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FUNCTIONAL CHARACTERISATION OF THE CGRP ANTAGONIST BIBN4096BS, IN SK-N-MC CELLS

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The 37-amino acid vasodilatory peptide calcitonin gene-related peptide (CGRP) has been implicated in the pathophysiology of migraine. We have recently developed a nonpeptide CGRP antagonist, BIBN4096BS, with subnanomolar affinity for primate CGRP-1 receptors. BIBN4096BS proved to be a valuable tool to dissect CGRP receptor pharmacology[1]. Interestingly, in functional assays in some tissue, the pK_B values for BIBN4096BS differ depending on the agonist used, α- or β-CGRP[2]. We have therefore examined the potency of BIBN4096BS to block cyclic AMP accumulation caused by α- or β-CGRP, in SK-N-MC human neuroblastoma cells. We have also used a novel radioligand, ^{125}I-Tyr^0-β-CGRP to determine the binding affinity of BIBN4096BS to probe whether a different β-CGRP receptor binding site exists.

^{125}I-Tyr^0-β-CGRP was custom synthesised by Biotrend. SK-N-MC were cultured as described[3]. Agonists (10^{-4} to 10^{-11} M) were given simultaneously with BIBN4096 BS (10^{-9} to 10^{-14} M), then incubated for 30 min at room temperature. Cyclic AMP was measured using Flashplates (NEN). Schild Plot analysis was carried out to determine pK_B values (Graph Pad Prism). Radioligand binding on SK-N-MC cell membranes was carried out as described[3]. All experiments were carried out with $n \geq 3$, in triplicate.

BIBN4096BS blocked the cAMP stimulation evoked by α-CGRP with very high potency (pK_B 13.4). BIBN4096BS was equally effective in blocking β-CGRP effects (pK_B 13.7). The slopes for both Schild plots were not different from unity. In binding experiments, BIBN4096BS displaced ^{125}I-Tyr^0-β-CGRP with an Ki value of 11.7, which is not significantly different to that previously determined using ^{125}I-h -α-CGRP.

BIBN4096BS is a highly potent full CGRP antagonist on SK-N-MC cells. The equipotency of BIBN4096BS versus α-or β-CGRP suggest that there are no differential binding sites present in this cell line.

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