Characterization of imidazoline receptors on porcine renal cortex membranes

Pattraporn Pukklay, Surin Plasen, Yupin Sanvarinda and Darawan Pinthong

Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

Abstract

Imidazoline receptors have been reported to play roles in kidney functions such as natriuresis and diuresis. The processes occurred in the segment of nephron, in particular, those that located in the cortex of kidney. The aim of this study is to characterize the subtype of imidazoline receptors (IR) and to determine receptor density and affinity of imidazoline receptors on porcine renal cortex membranes. From saturation binding assay, the maximum receptor density of IR on porcine renal cortex membranes labeled by [^{3}H]-clonidine was 390.2± 89.09 fmol/mg protein with Kd value of 9.69±3.8 nM. The maximum receptor density of I_2 receptor on porcine renal cortex membranes labeled by [^{3}H]-idazoxan was 655.6± 49.17 fmol/mg protein with Kd value of 8.49±1.29 nM. The result revealed that [^{3}H]-idazoxan binding sites (I_2 site) were 1.7 fold higher than those of [^{3}H]-clonidine binding whereas the affinities were comparable. In competitive binding assay, I_1 ligands, clonidine, rilmenidine, moxonidine, surprisingly competed with low affinities to I-site labeled by [^{3}H]-clonidine. The rank order of potency of competing ligands was : idazoxan (459±1.33 nM) > clonidine (730±1.31 nM) > rilmenidine (2,769±1.26 nM) > oxymetazoline (9,204±4.41 nM) > moxonidine = efaroxan (>10^5 nM). The result showed that this site differed from the typical I_1 sites. On the contrary, selective I_2 receptor ligand, idazoxan, competed with very high affinity to [^{3}H]-idazoxan binding site whereas I_1 receptor ligands, clonidine, rilmenidine, moxonidine, oxymetazoline and efaroxan also competed with very low affinity to I_2 site. The rank order of potency was : idazoxan (0.579±0.06 nM) > clonidine (16,100±0.15nM) > rilmenidine (18,900±3.4nM) > oxymetazoline (42,300±33.2 nM) > moxonidine = efaroxan (>10^5 nM). The results from this study suggested that the major imidazoline receptor subtype on porcine renal cortex is I_2 site. In conclusion, I_2 receptors are the main subtype exist on porcine renal cortex membranes which is suggested to be the functional receptors in kidney whereas I site labeled with [^{3}H]-clonidine is different from the typical I_1 site. This site may be a new subtype of imidazoline receptor.

Key words : Imidazoline Receptor/ Porcine Renal Cortex