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DYNAMICS OF ESTRADIOL AND TESTOSTERONE UPTAKE IN THE BRAIN OF ADULT MALE RATS

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SUMMARY

[^{3}H]-Estradiol (1.28 μmol) and [^{14}C]-testosterone (243 μmol) were given in an i.v. bolus and the concentration of both steroids in the central nervous system at different times was studied. Uptake and retention of phenolic steroids in the pituitary and the hypothalamus was higher than found in peripheral plasma indicating preferential concentration which persisted for more than 2 h. In contrast the uptake of testosterone and dihydrotestosterone was lower than could be expected from concentration in peripheral plasma and these compounds disappeared within 0.5 h after the pulse.

INTRODUCTION

During the last two decades several groups have reported data supporting the hypothesis that estrogens may act as gonadotropin regulators in males. As early as 1952 McCullagh and Schaffenbug[1] noted that estrogens were much more potent in inhibiting gonadal function than androgens. Lisk[2] used hormonal implants in the hypothalamus to demonstrate the superior action of estrogens in blocking gonadal activity. Korach and Muldoon[3] found no difference in estradiol binding to cytosol macromolecules in male and female hypothalami and suggested that estradiol may be the active hormone in males. Similar observations were also advanced by others [4–6].

Simultaneous intravenous injection of [^{3}H]-estradiol and [^{14}C]-testosterone into adult male rats resulted in significant concentration of both labels in the diencephalon whereas only estradiol, and not testosterone derived label, was found in the pituitary-hypothalamic area [7–10]. These experiments demonstrated that the binding of estrogenic hormones in the neuroendocrine tissues of males is significantly higher than that of androgens suggesting that these hormones play the major role in the control of physiological functions.

The studies described below were undertaken to define the dynamics of brain uptake of estradiol and testosterone following a single i.v. bolus with the expectation that a more complete understanding of these phenomena may aid in evaluating the physiological role of estradiol in the male rat.

EXPERIMENTAL

General experimental conditions were as described previously [10] but for the following modifications. Six male rats (age 65–70 days) were used per group (each time period). The dose per animal was 133 μCi of [2,4,6,7-^{3}H]-estradiol (SA 104 Ci/mmol) or 1.28×10^{-6} mol and 13 μCi of [4-^{14}C]-testosterone (SA 53.5 mCi/mmol) or 2.43×10^{-4} mol. Heparinized blood (6–10 ml) was collected under slight ether anesthesia by heart puncture. Both "free" (ethyl acetate extractable) and "conjugated" (ethyl acetate non-extractable) fractions were separated. One-half of the "free" extracts was used to measure the radioactivity present. The other 50% was subjected to double thin layer chromatography (t.l.c.): the first solvent system used was chloroform–acetone–petrol ether (6:1:4, by vol.), the second was chloroform–ethanol (95:5, v/v); both solvent systems were used on the same plate. The following cold standards were used at the same time: 4-androstene-3,17-dione (A), 17β-hydroxy-5α-androstan-3-one (DHT), estrone (E_1), estradiol (E_2), and testosterone (T).

To calculate the area under the time curve the time integral consistent for most of the tissues (8–32 min, three data points range) was used. The best straight line through all available data points yields an equation of the form: concentration (fmol/100 mg) = $Ae^{-\alpha(\text{time})}$, the evaluation of the integral from 8 to 32 min = $(A/\alpha)e^{-\alpha(32)} - (-A/\alpha)e^{-\alpha(8)}$. The concentrating of a particular hormone by a tissue was evaluated by two methods: the ratio of tissue to plasma levels (concentrating against circulating levels), and by comparing the ratios of the neuroendocrine tissue levels to the remainder of the brain (considered a dilute or neutral tissue), a comparison which would be unaffected by the limiting effect of the blood/brain barrier. The areas under the curve were divided by the dose expressed as fmol/100 mg mean body weight. The mean body weight was 275 g $\pm$ 1.2 and the concentration of [^{3}H]-estradiol was equal to 1.28×10^6 fmol while the concentration of [^{14}C]-testosterone was equal to 2.43×10^8 fmol (molar ratio = 190:1).

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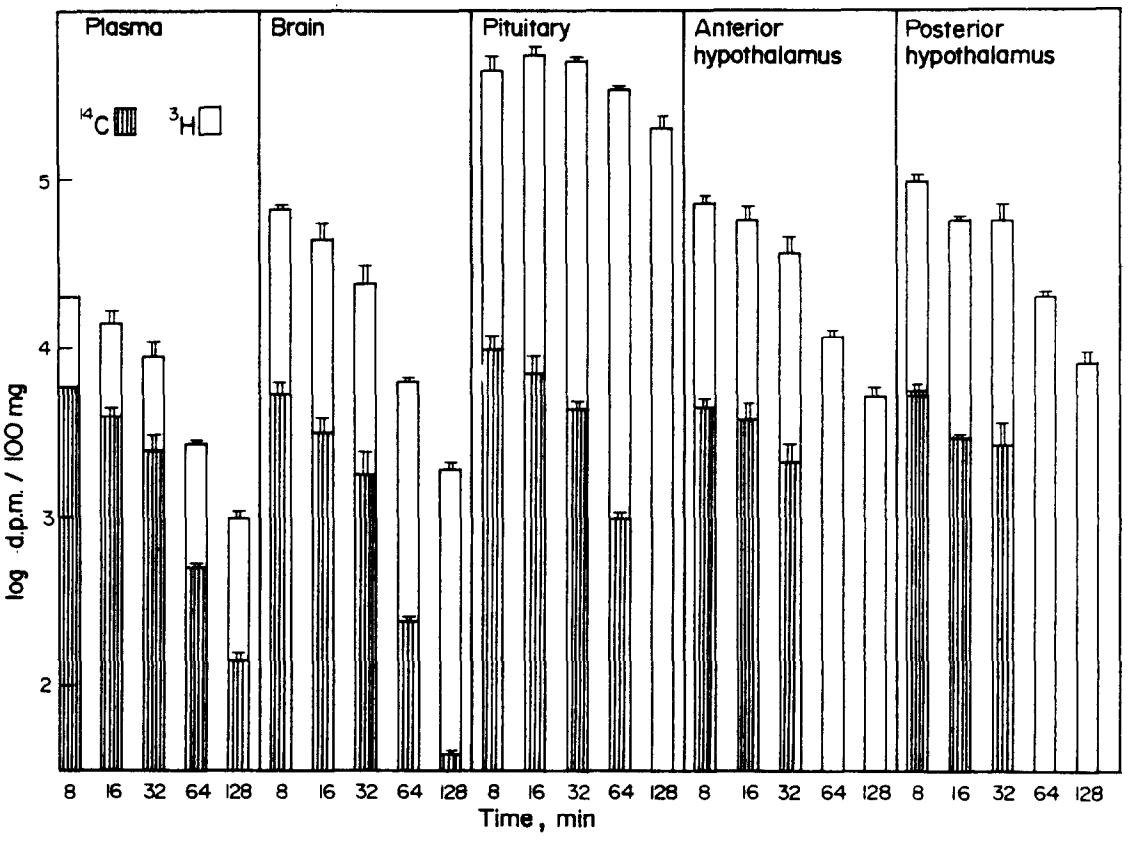

Fig. 1. Radioactivity concentration in various tissues of male rats following an i.v. bolus of bolus of [^{3}H]-estradiol (133 μCi—open bars) and [^{14}C]-testosterone (13 μCi—stippled bars) expressed as log d.p.m./100 mg wet tissue (ordinate) vs time in min after the bolus (abcissa).

RESULTS

Figure 1 illustrates graphically the total radioactivity detected in the five tissues studied expressed as log d.p.m./100 mg tissue wet weight (ordinate), detected at 8, 16, 32, 64 and 128 min pulse (abscissa). Estradiol (^{3}H) derived radioactivity was retained in all tissues during the observation period. The highest concentration was detected in the pituitary during 16–32 min after the injection (d.p.m./100 mg ± SEM were 542,390 ± 71,740 at 16 min, and 506,120 ± 37,890 at 32 min, respectively). The accumulations in anterior hypothalamus (71,000 ± 8475 d.p.m./100 mg) and posterior hypothalamus (95,680 ± 9506 d.p.m./100 mg) were highest 8 min after the i.v. injection; the same was true for plasma values (106,250 ± 7975 per 100 μl).

Isolation and purification yielded an estimate of the concentration of E_1, E_2, DHT and T present in the various tissues. Figures 2–4 show radioactivity present (d.p.m./100 mg wet tissue weight) between 8–128 min. Examination of the curves shows that [^{3}H]-estradiol was the principle hormone found in all tissues, except plasma where testosterone was the highest. All of the ^{3}H compounds had a higher level in the brain as compared to plasma with estradiol showing an almost sixteen-fold increase. The inverse was true regarding ^{14}C derived radioactivity with the exception of estradiol and DHT with [^{14}C]-estradiol being the highest with a four-fold increase.

DISCUSSION

Estradiol concentration in the peripheral plasma of female rats fluctuates with the estrus cycle, highest levels may reach about 30 pg/ml [11]; in male rats estradiol concentration is about 10 pg/ml [12]. Thus the hormone is present in both sexes in roughly the same concentration, except that in females the levels fluctuate whereas in males the release is apparently tonic. Testosterone concentration in male rats is about 1 ng/ml, about three times that of the concentration found in females [13] but a considerable fluctuation (2–5 fold) can take place [14]. Thus the estradiol:testosterone ratio in the peripheral plasma of male rats is about 1:200. The molar dosage ratio used in our studies (1:189) was selected to reflect this. We used a ^{14}C labelled testosterone of relatively low specific activity and thus had to sacrifice accuracy to detect low levels of this hormone and its metabolites.

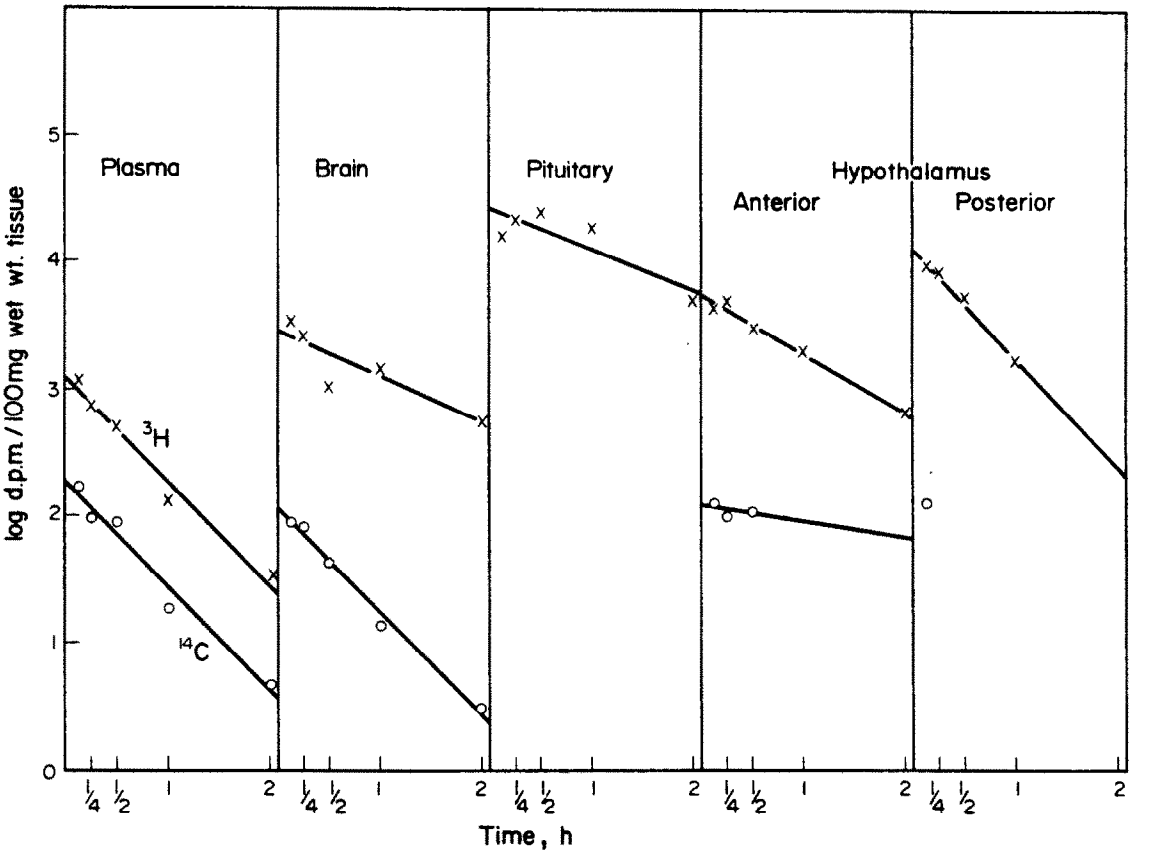

Fig. 2. Estrone concentration in tissues of adult male rats following an i.v. bolus of estradiol; pulse 8–128 min.

The metabolism of androgen into phenolic steroids by the CNS observed in our studies is in confirmation with results reported by others [15]. Likewise, the presence of estradiol receptor macromolecules in the hypothalamus and the pituitary has been documented in a number of species [for a review see 16, 17]. The observed higher uptake in the posterior hypothalamus as compared to the anterior portion is in agreement with earlier studies [18].

The first report that hypothalamic nuclei and the pituitary gland possess the ability to concentrate radioactive testosterone appeared 10 yr ago [19] yet there still appears to be a controversy whether or not the neuroendocrine tissue binds androgens. Several groups [16, 20–25] have shown *in vivo* and *in vitro* that radioactive androgens are bound by macromolecules in the cytosol fraction and nuclei of the anterior pituitary, hypothalamus, preoptic area and brain cortex, while others [3, 26, 27] detect only non-specific binding. The conflicting results could be caused by differences in experimental design; if androgen receptors are present in very small amounts and are labile, effects of temperature at which *in vitro* work is carried out, the amount and specific activity of steroid used, the manner of injection (some workers used intramuscular or intraperitoneal injection) and the time pulse are all of importance. Some groups see androgen binding in animals castrated 2 days prior to the experimental [28], others find no difference between intact animals and animals castrated 2–3 weeks [3], while still others report significant increase in the binding as a result of 2–3 week gonadectomy prior to the experiment [25]. We have elected to use intact animals in our studies to avoid this controversy.

Evaluation of results based solely on detected radioactivity provides only relative data since the specific activity of the two hormones used was markedly different. The concentration (fmol/100 mg wet tissue) and half-lives (in min) of the various steroids (both ^{3}H and ^{14}C) calculated from the regression lines (y – intercepts) are listed in Table 1. Because of the small number of points (3–5) available significant regression lines were occasionally difficult to obtain and the validity of some data (values indicated by an asterisk) may need revision.

It is seen from the table that the biological half-life of injected estradiol is significantly prolonged in the pituitary and the anterior hypothalamus. Determination of biological half-lives by themselves is not often a reliable estimate for uptake because $t_{1/2}$ values only

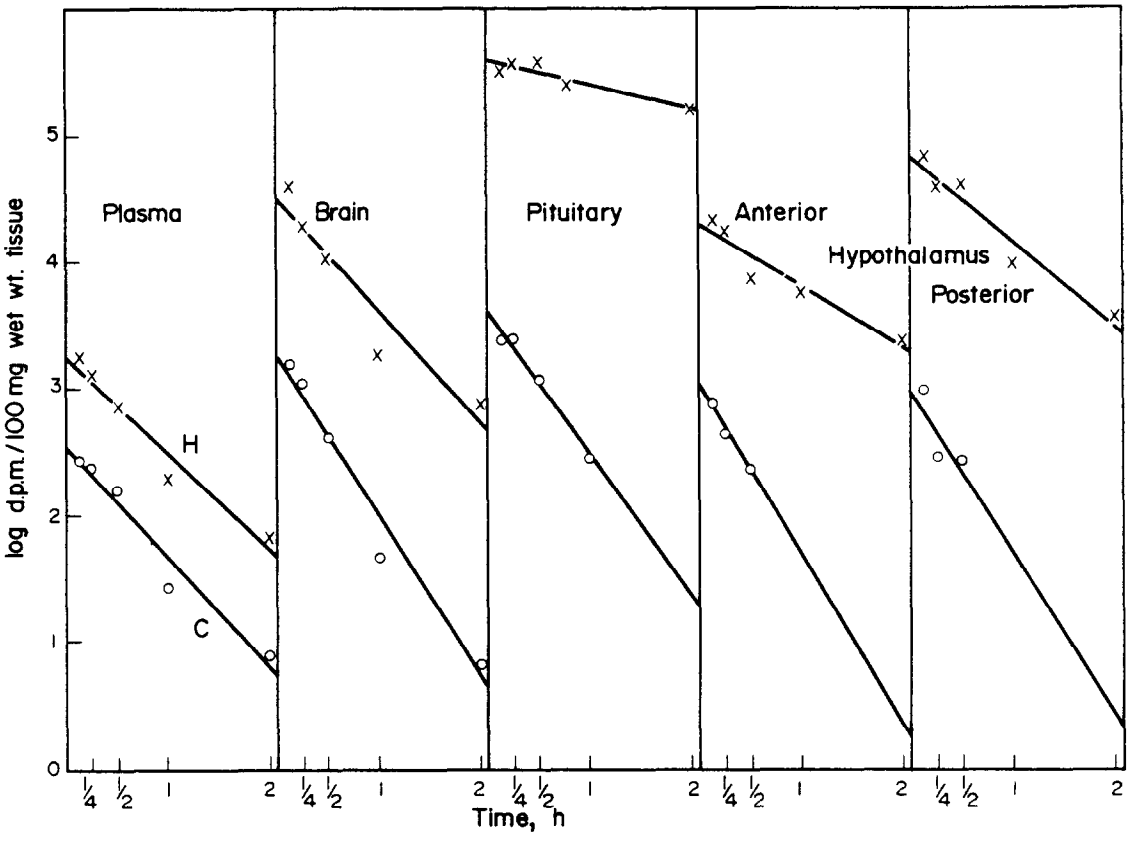

Fig. 3. Estradiol concentration in tissues of adult male rats following an i.v. bolus of estradiol; pulse 8–128 min.

reflect the terminal phase of the disappearance rate of a biexponential function. In our experiments the initial phase of the disappearance rate was not established (first sample 8 min after the pulse); hence, the pharmacokinetic parameters and the compartmental status of the tissue can be only estimated; if both exponential terms were positive then the tissue would belong to the central compartment (fast tissue) along with the plasma, and if one of the exponential terms were negative (possibility in the case of ^3H in the pituitary) then the tissue would belong to the peripheral (slow) compartment. The time course of metabolites is further complicated (even if the other exponential portion were present) because while metabolites are known to be tetraexponential ($Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} + De^{-\delta t}$), if the parent compound is known to be biexponential (represented by the α and β portions), the metabolites are frequently found to be also biexponential with hybrid constants representing the missing portions of the parents and the metabolites behavior in a two compartmental system. The terminal phase can reflect either the hybrid constant of the parent compound or of the metabolite, whichever is smaller [29].

A measure of the uptake which takes into consideration the total effect of level of hormone and its rate of decline without appealing to the particular answers of pharmacokinetic parameters, is provided by the time curves (Figs 2–4) and the ratios between the various hormones (Table 2).

It will be noted that the concentration of testosterone was significantly higher in the plasma but much more estradiol entered the brain than testosterone. This could be related to the fact that the blood–brain barrier is less permeable to testosterone than estradiol; the well known fact that testosterone in plasma circulates bound to blood carrier proteins could be a contributing factor. The $^{14}C/^3H$ and E_2/T ratio found in the brain (1.84) compared to a plasma ratio of 0.18 supports the hypothesis of a preferential estrogen blood-brain barrier as compared to testosterone. The overall estrogen/androgen ratio (Table 2) in the brain (3.90) is about eight times that seen in the blood (0.58). Assuming that the brain is not concentrating hormones but is merely reflecting the effects of the blood-brain barrier, then the aromatic steroids along with DHT are preferentially transported across the blood–brain barrier. Since we have not shown a preferential transport, the hypothesis that testosterone and androstenedione are blocked by the blood–brain barrier may be equally valid. The estrogen/androgen ratio in the neuroendocrine tissue, which was close to

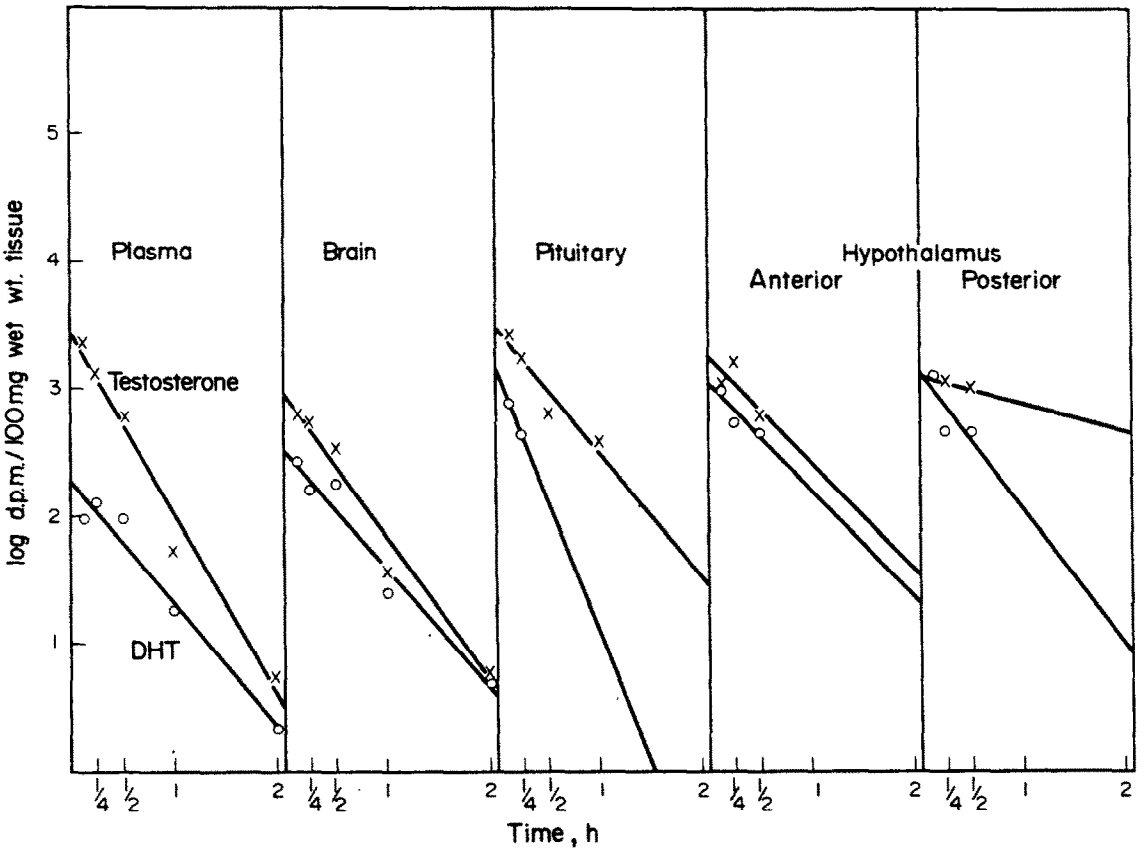

Fig. 4. Testosterone and 5α-dihydrotestosterone (DHT) concentration in tissues of adult male rats following an i.v. bolus of testosterone.

unity during the initial phase of the uptake shows that androgens are taken up by the tissue in significant amounts. The active species is probably DHT which was accumulated at the expense of T; the abundance of T in the plasma (DHT was about 7% of T values) is in agreement with previous reports [9].

The very high ratio between E_2/E_1 in the pituitary gland (308) indicates preferential binding of E_2 in this tissue. These findings are not in agreement with the results reported by others [30] who found the relative affinity of estrogen receptors in the pituitary of rats to be about the same for E_1 and E_2; however, this group used a cytosol fraction from female rats which may explain the difference.

The time course of the ^{14}C labelled steroids is of interest. In the present studies the sensitivity of detecting [^{14}C]-hormones, taking into account the amount of tissue available for determination was about 8 pmol for the pituitary, about 3 pmol for the hypothalamic tissues, and about 150 fmoles for the brain.

Table 1. Concentration and biological half-lives of various steroids in the central nervous system of male rats following a single i.v. bolus of estradiol and testosterone†

Steroid	Steroid concentration, fmol/100 mg wet wt. ($t_{1/2}$, min) Plasma	Brain	Pituitary	Ant H	Post H
Estradiol (^3H)	8 (25.0)	138 (21.6)	1754 (99.0)	84 (40.3)	292 (29.0)
(^{14}C)	3031 (22.0)	15,694 (15.0)	33,738 (17.0)	9034 (14.3)*	8487* (14.9)*
Estrone (^3H)	5 (23.5)	12 (53.8)*	115 (60.8)*	24 (42.3)	58 (22.1)
(^{14}C)	1541 (23.2)	960 (23.7)		1019 (154)*	
Testosterone (^{14}C)	23,701 (13.5)	7771 (16.9)	25,621 (19.7)*	15,484* (23.3)*	11,013 (90)
5α-Dihydrotestosterone (^{14}C)	1625 (20.0)	2947 (20.3)	12,124 (9.6)*	9363 (23.2)*	11,181* (18.4)*
4-Androstenedione (^{14}C)	3949 (16.7)	589 (20.8)		1440* (25.9)*	1120 (23.1)*

* Significance < 0.05.
† (Calculated values 8–128 min pulse).

Table 2. Ratios of androgens and estrogens in the neuroendocrine tissue of adult male rats (Integrated values 8–32 min)

Hormone Ratio	Tissues studied				
	Brain	Pituitary	Ant H	Post H	Plasma
Estrogens/androgens	3.9	0.9	0.4	0.4	0.6
E_2/T	2.0	1.4	0.6	0.8	0.1
E_2/DHT	5.4	1.3	1.0	0.8	1.9
E_2/E_1	16.3	309	8.7	151	2.0
T/DHT	2.6	2.1	1.7	1.0	14.6

Total ^{14}C detected in the pituitary prior to purification during the 8–64 min pulse was 81, 61, 37 and 8 pmol testosterone equivalents, respectively. The corresponding values for anterior hypothalamus (8–32 min interval) were 37, 32 and 18 and for posterior hypothalamus 47, 24 and 22, respectively. The rapid disappearance of this label from the endocrine tissue shows that the function of androgenic hormones is probably different from that of estrogens which are retained for a significantly longer period of time. It may be that androgens provide a stimulus for the synthesis of estradiol receptors but additional experiments are needed to establish this. The significant level of phenolic steroids in preference to androgens, and its persistence in brain tissues supports the hypothesis that estrogens play an important physiological or neuroendocrine role at the level of the male hypothalamo-pituitary axis.

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