The neurosteroid allopregnanolone modulates oxytocin expression in the hypothalamic paraventricular nucleus

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Blyth, Brian J., Richard L. Hauger, Robert H. Purdy, and Janet A. Amico. The neurosteroid allopregnanolone modulates oxytocin expression in the hypothalamic paraventricular nucleus. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R684–R691, 2000.—Virgin, ovariectomized rats exposed to 2 wk of sequential estradiol (E2) and progesterone (P) followed by P withdrawal have increased hypothalamic oxytocin (OT) mRNA and peptide levels relative to sham-treated animals. This increase is prevented if P is sustained. In the central nervous system, P is metabolized to the neurosteroid allopregnanolone (3α-oxo-5α-pregnan-20-one), which exerts effects by acting as a positive allosteric modulator of GABAA receptor/Cl−channel complexes. In the present study, ovariectomized rats that received sequential E2 and P for 2 wk followed by P withdrawal were administered allopregnanolone at the time of P withdrawal. Hypothalamic and plasma allopregnanolone concentrations, serum E2 and P concentrations, and hypothalamic OT mRNA levels were measured at death. Steroid-induced increases in OT mRNA were attenuated in animals treated with allopregnanolone at the time of P withdrawal. The results suggest that allopregnanolone plays an important modulatory role in steroid-mediated increases in hypothalamic OT.

A KEY FACTOR REGULATING the central expression of oxytocin (OT) in the rat is the pervading ovarian steroid milieu (1, 3, 7, 8, 17–20). If an ovariectomized rat is administered sequential estradiol (E2)-filled and progesterone (P)-filled implants for several weeks and the P implants are removed 36–48 h before death, the rat not only displays the onset of maternal responsiveness (4), but also has increased levels of OT mRNA in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei (8). Both E2 priming and P withdrawal are important for increases in central OT, because if E2 is omitted or P is maintained, levels of OT mRNA do not increase (8).

The mechanism of the ovarian steroid-mediated effects on OT is not fully understood. Steroids may regulate central nervous system (CNS) functions by intracellular receptor-mediated effects on the transcription of specific genes (21). Additionally, P and certain of its metabolites (neurosteroids) influence transmission by direct actions on neuronal membranes (11, 12). P is metabolized within the CNS and adrenal gland to 5α-dihydroprogesterone (DHP) via a 5α-reductase enzyme, and DHP is further converted to 3α-hydroxy-5α-pregnan-20-one [also known as allopregnanolone (A)], a neurosteroid that binds to GABA_A receptor/Cl−channels and enhances GABA tone (Fig. 1) (11, 12). GABA, an inhibitory neurotransmitter, is known to inhibit the OT system (5, 9).

Although the ovarian steroid-induced increases in OT mRNA levels occur in both the SON and the PVN (7, 8), the increase is greater in the PVN (7, 8). Therefore, to maximize the ability to identify changes in OT mRNA, we have focused this and our prior studies on the PVN. Recently, we reported that the effects of P on PVN OT mRNA are mediated not only via the P receptor (PR), but also the GABA system (18). If diazepam, a GABA_A-receptor agonist is administered when P is withdrawn in the E2-primed rat, OT mRNA levels do not increase (18). Diazepam and neurosteroids are believed to bind to separate sites on the GABA_A receptor and both agents enhance GABA tone by acting as positive allosteric modulators (11, 12). Additionally, administration of RU-486, a PR antagonist, also increased the levels of OT mRNA (18). These observations suggest that the regulation of OT mRNA levels by P is complex and involves both PR- and GABA-mediated mechanisms.

The ovarian steroid regimen that increases OT mRNA in the PVN and SON also alters GABA_A-receptor binding in the SON, as measured by [3H]-muscimol binding (2). Other ovarian steroid regimens tested did not decrease GABA_A-receptor binding in the SON. Of the brain regions examined, including the supraoptic, ventromedial, and medial preoptic nuclei and cingulate cortex, binding was only decreased in the SON, a major site of synthesis of OT within the CNS. Decreases in GABA_A-receptor binding may in part mediate the steroid-induced changes observed in SON OT expression.

Additionally, a change in the concentration of the neurosteroid A may alter GABA tone. Because brain and plasma levels of A positively correlate with plasma
oxidoreductase, may proceed in either direction.

Steroid treatments for experiments 1A and 1B were as follows:

- **Group 1**: Insert one 2-mm E2 implant
- **Group 2**: Insert three 30-mm P implants
- **Group 3**: Insert one 2-mm E2 implant and three 30-mm P implants
- **Group 4**: Insert three 30-mm P implants
- **Group 5**: Insert placebo

On the basis of prior publications, we have shown that maintenance of P attenuates OT mRNA levels. In this experiment, we were testing whether A attenuates or abolishes the increase in OT mRNA levels (1, 3, 7, 8, 17–20). In this experiment, we present the completeness of ovariectomy, blood drawn by jugular venipuncture was assayed for E2. Only those animals with circulating E2 concentrations that were near or less than the lower limits of detectability of the assay (≤5 pg/ml) were used in the experiments. At the time of arrival, animals were 6–11 wk of age (225–275 g body wt) and were acclimated to their surroundings for 7 days before a study. Animals were housed singly in stainless steel cages in a temperature (22°C) and humidity (60%)-controlled room with automatic light-dark cycles (lights on at 0700 and off at 1900). Rats had free access to water and food (Prolab, Rat, Mouse, Hamster 300, PMI Feeds, St. Louis, MO).

**MATERIALS AND METHODS**

Animals. Rats were ovariectomized by the supplier (Harlan Sprague Dawley, Indianapolis, IN) 1 wk before shipment to our facility. To verify the completeness of ovariectomy, blood drawn by jugular venipuncture was assayed for E2. Only those animals with circulating E2 concentrations that were near or less than the lower limits of detectability of the assay (≤5 pg/ml) were used in the experiments. At the time of arrival, animals were 6–11 wk of age (225–275 g body wt) and were acclimated to their surroundings for 7 days before a study. Animals were housed singly in stainless steel cages in a temperature (22°C) and humidity (60%)-controlled room with automatic light-dark cycles (lights on at 0700 and off at 1900). Rats had free access to water and food (Prolab, Rat, Mouse, Hamster 300, PMI Feeds, St. Louis, MO).

**Experiment 1** - blood and brain concentrations of A in rats. In this experiment, the dose of A (5 mg·kg⁻¹·day⁻¹) chosen was twice that which had previously been reported to induce anxiolysis in the rat (2–2.5 mg·kg⁻¹·day⁻¹ sc) (10). Brain tissue was removed at death, the hypothalami were extracted, and tissue A concentrations were measured by RIA. Trunk blood obtained at death was measured for A, E2, and P concentrations. Ovariectomized animals (n = 6 per group) were randomized to five treatments (Table 1). The sham group received empty capsules, and the A-alone group received A capsules. The remaining groups received E2 and P implants. In the E2/P+ group, the P implants remained in place until death. In both the E2/P− and E2/P−/A groups, P implants were removed 48 h before death. The E2/P− group was included to verify that A concentrations declined following P removal, whereas the E2/P−/A was included to verify that A concentrations were sustained. The E2/P− group was included to verify that A concentrations in animals in which P is sustained overlap levels in the A-supplemented group. The data were used to determine the dose of A to be used in experiment 2. P+ means P capsules were not removed, whereas P− means P capsules were removed. A+ means A capsules were not removed, whereas A− means A capsules were removed.

**Experiment 2** - Ovulatory OT mRNA concentrations. On the basis of the pilot data in experiment 1, the 5-mg·kg⁻¹·day⁻¹ dose of A appeared to be sufficient to achieve A concentrations equivalent to those in animals receiving sustained P implants. Additional animals were studied in this experiment to determine the effect on OT mRNA concentrations. Trunk blood was collected at death, and serum E2 and P and plasma A concentrations were measured (n = 8 animals per group). The hypothalami (n = 8 per group) were processed for OT mRNA by in situ hybridization (n = 32–35 tissue sections per group). Sufficient brain sections for in situ hybridization were obtained from four of the eight animals in each group. E2, P, and A concentrations of the animals from which brain sections were analyzed followed the trend of the entire treatment group. The E2/P+ group, which was included in experiment 1, was not included in experiment 2 because we have shown in prior publications that maintenance of P attenuates OT mRNA levels (1, 3, 7, 8, 17–20). In this experiment, we were testing whether A attenuates or abolishes the increase in OT mRNA levels that is induced by P withdrawal in the E2-treated group.

**Preparation and administration of steroid hormone implants.** Silastic tubing (ID 0.078 in., OD 0.125 in., Dow Corning, Midland, MI) cut to preselected length was packed with crystalline E2 or P (Sigma Chemical, St. Louis, MO) and prepared as previously described (7, 8). The doses used in the study are outlined in Table 1 and were chosen to replicate the concentrations of E2 and P achieved in prior experiments of a similar nature in this laboratory. A was purchased from Steraloids (Wilton, NH) and made into constant release capsules by Innovative Research (Sarasota, FL) at a dosage of 5 mg·kg⁻¹·day⁻¹. Sham capsules were prepared in the same manner but sealed empty. The subcutaneous placement and removal of capsules was done while animals received methoxyflurane inhalation anesthesia.

**Table 1. Steroid treatments for experiments 1A and 1B**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>Insert one 2-mm sham implant</td>
<td>Insert three 30-mm sham implants</td>
<td>Remove three 30-mm sham implants, insert placebo</td>
</tr>
<tr>
<td>A</td>
<td>Insert one 2-mm sham implant</td>
<td>Insert three 30-mm sham implants</td>
<td>Remove three 30-mm sham implants, insert placebo</td>
</tr>
<tr>
<td>E2/P−</td>
<td>Insert one 2-mm E2 implant</td>
<td>Insert three 30-mm P implants</td>
<td>Remove three 30-mm P implants, insert placebo</td>
</tr>
<tr>
<td>E2/P+</td>
<td>Insert one 2-mm E2 implant</td>
<td>Insert three 30-mm P implants</td>
<td>Insert placebo</td>
</tr>
<tr>
<td>E2/P−/A</td>
<td>Insert one 2-mm E2 implant</td>
<td>Insert three 30-mm P implants</td>
<td>Remove three 30-mm P implants, insert A</td>
</tr>
</tbody>
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A, allopregnanolone; E2, estradiol; P, progesterone. Animals in each group were killed on day 16.
Assays. Tail or trunk blood was collected into glass test tubes for E2 and P assays. Blood was centrifuged at 3,000 rpm for 15 min, and the serum was separated from the red blood cells (RBC) and stored frozen at −70°C until assay. Serum E2 and P concentrations were measured in duplicate by double-antibody RIA, using kits purchased from Diagnostic Products (Los Angeles, CA) and Diagnostic Systems Laboratories, (Webster, TX), respectively. The minimum detectable concentration of E2 and P in its respective assay was 5 pg/ml and 1 ng/ml. There is a 5% cross-reactivity of DHP, which can be reversibly metabolized from A (see Fig. 1) in the P assay (personal communication, Diagnostic Systems Laboratories). Synthetic A, in concentrations of 1–50 ng/ml, had no cross-reactivity in the P assay.

Plasma and hypothalamic A concentrations were measured using published methodology (13, 14). For plasma A determinations, blood was collected at death into polypropylene tubes containing 100 µl of EDTA (50 mg/ml) and immediately placed on ice. Within 10 min, the samples were centrifuged at 3,000 rpm at 4°C for 15 min. The plasma was separated from the RBCs, transferred to a clean polypropylene tube, and stored at −70°C until assay. The RIA had a minimum detectable concentration of A of 25 pg/ml and an intra-assay coefficient of variation of 8.5%. The assay has −15% cross-reactivity with P (14). The A concentrations are corrected for this cross-reactivity.

For A determinations in tissue, brains were immediately placed in a 1:1:1 acetonitrile-buffered saline (from Fisher Scientific, Fair Lawn, NJ) and immediately frozen on liquid nitrogen. For all others, brains were immediately frozen on dry ice. A coronal cut was made anterior to the optic chiasm, and a second coronal cut was made 5 mm caudally, just anterior to the mammillary bodies. This thick slice of hypothalamus contains the entire paired PVN (7, 8). This coronal section was placed on an iced Petri dish, and the cortex was trimmed from the hypothalamus. The hypothalamus were placed in pre-cooled polypropylene tubes containing 50% aqueous methanol and 1% acetic acid and homogenized. The homogenates were centrifuged at 1,200 rpm for 10 min at 4°C. The supernatant was applied to a C-18 silica cartridge (SEP-PAK, Waters Associates, Milford, MA) that had been equilibrated with 50% aqueous methanol and 1% acetic acid. The cartridge was then sequentially washed with 10-ml volumes of 50% aqueous methanol and 1% acetic acid, deionized water, and 50% aqueous methanol. The steroid fraction was eluted with 10 ml of 100% methanol and dried under nitrogen in a Savant condenser (Savant Instruments, Farmingdale, NY). Dried samples were stored at −70°C until assay.

In situ hybridization histochemistry. Serial frozen coronal sections 20-µm thick were taken through the region of the hypothalamus extending from the rostral PVN through the caudal PVN. Tissue sections 100-µm apart were thaw-mounted on gel-alum-coated slides, dried, and stored at −70°C until hybridization. Thaw-mounted sections were fixed in 4% paraformaldehyde, rinsed with 0.1 M phosphate buffer, acetylated in 0.25% acetic anhydride-0.1 M triethanolamine HCl, pH 8.0, dehydrated through a series of ethanol washes, and dried in air. After fixation, acetylation, dehydration, and delipidation, brain sections were hybridized by application of 70 µl/slide of hybridization buffer containing 50% formamide, 300 mM NaCl, 80 mM Tris HCl, pH 8.0, 1 mM EDTA, 10% dextan sulfate, 100 mM dithiotreitol (DTT), 1× Denhardt’s solution, and 35S-UTP-labeled OT antisense or sense (control) riboprobes at 45°C for 16 h. Slides were washed twice in 4× standard sodium citrate (SSC) and 2 mM DTT for 15 min each, and for 30 min in each of the following: RNaseA (2.5 µU/ml) and RNase buffer (7 mM Tris, 350 mM NaCl, 700 µM EDTA) at 37°C, RNase buffer and 1 mM DTT; 2× SSC and 1 mM DTT at room temperature, and 0.1× SSC and 1 mM DTT at 60°C. The final wash was for 3 min in 0.1× SSC at room temperature. After slides were dehydrated by transfer through 50% ethanol in 300 mM NH4Ac, 85% ethanol in 300 mM NH4Ac, and 100% ethanol, autoradiography was performed by dipping hybridized slides into NTB2 liquid emulsion (Eastman Kodak, Rochester, NY) diluted 1:2 with 600 mM NH4Ac. The coronal sections were then counterstained with cresyl violet and coverslipped.

35S-labeled riboprobes were used for in situ hybridization. The pGEM 4-OT-3c probe was transcribed in a 10-µl volume using directions from the manufacturer (Promega) with 35S-α-thiol UTP (12.5 mCi/ml) and SP 6 polymerase to yield a 160-bp OT cRNA (7, 8). The specific activity of the labeled probe was 1× 106 dpm/µg.

OT mRNA densitometric analyses focused on the PVN. Sequential slides containing sections with the peak number of labeled neurons in the PVN were counted. Both right and left PVN sections from each animal were included in the analysis. Although eight animals were included in each treatment group, only those sections from animals that were anatomically matched and were free of tears in the tissue were used in the final analysis. Sections from experimental and control animals were matched for rostral caudal level, and the sections analyzed corresponded to coronal plates 25–27 in the atlas of Swanson (16). With the use of these rigid inclusion criteria, brain sections from four of the eight animals in each treatment group were analyzed for PVN OT mRNA for a total of 32, 33, 32, and 35 sections in treatment groups sham, A alone, E2/P−, and E2/P−/A, respectively (Table 1). Data in a group were analyzed without knowledge of the experimental treatment. OT hybridization, exposure of autoradiographs to emulsion, and densitometric analysis were performed simultaneously. With the use of a calibrated microscope stage, fields from the appropriate sections of PVN were examined for OT mRNA using dark-field microscopy. Clusters of silver grains were imaged for integrated optical density measurement of PVN OT mRNA levels because the densely packed neurons in the PVN cannot always be resolved. Image fields were collected directly at ×10 using a high-sensitivity integrating three-chip Sony Camera (700 × 600 pixels), Coreco frame grabber board, and Optimus software program. Quantification of the number and size of silver clusters were done using the National Institutes of Health Image 1.58 software package for the Macintosh computer.

Statistical analysis. Results are expressed as means ± SE. Differences in serum E2 and P concentrations and plasma and hypothalamic A concentrations were determined by one-way ANOVA. When ANOVA indicated significant differences among groups within an experiment, pairwise comparisons between groups were made by post hoc Fisher’s protected least significant difference (PLSD) tests. For PVN OT mRNA data, a nonparametric test (Kruskal-Wallis) was used because of the variation in number of sections analyzed in each treatment group, and pairwise comparisons between groups were determined with the Mann-Whitney U test. Significance of correlation was determined with Fisher’s r to z transformation. All analyses were done on a Macintosh Power Computer with the Statview Statistics Package (Abacus Concepts, Berkley, CA). A confidence level of P < 0.05 was considered significant.

RESULTS

Experiment 1. The ovarian steroid treatments for experiment 1 are illustrated in Table 1. Serum E2 concentrations after ovariectomy, but before placement of E2-filled implants, were 7 ± 2 pg/ml, which is near
the lower limit of detectability of the E\textsubscript{2} assay (5 pg/ml). Serum P concentrations were 45 ± 4 ng/ml while P-filled implants were in place. After removal of P-filled implants, P concentrations declined to 9 ± 2 and 21 ± 3 ng/ml in the E\textsubscript{2}/P\textsubscript{−} and E\textsubscript{2}/P\textsubscript{−}/A groups, respectively. A concentrations in plasma and hypothalamus were higher in animals receiving A or P at the time of death (28–37 ng/ml and 4.9–7.9 ng/mg protein, respectively) than in animals receiving empty (sham) capsules or animals in which P capsules were removed before death (5–12 ng/ml and 1.1–1.8 ng/mg protein, respectively; ANOVA P < 0.0006; Fisher’s PLSD; Fig. 3). Plasma and hypothalamic A concentrations were positively correlated (r = 0.72, P = 0.0006) as were serum P and plasma A concentrations (r = 0.94, P < 0.0001; Fig. 3).

Experiment 2. In this experiment, brains were removed from additional animals and the abundance of OT mRNA was measured in the PVN by in situ hybridization histochemistry. Differences in PVN OT mRNA abundance were found among groups, Kruskal-Wallis (P < 0.0001). Pairwise differences, Mann-Whitney U test, are shown in Fig. 4. Compared with ovarioctomized rats receiving empty implants (sham), OT mRNA levels increased in both of the groups that received ovarian steroid-filled implants followed by removal of P-filled implants. However, the increase was attenuated (51% increase) in the rats receiving A at the time of P withdrawal (E\textsubscript{2}/P\textsubscript{−}/A group), whereas rats not receiving A at the time of P withdrawal (E\textsubscript{2}/P\textsubscript{−} group) had an increase of 87% relative to sham-treated controls. Compared with ovarioctomized rats receiving empty implants, A alone was without effect on OT mRNA levels. Representative photomicrographs of the PVN from animals treated with sham, E\textsubscript{2}/P\textsubscript{−}, and E\textsubscript{2}/P\textsubscript{−}/A regimens are shown in Fig. 5, A and B. Increased grain density was identified in regions of the PVN that are known to contain both magnocellular as well as parvocellular OT-expressing neurons.

To be certain that there was release of the steroid from the implants, assays for E\textsubscript{2}, P, and A were performed on trunk blood collected at death from animals in this experiment. At death, plasma A concentrations were higher in animals receiving A- or P-filled implants (19–20 ± 3 ng/ml) than in animals receiving empty (sham) capsules or animals in which P capsules were removed before death (8–12 ± 1 ng/ml; ANOVA P = 0.0005). Pairwise differences were found among the following: A alone vs. sham (P = 0.0066; A alone vs. E\textsubscript{2}/P\textsubscript{−} (P = 0.0003); E\textsubscript{2}/P\textsubscript{−}/A vs. sham (P = 0.01); and E\textsubscript{2}/P\textsubscript{−}/A vs. E\textsubscript{2}/P\textsubscript{−} (P = 0.0006, Fisher’s PLSD; Fig 3).

Mean (±SE) serum E\textsubscript{2} concentrations after ovarioctomy but before placement of E\textsubscript{2}-filled implants ranged from 4 to 6 ± 0.5 pg/ml, which is at or near the minimum detectable concentration of the E\textsubscript{2} assay (5

![Graph](http://ajpregu.physiology.org/ by 10.220.33.33 on October 14, 2017)

Fig. 2. Mean (±SE) concentrations of serum P (A), plasma A (B), and hypothalamic A (C) at death in experiment 1. Animals treated with P at time of death (E\textsubscript{2}/P (P+)) had higher P concentrations than each of the other treatment groups [P < 0.0001, Fisher’s protected least-squares difference (PLSD)]. Plasma and hypothalamic A levels were higher in animals receiving A or P at time of death than in animals receiving empty (sham) capsules or animals in which P capsules were removed before death (ANOVA P < 0.0001). In particular, A levels achieved by treatment with A capsules were equal to or higher than A levels achieved in animals treated with P capsules only. Pairwise comparison with Fisher’s PLSD showed the following significant differences between treatment groups for plasma and hypothalamic A concentrations, respectively: sham and A alone, P < 0.0001 and P < 0.0001; sham and E\textsubscript{2}/P\textsubscript{+}, P = 0.002 and P = 0.0002; sham and E\textsubscript{2}/P\textsubscript{−}/A, P < 0.0001 and P = 0.001; A alone and E\textsubscript{2}/P\textsubscript{+}, P = 0.04 and P = 0.008; A alone and E\textsubscript{2}/P\textsubscript{−}, P < 0.0001 and P < 0.0001; E\textsubscript{2}/P\textsubscript{+} and E\textsubscript{2}/P\textsubscript{−}, P < 0.0001 and P < 0.0001; E\textsubscript{2}/P\textsubscript{−} and E\textsubscript{2}/P\textsubscript{−}/A, P < 0.0001 and P < 0.0001. In hypothalamus only, A alone was significantly higher than E\textsubscript{2}/P\textsubscript{−}/A, P = 0.001.
modulation of OT expression in the E2-primed rat. Ovariectomized steroid-treated rat to study the role of P and mean (± SE) serum P concentrations declined to comparable levels in treatment groups receiving P-filled capsules. Serum P concentrations are < 25 ng/ml during the rat estrous cycle, except during proestrus when P concentrations peak briefly (~12 h) at 60 ng/ml.

The slightly higher concentrations of P in the E2/P−/A vs. E2/P− groups in experiment 1 are most likely due to the differences in the rate of decline in P between the two groups. We assayed synthetic A (purchased from Steraloids) in the P assay and found no cross-reactivity. Figure 1 also illustrates that A is not detected in the P assay because animals supplemented with A capsules (A alone) did not have significantly different levels of P than animals bearing empty capsules (sham). If A were detected in the P assay, then P levels should also be higher in the A alone vs. sham group. Thus A cross-reactivity is not the cause for the discrepancy in P levels between E2/P−/A− and E2/P− groups, and A is not detected in the P assay. The minor cross-reactivity of DHP in the P assay (~5%) is not likely the reason that levels of P are higher in animals treated with A. If this were so, then higher P concentrations should be found in every experiment in which animals are treated with A. This was not the case. For example, the P concentrations in animals treated only with A in experiment 1 were not different than sham animals. Moreover, there were no significant differences between P levels in the E2/P−/A− vs. E2/P− in experiment 2.

Declining P concentrations in the E2-primed rat increase hypothalamic OT expression, whereas maintenance of P attenuates this response. A concentrations are positively correlated with plasma P concentrations (6). When P implants are in place, A concentrations are higher than when P implants are removed. We determined the effect on OT mRNA levels of sustaining A at the time of P withdrawal. Animals treated with A (E2/P−/A) at the time of P withdrawal had an attenuated OT response relative to cohorts that did not receive A (E2/P−). Although decreased, OT mRNA
remained elevated above levels in both sham-treated animals and animals receiving only A.

One possible cause for the incomplete attenuation of OT may be insufficient administration of A, but this appears to be unlikely. We chose a dose of A (5 mg·kg$^{-1}·$day$^{-1}$) that is twofold greater than the dose that effectively induces anxiolysis (2–2.5 mg/kg) (10). A-induced anxiolysis is believed to be mediated by the GABA$_A$ receptor within the CNS. Administration was via constant-release capsules, which result in steady-state levels of the steroid. We measured A concentrations in plasma and hypothalamic extracts. The methods of collection and extraction used in this RIA have been previously tested and found to minimize oxidation and adherence to glass and plastic surfaces (14). The antiserum used for RIA has been extensively tested for specificity to A (14), and the minor cross-reactivity with P was factored into the assay result. The goal was to achieve plasma A concentrations in the treated animals that were equivalent to the levels in P-treated animals. Animals treated with A constant-release implants achieved plasma and hypothalamic concentrations of A (35–38 ng/ml and 4.9–7.9 ng/mg of protein, respectively) that were equal to or greater than animals in which P was maintained (28 ng/ml and 5.5 ng/mg protein, respectively). The plasma concentration of A in these animals was also nearly fourfold greater than previously reported levels in day 19 pregnant rats (9.8 ± 1.6 ng/ml) (6) a condition in which P levels are increased. The concentrations of A in plasma and hypothalamus were positively correlated, and both correlated with the serum P concentrations. Thus the dose of A appears to be sufficient to achieve concentrations equal to those of a high-P environment.

OT neurons in the PVN and SON are known to express $\alpha_1$- and $\alpha_2$-subunits, $\beta_2$- and $\beta_3$-subunits, and $\gamma_2$-subunit mRNAs of the GABA$_A$-receptor complex (5, 9). Plasticity in the GABA$_A$-subunit composition within hypothalamic OT neurons has been reported in the pregnant and lactating rat (5) but not yet studied in the steroid-treated rat. In the pregnant rat, $\alpha_1$-subunit expression and plasma P and A concentrations rise from days 1 to 19 of pregnancy and then decline (5). As $\alpha_1$-subunit expression declines, $\alpha_2$-subunit expression increases (5). The ratio of $\alpha_1$ to $\alpha_2$ subunits is positively correlated with the sensitivity of the GABA$_A$ receptor to allosteric modulation by A (5) and inversely with the activity of OT neurons. A relatively high level of A at a time when the GABA$_A$ receptor is most sensitive to its allosteric modulation is likely to increase GABA-mediated inhibition of OT neurons. Both GABA$_A$-subunit expression and A influence OT expression. We have recently identified that the steroid regimen that increases OT mRNA and peptide also alters GABA$_A$-receptor binding.

![Image](http://ajpregu.physiology.org/)

**Fig. 4.** Mean (±SE) relative abundance of paraventricular nucleus (PVN) oxytocin (OT) mRNA (A) and plasma A concentrations (B) in experiment 2. A levels in the bars indicating $n = 4$ were measured in rats in which brains were collected for OT mRNA hybridization. Mean relative abundance of PVN OT mRNA for each experimental group is expressed as a percentage of sham-treated group. Significant differences were found among groups, $P < 0.0001$, Kruskal-Wallis. Rats receiving E$_2$/P$^-$ and E$_2$/P$^-$/A treatment regimens, compared with sham-treated animals, had increases in PVN OT mRNA of 87% and 51%, respectively. OT mRNA levels in E$_2$/P$^-$/A group were significantly lower than in E$_2$/P$^-$ group, $P = 0.04$, Mann-Whitney U test. Pairwise differences in OT mRNA were also found between following: sham vs. E$_2$/P$^-$, $P < 0.0001$; sham vs. E$_2$/P$^-$/A, $P = 0.04$; A alone vs. E$_2$/P$^-$, $P < 0.0001$; A alone and E$_2$/P$^-$/A, $P = 0.001$, Mann-Whitney U test. A alone did not increase or decrease PVN OT mRNA compared with sham-treated animals. Plasma A was higher in animals receiving A or progesterone than in animals receiving empty (sham) capsules or animals in which P capsules were removed. (19–20 ± 3 ng/ml) than in animals receiving empty (sham) capsules or animals in which P capsules were removed (8–12 ± 1 ng/ml), ANOVA $P = 0.0005$. Pairwise differences in plasma A were found between the following: A alone vs. sham P = 0.006; A alone vs. E$_2$/P$^-$, P = 0.0003; E$_2$/P$^-$/A vs. sham, P = 0.01; E$_2$/P$^-$/A vs. E$_2$/P$^-$, P = 0.0006, Fisher's PLSD.
in the SON of the steroid-treated rat (2). In future studies, we will measure GABA<sub>A</sub> subunits in animals receiving a variety of steroid paradigms.

Perspectives

In the rat, rising E<sub>2</sub> and declining P levels enhance OT mRNA and peptide levels in the PVN and SON (7, 8). This same ovarian steroid exposure also optimally stimulates maternal behavior (4), which typically has its onset immediately prepartum in the rat, when hypothalamic OT expression is at a maximum. P withdrawal without E<sub>2</sub> priming, or E<sub>2</sub> alone without P withdrawal, will not increase OT mRNA in the PVN and SON (8) and are suboptimal steroid paradigms for inducing maternal behavior (4). The data suggest that ovarian steroid-induced increases in PVN OT mRNA may be important for the events of late pregnancy. The ability of OT neurons in the PVN and SON to change from a quiescent pattern to one of increased synthesis during late pregnancy represents an example of functional plasticity within the CNS. Coincident with the changes in OT are cyclical changes in E<sub>2</sub> and P that appear to regulate OT expression. A is known to increase GABA<sub>A</sub>-receptor currents in OT neurons of late-pregnant rats (5, 9), when high levels of A are present in the brain (6). Perhaps the increase in GABA-mediated mechanisms exerts an inhibitory influence on OT neurons. The decline in A concentrations coincident with the prepartum decline in P in the rat may disinhibit OT neurons and facilitate an increase in OT synthesis.

In summary, we conclude that A plays a modulatory role in the expression of hypothalamic OT in the virgin, ovariectomized steroid-treated rat. Sustained A in the absence of P partially attenuates steroid-induced increases in OT. Mechanistically, these effects are believed to be a result of the action of A as a positive allosteric modulator of the GABA<sub>A</sub> receptor. Conditions favoring high A levels result in increased GABA tone and OT inhibition, whereas conditions favoring low A levels result in decreased GABA tone and enhanced OT expression. These data provide evidence for an interaction between a peptide hormone and a major CNS neurotransmitter system. Understanding this system in the rat may provide insight into the neuroendocrine regulation of similar systems in the human.

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