Cloning and pharmacological characterization of the dog recombinant P2X7-receptor

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P2X7 receptors display considerable species differences in antagonist potency between the rodent and human orthologues (Hibell et al., 2001) which limits the pre-clinical evaluation of P2X7 receptor antagonists. In this study we have cloned and characterised the dog P2X7 receptor to determine if its agonist and antagonist sensitivity more closely resembles that of the human or rodent orthologues.

The dog P2X7 receptor was cloned from heart cDNA template using standard methods and the sequence confirmed from 4 different templates (heart, brain, ovary and testis). Recombinant receptors were characterised by measuring agonist-stimulated ethidium accumulation in HEK293 or U2OS cells stably expressing recombinant receptors in NaCl containing assay buffer as described previously (Fonfria et al., 2007). Data are the mean±s.e.m of 3-5 experiments. Statistical comparisons were performed using one-way ANOVA with Dunnett’s post test.

The dog P2X7 receptor sequence was 595 amino acids in length which is similar to the human, rat and mouse P2X7 receptors although it differed from other orthologues in possessing an additional threonine between positions 284 and 285 in the human sequence and missing the valine at position 538 in the human receptor. In functional studies, agonist-stimulated ethidium uptake in cells expressing the dog recombinant receptor was rapid in onset (detectable in 1 min) and reached a plateau after 30 min. There was no clear dependence of EC50 on time of incubation (p>0.05). ATP pEC50 values at dog, human, rat and mouse P2X7 receptors were 3.12±0.05, 3.06±0.04, 3.66±0.02 and 2.92±0.05, respectively (dog significantly different to rat, p<0.05). BzATP was a partial agonist compared to ATP at the dog P2X7 receptor (intrinsic activity 7.44±1.32%). BzATP pEC50 values at dog, human, rat and mouse P2X7 receptors were 4.67±0.04, 4.43±0.05, 5.30±0.08 and 4.06±0.03, respectively (dog significantly different to rat and mouse, p<0.05). At the dog P2X7 receptor, KN62 was a potent antagonist with a pIC50 of 7.44±0.24 which was similar to its pIC50 of 6.97±0.05 at the human P2X7 receptor but higher than at the rat P2X7 receptor (pIC50 < 5).

In this study we have cloned and functionally characterised the dog P2X7 receptor. Agonist potency and KN62 sensitivity of the dog receptor was different to the rodent orthologues but similar to the human P2X7 receptor. Further studies with additional antagonists will be required to confirm if dog P2X7 receptors are pharmacologically similar to human.

Fonfria, E. et al, (2007), In press

Hibell, AD. et al, (2001), J Pharmacol Exp Ther, 296, 947-957

Conflict of interest: The authors are employed by GlaxoSmithKline R&D Ltd