Endocrinol. Japon. 1967, **14** (3), 265~268

NOTES

EFFECT OF HYDROXYLATION BLOCKING AGENTS ON CORTICOIDS CONCENTRATION IN THE PIGEON ADRENALS

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SYNOPSIS

The influence of three corticoid-inhibitors (Amphenone, Metopirone and SU 9055) on the corticoid secretion of the domestic pigeon was studied on a comparative basis. The level of corticosterone was reduced considerably with Amphenone and Metopirone; SU 9055 induced a rise. The 'cortisol (?) level was reduced moderately with the three inhibitors. The findings on corticosterone only were statistically significant. The implications of these results have been briefly discussed.

The corticoid blocking agents (e.g., Amphenone, Metopirone, SU 9055) are being recently experimented in the field of mammalian endocrinology and have helped to gather valuable knowledge about the adrenocortical metabolism and function in human and other mammalian species (for details *vide* Brown, 1960 and Gaunt *et al.*, 1963). The use of these corticoid inhibitors have been rather restricted in the avian group. Thus, Newcomer (1959) observed that Amphenone failed to cause any perceptible changes in the adrenal Δ4-3 Ketocorticosteroids in white leghorn cockerels. Glick (1962) studied the effect of Amphenone on the leucocytes of chick and concluded that use of Amphenone did not inactivate the chicken adrenal cortex. The bursa, which is known to be reduced with glucocorticoids also atrophied after treatment with Amphenone and indicated its non-inhibition of the cortex. With Metopirone a reduction of corticosterone level was seen in the pheasant and the cockerel (Nagra *et al.*, 1963 and 1965). The effects of SU 9055 on avian corticoidogenesis is not yet known.

A trial of these drugs on a comparative basis might lead to interesting revelations on avian corticosteroid metabolism. Therefore, in the domestic pigeon, Amphenone, Metopirone and SU 9055 were administered almost simultaneously to make a comparative survey of effects on adrenal corticoids.

MATERIALS AND METHODS

Young adult pigeons approximately 90 days old were used for this study. The birds were divided into four groups, each containing nine. The first group was an untreated control one and three others were arbitrarily assigned for Amphenone, Metopirone and SU 9055 treatment. Amphenone to be used was dissolved in saline while Metopirone and SU 9055 were each dissolved in propylene glycol. Dosage schedule and treatment for each drug was 75mg/kg intravenously for an experimental period of one hour. Control birds for each experimental lot

Received for publication March 1, 1967.

were injected with the same amount of vehicle without the respective drug. These birds were also divided into three groups containing equal number of birds and were autopsied almost simultaneously with the experimental animals.

Adrenals from autopsied birds of each group were homogenised in saline medium and were extracted with acetone and petroleum ether for proteins and fats respectively. Subsequently, the dried samples were dissolved with ethyl acetate and were extracted with 0.2*N* sodium carbonate for the separation of pigments. The dried ethyl acetate extract was subsequently washed with methanol : chloroform mixture (1 : 1) and transferred into a test tube. The solvent was evaporated in a nitrogen atmosphere in a 40°C water bath. The residue was again dissolved in methanol : chloroform mixture (1 : 1) adding 6 drops at a time and applied on chromatographic paper.

The residue was transferred quantitatively to the starting line of the chromatography paper prepared in methanol. The chromatograms were developed at room temperature in dark following equilibriation for 18 hrs. with extract, standard and blank lanes in parallel. For each chromatogram, the reference standards used were authentic corticosterone and cortisol. The extract was chromatographed in the toluene : propylene glycol system (TPGd) (*vide* Zaffaroni *et al.*, 1950) on filter paper (Schleicher and Schnell No. 2043-A). The steroids were detected after color development of the corresponding spots of reference standards with Blue Tetrazolium (BT) reaction (Nowaczynski *et al.*, 1955). Corticosterone and cortisol were separated by the use of Isatin dye, Each absorbing area of the chromatogram was cut off and elution done by dripped overrunning of excised paper-strips with 30 m*l* of methanol. Blank and sample lanes of equal size from corresponding areas were eluted simultaneously. The elutes were evaporated under vacuo at 55°C and dried residue was transferred after dissolving in chloroform : methanol mixture (1 : 1); the solutions were then dried at 40°C water bath in a nitrogen atmosphere.

The eluted and dried steroids were dissolved in ethyl alcohol and to characterize further, color reaction was done with BT and NAOH solution according to the procedure of Recknagel and Litteria (1956). Quantitative measurement of the colored solutions was done in a Beckman spectrophotometer at 525mμ. The optical density readings were compared to a concentration curve similarly corrected for authentic corticosterone and cortisol.

RESULTS

Amphenone and other two compounds on their administration, produced anaesthetic and hypnotic effect on the pigeons to a varying degree. Anaesthetic effects were most pronounced with Amphenone and Metopirone. Birds became drowsy, lost their balance and sometimes lay prostrate. Most notable reactions were induced by Amphenone and SU 9055. The latter even proved toxic and lethal for two experimental birds. At autopsy, the spleen of some experimental birds (Amphenone and SU 4885 groups) was found highly enlarged. Statistically significant alterations in average relative adrenal weight occurred among birds with all three drugs (*vide* Table 1). Histological signs of stimulation were evident in interrenal cells following all three inhibitors. The cortex was moderately hypertrophied with Amphenone; Metopirone and SU 9055 caused extravassation, multiplication of cortical nuclei and lumina formation in the gland.

Table 1. Adrenal weights after stimulation with corticoid inhibitors

Treatment	Adr. wt.: Bd. wt. (Mean)	% Change
Control (9)*	5.06±1.90**	—
Amphenone (9)	6.70±1.74	+32.4
Metopirone (9)	6.88±4.24	+36.0
SU 9055 (9)	6.78±2.50	+34.0

* Number in parenthesis indicates the total number of pigeons used.

** Standard error of the Mean.

Table 2. Effect of corticoid-inhibitors on adrenal corticosteroids in pigeons

Group	Corticosterone μg/100mg of Adr.	% change	Cortisol (?) μg/100mg of Adr.	% change
Control (9)*	1.090± .05**	—	0.071± .02	—
Amphenone (9)	0.700± .03	−35	0.050± .01	−29
Metopirone (9)	0.700± .04	−35	0.048± .01	−32
SU 9055 (9)	1.200± .06	+10	0.063± .01	−11

* Number in parenthesis indicates the total number of pigeons used.

** Standard error of the Mean.

Estimation of corticoids

Following a single injection of 75mg/kg of SU 4885 (Metopirone) given intravenously, the corticosterone level in the adrenal fell to 35% of the control value of untreated pigeons as shown in Table 2. Amphenone also caused a suppression in the amount of corticosterone from control level which was quantitatively similar to that obtained with Metopirone. With SU 9055 administration, corticosterone rose as 10% of the control value. All these results were statistically significant ($P<.05$).

The effect of inhibitors on adrenal 'cortisol' (?) level is also shown in the Table 2. The finding of 'cortisol' as an avian corticosteroid component has been held as dubious. While earlier workers (Chester Jones *et al.*, 1959; Uristch and Deutsch, 1960) reported the presence of small amounts of cortisol in adrenal venous blood, others have not been able to confirm it (Nagra *et al.*, 1960). In his earlier study, de Roos (1960) reported minute quantities of cortisol in chicken adrenal *in vitro*, but in his later findings (de Roos, 1961) described it as an unknown compound 'X' which gives certain chromatographic behaviour as authentic cortisol. In the present investigation, a fraction was obtained from the pigeon adrenal which gave some chromatographic characters of authentic cortisol. However, we have used a different solvent system than that used by de Roos (1961). Moreover, he used TPTZ for identification, while we used BT reaction. In this preliminary study, it was not possible to further characterise the exact corticoidal nature of this 'unknown compound'. This fraction, however, showed certain characteristic results with the inhibitory drugs for corticoidogenesis. Compared to normal control values, a reduction was noted in the adrenal content of this fraction with Amphenone, Metopirone and SU 9055. The extent of reduction was 29%, 32% and 11% respectively for the three compounds. None of these results yielded statistical significance.

DISCUSSION

The chromatographic findings indicate that Amphenone and Metopirone had inhibitory effects on corticosterone secretion in the pigeon. Metopirone induced corticosterone inhibition has been reported also in other species of birds, i.e., the pheasant and the cockerel (Nagra *et al.*, 1963 and 1965). The findings on these avian species lead to the assumption that probably the mode of inhibition of Metopirone is much similar in birds as in mammals, i.e., by blocking 11β-hydroxylase system of corticoidogenesis (Jenkins *et al.*, 1959; Chart *et al.*, 1962) as already proposed by Nagra *et al.* (1965).

SU 9055, which has been shown to inhibit 17-α-hydroxylase system (Chart *et al.*, 1958 and 1962), was found to reduce cortisol formation only in mammals with a corresponding rise in corticosterone level (Jenkins *et al.*, 1959). In the pigeon, quite strikingly, a 10% increase in corticosterone value was noticed with SU 9055. Though this end result is similar to that in mammals in having no blockage, but rather an increase in corticosterone titre, nevertheless, the mode of action

of SU 9055 cannot be equally validated. Firstly, the absence of 17-hydroxylation system in avian corticoidogenesis is generally assumed (de Roos, 1961; Sandor *et al.*, 1963) and further no significant inhibition in the cortisol(?) fraction was noted. Since SU 9055 has been found to possess an inhibitory effect on 18-hydroxylation of aldosterone (Kahnt and Neher, 1962), it appears possible that SU 9055 caused blockage of aldosterone with a compensatory overproduction of corticosterone.

Rosenfeld and Bascom (1956) from their perfusion studies in calf adrenals proved that Amphenone inhibits corticoidogenesis at multiple sites in contrast with Metopirone and SU 9055 which bear rather selective actions. Therefore, it remains subject to further investigation how and at what levels Amphenone causes inhibition of avian steroidal pathways.

ACKNOWLEDGEMENTS

The gifts of Corticoid-inhibitors by Dr. J. J. Chart, of Ciba Pharmaceutical, New Jersey and Corticosterone and Cortisol by Rockefeller Foundation, N. Y., are heartily acknowledged.

REFERENCES

Brown, J. H. U. (1960). *Nature* **187,** 985.

Chart, J. J., H. Sheppard, M. J. Allen, W. L. Bencze and R. Gaunt (1958) *Experientia* **14,** 151.

Chart, J. J., H. Sheppard, T. Mowles and N. Howie (1962). *Endocrinology* **71,** 479.

Chester Jones, I., J. G. Phillips and W. N. Holmes In Comparative Endocrinology Ed. by A. Gorbman, John Willey, New York, p. 582~612 (1959).

de Roos, R. (1960). *Endocrinology* **67,** 719.

de Roos, R. (1961). *Gen. Comp. Endocrinol.* **1,** 494.

Gaunt, R., J. J. Chart and A. A. Renzi (1963). *Ann. Rev. Pharmacol.* **3,** 109.

Glick, B. (1962). *Poultry Sci.* **41,** 317.

Jenkins, J. S., J. W. Meakin and D. H. Nelson (1959). *Endocrinology* **64,** 572.

Kahnt, F. W., and R. Neher (1962). *Experientia* **18,** 499.

Nagra, C. L., G. J. Baum and R. K. Meyer (1960). *Proc. Soc. Exptl. Biol. Med.* **105,** 68.

Nagra, C. L., J. G. Birnie and R. K. Meyer (1963). *Endocrinology* **73,** 835.

Nagra, C. L., A. K. Sauers and H. N. Wittmaier (1965). *Gen. Comp. Endocrinol.* **5,** 69.

Newcomer, W. S. (1959). *Am. J. Physiol.* **196,** 276.

Nowaczynski, W., M. Goldner and J. Genest (1955). *J. Lab. Clin. Med.* **45,** 818.

Recknagel, R. O. and M. Litteria (1956). *Ibid.* **48,** 462.

Rosenfeld, G. and W. D. Bascom (1956). *J. Biol. Chem.* **222,** 565.

Sandor, T., J. Lamoureux and A. Lanthier (1963). *Endocrinology* **73,** 629.

Uristch, M. R. and N. M. Deutsch (1960). *Proc. Soc. Exptl. Biol. Med.* **104,** 35.

Zaffaroni, A., R. B. Burton and E. H. Keutmann (1950). *Science* **111,** 6.