62

Authors: K Yasuda, K Yoshida, R Sakakibara*, T Uchiyama*, T Hattori*, R Chess-Williams**, CR Chapple**
Institution: Dokkyo University, Chiba University*, Sheffield University**
Title: IDENTIFICATION OF b-ADRENOCEPTOR SUBTYPES IN BLADDER DOME, BLADDER BASE AND PROXIMAL URETHRA OF THE PIG BY RADIOLIGAND BINDING

Aims of study

β-adrenoceptors have been demonstrated in the bladder and urethra of several species including human. β-adrenoceptors are predominantly present in the bladder dome. It has been reported that β-adrenoceptors mediate relaxation of these smooth muscles in several species, and this relaxation may be mediated via β_1-, β_2- or β_3-receptor or a mixture of these subtypes. Recently mRNA encoding for the β_3-adrenoceptor has been found in the human detrusor along with that encoding for both the β_1 and β_2-adrenoceptors, and β_3-adrenoceptors have been suggested to have a role in mediating relaxation of the human bladder. However, the presence of β-adrenoceptor subtypes in the bladder base and urethra has not been reported. This study investigates the presence and ratio of β-adrenoceptor subtypes by radioligand binding study in the bladder dome, bladder base and proximal urethra of the female pig.

Methods

Cell membranes were obtained from bladder dome, bladder base and proximal urethra of female pig, with the mucosal and serosal layers removed. Tissues were pulverized and homogenized. Homogenates were centrifuged twice at 45,000 ×g for 10 minutes at 4^{o}C. Saturation experiments were conducted with 7 different concentrations (0.25 to 16 nM) of [^{3}H]DHA. Competition experiments with [^{3}H]DHA used different concentrations of unlabelled antagonists (β_1-selective antagonist CGP 20712A, β_2- antagonist ICI 118551 and β_3-antagonist SR59230A) to determine the drug affinity. Tissue homogenates were incubated (37^{o}C) with 1.3nM [^{3}H]DHA. Nonspecific binding represented [^{3}H]DHA bound in the presence of 1μM of unlabelled propranolol.

Results

In saturation binding study, Scatchard analysis of [^{3}H]DHA binding demonstrated a single population of binding sites with a mean dissociation constant (K_D) of 1.40±0.18nM, 1.22±0.18 and 29.2±0.52nM, respectively, and a density of 154.4±46.2 fmol/mg protein, 56.5±13.5 and 61.3±0.52 fmol/mg protein in the bladder dome, bladder base and proximal urethra, respectively.

In competition binding study, displacement of [^{3}H]DHA with the β_1-selective antagonist CGP20712A best fitted binding to a single receptor with a low affinity (pKi=5.02,4.17 and 4.91, respectively, in the bladder dome, bladder base and proximal urethra), suggesting that β_1-receptors were not present. Displacement binding with ICI 118551 (β_2-selective antagonist) best fitted a two site model in all lesions with only 21%, 20% and 28%, respectively, of binding sites having a high affinity (β_2-receptors). Displacement binding with SR59230A (β_3-selective antagonist) best fitted a two site model in bladder dome and base with 69% and 58%, respectively, of binding sites having a high affinity (β_3-receptors). In the urethra, displacement binding with SR59230A best fitted a one-site model, with a pKi value (7.22) relatively lower than that of high affinity site of bladder base (8.60) and urethra (8.45).

Conclusions

β_3-receptors appear to be predominantly present in the bladder dome, base and urethra of the female pig.