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SHORT COMMUNICATIONS

Effect of 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane *in vivo* on baseline and adrenocorticotropic hormone-induced steroid production in dog adrenal slices*

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A CERTAIN small amount of steroid production persists in the adrenal cortex of a hypophysectomized dog,[1] in adrenals perfused with buffer containing no ACTH,[2] and in adrenal slices incubated with no ACTH in the medium.[2,3] Therefore, steroidogenesis can be divided into baseline (no ACTH) and ACTH-stimulated parts. Conversion of adrenal cholesterol to pregnenolone must therefore take place at a constant baseline rate which can be increased by ACTH. An examination of Fig. 1 reveals that a drug which acts anywhere past the continuous conversion step of cholesterol to pregnenolone should block both baseline and ACTH-stimulated steroidogenesis. Compounds which have been shown to inhibit various steps past this point do inhibit baseline and ACTH-stimulated steroid production. Metyrapone, an inhibitor of 11β-hydroxylase, is an example of this type of drug.[6] Compounds, such as puromycin, which prevent steroidogenesis by inhibiting protein synthesis, do not acutely affect baseline steroid production.[7]

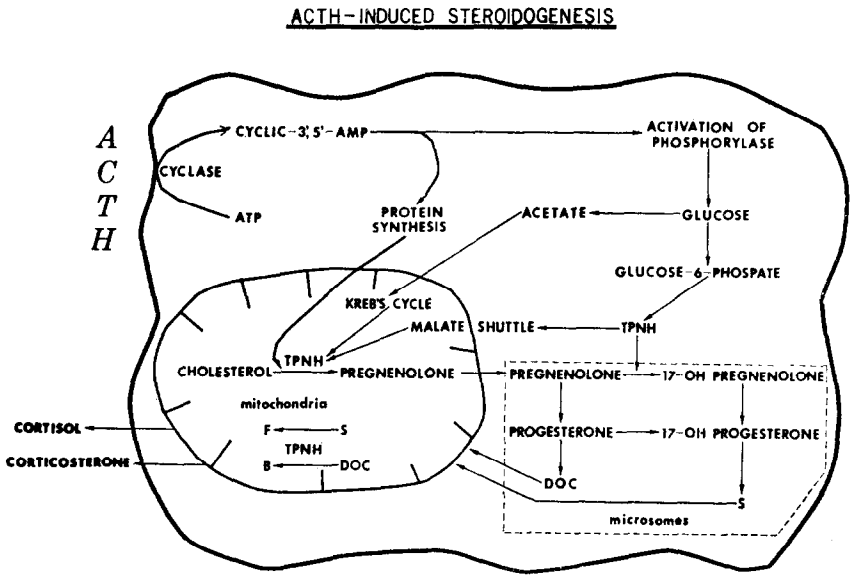

FIG. 1. Cell of adrenal cortex depicting ACTH-induced steroidogenesis. Detailed discussion of the pathways shown here may be found in papers by Bransome[4] and Simpson and Estabrook.[5]

It has been shown previously that 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD) has a direct action on the adrenal cortex of the dog[8,9] and man[10,11] to inhibit steroid production in response to ACTH. In order to define more closely the site of action of this drug in the

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steroid biosynthetic pathway, it was necessary to separate the effects of the drug on baseline steroid production and on ACTH-regulated steroidogenesis.

Male, mongrel dogs weighing 8–12 kg were fasted overnight while receiving water *ad lib*. One group of dogs received injections of glucocorticoids to suppress endogenous ACTH blood levels. Twenty mg of methyl prednisolone acetate (Upjohn) was given intramuscularly 18 hr prior to anesthetization. To supplement the action, 2 mg of dexamethasone sodium phosphate (Merck Sharp & Dohme) was administered intravenously after anesthetization with 30 mg/kg of pentobarbital sodium (Abbott). Another group of animals received no pretreatment with steroids. Control dogs of each group were then injected with drug solvent, 1:1 propylene glycol–95% ethanol. Drug-treated animals in each group received slow (1 ml/min) intravenous infusions of 60 mg/kg of *o,p'*-DDD (60 mg/ml) in this solvent.

Two hr after these injections, the adrenal glands were surgically removed, cleaned, sliced, preincubated and incubated in Krebs–Ringer bicarbonate (KRB) buffer.[12] Conditions for the incubation have been described previously.[3] After a 1-hr preincubation in KRB, the slices were incubated for 1 hr in either KRB containing 200 mg per 100 ml of glucose (KRBG) or KRBG plus 0·1 unit per ml of ACTH (U.S.P. Corticotropin reference standard).

The results of these studies are shown in Fig. 2. An examination of the preincubation values for nonsuppressed and suppressed control dogs indicates that treatment with methyl prednisolone (MP) was effective in reducing endogenous levels of ACTH in the adrenal slices. Control preincubation

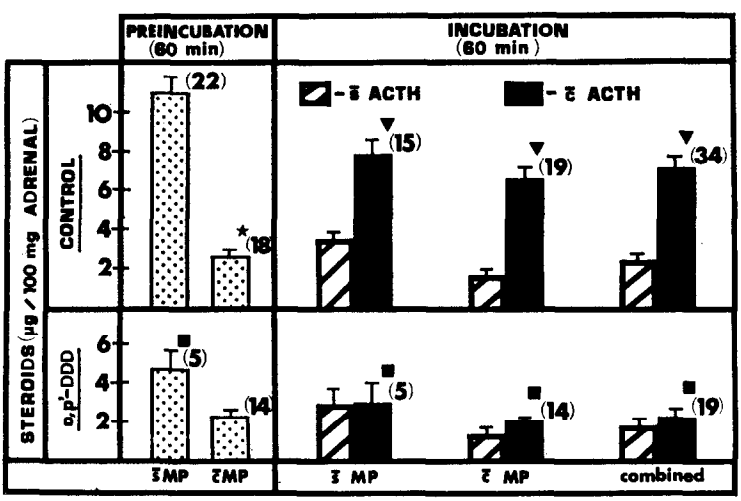

FIG. 2. Effect of *o,p'*-DDD (60 mg/kg, i.v.) on steroid production in adrenal slices obtained 2 hr after drug treatment. The number of dogs is shown in parentheses. Vertical lines represent one standard error of the mean. The triangle (▼) indicates significant difference between with and without ACTH ($P < 0.05$) using the *t*-test by the method of paired comparisons. The square (■) indicates a significant difference between control and *o,p'*-DDD-treated dogs ($P < 0.05$) using the *t*-test by the method of comparison of means. The asterisk indicates a significant difference with (c̄) and without (s̄) methyl prednisolone (MP) and dexamethazone treatment ($P < 0.05$) using the *t*-test by the method of comparison of means.

values for steroid production dropped from 11·61 ± 0·95 to 2·37 ± 0·31 µg/100 mg of adrenal after this treatment. The same pretreatment in *o,p'*-DDD-treated adrenals caused a similar drop (4·63 ± 1·55 to 2·17 ± 0·13) but to a lesser degree. When preincubation values from suppressed control and suppressed *o,p'*-DDD-treated adrenals are compared, there is no difference. Thus, *o,p'*-DDD treatment did not affect the level of baseline steroid production as estimated from preincubation steroid production values in maximally ACTH-suppressed dogs.

The most accurate estimation of baseline steroid production was obtained using adrenal slices from maximally ACTH-suppressed dogs incubated in the absence of ACTH. These values are shown as the striped bars in the middle graph on the right side of Fig. 2. In flasks incubated without ACTH, there

was no difference between control (1·54 ± 0·29) and *o,p'*-DDD-treated (1·33 ± 0·36) adrenals. This lack of effect on baseline steroid production was also indicated in results from nonsuppressed dogs (3·43 ± 0·30 and 2·83 ± 0·85) and in the combined steroid production results of all experiments, suppressed or not (2·37 ± 0·26 and 1·72 ± 0·32).

On the other hand, it is quite obvious that 2 hr of *o,p'*-DDD treatment *in vivo* completely blocked ACTH-induced steroidogenesis in dog adrenal slices. This can be seen very clearly in all of the graphs on the right side of Fig. 2.

The data presented here show that *o,p'*-DDD does not affect baseline steroid production at a time when ACTH-induced steroidogenesis is completely blocked. These findings suggest that *o,p'*-DDD interferes with the mechanism by which ACTH stimulates steroidogenesis, i.e. the activation of the conversion of cholesterol to pregnenolone.

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REFERENCES

1. F. R. SKELTON, in *The Adrenal Cortex*, p. 177. Harper, New York (1961).
2. M. M. HART and J. A. STRAW, *Biochem. Pharmac.* **20**, 1679 (1971).
3. M. M. HART and J. A. STRAW, *Biochem. Pharmac.* **20**, 257 (1971).
4. E. D. BRANSOME, JR., *A. Rev. Physiol.* **30**, 171 (1968).
5. E. R. SIMPSON and R. W. ESTABROOK, *Archs Biochem. Biophys.* **126**, 977 (1968).
6. R. GAUNT, J. J. CHART and A. A. RENZI, *Ergebn. Physiol.* **56**, 114 (1965).
7. J. J. FERGUSON, *J. biol. Chem.* **238**, 2754 (1963).
8. J. NICHOLS, in *The Adrenal Cortex*, p. 81. Harper, New York (1961).
9. A. CARZOLA and F. MONCLOA, *Science, N.Y.* **136**, 47 (1962).
10. A. L. SOUTHERN, S. TOCHIMOTO, L. STROM, A. RATNSCHNI, H. ROSS and G. GORDON, *J. clin. Endocr. Metab.* **26**, 268 (1966).
11. T. S. DANOWSKI, M. E. SARVER, C. MOSES and J. V. BONESSI, *Am. J. Med.* **37**, 235 (1964).
12. W. W. UMBRETT, R. H. BURRIS and J. F. STAUFFER, in *Manometric Techniques*, 4th edn, p. 132. Burgess, Minneapolis (1964).

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Effects of α-methylnoradrenaline on cardiac metabolism

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IT HAS been suggested that α-methylnoradrenaline (α-MNA) mediates the hypotensive action of α-methyldopa by acting as a false transmitter of less potency than the natural transmitter, noradrenaline.[1] More recently, it has been proposed that, although α-MNA may be less potent than noradrenaline on autonomic α-receptors, it may be at least equipotent to, or more potent than, noradrenaline on autonomic β-receptors.[2,3] A difference in affinity between α-MNA and noradrenaline for α- and β-receptors may well explain the hypotensive properties of α-methyldopa. A greater affinity of α-MNA for vascular β-receptors and a lesser affinity for vascular α-receptors would both decrease the hypertensive properties of α-MNA.