Dose related *in vitro* effects of ranitidine and cimetidine on basal and ACTH-stimulated steroidogenesis

C J KENYON, R FRASER, G G BIRNIE, J M C CONNELL, AND A F LEVER

From the MRC Blood Pressure Unit and Department of Medicine, Western Infirmary, Glasgow

SUMMARY Isolated bovine adrenocortical cells were incubated with and without 3 ng/ml ACTH, with various concentrations (10–1000 µg/ml) of either cimetidine or ranitidine. Cortisol, corticosterone, and deoxycorticosterone outputs were measured. Cimetidine and ranitidine at 320 and 1000 µg/ml inhibited ACTH-stimulated corticosterone and cortisol synthesis and cimetidine decreased basal cortisol synthesis. The inhibitory effects of cimetidine on cortisol synthesis were approximately 10 times greater than those of ranitidine. Cimetidine (1000 µg/ml), but not ranitidine increased deoxycorticosterone synthesis by ACTH-stimulated cells, indicating inhibition of 11β-hydroxylation in the adrenal steroidogenic pathway. Although doses of cimetidine and ranitidine which produce these *in vitro* effects are much greater than plasma concentrations in normal clinical use, they might be important in acutely ill patients given intravenous bolus injections of cimetidine, or if either antagonist were accumulated by the adrenal to produce high intracellular concentrations.

The imidazole H₂ receptor antagonist, cimetidine, prolongs the half-life of many drugs which are normally oxidized by the liver,^{1–3} by inhibition of the microsomal cytochrome P450-dependent monooxygenase system.⁴ Other imidazole drugs such as etomidate, ketoconazole and omeprazole (an inhibition of the gastric proton pump) have recently been shown to inhibit steroid synthesis by blocking cytochrome P450-dependent reactions in the adrenal cortex^{5–7} and it is possible that cimetidine inhibits adrenal steroidogenesis by a similar mechanism.

The dose related effects of cimetidine on basal and ACTH-stimulated cortisol, corticosterone, and deoxycorticosterone synthesis were compared with those of a non-imidazole H₂-receptor antagonist, ranitidine, which does not interfere with hepatic metabolism of other drugs⁸ and shows little affinity for hepatic cytochrome P450.

Methods

MATERIALS

Isolated zona fasciculata/reticularis cells were

prepared from minced slices of the inner adrenal cortices of six bovine adrenals by collagenase digestion using the method of Kenyon *et al.*⁶ adapted from Haning *et al.*¹⁰ Aliquots (200 µl) of a cell suspension containing 7 × 10⁵ cells/ml were added to 0.8 ml medium 199 containing various concentrations of cimetidine/ranitidine or vehicle, with or without 3 ng/ml ACTH (Synacthen, Ciba-Geigy). Cells were incubated at 37°C for one hour in an atmosphere of 95% O₂:5% CO₂, then centrifuged at 2000 g for 10 min at 0°C and the supernatant stored at –20°C. Cortisol was measured by direct radioimmunoassay using antiserum from the Scottish Antibody Production Unit. Cross reactivities with the cortisol antiserum were 0.2% for corticosterone, 0.03% for 11-deoxycorticosterone and 0.58% for 11-deoxycortisol. Corticosterone and deoxycorticosterone were measured by radioimmunoassay after extraction with methylene chloride followed by paper chromatography.¹¹ Statistical comparisons were by analysis of variance using Neuman-Keul's multiple range test.¹²

Results

The effects of increasing concentrations of ranitidine

Address for correspondence: Dr C J Kenyon, MRC Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT.

Received for publication 27 January 1986.

and cimetidine on cortisol, corticosterone, and deoxycorticosterone synthesis are shown in the Figure.

CORTISOL

Basal cortisol synthesis was inhibited by cimetidine at concentrations from 100 $\mu\text{g}/\text{ml}$ ($p < 0.05$) but not by ranitidine. Both cimetidine and ranitidine inhibited ACTH-stimulated cortisol synthesis. At concentrations of 320 and 1000 $\mu\text{g}/\text{ml}$ the effects of cimetidine were more marked than ranitidine ($p < 0.01$). The concentration of drug required for

50% inhibition of cortisol synthesis (IC_{50}) was 200 μg cimetidine/ml for basal and ACTH-stimulated and 1900 μg ranitidine/ml for ACTH-stimulated cells.

CORTICOSTERONE

The patterns of corticosterone inhibition with ranitidine and cimetidine were broadly similar to those of cortisol, although inhibitory effects of cimetidine at 320 ng/ml were not significantly different from ranitidine. Cimetidine at 1000 $\mu\text{g}/\text{ml}$ inhibited ACTH-stimulated corticosterone synthesis more than ranitidine ($p < 0.05$). IC_{50} s for ACTH-stimulated synthesis were 480 $\mu\text{g}/\text{ml}$ for cimetidine and 600 $\mu\text{g}/\text{ml}$ for ranitidine.

DEOXYCORTICOSTERONE

Deoxycorticosterone synthesis by cells treated with 1000 $\mu\text{g}/\text{ml}$ ranitidine was not stimulated by ACTH. In contrast cimetidine treated, ACTH-stimulated cells secreted 50% more deoxycorticosterone than ACTH-stimulated control cells and 12% more than ranitidine treated cells.

Discussion

The present finding that cimetidine *in vitro* inhibits both basal and ACTH-stimulated cortisol synthesis by isolated bovine adrenocortical cells is consistent with the observations of De Natale *et al*¹³ who noted that cimetidine treatment lowered plasma corticosterone in stressed and unstressed rats. These data are compatible with the theory that cimetidine directly inhibits adrenocortical function. The site of action of cimetidine's inhibitory effects is indicated from an analysis of the secretory pattern of other adrenocorticosteroids. Reduction of cortisol synthesis was associated with a similar change in corticosterone synthesis and an increase in deoxycorticosterone synthesis, indicating that cimetidine's effects are partly due to inhibition of the 11β -hydroxylation step. Other imidazole drugs (ketoconazole, etomidate and omeprazole) have effects on steroidogenesis which include inhibition of this cytochrome P450-dependent enzyme. Cimetidine's effect on the hepatic catabolism of other drugs is related to an interaction with cytochrome P450 and its inhibition of adrenal 11β -hydroxylase is probably attributable to the same mechanism. Ranitidine, however, a non-imidazole compound, has been shown to have no effect on hepatic cytochrome P450-dependent drug metabolism. Although steroidogenesis was slightly impaired by ranitidine in ACTH-treated cells, there was no evidence of 11β -hydroxylase inhibition. The difference between the ability of ranitidine and cimetidine to inhibit corticosterone synthesis is less marked than for

Figure Dose-related effects of cimetidine (●) and ranitidine (○) on cortisol, corticosterone and deoxycorticosterone synthesis by isolated bovine adrenocortical cells. Values shown are means ($\pm$ SEM, $n=8$) of steroid measurements in the incubation medium of cells treated with (solid lines) and without (broken lines) 3 ng/ml ACTH.

cortisol synthesis. The effects of cimetidine and ranitidine on basal corticosterone synthesis were not significantly different; cimetidine inhibition of ACTH-stimulated corticosterone synthesis was greater than that of ranitidine only at the highest drug concentration used. It is possible that cimetidine has a proportionately greater effect on 17 α -hydroxylase than ranitidine although the IC₅₀ for corticosterone inhibition by cimetidine is only slightly greater than that for cortisol. Corticosterone biosynthesis was more severely affected by ranitidine than cortisol biosynthesis while synthesis of corticosterone's precursor 11-deoxycorticosterone was not significantly inhibited. Further studies of this interesting discrepancy are required. The lack of effect by ranitidine on basal steroidogenesis and the very high concentration required for inhibitory effects on cortisol secretion indicated a non-specific interference in the mechanism of ACTH action unrelated to an interaction with cytochrome P450.

The IC₅₀ for ranitidine in these experiments is at least 4000 times greater than peak plasma concentrations during normal therapeutic use.¹⁴ *In vitro* studies with isolated perfused zona glomerulosa cells using a concentration of 0.35 μ g ranitidine/ml found no effect on ACTH-stimulated aldosterone synthesis but an increase in basal secretion and a much reduced angiotensin II response.¹⁵ More recently, Sancho *et al*¹⁶ have shown that 300 mg ranitidine/day for three days reduced plasma cortisol and aldosterone concentrations in sodium deplete, normal man. Perhaps differences both in the effects seen and concentration of drug required are related to the different species used in these studies.

The IC₅₀ for inhibition of cortisol synthesis by cimetidine is only 100-fold greater than peak plasma concentrations in normal use.¹⁷ De Natale *et al*¹³ have suggested that cimetidine is accumulated by the adrenal gland *in vivo*. If sufficiently high concentrations were achieved this could be of clinical importance, particularly in long term prophylactic treatment of intensive care patients. Similarly, bolus intravenous injections of cimetidine to treat acute gastric bleeding may transiently raise plasma concentrations high enough to blunt the normal adrenocortical response to haemorrhagic stress.

In summary, very high concentrations of cimetidine and ranitidine inhibit adrenal steroidogenesis *in vitro*. The effect is more marked for cimetidine than ranitidine as cimetidine inhibits the 11 β -hydroxylase in the steroidogenic pathway whereas ranitidine does not. Because ranitidine has been reported as being 10 times more potent than cimetidine as an inhibitor of the H₂-receptor,

cimetidine is more likely to have side effects on adrenocorticosteroid synthesis. These observations may be clinically important and merit further study.

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