varma DR: Characterization of α_1 -adrenoceptor subtype in rat myocardium, aorta and other tissues. *Br J Pharmacol* **119**, 269–276 (1996)
- 29 Noguchi H, Muraoka R, Kigoshi S and Muramatsu I: Pharmacological characterization of alpha 1-adrenoceptor subtypes in rat heart: a binding study. *Br J Pharmacol* **114**, 1026–1030 (1995)
- 30 Goetz AS, King HK, Ward SD, True TA, Rimele TJ and Saussy DJ: BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. *Eur J Pharmacol* **272**, R5–R6 (1995)
- 31 Saussy DLJ, Goetz AS, King HK and True TA: BMY 7378 is a selective antagonist of α_{1D} adrenoceptors (AR): evidence that rat vascular α_1 AR are of the α_{1D} subtype. *Can J Physiol Pharmacol* **72**, 323 (1994)
- 32 Kenny BA, Chalmers DH, Philpott PC and Naylor AM: Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol* **115**, 981–986 (1995)
- 33 Michel MC, Hanft G and Gross G: Radioligand binding studies of alpha 1-adrenoceptor subtypes in rat heart. *Br J Pharmacol* **111**, 533–538 (1994)
- 34 Esbenshade TA, Hirasawa A, Tsujimoto G, Tanaka T, Yano J, Minneman KP and Murphy TJ: Cloning of the human alpha 1d-adrenergic receptor and inducible expression of three human subtypes in SK-N-MC cells. *Mol Pharmacol* **47**, 977–985 (1995)
- 35 Wang S-N, Jerrel Fontenot H and Kennedy RH: α_{1D} -Adrenoceptors play a little role in the positive inotropic action of phenylephrine. *Eur J Pharmacol* **321**, 39–43 (1997)