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European Journal of Pharmacology 342 (1998) 85–92

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Differential α_1-adrenoceptor labeling by [^{3}H]prazosin and [^{3}H]tamsulosin

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Received 21 July 1997; revised 16 September 1997; accepted 10 October 1997

Abstract

The radioligand binding properties of [^{3}H]prazosin and [^{3}H]tamsulosin at α_1-adrenoceptors of several rat tissues, human prostate and cloned rat and human α_1-adrenoceptor subtypes were compared in Tris/EDTA buffer unless otherwise indicated. The affinity of [^{3}H]tamsulosin at tissue and cloned α_{1A}- and α_{1B}-adrenoceptors was somewhat greater and smaller, respectively, than that of [^{3}H]prazosin. In most rat tissues and at cloned rat α_{1A}- and α_{1B}-adrenoceptors, [^{3}H]tamsulosin had a smaller B_{max} than [^{3}H]prazosin. Studies with rat liver showed that this was due to considerably poorer labeling of agonist low affinity sites, while both radioligands detected similar numbers of agonist high affinity sites. Statistically significant differences in the number of binding sites for both ligands were not detected in HEPES or glycylglycine buffer, as the detectable receptor number for [^{3}H]prazosin and [^{3}H]tamsulosin tended to be smaller and greater, respectively, in these than in Tris/EDTA buffer. Among human α_1-adrenoceptor subtypes [^{3}H]tamsulosin labeled fewer sites than [^{3}H]prazosin for α_{1B}- but more sites for α_{1A}- and α_{1D}-adrenoceptors. We conclude that [^{3}H]prazosin and [^{3}H]tamsulosin do not detect the same number of α_1-adrenoceptors under a variety of conditions. This should be taken into account in the interpretation of data obtained with either radioligand. © 1998 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor; Tamsulosin; Prazosin; Radioligand binding

1. Introduction

α_1-Adrenoceptors mediate many physiologically important functions including contraction of vascular and prostatic smooth muscle (Ruffolo and Hieble, 1994). Therefore, α_1-adrenoceptor antagonists are used clinically in the treatment of arterial hypertension and benign prostatic hyperplasia. α_1-Adrenoceptor antagonists can also be radiolabeled and then used as radioligands for experimental studies. Prazosin was the first selective α_1-adrenoceptor antagonist in clinical use, and [^{3}H]prazosin has been widely used as the standard radioligand for the quantification and characterization of α_1-adrenoceptors. More recently, tamsulosin has been introduced clinically for the treatment of benign prostatic hyperplasia (Wilde and McTavish, 1996), and [^{3}H]tamsulosin has been introduced as a radioligand for the detection of α_1-adrenoceptors in prostate and other tissues (Yazawa et al., 1992). The use of [^{3}H]tamsulosin has resulted in the detection of α_1-adrenoceptors in tissues where this has not been possible using [^{3}H]prazosin or [^{125}I]BE 2254 (2-[β-(4-hydroxyphenyl)-ethyl-aminomethyl]-tetralone), e.g. in guinea pig liver (Garcia-Sainz et al., 1995), rat prostate (Yazawa and Honda, 1993) or human urethra (Taniguchi et al., 1997).

On the other hand, direct comparative studies of [^{3}H]prazosin and [^{3}H]tamsulosin have yielded surprising results: Thus, [^{3}H]tamsulosin was reported to label a greater or smaller number of α_1-adrenoceptors than [^{3}H]prazosin in rat hippocampus and spleen, respectively (Yazawa et al., 1992), while both ligands were reported to label a similar number of α_1-adrenoceptors in human prostate (Yamada et al., 1994). This implies that one of the two radioligands does not label all α_1-adrenoceptors and/or that one of them also labels additional sites which are not α_1-adrenoceptors. However, no comprehensive comparisons between radioligand binding properties of [^{3}H]prazosin and [^{3}H]tamsulosin have been presented. Therefore, we have performed a systematic side-by-side comparison of

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[^{3}H]prazosin and [^{3}H]tamsulosin in various rat tissues and human prostate and with cloned rat and human α_1-adrenoceptor subtypes.

2. Material and methods

2.1. Receptor sources

Male Wistar rats (250–350 g) were obtained from the local breeding facility at the Zentrales Tierlabor of the University of Essen. Human prostate was obtained from patients undergoing radical cystectomy. The expression vector plasmids pCMVα_{1a} containing the EcoR1-Pst1 2520 bp fragment of the rat α_{1D}-adrenoceptor cDNA and pcDV1Rα_{1b} containing a 2573 bp fragment including the entire coding region of the rat α_{1B}-adrenoceptor cDNA (Lomasney et al., 1991) were obtained from Dr. R.J. Lefkowitz (Durham, NC, USA). 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). All three constructs were transfected into COS-1 cells for transient expression using the dietyhlaminoethyl-dextran method with addition of chloroquine and dimethylsulfoxide steps as described previously (Michel and Insel, 1994). Rat-1 fibroblasts which had been transfected with cDNAs encoding the human α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors (Schwinn et al., 1995) were kindly provided by Dr. Geoff Johnston at Pfizer Central Research (Sandwich, Kent, UK). Membranes were prepared from rat and human tissues and cells expressing the cloned subtypes as described previously (Michel et al., 1993; Michel et al., 1996). The final pellets were resuspended in Tris/EDTA buffer (see below) except for the experiments where the effects of HEPES or glycylglycine buffer were tested.

2.2. Radioligand binding

Radioligand binding for [^{3}H]prazosin and [^{3}H]tamsulosin was performed as described recently (Michel et al., 1993, 1996). Briefly, experiments were performed in a total volume of 1000 μl during a 45-min incubation at 25°C. Unless otherwise indicated experiments were performed in binding buffer containing 50 mM Tris and 0.5 mM EDTA at pH 7.5. In some experiments, 20 mM HEPES or 10 mM glycylglycine was used as the binding buffer, or the binding buffer was supplemented with $MgCl_2$, GTP and/or NaCl as indicated. Incubations were terminated by rapid vacuum filtration over Whatman GF/C filters, and each filter was rinsed with 20 ml binding buffer. To avoid insufficient labeling of α_{1B}-adrenoceptors by [^{3}H]tamsulosin, the highest [^{3}H]tamsulosin concentration in all tissue saturation binding experiments was at least 2000 pM and at least 3–4 times the estimated K_d value for the respective tissue. In each experiment, [^{3}H]prazosin and [^{3}H]tamsulosin were tested in parallel. In time course experiments [^{3}H]tamsulosin binding (500 pM) to all three cloned human subtypes and to rat liver and spleen was in equilibrium at incubation times of 30–60 min (data not shown).

2.3. Chemicals

[^{3}H]Prazosin (specific activity 74 Ci/mmol) and [^{3}H]tamsulosin (also known as [^{3}H]YM 617, specific activity 44 Ci/mmol) were obtained from New England Nuclear (Dreieich, Germany). (−)-Noradrenaline bitartrate was obtained from Sigma (Deisenhofen, Germany). Phentolamine HCl was a gift from Novartis (Basel, Switzerland).

2.4. Data analysis

Saturation binding experiments were analyzed by fitting rectangular hyperbolic functions to the experimental data using the Inplot programme (Graphpad Software, San Diego, CA, USA). Competition binding experiments were analyzed by fitting mono- or biphasic sigmoidal functions to the experimental data; a biphasic model was accepted only when it resulted in a significantly better fit as judged by an *F*-test. Statistical significance of differences be-

Table 1
Comparison of [^{3}H]prazosin and [^{3}H]tamsulosin saturation binding parameters in rat tissues

Tissue	*n*	K_d, pM		NSB, % of total		B_{max}, fmol/mg protein		
		[^{3}H]prazosin	[^{3}H]tamsulosin	[^{3}H]prazosin	[^{3}H]tamsulosin	[^{3}H]prazosin	[^{3}H]tamsulosin	[^{3}H]tamsulosin as % of [^{3}H]prazosin
Rat liver	6	101 ± 18	370 ± 54[b]	3.2 ± 0.3	7.7 ± 1.2[b]	70.4 ± 8.5	39.4 ± 5.9[c]	54 ± 2[c]
Rat spleen	4	46 ± 4	226 ± 3[c]	5.0 ± 0.4	8.5 ± 0.3[b]	40.1 ± 6.0	30.2 ± 4.9[b]	75 ± 5[b]
Rat brain	5	93 ± 15	85 ± 6	3.8 ± 0.9	2.4 ± 0.4	107.2 ± 14.5	93.8 ± 13.1	88 ± 5
Rat kidney	5	71 ± 8	70 ± 11	6.6 ± 0.7	4.4 ± 0.7[a]	45.6 ± 8.0	37.8 ± 10.0	80 ± 6[a]
Rat lung	4	57 ± 8	113 ± 22[a]	15.5 ± 2.3	12.3 ± 0.9	32.5 ± 1.4	27.4 ± 1.5[b]	84 ± 4[b]
Rat heart	4	75 ± 20	120 ± 20	18.0 ± 4.0	17.8 ± 2.3	39.6 ± 3.0	23.9 ± 1.6[b]	61 ± 1[c]
Human prostate	5	189 ± 62	82 ± 32[a]	32.8 ± 4.7	14.2 ± 1.8[b]	55.8 ± 14.5	44.0 ± 6.5	83 ± 10

Data are means ± S.E.M. from the indicated number of experiments (*n*).
[a], [b] and [c]: $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, in a paired two-tailed *t*-test or in a two-tailed one-sample *t*-test, as appropriate.

Table 2
Comparison of [^{3}H]prazosin and [^{3}H]tamsulosin saturation binding parameters at cloned rat and human α_1-adrenoceptor subtypes

Tissue	n	K_d		B_{max}		
		[^{3}H]prazosin, pM	[^{3}H]tamsulosin, pM	[^{3}H]prazosin, fmol/mg protein	[^{3}H]tamsulosin, fmol/mg protein	[^{3}H]tamsulosin, % of [^{3}H]prazosin
Rat α_{1A}	4	152 ± 23	85 ± 22	12063 ± 1479	10810 ± 1550[a]	89 ± 2[a]
Rat α_{1B}	4	165 ± 47	511 ± 59[a]	15174 ± 987	8428 ± 1004[b]	56 ± 6[b]
Rat α_{1D}	4	132 ± 15	113 ± 4	1161 ± 44	1050 ± 39	91 ± 3
Human α_{1A}	5	130 ± 29	37 ± 6[a]	1042 ± 292	1359 ± 256[a]	147 ± 24
Human α_{1B}	5	127 ± 12	223 ± 9[b]	5960 ± 1096	5020 ± 856[a]	85 ± 32[b]
Human α_{1D}	5	90 ± 13	69 ± 3	147 ± 12	176 ± 11[a]	121 ± 6[a]

Data are means ± S.E.M. from the indicated number of experiments (n).
[a] and [b]: $P < 0.05$ and $P < 0.01$, respectively, in a paired two-tailed t-test or in a two-tailed one-sample t-test, as appropriate.

tween binding parameters of [^{3}H]prazosin and [^{3}H]tamsulosin was determined by paired, two-tailed *t*-tests. When the binding of a given ligand under multiple conditions was analyzed, repeated measures analysis of variance was performed; if this indicated that the variance between groups was significantly greater than that within groups, the groups were compared by Dunnett's multiple comparison test. All statistical calculations were performed with the Instat programme (Graphpad Software), and a $P < 0.05$ was considered significant.

3. Results

In saturation binding experiments in rat tissues and human prostate, [^{3}H]prazosin and [^{3}H]tamsulosin differed in three ways: Firstly, the affinity of [^{3}H]tamsulosin was significantly lower than that of [^{3}H]prazosin in tissues known to express a homogeneous population of α_{1B}-adrenoceptors, i.e. rat liver and spleen, while it was significantly higher in a tissue known to express mainly α_{1A}-adrenoceptors, i.e. human prostate; the affinities were not significantly different in tissues known to coexpress multiple α_1-adrenoceptor subtypes, e.g. rat brain, kidney and heart (Table 1). Secondly, the percentage of non-specific binding as defined by 10 μM phentolamine at the radioligand concentration closest to its K_d was similar in rat brain, lung and heart; in rat liver and spleen, [^{3}H]tamsulosin had significantly more non-specific binding than [^{3}H]prazosin, while in rat kidney and human prostate the opposite was true (Table 1). Thirdly, [^{3}H]tamsulosin had a smaller B_{max} than [^{3}H]prazosin in all tissues investigated although this did not reach statistical significance for rat brain or human prostate (Table 1). Expressed as a percentage of the [^{3}H]prazosin B_{max}, the [^{3}H]tamsulosin B_{max} ranged from 54% (liver) to 88% (brain).

We next investigated whether differences in K_d and/or B_{max} could be explained by subtype selectivity. For this purpose, cloned rat α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors were transiently expressed in COS cells. While [^{3}H]prazosin had similar affinity for all three rat α_1-adrenoceptor subtypes, [^{3}H]tamsulosin had highest affinity for the α_{1A}- and lowest for the α_{1B}-adrenoceptor (Table 2). Thus, the affinity of [^{3}H]tamsulosin at the rat α_{1B}-adrenoceptor was significantly lower than that of [^{3}H]prazosin, while it did not differ significantly in the other rat subtypes. While the two radioligands had similar B_{max} values at rat α_{1D}-adrenoceptors, [^{3}H]tamsulosin had a smaller B_{max} than [^{3}H]prazosin at cloned rat α_{1A}- and α_{1B}-adrenoceptors (89% and 56% of [^{3}H]prazosin values, respectively; Table 2).

Saturation binding experiments for both radioligands were also performed with the cloned human α_1-adrenoceptor subtypes stably expressed in rat-1 fibroblasts (Schwinn et al., 1995). While [^{3}H]tamsulosin and [^{3}H]prazosin had similar affinity at human α_{1D}-adrenoceptors, the K_d value of [^{3}H]tamsulosin was significantly smaller and greater than that of [^{3}H]prazosin at α_{1A}- and α_{1B}-adrenoceptors, respectively (Table 2). As seen in rats, [^{3}H]tamsulosin recognized a smaller density of human α_{1B}-adrenoceptors than [^{3}H]prazosin (Table 2). In contrast to the situation in rats, [^{3}H]tamsulosin labeled significantly more human α_{1A}- and α_{1D}-adrenoceptors than [^{3}H]prazosin (Table 2).

Further experiments were designed to elucidate why [^{3}H]tamsulosin labels fewer α_1-adrenoceptors than [^{3}H]prazosin under various conditions. These experiments were performed with rat liver, since the difference was very large in that tissue. We first studied whether the differences may relate to differential labeling of G-protein-modulated receptor affinity states. For this purpose, parallel saturation binding experiments with the two radioligands were performed in Tris buffer supplemented with 5 mM $MgCl_2$, with $MgCl_2$ + 10 μM GTP, and with $MgCl_2$ + GTP + 150 mM NaCl (Table 3). The affinities and B_{max} values of [^{3}H]prazosin and [^{3}H]tamsulosin in the

Table 3
Comparison of [^{3}H]prazosin and [^{3}H]tamsulosin saturation binding parameters in rat liver depending on buffer supplements

	[^{3}H]prazosin	[^{3}H]tamsulosin	% of [^{3}H]prazosin
K_d values			
+ $MgCl_2$	76 ± 6	368 ± 17	-
+ $MgCl_2$ + GTP	68 ± 6	612 ± 122	-
+ $MgCl_2$ + GTP + NaCl	68 ± 7	585 ± 93	-
B_{max} values			
+ $MgCl_2$	98.3 ± 7.9	55.0 ± 7.2[a]	55 ± 4[b]
+ $MgCl_2$ + GTP	97.0 ± 5.5	60.5 ± 7.0[a]	63 ± 8[b]
+ $MgCl_2$ + GTP + NaCl	92 ± 5.2	50.3 ± 6.4[a]	55 ± 7[b]

Saturation binding experiments were performed in Tris buffer (see Section 2 supplemented with 5 mM $MgCl_2$, 10 μM GTP and/or 150 mM NaCl as indicated with all conditions being tested in parallel within the same experiment. K_d values are expressed in pM and B_{max} values in fmol/mg protein or in % of paired [^{3}H]prazosin values (right column). Data are means ± S.E.M. of 4 experiments.
[a] $P < 0.05$ vs. [^{3}H]prazosin in a repeated measure ANOVA followed by a Dunnett's multiple comparison test.
[b] $P < 0.05$ vs. 100% in a two-tailed one-sample *t*-test.

Table 4
Competition of noradrenaline for [^{3}H]prazosin and [^{3}H]tamsulosin binding to rat liver

	[^{3}H]prazosin	[^{3}H]tamsulosin
Hill-slope	0.75 ± 0.04	0.48 ± 0.02[b]
$-\log K_{i\,high}$	7.27 ± 0.08	7.75 ± 0.13
$-\log K_{i\,low}$	5.91 ± 0.10	5.65 ± 0.18
% High affinity sites	19 ± 7	37 ± 4[a]

Experiments were performed in 5 mM $MgCl_2$-supplemented Tris buffer to facilitate formation of agonist high affinity states. Data are means ± S.E.M. of 4 experiments.
[a] and [b]: $P < 0.05$ and 0.01, respectively, in a paired, two-tailed *t*-test. A graphical representation of the data is shown in Fig. 1.

presence of the three supplements were similar to those determined in their absence (Table 1). Moreover, under all three conditions [^{3}H]tamsulosin labeled only 55–63% of the α_1-adrenoceptors detected by [^{3}H]prazosin (Table 3).

Competition experiments with noradrenaline in 5 mM $MgCl_2$-supplemented buffer were performed to compare labeling of agonist high and low affinity states by the two radioligands (Table 4, Fig. 1). In these experiments noradrenaline competition curves against [^{3}H]tamsulosin were shallower (smaller Hill-slopes) than against [^{3}H]prazosin. Moreover, noradrenaline had almost twice as many agonist high-affinity sites against [^{3}H]tamsulosin compared to [^{3}H]prazosin. In combination with the saturation binding data in $MgCl_2$-supplemented buffer (Table 3), it can be calculated that [^{3}H]prazosin and [^{3}H]tamsulosin labeled a similar number of agonist high affinity sites (18.7 vs. 20.4 fmol/mg protein) in rat liver, while [^{3}H]prazosin labels much more agonist low affinity sites than [^{3}H]tamsulosin (79.6 vs. 34.7 fmol/mg protein). Agonist affinities at the high and low affinity sites were similar with both radioligands.

It has been reported for α_2-adrenoceptors that binding properties of radioligands may depend on the buffer system being used (Deupree et al., 1996). Therefore, saturation binding experiments for [^{3}H]tamsulosin and [^{3}H]prazosin were compared in Tris/EDTA, HEPES and glycylglycine buffer (Table 5). [^{3}H]Prazosin had similar affinity in all three buffers. The affinity of [^{3}H]tamsulosin tended to be lowest in Tris/EDTA and highest in glycylglycine buffer, but these differences failed to reach statistical significance with the given number of experiments due to large data scatter. The number of detectable binding sites for [^{3}H]prazosin in glycylglycine buffer was significantly lower than that detectable in Tris/EDTA of HEPES buffer. On the other hand the number of detectable binding sites for [^{3}H]tamsulosin in glycylglycine buffer was significantly greater than in Tris/EDTA buffer. Moreover, receptor quantification by [^{3}H]tamsulosin in HEPES and glycylglycine buffer was more variable than in Tris/EDTA buffer. For both radioligands the number of binding sites detected in HEPES buffer was intermediate between those in Tris/EDTA and glycylglycine buffer. Thus, [^{3}H]tamsulosin labeled significantly fewer sites than [^{3}H]prazosin in Tris/EDTA but not in HEPES or glycylglycine buffer.

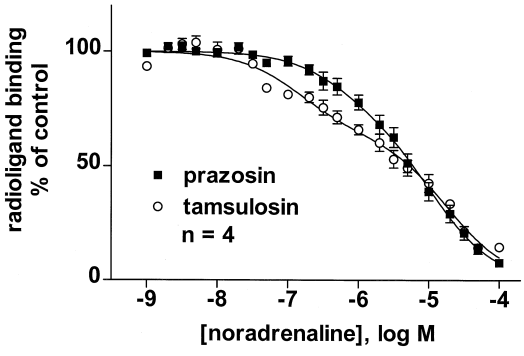

Fig. 1. Competition of noradrenaline for binding of [^{3}H]prazosin (filled squares) and [^{3}H]tamsulosin binding (open circles) to rat liver α_1-adrenoceptors in 5 mM $MgCl_2$-supplemented Tris/EDTA buffer. Data are mean ± S.E.M. of 4 parallel experiments. A quantitative analysis of the data is given in Table 4.

Table 5
Saturation binding parameters of [^{3}H]prazosin and [^{3}H]tamsulosin in three different buffers

	K_d, pM		B_{max}, fmol/mg protein	
	[^{3}H]prazosin	[^{3}H]tamsulosin	[^{3}H]prazosin	[^{3}H]tamsulosin
Tris/EDTA	50 ± 12	731 ± 214[a]	84.5 ± 1.7[d]	63.5 ± 6.4[a,c]
HEPES	42 ± 15	1402 ± 296[b]	77.5 ± 2.7[c]	96.8 ± 21.2
Gly–Gly	42 ± 13	2344 ± 896[a]	65.7 ± 4.8	109.7 ± 23.6

Data are means ± S.E.M. of 6 paired experiments.
[a] and [b]: $P < 0.05$ and 0.01, respectively, vs. [^{3}H]prazosin in a paired, two-tailed *t*-test.
c and d: $P < 0.05$ and 0.01, respectively, vs. data in glycylglycine (Gly–Gly) buffer in a repeated measures ANOVA followed by Dunnet's multiple comparison test.

4. Discussion

Radioligand binding studies are a powerful tool for the characterization and quantification of receptors. An optimal radioligand should have very low non-specific binding, high affinity and, most importantly, very high specificity for the receptors of interest. Non-specific binding may become limiting for receptor detection in radioligand binding studies when the receptor density is low. Yamada et al. (1994) have found that [^{3}H]tamsulosin has less non-specific binding than [^{3}H]prazosin in human prostate. The present study has confirmed these data and detected significant differences in non-specific binding between [^{3}H]prazosin and [^{3}H]tamsulosin in various tissues. However, neither radioligand had a consistently lower level of non-specific binding across all tissues.

It has been reported that the affinity of [^{3}H]tamsulosin was higher than that of [^{3}H]prazosin in human prostate (Yamada et al., 1994) but lower in rat spleen (Yazawa et al., 1992). The present study confirms and extends these observations. Thus, the affinity of [^{3}H]tamsulosin was similar to or higher than that of [^{3}H]prazosin in tissues expressing fairly large proportions of α_{1A}-adrenoceptors, e.g. rat cerebral cortex and kidney or human prostate, but was significantly lower than that of [^{3}H]prazosin in tissues known to express homogeneous populations of α_{1B}-adrenoceptors, e.g. rat spleen and liver. This is consistent with our data on cloned rat and human α_1-adrenoceptor subtypes: While the affinity of [^{3}H]tamsulosin was significantly smaller than that of [^{3}H]prazosin at α_{1B}-adrenoceptors, it was somewhat higher at α_{1A}-adrenoceptors (significant only for human subtypes with the given number of experiments) and similar for α_{1D}-adrenoceptors. Similarly

it has been reported from a comparison of bovine α_{1A}-, hamster α_{1B}- and rat α_{1D}-adrenoceptors stably expressed in human embryonic kidney (HEK) 293 cells that [^{3}H]tamsulosin has an order of affinity of $\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$ (Han et al., 1995). Thus, [^{3}H]tamsulosin has a qualitatively similar but quantitatively smaller selectivity for α_{1A}-adrenoceptors compared to its parent compound tamsulosin (Michel and Insel, 1994), while [^{3}H]prazosin, similar to prazosin (Laz et al., 1994), has no major subtype-selectivity. Despite the different affinity of [^{3}H]tamsulosin for α_1-adrenoceptor subtypes, the antagonist recognition profile of the subtypes and their sensitivity towards the alkylating agents chloroethylclonidine and SZL-49 (1-(4-amino-6,7-dimethoxy-2-quinazolnyl)-4-(2-bicyclo[2,2,2]-octa-2,5-dienyl-carbonyl)-piperazine) is similar upon use of [^{3}H]tamsulosin and of other radioligands (Han et al., 1995).

Another key feature in a useful radioligand is that it labels all receptors of interest and does not bind to sites distinct from these receptors. Under most experimental conditions, agonist radioligands only label a subset of receptors, i.e. those in the GDP-dependent, high-affinity state for agonists, while antagonists are usually believed to label all receptors (Bylund and Toews, 1993). Thus, it is generally assumed that the B_{max} derived from an antagonist saturation binding experiment corresponds to the density of receptors in the tissue or cell preparation under investigation. A notable exception to this rule is the use of highly subtype-selective antagonists, which specifically label only a certain subtype, e.g. the specific labeling of β_1- and β_2-adrenoceptors by [^{3}H]bisoprolol and [^{3}H]ICI 118,551 (erythro-(±)-1-(7-methyl-indan-4-yloxy)-3-isopropylaminobutan-2-ol), respectively (Lemoine et al., 1985; Wang et al., 1985). Recent studies have indicated that some antagonist radioligands detect different numbers of the same receptor when studied side-by-side, and this is not explained by the selective labeling of a receptor subtype. For example, the standard radioligands for α_2-adrenoceptors, [^{3}H]yohimbine and [^{3}H]rauwolscine, have repeatedly been found to yield smaller B_{max} values than newer radioligands including [^{3}H]RS 15385-197 (MacKinnon et al., 1992) and [^{3}H]RX 821002 (2-methoxy-idazoxan) (Erdbrügger et al., 1995) in direct side-by-side comparisons.

A similar situation has recently been described in the α_1-adrenoceptor field. Specifically, it has been found that the recently introduced [^{3}H]tamsulosin labels more receptors than the classically used [^{3}H]prazosin in rat hippocampus and fewer sites in rat spleen (Yazawa et al., 1992), while both ligands label a similar number of α_1-adrenoceptors in human prostate (Yamada et al., 1994). This implies that at least one of the two radioligands yields spurious results, i.e. labels not all α_1-adrenoceptors or labels additional sites which are not α_1-adrenoceptors. This possibility was systematically investigated in the present study. Our data confirm that the number of sites specifically being labeled by [^{3}H]prazosin and [^{3}H]tamsulosin can indeed differ depending on the receptor source being investigated. Our data with cloned receptors demonstrate that this may not be explained by selective labeling of only one or more subtypes of α_1-adrenoceptors by [^{3}H]tamsulosin. In this context it should also be mentioned that in the tissue saturation experiments the concentrations of [^{3}H]tamsulosin were chosen to a greater than 80% occupancy of all subtypes. Moreover, the subtype selectivity of [^{3}H]tamsulosin is smaller than that of its unlabeled parent compound and does not exceed 6-fold. The difference in labeled sites by the two radioligands can also not be explained by differential cellular environments because within a given cell type, i.e. COS or rat-1 cells, the α_1-adrenoceptor subtypes yielded different patterns. As [^{3}H]tamsulosin labeled fewer α_1-adrenoceptors than [^{3}H]prazosin in most models including rat liver, our further experiments focused on this tissue where the difference in B_{max} between both radioligands was particularly large.

In analogy to findings with other receptors, e.g. α_2-adrenoceptors (see above), we have explored whether differential recognition of G-protein-dependent receptor states and/or buffer conditions might explain the quantitatively different labeling of α_1-adrenoceptors by [^{3}H]prazosin and [^{3}H]tamsulosin. Our data demonstrate that experimental conditions which favour ($MgCl_2$ supplementation) or inhibit (GTP and NaCl supplementation) formation of agonist high affinity states of the α_1-adrenoceptor did not alter the radioligand binding properties of [^{3}H]prazosin or [^{3}H]tamsulosin. Similar observations have previously been made for the comparison of [^{3}H]rauwolscine and [^{3}H]RX 821002 binding to α_2-adrenoceptor subtypes (Erdbrügger et al., 1995). This makes it unlikely that the differential radioligand binding properties of [^{3}H]prazosin or [^{3}H]tamsulosin are explained by inverse agonism of one of them (Schütz and Freissmuth, 1992). Nevertheless, the differential α_1-adrenoceptor labeling of the two radioligands may be related to the ability to recognize agonist high and low affinity states. Thus, competition experiments with the full agonist, noradrenaline, revealed a greater proportion of receptors in the high affinity state for agonists when [^{3}H]tamsulosin rather than [^{3}H]prazosin was used as the radioligand. When absolute densities of agonist high and low affinity sites were calculated based on the combined results of the saturation and the noradrenaline competition experiments, we found that both radioligands label similar numbers of agonist high affinity states. On the other hand, [^{3}H]tamsulosin labels considerably fewer agonist low affinity sites, and this difference could quantitatively explain the different B_{max} of the two radioligands. This difference between [^{3}H]prazosin and [^{3}H]tamsulosin binding to α_1-adrenoceptors is distinct from our findings with [^{3}H]rauwolscine and [^{3}H]RX 821002 binding to α_2-adrenoceptors (Erdbrügger et al., 1995). Thus, in the comparison of the α_2-adrenoceptor radioligands, distinct B_{max} values could be explained by a better labeling of agonist

high affinity states by [^{3}H]RX 821002, while in the present comparison of the α_1-adrenoceptor radioligands, the explanation appears to be a better labeling of agonist low affinity states by [^{3}H]prazosin. In contrast, the ability of antagonist to compete for [^{3}H]tamsulosin binding to α_1-adrenoceptor subtypes does not appear to be different from that for other radioligands (Han et al., 1995). Whether the differential labeling of agonist low affinity sites of α_1-adrenoceptors affects the clinical profile of either drug cannot be decided from the present in vitro data, but this possibility should be kept in mind.

The α_1- and α_2-adrenoceptors also appear to differ with regard to the role of buffer systems in determining apparent B_{max} values. Thus, the difference in α_2-adrenoceptor B_{max} values between [^{3}H]rauwolscine and [^{3}H]RX 821002 was apparently independent of the buffer system (Erdbrügger et al., 1995; Deupree et al., 1996). In contrast, [^{3}H]tamsulosin labeled fewer α_1-adrenoceptors in rat liver in Tris/EDTA buffer but not in HEPES buffer; in glycylglycine buffer [^{3}H]tamsulosin even tended to label more sites than [^{3}H]prazosin, but this did not reach statistical significance due to a marked increase in data scatter in this buffer. Thus, Tris/EDTA buffer appears to favour [^{3}H]prazosin binding while glycylglycine buffer appears to favour [^{3}H]tamsulosin binding. While these data highlight the problem that B_{max} values from radioligand binding studies do not always reflect receptor densities, the reasons for these differences remain to be determined.

In conclusion we have demonstrated that the α_1-adrenoceptor radioligands [^{3}H]prazosin and [^{3}H]tamsulosin differ with regard to non-specific binding, affinity, sensitivity to buffer composition, and most importantly the number of sites they label. Differences in detected α_1-adrenoceptor numbers may at least partly be explained by a differential ability to recognize agonist low affinity states of the α_1-adrenoceptors. The overall pattern of differences between the two radioligands appears to be complex and to involve tissue- and receptor subtype-selective factors. This complexity has precluded a detailed analysis of the underlying mechanisms. Nevertheless our data clearly reject the hypothesis that the B_{max} from antagonist radioligand binding experiments always corresponds to the density of the receptor of interest. Caution may be necessary in the comparison of results obtained with different radioligands and/or buffer systems.

Acknowledgements

This work was supported in part by the intramural grant program of the University of Essen Medical School (IFORES-Programme). The skillful technical assistance of Mrs. E. Beisenbusch and Mrs. A. Gronewald is gratefully acknowledged. We thank Drs. Graham, Johnston and Lefkowitz for providing plasmids and cell lines.

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