CNS Region-Specific Oxytocin Receptor Expression: Importance in Regulation of Anxiety and Sex Behavior

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The oxytocin receptor (OTR) is differentially expressed in the CNS. Because there are multiple mechanisms by which the OTR can be transcriptionally induced, we hypothesized that differences in OTR expression may be explained by activation of distinct signal transduction pathways and may be critical for the control of anxiety and sex behaviors. To determine the regulation and functional significance of this expression, we infused female rats with modifiers of protein kinases before assaying for behavior and oxytocin receptor binding. In the ventromedial nucleus of the hypothalamus (VMH), estrogen-dependent induction of oxytocin receptors required protein kinase C activation, and oxytocin infused here promoted female sex behavior but had no effect on anxiety. In contrast, dopamine controlled tonic oxytocin receptor expression in the central nucleus of the amygdala (cAmyg) through activation of protein kinase A, and oxytocin infused here was anxiolytic but had no effect on female sex behavior. Therefore, we have identified brain region-specific regulation of the OTR in the VMH and cAmyg. Distinct signal transduction pathways regulating receptor expression and binding in each brain region may mediate in part the ability of oxytocin to exert these differential behavioral effects.

Key words: oxytocin; heterologous expression; amygdala; hypothalamus; lordosis; anxiety

The neuropeptide oxytocin (OT) is synthesized in neurons of the supraoptic and paraventricular (PVN) nuclei of the hypothalamus, which project to the posterior pituitary and release oxytocin into the bloodstream. This peripherally acting oxytocin is critical for the processes of lactation and parturition. Oxytocin neurons of the PVN also send projections to many regions within the brain, including the hippocampus, amygdala, and hypothalamus, in which oxytocin acts as a neurotransmitter involved in affiliative behaviors (Insel, 1992). Oxytocin receptors (OTRs) are found in many brain regions, including the central nucleus of the amygdala (cAmyg) and the ventromedial nucleus of the hypothalamus (VMH) in which they are heterologously expressed (Bale et al., 1995a,b). These two brain regions are critical components of neural circuits regulating distinct behavioral responses. The VMH is an important mediator of female sex behavior (Pfaff et al., 1994), whereas the cAmyg is a portion of the limbic system that coordinates fear and anxiety responses (Davis, 1997; Roozendaal et al., 1997; LeDoux, 1998). The exhibition of rat female sex behavior requires estrogen action in the brain, including the induction of OTRs in the VMH (McCarthy et al., 1994; Bale and Dorsa, 1995a,b; Bale et al., 1995a,b). In contrast, OTR levels in the cAmyg are unaffected by estradiol (Bale and Dorsa, 1995a), despite dense collections of estrogen-concentrating neurons (Pfaff and Keiner, 1973).

In vitro, OTR expression is increased by activation of protein kinase A (PKA) or protein kinase C (PKC) (Bale and Dorsa, 1998). Previously, we isolated and sequenced 4.5 kb of an upstream sequence of a rat genomic OTR clone from a rat testis library and identified crucial putative response elements, including a cAMP response element and several activator protein-1 (AP-1) sites (Bale and Dorsa, 1997). Because there are multiple mechanisms by which the OTR can be induced transcriptionally, we hypothesized that differences in OTR expression between the VMH and cAmyg may be explained by activation of distinct signal transduction pathways in these brain regions, and these may be critical for the control of anxiety and sex behaviors.

MATERIALS AND METHODS

Animals. Sprague Dawley female rats were obtained from Charles River (Kingston, NY) and housed under reversed light/dark cycle conditions (lights out at 10:00 A.M.) in the animal facility of the University of Maryland, Baltimore. All animals were ovariectomized under anesthesia [75 mg of ketamine + 2.5 mg of acepromazine/kg body weight (BW)] and allowed at least 1 week to recover before the experiment. All procedures were in accordance with the guidelines of the University of Maryland Institutional Animal Care and Use Committee.

Acute intracerebral infusions for oxytocin receptor binding. Animals were anesthetized (75 mg of ketamine + 2.5 mg of acepromazine/kg BW) and placed into a stereotaxic apparatus for site-specific infusions using a 1 μl Hamilton syringe with attached needle. Stereotaxic coordinates for infusion into the amygdala were 2.8 mm caudal to bregma, 3.75 mm lateral to the midline, and 8.00 mm below the surface of the brain. Coordinates for injections into the VMH were the same, except 0.75 mm lateral to the midline. These coordinates were based on Figure 29 of the Paxinos and Watson (1986) rat brain atlas. Bilateral infusions (1 μl per side) consisted of phorbol ester (200 nM; Sigma, St. Louis, MO), forskolin (10 μM; Sigma), H89 (200 μM; Calbiochem, La Jolla CA), bisindolylmalemide I (BIM, 200 μM; Calbiochem), or the dopamine D1 receptor antagonist *R*(+)-SCH-23390 hydrochloride (10 μg; Research Biochemicals, Natick, MA). Infusions were ~1 min in duration, and the injection cannula was left in place for an additional 30 sec before removal. At completion of the infusion, the incision was closed with surgical staples; the animal was treated with antibiotics and an analgesic and injected subcutaneously with either 2 μg of estradiol benzoate (EB) or oil vehicle in 0.1 cc of sesame oil before being returned to its home cage. To evaluate the

inhibitory effect of BIM on OTR expression in the VMH, EB treatment is necessary to induce a detectable level of OTRs in this brain region. No EB treatment is necessary for OTR expression in the cAmyg because endogenous levels are maximal without estrogen exposure, and therefore ovariectomized females were left untreated.

Oxytocin receptor binding. Eight hours after infusion, animals were anesthetized lightly with CO_2 before decapitation. Brains were removed rapidly, frozen briefly in isopentane, and stored at $-80°C$ until sectioning. Brains were cut on a cryostat (20 μm thick), and sections were mounted onto subbed slides and frozen at $-80°C$ until binding was performed. Slides were thawed before being washed twice for 10 min in 50 mM Tris buffer, pH 7.4, at room temperature. Sections were then incubated in buffer containing: 50 mM Tris, pH 7.4, 30 pM ^{125}I-Ornithine Vasotocin (OTA; DuPont NEN, Boston, MA), 10 mM $MgCl_2$, 0.1% BSA, and 0.05% bacitracin for 60 min at room temperature. Nonspecific binding was defined in adjacent sections that were exposed to both ^{125}I-OTA and 1 μM cold OT (Bachem, Torrance, CA). After the incubation period, slides were washed four times for 5 min each in a 50 mM Tris buffer containing 10 mM $MgCl_2$ at 4°C. Slides were dipped rapidly in deionized water and dried under cool air. Sections were then exposed to film for 3 d before being developed by standard photographic methods.

Autoradiography data analysis. Film autoradiographs were analyzed blind using a microcomputer-based image analysis system (MCID; Image Research, St. Catherines, Ontario, Canada). Oxytocin receptor binding optical density from autoradiographs was measured by subtracting nonspecific signal of adjacent sections from total signal to obtain the specific signal for each tissue section. The area of measurement for all samples was identical and was taken from the same region of the VMH or cAmyg as determined by choosing matching brain sections before analysis.

Repeated intracerebral infusions for behavioral testing. Animals were anesthetized (75 mg of ketamine + 2.5 mg of acepromazine/kg BW), placed into a stereotaxic apparatus, and implanted with a bilateral guide cannula (22 gauge; Plastics One, Roanoke, VA) into either the VMH or cAmyg using the same stereotaxic coordinates as for the acute infusions. The guide cannula extended 7.5 mm into the brain, and the internal dummy cannula extended an additional 0.5 mm. Animals were ovariectomized at the same time as the implantation of the cannula. At the time of infusion, awake animals were lightly restrained in a terry cloth towel, the dummy cannula was removed, and an injection cannula attached to a 1 μl Hamilton syringe via PE-10 tubing was inserted into the guide cannula. After infusions were performed in the same manner as described above, the fully awake animal was returned to its home cage.

Depending on the experiment, animals were infused with either the D1 antagonist SCH23390 (10 μg), the PKC inhibitor BIM (200 μM), or the PKC activator phorbol ester (PMA, 200 nM) in the morning (~9:00 A.M.) and the evening (~5:30 P.M.) on 2 consecutive days and again on the morning of the third day, for a total of five infusions. On the afternoon of the third day (the midpoint of the dark cycle), animals were infused with either oxytocin (1 μg) or saline vehicle 10–20 min before behavioral testing.

Female sex behavior testing. Ovariectomized females were treated with EB (in sesame oil vehicle, 2–5 μg/0.1 cc, s.c.) 48 and 24 hr before behavioral testing. At the midpoint of the dark cycle on the day of the test, animals were allowed to acclimate to a test arena before being tested manually for lordosis responsiveness by an investigator blinded to the particular treatment. Testing consisted of 10 trials of gently palpating the flanks and exerting pressure on the rump to induce a lordosis response. The degree of arching of the back and elevation of the rump was an indicator of the amplitude of the response and was assigned qualitative scores from zero (no lordosis) to three (maximal lordosis). Animals were pretested, infused with either oxytocin (1 μg) or saline vehicle, and post-tested within 10–20 min of infusion. Because animal variability prevents absolute numbers for lordosis response, results were calculated as the difference for each animal between pretest and post-test scores. For this and all other behavioral tests, the experimenter scoring the response was blinded to the experimental group. All animals were treated and tested at the same time by the same investigators.

Open-field testing. At the midpoint of the dark phase of the cycle, animals were placed for 5 min in the center of an open-field apparatus made of clear Plexiglas (100 cm long, 80 cm wide, and 50 cm high); the floor of the apparatus was divided into 30 equally sized squares ~16.5 cm per side. The movement of the animal was monitored such that each crossing over a line in the grid was noted. The squares around the perimeter were considered "outside," whereas all others were considered "inside." Data were expressed as the ratio of inside grid crossings to outside grid crossings. Because this was a test of novelty-induced anxiety, animals could not be pretested before drug treatment. Animals were tested in the apparatus 10–20 min after an infusion of oxytocin (1 μg) or saline. An increase in the inside crossings/outside crossings ratio is an indicator of decreased anxiety. Animals tested for anxiety after infusion into the cAmyg were not pretreated with EB because OTRs in this brain region are maximal without estrogen exposure.

Elevated plus maze testing. Immediately after the open-field test, animals were placed in the center of a standard elevated plus maze (EPM) apparatus of 50 cm height with 50-cm-long arms arranged perpendicularly. Two opposite arms were open, and two were enclosed with darkened Plexiglas walls. A 40 W bulb was suspended directly above the maze to provide even illumination to all arms. The number of entries and time spent in open and closed arms of the maze were recorded for 10 min with a digital recorder.

Histology. At the completion of the behavioral experiments, all animals were overdosed with Nembutal and perfused transcardially with ice-cold saline followed by 4% paraformaldehyde. Brains were removed, post-fixed for 1 hr, cryoprotected in 30% sucrose, and stored frozen at $-80°C$ until sectioned on a cryostat. Sections (50 μm thick) were collected throughout the area of the cannula implant and stained with cresyl violet for verification of correct placement.

Statistics. Binding data and lordosis responding were assessed for statistical significance by ANOVA, and individual comparisons were made by *post hoc* Kruskal–Wallis test. Open-field data expressed as a ratio were assessed by the nonparametric Mann–Whitney U test.

RESULTS

Experiment I: Assessment of OTR binding by receptor autoradiography after manipulation of PKA or PKC by infusion into the VMH or cAmyg

Ventromedial hypothalamus

Representative autoradiographs of oxytocin binding in the VMH after the various treatments are pictured in Figure 1. Quantification of binding showed that infusion of the PKC inhibitor, BIM (200 μM), significantly decreased OTR binding levels in the VMH compared with saline vehicle-infused, ovariectomized estradiol-replaced females (ANOVA; $p < 0.01$; $n = 4$) (Fig. 2*A*). Infusion of a phorbol ester (PMA, 200 nM) to mimic activation of PKC induced a significant increase in OTR binding in the absence of estrogen, compared with saline vehicle-infused ovariectomized controls ($p < 0.001$; $n = 4$–5) (Fig. 2*A*). Infusion into the VMH of H89 (200 μM; $n = 3$) to inhibit PKA in ovariectomized estradiol-replaced females had no effect on OTR binding, nor did infusion of forskolin (200 nM; $n = 3$) to enhance PKA in ovariectomized females (Fig. 2*B*)

Central nucleus of the amygdala

Representative autoradiographs of oxytocin binding in the cAmyg after the various treatments are pictured in Figure 3. Quantification of binding showed that infusion of H89, which inhibits PKA activity, significantly decreased OTR binding in this nucleus compared with vehicle-infused ovariectomized controls (ANOVA; $p < 0.01$; $n = 4$) (Fig. 4*A*). The cAmyg expresses dopamine D1 receptors that are positively coupled to adenylate cyclase and downstream activation of PKA. Infusion of the D1 antagonist SCH23390 significantly decreased OTR binding density in the cAmyg of ovariectomized females, compared with vehicle-infused controls ($p < 0.01$; $n = 3$–4) (Fig. 4*B*). Infusion of forskolin, an activator of PKA, combined with SCH23390 reversed the effects of the D1 antagonist, resulting in a return to near basal levels of OTR binding that was significantly greater than the SCH antagonist alone ($p < 0.05$; $n = 3$) (Fig. 4*B*). Infusion of the PKC inhibitor, BIM (200 μM), was without effect on OTR binding in the cAmyg of ovariectomized females (vehicle, 0.295 ± 0.07; BIM, 0.261 ± 0.09).
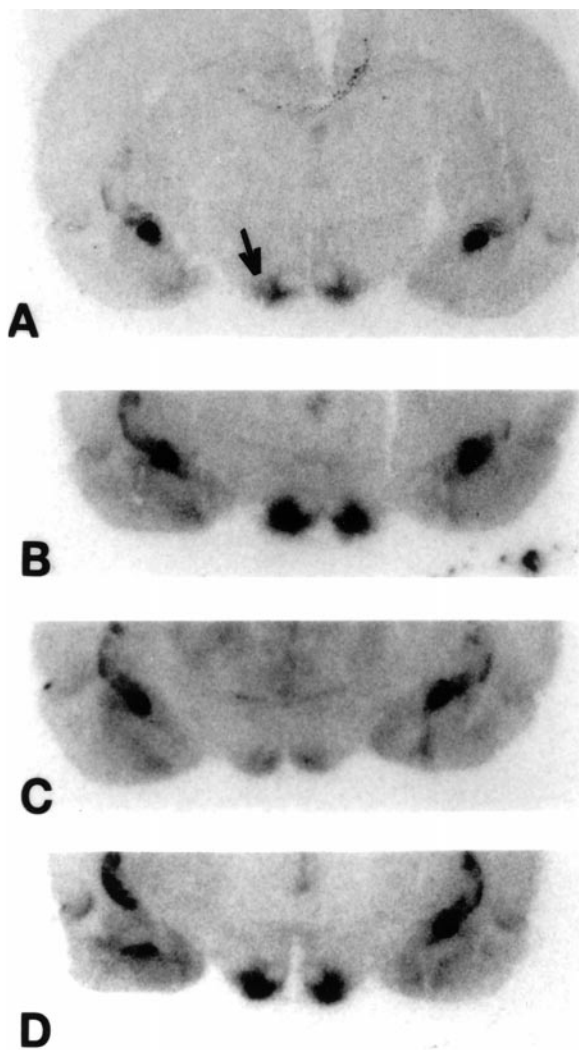
Figure 1. Representative autoradiographs illustrating oxytocin receptor binding in the VMH. Animals were pretreated with estradiol (2 μg) or oil vehicle by subcutaneous injection; infused centrally, directly into the VMH with BIM (inhibitor of PKC) or the phorbol ester, PMA, 8 hr before collection of the brains; and assayed for OTR binding by quantitative receptor autoradiography. Treatment groups were as follows: *A*, subcutaneous oil vehicle injection, saline vehicle infusion into the VMH; *B*, subcutaneous estradiol injection, saline vehicle infusion into the VMH; *C*, subcutaneous estradiol injection, BIM infusion into the VMH; *D*, subcutaneous oil vehicle injection, PMA infusion into the VMH. *Arrow* indicates region of VMH that was analyzed.

Experiment II: Assessment of site-specific behavioral effects of oxytocin infusion

Female sex behavior

Oxytocin infused into the VMH of females primed with a low dose of estradiol significantly increased lordosis responding (one-way ANOVA with repeated measures *post hoc* Kruskal—Wallis; $p < 0.001$; $n = 9$) (Fig. 5*A*) but had no effect on lordosis when infused into the cAmyg ($p > 0.5$; $n = 8$) (Fig. 5*B*). The VMH oxytocin-induced increase in lordosis was blocked by previous treatment with the PKC inhibitor, BIM ($p < 0.001$) (Fig. 5*A*). The ability of VMH-infused oxytocin to enhance lordosis responding was also evident in animals pretreated with the PKC activator PMA ($p < 0.01$), although there was no difference
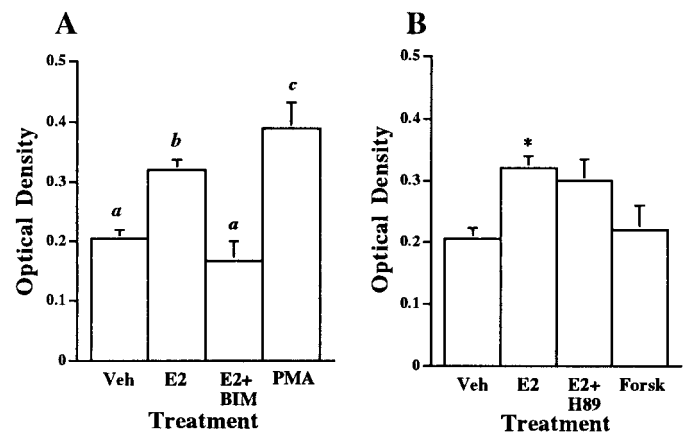
Figure 2. Quantification of OTR binding in the VMH. *A*, Optical density levels of OTR binding in the VMH of ovariectomized rats treated peripherally with estradiol (*E2*) or oil vehicle and centrally with inhibitors (*BIM*) or activators (*PMA*) of PKC. Treatment groups were *Veh* (subcutaneous oil vehicle injection, saline vehicle infusion into the VMH; $n = 4$), *E2* [subcutaneous estradiol (2 μg) injection, saline vehicle infusion into the VMH; $n = 7$], *E2 + BIM* [subcutaneous estradiol injection, BIM (200 μM) infusion into the VMH; $n = 4$], or *PMA* [subcutaneous oil vehicle injection, PMA (200 nM) infusion into the VMH; $n = 5$]. Brains were collected 8 hr after drug infusion and steroid treatment. Data were analyzed by one-way ANOVA and indicate a significant effect of treatment [$F_{(3,16)} = 12.01$; $p < 0.001$]; *post hoc* analysis indicates that *a* is significantly different from *b* ($p < 0.01$) and *c* ($p < 0.001$). *B*, When compared with the same Veh- and E2-treated groups as above, infusion of forskolin (*Forsk*) (200 nM; $n = 3$), an activator of PKA, or H89 (*E2 + H89*) (200 μM; $n = 3$), an inhibitor of PKA, into the VMH of ovariectomized females was without effect on OTR binding in the VMH. All values are ± SEM.

between animals pretreated with PMA or saline vehicle when they were given oxytocin (data not shown).

Open-field test

Oxytocin infusion into the cAmyg significantly increased open-field activity compared with controls, almost tripling the inside crossings/outside crossings compared with saline-infused animals (Mann–Whitney *U* test; $p < 0.01$; $n = 9$ for oxytocin-infused; $n = 6$ for saline-infused) (Fig. 6*A*). The D1 antagonist SCH23390 blocked this effect of oxytocin ($p < 0.01$; $n = 8$) (Fig. 6*A*). In contrast, oxytocin infusion into VMH had no effect on open-field activity when compared with saline-infused controls (Mann—Whitney *U* test; $p > 0.8$) (Fig. 6*B*). There was no difference in the total number of crossings between any groups, indicating no changes in general locomotor activity (VMH oxytocin infusion = 126.2 ± 49.4; saline = 126.0 ± 25.7; cAmyg oxytocin infusion = 112.3 ± 11.6; oxytocin + SCH23390 = 123.4 ± 14.1; cAmyg saline = 137.7 ± 19.8).

Elevated plus maze

Although infusion of oxytocin into the cAmyg appeared to increase the amount of time spent on the open arm of the plus maze and infusion of the D1 antagonist SCH23390 decreased the amount of time spent in the open arms when compared with oxytocin-infused controls (SCH23390 + oxytocin = 53.4 ± 6.6 sec, compared with oxytocin = 63.6 ± 12.0 sec and vehicle = 50.3 ± 11.3 sec), these differences failed to reach statistical significance. There were no differences in total arm entries when comparing oxytocin and saline infusion (oxytocin + SCH23390 = 14.4 ± 2.8; oxytocin = 10.9 ± 1.8; saline = 11.5 ± 1.8), again indicating no changes in general locomotor activity as a result of
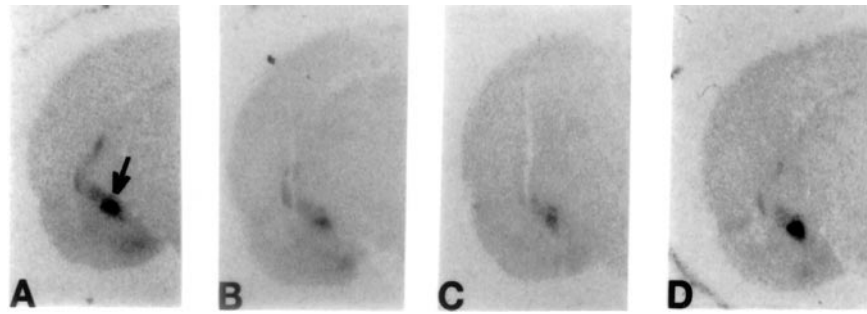
Figure 3. Representative autoradiographs illustrating OTR binding in the cAmyg. Animals were infused directly into the cAmyg, and brains were collected 8 hr later and assayed for OTR binding by quantitative receptor autoradiography. Treatments were as follows: *A*, saline vehicle infusion into the cAmyg; *B*, H89 (PKA inhibitor) infusion into the cAmyg; *C*, SCH23390 (dopamine D1 receptor antagonist) infusion into the cAmyg; or *D*, SCH23390 + forskolin infusion into the cAmyg. *Arrow* indicates region of cAmyg that was analyzed.
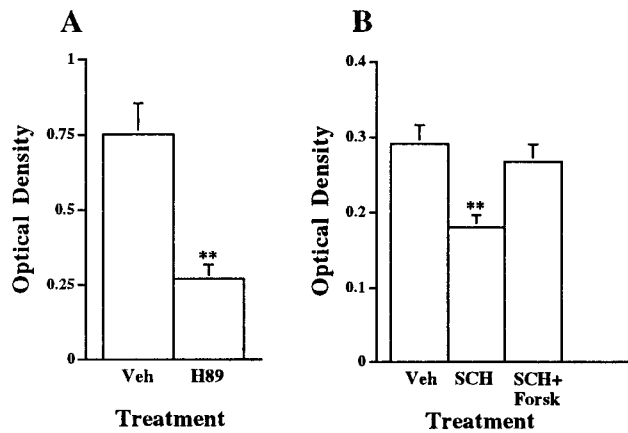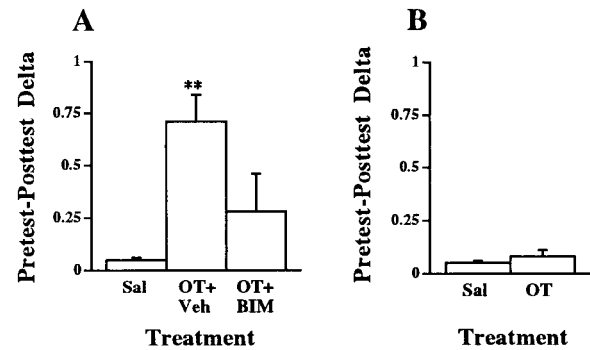

Figure 4. Quantification of OTR binding in the cAmyg. Ovariectomized rats were treated as follows: *A*, *Veh* 1 (saline vehicle for H89 infusion into the cAmyg; *n* = 7), and *H89* (H89 infusion into cAmyg; *n* = 5); *B*, *Veh* 2 (saline vehicle for SCH23390 infusion into the cAmyg; *n* = 2); *SCH* (SCH23390 infusion into the cAmyg; *n* = 3), or *SCH* + *Forsk* (SCH23390 + forskolin infusion into the cAmyg; *n* = 3). Brains were collected 8 hr after drug infusion. There was a significant effect of H89 infusion [ANOVA; $F_{(1,10)}$ = 13.96] and SCH23390 infusion [$F_{(3,7)}$ = 10.62; p < 0.01] when each was compared with its own controls. *Post hoc* analysis, **p < 0.01. All values are ± SEM. Previous treatment with estradiol was found to have no influence on the effectiveness of H89 treatment (data not shown).

Figure 5. Effects of oxytocin infusion into the VMH and cAmyg on female sex behavior. Animals were primed with low doses of estradiol for 2 d and then pretested for lordosis responding before infusion of oxytocin (*OT*; 1 μg) or saline (*Sal*) into the VMH or cAmyg. Post-testing was performed 10–20 min after infusion. Data are presented as the increase in lordosis amplitude between the pretest and post-test. *A*, For animals with indwelling cannulas in the VMH, oxytocin infusion significantly increased lordosis responding compared with animals either infused with saline or pretreated for 2 d with the PKC inhibitor, *BIM*. ANOVA with *post hoc* Kruskal—Wallis; **p < 0.001; n = 9 for all groups. These results show that oxytocin infusion into the VMH increases lordosis behavior and this behavior is blocked by pretreatment with BIM. *B*, For animals with indwelling cannulas in the cAmyg, there was no difference in lordosis responding between those infused with oxytocin (n = 5) versus saline [n = 4; $F_{(1,14)}$ = 0.25; p > 0.5]. All values are ± SEM.

oxytocin infusion. Oxytocin infusion into the VMH had no effect on EPM activity when compared with saline-infused controls (data not shown).

The location of the infusion sites based on cresyl violet-stained sections resulted in the exclusion of one animal for cAmyg because of improper cannula placement.

DISCUSSION

We report here the regionally specific regulation of OTR binding and its significance in the control of anxiety and sex behaviors. In the female rat VMH, OTR binding is reduced by up to 200-fold after ovariectomy and can be restored fully by replacement of estradiol (Johnson et al., 1989). A component of the estradiol-induced increase in OTRs involves increased levels of mRNA (Bale and Dorsa, 1995a,b), but whether this involves a genomic effect of estradiol is unknown. The mechanisms by which steroids can alter cellular functions have been expanded recently to include interactions with cytoplasmic signal transduction pathways (Zhou et al., 1996; Toran-Allerand et al., 1999). Our data indicate

that estradiol increases oxytocin receptor binding in the VMH via a PKC-dependent mechanism. Inhibition of PKC in the VMH blocked the ability of estradiol to increase OTR binding, whereas activation of PKC significantly increased OTR binding in the absence of estrogen. Therefore, the estradiol-induced increase in OTR binding in the VMH functions through activation of PKC and not through genomic actions of estrogen receptor directly at the OTR promoter.

In contrast to results in the VMH, OTR binding in the cAmyg is not regulated by estradiol (Bale et al., 1995a,b), and here we report that it is tonically maintained via a PKA-dependent mechanism involving the dopamine D1 receptor. This is the first evidence to suggest an interaction between the OTR and dopamine systems in the cAmyg. Inhibition of PKA in the cAmyg by H89 significantly decreased OTR binding when compared with controls. OTR binding was also reduced by antagonism of the dopamine D1 receptor by SCH23390. Tonic dopamine input into the cAmyg likely activates PKA through D1 receptors. The maintenance of OTR binding in the cAmyg appears specific to PKA

A **B**
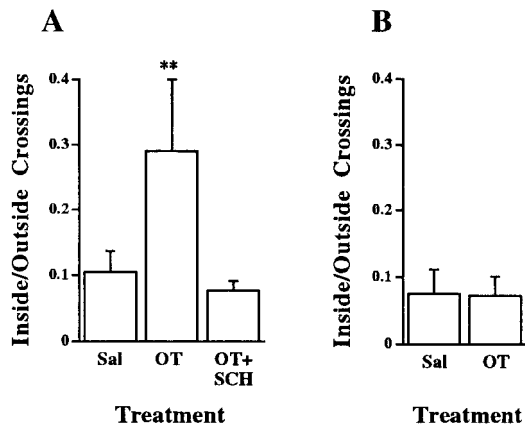
Figure 6. Effects of oxytocin infusion into the VMH and cAmyg on activity in an open field. Animals were placed into the center of an open-field testing apparatus within 10–20 min of infusion of oxytocin (1 μg) or saline. The ratio of inside/outside crossings is a useful overall indicator of activity in an open field. An increase in the ratio indicates reduced anxiety-like behavior because it demonstrates that the animals are more willing to explore the center of the open field. *A*, For animals with indwelling cannulas in the cAmyg and infused with oxytocin (*n* = 9), there was a significant increase in the ratio when compared with saline-infused controls (*n* = 6). This effect was blocked by pretreatment with the dopamine D1 receptor antagonist, SCH23390 (*n* = 8) (****p* < 0.01; ANOVA). There were no differences in overall activity between any groups. *B*, For animals with indwelling cannulas in the VMH, there was no effect of oxytocin infusion on activity in the open field.

because activation or inhibition of PKC had no effect at this brain site.

Oxytocin has been referred to as an "affiliative" hormone because of its intimate involvement in the regulation of behaviors involving attachment or close interaction between animals, including anxiety and sex behavior (Insel and Shapiro, 1992; McCarthy and Altemus, 1997). Consistent with previous reports (Schumacher et al., 1990; Schulze and Gorzalka, 1991), we found that infusion of oxytocin into the VMH increased female sex behavior as measured by lordosis responding. However, when PKC was inhibited, the enhancing effect of oxytocin on lordosis was blocked. The results of our binding studies suggest that the effect of PKC inhibition on lordosis may be caused by the consequent reduction of OTR expression in the VMH, although we cannot rule out possible interactions with other components necessary for a lordotic response. We attempted to increase OTR levels by activation of PKC with a phorbol ester, with the prediction that oxytocin effects on lordosis would be enhanced. Although we continued to observe a significant increase in lordosis amplitude and frequency after oxytocin infusion, there was no statistical difference in oxytocin effectiveness between females pretreated with a phorbol ester versus saline. This is likely attributable to a maximal level of OTR expression after the estrogen treatment. Estrogen has pleiotropic effects on lordosis responding that are not limited to an effect via increased OTR binding (Pfaff et al., 1994). Nonetheless, our data are the first to demonstrate that oxytocin stimulation of lordosis behavior involves the activation of PKC to increase OTR transcription. Previous studies have shown that PKC activation in the VMH can increase female sex behavior (Kow and Pfaff, 1998) but have not implicated a specific neurotransmitter. Estradiol may directly activate the phosphatidylinositol second messenger pathway leading to PKC activation (Mobbs et al., 1989) or act indirectly by inducing the expression of PKC isoforms or fos and jun complexes that can

then interact with PKC to stimulate transcription at AP-1 sites (Gaub et al., 1990) as are found in the OTR promoter (Bale and Dorsa, 1998). It cannot be overlooked that inhibition of PKC may also affect other processes within the VMH that may alter lordosis behavior.

In contrast to the VMH, the cAmyg is a component of the limbic system and is involved in "emotional" responses, including fear and defensive behaviors (LeDoux, 1998). Oxytocin has been reported to have anxiolytic (McCarthy et al., 1996) and antidepressant (Arletti and Bertolini, 1987) actions, as well as facilitating pair-bonding (Williams et al., 1994; Insel and Hulihan, 1995) and mother–infant interactions (Pedersen and Prange, 1979; Fahrbach et al., 1984). Any or all of these may involve oxytocin action on the cAmyg. These behavioral responses are modulated by estradiol, but are not dependent on estradiol, as is the case for VMH regulation of female sex behavior. The exact CNS site(s) at which oxytocin acts to exert an anxiolytic effect is unknown. The cAmyg is one of the nuclei of the amygdala that receives convergent information from several other amygdaloid regions. It has been proposed that the afferents of this nucleus generate behavioral responses that reflect the sum of neuronal activity produced by the amygdala complex (Pitkanen et al., 1997). These observations led us to speculate that adaptive selective pressure has been in favor of a constitutive expression of OTRs in the cAmyg to allow for rapid responses to stressful or emotional stimuli by reducing anxiety. The cAmyg receives direct dopaminergic innervation from the midbrain, and neurons of this nucleus innervate the dopamine neurons of the substantia nigra (Rouillard and Freeman, 1995). Conditioned stressors increase the activity of dopamine neurons projecting to several components of the amygdala, including the central nucleus (Coco et al., 1992). Our findings suggest that one function of increased dopamine release to the cAmyg is the maintenance and/or increase of OTR expression levels. Oxytocin is also released centrally in response to a wide variety of stressors (Samson et al., 1985; Nishioka et al., 1998). Therefore, the oxytocin and dopaminergic systems may act in concert to reduce anxiety in response to social and environmental stressors. We found that oxytocin infusion into the cAmyg increased open-field activity almost threefold and that this effect was not present in animals pretreated with a D1 receptor antagonist. On the basis of our binding results, it is likely that the level of OTRs had been sufficiently reduced by pretreatment with the dopamine antagonist such that the oxytocin infused into the cAmyg could no longer exert an anxiolytic effect. Anxiety testing using the same treatments did not reach significance in the elevated plus maze. We believe that the open-field test is of more ethological relevance here because female rodents must leave their burrow and enter into an open field to mate. Oxytocin is strongly implicated in the control of social behaviors, suggesting that the difference in magnitude of the anxiolytic effect between the open field and elevated plus maze may be a function of measuring different types of anxiety and their potential social context. In contrast to the cAmyg, oxytocin infusion into the VMH had no effect on anxiety responses. To our knowledge, this is the first report of a site-specific anxiolytic effect of oxytocin.

A remaining question is the mechanism by which heterogeneous regulation of OTR expression is achieved in the cAmyg and VMH. The potential for an undiscovered second OTR gene in the cAmyg cannot be ruled out, and there is considerable
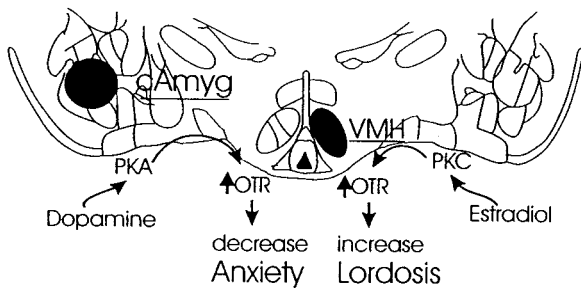
Figure 7. Summary diagram of effects observed on oxytocin receptor binding by estradiol and modulators of PKC and PKA and the resulting effects on behavior. Estradiol increases oxytocin receptor binding selectively in the VMN but not in the cAmyg. Estrogen action in the VMN involves PKC and results in increased female sex behavior as demonstrated by increased lordosis. Dopamine activation of the D1 receptor increases activation of PKA, resulting in increased or tonically maintained levels of oxytocin receptor binding in the cAmyg. Oxytocin acting in the cAmyg decreases anxiety. The use of distinct signal transduction pathways to regulate oxytocin receptor levels in the VMN and cAmyg may have evolved to allow for constitutive expression of oxytocin receptors in the cAmyg and an immediate response to possible life-threatening stimuli, as opposed to the hormonally constrained control of oxytocin receptors in the VMN that regulate female reproductive behavior.

circumstantial evidence to support this view (Bale et al., 1995a; Verbalis et al., 1995; Verbalis, 1999). An alternate hypothesis is based on the presence of three large TG-dinucleotide repeats in the OTR promoter (Bale and Dorsa, 1997). Submillimolar levels of calcium have been shown to regulate DNA structure at these TG-dinucleotide repeats in the other genes, causing an alteration in structure of the promoter and brain region-specific expression of the protein (Dobi and v Agoston, 1998). The heterologous regulation of OTR expression in the cAmyg and VMH may be a function of differences in availability of response elements in the promoter caused by induction of secondary structure.

In summary, we have identified brain region-specific regulation of the OTR in the VMH and cAmyg and have demonstrated that this heterologous regulation is critical for two different behavioral effects of oxytocin. Although the estrogen-dependent OTR expression in the VMH is induced by activation of the PKC pathway, the receptors in the cAmyg are dependent on PKA and are tonically activated through dopamine D1 receptors in this nucleus. In the cAmyg, oxytocin acts to decrease anxiety, but in the VMH it acts to stimulate female sex behavior. A schematic representation of these results is depicted in Figure 7. Sex receptivity in the female rat is dependent on appropriate levels and duration of exposure to estradiol, thereby temporally restricting its expression to coincide with the event of ovulation. In contrast, behavioral responses to anxiety-provoking stimuli such as novelty require a continuously primed neural substrate to allow for rapid adaptation. OTRs in the VMH and cAmyg appear to regulate these different behaviors, respectively, and the distinct signal transduction pathways regulating receptor expression and binding in each brain region may mediate in part the ability of oxytocin to exert these differential effects.

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