5-Methylurapidil may discriminate between α_1-adrenoceptors with a high affinity for WB4101 in rat lung

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The α_1-adrenoceptors of rat lung with a high affinity for [^{3}H]-prazosin were subdivided into two populations (high and low affinity sites) by WB4101 and 5-methylurapidil but the proportions were different between both drugs. After pretreatment with chlorethylclonidine, WB4101 recognized only high affinity sites, while 5-methylurapidil still detected high and low affinity sites. These results indicate that α_1-adrenoceptors with a high affinity for WB4101 are not homogeneous in the rat lung, suggesting the possible existence of a new α_1-adrenoceptor subtype in addition to α_{1A} and α_{1B} subtypes.

Keywords: α_1-Adrenoceptor subtype; 5-methylurapidil; WB4101; rat lung

Introduction According to recent subclassification of α_1-adrenoceptors, high affinity sites for [^{3}H]-prazosin or [^{125}I]-BE2254 are subdivided into two subclasses (α_{1A} and α_{1B}) (Morrow & Creese, 1986; Minneman, 1988; Hanft & Gross, 1989; Oshita *et al.*, 1991). The α_{1A} subtype shows high affinity for WB4101, benoxathian and 5-methylurapidil, and is relatively insensitive to an alkylating agent chlorethylclonidine (CEC), while the α_{1B} subtype has low affinity for the antagonists mentioned above and is potentially inactivated by CEC. However, recent molecular biological studies have demonstrated the presence of an additional α_1-adrenoceptor subtype (α_{1C}) with high affinity for prazosin (Schwinn *et al.*, 1990; Lomasney *et al.*, 1991). The present study in the rat lung shows that 5-methylurapidil displaces a different proportion of [^{3}H]-prazosin binding from that seen with WB4101 suggesting a possible existence of three distinct binding sites with high affinity for prazosin.

Methods The lungs of male of Wistar rats (250–450 g) were used in the present studies. After isolation of the large bronchi, the lungs were minced and homogenized in 20 volumes of ice cold buffer (Tris HCl 50 mM, NaCl 100 mM, EDTA 2 mM, pH 7.4) with a polytron (setting 8, 15 s × 2). The homogenates were filtered through 4 layers of gauze and centrifuged at 80,000 *g* for 30 min at 4°C. The pellets were resuspended in the same volume of assay buffer (Tris HCl 50 mM, EDTA 1 mM, pH 7.4), incubated for 15 min at 37°C, and centrifuged again as described above. The final pellets were resuspended in the assay buffer. In some studies the membrane preparations were incubated with 10 μM CEC for 30 min at 37°C and then washed once more with assay buffer before the binding experiments. Incubation with [^{3}H]-prazosin (76.6 Ci mmol^{-1}, NEN, Boston) was carried out in duplicate in a final volume of 1 ml at 30°C. After 45 min the membrane was filtered through Whatman GF/C filters by using a Brandel M30 cell harvester and the radioactivity retained on filters was determined by liquid scintillation counting. Prazosin (10 μM) was used to define non-specific binding. All values were expressed as mean ± s.e.mean. Other experimental conditions and data analysis methods were the same as those described previously (Oshita *et al.*, 1991).

Results *Saturation experiments:* The specific binding of [^{3}H]-prazosin (10–5000 pM) was concentration-dependent and saturated at 1000 pM. Scatchard plots of the binding data resulted in a straight line, suggesting a single class of binding sites ($pK_D = 9.83 \pm 0.16$, $B_{max} = 324 \pm 64$ fmol mg^{-1} protein). In the membranes pretreated with 10 μM CEC also, [^{3}H]-prazosin bound to a single site with an affinity similar to that of CEC-untreated membranes but the number of binding sites was reduced (46% reduction in B_{max}).

Displacement experiments: Unlabelled prazosin monophasically displaced the specific binding of 200 pM [^{3}H]-prazosin to CEC-untreated and -pretreated membranes, resulting in an affinity similar to the pK_D in the saturation experiments (Table 1). On the other hand, WB4101, benoxathian and 5-methylurapidil showed shallow displacement curves in CEC-untreated membranes. Computerized analysis revealed that the antagonists subdivided the binding sites into two populations; the proportion (approximately 65%) of WB4101 – or

Table 1 Inhibition of [^{3}H]-prazosin binding to α_1-adrenoceptors of rat lung by α_1-antagonists

Antagonist	n	*Slope factor*	$pK_{I\,high}$	$pK_{I\,low}$	% *high*
CEC-untreated					
Prazosin	3	0.86	9.47 ± 0.21	—	100
WB4101	3	0.52	9.82 ± 0.24	8.25 ± 0.38	67.6 ± 8.9
Benoxathian	3	0.58	9.61 ± 0.30	8.04 ± 0.16	63.0 ± 7.9
5-Methylurapidil	3	0.55	9.13 ± 0.11	7.25 ± 0.06	43.0 ± 3.2
CEC-pretreated					
Prazosin	3	0.92	9.21 ± 0.12	—	100
WB4101	3	0.96	9.32 ± 0.27	—	100
Benoxathian	3	0.98	9.89 ± 0.25	—	100
5-Methylurapidil	3	0.57	9.38 ± 0.35	7.07 ± 0.28	63.5 ± 3.4

Displacement curves were individually analysed by LIGAND programme. A two site fit was accepted only if it was significantly better than a one site fit.
Data shown are means ± s.e.mean. *n*: number of experiments.
$pK_{I\,high}$ and $pK_{I\,low}$: negative log of the equilibrium dissociation constants (−log M) at high or low affinity sites for antagonists tested.
% high: population binding at the high affinity site compared to the total specific binding sites.
CEC-pretreated: the rat lung membranes were pretreated with 10 μM chlorethylclonidine (CEC) and washed.

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benoxathian – high affinity sites was significantly higher than that (48%) for 5-methylurapidil ($P < 0.05$). In the membranes pretreated with 10 μM CEC, WB4101 and benoxathian recognized only the high affinity site. However, 5-methylurapidil still discriminated two distinct affinity sites, although the proportion of high affinity sites increased to 64% in CEC-pretreated membranes (Table 1).

Discussion In the rat lung, α_1-adrenoceptors were detected as a single population with high affinity for prazosin. However, displacement experiments with WB4101, benoxathian and 5-methylurapidil revealed that the sites consist of at least two distinct populations. According to the α_{1A}, α_{1B} subclassification (Minneman, 1988; Hanft & Gross, 1989), both the populations might simply be considered to correspond to α_{1A} and α_{1B} subtypes, respectively. In fact, the α_{1A}, α_{1B} discriminating drugs (WB4101 and benoxathian) clearly distinguished the high and low affinity sites in a similar way and the low affinity sites were completely inactivated by CEC. However, the proportion of the high affinity sites for WB4101 and benoxathian was approximately 20% higher than that for 5-methylurapidil in CEC-untreated membranes, and 5-methylurapidil still detected two distinct affinity sites in CEC-pretreated membranes. These results strongly suggest that the high affinity sites for WB4101 and benoxathian may not be recognized by 5-methylurapidil homogeneously. The existence of three distinct α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1C}) with a high affinity for prazosin has been demonstrated by molecular biological approach; the α_{1A} and α_{1C} subtypes show a high affinity for WB4101 and are less sensitive to CEC, as compared with the α_{1B} subtype (Lomasney *et al.*, 1991). The difference (20%) between the populations of high affinity sites for WB4101 and 5-methylurapidil observed in the present study may reflect the existence of an additional subtype (α_{1C} or unknown subtype). Therefore, the present results suggest that prazosin-high affinity sites of the rat lung consist of presumably three different α_1-adrenoceptor subtypes and that 5-methylurapidil may be a useful probe for the subdivision of WB4101-high affinity sites.

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(Received September 23, 1991
Accepted October 11, 1991)