Crutchley, D. J., Piper, P. J. (1974) Prostaglandin inactivation in guinea-pig lung and its inhibition. Br. J. Pharmacol. 52: 197–203
Curtis-Prior, P. B. (1974) Effects of the prostaglandin antagonist polyphloretin phosphate (PPP) on the metabolism of rat adipose tissue. Adv. Exp. Med. Biol. 63: 43
Curtis-Prior P. B. (1975) Prostaglandins and obesity. Lancet. i: 897–899
Curtis-Prior, P. B., Chan, Y. H. (1981) Effects of polyphloretin phosphate (PPP) and quinterenol on cyclic nucleotide phosphodiesterase activities. Pharmacol. Res. Commun. 13: 331–337
Curtis-Prior, P. B., Jenner, M. (1981) A component of polyphloretin phosphate (PPP) may be an alternative substrate for ATP-glycerol transferase. Experientia 37: 335–336
Curtis-Prior, P. B., Oblin, A. R. (1979) Variation of pre-incubation times of 'prostaglandin synthetase' preparations in relation to subsequent enzyme activity. Prostaglandins Med. 2: 314–320
Diczfalusy, E., Ferno, O., Fex, H., Hogberg, B., Linderot, T., Rosenberg, T. (1953) Synthetic high molecular weight enzyme inhibitors. I. Polymeric phosphates of phloretin and related compounds. Acta Chem. Scand. 7: 913–920
Eakins, K. E. (1971) Prostaglandin antagonism by polymeric phosphates of phloretin and related compounds. Ann. N. Y. Acad. Sci. 180: 386–395
Eakins, K. E., Karim, S. M. M. (1970) Polyphloretin phosphate—a selective antagonist for prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. Life Sci. 9: 1–5
Giessler, C., Mentz, P., Forster, W. (1977) The action of phospholipase A_2 on parameters of cardiac contraction, excitation and biosynthesis of prostaglandins. Pharmacol. Res. Commun. 9: 117–130
Juan, H., Lembeck, F. (1976) Polyphloretin phosphate reduces algesic action of bradykinin by interfering with E-type prostaglandins. Agents Actions 6: 646–650
Marrazzi, M. A., Matschinsky, F. M. (1972) Properties of 15-hydroxy-prostaglandin dehydrogenase; structural requirements for substrate binding. Prostaglandins 1: 373–388
Sanner, J. H. (1988) Eicosanoid antagonists. In: Curtis-Prior, P. B. (ed.) Prostaglandins: Biology and Chemistry of Prostaglandins and Related Eicosanoids. Churchill Livingstone, Edinburgh, p. 179
Sanner, J. H., Eakins, K. E. (1976) Prostaglandin antagonists. In: Karim, S. M. M. (ed.) Advances in Prostaglandins Vol 2. Prostaglandins: Chemical and Biochemical Aspects, MTP Press, Lancaster, p. 139
Takeguchi, C., Kohno, E., Sih, C. J. (1971) Mechanisms of prostaglandin synthetase. Biochemistry 10: 2372

J. Pharm. Pharmacol. 1990, 42: 662–664
Communicated January 18, 1990

© 1990 J. Pharm. Pharmacol.

Cortisone: a potent $GABA_A$ antagonist in the guinea-pig isolated ileum

JENNIFER ONG*, DAVID I. B. KERR*, HUGH R. CAPPER, GRAHAM A. R. JOHNSTON, *Department of Pharmacology, The University of Sydney, N.S.W. 2006, Australia*

Abstract—In the guinea-pig isolated ileum, cortisone at 0·001–10 nM induced a non-competitive, dose-dependent antagonism of $GABA_A$-receptor-mediated contractile responses to applied GABA, depressing the maximum contractile response to GABA (100 μM), without affecting contractile responses to acetylcholine or cholinergic twitch contractions. At higher concentrations (> 10 nM), cortisone depressed contractile responses to acetylcholine (10–100 nM) and cholinergic twitch responses to transmural stimulation. Cortisone is thus the most potent non-competitive antagonist at $GABA_A$-receptor complexes in the guinea-pig ileum. From molecular modelling, sterically there appeared little difference between cortisone and cortisol, the latter being an enhancer of $GABA_A$-receptor-mediated action in the ileum. However, there were significant differences in electrostatic potentials between the two steroids, due to the different levels of oxidation at C_{11} which may contribute to such opposing actions.

Several endogenous steroids are now known to be potent modulators at $GABA_A$-receptor complexes. In particular, A-ring reduced metabolites of progesterone such as 3α-hydroxy-5α-tetrahydroprogesterone potentiate GABA-activated chloride conductance (Majewska et al 1986), whilst the neurosteroid pregnenolone sulphate appears to be an endogenous antagonist at $GABA_A$-receptor complexes (Majewska & Schwartz 1987; Ong et al 1987a). Indeed this 3α-hydroxy substituent and saturated A-ring in the 5α configuration have been considered essential for modulation at $GABA_A$-receptor complexes (Gee et al 1987). However, cortisol, with a 3-oxo substituent on a 4-pregnene ring and a 17α-hydroxy group, is a highly potent modulator of $GABA_A$-receptor-mediated contractile responses in the guinea-pig isolated ileum, potentiating at low concentrations and inhibiting at higher doses (Johnston et al 1987; Ong et al 1987b; Andres-Trelles et al 1989). Majewska (1987) has found similar interactions of 17-hydroxy glucocorticoids at $GABA_A$-receptor complexes in rat brain synaptosomes. We now show that cortisone, which differs from cortisol only in the oxidation level at C_{11} (Fig. 1), does not potentiate, but instead is only a highly potent antagonist of GABA-induced ileal contractions, as previously reported in brief (Johnston et al 1987).

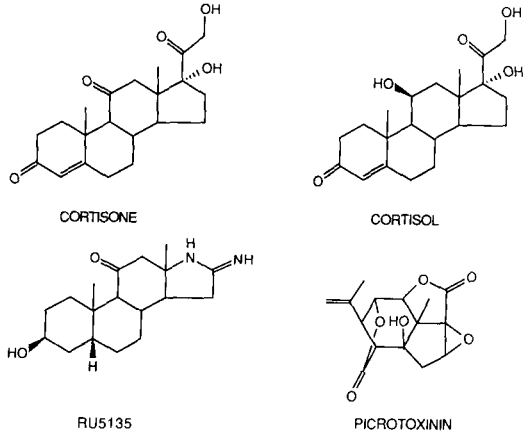

FIG. 1. Structures of cortisone, a potent non-competitive antagonist, cortisol, a potent potentiator, RU 5135, a steroid analogue which is a potent competitive antagonist and picrotoxinin, a non-competitive antagonist at $GABA_A$-receptor complexes in the guinea-pig isolated ileum.

*Present address: Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5000, Australia.
Correspondence to: J. Ong, Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5000, Australia.

Materials and methods

Guinea-pig isolated ileal preparation. Guinea-pigs of either sex, 200–300 g, were killed by a blow on the head and exsanguinated. Segments of ileal preparations, 3–4 cm in length, were quickly removed and mounted in a 20 mL organ bath containing oxygenated (95% O_2 and 5% CO_2) normal Krebs-bicarbonate solution as previously described (Ong et al 1987a). Longitudinal muscle ileal contractions were measured isometrically using an FT03 force transducer and displayed on a Grass polygraph. Repetitive cholinergic twitch contractions were elicited by ring electrodes using pulses at 0·1 Hz, 0·1 ms duration and submaximal voltage. Drugs were added at 20–30 min intervals, depending on the recovery of the tissue responses to control level, and cortisone was added at least 1–2 min before other agonists were tested. Drug volumes never exceeded 1% of the total bath volume. Cortisone was dissolved in methanol, the total concentration of the vehicle in the bath was between 0·001–0·01% which did not modify responses to GABA or transmural stimulation. All experiments were repeated in quadruplicate on at least 12 tissues from 3 different animals, and Student's *t*-test for paired and unpaired samples was used to assess the significance ($P < 0.05$) of differences between mean values of the dose-response effects.

Drugs used were cortisone, GABA and acetylcholine chloride (all from Sigma).

Molecular modelling. Molecular modelling was carried out using the Chem-X program, January 1989 version (Chemical Design Ltd, Oxford) on a miniVAX-II computer with an Evans & Sutherland PS300 high resolution colour graphics terminal and a Macintosh IIcx computer emulating Tektronix 4105 colour graphics terminal using VersatermPro and Tekalike emulation software. Molecular co-ordinates were obtained from the Cambridge Crystallographic Data Base, and the MM2 molecular dynamics program was used for structural optimization.

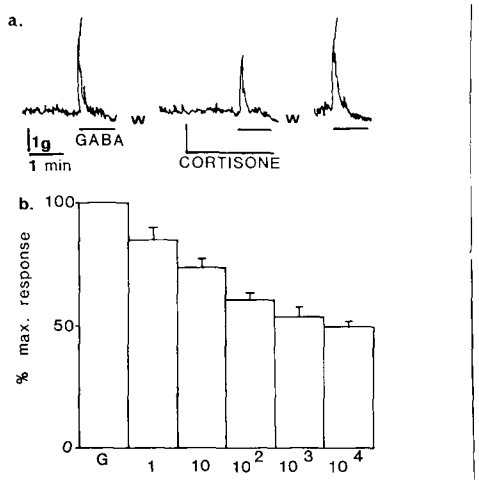

FIG. 2. Depression of contractile responses to GABA by cortisone in the guinea-pig isolated ileum, showing (a) antagonism of responses to GABA (EC50 = 10 μM) by cortisone (100 pM) followed by a recovery of the contraction to GABA after tissue wash-out (w), n = 12 and (b) dose-related antagonism of GABA-induced contraction (G; 10 μM) by cortisone, expressed as a percentage of the control contraction induced by GABA at 10 μM. Vertical bars indicate the means ± s.e.m. for 12 experiments performed. Student's *t*-test indicated significant differences ($P < 0.05$) between cortisone-treated and control tissues.

Results and discussion

Exogenously applied cortisone (0·001–10 nM) significantly antagonised $GABA_A$-receptor-mediated ileal contractile responses to GABA (10 μM, approximate EC50 GABA; Fig. 2) in a concentration-dependent manner, without affecting ileal twitch responses to transmural stimulation or contractile responses to acetylcholine (ACh; 10–100 nM). The antagonism by cortisone was non-competitive, with a dextral shift of the concentration-response curve for GABA, and depression of the maximum contractile response to GABA (100 μM; data not shown). However, higher concentrations of cortisone (> 10 nM) also depressed both contractile responses to ACh and cholinergic twitch contractions. Cortisone was not readily washed out, particularly if applied at higher concentrations (1–10 nM), when the control responses to GABA remained depressed even after 60 min.

From these results, cortisone is the most potent non-competitive antagonist of $GABA_A$-receptor-mediated contractile responses in the guinea-pig ileum yet found, with significant antagonism at 1 pM cortisone. By contrast, cortisol is a highly potent modulator, having potentiating actions at low concentrations and antagonizing actions at high concentrations (Ong et al 1987b; Andres-Trelles et al 1989). Both glucocorticoids differ from the progesterone metabolites, which also modulate $GABA_A$receptor complexes (Gee et al 1987), in possessing an unsaturated A-ring and a 3-oxo substituent in place of the 3α-hydroxy group of these metabolites. Cortisone and cortisol also have a 17-hydroxy substituent which appears to be an essential feature imparting increased potency to these glucocorticoids in modulating $GABA_A$-receptor complexes. Cortisone, in turn, differs from cortisol only in having an 11-oxo substituent instead of the 11β-hydroxyl substituent of the latter, this change being sufficient to convert the potent potentiating action of cortisol to the potent non-competitive antagonism of cortisone. On the other hand, the most potent competitive antagonist is the amidine steroid analogue RU 5135 (3α-hydroxy-16-imino-5β-17-aza-androstan-11-one, Fig. 1) first described by Hunt & Clements-Jewery (1981); RU 5135 inhibits muscimol and bicuculline binding to rat brain membranes at nM concentrations (Olsen 1984) and is a potent competitive antagonist of muscimol action (pA_2 8.31) in the cuneate nucleus (Simmonds & Turner 1985) and of $GABA_A$-receptor-mediated contractile responses (pA_2 8.0) in the guinea-pig ileum (Ong & Kerr 1989). The most widely used non-competitive $GABA_A$ antagonist is picrotoxinin (Fig. 1) which acts at μM concentrations (Curtis & Johnston 1974; Simmonds 1980; Ong & Kerr 1989).

Molecular modelling failed to reveal any structural similarities between cortisone, RU 5135 and picrotoxinin, and there is no experimental evidence to suggest that these compounds interact with the same active sites to antagonise $GABA_A$-receptor complexes. For cortisol and cortisone, however, whilst these differ sterically only slightly in ring C, as a result of the different oxidation levels at C_{11}, there were clear differences in electrostatic potentials resulting from these different levels of oxidation at C_{11}, as shown on the contour map (Fig. 3). Although cortisol and cortisone are superimposable at rings A, B and D, from the viewpoint of electrostatic potential, they evidently do not match when considering their behaviour at the $GABA_A$ potentiating site. Nevertheless, cortisol at higher concentrations behaves like cortisone in depressing GABA-induced ileal responses (Ong et al 1987b).

Both picrotoxinin and RU 5135 are potent convulsants on systemic administration to mammals (Curtis & Johnston 1974; Hunt & Clements-Jewery 1981) whereas cortisone administration, widely used in medical practice especially as an anti-inflammatory agent, does not produce such convulsions

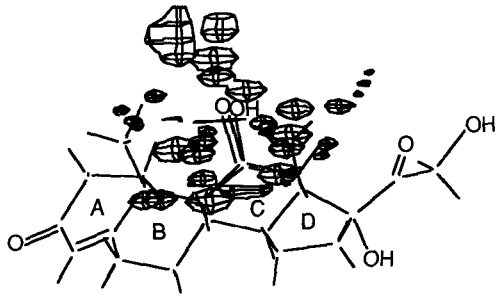

FIG. 3. Contour map of electrostatic potential differences between cortisol and cortisone showing areas with differences greater than 21 kJ mol^{-1} with a significance of 4 kJ mol^{-1}. The structures of the two steroids have been superimposed through rings A, B and D, and differ sterically only in ring C as a result of the different oxidation level at C_{11}.

(Haynes & Murad 1985). The lack of convulsant action of cortisone may be due to its rapid metabolism to cortisol; indeed, it is generally considered that corticosteroids with an 11-oxo substituent require reduction to 11-hydroxy compounds for their biological action (Haynes & Murad 1985). Alternatively, the degree of GABA antagonism (50%) achievable with cortisone, despite its high potency (1–10^4 pM), may not be sufficient to precipitate convulsions. Cortisone is, however, commonly anxiogenic at the clinical level, suggestive of $GABA_A$-receptor antagonism. The present study would indicate that a structurally specific site exists for cortisone, at least at $GABA_A$-receptor complexes of the enteric nervous system.

We thank the National Health and Medical Research Council of Australia for financial support, and the National Fellowships Advisory Committee for the award of a Queen Elizabeth II Fellowship to Jennifer Ong.

References

Andres-Trelles, F., Bibby, V., Lustman, S., Simmonds, M. A. (1989) Effects of cortisol on $GABA_A$ receptor-mediated responses compared in guinea-pig ileum and rat cuneate nucleus. Neuropharmacology 28: 705–708

Curtis, D. R., Johnston, G. A. R. (1974) Convulsant alkaloids. In: Simpson, L. L., Curtis, D. R. (eds). Neuropoisons, Their Pathological Actions, Volume 2—Poisons of Plant Origin. Plenum Press, New York, pp 207–248

Gee, K. W., Chang, W.-C., Brinton, R. E., McEwen, B. S. (1987) GABA-dependent modulation of the Cl^- ionophore by steroids in rat brain. Eur. J. Pharmacol. 136: 419–423

Haynes, R. C., Murad, F. (1985) Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of adrenocortical steroid biosynthesis. In: Goodman, A. G., Goodman, L. S., Rall, R. W., Murad, F. (eds). Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th edn, Macmillan Publishing Co., New York, pp 1459–1489

Hunt, P., Clements-Jewery, S. (1981) A steroid derivative, R 5135, antagonises the GABA/benzodiazepine receptor interaction. Neuropharmacology 20: 357–361

Johnston, G. A. R., Kerr, D. I. B., Ong, J. (1987) Stress, steroids and GABA receptors. In: Rand, M. J., Raper, C. (eds). Pharmacology, Int. Congr. Series, Vol. 750. Elsevier Science Publishers B. V. (Biomedical Division). Excerpta Medica. Amsterdam, pp 121–124

Majewska, M. D. (1987) Antagonist-type interaction of glucocorticoids with the GABA receptor-coupled chloride channel. Brain Res. 418: 377–382

Majewska, M. D., Schwartz, R. D. (1987) Pregnenolone-sulfate: an endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Ibid. 404: 355–360

Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L., Paul, S. M. (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232: 1004–1007

Olsen, R. W. (1984) Gamma-aminobutyric acid receptor binding antagonism by the amine steroid RU 5135. Eur. J. Pharmacol. 103: 333–337

Ong, J., Kerr, D. I. B. (1989) Modulation of spontaneous motility by $GABA_A$ receptor antagonism in the guinea-pig isolated ileum. Neurosci. Lett. 101: 203–208

Ong, J., Kerr, D. I. B., Johnston, G. A. R. (1987a) Pregnenolone and its sulphate modulate $GABA_A$-receptor-mediated contractile responses in the guinea-pig isolated ileum. Eur. J. Pharmcol. 142: 461–464

Ong, J., Kerr, D. I. B., Johnston, G. A. R. (1987b) Cortisol: a potent biphasic modulator at $GABA_A$-receptor complexes in the guinea-pig isolated ileum. Neurosci. Lett. 82: 101–106

Simmonds, M. A. (1980) Evidence that bicuculline and picrotoxin act at separate sites to antagonise gamma-aminobutyric acid in rat cuneate nucleus. Neuropharmacology 19: 39–45

Simmonds, M. A., Turner, J.P. (1985) Antagonism of inhibitory amino acids by the steroid derivative RU5135. Br. J. Pharmacol. 84: 631–635