J. steroid Biochem. Vol. 23, No. 2, pp. 235–238, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

SHORT COMMUNICATION

A SIMPLE *IN VITRO* APPROACH TO THE ESTIMATION OF THE BIOPOTENCY OF DRUGS AFFECTING ADRENAL STEROIDOGENESIS

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(*Received* 2 *July* 1984)

Summary—Dispersed guinea-pig adrenal cells can be maximally stimulated to secrete cortisol by adrenocorticotrophin (ACTH > 50 ng/l). Further, this stimulation appears to be specific to ACTH alone, with other naturally occurring chemicals (e.g. steroids, protein hormones) at supra-physiological concentrations being without effect on cortisol production. The effect of drugs of differing structure and therapeutic function (aminogluthethimide, metyrapone, trilostane, 17-ketotrilostane, danazol, epostane, megestrol acetate, stanozolol and etomidate) on ACTH-stimulated (50 ng/l) cortisol production has been tested in this system. All the drugs depressed steroid output in a similar dose-related fashion. The concentration of drug which inhibited cortisol output by 50% was (μmol/l, mean ± SEM): etomidate 0.097 ± 0.002: epostane 0.44 ± 0.02: 17-ketotrilostane 0.55 ± 0.04: trilostane 1.3 ± 0.1: metyrapone 3.5 ± 0.6: megestrol acetate, 11 ± 2: danazol 22 ± 2: aminogluthethimide 41 ± 5: stanozolol 50 ± 4. Thus, etomidate, an anaesthetic, is more potent than the established anti-steroidogenic drugs metyrapone, aminogluthethimide and trilostane. Further, direct anti-steroidogenic effects have been demonstrated for megestrol acetate and stanozolol for the first time. We conclude that this technique offers a promising new approach to the assessment of biological potency of drugs affecting endocrine tissues.

INTRODUCTION

Many biological systems can be maximally and specifically stimulated by a large dose of the factor(s) controlling their function. Under these conditions such systems should detect any agent (e.g. a drug) interfering with that biological process regardless of its site of action or of its structure.

The value of this approach has been investigated using dispersed guinea-pig adrenal cell preparations in which cortisol production is stimulated maximally by ACTH (> 50 ng/l: 15 pmol/l) [1]. Naturally occurring steroid and peptide hormones at supraphysiological concentrations have been shown to be without effect on basal or ACTH stimulated cortisol production. Further, no potentiation of the ACTH response was induced by low or high concentrations of angiotensin II, *N*-pro-opiocortin or λ-MSH [2, 3]. Thus, cortisol production by guinea-pig adrenal cells appears to be specifically controlled by ACTH. The inhibitory effect on cortisol production of 9 drugs with different chemical structures (Fig. 1) and sites of action is reported here. Three of the drugs tested (aminoglutethimide, metyrapone, trilostane) are used in the treatment of hyperadrenalism. The major plasma metabolite of trilostane, 17-ketotrilostane [4] has also been examined. A further drug, danazol, was introduced as an antigonadotrophic agent but has since been shown to interfere in both adrenal and gonadal steroidogenic function [5, 6]. Epostane (WIN 32729) has anti-steroidogenic effects on the ovary and is an abortifacient but also inhibits adrenal steroidogenesis [7]. Two other steroid analogues, megestrol acetate, a synthetic progestagen used in the treatment of prostatic cancer and stanozolol, an anabolic steroid, have also been evaluated.

Finally, the anaesthetic, etomidate, was tested. Recent evidence has suggested that it suppresses adrenocortical function *in vivo* [8, 9] and *in vitro* [10]. These effects may, in part, explain the apparent increase in mortality associated with the use of this drug in critically ill patients [11].

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EXPERIMENTAL

Full details of the preparation of the guinea-pig adrenal cells and of the characteristics of ACTH-stimulated cortisol secretion by this system are described elsewhere [1, 3].

Briefly, 2 male tricoloured guinea-pigs (500–700 g) were killed by cervical dislocation and the adrenals removed and cleaned of fat. The glands were chopped into 1 mm cubes using a McIlwain tissue chopper, the tissue pieces washed with Eagle's minimum essential medium (EMEM) and dispersed by mechanical agitation in EMEM containing trypsin at a concentration of 0.2%. The cells were collected by centrifugation (300 *g* for 5 min), and washed with Eagle's medium containing bovine serum albumin (BSA 0.5%), lima bean trypsin inhibitor (0.15%), calcium (8 mM) and ascorbate (2 mM). The above mixture served as the incubation medium. Finally, the suspension was filtered through nylon (100 μ) mesh. The cells (1×10^6 cells/ml) were then preincubated for 2 h at 37°C in an atmosphere of 100% O_2. After preincubation the cell suspension was centrifuged to remove any secreted cortisol and resuspended in fresh incubation medium.

In all experiments aliquots (40 μl) of cell suspension were dispensed into a 96 well-tissue culture plate and stimulated for 90 mins at 37°C with 50 ng/l ACTH (1–24) either alone or in combination with increasing concentrations of drug in a final ethanol concentration of 2.5%. Ethanol (2.5%) was also included as a control in the basal and ACTH-stimulated wells in the absence of drug. Duplicate samples (10 μl) were then assayed for cortisol by radioimmunoassay. Ethanol (2.5%) has a slight inhibitory effect on ACTH (50 ng/l)—stimulated cortisol secretion. In later work on the

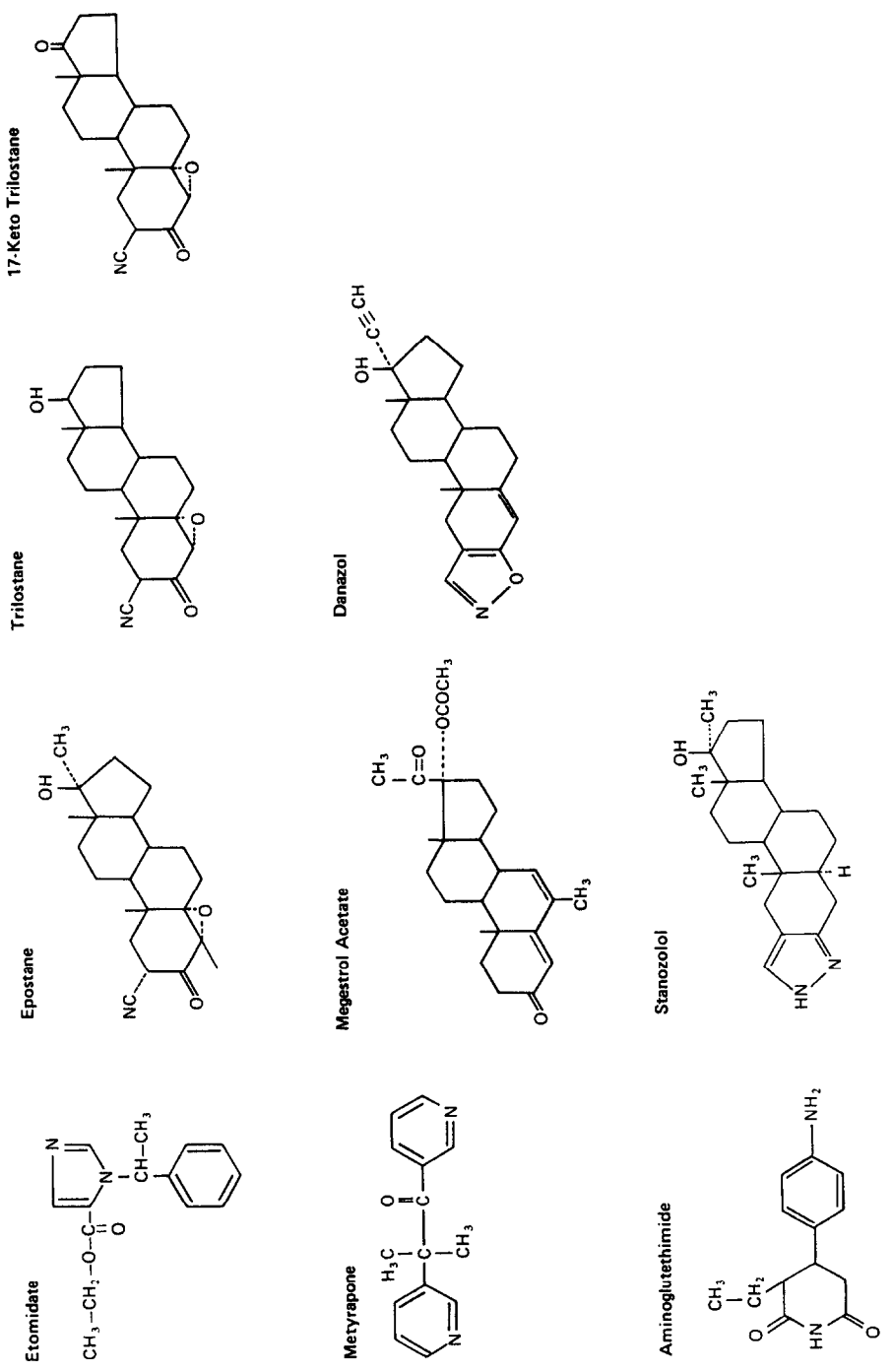

Fig. 1. The chemical structures of etomidate, epostane, trilostane, 17-ketotrilostane, metyrapone, megestrol acetate, danazol, aminoglutethimide and stanozolol.

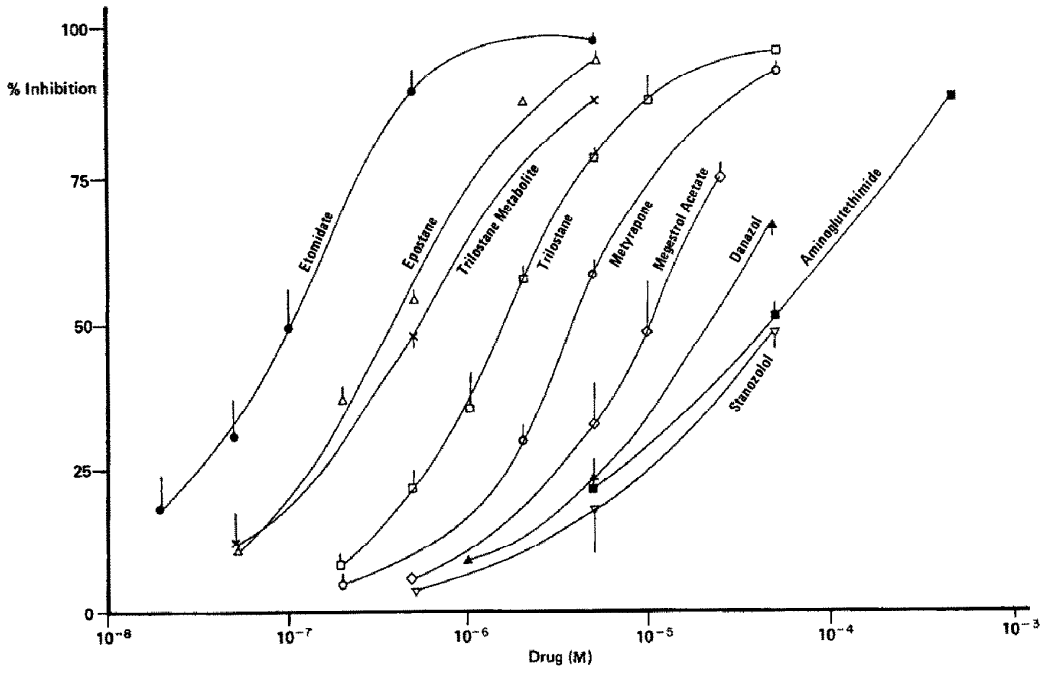

Fig. 2. Inhibition of ACTH (50 ng/l)—stimulated cortisol secretion by etomidate (●): epostane (△): 17-ketotrilostane (×): trilostane (□): metyrapone (○): megestrol acetate (◇): danazol (▲): aminoglutethimide (■) and stanozolol (▽). Each point is the mean ± SEM ($n = >8$ in all cases).

effect of anaesthetic agents on adrenal steroidogenesis [12] and on other determination of the sites of the anti-adrenal steroidogenic effect of certain drugs [13], we have used dimethylsulphoxide (DMSO, 2.5%) for incorporation of the drug into the cell suspension. DMSO (2.5%) has no significant effect on ACTH (50 ng/l)—stimulated cortisol secretion. Each experimental point was performed in at least duplicate wells: intra and inter-well coefficients of variation for this system were <6 and 10% respectively ($n = >100$) in both cases [3]. None of the drugs interfered in the cortisol radioimmunoassay at the concentrations employed.

RESULTS

Figure 2 shows the percent inhibition of ACTH-stimulated cortisol secretion by the drugs tested. The concentration of drug which inhibited cortisol secretion by 50% (ED_{50}) is shown in Table 1. Etomidate was the most potent drug (ED_{50}, 0.097 μmol/l) and stanozolol the least (ED_{50}, 50 μmol/l). All the drugs showed similar and steep dose reponse curves. For example, 50 μmol/l trilostane reduced ACTH-stimulated cortisol secretion by 20 ± 3-fold ($n = 9$).

Table 1. The concentration of drug which inhibited cortisol secretion by 50% (ED_{50}) is shown

Drug	ED_{50} (μmol/l)
Etomidate	0.097 ± 0.002 (4)
Epostane	0.44 ± 0.02 (3)
17-Ketotrilostane	0.55 ± 0.04 (3)
Trilostane	1.3 ± 0.1 (12)
Metyrapone	3.5 ± 0.6 (6)
Megestrol acetate	11 ± 2 (2)
Danazol	22 ± 2 (6)
Aminoglutethimide	41 ± 5 (5)
Stanozolol	50 ± 4 (2)

Results are expressed as mean ± SEM. Figures in parentheses are the number of estimations of the ED_{50}.

DISCUSSION

We have developed an *in vitro* system for the assessment of the relative adrenal anti-steroidogenic effect of drugs which is simple, quick and reproducible. Whether these potencies relate to the *in vivo* pharmacological effect of the drugs, where drug absorption, metabolism and presentation must all be considered, remains unclear at this stage. However, etomidate, an anaesthetic implicated in the deaths of critically ill patients, had the most potent anti-steroidogenic activity of all the drugs tested. This result confirms our earlier preliminary observation [14]. Thus this drug is 13, 36 and 423 times more potent than the established anti-adrenal drugs trilostane, metyrapone and aminoglutethimide. 17-Keto-trilostane, the major active plasma metabolite of trilostane, is 2.4 times more potent than the parent compound, a finding in good agreement with that observed with the cytochemical bioassay of these drugs [15]. Furthermore, anti-steroidogenic activity has been confirmed for danazol and epostane, and demonstrated for the first time for megestrol acetate and stanozolol, drugs which are administered for their other pharmacological effects.

In conclusion, we report a novel approach to the assessment of biological potency of drugs by exploiting some of the characteristics of dispersed adrenal cells. A similar method has been applied to the estimation of the biopotency of anti-thyroid drugs using dispersed porcine thyroid cells [16]. Work is also in progress on drug effects on testosterone secretion by isolated Leydig cell preparations. This type of technique may have wide application in the biological screening of new and existing compounds. Finally, this approach may lead to the development of satisfactory *in vitro* bioassays for drugs in plasma which may aid the design of optimal dose schedules and therapeutic monitoring of the physiologically active agents.

Acknowledgements—W.R.R., J.F., and A.L. gratefully acknowledge the financial assistance of the North West Regional Health Authority. R.M. similarly thanks Sterling-Winthrop Research and Development.

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