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ANTIHYPERTENSIVE ACTION OF 18,20-CYCLO-20,21-DIHYDROXY-4-PREGNEN-3-ONE, A STRUCTURAL ANALOGUE OF 18-HYDROXYDEOXYCORTICOSTERONE

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18-Hydroxydeoxycorticosterone (18-OH-DOC) is a mineralocorticoid secreted in greatly increased quantities under the influence of ACTH, not only in the rat, but also in man. The steroid has now been shown to have hypertensive properties in three species, namely the rat [1, 2], the dog [3, 4] and the sheep [5]. The hypertensive potency appears not to bear an obligatory relationship to its effect on electrolyte and water excretion, suggesting an additional action of the steroid, perhaps exerted at the level of the central nervous system. These attributes have strengthened the hypothesis that 18-OH-DOC might contribute to the etiology of some forms of hypertension and have focused our interest on the biological activity of a structurally closely related analogue, as a potential antagonist to adverse manifestations of 18-OH-DOC.

The derivative, 18,20-cyclo-20,21-dihydroxy-4-pregnen-3-one, differs from 18-OH-DOC only by the replacement of the 18,20-epoxylinkage with an 18,20-carbon bond. It was prepared from deoxycorticosterone acetate using a photochemical procedure [6] and tested in genetic hypertensive (SH) rats and in rats suffering from adrenal regeneration hypertension (ARH). Adult male SH and female ARH rats received an acute i.p. injection of 15 mg in a suspension of water and acacia. Blood pressure was measured by the tail cuff method and compared with the values obtained before injection as well as with the blood pressure of placebo-treated rats. The blood pressure of SH rats dropped from a preinjection value of 170 Torr to a minimum of 150 Torr within three hours after the injection and remained at that level for an additional 2 h (Fig. 1). The blood pressure of the ARH rats dropped from a preinjection value of 163 Torr to a value of 153 Torr 3 h, and of 140 Torr 5 h after injection. Both groups of animals regained their normal value blood pressure 24 h after administration of the drug. The average decline in blood pressure with time, derived by pooling all the individual differences, was not significant one hour, but highly significant 3 and 5 h after injection (Fig. 2).

These observations indicate that the analogue of 18-OH-DOC is capable of reducing the blood pressure of hypertensive rats at a concentration of 15 mg per rat, the only dose examined by us so far. This dose may not be inappropriate for effective competition since we have no information as yet on the rate of absorption of the i.p. administered compound,

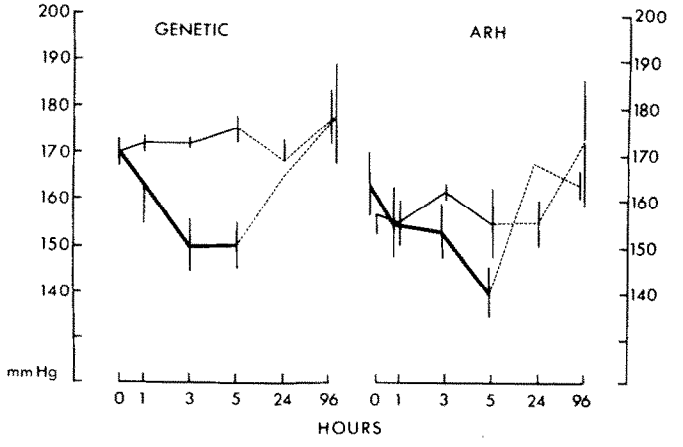

Fig. 1. Effects of 18,20-cyclo-20,21-dihydroxypregn-4-en-3-one on the blood pressure of genetic hypertensive (SH) rats, and rats suffering from adrenal regeneration (ARH). Heavy lines, steroid-treated animals, light lines, placebo-treated controls. Vertical bars indicate standard error. The steroid was administered i.p. in a suspension of water and acacia, at a dose of 90 mg/kg, or 15 mg/rat.

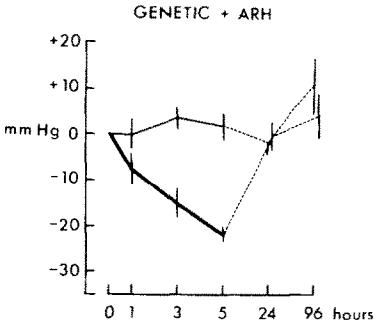

Fig. 2. Change in blood pressure, obtained by averaging all individual differences. Heavy lines, SH + ARH rats treated with steroid, light lines, SH + ARH rats treated with placebo. The changes at 3 and 5 h are highly significant ($P < 0.01$).

since the analogue is less lipophylic and hence presumably less readily accessible to steroid-sensitive receptor sites than 18-OH-DOC, and since measurements of 18-OH-DOC in rat adrenal vein blood indicate that maximally stressed animals could secrete 5 mg per day, assuming that the rate of secretion observed during the vein blood collection (10–30 min) can be maintained for 24 h [7]. *In vitro* the analogue specifically counteracts the inhibitory effects of 18-OH-DOC on aerobic glycolysis but does not modify the stimulation of glycolysis induced by deoxycorticosterone [8]. However, it remains to be established whether the 18,20-cyclo-compound exerts its antihypertensive effect by competing with a structurally related steroid or by some independent intrinsic action.

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DISCUSSION

Pasqualini. Do you have some data about the half life of 18-hydroxy-DOC and on its possible binding to specific plasma proteins.

Birmingham. I have no data on the half life but as far as the binding is concerned it is a very poor binder to corticosteroid binding globulin.

Ulick. Does the compound you described block the action of 11-deoxycorticosterone on sodium retention?

Birmingham. We have not tried it yet. The next experiment to do is to try it out on dogs rendered hypertensive with DOC or aldosterone or 18-OH-DOC. This should tell us something about the specificity of the blocker. The cyclo-compound is also similar in structure to aldosterone which, if you wish, you can write with an 18,20-epoxylinkage. What I think is interesting is to find that the cyclo-compound does not block the glycolytic effect of DOC on the adrenal *in vitro*, but, in contrast, it completely blocks the antiglycolytic effect of 18-OH-DOC and thus appears to exhibit specificity in its antagonism, at least in an *in vitro* system (Endocrine society, 57th annual meeting, Abstract No. 293, 1975).

Jones. It is because of your work that my laboratory looked at the effects of 18-hydroxy-DOC. As you mentioned we find that 18 hydroxy DOC antagonises the fast feedback action of corticosteroids when it is added to the hypothalamus *in vitro*. I was wondering whether there might be two mechanisms of action for 18-hydroxy-DOC. Firstly, it might have a direct effect on the hypothalamus. Secondly, 18-hydroxy-DOC might alter vascular responsiveness to noradrenaline and sympathetic tone, i.e. a peripheral effect. Has any work been carried out in this field?

Birmingham. No, not yet.

Crabbé. Did you look for the effect of 18-hydroxy-DOC on CRF release Dr. Jones? Does this steroid inhibit it?

Jones. No it does not inhibit. On the contrary it dysinhibits by antagonising the feedback effect of corticosteroids.

Crabbé. So that's in keeping with Dr. Birmingham's data.

Birmingham. Which is wonderful because I am so glad you did this. Have you measured the ACTH level, this is really the critical experiment that we should do.

Jones. No, we have not unfortunately.

Crabbé. Because of this, Dr. Birmingham, may I ask you whether the effects you have shown with 18 hydroxy DOC on thymus and liver are direct effects, or could they possibly be mediated through the release of corticosterone?

Birmingham. Yes, they could be. However, the antiinflammatory assay works also in the adrenalectomized rat.

Fraser. The marked effect of deoxycorticosterone on the drinking habit of the dog deserves discussion. Have you any information on changes in antidiuretic hormone levels in these experiments?

Birmingham. No, not really. We have suggestive evidence in the adrenalectomized rat of a vasopressin-like action of 18-OH-DOC, since in our bio-assay the retention of

water was out of proportion to the retention of sodium (*J. steroid Biochem.* **5** (1974) 789). But one has to watch out here, not only for differences between species but also within a species. Thus, in our dog experiments we got a statistically significant increase in saline intake in the first dog implanted with 18-OH-DOC, but in the second dog there was absolutely no effect at all on saline intake. The blood pressure was increased by 18-OH-DOC in both dogs.