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Reduction of Hypothalamic Vasopressinergic Hyperdrive Contributes to Clinically Relevant Behavioral and Neuroendocrine Effects of Chronic Paroxetine Treatment in a Psychopathological Rat Model

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The neuroendocrine and behavioral effects of chronic paroxetine treatment were investigated in two rat lines selectively bred for high anxiety-related behavior (HAB) or low anxiety-related behavior (LAB) emotionality. In addition to a characteristic behavioral phenotype with markedly passive stress-coping strategies, HAB rats show a hypothalamic vasopressinergic hyperdrive that is causally related to hypothalamic–pituitary–adrenocortical dysregulation as demonstrated in the combined dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test. A total of 8 weeks of chronic paroxetine treatment induced a more active coping strategy in the forced swim test in HAB rats only. In contrast, paroxetine-treated LAB rats did not change their swimming behavior. To investigate the neuroendocrine alterations linked to these behavioral changes, a combined DEX/CRH test was performed. In HAB rats, the paroxetine-induced behavioral changes towards more active coping strategies were accompanied by a normalization of the CRH-stimulated increase in corticotropin (ACTH) and corticosterone secretion. Concomitantly, the hypothalamic vasopressinergic hyperdrive was found to be reduced in HAB but not LAB rats, as indicated by a decrease in vasopressin mRNA expression, whereas vasopressin 1a receptor binding was unaffected. These findings provide the first evidence that the vasopressinergic system is likely to be critically involved in the behavioral and neuroendocrine effects of antidepressant drugs. This novel mechanism of action of paroxetine on vasopressin gene regulation renders vasopressinergic neuronal circuits a promising target for the development of more causal antidepressant treatment strategies. *Neuropsychopharmacology* (2003) **28,** 235–243. doi:10.1038/sj.npp.1300040

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INTRODUCTION

There is increasing evidence that neuropeptides are preferentially released and exert their main actions when neurons are strongly activated and under pathological conditions (Hökfelt *et al*, 2000). Accordingly, hyperactivity of central neuropeptidergic circuits such as corticotropin-releasing hormone (CRH) and vasopressin (AVP) neuronal systems is thought to play a causal role in the etiology and symptomatology of affective disorders (Hökfelt *et al*, 2000; Holsboer, 2000). In support of this, after prolonged stress, AVP is increasingly expressed and released from hypothalamic neurons in both humans and rodents (Antoni, 1993; Keck *et al*, 2000). Similarly, a markedly increased synthetic activity of hypothalamic AVP neurons has been described in depressed patients, which is reflected by increased AVP plasma concentrations (Purba *et al*, 1996; van Londen *et al*, 1997). Most recently, administration of a nonpeptide AVP vasopressin 1b (V_{1b}) receptor antagonist was shown to display anxiolytic and antidepressant-like effects in rodents (Griebel *et al*, 2002). AVP has been shown to exert both behavioral effects such as, for example, increased anxiety following intracerebroventricular administration, and to increase CRH-induced corticotropin (ACTH) secretion from pituitary corticotrope cells (Antoni, 1993; Landgraf *et al*, 1998; Bhattacharya *et al*, 1998; Insel and Young, 2000). In this context, profound changes in hypothalamic–pituitary–adrenocortical (HPA) system regulation have been demonstrated in major depression, and the combined dexamethasone (DEX) suppression/CRH challenge test has proven to be the most sensitive tool for detecting such altered HPA regulation (Holsboer, 2000). Moreover, we have previously provided data consistent with the possibility that normalization of an initial aberrancy in this test is predictive of a favorable antidepressant drug treatment response, and persistent HPA abnormality correlates with

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therapy resistance or relapse (Zobel *et al*, 1999, 2001). Therefore, assuming that normalization of an altered HPA setpoint is likely to be an essential mechanism for antidepressant drug action, the question arises as to the molecular mechanism underlying this readjustment. As CRH and AVP gene expressions are under glucocorticoid control, it has been hypothesized that corticosteroid signalling is impaired in major depression and may be improved via antidepressant drug treatment (Holsboer and Barden, 1996; Ma and Aguilera, 1999). In contrast to antidepressant-induced decreases in CRH, although suggested by *in vitro* studies, similar changes have not been shown for AVP or its receptors *in vivo* (Brady *et al*, 1991; Altemus *et al*, 1992; Butterweck *et al*, 2001; Stout *et al*, 2002). Thus, an appropriate animal model that reflects both significant psychopathological and neuroendocrine features of human depression with face, predictive and construct validity should be used to obtain predictions about the clinical condition in human depression (Geyer and Markou, 1995).

The present series of experiments was conducted to investigate the neuroendocrine and behavioral impact of the antidepresssant paroxetine, a serotonin reuptake inhibitor (SRI) commonly used to treat major depression, in two Wistar rat lines selectively bred for either high (HAB) or low (LAB) anxiety-related behavior (Liebsch *et al*, 1998a, b). Apart from the marked differences in their inborn emotionality (Liebsch *et al*, 1998a, b; Henniger *et al*, 2000; Ohl *et al*, 2001), it is the passive stress-coping behavior in the forced swim (FS) test that renders HAB animals a valuable model for investigating antidepressant treatment strategies (Liebsch *et al*, 1998b; Keck *et al*, 2001a, b; Lucki, 1997). Interestingly, the high emotionality of HAB rats is accompanied by an aberrant outcome of the DEX/CRH test (Keck *et al*, 2002). This phenomenon could be shown to be related to a hypothalamic AVP hyperdrive, thus confirming an early clinical postulate (von Bardeleben and Holsboer, 1989; Holsboer, 2000; Keck *et al*, 2002). Therefore, HAB rats, reflecting cardinal signs and symptoms prevalent in human anxiety disorders and depression, provide a tool to identify neuroendocrine and behavioral alterations associated with clinically efficacious antidepressant drug treatment.

MATERIALS AND METHODS

Animals

The animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Bavaria and the guidelines of the NIH. Experiments were carried out on outbred adult male HAB and LAB rats ($n = 40$; 345 ± 15 g body weight; 3 months of age) (Liebsch *et al*, 1998a, b). Rats were housed in groups of six in the breeding unit of the Max Planck Institute of Psychiatry under standard laboratory conditions until paroxetine treatment (12:12 h light/dark cycle with lights on at 0700 h, $22 \pm 1^{\circ}$C, 60% humidity, pelleted food and water *ad libitum*). During the 9 weeks of paroxetine treatment, rats were housed in groups of two to prevent social isolation stress. After surgery, the rats were housed singly in polycarbon cages ($23 \times 39 \times 36$ cm) until testing, and handled daily to reduce nonspecific stress during the experiments. To control for the increasing body weight, animals were weighed weekly during the experiments.

Antidepressant Drug Treatment

Paroxetine (Glaxo SmithKline, Harlow, UK) is a transisomeric phenylpiperidine with antidepressant properties induced by selective inhibition of the neuronal high affinity uptake of serotonin. Treatment of rats with paroxetine (9 weeks) occurred via the drinking water and antidepressant solutions were renewed every day. A paroxetine concentration of 75 mg/l was applied, resulting in a mean drug intake of approximately 5 mg/kg/day as measured by weighing the drinking bottles at each time of renewal.

Surgery

Paroxetine-treated HAB ($n = 10$) and LAB ($n = 10$) animals as well as the respective vehicle-treated animals of both lines ($n = 10$ each) underwent surgery 5 days before the experiment (in week 9). Surgery was performed under halothane anesthesia using aseptic procedures. The jugular vein was chronically catheterized for subsequent blood sampling as described previously (Keck *et al*, 2000, 2002). The catheter was exteriorized at the neck of the animal and filled with sterile saline containing gentamicin (30 000 IU/rat; Centravet, Bad Bentheim, Germany); 0.2 ml was infused into the animal.

Week 8: Behavioral Testing

FS test: On the day of testing, between 0800 and 1200 h, each rat was placed for 5 min into a cylindrical Plexiglas tank (40×18 cm), filled with tap water (23°C) up to a level of 27 cm (Keck *et al*, 2001a). During each swimming session, the following behaviors were scored by a trained observer blind to rat line and treatment by pressing preset keys on a personal computer: (i) time spent struggling, defined as strongly moving all four limbs, with the front paws breaking the water surface; (ii) time spent swimming, defined as moving all four limbs, swimming around in the tank or actively diving; and (iii) time spent floating, defined as remaining immobile with only occasional slight movements to keep the body balanced and the nose above water.

To control for putative changes in locomotor activity, animals were placed for 5 min in a novel environment ($50 \times 100 \times 40$ cm) and line crossings were counted.

Week 9: Experiment 1—Basal and Stress-Induced HPA System Activity

To monitor the release of ACTH and corticosterone into blood under basal conditions and in response to a combined physical/emotional stressor (5 min forced swimming), blood samples were taken from both paroxetine-treated and control freely moving HAB and LAB rats, beginning 5 days after surgery (5, 15, and 50 min after stress) (Keck *et al*, 2000, 2001a). Briefly, at the experimental day at 0700 h, the jugular venous catheter was connected via 50-cm PE-50 tubing to a plastic syringe filled with sterile heparinized saline (30 IU/ml; Ratiopharm, Ulm, Germany).

Thereafter, the rats remained undisturbed for 2 h to adapt to the equipment.

Week 9: Experiment 2—DEX/CRH Test

Three days after completion of experiment 1, the same animals were weighed at 0700 h. The DEX/CRH test was performed as previously described (Hatzinger *et al*, 1996; Keck *et al*, 2002). In brief, at 0800 h, the jugular venous catheter was connected via 50-cm PE-50 tubing to a plastic syringe filled with sterile heparinized saline (30 IU/ml; Ratiopharm, Ulm, Germany). Thereafter, the rats remained undisturbed. DEX (30 μg/kg, dissolved as phosphate in 0.9% saline; 0.5 ml/kg; Merck, Darmstadt, Germany) was administered intravenously (i.v.) at 1200 h, during the diurnal trough of HPA activity. To monitor the effects of DEX treatment on basal plasma concentrations of ACTH and corticosterone during the diurnal acrophase, four 0.2-ml blood samples were collected at 1800, 1830, 1900, and 1930 h. At 1931 h, CRH (50 ng/kg, 0.5 ml/kg i.v.; Ferring, Kiel, Germany) was injected. To assess the CRH-stimulated ACTH and corticosterone secretion, further blood samples were taken at 1940, 2000, 2020, and 2040 h.

Postmortem Analysis and Histology

Animals were killed with an overdose of halothane at the end of the experiments. Brains were removed, frozen in prechilled *n*-methylbutane on dry ice, and stored at −80°C. Adrenal glands were prepared and weighed immediately. The pituitary, adrenals, and other internal organs were visually examined to exclude animals with tumors from the statistical analysis.

Treatment of Blood Samples, Radioimmunoassays and High-Performance Liquid Chromatography of Paroxetine

Blood samples were collected in prechilled tubes containing EDTA and a protease inhibitor (10 μl aprotinin, Trasylol™, Bayer, Germany) and centrifuged (5 min, 4000 rpm, 4°C). Plasma samples were stored at −20°C until measurement. Plasma ACTH (50 μl) and corticosterone (10 μl) levels were measured using commercially available kits (Biochem, Freiburg, Germany) according to the respective protocols (sensitivity: ACTH, <1.0 pg/ml; corticosterone, <2.0 ng/ml). The intra- and interassay coefficients of variation were 7 and 10%, respectively.

Paroxetine detection: After thawing the plasma samples, 0.05 ml of an internal standard solution (protriptyline 10 μg/ml) and 0.4 ml of 2 M sodium carbonate buffer (adjusted to pH = 10.5 with NaOH) were added to 0.4 ml of plasma or homogenate and vortexed. A total of 5 ml *n*-hexane with 0.5% isoamyl alcohol concentrations were added and the samples were mixed for 30 min at room temperature. After centrifugation at room temperature for 15 min at 3000*g*, the organic layer was transferred to a tube containing 0.3 ml of 0.18 M phosphoric acid, mixed for 30 min and centrifuged at 3000*g* for 10 min. The organic layer was then discarded, and an aliquot of the aqueous phase was injected for chromatographic separation. The extraction recoveries were >90%. A Merck L-7480 fluorescence detector, and a Beckman gradient pump 126 Solvent Module equipped with a Beckman autoinjector 508 autosampler were used for the high-performance liquid chromatography analysis. Separations were made on a Luna 5 μ C18(2) 250 × 4.6 mm column (Phenomenex, Torrance, USA). The mobile phases A (acetonitrile–water, 43.6 mM orthophosphoric acid, 35.9 mM triethylamine (15:85)) and B (acetonitrile) were degassed for 15 min in an ultrasonic bath immediately before use. The column temperature was 60°C and the flow of the mobile phase was 1.0 ml/min. A mobile phase gradient 15–30% B was used for the chromatographic analysis of paroxetine. The substance was determined by fluorescence at the wavelength 295/365 nm.

In situ Hybridization Histochemistry

In situ hybridization histochemistry was performed exactly as previously described in detail in paroxetine-treated and control HAB and LAB rats ($n = 6$ each) (Müller *et al*, 2000). Briefly, consecutive frozen cryostat sections of the rat hypothalamus (14 μm) were alternately mounted on poly-L-lysine-coated slides for subsequent hybridization of AVP mRNA. Sections were stored at −20°C until use. The following specific oligonucleotide DNA probe was used for *in situ* hybridization: AVP(48-mer): 5′-GCA GAA GGC CCC GGC CGG CCC GTC CAG CTG CGT GGC GTT GCT CCG GTC-3′ (Müller *et al*, 2000). All sections were run in the same experiment under identical conditions. The slides were dipped in Kodak NTB2 emulsion diluted 1 : 1 in distilled water, exposed for 5 days at 4°C, and then developed in Kodak D19 solution. The developed slides were lightly counterstained with cresyl violet and examined using a Leica microscope with both bright- and dark-field condensors.

For evaluation of the hybridization signals, sections of the same level of the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus were chosen according to histological criteria. For objective evaluation, images of at least three sections per animal were scanned by a computer-assisted image analyzer (Optimas-Bioscan) fitted with a Zeiss Axioplan microscope and a Sony CCD camera. Quantitative analysis of mRNA expression was performed blind to rat line and treatment as previously described (Müller *et al*, 2000), using the Macintosh-based public domain image analysis program NIH image, version 1.6.1 (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov./nih-image). Quantitative analysis of AVP mRNA expression was performed as an automatic measurement of the optical density over each object (cell) automatically outlined, representing the density of silver grains per labelled cell. The mean for the optical density per cell was calculated for each animal.

Vasopressin 1a (V_{1a}) Receptor Autoradiography

Expression of V_{1a} receptors in the brains of paroxetine-treated and control HAB and LAB rats ($n = 6$ each) was detected by using the specific V_{1a} receptor ligand ^{125}I-HO-phenylacetyl^{1}-D-Tyr(Me)^{2}-Phe^{3}-Gln^{4}-Asn^{5}-Arg^{6}-Pro^{7}-Arg^{8}-NH_2 (^{125}I-HO-LVA) exactly as previously described (Barberis *et al*, 1995).

Quantitative autoradiography was performed on film autoradiographs as described before using computerized densitometry (Optimas 5.2, Optimas, Munich, Germany) in the lateral septum, ventromedial thalamic nucleus, and amygdalostriatal transition area (Keck *et al*, 2001b). Representative areas of the different brain regions were scanned by a digital camera. Care was taken to scan all images under identical light conditions. Optical densities were taken by measuring the mean gray value in the region of interest and blind to the treatment conditions, and presented as units of optical density. For all regions, 5–7 bilateral measurements were obtained for each animal, and weighted mean values of all groups were compared.

Statistical Analysis

Results are reported as means ± SEM. After CRH administration, ACTH and corticosterone responses were computed as the area under the concentration–time curve (AUC, arbitrary units) corrected for basal concentration (averaged plasma concentration of ACTH and corticosterone, respectively, between 1800 and 1930 h) using trapezoidal integration as described before (Keck *et al*, 2002). Statistical analysis was performed with a statistical software package (GB-Stat version 6.0, Dynamic Microsystems, Silver Spring, MD, USA). Statistical significance for plasma ACTH/corticosterone levels was determined by three-way ANOVA (line × treatment × time) with repeated measures on the last factor. Analysis of behavioral parameters was carried out using a two-way ANOVA (line × treatment). When appropriate, a *post hoc* Fisher's LSD test was used. The Mann–Whitney U-test was used for comparison of AUC values. The density of silver grains per labelled cell (AVP *in situ* hybridization) or the optical density of the region of interest (V_{1a} receptor autoradiography) were analyzed by Kruskal–Wallis one-way ANOVA followed by the Mann–Whitney U-test. Statistical significance was accepted if $P<0.05$.

RESULTS

All experimental animals were included in the statistical analysis as postmortem visual examination failed to reveal any organ pathologies. The weight gain of the rats, controlled weekly, was indistinguishable between groups (data not shown). The addition of paroxetine to the drinking water slightly reduced daily water intake during the first 3 weeks, but this reduction did not reach statistical significance (data not shown). Paroxetine plasma concentrations were found to be similar in HAB and LAB rats (60 ± 2 and 58 ± 4 ng/ml, respectively).

FS Test

A two-way ANOVA (line × treatment) revealed a significant line difference for the parameter time spent struggling ($F_{1,36}=39.7$, $P<0.0001$) and floating ($F_{1,36}=63.9$, $P<0.0001$), with a significant interaction with factor treatment for time spent struggling ($F_{1,36}=9.31$, $P=0.0043$) and floating ($F_{1,36}=31.6$, $P<0.0001$) (Figure 1). *Post hoc* analysis showed that the paroxetine-treated HAB animals showed significantly more struggling ($P<0.05$) than the vehicle-treated HAB controls and floated less ($P<0.01$). With respect to floating behavior, paroxetine-treated HAB rats were indistinguishable from LAB rats (Figure 1).

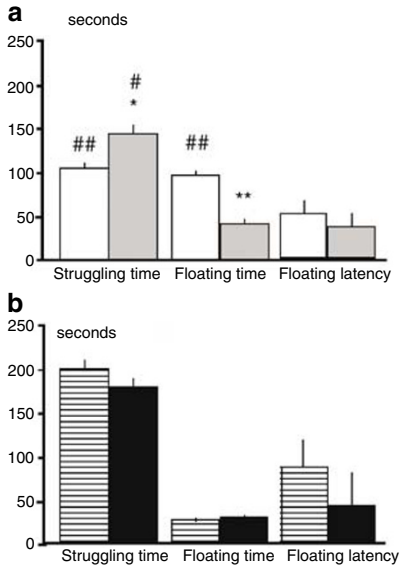

Figure 1 Behavioral measures obtained in the FS test in HAB (upper panel: vehicle, open bars, paroxetine, gray bars) and LAB (lower panel: vehicle, striped bars, paroxetine, black bars) rats ($n=10$ per group). Time during the 5-min testing session spent struggling and floating, respectively, and latency until the first floating reaction. Data are means+SEM. *$P<0.05$, **$P<0.01$ *vs* paroxetine treatment in HAB rats; # $P<0.05$, ## $P<0.01$ *vs* LAB.

Novel Environment

After long-term paroxetine, there was no effect on locomotor behavior as measured by the total number of line crossings made (HAB: vehicle, 4.5 ± 0.7; paroxetine, 3.1 ± 0.9; LAB: vehicle, 5.2 ± 0.5; paroxetine, 4.4 ± 0.8 line crossings).

Effects of Long-Term Paroxetine on Basal and Stress-Induced HPA System Activity

Basal plasma ACTH (HAB: vehicle, 91.4 ± 18.8; paroxetine, 64.9 ± 14.2 pg/ml; LAB: vehicle, 80.3 ± 8; paroxetine, 83.2 ± 6.9 pg/ml) and corticosterone (HAB: vehicle, 49.8 ± 20.8; paroxetine, 29.5 ± 5.5 ng/ml; LAB: vehicle, 35.6 ± 10.7; paroxetine, 27.8 ± 9.4 ng/ml) levels were comparable in the paroxetine-treated and control HAB and LAB rats (Figure 2). FS caused a significant increase in plasma ACTH (three-way ANOVA, factor: time; $F_{3,108}=83.6$, $P<0.0001$) and corticosterone ($F_{3,108}=269.4$, $P<0.0001$) in all groups. Plasma ACTH and corticosterone levels 5, 15, and 50 min after FS were statistically indistinguishable between the groups (Figure 2).

Effects of Long-Term Paroxetine on the DEX/CRH Test

After CRH, the release of ACTH stimulated by CRH was significantly greater in the vehicle-treated HAB than in the LAB rats (three-way ANOVA, line × treatment × time;

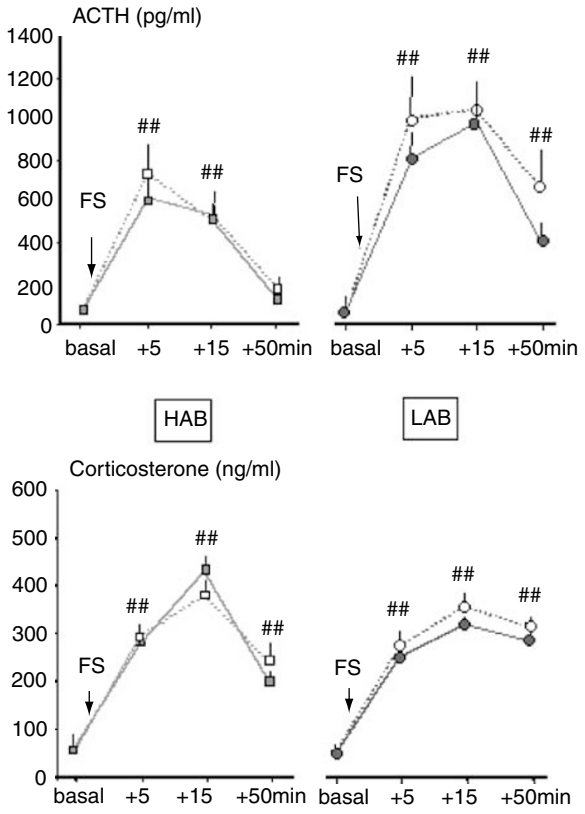

Figure 2 Effects of 5-min exposure to FS on plasma ACTH (upper panel) and corticosterone (lower panel) of vehicle-treated (dashed lines, open symbol) and paroxetine-treated (solid lines, dark symbol) male HAB (squares; left panel) and LAB (circles; right panel) rats ($n=10$ per group). Data are means+SEM. ## $P<0.01$ *vs* basal.

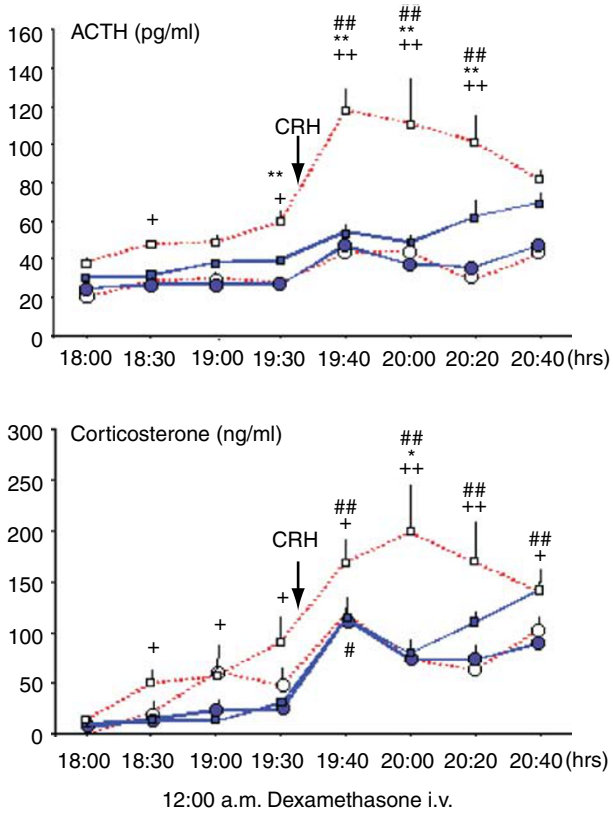

Figure 3 Plasma ACTH (upper panel) and corticosterone (lower panel) concentrations of vehicle-treated (dashed lines, open symbol) and paroxetine-treated (solid lines, dark symbol) male HAB (squares) and LAB (circles) rats ($n=10$ in each group) between 1830 and 2040 h during the combined DEX/CRH test. All rats were pretreated with DEX (30 μg/kg i.v.) at 1200 h. Data are means+SEM. # $P<0.05$, ## $P<0.01$ *vs* basal; * $P<0.05$, ** $P<0.01$ *vs* LAB; + $P<0.05$, ++ $P<0.01$ *vs* paroxetine-treated HAB rats.

$F_{7,252}=5.49$, $P<0.0001$; Figure 3), which was also reflected by higher AUC values (HAB: 3714 ± 779; LAB: 736 ± 141; $P<0.01$). The maximal rise of the plasma corticosterone concentration after CRH stimulation was significantly greater in the vehicle-treated HAB than in the LAB rats (line × time; $F_{7,252}=3.17$, $P=0.0031$). Again, this was also demonstrated by higher AUC values (HAB: 6440 ± 385; LAB: 5627 ± 258; $P<0.05$). Basal ACTH and corticosterone levels between 1830 and 1930 h significantly differed between paroxetine-treated and control HAB rats ($P<0.05$). Similarly, paroxetine treatment attenuated the CRH-stimulated increase in both ACTH and corticosterone secretion seen in the vehicle-treated HAB rats ($P<0.01$ and $P<0.05$, respectively). This effect was also reflected by lower AUC values for ACTH (vehicle: 3714 ± 779; paroxetine: 1793 ± 483; $P<0.01$). There were no statistically significant differences between paroxetine-treated and vehicle-treated LAB rats (Figure 3).

Expression of AVP mRNA within the PVN and Supraoptic Nucleus of Vehicle- and Paroxetine-Treated HAB and LAB Rats

Only a few scattered silver grains were detectable within the parvocellular neurons of the PVN synthesizing AVP in male vehicle- and paroxetine-treated HAB and LAB rats decapitated under basal conditions. In contrast, the density of silver grains per cell within the magnocellular part of the PVN was significantly higher in the vehicle-treated HAB rats than in the LAB rats (Kruskal–Wallis ANOVA followed by Mann–Whitney U-test; $P<0.01$) and decreased after paroxetine treatment in HAB rats ($P<0.05$; $n=6$ per group) (Figure 4a). There was no difference between paroxetine treated and control LAB rats. There were no differences in the supraoptic nucleus in either group (data not shown).

V_{1a} Receptor Autoradiography in Vehicle- and Paroxetine-Treated HAB and LAB Rats

Specifically labelled V_{1a}-binding sites were higher in the lateral septum of vehicle-treated HAB compared to LAB rats ($P<0.05$) (Figure 4b). In the other brain regions examined, there were no statistically significant differences between HAB and LAB animals. Paroxetine treatment had no influence on V_{1a}-receptor expression in either HAB or LAB rats (Figure 4b).

DISCUSSION

There is clearly a need for new antidepressant drugs that are pharmacologically distinct from the existing therapeutic agents in providing a greater rapidity of response by acting

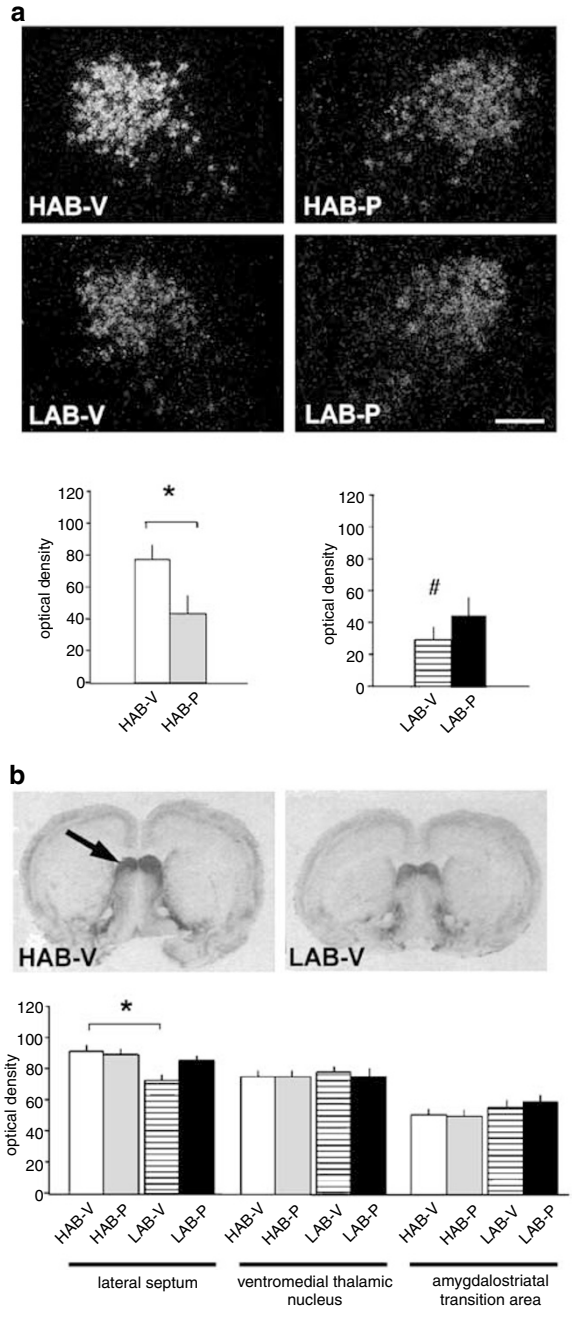

Figure 4 (a) AVP mRNA *in situ* hybridization. Upper panel: Representative sections showing the PVN of the hypothalamus (dark field). The density of silver grains per cell within the magnocellular part of the PVN was significantly higher in the vehicle-treated HAB (HAB-V) rats than in the LAB rats and decreased after paroxetine treatment in HAB rats (HAB-P) ($n = 6$ per group). There was no difference between paroxetine-treated (LAB-P) and control LAB (LAB-V) rats. Scale bar is 100 μm. Lower panel: Quantitative determination of the optical density per cell. Data are means+SEM. *$P < 0.05$, *vs* paroxetine-treated HAB rats. # $P < 0.05$ *vs* vehicle-treated HAB rats. (B) V_{1a} receptor autoradiography. Upper panel: Representative autoradiography displaying that specifically labelled V_{1a}-binding sites are higher in the lateral septum (arrow) of vehicle-treated HAB (HAB-V) compared to LAB (LAB-V) rats. In the other brain regions examined, there were no statistically significant differences between HAB and LAB animals. Paroxetine treatment had no influence on V_{1a}-receptor expression in either HAB (HAB-P) or LAB (LAB-P) rats. Lower panel: Quantitative determination of specific V_{1a}-binding densities. Data are means+SEM. *$P < 0.05$ *vs* vehicle-treated HAB rats.

more causally. However, it is still unclear by which pharmacological mode of action currently available antidepressants exert their clinical effects. In recent years, neuropeptidergic circuits known to be dysregulated in major depression like CRH and AVP neuronal systems have attracted attention as potential therapeutic targets (Hökfelt *et al*, 2000; Keck and Holsboer, 2001). In this study, we provide first evidence that paroxetine, a clinically well-established antidepressant, normalizes aberrant behavioral and neuroendocrine patterns in the psychopathological animal model of HAB rats. As it was recently demonstrated that a hypothalamic vasopressinergic hyperdrive accounts for the disturbance in HPA system regulation prevalent in these rats (Keck *et al*, 2002), the paroxetine-induced reduction of vasopressinergic overexpression indicates that this neuropeptidergic system may be critically involved in antidepressant drug action.

Long-Term Paroxetine Induces Active Stress-Coping Behavior

After 8 weeks of continuous paroxetine treatment, animals were tested in the FS test, which is considered to have predictive value for the efficacy of antidepressant treatments in humans (Lucki, 1997). As has been previously shown in response to the novel antidepressant treatment of repetitive transcranial magnetic stimulation (Keck *et al*, 2001a), paroxetine treatment markedly increased active stress coping in HAB rats only: HAB animals, innately displaying a markedly passive stress-coping strategy, struggled significantly more and floated less than the controls, indicating that these treatment-induced shifts towards active coping are determined by the rats' innate emotionality. In contrast, the behavior of LAB rats remained unchanged. In the same fashion, antidepressant effects are observed in depressed patients only, but not in normal controls, suggesting that the neurochemical effects of antidepressant drugs may be different in disturbed *vs* intact systems (Hökfelt *et al*, 2000). There was no change in general locomotor activity in HAB rats as measured in a novel environment, rejecting the possibility that paroxetine-induced changes in swimming behavior might be because of alterations in the rats' locomotion.

Long-Term Paroxetine Normalizes the Aberrant Outcome of the DEX/CRH Test

In up to 90% of depressed patients, the DEX/CRH test unveils even subtle stress hormone disturbances owing to central CRH and AVP overexpression, which, under basal conditions, are not necessarily reflected in peripheral plasma ACTH and corticosteroid concentrations (Holsboer, 2000). Correspondingly, basal and stress-induced plasma ACTH and corticosterone levels were comparable in the paroxetine-treated and control HAB and LAB rats during week 9 of paroxetine treatment. Similarly, in other studies, long-term treatment with the SRIs fluoxetine and paroxetine did not prevent adequate activation of the HPA system in response to severe stressors such as FS, immobilization or footshocks (Zhang *et al*, 2000; Connor *et al*, 2000; Stout *et al*, 2002). In contrast, we could show that after DEX pretreatment, basal ACTH levels at the onset of the activity

phase at 1930 h were significantly higher in vehicle-treated HAB rats when compared to LAB rats. After CRH stimulation, the release of ACTH and corticosterone was also significantly greater in the vehicle-treated HAB than in the LAB rats.

In patients suffering from major depression, the aberrant outcome in the DEX/CRH test can be normalized by effective antidepressant drug treatment (Holsboer, 2000; Zobel *et al*, 1999, 2001). Correspondingly, like in patients suffering from major depression (Nickel *et al*, 2003), paroxetine treatment attenuated the CRH-stimulated hypersecretion of both ACTH and corticosterone seen in the vehicle-treated HAB rats. Basal ACTH and corticosterone levels between 1830 and 1930 h also significantly differed between paroxetine-treated and control HAB rats. In contrast, like in the FS test, there were no statistically significant differences between paroxetine-treated and vehicle-treated LAB rats.

Long-Term Paroxetine Reduces Hypothalamic Vasopressinergic Hyperdrive

AVP is mainly synthesized in the magnocellular neurons of the hypothalamic PVN and the supraoptic nucleus. In addition, CRH and AVP are (co) localized in parvocellular neurons of the PVN (Tilders *et al*, 1993), representing the hypothalamic origin of the HPA system. It is well established that AVP potently synergizes with CRH to stimulate pituitary ACTH secretion (von Bardeleben and Holsboer, 1989; Gillies *et al*, 1982). In this context, there is strong evidence that AVP derived not only from parvocellular neurons of the PVN, but also from the magnocellular neurosecretory system, contributes to HPA system activity: AVP can be released from magnocellular neurons into the portal blood system, either by *en passant* release while passing through the internal zone of the median eminence or via short portal vessels from the posterior pituitary (Antoni *et al*, 1993; Wotjak *et al*, 1996a, b).

As has been hypothesized for human major depressive illness (Von Bardeleben and Holsboer, 1989), we could recently show that in the HAB rats the innate (magnocellular) vasopressinergic hyperdrive overrides the inhibitory action of DEX on ACTH release by enhanced AVP secretion, acting synergistically to the external CRH challenge in the DEX/CRH test (Keck *et al*, 2002). The CRH/AVP synergism is known to be physiologically relevant under conditions of long-term activation of the HPA system, such as chronic stress in rats (eg de Goeij *et al*, 1992), aging, and human depression (Purba *et al*, 1996; van Londen *et al*, 1997; Keck *et al*, 2000; Holsboer, 2000). It was therefore of interest to investigate whether chronic paroxetine treatment might be able to reduce the hypothalamic vasopressinergic overexpression in HAB rats, thereby normalizing the outcome of the DEX/CRH test. Indeed, basal AVP mRNA expression in the magnocellular neurons of the hypothalamic PVN, which was significantly higher in the control HAB rats than in the LAB rats, decreased after paroxetine treatment. Again, paroxetine was without effect in LAB animals. Interestingly, AVP mRNA was found to be unchanged in the magnocellular neurons of the hypothalamic supraoptic nucleus of paroxetine-treated rats of either line (data not shown), pointing towards the specificity of antidepressant treatment to regulate intra-PVN AVP gene expression. It should be noted, however, that under basal conditions the physiological expression of AVP in parvocellular neurons is very low (Tilders *et al*, 1993), and therefore subtle differences might have been below the detection limit in the present study.

So far, as opposed to CRH and its receptors (Keck and Holsboer, 2001), only indirect evidence for a role of AVP in the treatment of affective disorders came from the finding that fluoxetine treatment led to a reduction in cerebrospinal fluid concentrations of AVP in patients with major depression (de Bellis *et al*, 1993). In rodent experiments, using commercially obtained animals, a downregulation of AVP in response to chronic antidepressant drug treatment has not been reported (eg Butterweck *et al*, 2001). This finding is in accordance with our observation that paroxetine was without effect in LAB animals. Therefore, our findings in HAB rats, an animal model of psychopathology, underline the hypothesis that neuropeptides are only released at significant quantities under potentially pathogenic conditions, rendering peptidergic mechanisms promising targets for drug development (Hökfelt *et al*, 2000).

The V_{1a} receptor, which is highly expressed in the rat lateral septum, thalamic nuclei, and the amygdalostriatal transition area (Barberis and Tribollet, 1996), is well known to play a role in a variety of behavioral effects such as the modulation of emotionality and stress coping (Landgraf *et al*, 1998). Specifically, septal vasopressin has been shown to increase anxiety-related behavior in rats (Landgraf *et al*, 1995; Liebsch *et al*, 1996; Ebner *et al*, 1999). Accordingly, we found that in vehicle-treated HAB rats, displaying an increased inborn emotionality, V_{1a}-binding sites were higher in the lateral septum when compared to LAB rats, pointing towards an innate hyperdrive of the septal vasopressinergic system. However, despite its marked effects on stress-coping behavior, paroxetine had no influence on septal V_{1a}-receptor expression in either HAB or LAB rats.

Neurons in the PVN receive a moderate serotonergic innervation from the raphe nuclei (Sawchenko *et al*, 1983), and both electrophysiological and pharmacological studies indicate that these monoaminergic inputs play a critical excitatory role in the release of AVP at the neurohypophysis (eg Faull *et al*, 1993). Therefore, the possibility exists that the changes in AVP gene expression measured in the present study might be secondary to adaptive alterations in the serotonergic system (Raap and van de Kar, 1999). However, recent studies on the effects of serotonin or serotonin receptor 5-HT_{2A} activation indicate either no effect on AVP-containing neurons (Javed *et al*, 1999; van de Kar *et al*, 2001) or demonstrated an increase in intra-PVN AVP gene expression in a transgenic mouse model in which the inactivation of the monoamine oxidase-A gene results in increased amounts of serotonin in the brain (Vacher *et al*, 2002). Hence, it is rather unlikely that adaptive changes in the serotonergic system owing to long-term paroxetine account for the decrease in AVP gene expression in HAB rats.

Most antidepressants in current clinical use like paroxetine have well-documented effects on the disposition of biogenic amines that are readily demonstrable both *in vitro* and *in vivo*. However, converging lines of evidence such as

the delayed onset of action common to all antidepressants have led us beyond the monoaminergic synapse for strategies to improve antidepressant therapy. Using a psychopathological rat model, the present data suggest that neuroendocrine and behavioral antidepressant drug effects are at least in part mediated via modulation of AVP gene expression and that normalization of vasopressinergic neurotransmission after antidepressant drug treatment may lead to restoration of disease-related alterations. As administration of a V_1 receptor antagonist was recently shown to induce a normalization of HPA system activity in HAB rats, the effect of paroxetine on AVP gene expression is likely to be a primary mechanism of its antidepressant action rather than a secondary effect due to behavioral alterations (Keck *et al*, 2002). As AVP gene expression is suppressed by glucocorticoids (Antoni, 1993; Ma and Aguilera, 1999; Kim *et al*, 2001), a paroxetine-induced upregulation in glucocorticoid and mineralocorticoid receptor expression and binding capacity may be involved in our observations. Indeed, in the hippocampus, such upregulation has been demonstrated repeatedly in response to a variety of antidepressant drugs such as tricyclics and monoamine oxidase inhibitors irrespective of their primary pharmacological profile (Reul *et al*, 2000). Alternatively, paroxetine treatment may influence the intracellular cascade regulating AVP transcription. In addition, our results are consistent with the emerging consensus that under chronic stress and certain psychopathological conditions, AVP is a principal regulated variable that imparts stimulus-specific drive on the HPA system.

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