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IN VIVO UPTAKE AND METABOLISM OF [^{3}H]PROGESTERONE AND [^{3}H]5α-DIHYDROPROGESTERONE BY RAT CNS AND ANTERIOR PITUITARY: TISSUE CONCENTRATION OF PROGESTERONE ITSELF OR METABOLITES?

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SUMMARY

In mammals, progesterone has diverse effects which could result from the action of progesterone itself and/or specific metabolites. In neuro-endocrine tissues, progesterone is rapidly metabolized principally to 5α-dihydroprogesterone and 3α-hydroxy-5α-pregnan-20-one, and it is unclear whether progesterone and/or metabolite(s) mediate cellular responses. Below we report on the selective tissue accumulation of progesterone and certain of its metabolites. [^{3}H]-progesterone or [^{3}H]-5α-dihydroprogesterone (5α-DHP) was injected intravenously (i.v.) into ovariectomized rats for 10 or 30 min or into oestrogen-primed ovariectomized rats for 10 min. Isotopic dilution analyses of the accumulated tritium in plasma and selected tissues were made for progesterone, 5α-DHP, 3α-hydroxy-5α-pregnan-20-one, 20α-dihydroprogesterone, 20α-hydroxy-5α-pregnan-3-one and 5α-pregnane-3α, 20α-diol.

Significant differences between tissues were noted in terms of which steroidal compounds were accumulated above plasma and non-target tissue levels. The pituitary accumulated large amounts of [^{3}H]-5α-DHP from either injected steroid but progesterone levels did not differ from plasma and non-target tissues. The hypothalamus accumulated significant amounts of *both* 5α-DHP and progesterone in all groups. The midbrain-tectum accumulated 5α-DHP comparable to hypothalamus and pituitary levels in all groups after injection of *either* steroid. The pineal had the greatest accumulations of progesterone in all groups but there were no accumulations of 5α-DHP above non-target tissue levels following [^{3}H]-progesterone injections. The pineal also accumulated some 20α-metabolites. In uterus there was significant accumulation of progesterone only with oestrogen priming and little accumulation of 5α-DHP. These results and others suggest that the diverse effects of progesterone may result from progesterone *per se*, from metabolites or from combinations.

INTRODUCTION

Recent studies indicate that rat pituitary and brain tissues metabolize progesterone principally to 5α-dihydroprogesterone* (5α-DHP) and 3α-hydroxy-5α-pregnan-20-one [1–3] (for review see [1] and [2]), and that large amounts of 5α-DHP are selectively accumulated in hypothalamus and pituitary but not in uterus after injections of *either* [^{3}H]-progesterone or [^{3}H]-5α-DHP [4, 5]. These results and the reported progesterone-like effects of 5α-DHP on gonadotropin regulation [6–9], ovulation [10, 11] and sexual behavior [12–14] suggest that the conversion of progesterone to this metabolite may be an important component of the molecular mechanism(s) whereby progesterone exerts its effects on neuroendocrine tissues. On the other hand, the absence of a selective accumulation of 5α-DHP in the uterus is consistent with the reported inability of this steroid to elicit a uterine progestational response and the notion that it is progesterone *per se* which is the active form of the hormone in this tissue.

Progestone either alone or in combination with other hormones affects a variety of biological processes in mammals. These include effects on uterine function, gonadotropin regulation, ovulation, lactation, erythropoiesis, sexual behavior, brain excitability, and body temperature [1, 15]. If other steroids (i.e., metabolites) are responsible for the diverse biological effects of progesterone in different target tissues then there should be a differential and selective accumulation of progesterone and certain of its metabolites above plasma and non-target tissue levels.

In this article we will summarize our studies on the *in vivo* uptake, metabolism and accumulation of [^{3}H]-progesterone and [^{3}H]-5α-DHP and their metabolites by rat tissues after i.v. injections of [^{3}H]-progesterone and [^{3}H]-5α-DHP [1, 4, 5]. The biological relevance of these findings and the possible role of progesterone metabolites in mediating some of the neuroendocrine effects of progesterone will also be discussed. In the sections that follow experimental materials and methods will not be presented other than those needed for a general understanding since these details can be found in previous publications [1, 4, 5].

* The following nonstandard trivial names and abbreviations are used: oestradiol, 17β-oestradiol; 5α-dihydroprogesterone (5α-DHP), 5α-pregnane-3, 20-dione; 20α-dihydroprogesterone (20α-DHP), 20α-hydroxypregn-4-en-3-one; 5α-dihydrotestosterone (5α-DHT), 17β-hydroxy-5α-androstan-3-one; (3α-OH), 3α-hydroxy-5α-pregnan-20-one; (20α5α), 20α-hydroxy-5α-pregnan-3-one; (5α-DIOL), 5α-pregnane-3α,20α-diol.

Ovariectomized rats were used to maximize uptake. We also utilized ovariectomized-adrenalectomized rats but since the results were comparable they will not be presented here [4, 5]. Since the response to i.v. injection of progesterone in many target tissues is rapid [16–18] and injected progesterone undergoes rapid and multiple transformations [1, 17, 19] it is obviously important to know the early fate of progesterone when it reaches these tissues via the blood. Thus, a 10 min interval was chosen for most of these studies. Thirty min intervals were also investigated to see if there were any differences in the uptake, metabolism and retention when the time of exposure after injection of the [^{3}H]-steroid was lengthened. These studies were focussed upon pituitary and hypothalamus as well as extra-hypothalamic neural regions which may be progesterone target sites in the brain, including midbrain-tectum, cerebellum and pineal [20–27]. Uterine tissues were also taken to provide comparisons as a well-established progestin target tissue. In these studies we considered a [^{3}H]-steroid to be selectively accumulated if its tissue concentration was significantly greater than corresponding steroid concentrations in plasma and appropriate non-target tissues. Besides the usual choice of muscle for a non-target tissue, cerebral cortex was chosen as a putative non-target neural tissue to compensate for the expected affinity of the lipophilic steroids for the high lipid containing CNS tissues. Reverse isotopic dilution analyses of the accumulated tritium in plasma and in these selected tissues were made for progesterone, 5α-DHP, 3α-hydroxy-5α-pregnan-20-one (3α-OH), 20α-dihydroprogesterone (20α-DHP), 20α-hydroxy-5α-pregnan-3-one (20α5α), and 5α-pregnane-3α,20α-diol (5α-DIOL). Since oestrogen is a prerequisite for many of progesterone's effects [9, 24, 27–30] we also examined the influence of oestradiol pretreatment on the uptake and metabolism of these injected steroids.

EXPERIMENTAL

Materials and methods

Unless otherwise indicated materials and methods have been described [4, 5].

Animal and tissue preparation. Two groups of four or five ovariectomized adult Holtzman rats were injected via the tail vein with 40 μCi [^{3}H]-progesterone 10 days after surgery. The rats were sacrificed by exsanguination from the abdominal aorta either 10 min or 30 min after the injection. A third group of four or five animals was ovariectomized and injected subcutaneously (s.c.) with 2 μg oestradiol per day in 0.5 ml corn oil for three days prior to receiving injections of [^{3}H]-steroid. These animals were injected i.v. with [^{3}H]-progesterone 24 h after the last oestradiol injection and were sacrificed in the same manner as the other rats after a 10 min exposure to the [^{3}H]-steroid. [^{3}H]-5α-dihydroprogesterone (40 μCi) was injected into three other groups of animals following the same protocol. As described previously [4, 5] plasma and the following tissues were analyzed: anterior pituitary, medial basal hypothalamus, cerebral cortex, cerebellum, midbrain-tectum, pineal, muscle and uterus.

Analysis of plasma and tissue radioactivity. Processing of samples for identification and quantitation of [^{3}H]-steroids by reverse isotopic dilution analyses was the same as previously reported [4, 5]. The following unlabeled carrier steroids (200 μg each) were added: progesterone, 5α-DHP, 3α-hydroxy-5α-pregnan-20-one, 20α-DHP, 20α-hydroxy-5α-pregnan-3-one and 5α-pregnane-3α, 20α-diol.

Data analysis. All samples were randomized before being assayed. The mean d.p.m./mg fresh tissue was calculated for specific [^{3}H]-steroids and for total tissue tritium. Within each group each tissue was compared with plasma and muscle. Neuroendocrine tissues were also compared with cerebral cortex. Comparisons were also made between treatment groups. Statistical significance was determined within experimental groups by a one way anova with Fisher least significant difference *post hoc* test. Comparisons between treatment groups were done by analysis of variance followed by a Dunnetts *post hoc* test.

RESULTS

[^{3}*H*]-*Progesterone injections*

The results of the reverse isotopic dilution analyses for progesterone and its metabolites (5α-DHP, 3α-OH, 20α-DHP, 20α5α, and 5α-DIOL) in the tissues and plasma of the three groups injected with [^{3}H]-progesterone are presented in Fig. 1. As shown, there is extensive metabolism. Radioactivity was associated not only with progesterone but also with the other five carrier steroids, which represent the principal products of progesterone metabolism in these tissues [1–5, 31, 32].

Ten minute exposure. Nearly all of the total tissue tritium content [4, 5] was associated with the six carrier steroids (Fig. 1). The majority of the radioactivity in the samples was *not* unchanged progesterone. Of the remaining radioactivity, a large proportion was associated with the five progesterone metabolites, particularly 5α-dihydroprogesterone and to a lesser degree 3α-hydroxy-5α-pregnan-20-one.

As shown in Fig. 1, tissue levels of [^{3}H]-progesterone were not significantly greater than plasma and non-target tissue levels except for hypothalamus and pineal. [^{3}H]-5α-DHP predominated in the pituitary and, except for the pineal, was the other major [^{3}H]-steroid in neural tissues. Pituitary, hypothalamic, midbrain-tectum and cerebellar concentrations were greater than those of plasma, muscle and cerebral cortex. Muscle and uterine concentrations of [^{3}H]-5α-DHP were equivalent to plasma levels and were much less than the concentrations of progesterone in these tissues.

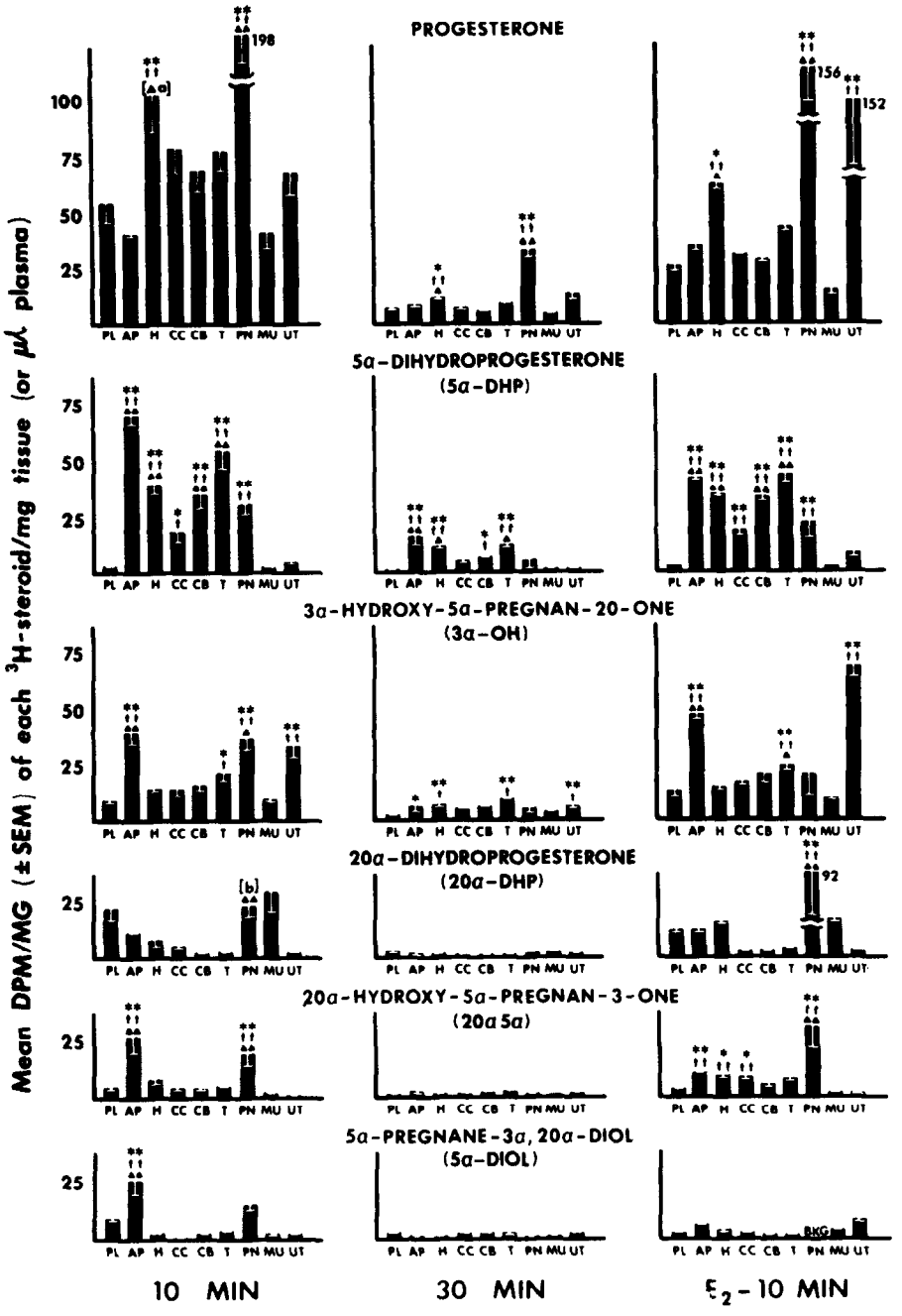


Fig. 1. Tissue and plasma [^{3}H]-steroid quantitation after injection of [^{3}H]-progesterone into ovariectomized rats after intervals of 10 minutes (histograms on left portions of figure), 30 min (middle portion of figure), or 10 min with oestradiol-primed ovariectomized rats (right portion of figure). The height of the solid bars indicate the mean amounts (±S.E.M., $N = 4$ or 5) of tritium associated with each of the designated carrier steroids on a d.p.m./mg tissue basis (or d.p.m./μl plasma) for each of the tissues abbreviated on the abscissa: PL, plasma; AP, anterior pituitary; H, hypothalamus; CC, cerebral cortex; CB, cerebellum; T, tectum (middlebrain-tectum); PN, pineal; MU, muscle; UT, uterus. The histograms for each carrier steroid for the 3 treatment groups are presented horizontally under the carrier steroid. The asterisks, daggers, and solid triangles indicate significance levels for the statistical comparisons between tissue and plasma, muscle or cerebral cortex, for each steroid as follows: *significantly greater than corresponding plasma levels ($P < 0.05$), **($P < 0.01$); †significantly greater than corresponding muscle levels ($P < 0.05$), ††($P < 0.01$); △ significantly greater than corresponding cerebral cortical levels ($P < 0.05$), △△($P < 0.01$). N.B. unless otherwise indicated significant comparisons with non-target tissues are shown only if indicated tissue levels of a particular steroid are also greater than corresponding plasma levels. (a) In two replicate experiments (each $N = 5$) similar results were obtained but in both experiments the mean results for hypothalamus were significantly greater than plasma ($P < 0.01$), muscle ($P < 0.01$) and cerebral cortical levels ($P < 0.01$) of unchanged progesterone. However, in the experiment shown, we obtained a p value of only 0.1, due to extreme variation in one of the 4 samples. (b) Not greater than plasma or muscle, but significantly greater than cerebral cortex.

Lesser amounts of [^{3}H]-3α-OH were found, but the concentrations of this steroid in pituitary and pineal were significantly greater than plasma and non-target tissue levels. The uterine concentration of 3α-OH was significantly higher than that in plasma and muscle and was five times greater than the concentration of 5α-DHP.

Only a small percentage of the total tissue tritium in the uterus, hypothalamus and other neural tissues (except pineal) was associated with 20α-DHP and its corresponding 5α-reduced metabolites, 20α5α and 5α-DIOL (Fig. 1). Tissue concentrations of these steroids were generally low and comparable to plasma except for the accumulation of [^{3}H]-20α5α in pituitary and pineal, [^{3}H]-20α-DHP in muscle and pineal, and 5α-DIOL in pituitary.

Thirty-minute exposure. As expected, the injected progesterone underwent rapid transformation. Thirty minutes after administration only 50–60% of the total tissue tritium [4, 5] was associated with the six carrier steroids (Fig. 1). Most of this radioactivity again was associated with progesterone, 5α-DHP and 3α-OH. Based upon t.l.c. scans [4], it is likely that the majority of the unidentified tritium was present as more polar metabolites, since much of this radioactivity was located near the origin.

Nonetheless, there persists, albeit at lower levels, the same relative [^{3}H]-steroid profile as in the 10 min

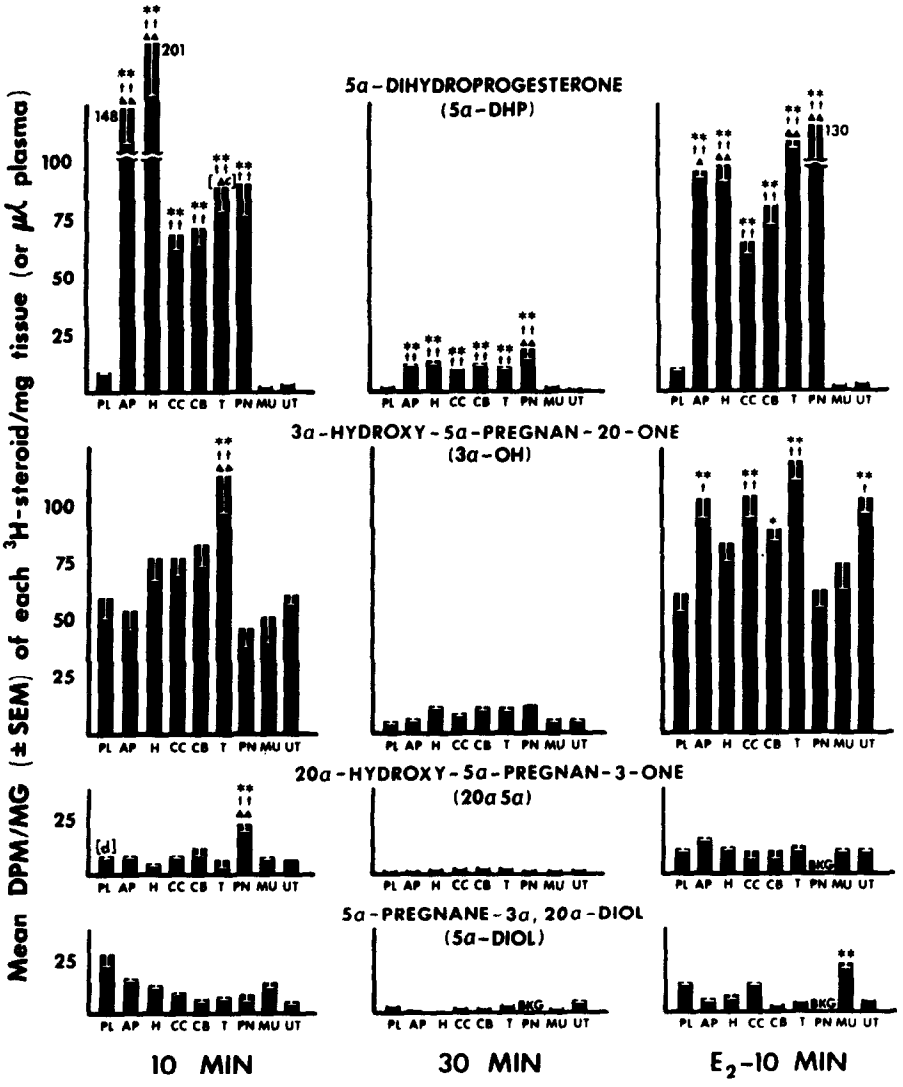


Fig. 2. Tissue and plasma [^{3}H]-steroid quantitation after injection of [^{3}H]-5α-dihydroprogesterone into ovariectomized rats after intervals of 10 min, 30 min, or 10 min with oestradiol primed ovariectomized rats. Please see legend to Fig. 1 for explanatory notes to this figure. The details are the same except for the footnotes and the additional abbreviation of BKG to designate that specific activity values were too near background to provide for meaningful calculations. (c) In two replicate experiments (each $N = 5$) similar results were obtained except that tectum levels of 5α-DHP were significantly greater than cerebral cortical levels ($P < 0.01$). (d) An error in our previous publication [4] mistakenly showed this value to be one-tenth the actual value. The present histogram value of 8.5 is correct.

study, except for the levels of 3α-OH and the 20α-compounds. Concentrations of progesterone in hypothalamus and pineal were still significantly greater than plasma, muscle and cerebral cortical levels. 5α-DHP levels in anterior pituitary, hypothalamus and midbrain-tectum were still greater than corresponding plasma, muscle and cerebral cortical levels.

Oestradiol pretreatment. There was no significant enhancement of the tissue accumulations of progesterone and its metabolites (Fig. 1) compared to the non-oestrogen treated 10 min group, except for pineal and uterus. In the pineal there was a 3-fold increase in the concentration of 20α-DHP and a two-fold increase in 20α5α. In the uterus there was a two-fold increase in the accumulation of progesterone, 3α-OH, and 5α-DIOL. The oestrogen-enhanced accumulation of [^{3}H]-progesterone by the uterus was also significantly greater (some 6–8 fold) than corresponding plasma and muscle concentration. Otherwise there was the same relative distribution of tissue radioactivity, except for a significant reduction in the amount of [^{3}H]-progesterone in all neural tissues examined and in pituitary concentrations of 5α-DHP, 20α5α and 5α-DIOL.

Although pituitary levels of 5α-DHP were decreased by oestrogen priming, the concentrations of 5α-DHP in the pituitary, hypothalamus, midbrain and cerebellum again were significantly greater than those in plasma and non-target tissues. Except for midbrain-tectum and pineal, the between tissue and plasma comparisons of 3α-OH were similar to the non-oestrogen treated 10 min group. Tectum levels were now significantly greater than plasma, muscle and cerebral cortical levels while pineal levels were not. The concentrations of 20α-DHP in the pineal were now significantly greater than plasma and non-target tissue levels.

[^{3}*H*]-5α-*Dihydroprogesterone injections*

The results of the isotopic dilution analyses for the same six [^{3}H]-steroids in tissues and plasma of the 3 groups injected with [^{3}H]-5α-DHP are summarized in Fig. 2. Two of these steroids, 5α-DHP and 3α-OH, accounted for most of the tissue radioactivity in all three groups. Small amounts of tritium were associated with 20α5α and 5α-DIOL, but tritium associated with progesterone or 20α-DHP was equivalent to background levels and therefore not shown in Fig. 2.

Ten-minute exposure. Ten minutes after an injection of [^{3}H]-5α-DHP, 80–90% of the total tissue tritium [4, 5] was associated with the four carrier steroids. 5α-DHP was the major [^{3}H]-steroid in pituitary, hypothalamus and pineal, while 3α-OH predominated in plasma, tectum, muscle and uterus (Fig. 2). Levels of 5α-DHP in pituitary and in all neural tissues were significantly greater than plasma and muscle levels. However, only the 5α-DHP concentrations in pituitary, hypothalamus and midbrain-tectum were also significantly greater than levels in cerebral cortex. Tissue levels of 3α-OH, 20α5α and 5α-DIOL generally did not differ from levels of plasma and non-target tissues except for the level of 3α-OH in midbrain and that of 20α5α in pineal.

Thirty-minute exposure. There was an appreciable reduction in total tissue tritium after 30 min [4, 5], with only 50–60% associated with the four carrier steroids (Fig. 2). Although concentrations of 5α-DHP in pituitary and neural tissues were still greater than those in plasma and muscle, only the pineal level was significantly greater than that of cerebral cortex. No tissue level of 3α-OH was significantly elevated above plasma and non-target tissue levels. Tissue levels of 20α5α and 5α-DIOL were too low to make any meaningful comparisons.

Oestradiol pretreatment. Oestradiol enhanced the accumulation of 5αDHP in pineal and of 3α-OH in pituitary and uterus. These levels were now greater than plasma and non target tissue levels. On the other hand, 5α-DHP levels in pituitary and hypothalamus were significantly reduced. Nonetheless, the levels of 5α-DHP in these tissues and in tectum were still significantly greater than plasma, muscle and cerebral cortical levels.

DISCUSSION

The results of these studies (Figs 1 and 2) clearly indicate that there are significant differences between tissues in their accumulation of [^{3}H]-progesterone, [^{3}H]-5α-DHP and their metabolites. The formation and accumulation of a particular metabolite may be indicative of an active intermediary or of an inactive metabolite representing a destruction of potency. To distinguish between these possibilities it is important to correlate accumulation with progesterone-like effects of these metabolites on the appropriate end-organ.

Several progesterone metabolites have progesterone-like effects on some, but not all, of the biological end-points traditionally associated with progesterone. 5α-DHP effects gonadotropin regulation and sexual behavior [2, 6–14]. 3α-hydroxy-5α-pregnan-20-one appears to be a more potent anesthetic than progesterone [33], but has weak or limited effects on gonadotropin regulation [2, 7–9, 11] and sexual behavior [2, 12–14] and no effect on uterine function [18, 34]. 5α-pregnane-3α,20α-diol has been reported to have central depressant effects [33, 35]. The more commonly known metabolite, 20α-DHP, has a number of progesterone-like effects [2, 16–18]. Since 20α-DHP can be converted back to progesterone, it is not clear whether this metabolite or progesterone is producing the effects. In contrast, the 5α-(and perhaps the 5β-)reduced metabolites do not appear to be acting by back conversion to progesterone, since this conversion does not occur in mammalian tissues [4, 5, 18].

To facilitate the discussion of the data, the similarities in the selective accumulation of specific steroids

in the three experimental groups is summarized for each tissue in Table 1.

As shown, the anterior pituitary accumulates 5α-DHP from injections of either progesterone or 5α-DHP but does not accumulate progesterone nor 20α-DHP derived from progesterone injections. There is some selective accumulation of 3α-OH derived from progesterone injections but not from 5α-DHP injections.

The accumulation of 5α-DHP by the pituitary appears to be physiologically relevant since, as noted above, 5α-DHP does have progesterone-like effects on gonadotropin regulation [2, 6–11]. Though, the lack of consistent retention of 3α-OH, particularly after 5α-DHP injections, suggests that conversion to 3α-OH may be an inactivation step since 3α-OH has limited effects on ovulation and gonadotropin regulation [2, 7–9, 11]. Since the formation of 3α-OH from 5α-DHP is reversible it is possible that 3α-OH is acting via a back conversion to 5α-DHP, analogous to the back-conversion of 5α-androstanediol to 5α-dihydrotestosterone whereby 5α-androstanediol mimics the effects of dihydrotestosterone [2, 36].

The pituitary also accumulates lesser but significant amounts of 20α5α and 5α-DIOL with the progesterone injections but not with 5α-DHP injections. We are not aware of any progesterone-like effects of these two metabolites on pituitary functions. Whether these steroids are active or inactive metabolites of progesterone or 20α-DHP in the pituitary is unclear at this time. As an expected feedback target site for progesterone, it is noteworthy that there was no pituitary accumulation of unchanged [^{3}H]-progesterone above plasma and non-target tissues in any treatment group.

The hypothalamus accumulates large amounts of 5α-DHP (Figs 1 and 2) in concentrations greater than plasma and non-target tissues in all treatment groups (Table 1) when either steroid is injected. The arguments cited above for the pituitary for the biological relevance of 5α-DHP as an active intermediary are relevant for the hypothalamus in gonadotropin regulation (via LRF) and also sexual behaviour (lordosis). Unlike the pituitary there is no significant accumulation of 3α-OH or the 20α-series of metabolites. The *major* difference in these two feedback tissues is that the hypothalamus accumulated unchanged progesterone in all animals injected with [^{3}H]-progesterone. The amounts are not as great as with the 5α-DHP accumulations, but are about two-fold greater than plasma and non-target tissue levels. Thus it may be that both progesterone and 5α-DHP play important roles in hypothalamic function either on the same or different processes (e.g. LHRH release and/or lordosis, etc.). Alternatively progesterone may be accumulated and then converted to the active 5α-DHP. Since 5αDHP is not converted to progesterone the converse does not seem likely.

With cerebellum, and cerebral cortex, the steroid levels were generally unremarkable, except for the accumulation of 5α-DHP in the cerebellum. A cerebellar role for progesterone in lordosis or gonadotropin feedback has been suggested [22, 37] but it is not well established at this time.

There was no significant progesterone accumulation in midbrain-tectum. The selective accumulations of 5α-DHP in midbrain-tectum may have biological relevance since a number of studies indicate that it has progesterone-like effects on lordo-

Table 1. Selective Tissue Accumulation of Progesterone (P), 5α-DHP and their Metabolites for all 3 Experimental Groups

Steroid Accumulated	After injection of	Tissues examined: Pituitary	Hypothalamus	Cerebellum	Midbrain-Tectum	Pineal	Uterus
Progesterone	[^{3}H]-P	No*	Yes*	No	No	Yes	E_2 only‡
	[^{3}H]-5α-DHP	No	No	No	No	No	No
5α-DHP	[^{3}H]-P	Yes	Yes	Yes†	Yes	No	No
	[^{3}H]-5α-DHP	Yes†	Yes†	No	Yes†	E_2 + 30 min	No
3α-OH	[^{3}H]-P	Yes†	No	No	E_2 only	10 min only§	Yes
	[^{3}H]-5α-DHP	No	No	No	10 min only	No	E_2 only
20α-DHP	[^{3}H]-P	No	No	No	No	Yes†	No
	[^{3}H]-5α-DHP	No	No	No	No	No	No
20α5α	[^{3}H]-P	Yes†	No	No	No	Yes†	No
	[^{3}H]-5α-DHP	No	No	No	No	10 min only	No
5α-DIOL	[^{3}H]-P	10 min only	No	No	No	No	No
	[^{3}H]-5α-DHP	No	No	No	No	No	No

* A yes answer is given only if the concentration of the specific [^{3}H]-steroid for that tissue is significantly greater than corresponding steroid concentrations in plasma and appropriate non-target tissues for the 3 experimental groups, i.e., the 10 min and 30 min untreated ovariectomized groups and the 10 min groups primed with oestradiol.

† Except for the 30 min group.

‡ Selective accumulation only in the Oestradiol (E_2)-treated group.

§ Selective accumulation only in the 10 min. untreated ovariectomized group.

sis [12–14]. This may be related to the possible role of the tectum in lordosis [27, 38]. 3α-OH is accumulated in the rats injected with progesterone only when the ovariectomized animals are treated with oestrogen. 3α-OH has weak progesterone-like effects on lordosis [12].

The profile of uptake and metabolism of progesterone and 5α-DHP by the pineal appears quite different from the other CNS regions. It selectively accumulated large amounts of progesterone in the presence or absence of oestrogen and retained appreciable amounts over the 30 min interval. Thus, the data clearly support a pineal-target tissue role for progesterone *per se*. The pineal had the highest levels of progesterone in the untreated 10 min and 30 min groups and levels were comparable to the uterus in the oestrogen-treated group. In contrast to some of the other neuroendocrine tissues there was no 5α-DHP accumulation above cerebral cortical levels with injection of [^{3}H]-progesterone. This is consistent with *in vitro* studies [3, 32] indicating limited conversion of progesterone to 5α-DHP in the pineal. However, the pineal accumulation of 5α-DHP after injection of 5α-DHP was significantly greater than plasma and non-target tissue levels when the rats were primed with oestrogen. The other major difference in this tissue was the higher accumulation of the 20α-series of metabolites after injections of either progesterone or 5α-DHP. In mammals a number of studies have indicated that progesterone and/or its metabolites may influence the activity of the pineal [32, 39]. Thus these results are consistent with the possibility that progesterone and certain of its metabolites (possibly the 20α-series) may modulate the activity of the pineal.

In muscle, the levels of progesterone, 5α-DHP and their metabolites were consistently equal to or lower than plasma. The formation and accumulation of 20α-DHP was expected since muscle has appreciable 20α-hydroxy steroid dehydrogenase activity [18].

In the uterus there was a significant accumulation of progesterone, but only after oestradiol priming. These data are consistent with previous reports that the prior action of oestradiol is needed for uterine progestational effects, that oestrogen increases uterine progesterone receptors [28–30, 40] and that it is progesterone itself that is selectively accumulated in the uterus. Oestrogen also increases ring A reduction *in vivo* and *in vitro* [41-43]. However, there was little accumulation of 5α-DHP from either injected progesterone or 5α-DHP. Although the uterus is capable of forming 5α-DHP [17, 18, 34] it does not selectively accumulate it. Thus, although a number of tissues possess 5α-reductase, for review [1, 2], the accumulation of 5α-DHP differs among tissues and mere comparisons of enzyme levels are obviously insufficient to predict tissue accumulations. This correlates well with previously published studies which demonstrated that 5α-DHP does *not* have any progesterone-like effects on a variety of uterine progesterone endpoints [9, 16–18, 34]. The levels of 3α-OH in uterus were consistently greater than those in muscle and plasma in all three treatment groups injected with progesterone. 3α-OH has been shown to be the major metabolite of progesterone in the uterus [41, 43–45]. However, since no uterine progesterone-like effects have been ascribed to 3α-OH [9, 16–18, 34] it may represent an inactive metabolite.

With the uterus, the effects of oestradiol in enhancing the accumulation of progesterone and 3α-OH, are quite clear. In contrast, oestradiol pretreatment did not increase the accumulation of progesterone by pituitary, hypothalamus or the other neural tissues examined over the levels found in the non-oestrogen treated group. These results are of interest since oestrogen priming has been reported to be necessary for the effects of progesterone on a variety of neuroendocrine and pituitary events [9, 24, 27, 46]. This may be indicative of a type of interaction of oestradiol with progesterone in these tissues which is different from that in the uterus.

The major tritiated constituents appear to have been identified in this study, particularly for the 10 min groups. From our previous *in vitro* studies on the metabolism of progesterone by these neural tissues [1, 3, 4] the unidentified tritium is likely to be minor *in situ* products. Alternatively they could represent uptake of blood borne derivatives from the liver and peripheral metabolism of the injected steroid (particularly in the 30 min study). Nonetheless, the remaining unidentified radioactivity in the 30 min studies and the small amounts in the 10 minute groups may be important and should not be overlooked. Apropos to this point is the role played by the limited conversion of testosterone to oestrogens in the hypothalamus [47].

Preliminary competition experiments (Karavolas, Hodges & O'Brien unpublished) in which animals received large doses of unlabelled steroid 5 minutes prior to [^{3}H]-progesterone or [^{3}H]-5α-DHP injections indicate that unlabelled 5α-DHP has little effect on hypothalamic or pituitary accumulations of [^{3}H]-progesterone or [^{3}H]-5α-DHP derived from [^{3}H]-progesterone injections. However, unlabelled progesterone dramatically reduced hypothalamic and pituitary accumulations of [^{3}H]-5α-DHP from [^{3}H]-5α-DHP injections. Further experiments should clarify these interactions as well as the specificity and saturability of the reported accumulations of progesterone and 5α-DHP.

Consistent with the view that a hormone or its active metabolite(s) is sequestered by target tissues in concentrations above those in plasma and non-target tissues, these studies suggest that metabolites of progesterone may be important components (or intermediaries) in some of progesterone's actions.

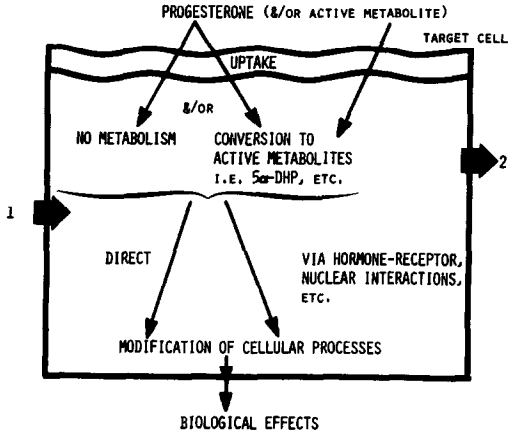
The mechanism of action of progesterone for a number of its effects in mammalian systems is unclear. The effects that ultimately lead to the apparent synthesis of specific proteins in its target tissues (particu-

larly uterus) appear to be acting through the cytoplasmic receptor-transcriptional model generally proffered for steroid hormones [48, 49]. Binding proteins that are specific receptors for progesterone are present in the cytoplasm of the uterus, vagina, breast and perhaps a few other organs [27, 50].

In neuroendocrine tissues there is no clear evidence for specific cytoplasmic or nuclear retention of progesterone *per se* [4, 51–53], except for the report of Seiki on the hypothalamus [54]. Although recent studies with the synthetic "progestin" 17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R5020) show cytosolic binding of this steroid, there is no clear evidence for the retention of the natural steroid, progesterone [53, 55, 56]. Our data suggest that in neural and pituitary tissues there is selective accumulation *in vivo* of progesterone itself or certain of its metabolites (particularly 5α-DHP and some of the 20α-metabolites) or combinations thereof.

The direct effects on isolated hypothalamic synaptosomes and other particulates [57], the rapid onset time for some neural effects (minutes and seconds) [3, 35, 58], and the lack of neuroendocrine nuclear retention [51] suggest that progesterone and perhaps other steroids may influence neural processes through mechanisms which do not involve the classical cytoplasmic receptor-transcriptional mechanism of action model.

Thus the present findings, together with others on the mode of action of progesterone, support a working hypothesis (Fig. 3) that the multiple and varied effects of progesterone on an end-organ may result from progesterone *per se* (e.g. as in the uterus), from one of its metabolites (e.g. 5α-DHP in the pituitary) or from combinations of progesterone and certain of its metabolites (e.g. as in the hypothalamus and perhaps the tectum and pineal). In the latter instance these steroids could *inter alia* affect the same or different cellular parameters within the same target tissue. It may be also that these steroids modulate neuroendocrine cellular changes by mechanisms other than *or* in addition to the classical steroidal transcriptional mode of action.



1. OTHER HORMONES, ETC. FOR NECESSARY INTERACTIONS OR PERMISSIVE EFFECTS
2. TERMINATION OF EFFECT: —▶DEGRADATION (INACTIVE METABOLITES, ETC.) —▶EXIT

Fig. 3. Diagrammatic representation of possible model(s) of progesterone's actions at target cells by either progesterone itself and/or progesterone metabolites such as 5α-dihydroprogesterone.

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