Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol16Issue1abst075P.pdf

Further characterisation of vascular responses to ligands of the calcium-sensing receptor family

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Introduction: We previously showed that the two positive allosteric modulators of calcium-sensing receptor (CaSR), calindol and cinacalcet are potent vasorelaxants, but CaSR may play a minor role in comparison with voltage-gated calcium channels[1]. Since then, a diverse range of endogenous and exogenous compounds, for example modulators structurally distinct from calindol and cinacalcet, and aromatic and basic L-amino acids, have been reported to activate CaSR and its related receptor, $GPRC_6$. Thus far, the vascular actions of these ligands are poorly characterised.

Method: Male Wistar rats (10-12 weeks) were killed by cervical dislocation and small mesenteric arteries from the gut were isolated for isometric tension recording. Relaxant responses in methoxamine-precontracted vessels are shown as mean±sem (n=4-6) and analysed by two-way analysis of variance or Student *t*-test, where appropriate.

Results: AC-265347 (AC), a more recently developed positive CaSR modulator, induced relaxation in mesenteric arteries (pEC_{50}=6.3±0.1, E_{max}=102±4%), which was more potent than calindol (pEC_{50}=6.0±0.1, E_{max}=100±5%, P<0.05) or cinacalcet (pEC_{50}=5.7±0.1, E_{max}=101±7%, P<0.01). However, only modest relaxation was seen with other novel positive CaSR modulators, S-methylglutathione (E_{max}=33±3%), Lglutathione (E_{max}=27±6%), L-tryptophan (1mM: 19.3±7.6%), or Lphenylalanine (1mM: -1.6±10%). Relaxation to AC was insensitive to endothelial removal (pEC_{50}=6.2±0.1, E_{max}=99±9%) or two structurally distinct negative CaSR modulators (+calhex231: pEC_{50}=6.3±0.1, E_{max}=105±9%; +NPS-2143: pEC_{50}=6.4±0.2, E_{max}=101±7%). However, it was reduced by a lower extracellular [Ca^{2+}] (0.5mM; pEC_{50}=6.1±0.1, E_{max}=95±11%, P<0.01), high extracellular [K^{+}] (60mM; pEC_{50}=5.5±0.3, E_{max}=96±14%, P<0.01), or desensitisation of Transient Receptor Potential Vanilloid type 1 receptor system by 10µM capsaicin (pEC_{50}=5.9±0.3, E_{max}=107±23%, P<0.01), similar to our previous findings with calindol and cinacalcet. Contractions to 3µM BayK8644, a voltage-gated Ca^{2+} channel activator, were also inhibited by AC (control: 3.1±0.9mN; +3µM AC: 0.9±0.3mN; P<0.05). On the other hand, L-arginine, a $GPRC_6$ agonist and a precursor of nitric oxide, induced endothelium-dependent relaxation (at 1mM; with endothelium: 46±5%, without endothelium: 16±5%, P<0.01), whereas the selective $GPRC_6$agonist, L-ornithine had little effect (1mM: 4±4%). Pretreatment with L-ornithine alone or in combination with other amino acids (L-tryptophan, Lphenylalanine, L-arginine and L-serine) also had no effect on CaSR-mediated relaxation to extracellular Ca^{2+} (data not shown).

Conclusion: The vascular profile of CaSR and $GPRC_6$ ligands is complex. Selected positive CaSR modulators induce mesenteric relaxation mainly through CaSR-independent mechanisms, whereas other CaSR/$GPRC_6$ ligands including L-amino acids have limited effect on vascular tone.

References:

(1) Thakore P and Ho WSV (2011). *Br J Pharmacol* **162**: 749-762