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THE EFFECT OF SOME ANTIPROSTATIC STEROIDS UPON CORTISOL PRODUCTION BY GUINEA-PIG ADRENAL CELLS STIMULATED BY ACTH

WILLIAM ROBERT ROBERTSON,* ANN LAMBERT, ROBERT MITCHELL, KEITH KENDLE† and VLADIMIR PETROW‡

Department of Chemical Pathology, University of Manchester, Hope Hospital, Salford, M6 8HD, U.K.; †Robert Gordon's Institute of Technology, School of Pharmacy, Aberdeen, AB9 1FR, U.K.; and ‡Department of Physiology, Duke University Medical Center, Durham, NC 27710, U.S.A.

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Abstract—The antiprostatic steroids 6-methylene-4-pregnene-3,20-dione (6-MP) (I), 17-alpha-acetoxy-6, 16-dimethylene-4-pregnene-3,20-dione (II), and melengestrol acetate (MGA) (III) were incubated with guinea-pig adrenal cells, both alone and maximally stimulated with ACTH. Cortisol output was then measured by RIA. Increased cortisol-like secretion was obtained with 6-MP in the absence of ACTH. In the presence of ACTH, cortisol-like steroid secretion was the sum of that seen with ACTH and 6-MP alone. It follows that 6-MP stimulates *in vitro* a cortisol-like steroid cross reacting with the cortisol antibody by a mechanism that by-passes ACTH. Steroid (II) weakly inhibited cortisol output. MGA, in contrast, proved to be a strong inhibitor of cortisol output (ID_{50} of 2.3 μmol/l). Its site of action was established by adding it to adrenal cells incubated with precursor steroids on the cortisol pathway. Conversion of 3β-hydroxysteroids to cortisol was inhibited whereas conversion of 3-keto steroids was not affected. It follows that MGA inhibits 3β-hydroxysteroid dehydrogenase.

The role of adrenal corticosteroids in prostate cancer has still to be elucidated. Nevertheless, the presence of glucocorticoid receptors in 62% of samples of prostate cancer metastases [1] appears to indicate a role for hydrocortisone in tumour growth. If this is indeed the case, then the effect of antiprostatic steroids upon adrenal corticosteroid production warrants determination.

Three closely related antiprostatic steroids shown in Fig. 1 are currently being studied in our laboratories‡ (see above) as potential therapeutic agents for both androgen-dependent and androgen-independent clones of the Dunning R 3327 prostatic adenocarcinoma (cf. Ref. 2). We have therefore determined their effects *in vitro* upon basal and ACTH-stimulated cortisol or cortisol-like secretion using dispersed guinea-pig adrenal cells [3]. In addition the site of the anti-steroidogenic effect of one of these anti-prostatic steroids [MGA (III)] was examined using a well-established method [4] based upon stimulation by the exogenous precursor steroids of the various steps leading to the biosynthesis of cortisol.

MATERIALS AND METHODS

Source of chemicals. Steroids [I] and [II] were prepared by methods previously described [5, 6] and purified by column chromatography onto silica and crystallization. Identity was confirmed by m.p., UV, i.r. and NMR. Purity was confirmed by TLC in two solvent systems.

* Correspondence to: Dr W. R. Robertson, Department of Chemical Pathology, Clinical Sciences Building, Hope Hospital, Eccles Old Road, Salford, M6 8HD, England, U.K.

ACTH (1–24) (Synacthen) was a gift from Dr McMartin (Ciba-Geigy, Horsham, U.K.).

Assessment of the in vitro *biopotency and site of action of the anti-prostatic steroids.* Guinea-pig adrenal cells were dispersed and cultured as described previously [3]. Aliquots (40 μl) of cell suspension were dispensed into a 96-well tissue culture plate and incubated for 90 min at 37° with increasing concentrations of anti-prostatic steroid in the presence and absence of ACTH (50 ng/l). The total volume of the incubate in each well was 80 μl and the final cell concentration was 0.75×10^6 cells/ml. After 90 min incubation duplicate 10 μl samples were taken and assayed for cortisol by specific radioimmunoassay [3]. Duplicate cultures were set up and three separate experiments were performed.

To assess the site of action of the anti-prostatic steroid (MGA) in the adrenal pathway, aliquots of adrenal cell suspension were stimulated for 90 min with the cortisol precursor steroids (all at 10^{-5} mol/l: pregnenolone, 17-alpha-hydroxypregnenolone, progesterone, 17-alphahydroxyprogesterone and 11-deoxycortisol, or with either dibutyryl cAMP (10^{-3} mol/l) or ACTH (100 ng/l) in the absence or presence of MGA (10^{-5} mol/l). Again, duplicate cultures were set up and duplicate 10 μl samples were taken from each well and assayed for cortisol. Two separate experiments were performed. Using an identical experimental system, the biopotency of 12 drugs in 54 separate experiments has been estimated [7]. In this series the coefficients of variation were <7% intra-well; <11% inter-well and <16% inter-assay.

The specificity of the cortisol antiserum (Scottish Antibody Production Unit, Carluke, U.K.) used in

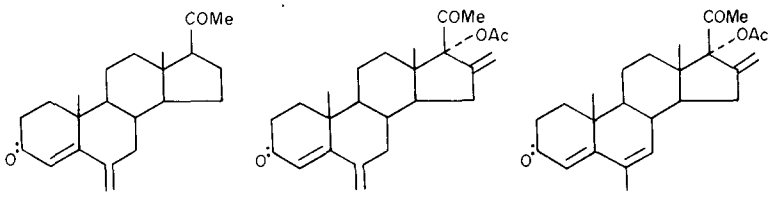

Fig. 1. The anti prostatic steroids 6-methylene-4-pregnene-3, 20-dione (6-MP) (I), 17 alpha-acetoxy-6, 16-dimethylene-4-pregnene-3,20-dione (II), and melengestrol acetate (MGA) (III).

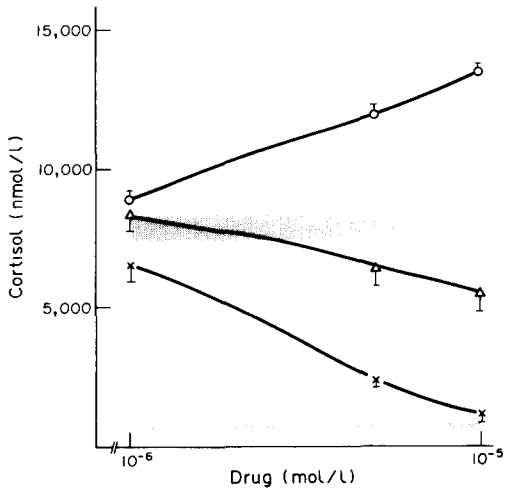

Fig. 2. Cortisol (or cortisol-like steroid) output (nmol/l) from adrenal cells maximally stimulated with 50 ng/l of (1–24) ACTH with increasing concentrations of steroids (I), (II) and (III). The error bars represent mean ± SD, N = 4. Steroid I (○), steroid II (△), steroid III (×). The upper and lower stippled areas represent the cortisol secretion (at 95% confidence level) in response to 50 ng/l ACTH (1–24) and no added ACTH, respectively.

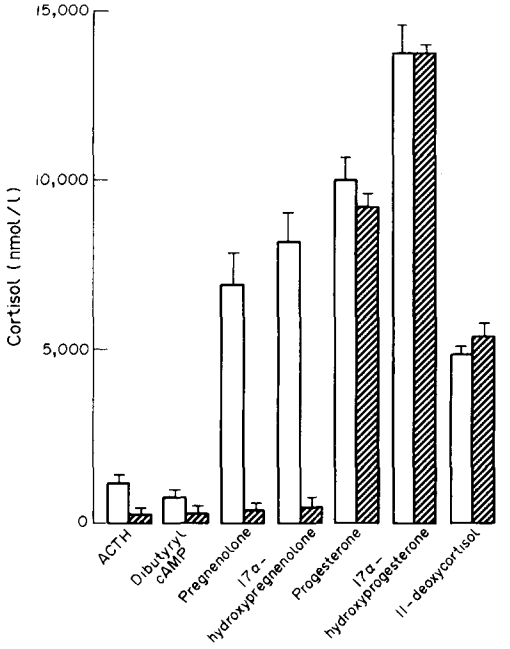

Fig. 3. Cortisol output (nmol/l) is shown in response to 100 ng/l of (1–24) ACTH, dibutyryl cAMP (10^{-3} mol/l) and precursor steroids pregnenolone (Pe), 17-alpha-hydroxypregnenolone (17OHPe), progesterone (Po), 17-alpha-hydroxyprogesterone (17OHPo) and 11-deoxycortisol (all at 10^{-5} mol/l in the presence (hatched columns) and absence (open columns) of MGA (10^{-5} mol/l). The columns represent mean ± SD, N = 4.

the cortisol radioimmunoassay has been reported elsewhere [8]. None of the anti-prostatic steroids interfered or crossreacted in the cortisol radioimmunoassay at the concentrations employed.

RESULTS

The effect of the steroids on cortisol output from dispersed guinea-pig adrenal cells

The effect of the three-anti-prostatic steroids on ACTH (50 ng/l)-stimulated cortisol (or cortisol-like steroid) secretion is shown in Fig. 2. 6-MP differed markedly from the other steroids in that cortisol-like steroid secretion in the presence of concentrations of 6-MP > 10^{-6} mol/l exceeded that stimulated by a saturating dose (50 ng/l) of ACTH. In the absence of ACTH, 6-MP provoked a concentration dependent increase in cortisol-like steroid secretion over the range 10^{-6} to 10^{-5} mol/l such that 10^{-5} mol/l 6-MP provoked a secretion of 4878 ± 866 nmol/l compared with 653 ± 39 nmol/l in the absence of drug. When the output in the presence of drug and ACTH is corrected for the output produced by drug alone, the values lie within the maximal stimulation error envelope, indicating an additive effect.

Steroids II and III (MGA) induced a concentration dependent inhibition of cortisol secretion over the range 10^{-6} to 10^{-5} mol/l (Fig. 2). Steroid II was a weak inhibitor and depressed ACTH 50 ng/l stimulated cortisol secretion by 31 ± 1% ($P < 0.05$) at 10^{-5} mol/l. In contrast, MGA inhibited more strongly and had an ID_{50} (concentration of drug which provoked 50% inhibition) of 2.3 ± 0.3 μmol/l. In the absence of ACTH, neither steroid II nor III stimulated basal cortisol secretion.

Investigation of the site of action of MGA

Figure 3 shows ACTH stimulated, dibutyryl

cAMP-stimulated and steroid precursor-provoked cortisol secretion by adrenal cells in the absence and presence of MGA 10^{-5} mol/l. The presence of the stimulators in the absence of MGA gave rise to a >10-fold increase in cortisol output over that seen in their absence. MGA depressed ACTH-stimulated and dibutyryl cAMP stimulated cortisol secretion by 83% and 76%, respectively. The steroid depressed cortisol secretion by >95% when the 3β-hydroxy steroids pregnenolone or 17-alphahydroxypregnenolone were present, but had no significant effect on steroid-stimulated cortisol production when the 3-keto-steroids progesterone, 17-alpha-hydroxyprogesterone and 11-deoxycortisol were added. These results indicate that the site of action of MGA is at the level of 3β-hydroxysteroid dehydrogenase.

DISCUSSION

6-MP differs from the other two steroids in showing a concentration dependent increase in the secretion of a cortisol-like steroid when incubated with guinea-pig adrenal cells. Furthermore, in the presence of ACTH, a further increase in cortisol-like steroid secretion is observed which is the sum of that obtained with ACTH and with 6-MP alone. As 6-MP does not cross-react with the antibody used in the cortisol RIA it is likely that the increase in cortisol output observed is due to direct stimulation of cortisol-like steroid production by 6-MP. A possible explanation is that 6-MP is hydroxylated by the adrenal to a cortisol-like product such as 6-methylene hydrocortisone which can cross-react in the RIA. This alternative is regarded as less likely as (i) 6-MP produces a small drop in adrenal weight on *chronic* administration to the rat [9] indicating increased glucocorticoid feedback and (ii) 6-methylene hydrocortisone is unlikely to be detected by the RIA since cortisone itself has <0.1% cross-reactivity [8].

The structural requirement for adrenal stimulation of cortisol-like steroid production must be rather specific as the closely related 6,16-dimethylenic steroid (II) had no cortisol precursor activity and indeed was a weak inhibitor of ACTH-stimulated adrenal steroidogenesis. MGA, in contrast, exhibited strong inhibitory activity with an ID_{50} of 2.3×10^{-6} mol/l. This steroid shows structural similarity to megestrol acetate which inhibits cortisol production *in vitro* with an ID_{50} of 1.1×10^{-5} mol/l [4].

By challenging the adrenal cells with cortisol precursor steroids (pregnenolone, 17-alpha-hydroxypregnenolone, 17-alpha-hydroxyprogesterone and 11-deoxycortisol) in the absence and presence of increasing concentrations of steroid, MGA has been demonstrated to a potent inhibitor of 3β-hydroxysteroid dehydrogenase. Megestrol acetate has previously been shown to be an inhibitor of this enzyme [4].

In conclusion, the effect of three structurally similar anti-prostatic steroids upon cortisol production by guinea-pig adrenal cells has been investigated. 6-MP (I) has been found to stimulate cortisol-like steroid output by an ACTH-independent mechanism. Steroid (II) weakly inhibited steroid output. MGA (III), in contrast, proved to be a strong inhibitor of ACTH-stimulated cortisol production (ID_{50} 2.3 μmol/l) and has been shown to act at the level of 3β-hydroxysteroid dehydrogenase.

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