004P α_1-ADRENOCEPTOR ANTAGONIST PROPERTIES OF THE β-ADRENOCEPTOR NON-CONVENTIONAL PARTIAL AGONIST CGP 12177A

N. Brahmadevara, A.M. Shaw & A. MacDonald, Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow G4 0BA.

CGP 12177A, described as a non-conventional partial agonist (potent β_1-/β_2-adrenoceptor antagonist with β-adrenoceptor agonist activity at higher concentrations (Kaumann, 1989)), produces relaxation of phenylephrine-constricted vascular smooth muscle at high concentrations. We have previously shown that this relaxation in rat aorta is not due to activation of β_3- or the low affinity state of β_1-adrenoceptors and we postulated that α_1-adrenoceptor blockade may be involved (Brahmadevara *et al.,* 2003). We therefore investigated the affinity of CGP 12177A for α_1-adrenoceptors in functional studies in rat aorta and in binding studies in rat cortex membranes.

Male Wistar rats (200 - 250 g) were stunned and killed by cervical dislocation before removal of the thoracic aorta. Ring preparations were suspended in Krebs physiological saline solution gassed with 95/5 % O_2/CO_2. at 37 ^oC for isometric recording. Tissues were incubated with CGP 12177A for 30 min before obtaining cumulative concentration-response curves (CRCs) to phenylephrine and U46619. Binding experiments were performed using [^{3}H]-prazosin (specific activity 77.0 Ci/mmol) in membranes of rat cortex. Non-specific binding was determined in the presence of phentolamine (25 μM) and separation of bound radioactivity was achieved by filtration through Whatman filters in a 24-well Brandel cell harvester. Saturation experiments were carried out to determine the K_d of [^{3}H]- prazosin and competition experiments were carried out with prazosin and CGP 12177A to determine IC_{50} and hence pK_i values. Data were analysed using Graph Pad Prism. Values are mean±s.e.mean.

In functional studies, pre-incubation with CGP 12177A (30 μM, 100 μM and 300 μM, n=6) produced parallel rightward shifts of the phenylephrine CRC with no reduction in the maximum responses. Schild regression analysis gave a pA_2 value of 5.23 with a slope of 0.97 (95% CL: 0.88 to 1.06) suggesting simple, competitive antagonism. In contrast, CGP 12177A (≤ 300 μM) had no effect on U46619-mediated contraction (pEC_{50}s: control, 7.58±0.01; CGP 12177A 300 μM, 7.61±0.03, n=6, $p>0.05$). In binding studies, saturation experiments with [^{3}H]-prazosin yielded a K_d of 0.16±0.02 nM and a B_{max} of 149.0±6.1 fmol/mg protein (n=3). In competition experiments, prazosin and CGP 12177A competed monophasically with [^{3}H]-prazosin binding, giving pK_i values of 9.83±0.12 (n=3) and 5.48±0.17 (n=3) respectively.

In conclusion, the pA_2 of CGP 12177A against phenylephrine obtained from functional studies agrees with the binding affinity of CGP 12177A obtained at α_1-adrenoceptors in rat cerebral cortex. The relaxant effect of CGP 12177A in phenylephrine-constricted rat aorta (Brahmadevara *et al.,* 2003) may therefore be attributed to an α_1-adrenoceptor blocking effect.

Kaumann, A.J. (1989) *Trends Pharmacol. Sci.,* **10,** 316 - 320.
Brahmadevara, N. *et al.,* (2003) *Br J. Pharmacol.,* **138**, 99-106.