Selectivity of Bevantolol Hydrochloride towards $\alpha$- and $\beta$-Adrenoceptor Subtypes in Rat Cerebral Cortex

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ABSTRACT — Selectivity of bevantolol hydrochloride (NC-1400) towards $\alpha$- and $\beta$-adrenoceptor subtypes of rat cerebral cortex was examined in binding experiments and compared with propranolol. Bevantolol biphasically displaced the $^3$H-dihydroalprenolol binding. The affinity of bevantolol to $\beta_1$-adrenoceptor was equal to that of propranolol. Bevantolol displaced $^3$H-prazosin binding monophasically but not $^3$H-$p$-aminoclonidine binding. These results suggest that bevantolol is a $\beta_1$-adrenoceptor antagonist with a relatively high affinity to $\alpha_1$-adrenoceptor subtypes.

$\beta$-Adrenoceptor antagonists are effective for the treatment of hypertension (1). Bevantolol (NC-1400) has been demonstrated to have a potent $\beta$-adrenoceptor blocking action, suggesting that it may be a promising anti-hypertensive drug (2). However, recent investigations have revealed that bevantolol has some interesting additional properties, for example, $\alpha_1$-adrenoceptor and Na channel blocking actions (3, 4).

The $\alpha$- and $\beta$-adrenoceptors were originally classified into $\alpha_1$ and $\beta_2$ subtypes and $\beta_1$ and $\beta_2$ subtypes, respectively (5, 6). More recently, $\alpha$-adrenoceptors have been further subdivided into subclasses (7–10). In the present study, we investigated the selectivity of bevantolol to each subtype of $\alpha$- and $\beta$-adrenoceptors in the rat cerebral cortex.

Radioligand binding experiments were done using the membrane fractions of rat cerebral cortex. Experimental procedures were essentially the same as those described previously (10, 11). $^3$H-Dihydroalprenolol ($^3$H-DHA), $^3$H-prazosin and $^3$H-$p$-aminoclonidine ($^3$H-PAC) were used for $\beta$, $\alpha_1$ and $\alpha_2$-adrenoceptor binding, respectively. Nonspecific binding was defined in the presence of 10 $\mu$M propranolol for $\beta$-adrenoceptors, 10 $\mu$M prazosin for $\alpha_1$-adrenoceptors and 10 $\mu$M clonidine for $\alpha_2$-adrenoceptors, respectively.

The following drugs were used: $^3$H-DHA (specific activity 111.5 Ci/mmol), $^3$H-prazosin (specific activity 76.6 Ci/mmol), $^3$H-PAC (specific activity 58.2 Ci/mmol), prazosin hydrochloride (Sigma, St. Louis, U.S.A.), yohimbine hydrochloride (Wako, Osaka, Japan), WB-4101 hydrochloride (Funakoshi, Tokyo, Japan), bevantolol hydrochloride (NC-1400) (Nippon-Chemiphar, Tokyo, Japan), clonidine hydrochloride, propranolol hydrochloride (Nacalai Tesque, Kyoto, Japan) and ICI-89,409 (1-(2-cynophenoxy)-3-$\beta$-(3-phenylureido)ethylamino-2-propanol) (a gift from ICI Pharma, Cheshine, U.K.).

Binding of the $\beta$-adrenoceptor antagonist $^3$H-DHA to the membrane fraction of rat cerebral cortex was examined at concentrations ranging from 30 pM to 4000 pM. The saturation experiments and the Scatchard plots indicated the binding of the ligand to a
single class of sites. The pKD value was 8.84 ± 0.06 (n = 3) and the Bmax was 232.5 ± 67.0 fmol/mg protein, which were in reasonable agreement with published values (12, 13). In displacement experiments, the binding of 1 nM 3H-DHA was inhibited by various β-adrenoceptor antagonists. Propranolol displaced the binding monophasically with a pKi value of 8.10 ± 0.07 (n = 3). On the other hand, displacement curves for bevantolol and ICI-89,409 were somewhat shallow (Fig. 1A). Computerized analysis of the displacement curves revealed that bevantolol displaced 66 ± 6% (n = 3) of 3H-DHA binding with a high affinity (pKi,high = 7.83 ± 0.31, n = 3) and the remaining 34%, with a low affinity (pKi,low = 6.23 ± 0.17, n = 3) and that ICI-89,409 displaced 79 ± 3% of 3H-DHA binding with a high affinity (pKi,high = 8.48 ± 0.28, n = 3) and the remaining 21% with a low affinity (pKi,low = 6.33 ± 0.17, n = 3). As ICI-89,409 has been reported to be a β1-selective antagonist (14), the result reveals that bevantolol can discriminate between β1- and β2-adrenoceptors as well as ICI-89,409 and further suggests that bevantolol is approximately 50-fold more selective towards β1-adrenoceptor than β2-adrenoceptor (Table 1). The affinity to β1-adrenoceptors of bevantolol was not significantly different from that of propranolol.

3H-Prazosin at 200 pM selectively bound to the α1-adrenoceptors of rat cortex membranes.

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**Fig. 1.** Displacement of 1 nM 3H-DHA binding (A) and 200 pM 3H-prazosin binding (B) to rat cerebral cortex membranes by bevantolol (●), propranolol (▲) and ICI-89,409 (○, A) or WB-4101 (○, B).
(specific binding: approximately 90% of the total binding), and the binding was monophasically inhibited by unlabeled prazosin ($pK_i = 10.16 \pm 0.03, n = 3$). Bevantolol also monophasically displaced the $^3$H-prazosin binding with a $pK_i$ value of $6.90 \pm 0.14$ ($n = 3$) (Fig. 1B). On the other hand, propranolol at concentrations up to 10$\mu$M failed to inhibit the binding. The displacement curves for WB-4101 were shallow, showing the presence of two distinct binding sites ($pK_i = 10.32 \pm 0.06$ and $8.49 \pm 0.13, n = 3$). Recently, Han et al. (15) and Oshita et al. (9) revealed that the two sites corresponded to $\alpha_{1A}$ and $\alpha_{1B}$ subtypes, respectively. Since bevantolol showed a monophasic displacement curve, the drug seems unable to discriminate between the two subtypes. Furthermore, the affinity constant for bevantolol ($pK_i = 6.90$) was higher than the $pA_2$ values ($4.77 \pm 0.07$) obtained in rabbit thoracic aorta (3), suggesting the possibility that bevantolol may be able to distinguish the central $\alpha_1$-adrenoceptors from the peripheral receptors. This point must be clarified in further studies.

$^3$H-PAC at concentrations ranging from 100 pM to 400 pM bound to the cortex membrane in a concentration-dependent manner ($pK_D = 9.18$). The specific binding was approximately 70% of the total binding at 1 nM $^3$H-PAC, which was monophasically displaced by clonidine ($pK_i = 8.66$). On the other hand, bevantolol and propranolol had no effect on the $^3$H-PAC binding. The lack of effect of bevantolol on prejunctional $\alpha_2$-adrenoceptors in the rat vas deferens has been reported (3).

$\beta$-Adrenoceptor blockers have long been used for the clinical treatment of hypertension, but their mechanism of action has not yet been clarified. Recently, we found that chronic administration of bevantolol or propranolol produces a remarkable increase in the $\beta_1$-adrenoceptor density of rat cerebral cortex (M. Takita et al., unpublished data), suggesting that the central $\beta$-adrenoceptor may be one of the sites of the antihypertensive action of $\beta$-adrenoceptor blockers. Since the present results show that, unlike propranolol, bevantolol has relatively high affinity not only to $\beta_1$-adrenoceptors but to $\alpha_1$-adrenoceptors, it is possible that the hypotensive effects may be caused by its blocking actions on both adrenoceptors in peripheral and central tissues.

REFERENCES


