J. steroid Biochem. Vol. 22, No. 1, pp. 97–102, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

IN VIVO SECRETION OF 3α-HYDROXY-5α-PREGNAN-20-ONE, A POTENT ANAESTHETIC STEROID, BY THE ADRENAL GLAND OF THE RAT

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(*Received* 27 *February* 1984)

Summary—3αOH-5α-Pregnan-20-one (allo-THP), a steroid with strong anaesthetic properties, was found to be secreted by the adrenal gland of the rat in quantities similar to those secreted by the rat ovary. From the hypnotic potencies established for this and other endogenous steroids there can be little doubt that the total amount of steroids with anaesthetic properties produced in a female rat are sufficient to exert a depressant action on certain cells of the brain.

In rats with intact adrenal glands a positive correlation existed between the adrenal secretion of allo-THP and pregnenolone or progesterone, whereas that between allo-THP and DOC was negative. This could be the result of a competition between the enzymes responsible for the oxidation and reduction of progesterone, the common precursor of allo-THP and DOC.

The possibility that allo-THP could have hypotensive actions was suggested.

INTRODUCTION

During a study on the role played by adrenal steroids in the development of "adrenal regeneration hypertension" [1] the presence in most adrenal blood samples of a lipophilic compound was noted whose chromatographic properties were different from those of pregnenolone and progesterone and of the C_{21}-hydroxylated corticosteroids. This compound had the same paperchromatographic mobilities as progesterone but gave on gas–liquid chromatography (GLC) a peak with the relative retention time (RRT) characteristic for 3αOH-5α-pregnan-20-one (allo-tetrahydroprogesterone: allo-THP). Allo-THP has previously been identified in ovarian venous blood of rats in quantities amounting to 50–90% of those of progesterone, depending on the phase of the oestrous cycle [2, 3].

Because of the increasing interest in the strong central depressant [4] and anaesthetic properties [5, 6] of the ring A-reduced progesterone metabolites, the secretion rates of allo THP by normal and enucleated, regenerated adrenal glands have been measured and form the subject of the present paper.

EXPERIMENTAL

Female Wistar rats (Canadian Breeding Laboratories, La Prairie, Quebec) were given a solution of 1% NaCl and 5% sucrose instead of drinking water. They were divided into 4 groups: the rats of the first group were sham operated controls. From the rats of the second group the right kidney and adrenal gland was removed [unilaterally nephrectomized and adrenalectomized (UAN)]. The rats of the third and fourth group were prepared as the UAN rats but in addition the left adrenal glands were enucleated and allowed to regenerate for 25–50 days. At the end of the experiment the mean weight of the regenerated glands was 14.2 ± 0.7 mg ($n = 25$), that of the intact left adrenals of the UAN rats 16.9 ± 1.2 mg ($n = 10$). The mean arterial blood pressure was measured by the tail cuff method. The values are given in Table 1. The rats with the regenerated adrenals fell into two groups, one normotensive group (AR-rats: adrenal regenerated) and one group which developed adrenal regeneration hypertension with final blood pressure values between 150 and 210 mm Hg (ARH rats: adrenal regenerated, hypertensive).

Adrenal venous blood was collected for 10–20 min under sodium pentobarbitone anaesthesia from the left gland (for details see [1]). The collection tubes contained small amounts of radio actively labelled steroids to enable the correction of the final results for losses occurring during the chemical procedures. For the correction of the values obtained for allo-THP the recoveries of radioactive progesterone was used. The whole blood samples were hemolized with water and extracted 3 times with ethylacetate. Each dried residue was first chromatographed on Whatman No. 42 filter paper in the toluene–propylene glycol system [7]. This sytem, separates corticosterone (B) from 18OH-deoxycorticosterone (18OH-DOC). The overflow which contained deoxycorticosterone (DOC), pregnenolone, progesterone and also allo-THP was rechromatographed in the B_3 system of Bush [8] in which DOC had an R_f value of about 0.6

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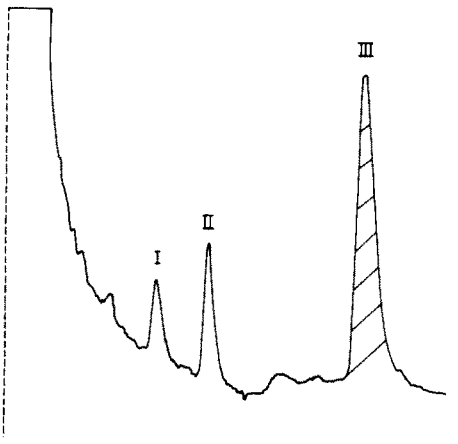

Fig. 1. 3αOH-5α-Pregnan-20-one (allo-tetrahydroprogesterone, allo-THP) and progesterone in rat adrenal venous blood. Gas–liquid chromatography tracing of an eluate of the progesterone region of a paper chromatogram (E_1 system of Eberlein and Bongiovanni) on which a purified extract of a sample of adrenal venous blood was developed (see Experimental). Retention times of peaks relative to that of cholestane (RRT 1); peak I: RRT 0.6 = allo-THP; peak II: RRT 0.8 = progesterone. The shaded peak III at RRT 1.45 is 11βOH-progesterone which was added as standard for quantitation.

whereas pregnenolone, progesterone and allo-THP travelled near the solvent front. The eluate of the solvent front was rechromatographed in the E_1 system of Eberlein and Bongiovanni[9]. The region containing pregnenolone and that containing progesterone + allo-THP was eluted separately, the eluates dried in a stream of N_2, the residues dissolved in 100 μl of ethanol and 10 μl removed for liquid scintillation counting to estimate the percentage of steroid lost. The remaining ethanol was again evaporated and the residue dissolved in 20 μl of a 10% solution of 11βOH-progesterone, the steroid used as internal standard for the quantitation by gas–liquid chromatography [GLC] (for details of GLC see [1]).

Figure 1 shows a GLC tracing of the eluate of the progesterone region from the E_1 chromatogram from a rat adrenal blood extract. The retention times given are relative to that of cholestane (RRT = 1). In addition to the progesterone peak (II, RRT 0.8) a smaller peak (I) can be seen at RRT 0.6, the retention time characteristic for allo-THP. The procedures for the identification of allo-THP in rat blood (including the formation of the trimethylsilyl ether and gas chromatography combined with mass-spectroscopy) [2, 3] and the methods used to measure the other adrenal steroids [1] have been previously described [1].

RESULTS

Allo-THP was detected in the adrenal venous blood extracts of 90% of the rats studied. In Table 1 the secretion rates of allo-THP in different rat groups and the mean blood pressure values are listed. The average hourly secretion rates ranged from 0.4 to 0.73 μg per gland, from 2–4 μg per 100 mg gland and from 0.28–0.93 μg per 100 g body weight. They were lowest in the rats with adrenal regeneration hypertension and increased in the order ARH < AR < UAN < controls. Table 2 shows the ratios of allo-THP secretion in relation to the secretion of the other steroids measured. The secretion rates of pregnenolone and progesterone were 3–7 times higher than those of allo-THP and the ratio was

Table 1. Adrenal secretion rates of 3αOH-5α-pregnan-20-one

Rat group	Secretion rates μg/left gland/h	μg/100 mg gland/h	μg/100 g b wt/h	Blood pressure (mm Hg)
Controls (13)	0.73 ± 0.14	3.99 ± 0.72	0.93* ± 0.17	128 ± 3 (10)
UAN (8)	0.64 ± 0.21	2.81 ± 0.87	0.53 ± 0.16 (7)	135 ± 4 (7)
AR (10)	0.49 ± 0.12	2.70 ± 0.61	0.36 ± 0.09	136 ± 6
ARH (13)	0.40 ± 0.11	2.04 ± 0.53	0.28 ± 0.08	165 ± 6

Mean values ± SEM; *Assumed that right gland secretes per mg tissue the same amount as left gland; Figures in parenthesis: number of observations; UAN: unilaterally adrenalectomized and nephrectomized; AR: adrenal regenerated, normotensive; ARH: adrenal regenerated, hypertensive; Co: controls; b wt: body weight; significance of differences in allo-THP secretion rates: (1) in μg/100 mg gland/h: Co vs ARH: $P < 0.05$; (2) in μg/100 g b wt/h: Co vs AR: $P < 0.02$; Co vs ARH: $P < 0.01$; significance of differences in blood pressure values ARH vs Co, UAN or AR: < 0.01 or less.

Table 2. Mean ratios of the adrenal secretion rates of various steroids over that of 3αOH-5α-pregnan-20-one (allo-THP) in individual blood samples of rats from different experimental groups

Group	Pregnenolone: allo-THP	Progesterone: allo-THP	DOC: allo-THP	18 OH-DOC: allo-THP	Corticosterone: allo-THP
Control	3.18 ± 0.37 (13)	4.31 ± 0.52 (13)	14.92 ± 3.86 (12)	116.5 ± 18.0 (13)	126.6 ± 18.8 (13)
UAN	3.62 ± 0.94 (8)	3.93 ± 0.51 (8)	11.11 ± 4.40 (8)	177.7 ± 64.2 (8)	232.0 ± 107.0 (8)
AR	3.54 ± 1.09 (10)	5.51 ± 1.62 (10)	29.03 ± 7.25 (10)	112.1 ± 32.0 (10)	124.8 ± 43.2 (10)
ARH	6.87 ± 2.88 (13)	6.03 ± 1.37 (13)	31.01 ± 9.10 (13)	150.5 ± 31.8 (13)	215.2 ± 49.5 (13)

For abbreviations see Table 1. Figures in parenthesis: number of steroid pairs.

Table 3. Correlation coefficients for the relation between the adrenal secretion rates of 3αOH-5α-pregnan-20-one (allo-THP) and various other steroids

Rat group	Pregnenolone: allo-THP	Progesterone: allo-THP	DOC: allo-THP	18OH-DOC: allo-THP	Corticosterone: allo-THP
Controls	+0.66 (13)	+0.48 (13)	−0.57 (12)	+0.38 (13)	+0.05 (13)
	$P < 0.02$	$P < 0.1$	$P < 0.05$	$P > 0.1$	$P > 0.1$
UAN	+0.89 (8)	+0.84 (8)	−0.14 (8)	+0.72 (8)	+0.65 (8)
	$P < 0.01$	$P < 0.01$	$P > 0.1$	$P < 0.05$	$P < 0.1$
AR	+0.73 (10)	+0.35 (10)	−0.01 (10)	+0.21 (10)	+0.60 (10)
	$P < 0.02$	$P > 0.1$	$P > 0.1$	$P > 0.1$	$P < 0.1$
ARH	+0.39 (12)	+0.43 (13)	−0.17 (13)	+0.53 (13)	+0.63 (13)
	$P > 0.1$	$P > 0.1$	$P > 0.1$	$P < 0.1$	$P < 0.02$

Abbreviations for rat groups as in Table 1. P: Levels of significance of correlation coefficients. Figures in parenthesis: number of pairs.

in both cases highest in the ARH group. The quantities of DOC secreted were 10–30 times, the quantities of 18OH-DOC and of B 100–200 times higher than those of allo-THP. Because of large individual variations the differences between the experimental groups were in no case significant.

In Table 3 the correlation coefficients for the relation between the secretion rates of allo-THP and various other steroids are given. The secretion of allo-THP correlated positively with those of pregnenolone and progesterone with P-values for the significance of r of < 0.02 or less on several occasions. In Fig. 2a the positive correlation between pregnenolone and allo-THP secretion in the control group is illustrated. Significant positive correlations were also seen between allo-THP and 18OH-DOC or corticosterone. In contrast, there was a significant negative correlation between the secretion of allo-THP and DOC in the same rats (see Fig. 2b). In the other groups the r values for allo-THP/DOC were also negative, but not significant.

DISCUSSION

The endocrinologist sees the adrenal cortex primarily as a source of hormones capable of modifying carbohydrate metabolism, of controlling inflammatory processes and of conserving body sodium. The extent to which this gland contributes hormones not confined to so-called gluco and mineralo corticoid activities is much less readily considered and deserves attention. These include steroids commonly associated with the ovary such as progesterone [e.g. 1, 11–14] or pregnenolone [e.g. 1, 11, 15] as well as other steroids devoid of gonodal or corticoid activity, which may nevertheless have a biological role, possibly related to their anaesthetic action.

In the present paper the secretion by the adrenal gland of the rat of a ring-A reduced progesterone derivative, allo-THP, has been described, for which a median hypnotic dose of 2.5 mg/kg has been established in the mouse [5] and rat [6]. This compares with a median anaesthetic dose for thiopentone sodium of 18 mg/kg in the mouse [16]. The detection of allo-THP in rat adrenal venous blood is not surprising as 5α-steroid-reductases have been demonstrated in the rat adrenal gland [e.g. 17, 18, 19, 20]. These papers also provide evidence that ACTH can inhibit the activity of the reducing enzymes. Kitay and his co-workers [21] put great emphasis on the regulatory

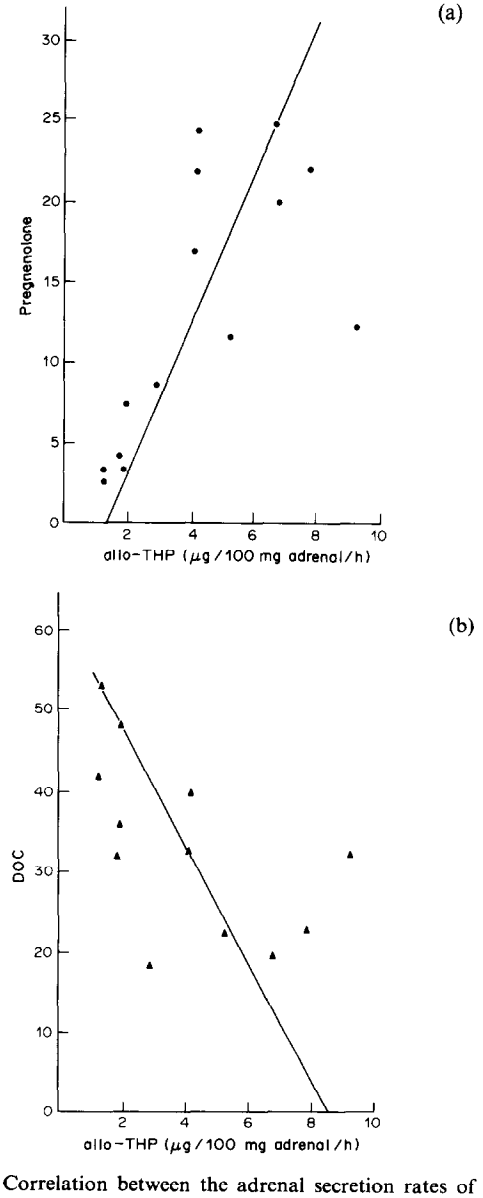

Fig. 2. Correlation between the adrenal secretion rates of 3αOH-5α-pregnan-20-one (allo-THP) and of Fig. 2a: pregnenolone; $r = +0.66$; Fig. 2b: DOC; $r = -0.57$. r = correlation coefficient.

Table 4. Progesterone and 3α-OH-5α-pregnan-20-one (allo-THP) in the ovary and adrenal gland of the rat: a comparison

Experimental condition		Progesterone	allo-THP	Ratio: Prog/allo-THP	Reference
Drinking fluid	Surgery	Secretion rate (μg/100 g tissue/h)			
		Ovaries			
Water	Ovarian blood collection, group 1*	4.5	1.1	4.1	(2)
Water	Ovarian blood collection, group 2	4.4	1.8	2.4	(2)
Water	Ovarian blood collection	4.7	not tested	—	(13)
		Adrenal glands			
Water	Adrenal blood collection	1.9	—	—	(31)
Water	Adrenal blood collection	1.4	—	—	(13)
0.9% NaCl, 4–6 weeks	Adrenal blood collection	6.2	—	—	(13)
1% NaCl in 5% sucrose, 3–7 weeks	Adrenal blood collection	14.0	4.0	3.5	present paper
		Tissue contents (μg/pair of glands)			
		Ovaries			
Water	No surgery	0.7	0.3	2.3	(22)
Water	No surgery	0.22	0.15	1.5	(2)
Water	Ovarian blood collection	1.2	0.8	1.5	(2)
		Adrenal glands			
Water	No stress	0.4	—	—	(23)
Water	Ovarian blood collection	0.8	—	—	(13)

Female rats, 130–220 g body weight. Samples were taken at different phases of the oestrous cycle, the values given are mean values throughout the cycle; *Samples only from early and late pro-oestrus; —: not tested.

effect of this phenomenon on corticosterone secretion by decreasing the formation of the ring-A reduced steroid metabolites in conditions of stress.

Table 4 allows a comparison between the production of allo-THP by the rat adrenal gland and the rat ovary. The amounts of allo-THP secreted per mg adrenal or mg ovary are of the same order of magnitude when measured in anaesthetized rats under the conditions of the surgical stress of adrenal- or ovarian blood collection and the ratios progesterone: allo-THP are similar for both glands. There were significant changes in allo-THP production by the ovary during the oestrous cycle [2, 22]. Investigations of cyclic variations of allo-THP by the adrenal gland are still to be carried out. Such variations may be expected because of the variations in adrenal progesterone contents during the cycle [23]. Table 4 shows also that the drinking of NaCl solutions for several weeks increases the adrenal secretion of progesterone.

Allo-THP secretion from the rat adrenal was measured under conditions in which the ACTH concentrations in the blood are elevated. In view of the inhibitory effect of ACTH on 5α-reductase it appears possible that under resting conditions the adrenal production of this steroid may be larger.

The negative correlation between allo-THP secretion and DOC secretion (see Fig. 2b) could be the result of a competition between the enzymes responsible for the reduction and oxidation of progesterone, the common precursor for allo-THP and DOC. The choice of pathways may be determined by the redox state of the tissue and by the concentrations of co-factors. Thus, in cow adrenal preparations the optimal concentrations of NADPH for the conversion of progesterone to 5α-allo-pregnanedione was 2 mM. The activity falls sharply at higher concentrations favoured by oxidative enzymes [18]. One way by which ACTH could effect the inhibition of the reductive steroid metabolism in the adrenal gland [17–21] is by control of NADPH synthesis, one of the earliest reported activities of ACTH [24]. In kinetic studies DOC has at times been found to behave as an end product rather than an intermediate metabolite [25], even though it is commonly considered to be the main precursor of the more oxygenated adrenocortical hormones. It was shown that 11β-hydroxylation [11, 26] or 18-hydroxylation [27] of progesterone can precede 21-hydroxylation in the rat adrenal.

It is tempting to speculate that the relatively low rate of allo-THP secretion in the hypertensive ARH rats reflects a role of this steroid in the homeostasis of blood pressure via an effect on the central nervous system. Experiments are therefore warranted to test for a prophylactic anti-hypertensive action of allo-THP or other ring A-reduced steroid derivatives which, in view of their lipophilic properties will readily cross the blood–brain barrier and exert their action at a central level. Other sites of action must also be considered. A decrease in the blood pressure of cats anaesthetized with Althesin, a steroid anaesthetic whose active component is 3α-OH-5α-pregnane-11,20-dione, was described [28].

The present observations have shown that, in the rat, steroids with central depressant actions are not only supplied by the ovaries [2, 3] and formed in certain brain regions [29, 30] but are also secreted by the adrenal gland. It appears likely that they are required for the physiological function of the brain.

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