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# Vasopressin, Oxytocin, Corticotrophin-Releasing Factor, and Sodium Responses During Fluoxetine Administration in the Rat

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**Hyponatremia has been observed in elderly patients treated with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. The pathogenesis of this effect is not known, but enhanced release of vasopressin (VP) and its renal actions may be a possible mechanism. Excess secretion of VP in combination with large fluid intake is known to induce hyponatremia. We determined if chronic fluoxetine administration in association with liberal fluid intake will induce hyponatremia via enhanced release of VP. We used a previously described model in which fluid intake is forced by administering rats a nutritionally balanced liquid diet. Male Sprague-Dawley rats in groups of 10 were randomized to solid and liquid diets, and each diet group administered daily ip injections of fluoxetine (10 mg/kg) or saline for 10 d. Water was given ad libitum to all groups. Daily weight, fluid and food intake, and urine output were measured. On d 10, rats were killed by rapid guillotine decapitation 1–3 h after injection. Trunk blood was collected for measurements of plasma VP and oxytocin (OT) and serum sodium (Na), BUN, creatinine, and glucose. Pituitary glands were assayed for VP and OT content. VP mRNA in the paraventricular and supraoptic nuclei (PVN and SON) and corticotrophin-releasing factor (CRF) mRNA in the PVN were measured by *in situ* hybridization histochemistry. Fluid intake was significantly higher in groups maintained on liquid vs solid diet ($p < 0.0001$), as was urine output ($p < 0.0001$). Fluoxetine-treated rats gained significantly less weight than placebo-treated rats ($p = 0.01$), in keeping with fluoxetine's anorexigenic properties. However, no significant differences were found among the groups in Na, plasma VP or OT, pituitary VP or OT, or PVN CRF or VP mRNA levels. We conclude that administration of fluoxetine to laboratory rats in the dose and duration used in this study does not significantly affect hypothalamic expression, pituitary stores, or peripheral secretion of VP.**

**Key Words:** Corticotrophin-releasing factor; fluoxetine; hyponatremia; neurohypophysis; oxytocin; posterior pituitary; serotonin reuptake inhibitor; sodium; vasopressin.

## Introduction

Hyponatremia has been observed in elderly patients treated with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine *(1–20)*. In those cases, the hyponatremia developed several days or weeks after starting the SSRI, and resolved with its discontinuation *(1–18)*. Most patients had no medical illnesses other than the psychiatric problems, and were not receiving additional medications that have been linked to the development of hyponatremia *(1–18)*.

The pathogenesis of the hyponatremia associated with SSRIs is not understood. Enhanced release of vasopressin, VP, and its renal actions of increasing free water absorption may be a possible mechanism. VP is synthesized primarily in the bilateral hypothalamic supraoptic and paraventricular nuclei (SON and PVN) and carried via axons to its storage site in the posterior pituitary gland *(21)*. VP is secreted into the peripheral circulation in response to rising plasma osmolality or decreased intravascular volume *(21)*. In addition to these physiological stimuli, certain medications can enhance the release of VP from the pituitary gland or its effects on the kidney *(21,22)*. In the rat, a single ip injection of fluoxetine (10 mg/kg) was reported to increase peripheral plasma VP levels above baseline for the 80-min sampling period after the injection *(23)*. VP and corticotrophin-releasing factor (CRF) concentrations were also increased in the hypophysial portal blood of these animals *(23)*. The

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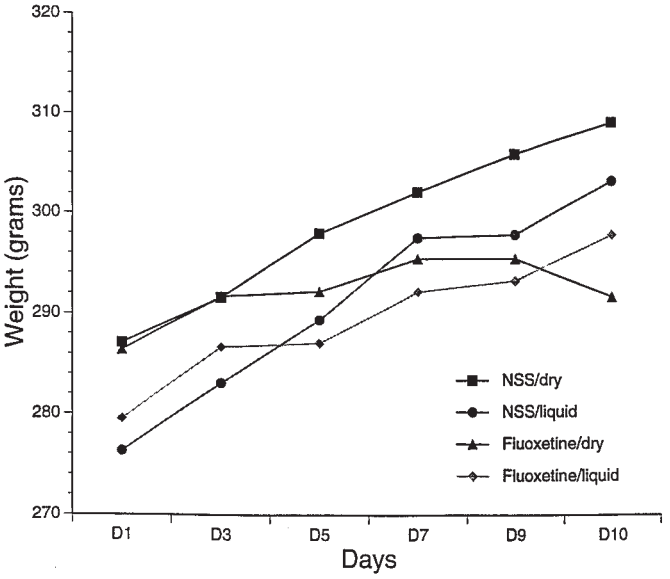


**Fig. 1.** Body weights of the animals in the four treatment groups during the 10-d study period. Although all animals gained weight during the study, animals receiving fluoxetine injections gained weight at a significantly lower rate than animals receiving placebo (saline) injections (ANOVA, $F = 3.9, p = 0.01$). Significant pairwise differences (Fisher's PLSD) were found among fluoxetine/dry vs saline/dry ($p = 0.005$), fluoxetine/dry vs saline/liquid ($p = 0.01$), and fluoxetine/liquid vs saline/dry ($p = 0.04$). Data represent the mean ± SEM of 10 animals/group.

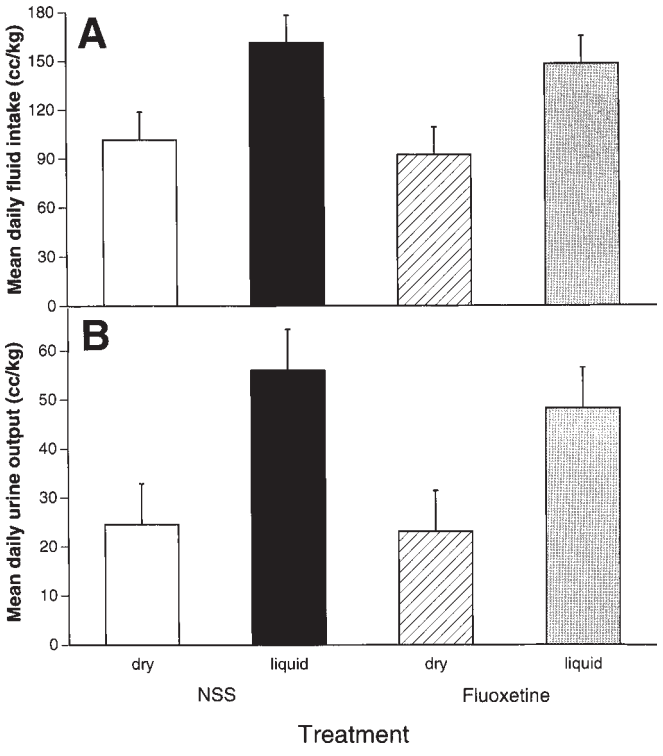


**Fig. 2.** Daily fluid intake (**A**) and urine output (**B**) among treatment groups. Animals receiving liquid diet had significantly higher fluid intake (ANOVA, $F = 81.4$, $p < 0.0001$) and urine output (ANOVA, $F = 14.6$, $p < 0.0001$) than matched cohorts receiving dry diet. For fluid intake, significant pairwise differences were noted between fluoxetine/liquid vs fluoxetine/dry ($p < 0.0001$) or saline/dry ($p < 0.0001$) and saline liquid vs saline/dry ($p < 0.0001$) or fluoxetine/dry ($p < 0.0001$) treated animals (Fisher's PLSD). For urine output, the following significant pairwise differences were observed: fluoxetine/liquid vs fluoxetine/dry ($p = 0.0002$) or saline/dry ($p = 0.004$) and saline/liquid vs saline/dry ($p < 0.0001$) or fluoxetine/dry ($p < 0.0001$) treated animals (Fisher's PLSD). No significant differences were found in either fluid intake or urine output between the two groups receiving the dry diet or the two groups receiving the liquid diet. Data represent the mean ± SEM of 10 animals in each group.

effects of chronic administration of fluoxetine on VP secretion have not yet been studied.

Enhanced VP secretion when combined with liberal fluid intake may result in hyponatremia *(21)*. The laboratory rat can be made to liberalize daily fluid intake by maintenance of a nutritionally balanced liquid diet in lieu of solid food *(24)*. Animals maintained on liquid diet have a higher daily fluid intake than cohorts maintained on rat chow, but do not develop hyponatremia unless an antidiuretic agent, either VP or an analog, such as desmopressin, DDAVP, is introduced *(24)*. Normonatremia is also maintained in animals receiving DDAVP and solid diet *(24)*. These observations illustrate that both excess VP and liberal fluid intake must be present for hyponatremia to develop.

We hypothesized that if chronically administered fluoxetine were to enhance release of VP, hyponatremia may develop during forced, but not standard, fluid intake. We thus examined the effect of chronic fluoxetine administration on serum sodium and osmolality, pituitary and plasma VP immunoreactive peptide, and PVN and SON VP messenger ribonucleic acid (mRNA) levels in rats maintained on standard vs forced fluid regimens. Since oxytoxin (OT) also plays a role in sodium and water metabolism in the laboratory rat *(25)*, we measured plasma and pituitary OT-immunoreactive peptide. CRF mRNA was also measured in the PVN of these animals because of the previous report that fluoxetine increased portal blood levels of CRF peptide *(23)*.

## Results

All groups gained weight during the study, but the rate of weight gain in the fluoxetine-injected animals was lower (ANOVA, $F = 3.9$, $p = 0.01$) than in saline-injected animals. The fluoxetine-injected animals maintained on a dry diet lost weight during the last few days of the study (Fig. 1), but the weight at sacrifice was greater than at the initiation of the study.

Mean daily fluid intake (Fig. 2A) and urine output (Fig. 2B) were significantly higher in groups receiving liquid vs dry diet (ANOVA, $F = 81.4$, $p < 0.0001$ and $F = 14.6$, $p < 0.0001$, respectively). These differences indicate a better hydration status in the groups receiving liquid diet.

There was a trend toward lower serum osmolality in the animals receiving the liquid vs dry diet (Table 1). In the fluoxetine-treated groups, serum osmolality was signifi-

**Table 1**
Serum Parameters in Rats

| Treatment group | Osmolality (mOsm/kg H2O) | BUN (mg/dl) | Creatinine (mg/dl) | Sodium (mEq/l) | Glucose (mg/dl) |
|---|---|---|---|---|---|
| NSS: dry | 293 ± 2 | 20 ± 1 | 0.62 ± 0.02 | 140 ± 1 | 128 ±4 |
| liquid | 293 ± 2 | 10 ± 1 | 0.58 ± 0.02 | 139 ± 1 | 124 ± 5 |
| Fluoxetine: dry | 298 ± 3 | 22 ± 2 | 0.64 ± 0.03 | 140 ± 1 | 130 ± 5 |
| liquid | 289 ± 1 | 11 ± 1 | 0.54 ± 0.01 | 139 ± 1 | 126 ± 4 |
| ANOVA F/P | 3.3 / 0.02 | 18.2 / 0.0001 | 3.3 / 0.02 | 0.6 / 0.56 | 0. 3/ 0.83 |

cantly lower in animals receiving liquid diet vs dry diet ($p < 0.003$, Fisher's protected least-significant difference [PLSD]). Since sodium, blood urea nitrogen (BUN), and glucose contribute to serum osmolality, we measured each of these parameters (Table 1). There were no significant differences in sodium or glucose among the groups, but BUN was significantly lower in liquid diet groups compared to matched cohorts receiving dry diet (ANOVA, $F = 18.2$, $p < 0.0001$). The levels of serum creatinine were also significantly lower in the liquid diet groups (Table 1). The group receiving fluoxetine injections and liquid diet had lower creatinine than fluoxetine-injected ($p < 0.006$) or saline-injected ($p = 0.027$) groups maintained on dry diet (Fisher's PLSD).

Serum levels of fluoxetine in animals receiving this agent ranged between 109 and 499 ng/mL and the levels of the active metabolite, desmethylfluoxetine, were 312–787 ng/mL, both of which are within or above normal therapeutic levels in humans.

Neither pituitary nor plasma VP peptide differed significantly among the groups (data not shown). Although there was a trend toward higher pituitary VP content in the groups maintained on liquid diet, this did not achieve significance (ANOVA, $F = 2.26$, $p = 0.1$). Similarly, plasma and pituitary OT peptide levels were not significantly different among the groups (data not shown).

We examined PVN and SON VP mRNA in both of the groups receiving dry diet. Since these nuclei are the major sites of synthesis of VP mRNA in the mammalian brain, a direct effect of fluoxetine on VP expression may be manifested as a change in VP mRNA levels within these nuclei. The results of *in situ* hybridization histochemistry of VP mRNA in the PVN and SON are shown in Fig. 3. No significant differences in VP mRNA were found between fluoxetine- and saline-treated animals. Sections through the PVN of these same animals were also hybridized to a $^{32}$P-labeled probe to rat CRF. No significant differences were noted in CRF mRNA between the fluoxetine- and saline-treated animals (data not shown).

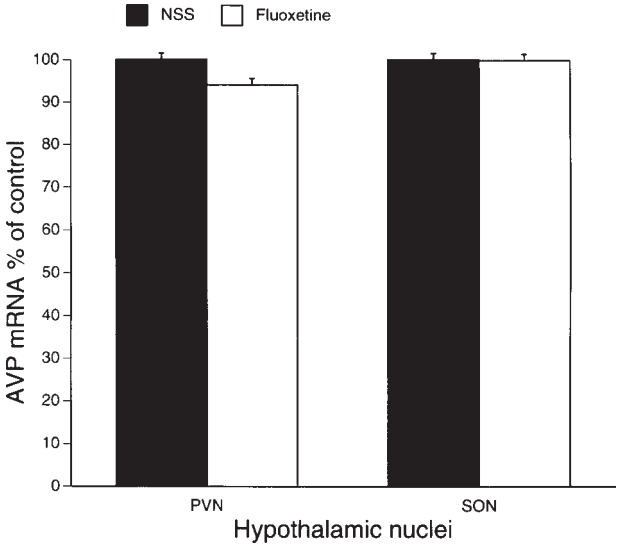


**Fig. 3.** Semiquantitative analysis of *in situ* hybridization of VP mRNA in the PVN and SON of fluoxetine- and saline-injected animals receiving dry diet. Data are presented as a percent of the saline-injected animals and represent the mean ± SEM of 4–6 readings/animal in the PVN and 8–12 readings/animal in the SON. Four animals were studied in each group.

## Discussion

Hyponatremia is a common problem among the elderly, and most cases of hyponatremia associated with the use of SSRIs have been in aged patients *(1–17,19)*. Approximately 30 cases have been reported in the medical literature of

hyponatremia associated with the use of SSRIs *(1–20)*. In most instances, no identifying factor other than the SSRI or the underlying psychiatric problem was noted *(1–18)*. Although most of these cases appeared to meet the criteria for syndrome of inappropriate antidiuresis *(1–20)*, in the majority of instances, VP levels were not measured to confirm the clinical impression. Thus, whether VP was causative in many of these cases remains uncertain. Since fluoxetine has been reported to induce acute release of VP in the laboratory rat *(23)*, we questioned whether enhanced VP release was a contributory factor. We utilized a previously described model of forced hydration in the rat *(24)*, because enhanced secretion of VP is not sufficient to cause hyponatremia without concurrent excesses of hypotonic body fluids *(24)*. In our study, the animals receiving the liquid diet had significantly greater fluid intake and urine output as well as lower serum creatinine and BUN, attesting to the effectiveness of the hydration paradigm. Despite the enhanced fluid intake, hyponatremia did not develop, and the VP system of the rat appeared to be unaffected by chronic fluoxetine administration. Levels of plasma and pituitary VP peptide and PVN and SON VP mRNA were not significantly different between fluoxetine- and placebo-treated animals. In the laboratory rat, administration of fluoxetine for 10 d does not alter the hypothalamic expression, the pituitary storage, or the peripheral release of VP.

Others reported enhanced VP secretion in the hypophysial portal and peripheral circulations of rats administered a single dose, 10 mg/kg body wt, of fluoxetine *(23)*. The increase in circulating levels of VP was sustained for the 80-min sampling time in that study. The results suggested that fluoxetine may affect the VP axis at both the hypothalamic and pituitary levels. In the present study, we used the same dose of fluoxetine ip daily for 10 d to determine if the acute effect on VP is sustained, and whether it can lead to hyponatremia with adequate fluid intake. Chronic fluoxetine injections had no effect on VP mRNA or peripheral VP release in our study. The reasons for these discordant findings are not yet understood, since the doses administered in both studies were comparable. The levels of fluoxetine and its metabolites achieved during our study were in the range considered to be therapeutic or higher for humans treated with this agent *(26)*. Possibly there are differences between the acute and chronic effects of fluoxetine on the VP system, but how this occurs is not understood.

In the laboratory rat, but not in humans, OT has also been found to affect sodium and water balance. Reports have shown that stimuli known to increase VP release, such as hyperosmolality *(27)*, hemorrage *(28)*, or induction of hypovolemia via administration of polyethylene glycol (PEG) *(25)*, also increase OT secretion in the rat. Hypoosmolality, which inhibits VP release, also diminishes OT secretion *(25)*. To determine whether fluoxetine altered the OT system, we compared plasma and pituitary OT peptide levels in rats administered fluoxetine vs placebo. No significant differences were found in OT between these two treatments in either solid or liquid diet groups.

Because a prior study had reported an acute increase in hypophysial portal blood concentrations of CRF *(23)*, we measured CRF mRNA in the PVN of animals treated with fluoxetine and placebo injections. The PVN is the major site of synthesis of CRF that reaches the portal circulation *(29)*. Because CRF is an anorexigen in rats *(30,31)*, an increase in CRF concentrations may be one way by which fluoxetine diminishes food intake. In contrast to the report of an increase in CRF peptide with acute fluoxetine administration, no significant increase in CRF mRNA was found after 10 d of fluoxetine administration.

Hyponatremia associated with SSRIs has been most often reported in elderly and hospitalized patients *(1–20)*. Many elderly or hospitalized patients are catabolic secondary to illnesses or reduced caloric intake. Fluoxetine diminishes food intake *(32)*. In our study, the fluoxetine-treated animals gained less weight than their placebo-treated cohorts. Marked caloric restriction can cause weight loss owing to excretion of water generated from tissue catabolism. Sodium loss also occurs *(33)*. Despite the sodium loss, hyponatremia typically does not occur in calorically restricted individuals unless VP levels are increased *(33)*. Since fluoxetine does not appear to increase VP secretion, it is unlikely that caloric restriction is the etiological factor for the hyponatremia associated with SSRIs.

Certain psychiatric conditions have been associated with the development of hyponatremia *(34)*. In some cases of hyponatremia, no abnormality other than the psychiatric condition could be identified *(34)*. The possibility exists that the psychiatric illness itself may be the etiological cause of the hyponatremia in patients treated with SSRIs, but the mechanism by which this occurs is poorly understood. If VP were to be increased by psychiatric stress, an alteration in VP may be the result of the psychiatric disease rather than the SSRI. Recently, VP release has been reported in response to recombinant IL-6, which is known to be released with stress *(35)*. Whether cytokines are released by psychiatric illness, alter VP, or induce hyponatremia in psychiatric illness remains to be determined.

We conclude that administration of fluoxetine to laboratory rats in the dose and duration used in this study does not significantly affect hypothalamic VP or CRF mRNA levels, or the pituitary or plasma levels of VP or OT peptides. Although we do not yet know whether fluoxetine will alter VP secretion in elderly individuals, the findings in this study suggest that enhanced release of VP secondary to a direct effect of fluoxetine on the VP system seems unlikely.

## Materials and Methods

### *Animals*

Male Sprague-Dawley rats (weighing 250–300 g) were obtained from Harlan-Sprague-Dawley (Indianapolis, IN)

and were housed individually in stainless-steel cages in a temperature (22°C), humidity (67%), and light (lights on, 0700–1900 h) controlled room. Animals were acclimated to their surroundings for 4 d prior to the experimental period. Rats were randomly assigned in groups of 10 to one of the following 10-d treatment regimens: group 1—solid chow (dry diet) + daily injection of 0.9% sodium chloride (normal saline solution, NSS) as placebo; group 2—liquid diet + daily injection of 0.9% NaCl as placebo; group 3—solid chow (dry diet) + daily fluoxetine injection; group 4—liquid diet + daily fluoxetine injection.

Fluoxetine was kindly provided by Eli Lilly and Company (Indianapolis, IN) and was dissolved in 0.9% NaCl solution. All groups had free access to water before and throughout the study. Daily fluid intake was measured for each animal. Animals receiving liquid diet were acclimated to this feeding for 2 d prior to the start of the protocol. Animals were weighed every other day and injected daily (10 mg/kg ip), including the date of sacrifice, between 0800 and 1000 h. At the end of the 10 d, animals were killed by rapid guillotine decapitation between 1100 and 1300 h. Trunk blood was collected into heparinized tubes for determination of plasma VP and OT, and into nonheparinized tubes for determination of serum sodium, osmolality, creatinine, and glucose. The entire pituitary gland was removed and placed in ice cold 0.1 *N* HCl acid and frozen at –20°C for determination of VP and OT-immunoreactive peptide content. Whole brains were rapidly removed from animals and immediately placed on dry ice and stored at –80°C until sectioning with a cryostat. Urine was collected for volume on the last 2 d of the protocol.

### *Radioimmunoassays of VP and OT in Plasma*

A starting volume of 500 µL of plasma was extracted for each peptide using the acetone- ether method previously described from this laboratory *(36,37)*. The final extraction step was performed in glass tubes. After the ether phase was removed, the remaining extract was air-dried. The extracts were frozen at –20°C and assayed within 1 wk. The extract was reconstituted in 250 µL assay buffer and duplicate 50-µL aliquots, and single 10-µL aliquots of extracts were measured for OT and VP in their respective assays. The antisera for OT and VP were generated in rabbits and their characteristics previously published *(36,37)*. The final dilution of VP antiserum was 1:700,000 and of OT antiserum 1:125,000. Assay buffer was 0.1*M* phosphate-buffered saline with 1 mg/mL bovine serum albumin (BSA) and 1 mg/mL sodium azide. Samples were incubated for 24 h at 4°C with 50 µL of the antiserum dilution and 2500 cpm/min (50 µL) of either 125 I OT or 125 I VP were added followed by additional incubation for 5 d. Antibody-bound VP and OT were separated using γ-globulin and 25% PEG. The sediments in each tube were counted. The assays for OT and VP had minimum sensitivities of 1 and 10 pg/tube, respectively. All samples were assayed simultaneously.

### *Pituitary VP and OT Contents*

The pituitary glands from each animal were individually homogenized in 1 mL of 0.1 *N* HCl. The homogenates were diluted 1:10,000 in assay buffer, and 50-µL aliquots of each sample were measured in duplicate in the assays for OT and AVP using the same methods described above for plasma, except the final dilution of the VP antiserum was 1:400,000.

### *Serum Sodium, Osmolality, Creatinine, Glucose, Urea, Nitrogen, and Fluoxetine Levels*

Serum parameters were measured by the Clinical Chemistry Laboratory, University of Pittsburgh Medical Center, as outlined below. Serum sodium and creatinine were measured by direct potentiometry and two-point rate test, respectively, using Ekta Chem Clinical Chemistry slides (Johnson & Johnson Clinical Diagnostics, Rariton, NJ). Serum osmolality was measured by freezing-point depression. Blood urea nitrogen was measured by urease reaction and glucose by glucose oxidase reaction using the Vitros Trislide Technique (Johnson & Johnson). Levels of fluoxetine and its active metabolite, desmethylfluoxetine, were measured by HPLC equipped with a variable-wavelength detector.

### *In Situ Hybridization Histochemistry*

Serial 20-µm frozen coronal sections were taken through the region of the hypothalamus extending from the rostral through the caudal PVN and SON. Tissue sections were thaw-mounted onto gel alum-coated slides, dried, and stored at –70°C until hybridization. Hybridization was performed using techniques that have been extensively detailed previously *(38)*. Briefly, slides were fixed in 4% paraformaldehyde, acetylated in 0.25% acetic anhydride–0.1*M* triethanolamine HCl, pH 8.0, dehydrated through a series of ethanol exposures, and dilipidated in chloroform. Brain sections were hybridized by application of 70 µL/slide of hybridization buffer and $^{35}$S-UTP-labeled antisense or sense (control) riboprobes at 45°C for 16 h. The AVP riboprobe was derived from the rat pGEM 3-AVP 3c clone (provided by Thomas Sherman, Georgetown University). The CRF riboprobe was provided by Kelly Mayo. Transcription of linearized plasmid was done according to the directions of the manufacturer (Promega) and the addition of $^{35}$S-α-thiol-UTP (12.5 mCi/mL) and SP 6 polymerase. The specific activity of the labeled probe was $1 \times 10^6$ cpm/µg. Slides were washed successively in SSC and dehydrated by transfer through ethanol. Autoradiography was performed by dipping hybridized slides into NTB2 liquid emulsion (Eastman Kodak, Rochester, NY) diluted 1:2 with 600 m*M* ammonium acetate. Sections were dried and transferred to a light-tight box for 4 d, (OT) and 10 d (CRF), developed in D-19 (Kodak), placed in Kodak fixer, and counterstained with cresyl violet.

For quantitation, the cresyl violet-stained slides for each animal were examined under a light microscope, and the sections from each animal that contained the maximal

extent of the PVN on each side of the brain were chosen for analysis. Sections from experimental and control animals were matched for rostral caudal level without knowledge of the experimental treatment. All sections included in the analysis were hybridized, exposed, and analyzed in the same assay. Labeled sections were placed on a calibrated microscope stage, brought to focus, and sections of PVN or SON labeled for the mRNA species of interest were viewed using dark-field microscopy. Clusters of silver grains were imaged for integrated optical density measurements. This analysis was deemed appropriate for the PVN and SON in which densely packed neurons cannot always be resolved. Image fields were collected directly at X10 using a high sensitivity, integrating three chip Sony camera ($700 \times 600$ pixels), Corecco frame board, and Optimus software program. Quantitation of the density of silver clusters was done using NIH Image software package for Macintosh.

### *Statistical Analysis*

Results are expressed as mean ± SEM. Statistical significance was tested by two way analysis of variance (ANOVA). In cases in which ANOVA indicated statistical significance, post hoc comparisons were done by Fisher's protected least-significant difference (PLSD) test, or in the case of *in situ* hybridization results, Mann-Whitney U test. The level of significance was set at $p < 0.05$.

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