Neonatal Treatment of Rats with the Neuroactive Steroid Tetrahydrodeoxycorticosterone (THDOC) Abolishes the Behavorial and Neuroendocrine Consequences of Adverse Early Life Events

Vladimir K. Patchev,* Alexandra Montkowski,* Daniela Rouskova,* Lajos Koranyi,‡ Florian Holsboer,* and Osborne F.X. Almeida*

**Department of Neuroendocrinology, Max Planck Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany; ‡Department of Clinical and Experimental Laboratory Medicine, Postgraduate Medical School, Haynal University of Health Sciences, 1135 Budapest, Hungary*

Abstract

Stressful experience during early brain development has been shown to produce profound alterations in several mechanisms of adaptation, while several signs of behavioral and neuroendocrine impairment resulting from neonatal exposure to stress resemble symptoms of dysregulation associated with major depression. This study demonstrates that when applied concomitantly with the stressful challenge, the steroid $GABA_A$ receptor agonist 3,21-dihydropregnan-20-one (tetrahydrodeoxycorticosterone, THDOC) can attenuate the behavioral and neuroendocrine consequences of repeated maternal separation during early life, e.g., increased anxiety, an exaggerated adrenocortical secretory response to stress, impaired responsiveness to glucocorticoid feedback, and altered transcription of the genes encoding corticotropin-releasing hormone (CRH) in the hypothalamus and glucocorticoid receptors in the hippocampus. These data indicate that neuroactive steroid derivatives with GABA-agonistic properties may exert persisting stress-protective effects in the developing brain, and may form the basis for therapeutic agents which have the potential to prevent mental disorders resulting from adverse experience during neonatal life. (*J. Clin. Invest.* 1997. 99:962–966.) Key words: neurosteroid • maternal separation • corticosterone • corticotropin-releasing hormone • glucocorticoid receptor

Introduction

The neuroactive steroid 3,21-dihydroxypregnan-20-one (tetrahydrodeoxycorticosterone, THDOC)[1] is a potent endogenous modulator of the γ-aminobutyric acid ($GABA_A$) receptor and displays significant sedative, antiaggressive, and anxiolytic properties (1–4). Its concentration in the brain is reportedly augmented during exposure to stress (5). Previous studies have thus suggested that THDOC and other chemically related steroid compounds might serve as endogenous stress-protective agents (6, 7). This view was further confirmed by studies with the related ring-A-reduced steroid 3α-hydroxy-5α-pregnan-20-one (tetrahydroprogesterone, THP) which proved efficacious in counteracting anxiety induced by corticotropin-releasing hormone (CRH), in addition to acting at central sites to dampen the activity of the hypothalamo-pituitary-adrenal (HPA) axis (8, 9).

While the studies summarized above were all carried out in adult animals, little is known about the role of neurosteroids in the developing brain. However, a recent paper describing the ability of neurosteroids to influence GABA-ergic mechanisms in the infant brain (10) led us to examine whether neonatal administration of THDOC can produce long lasting changes in the neurochemical substrates involved in the regulation of behavioral and neuroendocrine processes, in particular those related to anxiety and stress. The present experiments, conducted in rats, were performed in light of the literature suggesting that stressful experiences during perinatal life result in profound and irreversible alterations in the mature organism's behavioral and neuroendocrine responses to stress, such as increased anxiety (11, 12), elevated synthesis of hypothalamic CRH (13, 14), and decreased responsiveness of the HPA axis to glucocorticoid negative feedback (13).

In this study, THDOC was administered to infant rats during the first 10 postnatal days before repeated separation from their mothers (a well-established paradigm for induction of stress during early development; c.f., references 15–17); the animals were subsequently tested as adults to examine whether THDOC given alone or concomitantly with the neonatal stress had produced any long lasting behavioral and neuroendocrine alterations. Specifically, we assessed anxiety, diurnal, and stress-induced adrenocortical secretion, the ability of exogenous glucocorticoids to attenuate the humoral response to stress, and the steady state expression of genes encoding CRH and glucocorticoid receptors in pertinent brain areas that contribute to the regulation of the HPA axis.

Methods

Animals. Pregnant Wistar dams (Max Planck Institute of Biochemistry, Martinsried, Germany) were single-housed under controlled illumination (12/12 h, lights on at 7:00 am) and ambient temperature (24°C), and had free access to food and water. Within 6–8 h after delivery, litters were culled to six male pups each. Separate groups of infant rats were used for each of the experimental treatments described below.

Address correspondence to V.K. Patchev, M.D., Ph.D., Department of Neuroendocrinology, Max Planck Institute of Psychiatry, Clinical Institute, Kraepelinstr. 2, 80804 Munich, Germany. Phone +4989-3062-2524; FAX: +4989-3062-2461.

Received for publication 25 September 1996 and accepted in revised form 16 December 1996.

1. *Abbreviations used in this paper:* CORT, corticosterone; CRH, corticotropin-releasing hormone; DEX, dexamethasone; GABA, γ-aminobutyric acid; GR, glucocorticoid receptor; HPA, hypothalamo-pituitary-adrenal; PVN, paraventricular nucleus; THDOC, tetrahydrodeoxycorticosterone (3,21-dihydroxypregnan-20-one); USV, ultrasonic vocalization.

J. Clin. Invest.

0021-9738/97/03/0962/05 $2.00
Volume 99, Number 5, March 1997, 962–966

Determination of the anxiolytic dose of THDOC in infant rats. A pilot study was undertaken in order to define an effective anxiolytic dose of THDOC in infant rats. All steroids tested were purchased from Steraloids (Wilton, NH). THDOC and its parent steroid corticosterone (CORT) were solubilized in an aqueous solution of 2-hydroxypropyl-β-cyclodextrin (RBI, Natick, MA), and two different doses (20 and 40 μg/rat) or vehicle were subcutaneously injected in 7-d-old pups 30 min before separating them from their dams. Each individual was placed in a plastic box without bedding, and episodes of ultrasonic vocalization (USV) in the frequency range of 40–42 KHz were transformed to audible signals with a bat detector (QMC Instruments, London, UK) and tape-recorded online (Revox B77 MK II) for a period of 5 min. Tape recordings were transcribed with a polygraph recorder (Nihon Kohden, Tokyo, Japan), and the number of USV episodes produced by each individual was counted.

Neonatal stress and chronic THDOC administration. Between postnatal days 2 and 10, pups were separated from their dams for 8 h (10:00 am–6:00 pm) on every second day (total five sessions), while subcutaneous injections of either vehicle (corn oil) or THDOC in a volume of 0.1 ml were given daily for 10 consecutive days immediately prior to the separation sessions. Based on the results in the above-described pilot experiment, THDOC was applied in a daily dose of 20 μg/rat. Vehicle-injected animals that were left undisturbed with their dams (apart from a weekly change of the bedding) served as controls. Rats were weaned at the age of 21 d, and housed in groups of six for the rest of the experiment.

Assessment of anxiety in the elevated plus-maze. 1 d before experimentation, the animals were transferred to the testing room to allow acclimation to the new environment. Observations were performed between 9:00 am and noon at the age of 75–80 d by an investigator who was unaware of the treatment conditions. Rats were individually placed in the center of the elevated plus-maze, and the behavior was monitored over a period of 5 min using a video camera and automatic data logger. The percent ratios between the number of entries and time spent in the open compartments versus total number of entries and time spent in both types of arms was computed for each individual and used as a measure of anxiety, i.e., rats displaying higher preference for the open compartments are considered less anxious and vice versa (18).

Basal and stress-induced adrenocortical secretion in adult rats. These studies were performed between 85 and 100 d of age. Circulating CORT levels were determined by radioimmunoassay (ICN, Costa Mesa, CA) in serum samples obtained through tail skin incisions. For measurements of basal diurnal CORT secretion, blood was collected on two different days between 7:00 and 9:00 am and 7:00 and 9:00 pm, respectively. 1 wk later, acute stress-induced release of CORT was measured after exposing the animals to a series of intermittent air puffs (delivered with a hair dryer) for 2 min. Blood samples were withdrawn 20 min after termination of the stress session. Finally, the efficacy of dexamethasone (DEX) in attenuating stress-induced CORT secretion was tested after 7 d of recovery from the previous experiment. Dexamethasone (Fortecortin; Merck, Darmstadt, Germany) was injected at a dose of 50 μg/kg 30 min before the stress procedure, and blood samples were collected 20 min after termination of the stress procedure. All procedures were in compliance with national regulations on animal welfare.

In situ hybridization histochemistry. 3 wk after the last blood collection, the rats were killed by decapitation, brains were rapidly removed from the skull, snap-frozen by brief immersion in prechilled isopentane, and stored at −80°C until sectioning. Coronal cryosections (14 μm) were thaw-mounted on gelatine-coated slides; the nucleotide probes used, and the procedures of fixation, permeabilization, and delipidation are described elsewhere (19). Autoradiographs from hybridized sections were generated by exposure to Hyperfilm β-max (Amersham International; Little Chalfont, UK) for periods of various duration. Specificity of the hybridization signals was monitored using adjacent sections which were hybridized with the corresponding sense probes.

Autoradiographic signals in brain regions of interest were quantified by computer assisted densitometry (NIH Image 1.52; National Institute of Mental Health, Bethesda, MD) with automatic background subtraction. Optical densities were converted into microcuries per gram tissue by a third order polynomial equation using calibration curves generated from co-exposed radioactive standards (ARC Inc.; St. Louis, MO). Four measurements in two consecutive sections were performed in each animal, and individual means were calculated.

Statistics. Comparisons between behavioral data were made by nonparametric one-way ANOVA followed by the Wilcoxon-Mann-Whitney test for pairs of independent groups, when appropriate. Results from hormone measurements and autoradiographic densitometry were analyzed by one-way ANOVA and, subsequently, by the Student-Neumann-Keuls test. The minimal level of significance was preset at $P \leq 0.05$.

Results

THDOC dose-dependently suppresses USV in infant rats. When separated from their dams, vehicle-treated infant rats vocalized in the frequency range of 40–42 KHz once to twice per second during the entire observation period of 5 min. Pretreatment with 20 μg of THDOC significantly reduced, while the dose of 40 μg completely abolished, episodes of USV (Fig. 1, *A* and *B*). In both doses tested, CORT significantly suppressed USV; however, its effects failed to display signs of dose dependency.

THDOC attenuates the behavioral consequences of neonatal maternal deprivation. Results from the plus-maze tests for anxiety in adult rats are shown in Fig. 2, *A* and *B*. Subjects that had received vehicle injections at the time of maternal separation during infancy showed significantly higher levels of anxiety as compared to their nondeprived counterparts, as indicated by the fact that they spent a significantly shorter time in the open arms of the plus-maze. Daily administration of 20 μg THDOC abolished the anxiogenic after-effects of neonatal stress, and nondeprived rats which had received THDOC treatment tended to show lower levels of anxiety, although this

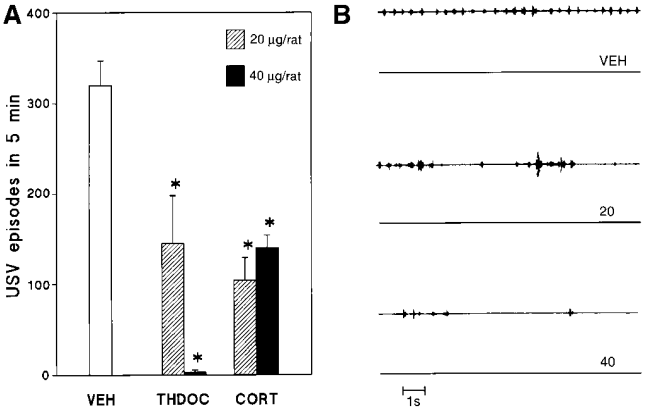

Figure 1. Anxiolytic effects of pretreatment with two different doses (20 and 40 μg/rat; hatched and solid bars, respectively) of THDOC and CORT, as determined by the suppression of maternal separation-induced USV in 7-d-old rats. (*A*) Each bar represents the mean±SEM of four individuals; * significant differences as compared to vehicle. (*B*) Excerpts of polygraph recordings of USV in rats injected with either vehicle or two doses of THDOC; the time-bar corresponds to 1 s.

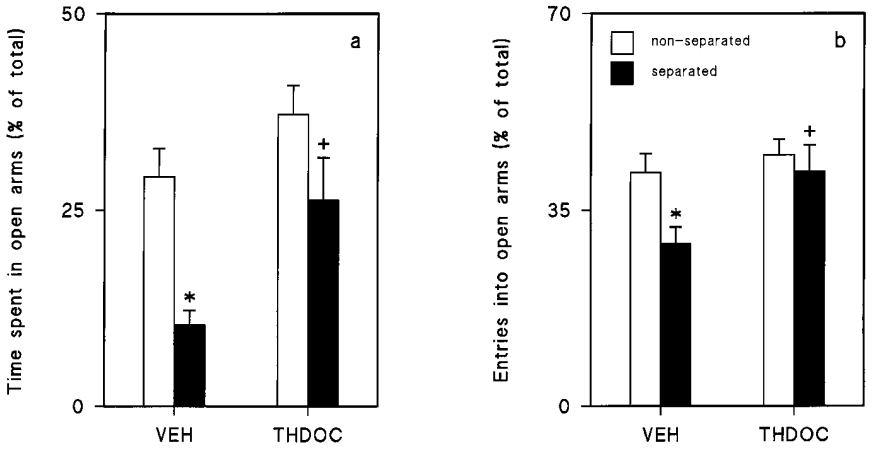

Figure 2. Effects of chronic maternal separation (*solid bars*) and daily treatment with 20 μg THDOC or vehicle on indices of anxiety, as measured in the elevated plus-maze. (*A*) shows the time spent in, and (*B*) displays the number of entries into, the open compartments of the maze. Each bar represents the mean± SEM of six individuals. * significant effects between maternally deprived and nondeprived rats (*open bars*); $^{+}$significant effects of THDOC treatment in maternally separated animals.

tendency did not prove to be statistically significant. The entries into the open arms (Fig. 2 *B*) displayed changes that were generally parallel to those of behavioral anxiety.

Effects of maternal deprivation and THDOC treatment on basal and stress-induced adrenocortical secretion and responsiveness to glucocorticoid feedback. Resting and nighttime levels of CORT were similar in all groups of animals, with no apparent influence of either maternal separation or THDOC treatment (data not shown). Exposure to air-puff stress resulted in a significantly elevated CORT secretion in rats that had received vehicle during sessions of maternal separation (Fig. 3 *A*). In contrast, all groups of animals (maternally deprived and nondeprived) that were injected with THDOC showed a CORT response to the stressful stimulus that was similar to that measured in control (nondeprived, vehicle treated) subjects.

Pretreatment of rats with DEX (50 μg/kg) before exposure to the air-puff stress resulted in a significant attenuation of the CORT response to stress in all animals; however, the suppressive effect of DEX was significantly weaker in maternally deprived and vehicle-injected rats, when compared to all other groups. This effect of maternal separation was not present in rats that had received concomitant THDOC treatment (Fig. 3 *B*).

THDOC attenuates neonatal stress-induced alterations in the gene expression of CRH and corticosteroid receptors in the brain. The hybridization signal for CRH mRNA in the hypothalamic paraventricular nucleus (PVN) was significantly elevated in rats which had experienced maternal separation and received vehicle treatment (Fig. 4 *A*). This effect of maternal deprivation was abolished in rats injected with THDOC. In rats which were not subjected to neonatal stress, THDOC failed to produce significant changes in CRH gene transcription.

Maternally deprived, vehicle-treated rats displayed significantly lower hybridization signal for glucocorticoid receptor (GR) mRNA, as compared to their nonseparated counterparts, and this effect of maternal deprivation was significantly attenuated by concomitant THDOC treatment. However, GR mRNA levels in neonatally stressed rats were still significantly lower than those seen in controls. THDOC alone failed to significantly alter the gene expression of GR in the hippocampus (Fig. 4 *B*).

Discussion

Chronic stress in the form of intermittent maternal deprivation during early postnatal life has been repeatedly shown to induce profound and irreversible alterations in neuroendocrine and behavioral mechanisms of adaptation. Thus, when tested as adults, rats that had been subjected to maternal separation in infancy show abnormal endocrine responses to stress and alterations in central mechanisms controlling HPA activity (13,

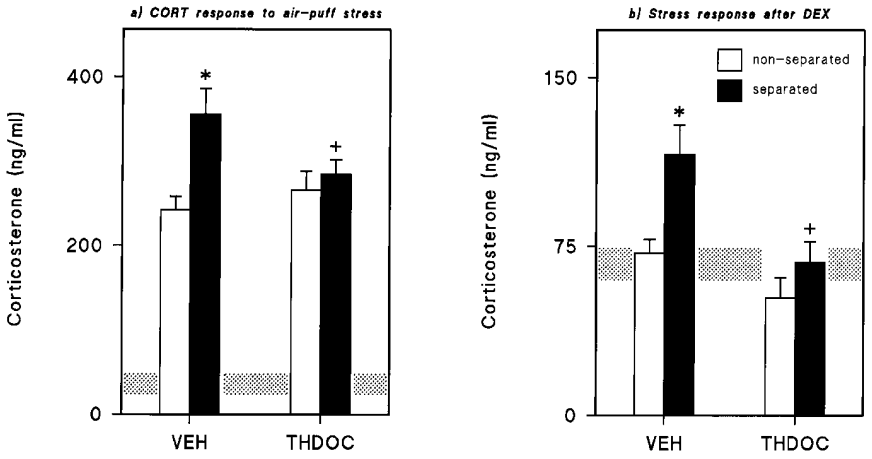

Figure 3. Adrenocortical secretory response to emotional stress (*A*) and its suppression by pretreatment with 50 μg/kg dexamethasone (*B*) in rats which were exposed to repeated maternal separation (*solid bars*) and received daily injections of THDOC or vehicle during the first 10 d of life. Shaded area indicates the range (±SEM) of basal CORT secretion (*A*) and DEX-induced suppression in naive age- and sex-matched rats (*B*). Each bar represents the mean±SEM of 5–6 determinations. * significant effects of maternal deprivation; $^{+}$significant effects of THDOC administration versus the corresponding vehicle-treated group.

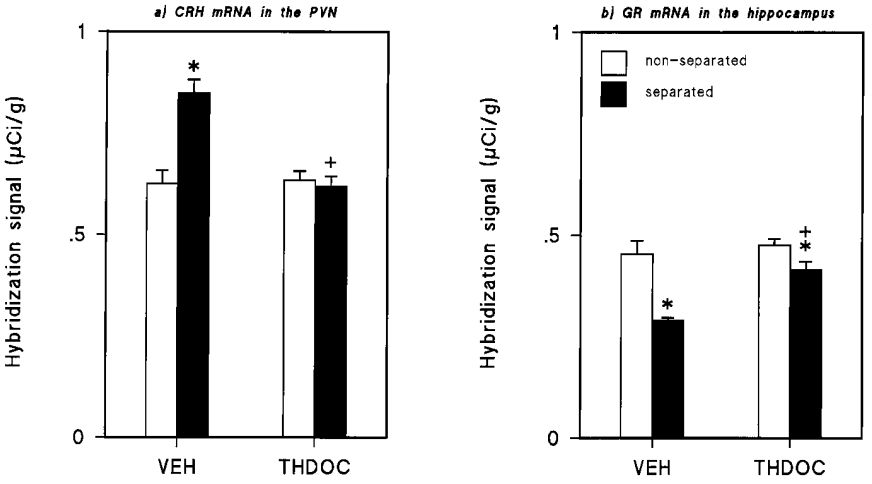

Figure 4. Changes in mRNA levels encoding CRH in the PVN (*A*) and GR (*B*) in the hippocampal subfield CA_{1-2}, resulting from repeated maternal separation (*solid bars*) and concomitant vehicle or THDOC treatment during early infancy. * significant differences between maternally deprived and nonseparated rats (*open bars*); $^{+}$significant effects of THDOC versus vehicle treatment in the corresponding group. Bars represent means±SEM of five individuals.

14, 20, 21), and aberrant behaviors (11, 22–24). Several of these symptoms resemble the neuroendocrine and behavioral disturbances that are characteristic of mood disorders (e.g., major depression), leading to the suggestion that the maternal deprivation model might be suitable for studying at least some of the mechanisms that may contribute to the pathogenesis of affective and stress-related diseases (20, 25). Indeed, hyperactivity of CRH-producing neuronal populations and impaired efficacy of glucocorticoid-mediated feedback on the HPA axis are considered to be reliable hallmarks and, even causal factors, of disturbed neuroendocrine regulation and cognitive-emotional impairment associated with these disorders (26, 27).

In this study, the assumption that perinatal stress may result in an imbalance between the hypophysiotropic "drive" (exerted by hypothalamic CRH) and the "restraint" mediated by hippocampal glucocorticoid receptors is corroborated by the observations that maternally deprived rats display (*a*) an increased number of CRH mRNA transcripts in the PVN, and (*b*) a reduction in the expression of GR-encoding mRNA in the hippocampus. Neonatally stressed rats also displayed increased anxiety when placed in a novel environment in adulthood. Thus, repeated maternal deprivation during early development produces disruptions in both neuroendocrine and behavioral functions. This pathophysiological "background" led us to make an evaluation of the ability of the neuroactive steroid THDOC to influence these long-term consequences of neonatal stress.

The pilot study provided clear evidence for the anxiolytic efficacy of THDOC in infant rats. In both doses tested, THDOC suppressed USV which is considered to be a reliable sign of anxiety in maternally deprived rats (28). Interestingly, the parent steroid CORT also displayed anxiolytic properties; however, no dose dependency could be established for the action of this compound. It is pertinent to note that, in several subjects, pretreatment with the higher dose of 40 μg THDOC resulted in diminished locomotor activity during the observation period. Thus, the probability that this dose may have a somnogenic effect led us to the decision to select the dose of 20 μg/rat in the subsequent experiments involving chronic administration of THDOC.

The present data suggest that, when administered concomitantly with maternal deprivation, THDOC counteracts the long-lasting behavioral and neuroendocrine alterations induced by this stressor, such as increased anxiety, exaggerated adrenocortical response to emotional stress, decreased responsiveness to the suppressive action of dexamethasone, increased levels of CRH mRNA in the PVN, and diminished numbers of GR-encoding transcripts in the hippocampus. These findings strongly suggest that, as reported previously (6–9), ring-A-reduced steroid hormone derivatives may act on several mechanisms that subserve the regulation of the behavioral and neuroendocrine response to stress. In addition, the present results provide the first evidence for persistent transcriptional regulatory effects by neurosteroids. The demonstration that the developing rat brain is responsive to the pharmacological effects of neurosteroids (10), and the well-known fact that the HPA regulatory mechanisms mature during infancy (29–32), while being liable to environmental influences (21, 33–35), allow us to assume that pharmacological doses of neurosteroids may affect the development of a variety of neural circuits of adaptive relevance. Thus, our results suggest that THDOC and related compounds may protect the developing brain against adverse emotional challenges. It is also pertinent to note that, when applied alone, THDOC failed to produce significant changes in most of the parameters of interest (at least, not in the dose used in this study). With regard to therapeutic implications of neurosteroids, the latter finding gains additional importance in view of reports suggesting that other GABA-ergic agonists (for example, benzodiazepines), may induce behavioral alterations, when administered during perinatal life (36–38).

While the effects of THDOC on behavioral anxiety and endocrine responsiveness to stress can be attributed to acute sedation (i.e., altered perception of the stressful situation during infancy), the mechanisms through which neurosteroids may induce long-term changes in the gene transcription of neuropeptides and corticosteroid receptors remain obscure. In accordance with recent hypotheses, neurosteroid-induced long-term modulation of CRH and GR gene transcription, as reported herein, could be ascribed to either persistent alterations in GABA-ergic transmission, or to "promiscuous" interactions of neurosteroids with putative steroid hormone receptors or related transcriptional factors. The former possibility appears plausible in view of results showing that various $GABA_A$ receptor agonists can produce changes in neuropeptide gene transcription (39–41). As to the alternative of neurosteroid in-

teractions with steroid hormone receptors, strong evidence that THP and THDOC, upon oxidation to the corresponding dihydro-derivatives, may bind to the progesterone receptor, has been shown in an in vitro study (42).

In summary, the results of this study indicate that chronic treatment with the neuroactive steroid THDOC during early development counteracts behavioral and neuroendocrine dysregulation induced by adverse early life events, while not producing major changes in neural mechanisms of adaptive relevance, when applied alone. Our findings, thus, suggest that exogenously administered neurosteroids may alleviate the consequences of adverse environmental challenges (such as emotional stress) inflicted during early brain development.

Acknowledgments

The expert assistance of Julia Deicke and Dieter Fischer is gratefully acknowledged.

L. Koranyi was supported by a visiting scholarship of the Deutsche Akademischer Austausch-Dienst; V. Patchev is the recipient of a research award of the Theodore and Vada Stanley Foundation.

References

1. Majewska, M.D., N.L. Harrison, R.D. Schwartz, J.L. Barker, and S.M. Paul. 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science (Wash. DC)*. 232:1004–1007.

2. Mendelson, W.B., J.V. Martin, M. Perlis, R. Wagner, M.D. Majewska, and S.M. Paul. 1987. Sleep induction by an adrenal steroid in the rat. *Psychopharmacology.* 93:226–229.

3. Crawley, J.N., J.R. Glowa, M.D. Majewska, and S.M. Paul. 1986. Anxiolytic activity of an endogenous adrenal steroid. *Brain Res.* 398:382–385.

4. Kavaliers, M. 1988. Inhibitory influences of the adrenal steroid, 3α, 5α-tetrahydroxy-corticosterone on aggression and defeat-induced analgesia in mice. *Psychopharmacology.* 95:488–492.

5. Purdy, R.H., L.A. Morrow, P.H. Moore, Jr., and S.M. Paul. 1991. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natl. Acad. Sci. USA.* 88:4553–4557.

6. Paul, S.M., and R.H. Purdy. 1992. Neuroactive steroids. *FASEB J.* 6: 2311–2322.

7. Majewska, M.D. 1992. Neurosteroids: endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. *Prog. Neurobiol.* (*Oxf*). 38:379–395.

8. Patchev, V.K., M. Shoaib, F. Holsboer, and O.F.X. Almeida. 1994. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience.* 62:265–271.

9. Patchev, V.K., A.H.S. Hassan, F. Holsboer, and O.F.X. Almeida. 1996. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology.* 15:533–540.

10. Zimmerberg, B., S.A. Brunelli, and M.A. Hofer. 1994. Reduction of rat pup ultrasonic vocalizations by the neuroactive steroid allopregnanolone. *Pharmacol. Biochem. Behav.* 47:735–738.

11. Hofer, M.A. 1976. Studies on how early maternal separation produces behavioral changes in young rats. *Dev. Psychobiol.* 9:189–206.

12. Janus, K. 1987. Effects of early separation of young rats from the mother on their open-field behavior. *Physiol. Behav.* 40:711–715.

13. Plotsky, P.M., and M.J. Meaney. 1993. Early postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Mol. Brain Res.* 18:195–200.

14. Ladd, C.O., M.J. Owens, and C.B. Nemeroff. 1996. Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology.* 137:1212–1218.

15. Suhecki, D., D.Y. Nelson, H. Van Oers, and S. Levine. 1995. Activation and inhibition of the hypothalamic-pituitary-adrenal axis of the neonatal rat: effects of maternal deprivation. *Psychoneuroendocrinology.* 20:169–182.

16. Walker, C.-D., K.A. Scribner, C.S. Cascio, and M.F. Dallman. 1991. The pituitary-adrenocortical system of neonatal rats is responsive to stress throughout development in a time-dependent and stressor-specific fashion. *Endocrinology.* 128:1385–1395.

17. Winslow, J.T., and T.R. Insel. 1991. The infant separation paradigm: a novel test for novel anxiolytics. *Trends Pharmacol. Sci.* 12:402–404.

18. Dawson, G.R., and M.D. Tricklebank. 1995. Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends Pharmacol. Sci.* 16:33–36.

19. Patchev, V.K., S. Hayashi, C. Orikasa, and O.F.X. Almeida. 1995. Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB J.* 9:419–423.

20. Kalin, N.H. 1989. The HPA system and neuroendocrine models of depression. *In* Animal Models of Depression. G.F. Koob, C.L. Ehlers, and D.J. Kupfer, editors. Birkhauser Boston, Cambridge, MA. 57–73.

21. Meaney, M.J., S. Bhatnagar, S. Larocque, C. McCormick, N. Shanks, S. Sharma, J. Smythe, V. Viau, and P.M. Plotsky. 1993. Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic CRF system. *Ann. NY Acad. Sci.* 697:70–85.

22. Stanton, M.E., K.M. Crofton, and C. Lau. 1992. Behavioral development following daily episodes of mother-infant separation in the rat. *Fund. Applied Toxicol.* 19:474–477.

23. Kaneko, W.M., E.P. Riley, and C.L. Ehlers. 1994. Behavioral and electrophysiological effects of early repeated maternal separation. *Depression.* 2: 43–53.

24. Matthews, K., L.S. Wilkinson, and T.W. Robbins. 1996. Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. *Physiol. Behav.* 59:99–107.

25. McKinney, W.T., E. Moran, and G.W. Kraemer. 1984. Separation in non-human primates as a model of human depression: neurobiological implications. *In* Neurobiology of Mood Disorders. R. Post, and J. Ballenger, editors. Williams & Wilkins, Baltimore, MD. 393–406.

26. Owens, M.J., and C.B. Nemeroff. 1991. The physiology and pharmacology of corticotropin-releasing factor (CRF). *Pharmacol. Rev.* 43:425–473.

27. Holsboer, F., D. Spengler, and I. Heuser. 1992. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. *Prog. Brain Res.* 93:385–417.

28. Noirot, E. 1972. Ultrasounds and maternal behavior in small rodents. *Dev. Psychobiol.* 5:371–387.

29. Sapolsky, R.M., and M.J. Meaney. 1986. Maturation of the adrenocortical response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res. Rev.* 11:65–76.

30. Sarrieau, A., S. Sharma, and M.J. Meaney. 1988. Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. *Brain Res.* 471:158–162.

31. Rosenfeld, P., D. Suchecki, and S. Levine. 1992. Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci. Biobehav. Rev.* 16:553–568.

32. Rosenfeld, P., J.A.M. van Eekelen, S. Levine, and E.R. de Kloet. 1993. Ontogeny of corticosteroid receptors in the brain. *Cell. Mol. Neurobiol.* 13:295–319.

33. Plotsky, P.M., and M.J. Meaney. 1993. Postnatal handling alters hypothalamic CRF mRNA, synthesis, and release. *Mol. Brain Res.* 18:195–200.

34. Bhatnagar, S., and M.J. Meaney. 1995. Hypothalamic-pituitary-adrenal function in chronic intermittently cold-stressed neonatally handled and non handled rats. *J. Neuroendocrinol.* 7:97–108.

35. O'Donnell, D., S. Larocque, J.R. Seckl, and M.J. Meaney. 1994. Postnatal handling alters glucocorticoid, but not mineralocorticoid messenger RNA expression in the hippocampus of adult rats. *Mol. Brain Res.* 26:242–248.

36. Kellogg, C.K. 1988. Benzodiazepines: influence on the developing brain. *Prog. Brain Res.* 73:207–228.

37. Tucker, J.C. 1985. Benzodiazepines and the developing rat: a critical review. *Neurosci. Biobehav. Rev.* 9:101–111.

38. Schroeder, H., A.-C. Humbert, V. Koziel, D. Desor, and A. Nehlig. 1995. Behavioral and metabolic consequences of neonatal exposure to diazepam in rat pups. *Exp. Neurol.* 131:53–63.

39. Rage, F., S. Jalaguier, C. Rougeot, and L. Tapia-Arancibia. 1993. GABA inhibition of somatostatin gene expression in cultured hypothalamic neurones. *Neuroreport.* 4:320–322.

40. Reimer, S., and V. Höllt. 1991. GABAergic regulation of striatal opioid gene expression. *Mol. Brain Res.* 10:49–54.

41. Imaki, T., and W. Vale. 1993. Chlordiazepoxide attenuates stress-induced accumulation of corticotropin-releasing factor mRNA in the paraventricular nucleus. *Brain Res.* 623:223–228.

42. Rupprecht, R., J.M.H.M. Reul, T. Trapp, B. van Steensel, W. Zieglgänsberger, K. Damm, and F. Holsboer. Progesterone receptor mediated effects of neurosteroids. *Neuron.* 11:523–530.