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PHARMACOLOGY AND DESENSITISATION OF THE HUMAN RAMP3 AND RAT CALCITONIN RECEPTOR-LIKE RECEPTOR COMBINATION

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Adrenomedullin (ADM) is a multifunctional regulatory peptide with its effects mediated by specific receptors. Here we examined the characteristics of the ratCRLR/humanRAMP3 combination stably expressed in HEK 293 cells with respect to binding of calcitonin family peptides and desensitisation of the ADM cAMP response following pre-exposure to ADM or other peptides. HEK 293 cells were stably transfected with rat CRLR and human RAMP3. Northern blot analysis of RNA extracted from these cells showed high expression of CRLR and RAMP3 and barely detectable levels of RAMPs 1 and 2. ^{125}I-ratADM binding to RAMP3/CRLR was competed for by unlabelled rat ADM with an IC_{50} of 9 nM. CGRP and other calcitonin family members competed with ^{125}I-ratADM more weakly with the following IC_{50} values; α-CGRP 168 nM, β-CGRP 69 nM, $CGRP_{8-37}$ 61 nM, cys(ACM2,7)CGRP 270 nM, human ADM_{22-52} 220 nM, rat amylin 117 nM, rat calcitonin >1000 nM. Both ADM and CGRP caused elevation of cAMP with ADM being the most potent (EC_{50} 1.5 ± 1.3 nM vs. 18.0 ± 1.1 nM, n = 3). A 2-h preincubation of these cells with 100 nM ADM resulted in a 93 ± 1% (n = 3) reduction in the subsequent cAMP response to 100 nM ADM. A similar effect was seen with ADM_{13-50} but α-CGRP was less effective at this concentration. The desensitisation caused by ADM was not mimicked by 1 mM dibutryl cAMP nor inhibited by pretreatment with the protein kinase A inhibitor H-89 (100 to 500 nM). Thus the ratCRLR/humanRAMP3 combination forms a receptor with higher affinity for ADM than CGRP which is potently desensitised by ADM. This desensitisation was not due to the activation of PKA.