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

Marcus Schindler and Henri Doods

Boehringer Ingelheim Pharma KG, Cardiovascular Research, 88397 Biberach, Germany

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 -Tyr⁰- β -CGRP to determine the binding affinity of BIBN4096BS to probe whether a different β -CGRP receptor binding site exists.

^{125}I -Tyr⁰- 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 -Tyr⁰- β -CGRP with an K_i 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.

REFERENCES

1. Wu, D. et al. (2000) *Eur. J. Pharmacol.* 400, 313–319.
2. Wu, D. et al. (2001) this meeting.
3. Doods, H. et al. (2000) *Br. J. Pharmacol.* 129, 420–423.