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OESTROGEN–PROGESTIN REGULATION OF FEMALE SEXUAL BEHAVIOR IN GUINEA PIGS

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SUMMARY

The display of female sexual behavior in guinea pigs is strongly correlated with the concentration of cytoplasmic progestin receptors in hypothalamic-preoptic area-septum (HPS). These progestin receptors increase in concentration in the HPS after a period of oestrogen priming. The synergistic actions of oestrogen and progestin may not only influence noradrenergic transmission, but noradrenergic transmission may influence the degree of synergy between oestrogen and progestin.

INTRODUCTION

About forty years ago the discovery was made that progesterone synergizes with oestradiol to facilitate female sexual behavior in rodents such as rats, hamsters, and guinea pigs [1–3]. This synergism occurs not only under experimental conditions (females ovariectomized and treated with oestradiol and progesterone) but during the course of normal estrous cycles with no exogenous hormones required. The predictable nature of the effects of oestradiol and progesterone on female sexual behaviour of rodents, while of limited interest in itself, has broader significance insofar as it can be used to yield insight into the cellular actions of hormones on brain tissues involved in the expression of behaviour. In this paper, we discuss some molecular mechanisms through which oestradiol and progesterone synergize to facilitate sexual behaviour in female guinea pigs.

The first point that should be made is that oestradiol facilitates expression of female sexual behavior in all non-primate animals studied so far [1, 4]. However, the degree to which progesterone synergizes with oestradiol to further facilitate female behaviour varies greatly among species. For example, progesterone does not appear to facilitate expression of female behaviour in oestrogen primed rabbits and cats [4]. In rats, oestradiol is sufficient for full facilitation of female behaviour [5], but a much lower dose of oestradiol can be used if progesterone is given about 24–60 h after oestradiol [1, 2]. In guinea pigs, oestradiol alone, even in high doses does not consistently facilitate the full expression of female behaviour [6]. Addition of progesterone injection 24–60 h after oestradiol administration invariably results in display of full female behaviour in ovariectomized guinea pigs [6]. Thus, the guinea pig is an especially appropriate animal in which to study the synergism between oestradiol and progesterone in neural tissue. Our current strategy in the study of this synergism involves the observation of behavioural effects of oestrogen and progestin treatment. Attempts are then made to correlate the behavioural observations with changes in the concentration of progestin receptors in brain after oestrogen treatment.

METHODS

Hartley strain adult females, ovariectomized at least 2 weeks prior to experimentation are used. Steroid hormones are injected subcutaneously in oil vehicle in all cases.

Behavioural tests for female sexual behaviour consist of hourly attempts to elicit the lordosis response by manual stimulation of the back and perineum [7]. Such tests begin after the animal has been primed with oestrogen. They continue for 10–12 h after injection of progesterone to oestrogen primed subjects. Behavioural tests and biochemical determinations are made on separate batches of animals.

Measurements of progestin receptors are performed as described [8]. The technique involves removal and dissection of brain tissue of oestrogen-treated guinea pigs, followed by homogenization in buffer (10 mM Tris–HCl, 1.5 mM Na_2EDTA, 10% glycerol, 12 mM monothioglycerol, pH 7.4; TEGT). The homogenates are centrifuged for 1 h at 48,000 *g*, and the supernatant incubated with 0.4 nM [17α-methyl-^{3}H]-R5020 (specific activity = 86 Ci/mmol) ± a 50× excess of unlabelled R5020 (R5020 is a synthetic progestin with the formula 17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione; this substance has a high affinity for progestin receptors and is a very potent facilitator of female sexual behaviour in oestrogen primed guinea pigs). After 4 h, bound and free [^{3}H]-R5020 are separated by gel filtration on Sephadex LH-20 columns. Thirty minutes after application of sample to the column, bound [^{3}H]-R5020 is eluted into scintillation vials with 800 μl TEGT. Ten milliliters of toluene scintillation fluid are added and the radioactivity is counted in a liquid scintillation counter. Results are expressed as fmol/mg protein.

PROGESTIN RECEPTORS IN THE BRAIN

(a) *Temporal properties*

As the facilitatory actions of progesterone on female sex behaviour of guinea pigs do not occur in

Table 1. Correlation between temporal properties of oestrogen-priming for lordosis and oestrogen-priming for induction of progestin receptors in guinea pigs

Lordosis			Progestin receptors	
Hours of oestrogen-priming	N	% Showing lordosis	Hours of oestrogen-priming	fmol [^{3}H]-R5020 specifically bound/mg protein in HPS
0	10	0	0	10.3
11	20	15.0	14	10.5
18	8	75.0	24	13.5
39	32	96.9	40	16.0
60	31	83.9	64	19.4
70	7	85.7	—	—
88	8	37.5	88	12.8
94	8	25.0	—	—

Behavioural data drawn from Feder *et al.*[17] and receptor data from Blaustein and Feder[8]. The estrogen-priming dose was 1.6 μg E_2B in oil vehicle given subcutaneously to ovariectomized guinea pigs.

the absence of prior exposure to oestradiol (or certain other oestrogens), it seems logical to suppose that part of the action of oestradiol involves priming of progesterone sensitive elements of the nervous system (i.e. progestin receptors). The first question to ask about these putative elements involves time. How long does oestrogen have to act before it "primes" or "activates" progestin-sensitive elements. How long after activation of these elements does female behaviour begin to occur and once these elements are primed, how long do they continue to be active?

Behavioural observations indicate that when ovariectomized guinea pigs are given an oestradiol benzoate (E_2B) injection (1.6 μg/animal) and a subsequent injection of 0.5 mg progesterone (P) they begin to display lordosis about 4 h after P. However, this synergism between the two steroids is evident only when the interval between injections exceeds 11 h. With an 18 h interval the synergy is almost optimal and with a 40 h interval the synergy is optimal [9, 10] (Table 1). These data suggest that it takes from 11 to 18 h for oestradiol to prime progesterone-sensitive elements in the nervous system. To test this suggestion, a separate group of ovariectomized guinea pigs were given 1.6 μg E_2B. They were killed 14, 24, 40, 64, or 88 h after this injection, and their brains removed and analyzed for progestin receptor content. Results showed that at 14 h after E_2B there was no increase in hypothalamic-preoptic area-septum (HPS) concentration of cytoplasmic progestin receptors. At 24 h the concentration of cytoplasmic progestin receptor in HPS had increased by 31%, at 40 h by 70% and at 64 h by 97%. Concentrations of the progestin receptor at 88 h were only slightly above baseline levels (Table 1). These data show clearly a correlation between time requirements for oestrogen effects on behaviour and for oestrogen effects on cytoplasmic progestin receptors. That is, it takes $>11 < 19$ h for oestrogen to prime the CNS in such a way that it is responsive to facilitatory actions of progesterone, and it takes $>14 < 24$ h for oestrogen to cause an increase in HPS cytoplasmic progestin receptors. The oestrogen priming of behavioral substrates declines between 60 and 88 h after E_2B injection, and this corresponds to a reduction in oestrogen effect on HPS progestin receptors at 60–88 h after E_2B.

When progestin receptors are optimally activated by oestrogen, an injection of P will facilitate lordosis within 3–4 h. The behaviour continues for about 8 h then ceases [6]. Correspondingly, injection of P to an optimally oestrogen-primed guinea pig causes rapid depletion of HPS and midbrain cytoplasmic progestin receptors (presumably because the receptors are translocated to the nucleus) [8]. On the basis of work with uterine tissue it is expected that the translocated receptor is no longer present within the cell nucleus after about 12 h [11].

(b) *Anatomical distribution*

Previous work with steroid-filled cannulae implanted intracerebrally indicated that the medial basal hypothalamus (MBH) is the primary site of action of oestrogen for facilitation of lordosis behavior in female guinea pigs [12]. Furthermore, a narrow basal strip of MBH seemed to mediate the synergistic effects of progesterone on facilitation of behaviour [13].

Although studies with brain homogenates indicated that there was a somewhat greater uptake of tritiated progesterone in the hypothalamus than in the cerebral cortex, the uptake of [^{3}H]-progesterone into brain homogenates did not appear to be influenced by prior oestrogen treatment and did not appear to involve a saturable mechanism [14]. Studies of nuclear fraction uptake of [^{3}H]-progesterone, using scintillation-counting techniques also yielded negative results with no nuclear uptake of [^{3}H]-progesterone apparent in any brain area [15]. These results were discouraging, but alternative methods suggested that there was indeed a saturable, oestrogen-inducible receptor system for progestin in the brain of the female guinea pig. For example, autoradiographic analysis of guinea pig brain demonstrated selective uptake of radioactivity in the nuclear fraction of the

Table 2. Correlation between anatomical distribution of sensitive sites for facilitation of lordosis and sites of oestrogen-inducible progestin receptor production in guinea pig brain

Lordosis		Progestin receptors	
Site of E_2B implant	Lordosis behaviour present	Progestin-receptor measured in:	fmol [^{3}H]-R5020 specifically bound/ mg protein ($\bar{X} \pm$ S.E.M.)
Medial basal hypothalamus	Yes	Hypothalamus	30.4 ± 2.3
Preoptic area	Little or none	Preoptic area-septum	21.1 ± 1.5
Midbrain	No	Midbrain	9.1 ± 0.5
		Cortex	7.8 ± 0.3
		Amygdala	7.0 ± 0.4
		Cerebellum	3.1 ± 0.2

Behavioural data drawn from Morin and Feder[13]. In this study progesterone was implanted with 27 gauge cannulae after 36 h of E_2B priming. Receptor data drawn from Blaustein and Feder[8] in which 10 μg E_2B was given subcutaneously in oil vehicle, and a 40 h interval of priming was allowed.

arcuate nucleus and portions of the preoptic area. This uptake mechanism was enhanced by oestrogen pretreatment and was saturable, as shown by pretreatment with unlabelled progesterone [16].

Another successful methodology is that utilized in the present report. This involves the use of the synthetic progestin, R-5020, to detect progestin receptor. This progestin is even more effective than progesterone as an oestrogen synergist in the induction of female behaviour in guinea pigs [17] and rats [18]. From this, we predicted and have shown that R-5020 has a higher affinity for putative progestin receptors in brain and is a more sensitive detector of such receptors. Oestrogen inducible, saturable, progestin receptors in cytoplasm were found in highest concentration in the HPS, with detectable, but lower concentrations in the midbrain. The amygdala and cerebellum did not contain oestrogen-inducible cytoplasmic progestin receptors (Table 2).

(c) *Chemical specificity*

The information discussed thus far suggests a long-term (approx. 14–18 h) action of oestrogen on induction of cytoplasmic progestin receptors in the HPS. We have not yet tested oestrogens other than oestradiol (E_2) for their ability to induce production of progestin receptors. However, we have evidence that oestrone, oestriol, and dimethylstilboestrol (DES) are all capable of priming female guinea pigs to respond behaviourally to progesterone administration [19, 20]. Therefore, we predict that these compounds would induce progestin receptor production. On the other hand, corticosterone [21, 20] fails to prime guinea pigs for a behavioural response to progesterone and we predict that this compound would not induce progestin receptor production. Some synthetic "anti-oestrogens" may occupy an intermediate position. For example, administration of enclomiphene (ENC) at 39, 20 and 11 h prior to progesterone fails to stimulate behavioural responsiveness to progesterone. However, administration of ENC at 39 and 20 h in conjunction with injection of E_2B at 11 h prior to progesterone, induces behavioural responsiveness to progesterone in >60% of females [10]. The exposure of the brain to 11 h of E_2B priming in the absence of prior ENC fails to mediate a behavioral response to progesterone, these results suggest that ENC may start, but fail to complete, a process necessary for induction of progestin receptors in brain.

The foregoing data suggest that there is specificity with respect to the compounds that are able to induce increased concentration of progestin receptors in HPS, and to a lesser extent, in midbrain. Another question about specificity concerns the nature of the compounds that bind to progestin receptors. Blaustein and Feder[8] have tested this aspect of specificity, and the results are shown in Table 3. It is interesting to note that there is a very good correlation between affinity for the progestin receptor and potency of behavioural effect in oestrogen primed females with those compounds that have been tested with our methods (Table 3).

POSSIBLE MECHANISMS OF THE OESTROGEN–PROGESTIN INTERACTION IN BRAIN

The data discussed thus far reveal strong correlations between the display of female sexual behaviour in oestrogen primed guinea pigs given progesterone and the concentration of HPS cytoplasmic progestin receptors in oestrogen primed guinea pigs. These correlations exist with regard to temporal considerations, anatomical distribution and chemical specificity. What do these correlations suggest about the nature of oestrogen–progestin synergism in brain?

On the basis of previous work and the data discussed in this report, we propose the following model for oestrogen–progestin synergism in guinea pigs. First, oestrogen is selectively taken up in certain HPS cells [22]. It is translocated to the nuclei of these cells [15]. The prolonged action of oestrogen in the nucleus is apparently necessary for behavioural facilitation. This can be inferred from several studies. For example, anti-oestrogens administered many h (e.g.

Table 3. Correlation between potency of compounds that facilitate lordosis and relative binding affinities (RBA) of compounds for progestin receptor in HPS of oestrogen-primed guinea pigs

Lordosis		Progestin receptor	
Compound	Dose required to facilitate lordosis in >50% of animals	Compound	RBA
Progesterone	32 μg	Progesterone	100
R-5020	10 μg	R-5020	224
5α-Dihydroprogesterone	627 μg	5α-Dihydroprogesterone	20
Corticosterone	500 μg	Corticosterone	2
RU-2858	Ineffective (up to 32 μg)	RU-2858	0.1
17α-Hydroxyprogesterone	Ineffective (up to 10 mg)	17α-Hydroxyprogesterone	0.4

Behavioural data from Wade and Feder[14], Feder *et al.*[17], and Wilcox and Feder (Unpublished). For behavioural tests, females were primed with 1.6 μg–6.6 μg E_2B and given progestin 36–40 h later. The progestins were given in oil vehicle. When 5α-dihydroprogesterone is given in alcohol vehicle the dose required is only about 30 μg. Receptor data from Blaustein and Feder[8]. Priming was accomplished by injecting 10 μg E_2B.

40 h) after E_2B prevent behavioural facilitation, presumably by competing with oestradiol for binding sites on oestrogen receptors [10, 23, 24]. Oestrogens, such as oestriol, that have a short residence period in the nuclear fraction of guinea pig HPS cells [25] appear to be much more effective in facilitation of sex behaviour when they are administered in repeated small doses than when they are given as a single large dose [19, 20]. Presumably, the oestrogen–receptor complex translocated to the nucleus causes increased mRNA synthesis with consequent increases in translation of components of progestin receptor molecules. The progestin receptors in the cytoplasm then form complexes with endogenous or exogenous progestins, and these complexes are translocated to nuclei of the same HPS cells. The way in which the translocation of steroid-receptor complexes facilitate display of sex behaviour is not known. Two possibilities are that the translocated receptors induce a change in neurotransmitter synthesis in the steroid-responsive neuron and/or induce a process that leads to a change in permeability of steroid-responsive neuron's soma membrane. This change could alter responsiveness of the neuron to neurotransmitters from presynaptic cells.

Recent work with neurotransmitters in this laboratory does not yet allow a decision as to which of these alternatives is valid. However, this work strongly implicates the noradrenergic system as a dominant factor in steroid-induced female behaviour. For example, stimulation of noradrenergic receptors (with systemically administered clonidine) facilitates lordosis in oestrogen-primed guinea pigs whether they are given progesterone or not [26, 27]. Furthermore, blockade of norepinephrine (NE) activity by administration of a NE synthesis blocker (U-14, 624) or a NE receptor blocker (phenoxybenzamine) suppresses female sexual behaviour in ovariectomized females given E_2B and progesterone 40 h later [28]. Although subject to alternative explanations, these data indicate that NE transmitter activity affects the steroid-sensitive nerve cells involved in sex behavior. To test this idea further, we have administered U-14, 624 or phenoxybenzamine to ovariectomized guinea pigs primed with E_2B. Both drugs appear to suppress induction of progestin receptors by E_2B in HPS cells by about 30% as measured by our assay (Nock, Blaustein and Feder, unpublished). These preliminary data suggest that NE from presynaptic neurons has a permissive or a facilitatory effect on oestrogen target neurons' ability to produce progestin receptor. However, this preliminary finding may be due to artifacts such as drug stimulation of progestin from the adrenals (with resultant depletion of progestin receptors). The drugs may also act by alteration of the affinity of progestin for its receptor or by interference with oestrogen uptake, binding, translocation or oestrogen receptor production. These alternative possibilities are being evaluated.

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