when the molecule was reassembled. This limited the number of mutations that could be tested. Methods for oocyte harvesting, RNA injection, whole cell and single-channel recording have been reported [R. H. Joho *et al.*, above; J. R. Moorman *et al.*, *Am. J. Physiol.* **253**, H985 (1987)]. Single channel current traces were idealized with a program written by A. M. J. Van Dongen and implemented by H. Nguyen. The algorithm uses a dI/dt threshold for determining transitions between open and closed states. The idealized traces were used to construct frequency histograms of closed times, burst durations, and waiting times to first opening. The data were represented by a single or sum of two exponentials model where the parameters were estimated with a maximum likelihood technique. Cumulative distributions of waiting time to first opening were corrected for the number of channels in the patch.
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19. In these electrostatic models we assume that the charged regions lie within a Debye length of one another in the cytoplasm or that they lie within hydrophobic regions of low dielectric constant. A nonelectrostatic explanation may also apply in which removal of lysines or the arginine changes the sensitivity of the mutant channels to endogenous proteases, which in turn leads to altered channel gating.
20. We thank H. Silberberg for preparation of the mutant mRNAs, K. Kasper for DNA sequencing, and G. Schuster for oocyte injection. Supported by research grants KL-01858 (J.R.M.), HL-36930 and NS-23877 (A.M.B.) from the NIH, the Advanced Technology Program of the State of Texas (R.H.J.) the Kempner Foundation and the Sealy Memorial Endowment (J.R.M.), and the American Heart Association, Texas affiliate (R.H.J. and G.E.K.).

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Behavioral Effects of Progesterone Associated with Rapid Modulation of Oxytocin Receptors

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The ventromedial nuclei of the hypothalamus (VMN) are important for the control of feminine mating behavior, and hormone action within these nuclei has been causally related to behavior. Estradiol induces receptors for oxytocin in the VMN and in the area lateral to these nuclei over the course of 1 to 2 days, and progesterone causes, within 30 minutes of its application, a further increase in receptor binding and an expansion of the area covered by these receptors lateral to the VMN. The rapid progesterone effect appears to be a direct and specific effect of this steroid on the receptor or membrane, because it was produced in vitro as well as in vivo and was not mimicked by a variety of other steroids. The effect of progesterone occurred in the posterior part of the VMN, where oxytocin infusion facilitated feminine mating behavior; it did not take place in the anterior part of the VMN, where oxytocin infusion had no effect on mating behavior.

IT IS GENERALLY ACCEPTED THAT STEROID hormones can regulate cellular functions in the brain and certain behaviors by modulating gene expression after binding to intracellular receptors (*1, 2*). Steroid hormones also rapidly affect the excitability of brain cells in vitro or when applied electrophoretically by changing ion conductances, the release of neurotransmitters, or the characteristics of neurotransmitter receptors (*3*). These rapid hormone effects can be observed in brain regions such as the cerebellum and the cortex that contain no measurable intracellular steroid receptors (*4*). The physiological significance of these observations remains uncertain (*5*) because no rapid membrane effects of steroids have been causally related to the activation of a behavioral response. We now show the modulation of estrogen (E)-induced oxytocin receptors by progesterone (P), a rapid steroid effect that appears to be involved in the facilitation of a complex behavior, feminine mating (lordosis) behavior in the rat (*6*).

Progesterone induces an expansion of the area covered by oxytocin receptors in the ventromedial hypothalamus of female rats primed with E (*7*). This spread of oxytocin binding occupies the area lateral to the VMN, which contains oxytocin-immunoreactive fibers and the long dendrites and axons of VMN neurons (*7, 8*). Oxytocin infused into the area lateral to the VMN facilitates lordosis behavior only in female rats primed sequentially with E and P and not in females treated with E alone (*7*). This behavioral effect can be blocked by a specific antagonist of oxytocin (*9*). We have examined this modulation of oxytocin receptor binding by P and its relation to lordosis behavior.

Oxytocin receptor binding to brain sections was quantified by in vitro receptor autoradiography (*10*). Iodinated [$d(CH_2)^5$, Tyr(Me)^2, Thr^4, Tyr-NH$_2$^{9}] ornithine vasotocin analog ([^{125}I]OVTA) was used as a ligand for the central oxytocin receptor because of its high specificity, affinity (K_D, 50 pM), and specific activity (2200 Ci/mmol) (*11*). Initially, we examined the regional distribution of oxytocin receptor binding within the ventromedial hypothalamus and changes in this binding induced by P in ovariectomized (OVX) and adrenalectomized (ADX) female rats primed with estradiol benzoate (EB). Brains of EB-primed females were sampled for oxytocin receptor autoradiography at different times after the injection of vehicle (EB only) or P (EB + P) (*12*). In the anterior part of the ventromedial hypothalamus, oxytocin receptor binding was limited to the cell body region of the VMN and was not affected by P treatment (Figs. 1 and 2). In the posterior ventromedial hypothalamus of EB-primed females, oxytocin receptor binding occurred in the ventrolateral parts of the VMN but also extended into the area lateral to the nuclei (Fig. 1). In the posterior VMN, P treatment only slightly increased the oxytocin receptor binding in the cell body region (5 to 8%), but it increased oxytocin receptor binding by more than 25% in the surrounding neuropil (Figs. 1 and 2). This increase in binding density was accompanied by an expansion of the area covered by oxytocin receptors. This effect of P could be observed within 30 min and it lasted for at least 8 hours (Fig. 2).

Because P increases the synthesis of new proteins in the VMN (*2, 13*), we determined whether the increase in oxytocin receptor binding produced by P involves the rapid synthesis of new receptors (*14*). We tried to block the effects of P with the protein synthesis inhibitor anisomycin. Fifteen minutes before injecting 0.5 mg of P or vehicle, EB-primed females were injected with saline or with 100 mg of anisomycin per kilogram of body weight in saline (*15*). This amount of anisomycin inhibits cerebral protein syn-

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thesis for 4 to 6 hours and is sufficient to block the induction of proteins by E, such as hypothalamic intracellular P receptors (*16*). Brains were sampled for receptor autoradiography 4 hours after P treatment. Anisomycin did not reduce the effects of P on the density and distribution of oxytocin receptors in the area lateral to the caudal VMN. [Binding: EB (n = 7), 9.9 ± 1.5; EB + P (n = 6), 12.2 ± 1.4*; EB + anisomycin + P (n = 4), 12.0 ± 0.41* fmol of protein per milligram. Area: EB, 1.99 ± 0.13; EB + P, 2.54 ± 0.38*; EB + anisomycin + P, 2.64 ± 0.52* mm^2; mean ± SD, *$P \leq 0.05$ when compared to EB by Duncan multiple-range tests.] Thus, the effects that P exerts on hypothalamic oxytocin receptor binding do not appear to involve protein synthesis and may be due to a direct effect of the hormone on the receptor or on the cell membrane.

This hypothesis was explored by incubating nonfixed but previously dried and frozen brain sections from EB-primed females (*17*), corresponding to the caudal VMN, for 1 hour in the presence of [^{125}I]OVTA and low concentrations of P, 5α-dihydroprogesterone (5α-DHP), 3α-hydroxy-5α-pregnane-20-one (3α-ol DHP), cholesterol, or estradiol. Progesterone, but none of the other steroids, increased the area covered by oxytocin receptor binding in vitro (Fig. 3). As after its in vivo administration, P also increased the density of oxytocin binding in the area lateral to the VMN (EB, 11.70 ± 0.57; EB + in vitro P, 14.90 ± 0.56 fmol of protein per milligram; mean ± SD, $t(9)$ = 3.19, $P \leq 0.001$ by paired two-tailed t test). Thus, P directly affects the oxytocin receptor or the cell membrane, because protein synthesis is not possible in dried and frozen brain sections. Whereas E induction of oxytocin receptors in the VMN is most likely due to a synthesis of new receptors (*18*), the rapid modulation by P is likely to be a membrane effect. Moreover, we found that the amplitude of the P effect is dependent on the amount of E priming. The expansion of oxytocin receptor binding in response to P was greater in females primed with a high dose of EB (50 μg) than in females primed with a low dose of EB (10 μg) (*19*).

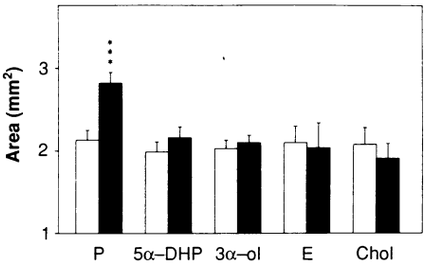

Fig. 3. Effect of P, 5α-DHP, 3α-ol, estradiol-17β (E), or cholesterol (chol) on the oxytocin receptor field in the caudal ventromedial hypothalamus when applied in vitro to previously dried brain sections of E-primed females. Adjacent brain sections from ten females were dried under a fan for 30 min and then incubated in the absence (open columns) or in the presence of hormone (closed columns) (duplicates for each rat). The incubation conditions were similar to those described in (*10*). All steroids were added to the incubation medium in ethanol (final concentration, 0.5%). Control sections were incubated in the presence of 0.5% ethanol without steroid. The final concentrations of steroids were 10^{-7} M for the progestagens and cholesterol and 10^{-9} M for E (*32*) (mean ± SD, ***$P \leq 0.001$ when compared to the corresponding controls by paired two-tailed t test).

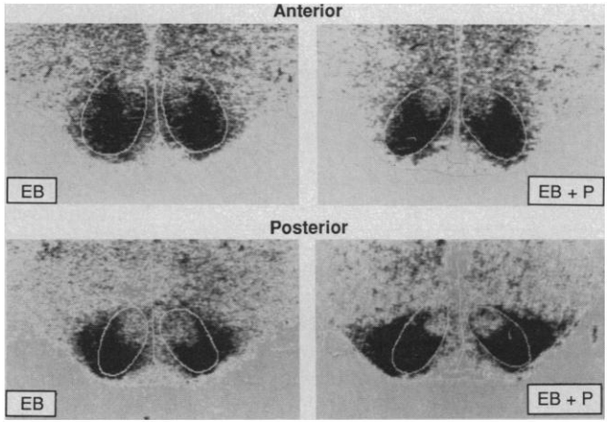

Fig. 1. Representative autoradiograms of oxytocin receptor binding in the anterior (top) and posterior (bottom) ventromedial hypothalamus. The outlines of the VMN were determined by apposing stained sections to the autoradiograms. The anterior and the posterior planes of the ventromedial hypothalamus corresponded to coronal plates 27 to 28 and 30 to 31, respectively, of the stereotactic atlas of Paxinos and Watson (*31*). Female rats were treated with EB alone (EB) or with EB followed 48 hours later by P (EB + P). Brains were sampled 4 hours after P treatment. Magnification, ×15.

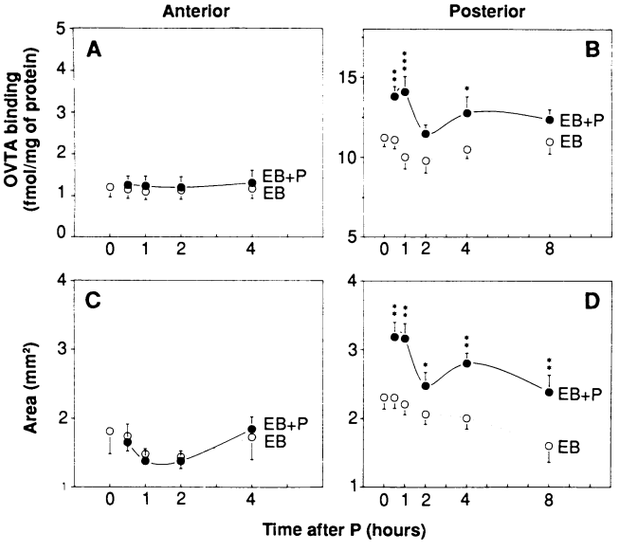

Fig. 2. Effect of P on oxytocin receptor binding at 30 min in the zone lateral to the VMN (**B**) and on the area covered by oxytocin receptors (**D**) and effect of P on oxytocin binding in the anterior ventromedial hypothalamus (**A** and **C**). Brains from E-primed females were sampled for receptor autoradiography at different times after the injection of vehicle (EB, open circles) or P (EB + P, filled circles). Each data point corresponds to the mean ± SD from eight (0, 1, 2, and 4 hours) or four (30 min and 8 hours) animals (***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$ when compared to the corresponding EB group by Duncan multiple range tests).

In parallel studies, we localized the site of action of oxytocin in the facilitation of lordosis behavior. The behavioral effects of oxytocin were observed after the bilateral infusion of the peptide into the areas lateral to the VMN (*20*). One hour after the infusion, females were tested for the occurence of lordosis behavior (*21*). Oxytocin facilitated lordosis behavior in females primed with both EB and P only when it was infused bilaterally into the area lateral to the caudal VMN (lordosis scores: saline (n = 12), 0.54 ± 0.14; oxytocin (n = 15), 2.15 ± 0.16; mean ± SEM, $P \leq 0.001$ by Mann-Whitney test). By contrast, oxytocin did not facilitate lordosis behavior when infused into the anterior part of the ventromedial hypothalamus, where the E-induced oxytocin receptors are not modulated by P treatment [lordosis scores: saline (n = 12), 0.41 ± 0.07; oxytocin (n = 17), 0.46 ± 0.08; mean ± SEM, $P > 0.7$ by Mann-Whitney test]. The sites where oxytocin increased female receptivity corresponded to the area where P rapidly modulated oxytocin receptor binding (Fig. 4). We have also reexamined stained sections for the neuroanatomical localization of oxytocin infusion sites from a previous study (*7*). Animals that showed facilitation of lordosis behavior in response

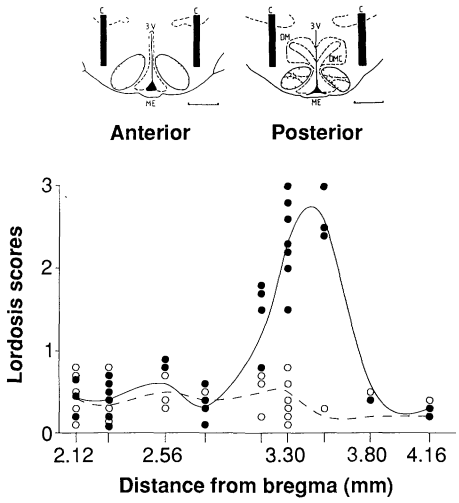

Fig. 4. Effect of oxytocin on lordosis responding 1 hour after infusion lateral to the posterior VMN (top, dotted nuclei), where P modulates oxytocin receptor binding, and into the anterior ventromedial hypothalamus (*20*). Fifty-six females primed with EB + P were infused bilaterally with oxytocin (100 ng dissolved in 0.5 μl of saline per site) (filled circles, solid line corresponding to a tenth order polynomial regression) or with saline alone (open circles, dotted line corresponding to a third order polynomial regression). C, cannula; DM, dorsomedial hypothalamic nuclei; DMC, area compacta of the DM; ME, median eminence; 3V, third ventricle. Scale bars, 1 mm.

to oxytocin had infusion sites located in the posterior ventromedial hypothalamus.

Our behavioral observations indicate that the rapid modulation of oxytocin receptor binding by P may be involved in the facilitation of female mating behavior. First, oxytocin facilitated lordosis behavior only in females primed with both E and P (*7*). Second, P increased both oxytocin receptor binding and lordosis responding within 30 min. Finally, oxytocin facilitated lordosis behavior only when it was infused into the caudal part of the ventromedial hypothalamus, where the E-induced oxytocin receptors are modulated by P. These data suggest that P activates oxytocin receptors located in the neuropil surrounding the caudal VMN.

There are two possible mechanisms for these effects of P. P could change the conformation of oxytocin receptors on the dendrites, perhaps from an inactive low-affinity state to an active high-affinity state. This shift would explain both the increase in receptor binding and extension of the oxytocin receptor field. A similar mechanism has been proposed for the central γ-aminobutyric acid A ($GABA_A$) receptor (*22*), and different interconvertible affinity states have been described for the oxytocin receptor (*23*). Progesterone could produce these effects by binding directly to the receptor (24) or by modifying its lipid environment by intercalating into the lipid bilayers (*25, 26*).

Alternatively, P could increase the mobility of oxytocin receptors within the membrane of dendrites of laterally projecting VMN neurons by causing changes in membrane fluidity. A fluidization of the neuronal membrane by P may also expose a reserve of oxytocin binding sites that were previously inaccessible (*28*). Changes in the mobility of receptors might also involve transport within neuronal processes (*29*). The extent to which receptor mobility can occur under the conditions of our in vitro assay is not known. Regardless of the mechanism, the effect of P is limited to a zone lateral to the caudal VMN, which is part of the neuronal network involved in the regulation of female mating behavior.

These results show a specific and rapid steroid effect on the neuronal membrane that is involved in the activation of a behavioral response. However, rapid membrane effects of P are unlikely to be sufficient for the facilitation of lordosis behavior. Although the rapid effects of P on oxytocin receptor binding in the posterior ventromedial hypothalamus appear to be a key step in the facilitation of lordosis, the regulation of gene expression by P bound to intracellular receptors is also necessary for the facilitation of female mating behavior (*2, 16, 30*).

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14. Protein synthesis occurs throughout the dendritic tree and tritiated amino acids are incorporated into dendritic proteins within 10 min [O. Steward *et al.*, *Mol. Neurobiol.* **2**, 227 (1988)]. The mRNA coding for the nicotinic acetylcholine receptor of the neuromuscular junction accumulates at the site of receptor insertion [J. Merlie and J. R. Sanes, *Nature* **317**, 66 (1985)], indicating that complex proteins like neurotransmitter receptors can be rapidly synthesized at the synapse.
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19. OVX and ADX rats were injected with 10 μg of EB or with 50 μg of EB followed 48 hours later by an injection of vehicle (EB group) or 0.5 mg of P (EB + P group). Brains were sampled 4 hours after the last injection. The area covered by oxytocin receptors within the caudal ventromedial hypothalamus was determined by in vitro receptor autoradiography. Area after priming with 10 μg of EB: EB females (n = 7), 2.19 ± 0.18; EB + P females (n = 4), 2.80 ± 0.22** mm^2 (28% increase). Area after priming with 50 μg of EB: EB females (n = 4), 1.98 ± 0.21; EB + P females (n = 4), 3.19 ± 0.30** mm^2 (61% increase), mean ± SD, ** $P \leq 0.01$ when compared to the corresponding EB group by Duncan multiple range tests. Both the effect of the P treatment ($P \leq 0.001$, F = 70.3, df = 1) and the interaction between dose of EB and P treatment ($P \leq 0.001$, F = 14.16, df = 1) were significant (two-

way ANOVA).
20. One week after OVX, we sterotactically implanted bilateral guide cannulas (22 gauge) in 3-month-old female Sprague-Dawley rats under Chloropent anesthesia; the tip of the cannula was located 1.0 mm above the injection site (dorsal to the lateral ventromedial hypothalamus). After surgery, females were kept for 2 weeks as in (*12*) and then injected subcutaneously with 10 μg of EB in 100 μl of sesame oil (9:30–10:00 a.m.) followed 48 hours later by an injection of 0.5 mg of P in 100 μl of propylene glycol. Four hours after P, each side of the ventromedial hypothalamus was infused through a 28-gauge double inner cannula during 1 min with 0.5 μl of either saline or saline containing 100 ng of oxytocin (Peninsula). This dose of oxytocin is low and facilitates female mating only after E + P priming (*7*).
21. All females received 12 mounts by a male, the last 10 of which were scored. Lordosis scores (LS) were computed by rating the response of the female to a mount from 0 to 3, representing gradations from the absence of dorsiflexion (LS = 0) to a maximal dorsiflexion (LS = 3). The final lordosis quality scores (QS) were computed as Σ LS/10 mounts [D. F. Hardy and J. F. DeBold, *Horm. Behav.* **2**, 287 (1971)]. Lordosis scores were measured by two independent observers who were blind to the infusion conditions, and the results presented correspond to the mean of both observations.
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32. These concentrations of steroids correspond to plasma concentrations during proestrus [M. S. Smith, M. E. Freeman, J. D. Neill, *Endocrinology* **96**, 219 (1975); L. G. Nequin, J. Alvarez, N. B. Schwartz, *Biol. Reprod.* **20**, 659 (1979)]. However, such high concentrations of P might not occur within the brain because serum concentrations of free P are in the nanomolar range (*5*). That is, only steroids that are not bound to plasma proteins are thought to diffuse through the membrane of brain target cells. This conclusion is also supported by the observation that cerebrospinal fluid concentrations of P correspond to free serum concentrations of P (*5*). However, concentrations of P in the hypothalamus and in the cerebral cortex are similar to total serum concentrations of P and can reach 10^{-7} M after ovulation [M. Bixo, T. Bäckström, B. Winblad, G. Selstam, A. Andersson, *Acta Physiol. Scand.* **128**, 241 (1986); M. Bixo, T. Bäckström, S. Cajander, B. Winblad, *Acta Path. Microbiol. Scand. Sect. A* **94**, 297 (1986)].
33. Supported by grant NS-07080 (B.S.M.) and HD-05751 (D.P.). M.S. was supported by a grant from the European Molecular Biology Organization (EMBO) and by NIH TWO4103. H.C. was supported by a fellowship of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

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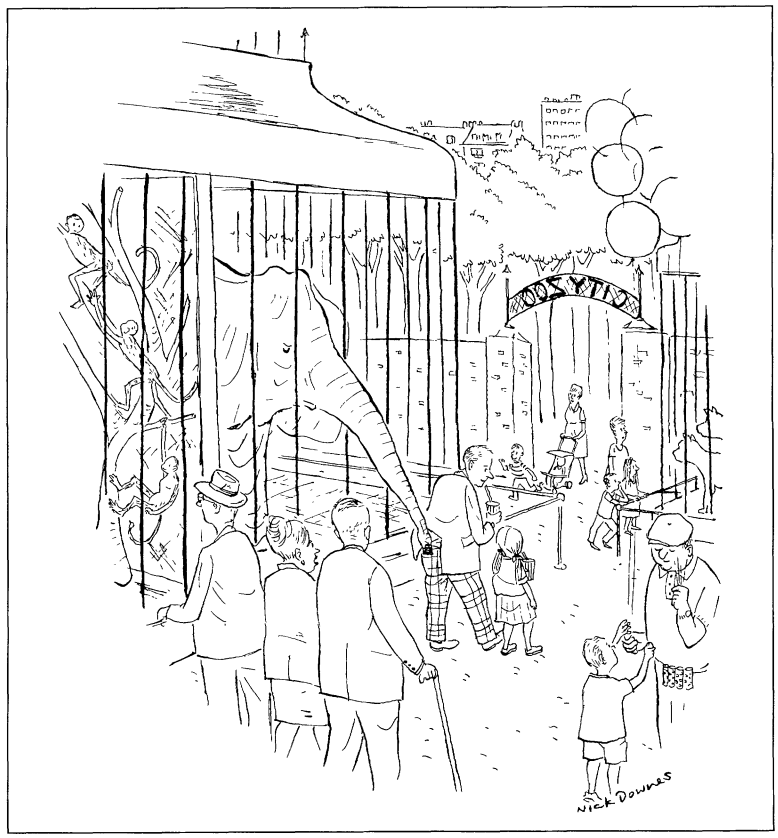

" It's a shame what happens to them under these conditions."