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Effects of sertraline on regional neuropeptide concentrations in olfactory bulbectomized rats

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Abstract

Corticotropin-releasing factor (CRF) and thyrotropin-releasing hormone (TRH) are two neuropeptides that exhibit increased cerebrospinal fluid (CSF) concentrations during major depressive episodes while somatostatin (somatotropin-release inhibiting factor, SRIF) is decreased. Clinical and basic research findings indicate that clinically effective antidepressant therapies often normalize the indicators of CRF and TRH hypersecretion as well as SRIF hyposecretion. The olfactory bulbectomized (OBX) rat is used to screen potential antidepressant drugs for clinical efficacy. This model requires chronic administration of the antidepressant drug to normalize OBX-induced behaviors such as increased locomotion in a novel environment. This report describes the regional brain concentration changes in CRF, TRH and SRIF produced by OBX and demonstrates the ability of the selective serotonin re-uptake inhibitor and antidepressant drug, sertraline (10 mg/kg), to normalize certain of these alterations in regional neuropeptide concentrations as well as normalizing OBX-induced increases in locomotor activity. OBX-induced increases in CRF concentrations in the hypothalamus and bed nucleus of the stria terminalis were specifically and significantly decreased by sertraline. OBX-induced increases in TRH concentrations in the hypothalamus were reversed by sertraline. The concentration of SRIF was significantly reduced by OBX in the anterior caudate and the piriform cortex, but sertraline reversed these changes only in the anterior caudate.

Keywords: Corticotropin-releasing hormone (CRF); Thyrotropin-releasing hormone (TRH); Somatotropin-release inhibiting factor (somatostatin, SRIF); Corticosterone; Locomotor activity; Selective serotonin re-uptake inhibitor; Antidepressant drugs; Major depressive disorder

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid neuropeptide that functions as a releasing factor in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis and as a neurotransmitter in a variety of extrahypothalamic brain regions (see De Souza and Grigoriadis, 1995, for review). Administration of synthetic CRF directly into the brain of laboratory animals initiates behavioral responses that are somewhat analogous to symptoms of human major depressive disorder (MDD) including changes in sleep architecture, decreased feeding, exploratory and sexual behaviors and increased anxiety (Butler et al., 1990; Kalin et al., 1983; Koob and Britton, 1990). In laboratory animals, presentation of stressful stimuli alters the regional concentrations of CRF in brain (Chappell et al., 1986) and microinjection of CRF onto noradrenergic neurons of the locus coeruleus increases their firing rate (Valentino, 1990). Two types of G-protein linked receptors for CRF have been described that are linked to adenylate cyclase as a second messenger (Chalmers et al., 1995) and a binding protein for CRF (Potter et al., 1992) allows rapid increases in synaptic availability upon physiologic demand. Patients with MDD have alterations in the regulation of the HPA axis including hypersecretion of cortisol from the adrenals (Sachar et al., 1973), decreased responsiveness of pituitary CRF receptors (Holsboer et al., 1987) mediating the release of adrenocorticotropin (ACTH) and increased numbers of neurons containing CRF mRNA in the paraventricular nucleus of the hypothalamus (Raadsheer et al., 1995). Additionally, MDD patients (Nemeroff et al., 1984; Banki et al., 1987), posttraumatic stress disorder patients (Bremner et al., 1997) and successful suicides (Arato et al., 1986) demonstrate increased concentrations of CRF in the cerebrospinal fluid (CSF) and decreased binding of CRF in the frontal cerebral cortex is observed in suicides compared

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to homicide or accidental death (Nemeroff et al., 1988). Antidepressant therapies such as electroconvulsive shock (Nemeroff et al., 1991) and antidepressant drugs (DeBellis et al., 1993; Heuser et al., 1998) reverse the increased CSF concentrations of CRF in major depression. Thus CRF is apparently hypersecreted during MDD and many of the indicators of this hypersecretion are normalized with symptom relief.

Thyrotropin-releasing hormone (TRH) is a tripeptide with the sequence pyroGlu–His–Pro–amide. TRH in the hypothalamus regulates the release of thyroid stimulating hormone (TSH) and prolactin from the anterior pituitary but functions as a neurotransmitter in extrahypothalamic brain regions (see Mason et al., 1995, for review). TRH receptors are found on postsynaptic membranes, are G-proteins with seven transmembrane spanning sequences and are linked to inisitol phosphate second messengers. TRH is also able to regulate neurons with which it communicates by transport of a TRH-receptor complex from the postsynaptic neuronal membrane to the cell nucleus. TRH administered to laboratory animals often causes hyperthermia and reverses the central nervous system depressant effects of drugs such as alcohol and barbiturates (Bissette et al., 1978; Kalivas and Horita, 1983). Patients with MDD demonstrate down-regulation of pituitary receptors for TRH (Shelton et al., 1993) and such patients also show a decrease in the amplitude of nocturnal TSH secretion and nocturnal hyperthermia (Souetre et al., 1988). These alterations are often normalized by treatments that relieve depressive symptoms and intravenous (Bunevicius and Matulevicius, 1993) or intrathecal (Marangell et al., 1997; Callahan et al., 1997) administration of exogenous TRH produces transient antidepressive effects. TRH concentrations in CSF are elevated in MDD (Kirkegaard et al., 1979; Banki et al., 1988) and administration of electroconvulsive shock (Kubek et al., 1985) or antidepressant drugs (Lighton et al., 1985; Przegalinski and Jaworska, 1990) to laboratory animals increases the concentration of TRH in certain brain regions. The available evidence therefore suggests that TRH is also hypersecreted in neuroendocrine circuits during episodes of major depression and may normalize upon relief of depressive symptoms.

Somatotropin-release inhibiting factor (SRIF, somatostatin) is a hypophysiotropic hormone that inhibits release of a variety of pituitary hormones and plays a role as an inhibitory neurotransmitter in extrahypothalamic brain regions (see Rubinow et al., 1995, for review). Somatostatin is produced as either a cyclic 14 amino acid peptide or as a longer 28 amino acid form that contains the shorter cyclic version. At least five molecular forms of somatostatin receptors have been identified and all are G-protein linked, seven transmembrane receptor proteins. Somatostatin is often colocalized with the inhibitory transmitter, gamma-amino butyric acid, and SRIF is the third most abundant neuropeptide in the cortex after vasoactive intestinal peptide and cholecystokinin. Postmortem brain and CSF concentrations of SRIF are reduced in a variety of diseases with cognitive impairment, including Alzheimer's disease and MDD (Bissette et al., 1986; Rubinow, 1986). Depressed subjects treated with electroconvulsive therapy demonstrate partial reversal of the SRIF deficit in CSF (Nemeroff et al., 1991) and SRIF levels in the hippocampus are increased in laboratory animals treated with electroconvulsive shock (Orzi et al., 1990). Microinjection of SRIF into brain alters behavior in laboratory animals (Nemeroff et al., 1987) and treatment of laboratory animals with antidepressant drugs alters brain concentrations of SRIF (Kakigi et al., 1992) and SRIF receptors (Gheorvassaki et al., 1992). These drugs alter SRIF content by two distinct mechanisms (Prosperini et al., 1997), an initial release from storage vesicles after a single acute administration and a decrease in synthesis of SRIF mRNA after 14 days of chronic administration. Thus, somatostatin may be a state marker for depressive symptoms and may also be a target for the therapeutic actions of antidepressant drugs.

In order to determine whether certain brain circuits employing CRF, TRH or SRIF respond specifically to chronic treatment with antidepressant drugs, the response of olfactory bulbectomized (OBX) rats to several weeks of treatment with the selective serotonin re-uptake inhibitor antidepressant drug, sertraline (ZOLOFT, Pfizer, Groton, CT), was examined. The OBX rat is a useful model to screen for the clinical efficacy of antidepressant drug candidates and responds to selective serotonergic and noradrenergic re-uptake inhibitors as well as monoamine oxidase inhibitors (see Kelly et al., 1997, for recent review). Some proponents of the OBX paradigm argue that there is good face validity with human depressive symptoms, especially agitated depression. Others argue that the behavioral normalization of OBX rats to chronic antidepressant drug administration is more of a predictive screen for clinical antidepressant efficacy rather than a model for depressive pathophysiology. Unlike some other screens, the OBX model requires chronic treatment to restore normal behavior on a time frame similar to that observed in clinical usage of antidepressant drugs and this temporal aspect does have face validity with human responses to antidepressant drugs. These rats do not, however, exhibit convincing anhedonia or other behaviors analogous to inappropriate feelings of guilt or sadness and do not suicide, so they cannot be considered as truly depressed in human terms. The OBX rat exhibits increased locomotor activity in an open field and fails to acquire a passive avoidance response relative to sham-operated controls. Both of these behaviors are normalized by chronic treatment with antidepressant drugs. We have used this behavioral normalization as an indicator that CNS alterations have occurred and that relevant neurochemical alterations are likely present. Here we report the regional brain concentrations of CRF, TRH and SRIF in OBX and sham-OBX rats treated for 5 weeks with sertraline when compared to vehicle-treated controls.

2. Methods

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Duke University Medical Center, Durham, NC. Adult, male Sprague–Dawley rats (220 g body weight) were ordered from Zivic-Miller Laboratories (Zellinople, PA). OBX or sham surgery was conducted under halothane general anesthesia by the supplier and rats were shipped 2 days after surgery. Surgery consisted of drilling two bilateral 2 mm burr holes approximately 1 mm anterior to the bregma cranial suture and 2 mm lateral to midline. Olfactory bulbs were carefully sectioned from anterior frontal cortex by scalpel and bulb tissue gently aspirated. Burr holes were closed with bone wax and the animals allowed to recover. Shams were anesthetized with halothane and burr holes drilled and closed, but no tissue was sectioned or aspirated.

Animals were shipped to the laboratory and were individually housed in an AAALAC-approved animal facility with free access to lab chow and tap water. They were handled daily during weight assessment and habituation to the euthanasia procedure between the hours of 8 a.m. and 10 a.m. Beginning 1 week after surgery, animals were tested for locomotor activity in photocell cages (Columbus Instruments, Columbus, OH). Locomotor activity was assessed each week between the hours of 10 a.m. and 2 p.m. with lights on and a normal light/dark 12 h photoperiod beginning at 6 a.m. and 6 p.m., respectively. Locomotor activity cages ($n=8$) consisted of clear Plexiglas chambers (43.2×44.4 cm) with 15 photocells on each x and y axes. Photocells were 2.65 cm apart, the beam width was 0.32 cm and beam wavelength was 940 nm. Locomotor activity data was expressed as total horizontal activity (total beam interruptions) and ambulatory activity (adjacent beam interruptions); vertical activity was not measured. Activity was measured for 30 min for each animal and computed as number of photocell counts in 5-min epochs. Two weeks after surgery, sham and OBX animals were randomly assigned to drug or vehicle groups and were injected daily with either sertraline (10 mg/kg ip) or 0.9% sodium chloride vehicle. Sertraline (ZOLOFT) was a gift from Pfizer.

Locomotor activity was assessed once per week for the next 3 weeks. On the third week of drug treatment, the sertraline-treated OBX rats no longer had significantly increased locomotor activity relative to the sham vehicle or sham sertraline groups while the locomotor activity of the OBX-vehicle group remained significantly increased relative to the sham groups. After another 2 weeks of sertraline treatment, all animals were euthanized by guillotine decapitation without anesthesia. This procedure is necessary to prevent corticosterone secretion due to involuntary loss of consciousness in anesthetized animals and is conditionally approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. During daily body weight determination for drug dosage, all animals were briefly introduced to the guillotine in the same room as the euthanasia procedure would be conducted. This habituation and the use of a fume hood to store carcasses and blood samples during the procedure largely prevents the increased corticosterone secretion due to the euthanasia procedure (Bissette, 1997).

Trunk blood was collected on ice for measurement of corticosterone. Refrigerated blood samples were centrifuged at low speed to obtain serum and the ICN Biomedicals (Costa Mesa, CA) corticosterone assay kit was used to measure corticosterone concentrations. This double antibody assay has a sensitivity of 50 ng/ml and an intra-assay variance of between 4% and 8% and was used according to the manufacturer's instructions except for the modification of using the primary antisera at half strength. The assay was performed in duplicate samples and the average of these two samples was used for statistical analysis.

Brains were rapidly removed from the skull and were frozen for subsequent dissection. Absence of olfactory bulbs was visually confirmed in the OBX rats and any damage to the frontal cortex was noted. Brains were thawed to -20°C and were carefully dissected on an inverted petri dish over ice into the following regions: anterior caudate (without globus pallidus or putamen), amygdala, anterior septum, bed nucleus of the stria terminalis, cerebellum, cingulate cortex, dorsal brainstem, entorhinal cortex, hippocampus, hypothalamus, locus coeruleus, median raphe, medial septum, nucleus accumbens, olfactory tubercles, posterior caudate, piriform cortex, substantia nigra and ventral brainstem. Tissue was weighed and then homogenized with 10–20 volumes of ice cold 1 N HCl. Homogenates were centrifuged under refrigeration and supernatants were collected. Pellets were dissolved in 1 N NaOH and proteins assayed by the Folin phenol reagent using a Technicon autoanalyzer. Supernatants were aliquoted into 10×75 mm borosilicate glass tubes for radioimmunoassay of CRF and TRH.

Radioactive tracers for the TRH, SRIF and CRF assays were synthesized by the Bolton–Hunter reaction using synthetic TRH (Bachem, Torrance, CA) Tyr^0–SRIF and Tyr^0–CRF (Peninsula, Belmont, CA) and ^{125}Iodine (New England Nuclear, Boston, MA). Tracer was purified by HPLC and fractions were characterized for peak immunoreactivity using two or more concentrations of primary antibody. Assays were conducted with primary rabbit antisera for CRF (recognized 33–41 region of CRF, $IC_{50}=35$ pg, sensitivity = 0.625 pg, intra-assay variability 6%) obtained from Peninsula Labs (Belmont CA) and TRH (recognizes native molecule and-His-substitutions, $IC_{50}=25$ pg, sensitivity 0.625 pg, intra-assay variability 4%) from Arnel Products, New York. SRIF assays were conducted with primary sheep antisera for SRIF (recognizes C-terminal 8–11 residue region of SRIF, $IC_{50}=30$ pg, sensitivity = 0.625 pg, intra-assay variability 5%) which was a generous gift from W. Vale, Salk Institute for Neurobiology, La Jolla, CA. Primary antisera concentration was adjusted to bind 25% of radioactive tracer in the absence of any unlabelled peptide. Standard curves were constructed by

serial dilution from synthetic peptides and ranged from 5120 to 0.625 pg. Aliquots of supernatant extracts were lyophilized and reconstituted in 200 μl of radioimmunoassay buffer (0.1 M NaCl, 0.1 M sodium phosphate, 1.0 mM EDTA, 1.0 mM sodium azide, 0.1% Triton detergent, 0.1% gelatin, pH 7.4). Primary antisera was also diluted with this buffer with 1.0% normal rabbit serum. The CRF, TRH and SRIF radioimmunoassays were performed as displacement assays over a period of 4 days: Day 1 — add buffer to lyophilized tissue samples, prepare standard curves, add primary antisera, incubate at 4°C for 24 h. Day 2 — Add radioactive tracer (20,000 cpm) to all tubes, incubate at 4°C for 24 h. Day 3 — add 2nd antibody (goat anti-rabbit for CRF and TRH and rabbit anti-sheep for SRIF, Arnel Products) to all tubes, incubate at 4°C for 24 h. Day 4 — centrifuge to separate bound from free tracer, aspirate supernatant, count remaining radioactivity on an LKB Rack-gamma gamma counter (80% efficiency). Total sample amounts of neuropeptide were calculated from aliquot concentrations and were divided by total sample protein to allow reporting regional neuropeptide concentrations as pg/mg protein.

Statistical analysis was performed with Statview and Superanova software (Abacus Concepts, now SAS Institute, Cary, NC) for the Macintosh computer. Locomotor activity was compared across experimental groups with Student Newman–Keuls test after a three-way mixed ANOVA (surgery × drug × weeks) indicated significant differences among groups and regional brain concentrations of CRF, TRH and SRIF were compared across experimental groups with Student Newman–Keuls test after two-way ANOVA (surgery × drug) indicated such differences were present. Correlations were sought between locomotor activity scores and significant peptide concentration changes only in brain regions known to mediate locomotor behavior and between significant neuropeptide alterations appearing in a single region. For these correlations we used the Spearman rank correlation.

3. Results

The locomotor activity of the OBX-vehicle and OBX-sertraline groups was significantly elevated ($P \leq .05$) compared to the sham-vehicle and sham-sertraline groups immediately before and for 2 weeks after beginning sertraline treatment (Fig. 1). After 3 weeks of sertraline treatment, the OBX-sertraline group no longer had significantly elevated locomotor activity relative to the sham groups while the OBX-vehicle group's locomotor activity remained significantly elevated. The figure shows the average ± S.E.M. of the 30-min weekly trials for the four groups of rats.

Corticosterone concentrations in serum were somewhat elevated in the OBX-sertraline rats relative to the sham

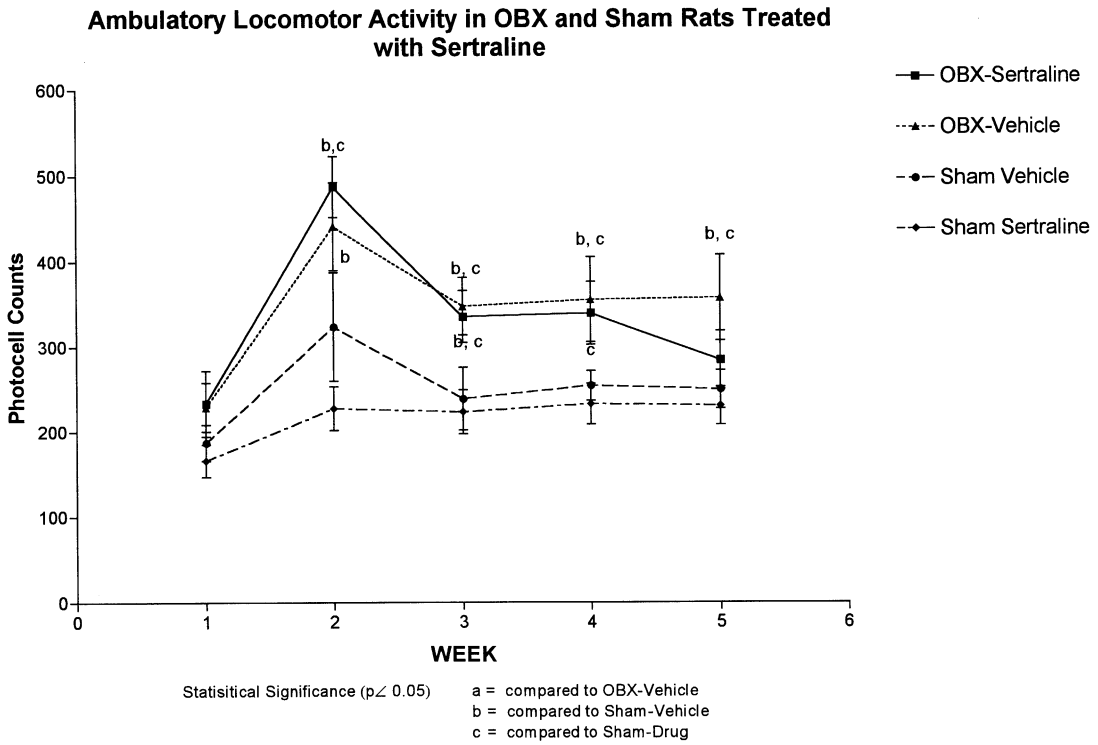

Fig. 1. Average 30 min ambulatory activity photocell counts ± S.E.M. over a 5-week period. Sertraline treatment began the day after the second week's activity measure. Statistical significance was calculated with Student Newman–Keuls post hoc test after ANOVA. Abbreviations: OBX-drug = olfactory bulbectomized treated with sertraline, OBX-vehicle = olfactory bulbectomized treated with saline vehicle, Sham-vehicle = sham-operated control treated with saline vehicle, Sham-drug = sham-operated treated with sertraline.

Table 1
Regional concentration of corticotropin releasing factor in OBX and sham rats treated with sertraline (mean ± S.E. in pg/mg protein)

Brain regions	OBX-sertraline	OBX-vehicle	Sham-vehicle	Sham-sertraline
Amygdala	6.9 ± 0.40	7.42 ± 0.63	7.26 ± 0.59	5.55 ± 0.47[a,b]
Anterior septum	33.7 ± 2.6	33.2 ± 2.15	40.0 ± 2.6	42.7 ± 7.27
Bed nucleus stria terminalis	127.7 ± 8.69	184.0 ± 22.3[c]	119.3 ± 7.78[a]	137.6 ± 6.76[a]
Entorhinal cortex	2.49 ± 0.23	2.83 ± 0.16	3.19 ± 0.27	3.43 ± 0.33[c]
Hypothalamus	80.1 ± 3.66	101.1 ± 6.53[c]	59.4 ± 4.95[a,c]	66.5 ± 3.12[a,c]
Locus coeruleus	42.3 ± 4.63	41.9 ± 6.62	45.1 ± 5.32	50.4 ± 8.56
Median raphe	58.1 ± 3.94	46.8 ± 4.48[c]	28.7 ± 2.58[a,c]	23.3 ± 1.88[a,c]
Medial septum	126.3 ± 33.6	60.8 ± 4.20[c]	54.5 ± 2.62[c]	56.3 ± 3.20[c]

[a] Statistical significance ($P \leq .05$), compared to OBX-vehicle.
[b] Statistical significance ($P \leq .05$), compared to sham-vehicle.
[c] Statistical significance ($P \leq .05$), compared to OBX-sertraline.

groups or OBX-vehicle group, but this increase did not reach statistical significance. Absolute values (mean ± S.D. in ng/ml) for corticosterone concentrations were: OBX-vehicle (29.0 ± 69.5, $n = 17$), OBX-sertraline (41.8 ± 133.9, $n = 16$), sham-vehicle (22.6 ± 71.0, $n = 15$) and sham-sertraline (22.4 ± 56.4, $n = 15$). These values were lower than those previously reported for OBX animals and this effect was most likely due to the 6 weeks of habituation to the guillotine procedure and the precautions to remove the odor of blood that were employed during euthanasia.

The effects of OBX and 5 weeks of sertraline treatment on regional CRF concentrations are shown in Table 1. The OBX procedure raised CRF concentrations in several brain regions, but only the hypothalamus (+70%), bed nucleus of the stria terminalis (+54%) and median raphe (+63%) achieved statistical significance at the $P \leq .05$ level. Sertraline was able to significantly reduce the OBX-induced increase in CRF in the hypothalamus (Fig. 2) and bed nucleus of the stria terminalis but further elevated CRF in the median raphe relative to vehicle-treated OBX rats. Sertraline also significantly elevated CRF in the medial septum of OBX rats relative to OBX-vehicle (+108%), sham-vehicle (+132%) and sham-sertraline (+124%) groups. These effects of sertraline were specific for the OBX rats as the sham-sertraline controls did not exhibit similar changes in either magnitude or direction compared to sham-vehicle controls. The OBX-induced decreases of CRF in the anterior septum (−17%) did not achieve statistical significance relative to sham controls. The only nonspecific effect of sertraline in the sham group to reach statistical significance was a decrease in CRF concentration in the amygdala (−24%) relative to sham-vehicle control.

The effects of OBX and sertraline treatment on regional TRH concentrations are shown in Table 2. Significant elevations in TRH concentrations were produced by OBX in the hypothalamus (+63%), median raphe (+313%) and medial septum (+54%) relative to the sham-vehicle controls. Increased concentrations of TRH in OBX rats that

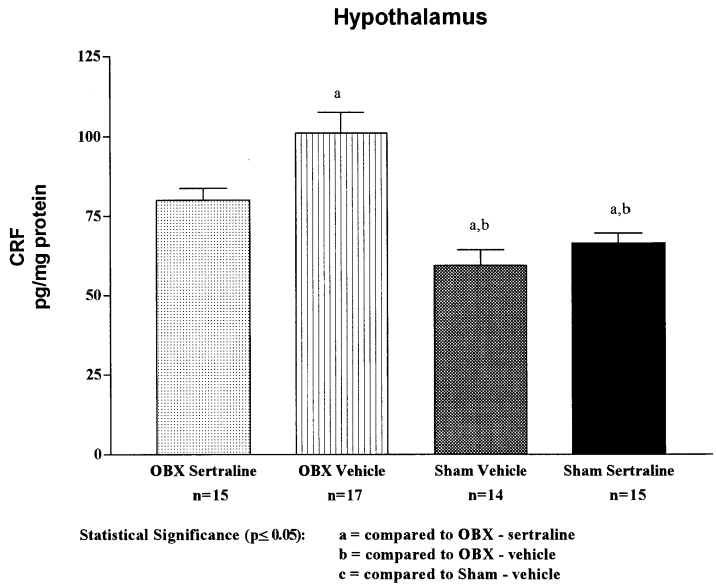

Fig. 2. Group mean ± S.E.M. hypothalamic concentrations of CRF in sertraline- and vehicle-treated rats. Statistical significance was calculated with Student Newman–Keuls post hoc test after ANOVA. Abbreviations: OBX-drug = olfactory bulbectomized treated with sertraline, OBX-vehicle = olfactory bulbectomized treated with saline vehicle, Sham-vehicle = sham-operated control treated with saline vehicle, Sham-drug = sham-operated treated with sertraline.

Table 2
Regional concentration of thyrotropin releasing hormone in OBX and sham rats treated with sertraline (mean ± S.E. in pg/mg protein)

Brain regions	OBX-sertraline	OBX-vehicle	Sham-vehicle	Sham-sertraline
Anterior caudate	15.9 ± 1.98	17.6 ± 2.17	14.7 ± 2.48	12.2 ± 1.54
Amygdala	14.6 ± 1.47	15.1 ± 1.90	14.7 ± 2.53	9.72 ± 0.99[a]
Anterior septum	52.9 ± 6.01	50.6 ± 3.59	48.1 ± 6.15	48.0 ± 4.14
Bed nucleus stria terminalis	177.5 ± 24.1	168.7 ± 19.2	170.2 ± 30.0	179.5 ± 27.7
Dorsal brainstem	105.3 ± 10.1	61.2 ± 3.86[b]	58.5 ± 4.28[b]	50.3 ± 7.87[b]
Entorhinal cortex	3.92 ± 0.94	4.87 ± 0.54	3.35 ± 0.59	4.17 ± 1.06
Hippocampus	5.86 ± 1.13	2.96 ± 0.37[b]	4.41 ± 0.75	5.24 ± 0.47[a]
Hypothalamus	282.7 ± 30.4	391.6 ± 34.2[b]	240.7 ± 38.4[a]	237.2 ± 21.3[a]
Locus coeruleus	56.0 ± 5.95	75.8 ± 9.98	81.9 ± 11.6	95.8 ± 23.0[b]
Median raphe	42.8 ± 2.70	68.2 ± 3.16[b]	16.5 ± 2.10[a,b]	13.3 ± 1.30[a,b]
Medial septum	210.7 ± 41.6	156.9 ± 16.6	102.2 ± 14.8[a]	147.4 ± 31.5
Nucleus accumbens	31.6 ± 3.93	27.4 ± 2.88	29.6 ± 2.48	23.4 ± 1.82
Olfactory tubercles	10.5 ± 3.82	11.3 ± 3.31	60.3 ± 40.9	94.3 ± 50.6
Posterior caudate	33.9 ± 2.99	30.9 ± 3.02	34.7 ± 4.63	41.5 ± 3.63[a]
Piriform cortex	23.1 ± 4.39	17.1 ± 1.90	16.0 ± 3.47	26.0 ± 3.43[c]
Substantia nigra	3.92 ± 0.61	4.89 ± 0.65	4.97 ± 0.59	5.98 ± 0.94[b]
Ventral brainstem	91.3 ± 11.8	77.7 ± 10.4	121.6 ± 30.7	87.8 ± 11.9

[a] Statistical significance ($P \leq .05$), compared to OBX-vehicle.
[b] Statistical significance ($P \leq .05$), compared to OBX-sertraline.
[c] Statistical significance ($P \leq .05$), compared to sham-vehicle.

were observed in the entorhinal cortex (+45%) and decreased TRH concentrations in the hippocampus (−33%), olfactory tubercles (−81%) and ventral brainstem (−36%) of OBX rats did not reach statistical significance. Sertraline treatment was able to significantly reverse TRH increases produced by OBX in the hypothalamus (Fig. 3) and median raphe, but further elevated TRH in the medial septum (+34%) relative to vehicle-treated OBX rats. Non-specific, statistically significant elevations of TRH due to sertraline were observed for the sham-sertraline groups in the piriform cortex (+63%) compared to sham-vehicle. Sertraline also significantly increased the concentration of TRH in the dorsal brainstem of OBX rats relative to the OBX-vehicle (+72%), sham-vehicle (+80%) and sham-sertraline (+109%) groups.

The regional brain concentrations of SRIF in the four experimental groups are shown in Table 3. The OBX procedure significantly decreased SRIF concentrations in the anterior caudate (−40%) of vehicle-treated rats relative to sham-OBX vehicle-treated controls. Sertraline treatment

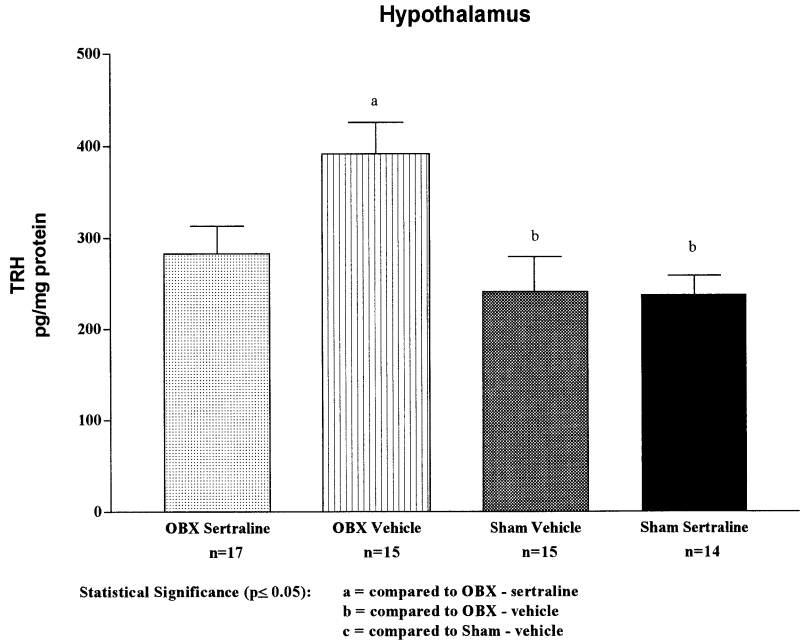

Fig. 3. Group mean ± S.E.M. hypothalamic concentrations of TRH in sertraline- and vehicle-treated rats. Statistical significance was calculated with Student Newman–Keuls post hoc test after ANOVA. Abbreviations: OBX-drug = olfactory bulbectomized treated with sertraline, OBX-vehicle = olfactory bulbectomized treated with saline vehicle, Sham-vehicle = sham-operated control treated with saline vehicle, Sham-drug = sham-operated treated with sertraline.

Table 3
Regional concentration of somatostatin in OBX and sham rats treated with sertraline (mean ± S.E. in pg/mg protein)

Brain regions	OBX-sertraline	OBX-vehicle	Sham-vehicle	Sham-sertraline
Anterior caudate	349.9 ± 27.1	301.6 ± 33.0	422.3 ± 36.2[a]	321.3 ± 29.9[b]
Amygdala	469.7 ± 39.4	482.6 ± 32.4	262.8 ± 30.2[a,c]	305.1 ± 28.6[a,c]
Anterior septum	1574.4 ± 168.7	1216.8 ± 90.5	1391.4 ± 157.5	1235.5 ± 139.6
Bed nucleus stria terminalis	1139.0 ± 144.0	1197.1 ± 115.8	849.4 ± 113.0	1097.6 ± 99.1
Cingulate cortex	157.9 ± 24.1	145.0 ± 20.8	196.2 ± 28.2	164.6 ± 15.4
Dorsal brainstem	278.5 ± 22.4	150.4 ± 15.7	121.3 ± 10.6[c]	116.7 ± 11.1[c]
Entorhinal cortex	66.9 ± 6.52	75.2 ± 6.87	71.1 ± 5.71	78.7 ± 8.31
Hippocampus	104.7 ± 13.2	94.7 ± 7.39	97.0 ± 19.2	89.8 ± 11.4
Hypothalamus	2063.3 ± 184.2	2013.2 ± 222.7	1796.8 ± 253.9	2175.4 ± 150.2
Locus coeruleus	741.4 ± 76.3	995.6 ± 106.6[a]	808.2 ± 88.7	769.7 ± 77.8
Median raphe	1014.8 ± 66.4	720.6 ± 55.4[c]	747.7 ± 78.8[c]	611.4 ± 42.1[c]
Medial septum	1257.2 ± 360.1	664.0 ± 67.6[c]	566.5 ± 87.4[c]	686.6 ± 143.2[c]
Nucleus accumbens	734.7 ± 136.8	801.9 ± 99.5	689.4 ± 115.7	628.7 ± 64.0
Olfactory tubercles	1015.7 ± 94.0	1542.5 ± 168.2[c]	1107.7 ± 128.6	1735.8 ± 233.7[b,c]
Posterior caudate	434.8 ± 36.9	516.0 ± 41.5	513.3 ± 58.4	569.3 ± 44.8[c]
Piriform cortex	257.8 ± 43.5	247.7 ± 54.6	557.9 ± 104.6[a,c]	341.3 ± 42.0[b]

[a] Statistical significance ($P \leq .05$), compared to OBX-vehicle.
[b] Statistical significance ($P \leq .05$), compared to sham-vehicle.
[c] Statistical significance ($P \leq .05$), compared to OBX-sertraline.

reversed most of this OBX effect in the anterior caudate (Fig. 4) to the point where statistical significance was lost although SRIF concentrations remained slightly decreased (−20%) relative to sham-OBX vehicle-treated controls. Sertraline did not increase SRIF in the sham-OBX controls, indicating the effect in the anterior caudate was specific for OBX rats. The OBX procedure significantly increased SRIF concentrations in the amygdala (+84%) and significantly decreased SRIF in the piriform cortex (−56%). These region's alterations in SRIF concentrations were not normalized by sertraline. The olfactory tubercles also had elevations in SRIF concentrations after OBX (+39%;

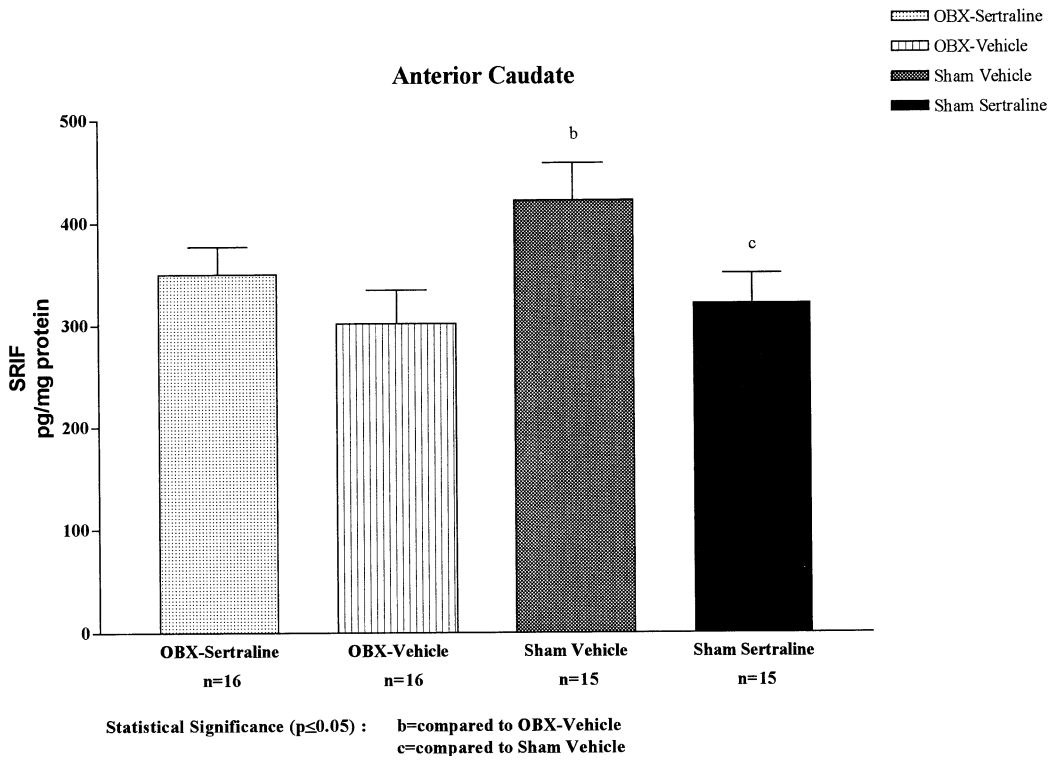

Fig. 4. Group mean ± S.E.M. anterior caudate concentrations of somatostatin (SRIF) in sertraline- and vehicle-treated rats. Statistical significance was calculated with Student Newman–Keuls post hoc test after ANOVA. Abbreviations: OBX-drug = olfactory bulbectomized treated with sertraline, OBX-vehicle = olfactory bulbectomized treated with saline vehicle, Sham-vehicle = sham-operated control treated with saline vehicle, Sham-drug = sham-operated treated with sertraline.

$P \leq .06$) and this increase was reversed by sertraline in the OBX rats. The concentration of SRIF was significantly elevated (+57%) in the sham-OBX rats treated with sertraline, indicating the effect of sertraline in reducing the OBX-induced elevations in SRIF concentrations in the olfactory tubercles were specific for the OBX rats.

Sertraline significantly increased SRIF concentrations in the median raphe (+41%) and medial septum (+89%) of OBX rats compared to OBX-vehicle animals but these regions did not demonstrate alterations in SRIF concentrations due to the OBX procedure. However, sertraline did not produce such changes in the sham-OBX rats in these regions. The locus coeruleus concentrations of SRIF were significantly reduced by sertraline in the OBX rats compared to OBX-vehicle rats, but no effects of OBX were observed in this region when the OBX-vehicle rats were compared to the sham-OBX vehicle-treated controls. Sertraline significantly increased SRIF concentrations in the dorsal brainstem of OBX rats compared to either sham-OBX vehicle or sham-OBX sertraline-treated rats and significantly increased SRIF in the posterior caudate of sham-OBX rats compared to OBX-sertraline rats.

4. Discussion

In discussing the regional neuropeptide changes in OBX rats relative to sham controls, we have chosen to use the terms "therapeutic" and "side-effects" to indicated alterations in neuropeptide concentrations that were produced by OBX and normalized by sertraline ("therapeutic") versus those produced by sertraline in the sham group and not seen in the OBX group ("side-effects"). These terms should not be interpreted as describing neuropeptide alterations that are congruent with those occurring in human depressive pathophysiology, but are limited to the alterations seen in this laboratory animal model. The design of this study was chosen intentionally to allow discrimination between the "nonspecific" effects of sertraline on regional neuropeptide circuits in the sham-operated animals with those that were "specific" for the OBX animals. Comparing the sertraline-treated OBX and sham groups to their respective vehicle-treated controls allows evaluation of whether the effect of sertraline was seen only in the OBX rats ("therapeutic" effect) or whether it was a general regional response ("side effect") of the neuropeptide's regional regulation by the drug. As normal human subjects without major depressive symptoms do not experience alterations in mood after chronic treatment with clinically efficacious antidepressant drugs, but do experience the same side effects reported by depressed patients, the response of neuropeptide circuits to sertraline treatment in OBX rats would not be considered specific if the sham-sertraline animals also had alterations in neuropeptide concentration that were in a similar direction and magnitude as those seen in the sertraline-treated OBX rats. Much of the data previously published on neuropeptide responses to antidepressant drug treatment has used otherwise normal animals and would not be able to discern specific effects from nonspecific effects (Brady et al., 1992; Owens et al., 1989; Nemeroff et al., 1988). While not claiming that a specific or "therapeutic" regional neuropeptide response to sertraline by the OBX rat was responsible for the behavioral normalization observed, it may be more than coincidental. As this experiment was limited to one treatment time point, the ability of such treatment to sustain the normalization of the neuropeptide circuits demonstrating specific or "therapeutic" responses cannot be predicted at present. However, this could be tested across several time points in future experiments. The time course of such changes after the OBX surgery will obviously affect regional neuropeptide concentrations and their responses to antidepressant drugs. The regional neuropeptide concentration changes found at the time point addressed in this experiment (7 weeks after surgery and 5 weeks after initiation of sertraline treatment) probably represent chronic responses to the surgery and drug treatments rather than acute effects of the last drug injection, as animals were treated daily for 5 weeks and euthanasia was not performed until 22 h after the last drug injection.

The normalization of increased locomotor behavior in the OBX rats treated with sertraline occurred after 3 weeks of daily administration. This time frame is generally what has been described for other studies where OBX rats were treated with other clinically effective antidepressant drugs (see Kelly et al., 1997, for recent review; van Riezen and Leonard, 1990; Jesberger and Richardson, 1985). That this behavioral normalization occurs only after chronic treatment supports this model as similar to clinical experience in relief of depressive symptoms and suggests that some of the physiological responses in the model may be similar to that observed in depressed patients (Slotkin et al., 1999). Animals were only tested for locomotor activity once per week for 1/2 h to prevent the animals from habituating to the photocell cages. As the most obvious differences in locomotor activity among the various groups was seen during the first 10 min, a shorter period of locomotor activity testing may be adequate for future experiments using OBX rats as well as being less likely to induce habituation to the test chamber. A recent paper (Mar et al., 2000) suggests that increased rates of habituation underlie antidepressant effects on OBX-induced behaviors, although no evidence of such effect was seen across the different 5-min epochs of the 30-min locomotor activity trials in the sertraline-treated OBX rats relative to either sham control group or the OBX-vehicle group. Animals were also tested during the daylight hours to maximize differences among groups due to the relative decreases in locomotor activity in normal animals during this period of the day.

The levels of corticosterone in the blood of the OBX and sham animals at euthanasia were less than have been previously reported. The reported elevation (Marcilhac et al., 1999b; Cairncross et al., 1977; Cattarelli and Demael,

1986) of basal levels of corticosterone in OBX rats is somewhat controversial and has not always been observed in the published literature (Arnold and Meyerson, 1990; Broekkamp et al., 1986; Montilla et al., 1977; O'Connor and Leonard, 1984). OBX rats are not able to use their primary sensory apparatus, olfaction, and are easily startled if not handled gently and often. To minimize such effects, these animals were introduced to the guillotine every day for 6 weeks when obtaining daily body weight. This procedure occurred well before drug injection to prevent the animal from associating the guillotine with injection and was performed with due attention to the animals' comfort. These precautions were probably responsible for the low corticosterone levels seen in the OBX and control groups. The statistically nonsignificant elevation of corticosterone concentrations in the OBX-sertraline group was not correlated with hypothalamic concentrations of CRF nor were the corticosterone concentrations in the other groups. This disparity is also seen in depressed clinical populations with CSF levels of CRF and TRH correlating poorly with basal or stimulated blood levels of corticosterone or thyrotropin and thyroid hormones, although the hypothalamic contribution of CRF and TRH to human CSF levels is not known with adequate accuracy. Within groups, animals were euthanized in random order to prevent confounds from blood odor from influencing one group more than another. Because the sham-sertraline group did not have the higher levels seen in the OBX-sertraline group, it is unlikely that the effect of increasing corticosterone levels in the OBX-sertraline group was due to sertraline treatment. In fact, in reports where OBX was associated with increased levels of corticosterone, antidepressant drugs attenuated this effect (Cairncross et al., 1978, 1979). This effect of antidepressants on the HPA axis is also seen in normal rats treated with the selective serotonin re-uptake inhibitor, citalopram (Jensen et al., 1999). These researchers saw desensitization of the HPA axis as measured by decreased corticosterone and ACTH secretion as well as reduced levels of proopiomelanocortin in the hypothalamic paraventricular nucleus to chronic (14 days), but not acute (1 day), citalopram treatment.

Among the several brain regions exhibiting alterations in CRF and TRH concentrations in the OBX rats, the hypothalamus stands out. Both CRF and TRH were significantly elevated in the hypothalamus of OBX-vehicle rats relative to sham-vehicle controls and both of these neuropeptides were specifically decreased by sertraline only in the OBX-sertraline group. Neither CRF nor TRH hypothalamic concentrations were significantly altered in the sham-sertraline groups relative to the sham-vehicle group. Thus the effects of sertraline on the hypothalamic concentrations of these neuropeptide releasing factors were specific for the manipulated OBX groups and may respond similarly in depressed human subjects treated with sertraline for similar periods. A previous report (Marcilhac et al., 1999a) did not observe an increase in immunohistochemical CRF or CRF mRNA in the median eminence of the hypothalamus but did find an increase in vasopressin in OBX rats. Others (Heilig and Ekman, 1995) see no regional brain alterations in CRF immunoreactivity after chronic (6 weeks) treatment with citalopram or desipramine in otherwise normal Wistar rats. However, most of the available literature supports an attenuation of activity of the HPA axis by antidepressant drugs (Holsboer and Barden, 1996; Reul et al., 1993, 1994; Shimoda et al., 1988). In another study, surgical bulbectomy did not alter TRH levels or TRH receptor number 1 month later in the hypothalamus, hippocampus, amygdala or olfactory tubercles (Sharif, 1988). In the present study, the hypothalamus was dissected as a single block of tissue from the rat brains and did not contain the median eminence, which was usually torn off with the pituitary during removal of the brain from the skull. The hypothalamic paraventricular nucleus and periventricular nucleus are the nuclei within the hypothalamus that contain the highest concentrations of CRF and TRH, respectively, and are the presumed sites of sertraline normalization of these two distinct neuropeptide releasing factors in OBX rats.

Unlike CRF and TRH, SRIF concentrations in the hypothalamus were not significantly altered by OBX or subsequent sertraline treatment. This finding highlights the specificity of the observed CRF and TRH concentration elevations in this region and limits the congruence of OBX-induced alterations in neuropeptides in this region with those seen in major depressive CSF to TRH and CRF. As SRIF in CSF of depressed subjects is decreased relative to nondepressed groups (Bissette et al., 1986), a decrease in SRIF hypothalamic concentrations would be required to reflect the depressed CSF changes for this peptide.

The close anatomical association of CRF, TRH and SRIF neuronal populations within the hypothalamus (Liao et al., 1992) and their reciprocal regulation (Aguila and McCann, 1985) is well known. In this group of rats the correlation of hypothalamic concentrations of TRH and CRF was statistically significant (r=.601, $P \leq .001$) across all treatment groups. However, when TRH and CRF hypothalamic correlations were sought within treatment groups, the statistically significant correlations were found to be due to the groups not treated with sertraline (OBX-vehicle r=.684, $P \leq .003$; OBX-sertraline r=.393, $P \leq .15$; sham-vehicle r=.665, $P \leq .006$; sham-sertraline r=.211, $P \leq .498$). Apparently sertraline treatment alters the coregulation of these hypothalamic neuropeptide circuits in a manner that disturbs the normal correlation. Whether this action could explain some of the therapeutic effects of antidepressant drugs in general or is specific for this class of antidepressant drug or this specific compound will require further research using other classes and formulations of antidepressant drugs.

Because sertraline blocks the re-uptake of serotonin and serotonin neurons comprise the bulk of neurons in the median raphe nucleus, the partial reversal of OBX-induced increases in the concentration of TRH in this region are probably due to this effect of sertraline. This "therapeutic"

effect may be considered specific as sertraline did not increase the median raphe concentration of TRH in the sham-sertraline group. TRH was reported to be colocalized with serotonin in the medullary raphe region and this may have contributed to sertraline's effect in the mesencephalic median raphe as well. Unlike the median raphe, in the medullary raphe a decrease in TRH would be predicted in the sham group based upon the effect of selective serotonin re-uptake inhibitors on messenger RNA levels of prepro-TRH (Riley et al., 1993), where treatment with either fluoxetine or zimelidine for 14 days in otherwise normal rats lowers TRH mRNA in this region.

Unlike TRH, CRF was further elevated in the median raphe by sertraline. Although not a "therapeutic " effect, this response was specific for the OBX rats, as the sham-sertraline animals had a slight decrease rather than an increase in CRF concentrations in this region. An increase in serotonin-related neuronal markers in the frontal cortex 12 weeks after OBX surgery has been previously reported (Zhou et al., 1998) and was presumed to result from raphe responses to disruption of frontal cortex innervation. Further differences between TRH and CRF responses to OBX were seen in the bed nucleus of the stria terminalis, where OBX induced increases in CRF but not TRH. This CRF increase was significantly decreased by sertraline in the OBX rats, while sertraline induced a slight increase in CRF in the sham rats. Because the CRF circuits in the bed nucleus communicate with both the hypothalamus and amygdala, the increase in CRF in this region may be due to an increased perception of stress among the OBX rats. That this regional increase in CRF content is specifically normalized by sertraline may indicate a similar effect in humans, although such an explanation remains speculative in the absence of data demonstrating alterations of CRF synaptic availability in this region in humans with MDD.

Unlike the more specific responses to sertraline observed on CRF and TRH concentrations in the hypothalamus and median raphe, the medial septum (which contains both medial and lateral septal nuclei in this dissection) was altered in a more nonspecific fashion. CRF concentrations in this region were not elevated by the OBX procedure alone, but sertraline elevated CRF in the OBX group only. The OBX procedure did elevate TRH concentrations in this region and this effect was further increased by sertraline in the OBX-sertraline group. However, the sham-sertraline group also demonstrated elevations of TRH in the medial septum, thus the response to sertraline in the OBX group cannot be considered specific. Apparently, sertraline elevates the concentration of TRH in this region after 5 weeks of daily administration regardless of whether OBX was performed.

Among the other regions where neuropeptide concentration changes were observed, but did not reach statistical significance at the $P \leq .05$ level, the entorhinal cortex deserves comment. In a previous pilot study of the effects of OBX on this region without antidepressant drug treatment, TRH concentrations were elevated over 200% at 4 weeks after OBX relative to sham-operated controls (data not shown). Euthanasia in the present experiment did not occur until 7 weeks after the OBX procedure and TRH was only elevated 45% in the OBX-vehicle group compared to the sham-vehicle group. Thus, the entorhinal cortex (which receives afferents from the olfactory bulbs) responds to OBX with a transient increase in TRH concentrations that are attenuated with time. Whether this response is due to deafferentation of olfactory bulb innervation remains to be confirmed, but TRH is thought to act as a trophic agent during development and a similar action may be triggered by loss of normal input from the olfactory bulbs. This response was specific for TRH as CRF does not exhibit such changes in OBX rat entorhinal cortex, in fact CRF was decreased by OBX and further decreased by sertraline in this region although these changes did not approach statistical significance.

There were few changes in CRF and TRH concentrations in the other regions examined that would inform mechanisms relevant to either the behavioral normalization or the regional regulation of these neuropeptides. For CRF in the amygdala and TRH in the piriform cortex, there were no effects of OBX but sertraline treatment increased the neuropeptide concentration in both the OBX and sham groups receiving treatment. Such nonspecific changes may indicate serotonin regulation of the neuropeptide circuits in these regions but the relevance of these changes to either the therapeutic or behavioral aspects of this model remain obscure. A previous report did not observe changes in CRF or TRH mRNA in the piriform cortex or other forebrain regions of OBX rats using in situ hybridization (Holmes et al., 1998).

Of the brain regions investigated in this experiment, only the anterior caudate demonstrated a "specific" normalization of SRIF after sertraline treatment. Although the decrease in anterior caudate SRIF concentrations produced by OBX were only partially reversed by 5 weeks of sertraline treatment, this effect may be considered specific because sertraline's effect in the sham control group was in the opposite direction. Previous reports have usually, but not always, associated increased locomotor activity with increased synaptic availability of SRIF in striatal structures. Thus, microinjection of SRIF into the nucleus accumbens region increases locomotor activity (Raynor et al., 1993) as does intracerebroventricular administration of SRIF (Vecsai and Widerlov, 1988). This response may be more pharmacological than physiological because temporary depletion of endogenous SRIF after chronic cysteamine treatment does not significantly alter locomotor activity (Justino et al., 1997) and acute cysteamine treatment is associated with only slight increases in locomotor activity (Vecsai et al., 1989). When assessing regional SRIF concentrations by radioimmunoassay, the mechanism by which concentrations are altered is not defined. Thus the decreased SRIF concen-

trations in the anterior caudate of OBX rats could indicate decreased synthesis and subsequent decreased inhibition of striatal circuits receiving SRIF released from presynaptic neurons. This could conceivably contribute to the increased locomotor activity seen after OBX surgery. However, the correlation of individual rat locomotor activity in the final behavioral test with anterior caudate SRIF concentrations in the OBX-vehicle group was $r=.118$, $P \leq .45$, ns. This lack of significant correlation does not support the interpretation of anterior caudate SRIF contributing to locomotor activity levels. If the earlier reports of increased locomotor activity after SRIF is applied directly to the caudate are accurate, the decreased SRIF concentrations in the anterior caudate would most likely represent increased SRIF release relative to synthesis in these neurons.

Among the other regions demonstrating OBX-induced alterations in SRIF concentration, the amygdala deserves comment. This brain region is activated by emotional stimuli as part of the limbic system circuitry and a recent report documents long-term (up to 64 days postlesion) increases in c-Jun immediate-early gene expression in the basolateral nucleus of the amygdala in OBX rats (Wrynn et al., 2000). Increased SRIF concentrations in the amygdala have been associated with stress; sequential removal of group housed rats from their cage during euthanasia induces increased SRIF concentrations in the amygdala of the last two rats removed (Brodin et al., 1994) and whole-body shaking of rats also increases SRIF concentrations in the amygdala (Nakamura et al., 1994). Immobilization stress also activates the immediate early gene expression of c-fos in SRIF neurons in the central nucleus of the amygdala (Honkaniemi, 1992), demonstrating the potential induction of SRIF expression by c-fos activation induced by stress. The OBX procedure significantly increased SRIF (+184%) in the amygdala of the rats in the present study, but this was not altered by sertraline treatment. If this increase in SRIF concentration is due to increased synthesis associated with activation of either c-Jun or c-Fos expression, it is not regulated by sertraline-treatment. This would indicate that sertraline does not reverse stress-induced SRIF changes in general and further distinguish the observed effects on CRF and TRH.

In summary, the OBX model presents a useful platform for investigating antidepressant effects on neurotransmitters thought to play a role in MDD. The identification of brain regions exhibiting specific and "therapeutic" effects is the first step in elucidating the putative mechanism of antidepressant action in a clinical setting. The next step is to identify the neurons projecting to these regions that provide the neuropeptide-containing terminals that are responsible for the regional concentration changes. These neurons can then be examined for the ability of different classes of clinically effective antidepressants to regulate the production and synaptic availability of the respective neuropeptide. Such information may identify circuits of interest for development of more specific antidepressants and lead to drugs that are able to relieve depressive symptoms in wider populations or in shorter periods of time than the currently available drugs.

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