Free and conjugated steroids in CSF, plasma and urine

Material	Time	ml	cpm $^3H/^{35}S$ per 10 ml CSF or plasma and 100 ml urine							
			Free steroids	R	Sulphatides	R	Sulphates	R	Glucurono-sides	R
CSF										
(1)	30 min	8.9	6	>100	1,370	13.2	32	16.0	2	>100
			0		105		2		0	
(2)	165 min	6.0	0		227	16.2	10		0	
			0		14		0		0	
Plasma										
(1)	5 min	5.8	189	>100	85,200	14.6	11,900	14.7	32	>100
			0		5,830		810		0	
(2)	30 min	18.5	923	>100	62,000	14.6	2,840	14.8	51	>100
			0		4,240		191		0	
(3)	60 min	5.0	133	>100	52,700	14.9	1,130	15.1	28	>100
			0		3,530		75		0	
(4)	90 min	6.0	40	>100	45,700	15.2	962	15.3		
			0		3,010		63			
(5)	120 min	21.0	18	>100	40,500	15.6	733	15.9		
			0		2,590		46			
(6)	165 min	9.8	11	>100	28,600	16.9	680	16.2		
			0		1,690		42			
Urine										
(1)	0–4 h	260	19,600	>100			620,000	22.0	32,800	>100
			32				28,200		63	
(2)	4–12 h	180	15,100	>100			230,000	30.4	43,600	>100
			0				7,630		14	
(3)	12–24 h	90	12,600	>100			289,000	38.3	69,400	>100
			102				7,550		31	

sulphates approximated 1:2:22. A steady increase in the isotope ratio R of urinary steroid sulphates from 22.0 in the first portion to 30.4 in the second and 38.3 in the third portion reflects a substantial (22.7%, 51.3% and 61.4%) hydrolysis of sulphoconjugates and resulphurylation of liberated steroids in the course of the experiment. Such findings, as well as the quantitative distribution of metabolites in the different fractions from urine, were in close agreement with previous results, obtained after i.v. administration of double-labelled DHEA sulphate[4,5].

From the data presented it was concluded that only lipophile steroid sulphoconjugates, e.g. steroid sulphatides, may pass from blood to CSF within a reasonable period of time. However, a certain blood/CSF barrier seems to exist even for these compounds.

Zusammenfassung. Nach i.v. Injektion von 7α-H-DHEA-^{35}S-sulfat wurden Liquor, Plasma und 24-Stundenharn eines Mannes auf freie und konjugierte, markierte C_{19}- und C_{18}-Steroide untersucht. Es zeigte sich, dass schon 30 Minuten nach Versuchsbeginn im Liquor fast nur doppelt-markierte Steroid-sulfatide mit praktisch unverändertem Isotopenverhältnis $^3H/^{35}S$ enthalten waren. Da weiterhin DHEA und seine Metaboliten im Liquor eine weitaus niedrigere spezifische ^3H-Aktivität besassen als die entsprechenden Verbindungen im Plasma, ist anzunehmen, dass der Übertritt von lipophilen Steroidsulfatiden zwar verhältnismässig rasch, aber nur in begrenztem Umfang erfolgte.

G. W. Oertel, H. Gumpert, P. Knapstein und D. Vogt

Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik und Neurochirurgische Universitätsklinik, D-65 Mainz (Germany), 15 October 1969.

The Effect of Castration and Hypophysectomy on the Content of Noradrenaline and Serotonin in the Hypothalamus of the Rat

Recently it has been shown that the castration of male rats has an influence on the formaldehyde induced fluorescence of the primary catecholamines of the hypothalamus[1]. Castration increases the turn-over rate of noradrenaline (NA) in the hypothalamus[2–4]. The hypophysectomy of short duration (5 days) has no effect on the catecholamine fluorescence in the hypothalamus[1]. In the present work, quantitative confirmation has been tried by estimating chemically NA and serotonin (5-HT) of the hypothalamus and cerebral cortex in male and female rats after castration or the hypophysectomy of long

[1] M. Hyyppä, Z. Zellforsch. *98*, 550 (1969).
[2] F. Anton-Tay, R. W. Pelham and R. Wurtman, Endocrinology *84*, 1489 (1969).
[3] A. O. Donoso, M. B. de Guitierrez Moyano and R. C. Santolaya, Neuroendocrinology *4*, 12 (1969).
[4] J. A. Coppola, J. Reprod. Fert., Suppl. *4*, 35 (1968).

duration (3 months). Because an augmentation of the storage of FSH-RF has been shown after castration or hypophysectomy[5,6], it is of interest to examine whether NA or 5-HT are involved like dopamine (D)[7,8,9] in the feedback systems which regulate FSH secretion through FSH-RF.

Material and method. 40 adult male albino rats were castrated. These and 40 control rats were killed after 3 weeks for the determination of NA and 5-HT. Correspondingly 13 female rats were hypophysectomized[10] at the age of 21 days, and these were killed 3 months later together with 9 control rats. The content of NA and 5-HT in the hypothalamus and cerebral cortex was measured[11]. After hypophysectomy 3 hypothalami were examined using the fluorescence histochemical method of FALCK and HILLARP for primary catecholamines[1].

Results and discussion. (1) The effect of castration. No significant effect of castration was seen on the content of NA and 5-HT in the anterior or posterior hypothalamus war in the cerebral cortex of male rats (Table I). Previously an increase of the content of NA has been demonstrated in the anterior part of the hypothalamus after castration[12]. This observation has not been confirmed by other investigators[13]. It is more obvious that only the turn-over rate of NA has increased following castration, which results in an augmentation of the FSH level[2]. When the increase of the intensity of the formaldehyde induced fluorescence in some tubero-infundibular cell bodies has been observed after castration, it may be as a result of the alteration of the D content in the region which regulates the gonadotrophin secretion. This occurs simultaneously with the increase of FSH-RF[7]. Thus hypothalamic catecholamines are involved in the regulation and release of FSH-RF.

(2) The effect of hypophysectomy. The hypophysectomy of long duration (3 months) caused a significant increase of the content of NA in the anterior and posterior hypothalamus of female rats, but it had no effect on the NA content in the cerebral cortex. Also the amount of 5-HT increased almost significantly in the posterior hypothalamus (Table II). No marked changes were seen histochemically in the intensity of the formaldehyde induced fluorescence. It has been observed that hypophysectomy alone causes a non-significant augmentation of FSH-RF in male rat, but this might be an effect of the function of the negative long-feedback[5]. In male[1], as well as in female rat[14], the hypophysectomy of short duration had no influence on the fluorescence intensity in the tubero-infundibular region. Whether this is due to the failure of changing the content of D in this system, is difficult to demonstrate without quantitative estimations. These are in progress.

The results obtained from this study are in favour of the earlier observations concerning the effect of FSH on the metabolism of NA in the central nervous tissue[2]. When the pituitary has been removed, it seems that the hypothalamic stores of NA increase. Perhaps their catabolism is then delayed. It seems likely that the short-feedback has an influence on the NA level, but the long-feedback seems to function independently of the hypothalamic NA. The feedback effects on the 5-HT are not so clear as on NA and D, though the role of 5-HT in the regulation of the ovulation[15] has been stated[16].

Table I. The effect of castration on the hypothalamic NA and 5-HT in male rats

Region	NA		5-HT	
	Normal	Castrated	Normal	Castrated
Anterior hypothalamus	1.96 ± 0.39 (9)	1.97 ± 0.52 (13)	1.11 ± 0.04 (6)	1.11 ± 0.34 (11)
Posterior hypothalamus	1.52 ± 0.49 (13)[a]	1.15 ± 0.36 (11)	0.96 ± 0.15 (9)	0.98 ± 0.24 (12)
Cerebral cortex	0.29 ± 0.09 (12)	0.25 ± 0.06 (11)	0.35 ± 0.09 (9)	0.35 ± 0.08 (12)

Means μg/g ± S.D. [a] Almost significant difference from normal ($P \leq 0.05$).

Table II. The effect of hypophysectomy on the hypothalamic NA and 5-HT in female rats

Region	NA		5-HT	
	Normal	Hypophysectomized	Normal	Hypophysectomized
Anterior hypothalamus	1.07 ± 0.22 (3)	2.15 ± 0.30 (4)[b]	1.17 ± 0.36 (3)	1.28 ± 0.10 (4)
Posterior hypothalamus	1.28 ± 0.41 (3)	2.52 ± 0.22 (4)[b]	1.10 ± 0.20 (3)	1.65 ± 0.22 (4)[a]
Cerebral cortex	0.46 ± 0.33 (3)	0.45 ± 0.22 (4)	0.71 ± 0.11 (3)	0.66 ± 0.04 (4)

Mean μg/g ± S.D. 3–4 samples were pooled, in brackets the number of extracts. [a] Almost significant difference from normal ($P \leq 0.05$). [b] Significant difference from normal ($P \leq 0.01$).

Zusammenfassung. Über die Wirkung der Gehirn-Catecholamine auf die endokrinen Funktionen wird ein gültiger Befund erhoben.

M. HYYPPÄ and M. VALAVAARA

Departments of Anatomy and Medical Chemistry, University of Turku, Turku 3 (Finland), 6 October 1969.

[5] M. MOTTA, Proc. Third Int. Congr. Endocr., Mexico 1968, in press (1969).
[6] M. HYYPPÄ and M. MOTTA, Scand. J. clin. Lab. Invest. *23*, Suppl. 108, *39* (1969).
[7] M. HYYPPÄ and M. LORENZ, Acta Endocr., Copenh. Suppl. *138*, 192 (1969).
[8] J. A. KAMBERI, H. P. G. SCHNEIDER and S. M. MCCANN, *The Endocrine Society*, Program of the Fifty-First Meeting 1969, p. 161.
[9] K. FUXE, T. HÖKFELT and O. NILSSON. Neuroendocrinology *5*, 107 (1969).
[10] G. FALCONI and G. L. ROSSI, Endocrinology *74*, 301 (1964).
[11] R. P. MAICKEL, R. H. COX JR., J. SAILLANT and F. P. MILLER, Int. J. Neuropharmac. *7*, 275 (1968).
[12] A. O. DONOSO, F. J. E. STEFANO, A. M. BISCARDI and J. CUKIER, Am. J. Physiol. *212*, 737 (1967).
[13] R. SANDLER, Endocrinology *83*, 1383 (1968).
[14] M. HYYPPÄ, unpublished data (1968).
[15] C. KORDON, Neuroendocrinology *4*, 129 (1969).
[16] Supported by a grant from The Finnish Medical Society 'Duodecim'.