Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 945–947. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

THE APPEARANCE OF DIHYDROTESTOSTERONE-^{3}H IN THE DIENCEPHALON OF NEONATAL RATS AFTER SUBCUTANEOUS INJECTION OF TESTOSTERONE-^{3}H

J. CHAMBERLAIN
Searle Scientific Services, Lane End Road, High Wycombe, England

and

A. W. ROGERS
MRC Neuroendocrinology Unit, Department of Human Anatomy University of Oxford, South Parks Road, Oxford, England

(*Received* 5 *May* 1972)

SUMMARY

Tritium labelled testosterone was injected into neonatal female rats. Tritium labelled steroids were extracted from the blood and organs of rats sacrificed 20 and 40 min after injection and identified by paper and thin-layer chromatographic procedures using authentic reference compounds. Only labelled testosterone was recovered from the blood in both experiments, whereas 25 and 10% of the radioactivity in the diencephalon was identified as 5α-dihydrotestosterone. In each case most of the label was recovered as polar metabolites.

INTRODUCTION

IT IS NOW well established that sexual differentiation in the rat takes place in the neonate under the influence of endogenous or exogenous testosterone [1]. Recently several reports [2–4] have indicated that the active form of the hormone at the cellular level is 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one). In the present work we have investigated the distribution and chemical nature of the labelled compounds found after subcutaneous injection of tritiated testosterone into female rats 96–120 h after birth. This communication will show that dihydrotestosterone appears in the diencephalon of the treated rats.

Two female rats between 96 and 120 h old were injected subcutaneously with 50 μCi (10 μg) [^{3}H]-testosterone in ethyl oleate and sacrificed after 20 and 40 min, respectively. Blood was collected from the neck. The diencephalon, cerebral hemispheres, pituitary and ovaries were removed for analysis. Counting of radioactivity in blood and tissues and identification of metabolites by thin-layer and paper chromatography were as previously described [5]. A summary of these results is in Table 1.

Detailed examination of the extracts of diencephalon was as follows: The crude alcoholic extract was chromatographed on Silica Gel G (benzene-ethyl acetate, 4 : 1 v/v). Zones of silica gel (0·33 in) were scraped from the plate and eluted with ethanol. Aliquots of the ethanol extracts were counted and the resulting radiochromatogram for experiment I is shown in Fig. 1.

The pattern of radioactivity was similar for the two experiments; peak B in

Table 1. Testosterone and its metabolites in blood and tissues of neonatal female rats after subcutaneous injection of testosterone-[^{3}H].

Sample	Total Radioactivity (cpm)	Polar Metabolites	T	DHT	A	5α-A
			%			
Blood I	85,000	0	100	0	0	0
II	10,000	0	100	0	0	0
Diencephalon						
I	3,500	50	25	25	0	0
II	12,000	57	23	20	0	0
Ovaries II	4,800	0	82	0	18	0
Cerebral Hemispheres						
I	6,800	0	86	(10)	0	4
II	1,100	24	33	(33)	(8)	2
Pituitary II	4					

Key: I indicates sample from rat killed 40 min post injection; II indicates sample from rat killed 20 min post injection; T = Testosterone; DHT = 5α-dihydrotestosterone; A = Androstenedione; 5α-A = 5α-Androstanedione.

Figures in parentheses indicate partial characterization only.

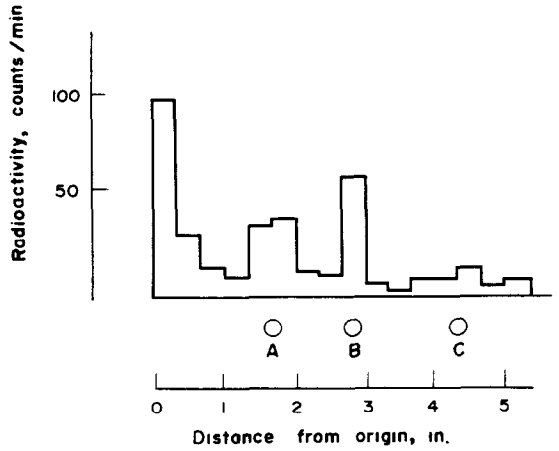

Fig. 1. Distribution of radioactivity of diencephalon extract after chromatography on silica gel G (benzene–ethyl acetate 4 : 1 v/v). A = Testosterone; B = 5α Dihydrotestosterone; C = 5α Androstanedione.

each case had the same R_f value (0·51) as standard 5α-dihydrotestosterone run on the same plate.

Aliquots of material from Peak B of experiment I were respectively oxidised and acetylated, and these derivatives and the untreated material rechromatographed on Silica Gel G (chloroform-ether, 9 : 1 v/v). Radioactivity was located on the plates by scraping 0·5 in. zones of silica gel directly into counting vials. The

radioactivity of the free material was located in the same zone as standard 5α-dihydrotestosterone (Average $R_F = 0{\cdot}45$), that of the acetylated derivative in the same zone as 5α-dihydrotestosterone acetate (Average $R_F = 0{\cdot}75$) and that of the oxidised derivative in the same zone as 5α-androstanedione (Average $R_F = 0{\cdot}65$).

An aliquot of material from peak B of experiment II was oxidised and this product and a further aliquot of the untreated material chromatographed in the paper partition system petrol ether: methanol: water, 5 : 4 : 1 R_F [5]. All radioactivity was located with standard 5α-dihydrotestosterone in the case of the untreated material ($R_F = 0{\cdot}30$), and with 5α-androstanedione in the case of the oxidised aliquot ($R_F = 0{\cdot}50$).

To the remainder of peak B material of experiment II was added 5 μg carrier 5α-dihydrotestosterone, and the mixture was chromatographed on Silica Gel G (benzene-ethyl acetate, 3 : 1 v/v). Zones of silica gel (0·1 in.) were eluted with ethyl acetate and the eluted zones assayed for 5α-dihydrotestosterone by gas chromatography and for radioactivity. Successive eluted zones of silica gel gave specific activities of 360, 385 and 350 cpm/μg steroid.

These results show that 5α-dihydrotestosterone is found in the diencephalon of 4-day old female rats, injected subcutaneously with testosterone. Though the characterisation remains incomplete in the extracts from cerebral hemispheres, it is probable that the same compound occurs here in similar yield. No evidence of its presence, on the other hand, could be found either in peripheral blood or in the ovaries.

The interpretation of these findings remains rather speculative. Previous work [7] failed to demonstrate any concentration of radioactivity above blood level by the brain in similar neonatal rats injected with [^{3}H]-testosterone. The subcutaneous injection of 100 μg 5α-dihydrotestosterone into 4-day female rats fails to androgenise them (Brown–Grant, Munck and Naftolin, unpublished data). It is possible, however, that the mode of action of the hormone in these animals requires its availability as testosterone, with intracellular conversion to 5α-dihydrotestosterone.

It is interesting to find the production of this compound from testosterone in the brains of neonatal rats – a conversion described for known target organs.

REFERENCES

1. Harris G. W.: *Endocrinology* **75** (1964) 627.
2. Bruchovsky N. and Wilson J. D.: *J. biol. Chem.* **243** (1968) 2012.
3. Anderson K. M. and Liao S.: *Nature* **219** (1968) 277.
4. Baulieu E. E., Lasnitzski I. and Robel P.: *Nature* **219** (1968) 1155.
5. Chamberlain J., Jagarinec N. and Ofner P.: *Biochem J.* **99** (1966) 610.
6. Bush I. E.: *Biochem J.* **50** (1952) 370.
7. Sherratt M., Exley D. and Rogers A. W.: *Neuroendocrinology* **4** (1969) 374.
8. Gloyna R. E. and Wilson J. D.: *J. clin. Endocr.* **29** (1969) 970.