Selective Interaction of DG-5128 with a Low Agonist Affinity State of Alpha-2 Adrenoceptor

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Abstract—Using rat cerebral cortex membranes, the inhibitory effect of DG-5128 against (3H)-clonidine binding was compared between low (a2L) (in the presence of EDTA) and high (a2H) affinity states (in the presence of excess magnesium) of alpha2-adrenoceptor for agonists. The K<sub>i</sub> value (pK<sub>i</sub>=6.79) of DG-5128 in the a2L state was 6.4 times higher than the value in the a2H state. Thus, DG-5128 produces alpha2-adrenoceptor antagonism through the selective interaction with an a2L state of the receptor.

Recently, we have found that DG-5128 (2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesqui-hydrate), a new hypoglycemic agent (1-4), is a preferential and specific alpha2-antagonist (5, 6). It has been demonstrated that alpha2-adrenoceptors exist as a low affinity (a2L) state and a high affinity (a2H) state for agonists in broken cells, but the receptors in intact cells correspond to the a2L state (7). The present study was performed to compare the affinities of DG-5128 between the two affinity states of alpha2-adrenoceptors, in order to further clarify the binding properties of this antagonist.

Adult male Wistar rats (300–400 g) were decapitated, and the cerebral cortex carefully dissected. The tissue was homogenized in ice-cold Tris/HCI buffer (50 mM, pH 7.7 at 25°C), using a Polytron. The homogenate was centrifuged (62,700×g, 15 min), and the pellet was washed twice by suspension and recentrifugation. The final membrane suspension was prepared in the Tris/HCI buffer containing 1 mM EDTA. For saturation studies, the membranes were incubated (25°C) with 0.1–2.4 nM of (3H)-clonidine (NEN, 20.5 Ci/mmol) in a final 2-ml volume of Tris/HCI buffer containing EDTA (1 mM). For drug competition studies, the membranes were incubated with 1.28±0.02 nM of the radioligand and varying concentrations of antagonist. All assays were conducted in duplicate. After 30 min incubation, the binding reaction was terminated by rapid filtration through Whatman GF/B filters with 4×5 ml rinses of ice-cold buffer. The radio-ligand retained on the filters was extracted by scintillation fluid, and the tissue bound radioactivity was measured. Specific radio-ligand binding was defined as the binding displaceable by 10 μM of (-)norepinephrine. Protein was determined by the method of Lowry et al. (8). Saturation and drug competition curves were analyzed by Scatchard transformation (9) and log-probit analysis (10), respectively. The statistical evaluation was examined by Student's t-test. DG-5128 was generously donated by Daiichi Seiyaku Co., Ltd. (Tokyo, Japan).

(3H)-clonidine bound to a single class of binding sites in rat cerebral cortex membranes. The apparent dissociation constant (K<sub>D</sub>) and maximum number of binding sites (B<sub>max</sub>) determined in the presence of EDTA were 1.24±0.10 nM and 106.9±6.6 fmol/mg protein (n=3), respectively. (3H)-clonidine also bound to a single population of binding sites when MgCl<sub>2</sub> was added to the assay medium (a final concentration of 10 mM in excess of EDTA). However, the K<sub>D</sub> and B<sub>max</sub> values were significantly (P<0.05) changed to 0.77±0.02 nM and 210.1±7.8 fmol/mg protein (n=3), respectively. These results
indicate that alpha2-adrenoceptors exhibit preferentially the \( \alpha_{2L} \) state in the presence of EDTA and the \( \alpha_{2H} \) state in the presence of magnesium, respectively, as described by Glossmann and Hornung (11).

DG-5128 and yohimbine inhibited the specific (3H)-clonidine binding in the presence of EDTA with \( K_i \) values of 162 nM (\( pK_i = 6.79 \)) and 12.0 nM (\( pK_i = 7.92 \)), respectively (Table 1). However, the competition curves for both the two antagonists shifted to the right by the addition of excess magnesium (Fig. 1), and the \( K_i \) values changed to 1030 nM (\( pK_i = 5.99 \)) and 46.4 nM (\( pK_i = 7.33 \)), respectively. Thus, magnesium produced 6.36-fold and 3.87-fold shifts of the \( K_i \) values for DG-5128 and yohimbine, respectively (Table 1).

The different affinities of antagonist between the two agonist affinity states of alpha2-adrenoceptors were first reported by Glossmann and Hornung (11) and Salama et al. (12), both groups using the same tissue. These authors pointed out that the effects of magnesium on the antagonist affinities were not always equal among the antagonists examined. In the present experiments, also, the competition curve of DG-5128 was markedly shifted to the right by magnesium, as compared with the case of yohimbine. Thus, it is likely that DG-5128 interacts with the \( \alpha_{2L} \) state (in the presence of EDTA) more effectively than with the \( \alpha_{2H} \) state (in the presence of excess magnesium).

As described by Salama et al. (12), yohimbine exhibited a complex interaction with alpha2-adrenoceptors. The competition curve was heterogeneous (\( n_H: 0.80 \pm 0.03 \)) in the \( \alpha_{2L} \) state (with EDTA present), whereas it was homogeneous (\( n_H: 0.92 \pm 0.07 \)) in the \( \alpha_{2H} \) state (with magnesium present) (Fig. 1, Table 1). In contrast, DG-5128 exhibited a homogeneous competition (\( n_H: \) close to...
unity) regardless of the $\alpha_{2L}$ or $\alpha_{2H}$ states (Fig. 1, Table 1), suggesting the absence of cooperative interaction.

Consequently, all these evidences indicate that DG-5128 exerts specific $\alpha_2$ antagonism through the selective and homogeneous interaction with a low agonist affinity state of the $\alpha_2$-adrenoceptors.

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