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Comparison of guinea-pig, bovine and rat α_1-adrenoceptor subtypes

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1 To elucidate a possible role of species differences in the classification of α_1-adrenoceptor subtypes, we have characterized the α_1-adrenoceptors in guinea-pig spleen, kidney and cerebral cortex and in bovine cerebral cortex using concentration-dependent alkylation by chloroethylclonidine and competitive binding with 5-methylurapidil, methoxamine, (+)-niguldipine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085, tamsulosin and (+)-tamsulosin. Rat liver α_{1B}-adrenoceptors were studied for comparison. Chloroethylclonidine-sensitivity and (+)-niguldipine affinity were also compared at cloned rat and bovine α_{1a}-adrenoceptors.

2 Chloroethylclonidine concentration-dependently inactivated α_1-adrenoceptors in all five tissues. While chloroethylclonidine inactivated almost all α_1-adrenoceptors in rat liver and guinea-pig kidney and brain, 20-30% of α_1-adrenoceptors in guinea-pig spleen and bovine brain were resistant to alkylation by 10 μM chloroethylclonidine. With regard to concentration-dependency guinea-pig kidney and brain were approximately 10 fold less sensitive than guinea-pig spleen or rat liver.

3 In rat liver, all drugs tested competed for [^{3}H]-prazosin binding with steep and monophasic curves. Drug affinities were relatively low and resembled most closely those of cloned rat α_{1b}-adrenoceptors.

4 In guinea-pig spleen, all drugs tested competed for [^{3}H]-prazosin binding with steep and monophasic curves. Drug affinities were relatively low and resembled most closely those of cloned rat α_{1b}-adrenoceptors.

5 In guinea-pig kidney most drugs tested competed for [^{3}H]-prazosin binding with steep and monophasic curves and had relatively low drug affinities close to those of cloned rat α_{1b}- and α_{1d}-adrenoceptors. However, noradrenaline and tamsulosin had consistently biphasic competition curves recognizing 36-39% high and 61-64% low affinity sites.

6 In guinea-pig cerebral cortex, all drugs tested competed for [^{3}H]-prazosin binding with shallow and biphasic curves. While most drugs recognized approximately 25% high affinity sites, tamsulosin and noradrenaline recognized approximately 50% high affinity sites. Drug affinities at the high and low affinity sites except those for tamsulosin and noradrenaline resembled those at cloned α_{1a}- and α_{1b}-adrenoceptors, respectively.

7 In bovine cerebral cortex all drugs tested except for noradrenaline competed for [^{3}H]-prazosin binding with shallow and biphasic curves. All drugs recognized approximately 70% high affinity sites. Drug affinities at the high and low affinity sites resembled those at cloned α_{1a}- and α_{1b}-adrenoceptors, respectively. Noradrenaline competition curves in bovine cerebral cortex were steep and monophasic.

8 When cloned rat and bovine α_{1a}-adrenoceptors transiently expressed in COS cells were studied in a direct side-by-side comparison, both species homologues had similar chloroethylclonidine-sensitivity and (+)-niguldipine affinity.

9 We conclude that properties of bovine α_{1A}- and α_{1B}-adrenoceptors are very similar to those of other species such as rat. α_1-Adrenoceptor subtypes in guinea-pigs resemble α_{1A}- and α_{1B}-adrenoceptors in other species but chloroethylclonidine sensitivity and competition binding profiles of noradrenaline and tamsulosin are not compatible with previously established α_1-adrenoceptor subtype classification.

Keywords: α_1-Adrenoceptor subtypes; rat, guinea-pig, bovine-adrenoceptors; chloroethylclonidine; (+)-niguldipine; noradrenaline; tamsulosin

Introduction

α_1-Adrenoceptors were originally subclassified according to pharmacological criteria into two subtypes, α_{1A} and α_{1B} (Minneman, 1988). In receptor cloning studies three distinct cDNAs encoding α_1-adrenoceptor subtypes have been detected (Cotecchia *et al.*, 1988; Schwinn *et al.*, 1990; Lomasney *et al.*, 1991). Human homologues of these subtypes have also been cloned (Forray *et al.*, 1994; Schwinn *et al.*, 1995). The alignment of pharmacologically defined and cloned subtypes has been controversial for some time (for review see Michel *et al.*, 1995). Based on a proposal by Ford *et al.* (1994) it has now been agreed by the IUPHAR nomenclature committee that α_{1A}-adrenoceptors (previously designated α_{1c}), α_{1B}-adrenoceptors and α_{1D}-adrenoceptors (previously designated α_{1a}- or $\alpha_{1a/d}$-adrenoceptors) are recognized; this nomenclature will be used throughout this manuscript; tissue and cloned α_1-adrenoceptor subtypes will be referred to by upper case and lower case subscripts, respectively.

Some of the controversy about α_1-adrenoceptor subtype nomenclature resulted from the fact that the original cloning of the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors was achieved from different species, i.e. hamster, cow and rat (Cotecchia *et al.*, 1988; Schwinn *et al.*, 1990; Lomasney *et al.*, 1991). In particular the original description of the bovine α_{1a}-adrenoceptor contained a surprisingly low affinity for the most α_{1A}-adrenoceptor-selective antagonist, (+)-niguldipine, and a surprisingly high sensitivity to alkylation by chloroethylclonidine (Schwinn *et al.*,

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1990; Schwinn & Lomasney, 1992). Moreover, the α_1-adrenoceptors in guinea-pig liver had characteristics similar to α_{1A}-adrenoceptors, but mRNA prepared from this tissue hybridized to a probe prepared from the rat α_{1d}- but not the bovine α_{1a}- or hamster α_{1b}-adrenoceptor (Garcia-Sainz *et al.*, 1992). These were the main reasons why the bovine clone was not originally recognized to encode an α_{1A}-adrenoceptor.

The alignment of pharmacologically defined and cloned α_1-adrenoceptor subtypes was mainly facilitated when all three subtypes were cloned within a species, i.e. rat (Forray *et al.*, 1994) and man (Laz *et al.*, 1994; Schwinn *et al.*, 1995). Nevertheless it has remained unclear whether the original discrepancies with data obtained in guinea-pigs and in cows are due to species differences of α_1-adrenoceptor subtype characteristics. Therefore, we have studied α_1-adrenoceptor binding properties of bovine cerebral cortex and guinea-pig spleen, kidney and cerebral cortex using [^{3}H]-prazosin competition binding studies. For this purpose a panel of nine subtype-selective drugs was used which we have previously characterized using identical methods in rat tissues (Büscher *et al.*, 1994; Michel *et al.*, 1993a; 1994) and with cloned subtypes (Büscher *et al.*, 1994; Michel & Insel, 1994). Additionally the quantitative sensitivity of guinea-pig and bovine α_1-adrenoceptors to alkylation by chloroethylclonidine was determined. Some of our data prompted us to perform similar experiments in a well defined model of homogeneous α_{1B}-adrenoceptors, rat liver (Gross *et al.*, 1988; Minneman *et al.*, 1988; Han & Minneman, 1991), and to investigate cloned rat and bovine α_{1a}-adrenoceptors transiently expressed in COS cells with regard to (+)-niguldipine affinities and chloroethylclonidine sensitivity.

Methods

Tissue preparation

Male Wistar rats (150–250 g) and guinea-pigs (200–300 g) were obtained from Lippische Versuchstierzucht (Exertal, Germany). Bovine brains were obtained from the local slaughterhouse. Rat and guinea-pig tissues were macroscopically freed from surrounding connective tissue, rapidly frozen in liquid nitrogen, stored at −70°C, and membranes were prepared on the day of the experiment. Membranes from bovine cerebral cortex were prepared freshly, and the membrane preparation was frozen in liquid nitrogen and stored at −70°C. Bovine and guinea-pig brain tissue was homogenized with 10 strokes at 1500 r.p.m. in a motor-driven glass homogenizer with a teflon pestle in ice-cold 20 mM $NaHCO_3$ solution. Rat liver and guinea-pig spleen and kidney were homogenized with an Ultra-Turrax once for 10 s at full speed and thereafter twice for 20 s each at 2/3 speed. The homogenates were centrifuged twice for 20 min each at 50,000 *g* at 4°C, and the final pellets were resuspended in binding buffer (see below) at protein concentrations of 2–3 mg ml^{-1} (rat liver, guinea-pig and bovine cerebral cortex) or 3–5 mg ml^{-1} (guinea-pig kidney and spleen). Protein concentration was determined by the method of Bradford (1976) with bovine IgG used as the standard.

α_1-Adrenoceptor transfection

The plasmid pBCα_{1c} which contains the entire coding region of the bovine α_{1a}-adrenoceptor (Schwinn *et al.*, 1990) was obtained from Dr S. Cotecchia (Lausanne, Switzerland). The plasmid pMT2'α_{1c} which contains the entire coding region of the rat α_{1a}-adrenoceptor (Perez *et al.*, 1994) was obtained from Dr R.M. Graham (Sydney, Australia). Both constructs were transfected into COS-1 cells for transient expression using the DEAE-dextran method with addition of chloroquine and dimethylsulphoxide steps as described previously (Suryanarayana & Kobilka, 1991; Michel & Insel, 1994). Four days after transfection, cells were harvested, resuspended in ice-cold buffer A and homogenized by a Tissuemizer for 10 s at full speed followed twice for 20 s at 2/3 speed. The homogenate was centrifuged for 20 min at 50,000 *g* and the resulting pellet was resuspended in binding buffer A at a concentration of 0.6–2 mg ml^{-1}.

Radioligand binding

Radioligand binding with [^{3}H]-prazosin as the ligand was performed as previously described (Michel *et al.*, 1993a). Briefly, aliquots of the membrane suspensions (100 µl) were incubated in a total volume of 1000 µl of binding buffer (50 mM Tris, 0.5 mM EDTA at pH 7.5) for 45 min at 25°C. In competition binding experiments with agonists 100 µM GTP was always added to prevent GDP-dependent formation of agonist high-affinity states. The incubation was terminated by rapid vacuum filtration over Whatman GF/C filters, and each filter was washed twice with 10 ml binding buffer. Following drying of the filters for 1 h at 60°C, 4 ml scintillator (Quickszint 1, Zinsser, Frankfurt, Germany) was added to each filter, and after vigorous shaking of each sample, radioactivity on the filters was quantified in a scintillation counter at 42% efficiency. Non-specific binding was defined as binding in the presence of 10 µM phentolamine. In some experiments membrane preparations were treated with the indicated concentrations of chloroethylclonidine or vehicle for 30 min at 37°C followed by two washout centrifugations prior to incubation with the radioligand.

Chemicals

Dye reagent for the protein assay was purchased from BioRad (Munich, Germany), oxymetazoline HCl, methoxamine HCl and (−)-noradrenaline bitartrate from Sigma (Munich, Germany), chloroethylclonidine HCl from Research Biochemicals Inc. (Natick, MA, U.S.A.) and [^{3}H]-prazosin (specific activity 80 Ci mmol^{-1}) from New England Nuclear (Dreieich, Ger-

Table 1 [^{3}H]-prazosin saturation binding parameters

Tissue	n	K_d (pM)	B_{max} (fmol mg^{-1} protein)	*Non-specific binding* (% of total)
Rat liver	3	244±24	106±4	14±2
Guinea-pig spleen	4	81±7	47±4	19±3
Guinea-pig kidney	4	114±34	12±3	35±10
Guinea-pig cerebral cortex	3	215±20	129±21	8±1
Bovine cerebral cortex	3	103±1	89±8	28±1

Data are derived from [^{3}H]-prazosin saturation binding experiments with 6 ligand concentrations. Non-specific binding was calculated in each experiment at the ligand concentration closest to the calculated K_d value. Data are mean±s.e.mean of the indicated number (*n*) of experiments.

many). The following drugs were gifts of the respective companies: tamsulosin HCl ((−)-isomer, formerly known as YM 617) and its stereoisomer (+)-tamsulosin HCl (Yamanouchi Pharmaceutical Co., Tokyo, Japan), phentolamine HCl (Ciba Geigy, Basel, Switzerland), 5-methylurapidil HCl and (+)-niguldipine (Byk Gulden, Konstanz, Germany), SDZ NVI-085 ((−)-(4a**R**,10a**R**)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methyl- thio-2H-naphth 2,3,b-1,4-oxazine HCl) Sandoz, Basel, Switzerland).

Data analysis

Data are shown as means ± s.e.mean of n experiments. Saturation binding experiments were analyzed by fitting rectangular hyperbolic functions to the experimental data. Competition binding experiments were analyzed by fitting mono-, bi- and triphasic sigmoidal functions to the experimental data; a two- or three-site fit was accepted only when it resulted in a significant improvement of the fit compared to a one- or two-site fit, respectively, as assessed by an F-test. K_i values were calculated from the IC_{50} values in the binding and functional experiments according to the equation

$$K_i = IC_{50}/1 + (L/K_d)$$

where L is the concentration of radioligand and K_d is its affinity. All curve fitting procedures were performed using the InPlot programme (GraphPAD Software, San Diego, Calif., U.S.A.). Statistical significance of differences was assessed by two-tailed t tests using the InStat programme (GraphPAD Software) and a $P<0.05$ was considered significant. Drug affinities determined in the tissues competition binding studies were compared to those found previously with cloned α_1-adrenoceptor subtypes (Büscher *et al.*, 1994; Michel & Insel, 1994) by correlation analysis; the slope factor and the squared correlation coefficient (r^2) of these analyses are given.

Results

[^{3}H]-prazosin saturation binding and effects of chloroethylclonidine treatment

Saturation binding experiments were performed in rat liver, bovine brain, and guinea-pig spleen, kidney and brain membranes. Specific [^{3}H]-prazosin binding in all five tissues was saturable and of high affinity with K_d values ranging between 80 and 250 pM (Table 1). α_1-Adrenoceptor density was greatest in guinea-pig cerebral cortex and smallest in guinea-pig kidney (Table 1). A small receptor density was associated with relatively high percentages of non-specific binding in some tissues, particularly in guinea-pig kidney (Table 1). Despite considerable efforts we were unable to detect quantifiable specific [^{3}H]-prazosin binding in membrane preparations from guinea-pig liver (data not shown).

Chloroethylclonidine treatment concentration-dependently reduced detectable α_1-adrenoceptor density in all five tissues (Figure 1). Treatment with 10 μM chloroethylclonidine almost completely inactivated α_1-adrenoceptors in rat liver and guinea-pig kidney and cerebral cortex. In contrast, 20–30% of α_1-adrenoceptors in guinea-pig spleen and bovine brain were resistant to inactivation by 10 μM chloroethylclonidine. For a 50% inactivation of α_1-adrenoceptors approximately 10 times higher chloroethylclonidine concentrations were required in guinea-pig kidney and brain compared to guinea-pig spleen and rat liver. While K_d values for [^{3}H]-prazosin could not reliably be calculated under conditions where almost all α_1-adrenoceptors were alkylated, no major change of K_d values was observed upon chloroethylclonidine treatment in the other experiments (data not shown). Chloroethylclonidine treatment also concentration-dependently reduced detectable density of cloned α_{1a}-adrenoceptors with rat and bovine receptors having similar sensitivity (Figure 2).

[^{3}H]-prazosin competition binding

In rat liver, 5-methylurapidil, methoxamine, (+)-niguldipine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085 and tamsulosin and (+)-tamsulosin competed for [^{3}H]-prazosin binding with steep and monophasic curves (Figure 3, Table 2). The affinities of all compounds at rat liver α_1-adrenoceptors were relatively low (Table 2) and correlated much better with those at cloned α_{1b}-adrenoceptors (slope 1.02 ± 0.09, $r^2 = 0.948$) than those at cloned α_{1a}- or α_{1d}-adrenoceptors (slope

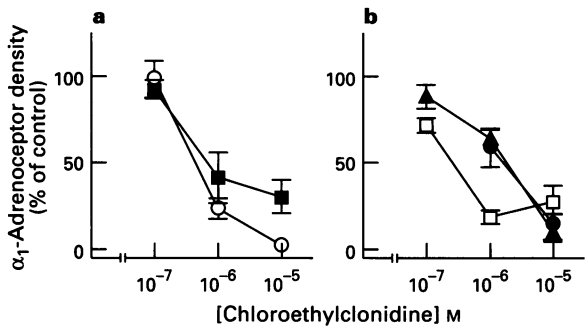

Figure 1 Inactivation of tissue α_1-adrenoceptors by chloroethylclonidine. Rat liver (○) and bovine brain (■) (a) and guinea-pig spleen (□), kidney (▲) and brain (●) (b) membranes were treated with the indicated concentrations of chloroethylclonidine for 30 min at 37°C. Following two washout centrifugations density (B_{max}) of remaining α_1-adrenoceptors was determined by [^{3}H]-prazosin saturation experiments. In some cases receptor alkylation by chloroethylclonidine was too extensive to allow reliable B_{max} determinations from fitting of the saturation binding data; in these cases the amount of specific binding at the highest [^{3}H]-prazosin concentration was assumed to represent B_{max}. Data are expressed as % of control (see Table 1), i.e. membranes which were treated identically in the absence of chloroethylclonidine within the same experiment, and are means ± s.e.mean of 4–5 experiments. Chloroethylclonidine treatment had no major effect on [^{3}H]-prazosin affinities (K_d values) in any tissue in all cases where saturation curves could be analyzed.

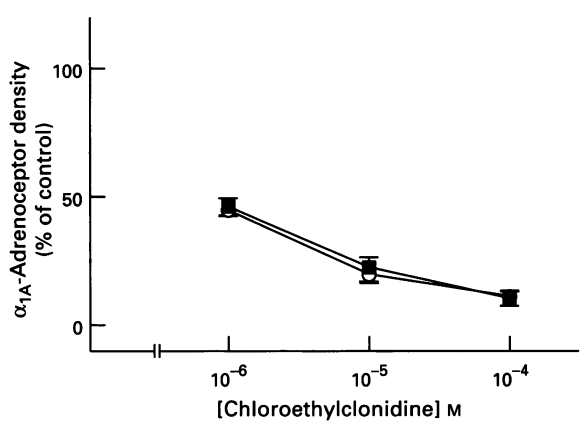

Figure 2 Inactivation of cloned rat and bovine α_{1a}-adrenoceptors by chloroethylclonidine. Membranes from COS cells transiently expressing cloned rat (○) and bovine α_{1a}-adrenoceptors (■) were treated with the indicated concentrations of chloroethylclonidine for 30 min at 37°C. Following two washout centrifugations, density (B_{max}) of remaining α_1-adrenoceptors was determined by [^{3}H]-prazosin saturation experiments. Data are expressed as % of control (3899 ± 242 and 3599 ± 326 fmol mg^{-1} protein for rat and bovine receptor, respectively), i.e. membranes which were treated identically in the absence of chloroethylclonidine within the same experiment, and are means ± s.e.mean of 4 experiments ($n = 3$ for the highest chloroethylclonidine concentrations at the bovine receptor). [^{3}H]-prazosin affinities (K_d values) were not significantly changed by chloroethylclonidine for either species homologue.

1.03±0.22, $r^2=0.760$ and slope 0.94±0.15, $r^2=0.848$, respectively), confirming the presence of a homogeneous population of α_{1B}-adrenoceptors in rat liver.

In guinea-pig spleen all drugs tested also competed for [^{3}H]-prazosin binding with steep and monophasic curves, and the calculated affinities were relatively low (Figure 4, Table 3). The affinities of all compounds at guinea-pig spleen α_1-adrenoceptors correlated much better with those at cloned α_{1b}-adrenoceptors (slope 1.01±0.08, $r^2=0.954$) than those at cloned α_{1a}- or α_{1d}-adrenoceptors (slope 1.04±0.20, $r^2=0.798$ and slope 0.91±0.16, $r^2=0.825$, respectively), indicating that guinea-pig spleen also contains a homogeneous population of α_{1B}-adrenoceptors.

In guinea-pig kidney similar results were obtained for most compounds (Figure 5, Table 4) but the overall data scatter was greater than in the other tissues investigated, possibly due to the smaller signal/noise ratio in this tissue (see above). However, noradrenaline and tamsulosin competed for [^{3}H]-prazosin binding with shallow and biphasic curves (Hill-slopes 0.65 and 0.63, respectively; Figure 5, Table 4). In such biphasic fits approximately 36–39% of all guinea-pig kidney α_1-adrenoceptors had high affinity for noradrenaline and tamsulosin with remaining sites having low affinity ($-\log K_{i\ high}$ 7.4 and 9.4 and $-\log K_{i\ low}$ 4.8 and 7.5, respectively). The affinities of all compounds (except for noradrenaline and tamsulosin) at guinea-pig kidney α_1-adrenoceptors correlated similarly well with those at cloned α_{1b}-adrenoceptors (slope 1.10±0.19, $r^2=0.865$) and α_{1d}-adrenoceptors (slope 0.87±0.14, $r^2=0.889$), but worse with those at cloned α_{1a}-adrenoceptors (slope 1.23±0.24, $r^2=0.786$); overall the correlation with cloned α_{1b}- or α_{1d}-adrenoceptor affinities generally was not as tight as that found for rat liver or guinea-pig spleen. Thus, the α_1-adrenoceptors in guinea-pig kidney are similar to but in some ways distinct from classical α_{1B}-adrenoceptors.

In guinea-pig cerebral cortex membranes, all drugs competed for [^{3}H]-prazosin binding with shallow and biphasic curves (Figure 6, Table 5). While most drugs recognized approximately 25% high affinity sites, noradrenaline and tamsulosin recognized approximately 50–51% high affinity sites. Drug affinities (except for noradrenaline and tamsulosin) at the high affinity sites of guinea-pig cerebral cortex correlated better with those at cloned α_{1a}-adrenoceptors (slope 0.85±0.10, $r^2=0.938$) than those at cloned α_{1b}- or α_{1d}-adrenoceptors (slope 0.75±0.10, $r^2=0.918$ and slope 0.49±0.17, $r^2=0.638$, respectively). In contrast drug affinities at the low affinity sites of guinea-pig cerebral cortex (except for noradrenaline and tamsulosin) correlated best with those at cloned α_{1b}-adrenoceptors (slope 1.03±0.09, $r^2=0.967$) and less so with those at cloned α_{1a}- or α_{1d}-adrenoceptors (slope 1.12±0.15, $r^2=0.917$ and slope 0.75±0.15, $r^2=0.843$, respectively). Thus, guinea-pig cerebral cortex appears to contain α_{1A}- and α_{1B}-like adrenoceptors but their interaction with noradrenaline and tamsulosin is distinct from those in most other tissues.

In bovine brain membranes all drugs except for noradrenaline competed for [^{3}H]-prazosin binding with shallow and biphasic curves in which approximately 70% of all sites had

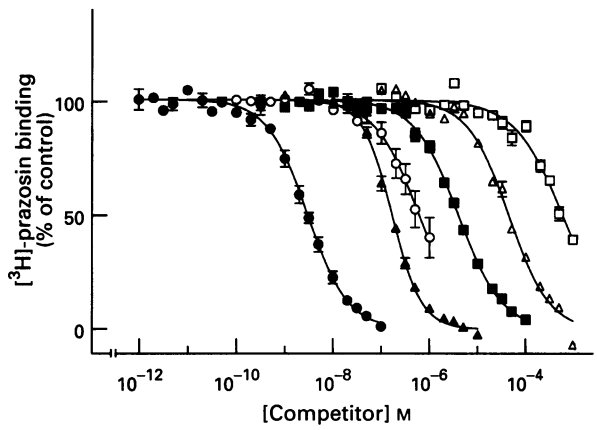

Figure 3 Competition binding in rat liver membranes. Competition curves are shown for tamsulosin (●), phentolamine (▲) (+)-niguldipine (○), noradrenaline (■), SDZ NVI-085 (△) and methoxamine (□). Data are means±s.e.mean of 3 experiments. A numerical analysis of these data is given in Table 2.

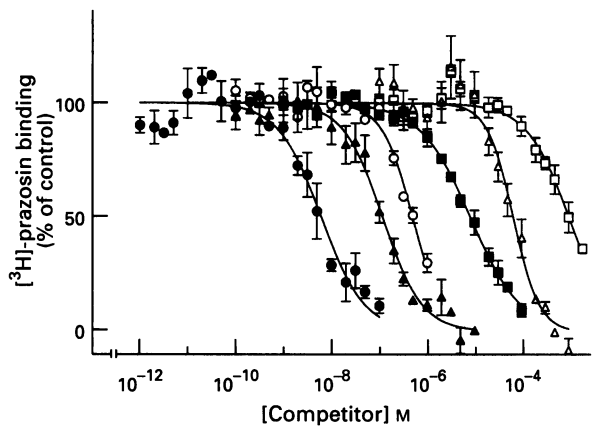

Figure 4 Competition binding in guinea-pig spleen membranes. Competition curves are shown for tamsulosin (●), phentolamine (▲), (+)-niguldipine (○), noradrenaline (■), SDZ NVI-085 (△) and methoxamine (□). Data are means±s.e.mean of 3 experiments. A numerical analysis of these data is given in Table 3.

Table 2 Drug affinities in rat liver

Drug	n	n_H	pK_i
5-Methylurapidil	3	0.92±0.10	6.28±0.39
Methoxamine	3	0.84±0.06	3.92±0.01
(+)-Niguldipine	3	1.00±0.05	6.90±0.10
Noradrenaline	3	0.95±0.04	6.11±0.01
Oxymetazoline	3	1.08±0.01	6.55±0.02
Phentolamine	3	1.41±0.05	7.53±0.03
SDZ NVI-085	3	1.01±0.02	4.88±0.04
Tamsulosin	3	1.04±0.01	9.21±0.06
(+)-Tamsulosin	3	1.10±0.06	6.87±0.09

Data are derived from competition binding experiments with 21 narrowly spaced concentrations of competitor. Agonist competition experiments were performed in the presence of 100 μM GTP. Data are mean±s.e.mean of the indicated number (n) of experiments. A graphic representation of the competition binding data is given in Figure 3.

Table 3 Drug affinities in guinea-pig spleen

Drug	n	n_H	pK_i
5-Methylurapidil	4	1.22±0.15	6.07±0.02
Methoxamine	3	1.09±0.04	3.74±0.01
(+)-Niguldipine	3	1.38±0.11	7.14±0.05
Noradrenaline	3	0.82±0.07	5.95±0.08
Oxymetazoline	3	1.13±0.34	6.59±0.11
Phentolamine	3	1.27±0.17	7.69±0.05
SDZ NVI-085	3	1.59±0.21	4.99±0.09
Tamsulosin	3	1.00±0.09	9.00±0.15
(+)-Tamsulosin	3	1.30±0.06	7.03±0.02

Data are derived from competition binding experiments with 21 narrowly spaced concentrations of competitor. Agonist competition experiments were performed in the presence of 100 μM GTP. Data are mean ± s.e.mean of the indicated number (n) of experiments. A graphic representation of the competition binding data is given in Figure 4.

Table 4 Drug affinities in guinea-pig kidney

Drug	n	n_H	$pK_{i\ high}$	$pK_{i\ low}$	*% high*
5-Methylurapidil	3	1.80±0.08	6.18±0.07	–	–
Methoxamine	4	1.38±0.47	4.05±0.21	–	–
(+)-Niguldipine	4	1.58±0.27	6.78±0.11	–	–
Noradrenaline	8	0.73±0.14	7.37±0.35	4.81±0.05	36±7
Oxymetazoline	3	1.15±0.34	6.28±0.13	–	–
Phentolamine	3	1.33±0.46	7.79±0.19	–	–
SDZ NVI-085	3	1.33±0.15	5.35±0.09	–	–
Tamsulosin	5	0.63±0.09	9.43±0.19	7.53±0.33	39±9
(+)-Tamsulosin	3	1.09±0.20	7.31±0.05	–	–

Data are derived from competition binding experiments with 21 narrowly spaced concentrations of competitor. Agonist competition experiments were performed in the presence of 100 μM GTP. Pseudo-Hill slopes (n_H) refer to data collected for a monophasic fit. When a two-site model explained the data significantly better this was accepted and data for pK_i high, pK_i low and percentage of high affinity sites (% high) were calculated. Data are mean ± s.e.mean of the indicated number (n) of experiments. A graphic representation of the competition binding data is given in Figure 5.

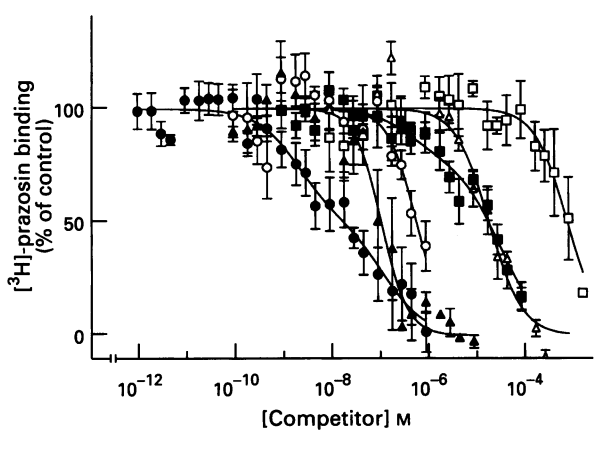

Figure 5 Competition binding in guinea-pig kidney membranes. Competition curves are shown for tamsulosin (●), phentolamine (▲), (+)-niguldipine (○), noradrenaline (■), SDZ NVI-085 (△) and methoxamine (□). Data are means ± s.e.mean of 3–8 experiments. A numerical analysis of these data is given in Table 4.

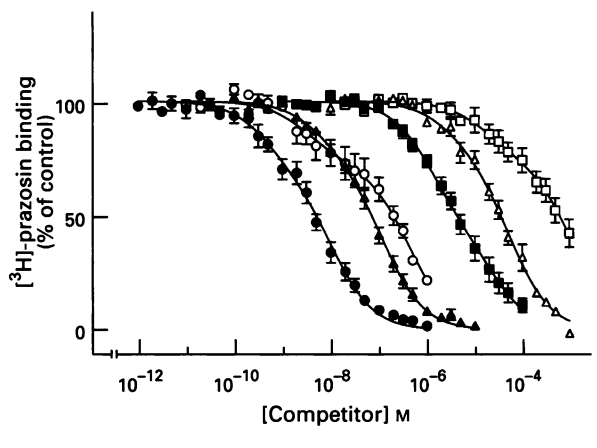

Figure 6 Competition binding in guinea-pig cerebral cortex membranes. Competition curves are shown for tamsulosin (●), phentolamine (▲), (+)-niguldipine (○), noradrenaline (■), SDZ NVI-085 (△) and methoxamine (□). Data are means ± s.e.mean of 3–8 experiments. A numerical analysis of these data is given in Table 5.

high affinity for the test compounds (Figure 7, Table 6). In contrast noradrenaline competition curves were steep and monophasic with relatively low affinity (Figure 7, Table 6). The drug affinities (except for noradrenaline) at the bovine brain high affinity sites correlated better with those at cloned α_{1a}-adrenoceptors (slope 1.03±0.12, $r^2=0.921$) than those at cloned α_{1d}-adrenoceptors (slope 0.91±0.17, $r^2=0.831$); correlation with affinities at the cloned α_{1b}-adrenoceptors (slope 1.01±0.04, $r^2=0.993$) was at least as good as that with the α_{1a}-adrenoceptors but affinities were generally 1.5 log units lower at the cloned α_{1b}-adrenoceptor than at the high affinity sites in bovine cerebral cortex. Drug affinities at the low affinity sites in bovine cerebral cortex correlated better with those at the cloned α_{1b}-adrenoceptor (slope 1.12±0.07, $r^2=0.982$) than

Table 5 Drug affinities in guinea-pig cerebral cortex

Drug	n	n_H	$pK_{i\ high}$	$pK_{i\ low}$	*% high*
5-Methylurapidil	5	0.63±0.06	8.27±0.37	6.52±0.23	31±4
Methoxamine	5	0.57±0.08	5.48±0.33	3.53±0.06	23±6
(+)-Niguldipine	3	0.54±0.13	8.93±0.08	6.86±0.07	27±9
Noradrenaline	9	0.75±0.05	6.55±0.09	5.15±0.15	50±8
Oxymetazoline	4	0.82±0.11	8.02±0.19	6.27±0.03	22±3
Phentolamine	4	0.77±0.02	8.86±0.16	7.50±0.03	25±4
SDZ NVI-085	4	0.83±0.03	6.16±0.16	4.68±0.02	21±7
Tamsulosin	8	0.78±0.06	9.53±0.45	7.83±0.31	51±10
(+)-Tamsulosin	4	0.69±0.08	8.49±0.17	6.72±0.07	19±4

Data are derived from competition binding experiments with 21 narrowly spaced concentrations of competitor. Agonist competition experiments were performed in the presence of 100 μM GTP. Pseudo-Hill slopes (n_H) refer to data collected for a monophasic fit. When a two-site model explained the data significantly better this was accepted and data for pK_i high, pK_i low and percentage of high affinity sites (% high) were calculated. Data are mean±s.e.mean of the indicated number (n) of experiments. A graphic representation of the competition binding data is given in Figure 6.

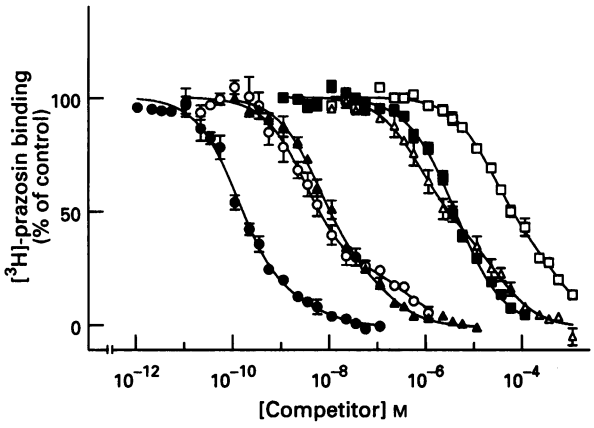

Figure 7 Competition binding in bovine cerebral cortex membranes. Competition curves are shown for tamsulosin (●), phentolamine (▲), (+)-niguldipine (○), noradrenaline (■), SDZ NVI-085 (△) and methoxamine (□). Data are means±s.e.mean of 3–10 experiments. A numerical analysis of these data is given in Table 6.

those at the cloned α_{1a}- or α_{1d}-adrenoceptor (slope 1.11±0.18, $r^2=0.869$ and slope 1.03±0.16, $r^2=0.869$, respectively). Thus, bovine cerebral cortex appears to contain typical α_{1A}- and α_{1B}-adrenoceptors.

In membranes from COS-1 cells expressing cloned rat or bovine α_{1a}-adrenoceptors (+)-niguldipine competed for [^{3}H]-prazosin with high affinity (−log K_i 9.79±0.10 and 10.25±0.06; $n=4$; Figure 8) indicating the absence of major species differences with regard to (+)-niguldipine affinity.

Discussion

While α_1-adrenoceptors were originally subclassified according to pharmacological criteria into two receptor subtypes, α_{1A} and α_{1B} (Minneman, 1988), three distinct α_1-adrenoceptor subtypes have been cloned (Bylund *et al.*, 1994). Extensive pharmacological characterization of expressed cloned subtypes and their comparison with natively expressed subtypes within the same species has now allowed the alignment of pharmacologically defined and cloned subtypes. Thus, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors are now recognized (Ford *et al.*, 1994; Michel *et al.*, 1995). This alignment, however, has remained controversial for some time mainly due to data obtained with the cloned bovine α_{1a}-adrenoceptor (Schwinn *et al.*, 1990; Schwinn & Lomasney, 1992) and with guinea-pig liver (Garcia-Sainz *et al.*, 1992). In particular it had been reported that the cloned bovine α_{1a}-adrenoceptor (previously designated α_{1c}) had greater chloroethylclonidine-sensitivity and at least 100 fold lower (+)-niguldipine affinity (Schwinn *et al.*, 1990; Schwinn & Lomasney 1992; Goetz *et al.*, 1994) than pharmacologically defined α_{1A}-adrenoceptors in rats (Boer *et al.*, 1989; Michel *et al.*, 1994). Moreover, the α_1-adrenoceptors in guinea-pig liver had characteristics similar to α_{1A}-adrenoceptors in radioligand binding studies, but mRNA prepared from this tissue hybridized to a probe prepared from the rat α_{1d}- but not the bovine α_{1a}- or hamster α_{1b}-adrenoceptor (Garcia-Sainz *et al.*, 1992). Therefore, we have studied α_1-adrenoceptor binding properties of guinea-pig spleen, kidney and cerebral cortex, and of bovine cerebral cortex using concentration-dependent alkylation by chloroethylclonidine and competition binding with a panel of drugs we have previously characterized in rat tissues and with cloned receptor subtypes (Michel *et al.*, 1993a; 1994; Michel & Insel, 1994; Büscher *et al.*, 1994); this panel includes 5-methylurapidil, methoxamine, (+)-niguldipine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085, tamsulosin and (+)-tamsulosin. Additionally, rat liver was studied as a well-defined model of α_{1B}-adrenoceptors and comparisons of cloned rat and bovine α_{1a}-adrenoceptors were performed with regard to chloroethylclonidine sensitivity and (+)-niguldipine affinity.

Numerous previous studies have identified a homogeneous population of α_{1B}-adrenoceptors in rat liver (Gross *et al.*, 1988; Minneman *et al.*, 1988; Han & Minneman, 1991). In our study all test compounds including the agonists noradrenaline, methoxamine and SDZ NVI-085 (in the presence of 100 μM GTP) had steep and monophasic competition curves in rat liver. The calculated affinities for all drugs were relatively low and matched those previously reported from our lab for another model system of homogeneous α_{1B}-adrenoceptors, rat spleen (Michel *et al.*, 1993a) and for cloned rat α_{1b}-adrenoceptor (Michel & Insel, 1994). Chloroethylclonidine treatment concentration-dependently inactivated α_1-adrenoceptors in rat liver, and full inactivation was achieved with 10 μM chloroethylclonidine. Taken together our data confirm that rat liver is a model system for a homogeneous α_{1B}-adrenoceptor population.

Despite extensive efforts we were unable to label guinea-pig liver α_1-adrenoceptors using [^{3}H]-prazosin, and other investigators have also been unsuccessful in this respect (Minneman, personal communication). Therefore, we have studied α_1-adrenoceptors in three other guinea-pig tissues, spleen, kidney and cerebral cortex. In guinea-pig spleen all drugs tested competed for [^{3}H]-prazosin binding with steep and monophasic curves indicating the presence of a homogeneous population of receptors. The relatively low affinity of all compounds resembled most closely those of cloned rat α_{1b}-adrenoceptors (Michel & Insel, 1994) and were nearly identical

Table 6 Drug affinities in bovine cerebral cortex

Drug	n	n_H	$pK_{i\ high}$	$pK_{i\ low}$	*% high*
5-Methylurapidil	7	0.67±0.09	8.51±0.28	6.49±0.12	66±5
Methoxamine	3	0.70±0.01	5.38±0.06	3.88±0.19	64±5
(+)-Niguldipine	3	0.67±0.03	9.27±0.16	7.06±0.13	77±1
Noradrenaline	10	0.91±0.03	6.06±0.03	–	–
Oxymetazoline	3	0.73±0.02	7.93±0.04	6.21±0.09	81±1
Phentolamine	3	0.74±0.04	8.97±0.08	7.43±0.27	78±4
SDZ NVI-085	3	0.62±0.02	6.67±0.12	5.09±0.12	59±5
Tamsulosin	4	0.80±0.11	10.78±0.09	8.78±0.37	83±3
(+)-Tamsulosin	4	0.69±0.04	8.56±0.27	6.80±0.18	67±9

Data are derived from competition binding experiments with 21 narrowly spaced concentrations of competitor. Agonist competition experiments were performed in the presence of 100 μM GTP. Pseudo-Hill slopes (n_H) refer to data collected for a monophasic fit. When a two-site model explained the data significantly better this was accepted and data for pK_i high, pK_i low and percentage of high affinity sites (% high) were calculated. Data are mean±s.e.mean of the indicated number (n) of experiments. A graphic representation of the competition binding data is given in Figure 7.

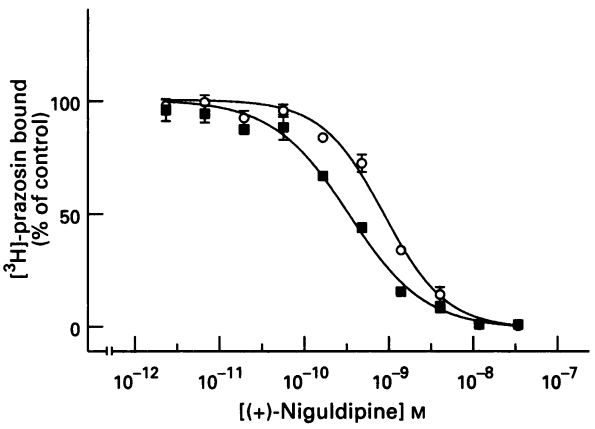

Figure 8 Competition binding of (+)-niguldipine for cloned rat and bovine α_{1A}-adrenoceptors. Experiments were performed on membranes from COS cells transiently expressing cloned rat (○) or bovine α_{1A}-adrenoceptors (■). Data are means±s.e.mean of 4–6 experiments.

to those we have found in rat liver in the present study. Recent functional data (Eltze, 1994) also demonstrate that contraction of guinea-pig spleen is mediated by an α_{1B}-adrenoceptor based on experiments using chloroethylclonidine and various antagonists; the potencies (pK_b values) for 5-methylurapidil, (+)-niguldipine, phentolamine and tamsulosin in that study were similar to the $-\log K_i$ values obtained in the present study. In our study chloroethylclonidine was similarly potent in guinea-pig spleen and α_{1B}-adrenoceptors in rat liver (present study) or rat spleen (Michel *et al.*, 1993b) but in contrast to the two rat tissues a full inactivation was not achieved by 10 μM chloroethylclonidine. Thus, guinea-pig spleen appears to contain a homogeneous population of α_{1B}-like -adrenoceptors but they are not as efficiently alkylated by chloroethylclonidine as the rat α_{1B}-adrenoceptor.

The α_1-adrenoceptor in guinea-pig kidney was difficult to characterize due to a small receptor number and thus small signal/noise ratio. Our data indicate that it is generally α_{1B}-adrenoceptor-like but distinct in some aspects: as in rat liver and guinea-pig spleen, 5-methylurapidil, methoxamine, (+)-niguldipine, oxymetazoline, phentolamine, SDZ NVI-085 and (+)-tamsulosin had steep and monophasic competition curves indicating the presence of a homogeneous α_1-adrenoceptor population. The affinities of these drugs were similar to those in rat liver, guinea-pig spleen or with cloned α_{1b}-adrenoceptors. However, noradrenaline and tamsulosin surprisingly competed for [^{3}H]-prazosin binding with shallow and biphasic curves, indicating the possible coexistence of two subtypes in an approximate 35–40/65–60% ratio in guinea-pig kidney. To the best of our knowledge, no subtype of α_1-adrenoceptors has been proposed until now which differs from rat α_{1B}-adrenoceptors based on tamsulosin and noradrenaline affinities but is not discriminated by 5-methylurapidil, methoxamine, (+)-niguldipine, oxymetazoline, phentolamine, SDZ NVI-085 and (+)-tamsulosin. Moreover, 10 μM chloroethylclonidine inactivated guinea-pig kidney α_1-adrenoceptors as efficiently as the α_{1B}-adrenoceptors in rat liver (present study) or spleen (Michel *et al.*, 1993b); however, chloroethylclonidine was approximately 10 times less potent than in rat liver and spleen or in guinea-pig spleen. Thus, the α_1-adrenoceptor in guinea-pig kidney resembles an α_{1B}-adrenoceptor with regard to affinities for most drugs but differs in noradrenaline and tamsulosin affinity and chloroethylclonidine-sensitivity.

In guinea-pig cerebral cortex, all drugs exhibited shallow and biphasic competition curves indicating the presence of at least two α_1-adrenoceptor subtypes. Most compounds indicated that one of them represents approximately 25% of all receptors and has drug affinities similar to the α_{1A}-adrenoceptors in rat cerebral cortex (Michel *et al.*, 1993a; Büscher *et al.*, 1994) or cloned bovine α_{1a}-adrenoceptors (Büscher *et al.*, 1994; Michel & Insel, 1994). The other represents approximately 75% of all receptors and has drug affinities similar to those at α_{1B}-adrenoceptors in guinea-spleen and rat liver (see above), rat spleen or cloned rat α_{1b}-adrenoceptors (Michel *et al.*, 1993a; Büscher *et al.*, 1994; Michel & Insel, 1994). However, tamsulosin and noradrenaline again behaved differently from the other drugs. Thus, both drugs had similar biphasic competition curves but recognized approximately 55% high affinity sites. The aberrant behaviour of noradrenaline and tamsulosin in guinea-pig brain also confirms that its biphasic competition curves in guinea-pig kidney are not related to the small signal/noise ratio in that tissue. Although 25% of the receptors in cortex were α_{1A}-like, 10 μM chloroethylclonidine caused an almost complete inactivation; in contrast this chloroethylclonidine concentration had not inactivated α_{1A}-adrenoceptors in rat cerebral cortex or kidney (Michel *et al.*, 1993a). On the other hand, chloroethylclonidine was less potent in guinea-pig cerebral cortex than in rat liver and spleen or guinea-pig spleen (see above). Thus, guinea-pig cerebral cortex appears to express an α_{1A}- and an α_{1B}-like -adrenoceptor in an approximate 25:75% ratio, but data with tamsulosin, noradrenaline and chloroethylclonidine do not readily fit this concept.

Thus, the question exists why noradrenaline and tamsulosin recognized an α_1-adrenoceptor subtype in guinea-pig kidney and cerebral cortex which is not recognized by a panel of seven other subtype-selective drugs. While we do not have a definitive answer to this question, out data can exclude several obvious possibilities. Firstly, biphasic noradrenaline competition curves are unlikely to represent G-protein-dependent agonist high and low affinity states because our experimental conditions routinely included a high concentration of GTP, and

because under identical conditions noradrenaline competition curves were steep and monophasic in rat spleen and cerebral cortex (Michel *et al.*, 1993a), rat liver and guinea-pig spleen (present study). Moreover, methoxamine and SDZ NVI-085 did not show a distinct binding pattern despite being agonists, and tamsulosin recognized a similar percentage of high affinity sites as noradrenaline although it is an antagonist. Secondly, noradrenaline is selective for cloned α_{1d}- relative to α_{1a}- or α_{1b}-adrenoceptors (Forray *et al.*, 1994; Laz *et al.*, 1994; Michel & Insel, 1994; Schwinn *et al.*, 1995) while tamsulosin is selective for α_{1A}- over α_{1B}-adrenoceptors and has similar affinity for α_{1a}- and α_{1d}-adrenoceptors (Michel *et al.*, 1993a; Michel & Insel, 1994). However, differential recognition of one of these subtypes is unlikely to explain our observations since our panel of test compounds included others which are also selective for α_{1a}- and α_{1d}- relative to α_{1b}-adrenoceptors in our hands, such as methoxamine, SDZ NVI-085 and (+)-tamsulosin (Michel & Insel, 1994). Finally, noradrenaline also fails to discriminate α_{1A}- and α_{1B}-adrenoceptors natively expressed in tissues such as rat cerebral cortex, and tamsulosin and (+)-tamsulosin exhibit a similar α_1-adrenoceptor subtype profile in other models (Michel *et al.*, 1993a; Michel & Insel, 1994). Thus, further studies possibly involving the cloning of guinea-pig homologues of the known α_1-adrenoceptor subtypes are necessary to determine whether the receptors in this species display unusual characteristics or whether an additional yet undefined subtype exists. Before this question is resolved, the guinea-pig should only be used with great caution as an α_1-adrenoceptor subtype model system.

In bovine cerebral cortex all drugs tested except for noradrenaline competed for [^{3}H]-prazosin binding with shallow and biphasic curves. All of these drugs detected a similar percentage of high affinity sites. Since noradrenaline does not discriminate α_{1a}- and α_{1b}-adrenoceptors in rats or man (Forray *et al.*, 1994; Laz *et al.*, 1994; Schwinn *et al.*, 1995) and since the affinities of the other test compounds at the high and low affinity sites were similar to those previously described in our lab for cloned α_{1a}- and α_{1b}-adrenoceptors (Michel & Insel, 1994), we conclude that the α_1-adrenoceptor subtypes found in bovine cerebral cortex have similar pharmacological characteristics as the α_{1A}- and α_{1B}-adrenoceptors found in other species such as rat or man. In particular, (+)-niguldipine recognized the same percentage of high and low affinity sites as the other subtype-selective drugs, and its affinities are similar to those at α_{1A}- and α_{1B}-adrenoceptors in other species such as rat or man (Boer *et al.*, 1989; Hanft & Gross, 1989; Han & Minneman, 1991; Michel *et al.*, 1992). Thus, our data do not support the idea that (+)-niguldipine affinities at bovine α_1-adrenoceptor subtypes differ from those in other species.

In contrast it has been reported that (+)-niguldipine has only low affinity for cloned bovine α_{1a}-adrenoceptors (Schwinn & Lomasney, 1992; Goetz *et al.*, 1994). On the other hand it has been found that (+)-niguldipine has high affinity at cloned rat (Laz *et al.*, 1994; Perez *et al.*, 1994) or human (Forray *et al.*, 1994; Weinberg *et al.*, 1994) α_{1a}-adrenoceptors. Therefore, we have compared the affinity of (+)-niguldipine at cloned rat and bovine α_{1a}-adrenoceptors directly. Our data demonstrate that the affinity of bovine α_{1a}-adrenoceptors for (+)-niguldipine is at least as high as that of its rat homologue. While we are not aware of other direct comparisons between rat and bovine α_{1a}-adrenoceptors, it should be noted that other recent studies also have detected relatively high (+)-niguldipine affinities at bovine α_{1a}-adrenoceptors (Testa *et al.*, 1995). While we cannot explain the discrepancies among reported (+)-niguldipine affinities at cloned bovine α_{1a}-adrenoceptors, our data do not support the idea that cows differ from other species in this respect.

In bovine brain, chloroethylclonidine concentration-dependently inactivated α_1-adrenoceptors as it did in the other tissues investigated. In bovine brain approximately 30% of receptors remained unaffected by chloroethylclonidine when tested at 10 μM, but its potency was less than in rat α_{1B}-adrenoceptor tissues such as liver (present study) or spleen (Michel *et al.*, 1993b). In contrast the competitively acting drugs had recognized approximately 70% high affinity sites (putative α_{1A}-adrenoceptors) in this tissue. Thus, the possibility exists that the bovine α_{1A}-adrenoceptor is more chloroethylclonidine-sensitive than that of other species. To test this hypothesis directly, we have quantitatively compared the chloroethylclonidine sensitivity of cloned rat and bovine α_{1a}-adrenoceptors. Our results suggest that this receptor has a very similar chloroethylclonidine-sensitivity in both species. While it has originally been assumed that α_{1A}-adrenoceptors are resistant to the alkylating effects of chloroethylclonidine (Minneman *et al.*, 1988), more recent data suggest that chloroethylclonidine-sensitivity is a quantitative rather than a qualitative feature of α_1-adrenoceptor subtypes among which the α_{1a}-adrenoceptor is the least sensitive (Forray *et al.*, 1994; Laz *et al.*, 1994; Schwinn *et al.*, 1995). While the degree of α_{1a}-adrenoceptor inactivation is greater in our study than in previous reports (Forray *et al.*, 1994; Laz *et al.*, 1994; Schwinn *et al.*, 1995), it should be noted that we have used longer incubation times than those authors and that the alkylating chloroethylclonidine effects at a given subtype depend on time, concentration and incubation temperature. Thus, our data suggest that bovine α_{1A}- and α_{1B}-adrenoceptors are pharmacologically similar to those in other species such as rat or man with regard to the affinities of competitively acting drugs.

A general finding of our study was that chloroethylclonidine sensitivity of α_1-adrenoceptor subtypes does not always correspond to the presence of α_{1A}- and α_{1B}-like adrenoceptors as defined by competitively acting drugs. Thus, model systems which appear otherwise similar (e.g. rat liver and spleen and guinea-pig spleen and kidney) may differ with regard to the efficiency and/or potency of chloroethylclonidine to cause inactivation. This could partly result from the small signal/noise ratio upon chloroethylclonidine treatment, particularly in tissues with a low receptor expression density. Thus chloroethylclonidine, particularly when used at a single concentration, may give misleading information with regard to α_1-adrenoceptor subtype characterization. This problem is further enhanced since chloroethylclonidine may also inactivate α_{1A}- and α_{1D}-adrenoceptors (Schwinn *et al.*, 1995) and α_{2A}-and α_{2C}-adrenoceptors (Michel *et al.*, 1993b) under certain conditions, and may also have competitive antagonistic effects at α-adrenoceptor subtypes which are not alkylated (Michel *et al.*, 1993b).

Taken together our data demonstrate that the pharmacological characteristics of α_1-adrenoceptor subtypes are quite similar in rats, cows and man, and that species differences are not readily detectable among them. In contrast, data in guinea-pigs are not fully explained by the current α_1-adrenoceptor subtype classification. Clear differences are detectable for noradrenaline and tamsulosin in competition binding studies and for chloroethylclonidine sensitivity. While the reasons for these differences are not clear, our data suggest that attention should be paid to possible species heterogeneity in future studies, in particular when guinea-pigs are used.

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