Rapid Changes in Monoamine Levels Following Administration of Corticotropin-Releasing Factor or Corticosterone Are Localized in the Dorsomedial Hypothalamus

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Monoaminergic systems are important modulators of the neuroendocrine, autonomic, and behavioral responses to stress-related stimuli. The male roughskin newt (Taricha granulosa) was used as a model system to investigate the effects of corticotropin-releasing factor (CRF) or corticosterone administration on tissue concentrations of norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) in microdissected brain areas. Intracerebroventricular infusion of 25 or 50 ng of CRF increased locomotor activity and site-specifically increased dopamine concentrations within the dorsomedial hypothalamus 30 min after treatment when compared to vehicle-treated controls. In further studies, male newts were treated as follows: (1) no injection, no handling, (2) saline injection, or (3) 10 mg corticosterone and then placed in a novel environment. Monoamine and monoamine metabolite concentrations were similar in the unhandled and saline-injected controls 20 min after treatment. In contrast, corticosterone-injected newts had elevated concentrations of dopamine, serotonin, and 5-HIAA in the dorsomedial hypothalamus (a region that contains dopamine- and serotonin-accumulating neuronal cell bodies in representatives of all vertebrate classes) but not in several other regions studied. These site-specific neurochemical effects parallel neurochemical changes observed in the dorsomedial hypothalamic nucleus of mammals following exposure to a variety of physical and psychological stress-related stimuli. Therefore, these changes may reflect highly conserved, site-specific neurochemical responses to stress and stress-related neurochemicals in vertebrates. Given the important role of the dorsomedial hypothalamus in neuroendocrine, autonomic, and behavioral responses to stress, and a proposed role for this region in fast-feedback effects of glucocorticoids on the hypothalamo-pituitary-adrenal axis, these stress-related monoaminergic changes are likely to have important physiological or behavioral consequences. © 2001 Academic Press

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Corticotropin-releasing factor (CRF) and glucocorticoid hormones (corticosterone, cortisol) are important factors in the physiological and behavioral responses to stress. CRF has a well-established hypophysiotropic role (Vale, Spiess, Rivier, and Rivier, 1981) in addition to diverse neurotransmitter or neuromodulator actions within the central nervous system (Dunn and Berridge, 1990). Included in these actions are effects on noradrenergic (reviewed by Koob, 1999), dopaminergic (Kalivas, Duffy, and Latimer, 1987), and serotonergic neuronal activity (Price, Curtis, Kirby, Valentino, and Lucki, 1998; Kirby, Rice, and Valentino, 2000; Lowry, Rodda, Lightman, and Ingram, 2000). Rapid electrophysiological effects of CRF, highly correlated with changes in behavioral activity, also have been described in medullary and brainstem raphé neurons of Taricha granulosa (Lowry, Rose, and Moore, 1996b). Corticotropin-releasing factor can stimulate behavioral activity in rodents (Koob and Bloom, 1985) and in T. granulosa (Moore, Roberts, and Bevers, 1984;
Lowry et al.

...Lowry, Deviche, and Moore, 1990; Lowry and Moore, 1991; Lowry et al., 1996b). The behavioral effects of CRF are context-dependent (Koob and Bloom, 1985), consistent with a role for CRF in modulating the excitability of behavioral circuits recruited in response to diverse social and environmental challenges.

The glucocorticoid hormone corticosterone (CORT) regulates transcriptional activity in the central nervous system by binding to soluble corticosteroid receptors; CORT also rapidly modulates neuronal excitability by interacting with receptors in neuronal membranes. For example, iontophoretically applied or microinjected CORT or cortisol rapidly alters spontaneous and evoked firing rates or other electrophysiological properties of neurons in mammalian hypothalamic regions containing cerebrospinal fluid-contacting, serotonin- and dopamine-accumulating neuronal cell bodies, the paraventricular organ, and the nucleus infundibularis dorsalis (for references, see Lowry, Renner, and Moore, 1996a).

**MATERIALS AND METHODS**

Adult male roughskin newts (T. granulosa) were collected locally (Benton Co., OR) from freshwater ponds. Animals were maintained in the laboratory for approximately 48 h in holding tanks containing dechlorinated tap water. Animals used in Experiments I and II were held in a room with controlled photoperiod (14 h light: 10 h dark; lights on at 0630 h) and temperature (19°C). For Experiment III, animals were maintained in a room with natural photoperiod and an ambient temperature of 23°C. Newts were not handled or disturbed until immediately prior to treatment. Animals were treated in accordance with the principles and procedures of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication 80-23, Revised 1985, Office of Science and Health Reports, DRR/HIH, Bethesda, MD 20205).

**Experiments I and II**

Subjects received intracerebroventricular (icv) injections of either amphibian Ringer’s solution or vehicle containing synthetic ovine CRF (a gift from Drs. Wylie Vale and Jean Rivier, Salk Institute for Biological Studies, San Diego, CA). Intracerebroventricular injections of CRF (Experiment I, 25 ng; Experiment II, 50 ng; n = 10) were given through a 0.5-mm hole at the junction of the parietal and frontal bones in the cranial midline using a microsyringe with a tip diameter of 50 μm as described in Moore and Miller (1983). Solutions (2 μl) were infused into the third ventricle over a period of 5 s. Following icv injection newts were isolated in temporary circular holding tanks (25 cm in diameter) containing 5 L of dechlorinated tap water, until behavioral testing. For behavioral testing, newts were placed individually in a dechlorinated water-filled (10 cm in depth) circular runway with an inside diameter of 70 cm and an outside diameter of 85 cm; each testing arena was marked with radial lines to define 16 equal sectors. Newts were placed in the testing arena 30 min after treatment, which matches the postinjection time period used previously with T. granulosa (Lowry et al., 1990; Lowry and Moore, 1991). Starting 4 min after...
placement, locomotor activity was quantified by counting the total number of lines crossed during 3 consecutive min. Locomotion consisted of a combination of walking and swimming movements.

Experiment III

Subjects received one of the following treatments: (1) no injection, no handling, (2) saline injection, or (3) injection of 10 μg CORT (Sigma, St. Louis, Missouri). Animals (n = 12) were injected intraperitoneally (ip) with a volume of 0.1 ml. Following treatment, newts were isolated in temporary circular holding tanks as described above, but were not tested for locomotor activity; previous studies in *T. granulosa* indicate that ip injection of CORT does not alter locomotor activity (Moore et al., 1984). In order to investigate rapid effects of CORT on monoaminergic metabolism, animals were killed 20 min after injection, matching the postinjection time period used previously in *Taricha* (Orchinik et al., 1991).

Tissue Preparation

Immediately after behavioral testing (Experiments I and II) or 20 min after injection (Experiment III), newts were killed by decapitation. The brain and rostral spinal cord were rapidly dissected (1–2 min) and embedded in Tissue-Tek Optimal Cutting Temperature Compound (Baxter Diagnostics, Inc., Redmond, WA), frozen on dry ice, and stored at −80°C until sectioning. Serial 300-μm sections of frozen brain were cut at −12°C in a cryostat. Sections were placed on gelatin-coated glass slides, collectively thaw-mounted by briefly warming, and then the slide was refrozen rapidly. Slides were sealed in slide boxes and stored at −80°C until microdissection.

Microdissection and Neurochemical Measurements

Microdissection of individual brain regions was accomplished using the methods described for the microdissection of mammalian brain tissues (Palkovits, 1973; Palkovits and Brownstein, 1982), as previously applied in *T. granulosa* (Zoeller and Moore, 1986; Lowry et al., 1996a). Microscope slides were placed on a cold stage (Thermoelectric Cold Plate, TCP-2, Thermoelectrics Unlimited, Inc.) and maintained at −10°C. Tissue punches (300 μm i.d.) were expelled into 60 μl of acetate buffer (pH 5) containing internal standard, 0.5 × 10−7 M α-methyl dopamine (Experiments I and II), or 3.2 × 10−8 M 3,4-dihydroxy benzylamine hydrobromide (Experiment III) and then stored at −80°C until analyzed for monoamine content. A microdissection atlas described previously for *T. granulosa* (Lowry et al., 1996a) was used. Microdissected brain regions included, in Experiments I and II, the dorsomedial hypothalamus (region containing the paraventricular organ; DMH), dorsal tegmentum mesencephali (DTM), isthmic tegmentum (IST), dorsolateral hypothalamus (region containing the nucleus infundibularis dorsalis; LH), medial pallium (MP), raphé region (R), septum (S), thalamus (TH), trigeminal tegmentum (TT), and ventral striatum (VS), and in Experiment III, the amygdala pars medialis (Northcutt and Kicliter, 1980; APM), DMH, LH, nucleus accumbens (NA), nucleus of the solitary tract (NTS), preoptic area (POA), R, and VS.

Tissue concentrations of norepinephrine, epinephrine, dopamine (DA), DOPAC, serotonin (5-hydroxytryptamine, 5-HT), and 5-HIAA were analyzed using HPLC–ED following methods previously described (Renner and Luine, 1984; Renner, Allen, and Luine, 1986; Lowry et al., 1996a). Briefly, the tissue punches were thawed and centrifuged at 15,000 g for 2 min. The supernatant was treated with 2 μl of 0.02% ascorbate oxidase (Boehringer Mannheim) to minimize ascorbic acid contributions to the solvent front (McKay et al., 1984) and directly injected into a Waters chromatographic system (Waters Associates, Inc.). Chromatographic separation was accomplished using a C-18, 4-μm radial compression cartridge and a mobile phase consisting of (wt/vol) 0.84% sodium acetate, 1.2% citric acid, 0.015% octanesulfonic acid sodium salt (Eastman Kodak), and 0.02% disodium EDTA in (vol/vol) 15% methanol in water. Electrochemical detection was provided by a laboratory-built potentiostat and a glassy carbon electrode (Bioanalytical Systems) set at +0.7 V with respect to an Ag/AgCl reference electrode. The tissue pellet was dissolved in 0.3 N NaOH and analyzed for protein content according to the method of Bradford (1976).

The pg/cm peak heights of known concentrations of the standards were determined from the mean peak heights of three chromatograms for each respective standard. The internal standard was injected three times to determine the peak height for 100% sample recovery. Amine concentrations were calculated from the standard values and corrected for percentage of recovery and injection volume using a Waters 730 Data Module. The amine concentrations were divided by μg protein to yield pg amine/μg protein. All sam-
samples were analyzed blind and in a fully randomized manner using codes for animals and treatments.

Statistical Analyses

For analysis of behavioral data, the Mann–Whitney U test was used to test for statistical differences in locomotor activity between treatment groups (Siegel, 1956). Two-tailed probabilities \( P < 0.05 \) were considered statistically significant. Differences between mean tissue concentrations of monoamines were analyzed using two-way factorial analysis of variance (ANOVA; Statistica, Tulsa, OK), with drug treatment and brain region treated as independent variables. We performed two-way ANOVA for each of the following variables: norepinephrine, epinephrine, DA, DOPAC, the DOPAC:DA ratio, 5-HT, 5-HIAA, and the 5-HIAA:5-HT ratio. Data for 25 ng CRF and 50 ng CRF and CORT studies were analyzed separately. When main effects of treatment or interactions between treatment and brain region were significant, we performed posthoc analyses using Tukey’s honestly significant difference (HSD) test. Preliminary analyses in Experiment III indicated that there were no differences between the two control groups (uninjected vs saline injected), so the data for these controls were combined for further statistical analyses. Correlational analyses were performed using Pearson correlation statistics (SYSTAT for Windows: Statistics, Version 5 Edition. Evanston, IL: SYSTAT, Inc., 1992). An overall significance level of \( \alpha = 0.05 \) was used for all tests.

For neurochemical measurements, outlier values were identified using the Grubbs method and were not included in the final statistical analysis or reported values (two-tailed probability, \( P < 0.05 \); Grubbs and Beck, 1972). In Experiment I, of 1200 possible measurements (10 brain regions \( \times 6 \) neurochemical measures \( \times 2 \) treatments \( \times n = 10 \)), 12 (1.00%) were excluded as statistical outliers. In Experiment II, of 1200 possible measurements, 16 (1.33%) were excluded as statistical outliers. In Experiment III, of 1728 possible measurements (8 brain regions \( \times 6 \) neurochemical measures \( \times 3 \) treatments \( \times n = 12 \)), 22 (1.27%) were excluded as statistical outliers.

RESULTS

In Experiments I and II, icv injection of CRF stimulated locomotor activity relative to saline-injected controls and the behavioral effects of CRF were associated with site-specific increases in tissue concentrations of DA, but no other monoamines or metabolites (Figs. 1 and 2). In Experiment III, CORT administration resulted in site-specific increases in tissue concentrations of DA, 5-HT, and 5-HIAA (Fig. 3). Of particular interest, the effects of both CRF and CORT were localized to the DMH. Detailed descriptions of the results follow.

CRF increased locomotor activity in a dose-dependent manner (two-tailed probability \( P < 0.05 \); Mann–Whitney U test). Injection of 25 ng CRF icv (Experiment I) increased locomotion from 9.0 ± 3.3 to 23.5 ± 6.0 crossings/3 min, while 50 ng CRF icv (Experiment II) increased locomotion from 10.7 ± 3.2 to 37.5 ± 4.6 crossings/3 min (\( n = 10 \)). In Experiment I, there was also a significant interaction between drug treatment and brain region on the mean tissue levels of DA (\( F(9, 152) = 2.538; P = 0.0097 \)). Posthoc analyses revealed that mean levels of DA were significantly higher in the DMH of CRF-treated animals than of control animals, but there were no significant treatment effects on DA levels in any of the other brain regions examined (Fig. 1). There was a significant interaction between drug treatment and brain region on the tissue levels of DOPAC (\( F(9, 130) = 2.392; P = 0.0153 \)). However, there were no significant differences in the mean levels of DOPAC between CRF- and saline-injected animals in any of the individual brain regions examined. There were no significant drug treatment effects and no significant interactions between drug treatment and brain region on mean tissue levels of norepinephrine, epinephrine (data not shown), 5-HT, and 5-HIAA (Fig. 1) or the ratios between DOPAC:DA or 5-HIAA:5-HT (data not shown).

Analysis of data following injection of the higher dose of CRF (50 ng, Experiment II) also indicated a significant interaction between drug treatment and brain region on the mean tissue levels of DA (\( F(9, 166) = 2.064; P = 0.0355 \)). Again, mean levels of DA were significantly higher in the DMH of CRF-treated animals than of control animals, but there were no significant differences in any of the other brain regions examined (Fig. 2). In contrast to Experiment I, overall DOPAC levels were lower in CRF (50 ng)-treated animals than in controls (\( F(1, 142) = 4.312; P = 0.0396 \)); there was no interaction between treatment and brain region. There were no significant drug treatment effects and no significant interactions between drug treatment and brain region on mean tissue levels of norepinephrine, epinephrine (data not shown), 5-HT, and 5-HIAA (Fig. 2) or the ratios between DOPAC:DA or 5-HIAA:5-HT (data not shown).

Acute treatment with CORT (Experiment III; 10 \( \mu \)g)
increased the mean tissue concentrations of DA, 5-HT, and 5-HIAA in a site-specific manner. There was a significant interaction between drug treatment and brain region on the tissue levels of DA ($F(7, 256) = 3.082; P = 0.0039$). Posthoc tests revealed that the mean levels of DA were significantly higher in the DMH of CORT-treated animals than of controls; there were no significant treatment effects on DA in the other brain regions examined (Fig. 3). There was a significant main effect of drug treatment on 5-HT levels ($F(1, 254) = 4.942; P = 0.0271$), and the interaction between treatment and brain region was nearly significant ($F(7, 254) = 1.992; P = 0.0566$). Posthoc analyses revealed that 5-HT levels were higher in the DMH of CORT-treated animals than in the DMH of controls; there were no significant treatment effects on 5-HT in any of the other brain regions examined. There was a significant main effect of drug treatment on 5-HIAA levels ($F(1, 254) = 3.938; P = 0.0484$), and the interaction between treatment and brain region was nearly significant ($F(7, 254) = 1.914; P = 0.0683$). Posthoc analyses indicated that 5-HIAA levels were higher in the DMH of CORT-treated animals than of controls; there were no significant treatment effects on 5-HIAA in any of the other brain regions examined. There were no significant drug treatment effects and no significant interactions between treatment and brain region on mean tissue levels of norepinephrine, epinephrine (data not shown), or DOPAC (Fig. 3) or the ratios between DOPAC:DA or 5-HIAA:5-HT (data not shown).

**Correlations among Locomotor Activity, DA, and 5-HT**

CRF and CORT had site-specific effects on tissue levels of DA and 5-HT within the DMH. We performed additional statistical tests on CRF- and CORT-injected animals to evaluate the possibility that the changes in DA and 5-HT concentrations were correlated and further, that these changes in tissue concentrations of DA or 5-HT were correlated with locomotor activity scores in individual animals. In a correlational analysis using all animals from Experiments I and II, there was no significant correlation between either DA ($r = 0.28; P > 0.05$) or 5-HT ($r =$...
0.27; \( P > 0.05 \)) and locomotor activity, indicating that CRF-induced elevations of DA in the DMH were not predictive of CRF-induced behavioral changes. In contrast, absolute levels of DA and 5-HT within the DMH of individual animals were positively correlated (\( r = 0.85; \ P < 0.0001; \) Fig. 4A), suggesting that DA and 5-HT levels, although not correlated with locomotor activity, may be coregulated within the DMH. Subsequent analysis of the relationship between tissue concentrations of DA and 5-HT within the DMH of control and CORT-treated animals from Experiment III also revealed a highly significant correlation (\( r = 0.85; \ P < 0.0001; \) Fig. 4B). Thus animals with high concentrations of DA in the DMH invariably had high concentrations of 5-HT in the DMH, again suggesting that DA and 5-HT levels may be coregulated within this brain region.

**DISCUSSION**

Administration of either CRF or CORT had site-specific effects on concentrations of monoamines or monoamine metabolites in the brains of adult male *T. granulosa*, compared to saline-injected controls. Central injection of 25 or 50 ng CRF increased tissue concentrations of DA, while peripheral injection of CORT increased tissue concentrations of DA, 5-HT, and 5-HIAA. There was a remarkable degree of neuroanatomical specificity of these actions. Significant effects of CRF or CORT on DA and 5-HT concentrations were found exclusively within the DMH. These data are consistent with previous studies demonstrating rapid electrophysiological and behavioral effects of CRF or CORT in *Taricha* (Moore and Miller, 1984; Lowry et al., 1990, 1996b; Orchinik et al., 1991; Rose et al., 1993). Although concentrations of DA and 5-HT within the DMH were not correlated with locomotor activity, it remains likely that neurochemical changes within the DMH have context-dependent consequences for behavioral, autonomic, or neuroendocrine responses to specific social or environmental challenges.

**Stress-Related Stimuli Elevate DA and 5-HT Concentrations in the DMH of Nonmammalian and Mammalian Vertebrates**

The unique sensitivity of the DMH to CRF and CORT was surprising. It is possible that site-specific
effects of CRF were a consequence of the proximity of the DMH to the icv injection site. However, there were no effects of CRF injection on monoamine or monoamine metabolite concentrations within the thalamus, which, in Taricha, is also in direct contact with the third ventricle. Also arguing against such an interpretation, systemic injection of CORT produced similar site-specific effects on DMH monoamines. Several studies in mammals also have described site-specific effects of stress-related stimuli on tissue concentrations of DA and 5-HT within the DMH. For example, ip injection of CORT increases tissue concentrations of 5-HT and 5-HIAA in the rat mediobasal hypothalamus, a structure that includes the DMH, within 30 min of treatment (Losada, 1988). In addition, acute immobilization of rats increases DA (Kvetn’ansky, Palkovits, Mitro, Torda, and Mikulaj, 1977; Kvetn’ansky, Daher, Čulman, Opršalová, and Kiss, 1983; Beaulieu, Di Paolo, Côté, and Barden, 1987) or 5-HT (Čulman, Kvetn’anský, Torda, and Murgaš, 1980) concentrations in the DMH, but not in other hypothalamic brain regions, including the paraventricular nucleus of the hypothalamus (PVN). Stress-induced elevations of DA and 5-HT within the DMH can persist up to 12–24 h following presentation of a stress-related stimulus, suggesting that stress-induced accumulation of DA and 5-HT within the DMH may have long-lasting effects on neural function (Shekhar, Katner, Rusche, Sajdyk, and Simon, 1994; Sajdyk, Katner, and Shekhar, 1997). The stress-induced elevation of DA concentration within the DMH does not appear to be due to increased synthesis or to decreased rates of intraneuronal or extraneuronal metabolism (Kvetn’ansky et al., 1983; Sajdyk et al., 1997). Taking these observations from mammalian studies into account, stress-induced elevations of DA concentration in the DMH are likely to involve either (1) a decrease in release or (2) an increase in DA accumulation via mechanisms independent of de novo synthesis.

Mechanisms for CRF- and CORT-Induced Accumulation of DA and 5-HT in the DMH

From these studies, we cannot determine if CRF and CORT are acting directly within the DMH, indirectly via central nervous system sites, or even through pe-
ripheral mechanisms to increase monoamine levels. In favor of a direct action of CRF on the DMH are the following: (1) There are CRF-containing cell bodies and neuronal fibers within the DMH of mammalian (Swanson, Sawchenko, Rivier, and Vale, 1983; Merchenthaler, Hynes, Vigh, Schally, and Petrusz, 1984; Champagne, Beaulieu, and Drolet, 1998) and nonmammalian vertebrates (Mancera, López Avalos, Pérez-Figares, and Fernández-Llebrez, 1991). (2) Evidence suggests that CRF₁ and CRF₂ receptors as well as CRF binding protein are expressed in the rat DMH (Bittencourt and Sawchenko, 2000; Potter, Behan, Linton, Lowry, Sawchenko, and Vale, 1992). Therefore, the machinery is present for CRF to act directly within the DMH to elevate DA concentrations. However, it is possible that icv injections of CRF may stimulate DA accumulation in the DMH indirectly through activation of the hypothalamo–pituitary–adrenal (HPA) axis and the consequent rise in plasma CORT levels. The rat DMH has intracellular corticosteroid receptors (both type I mineralocorticoid and type II glucocorticoid receptors; Cintra, Fuxe, Wikstrom, Visser, and Gustafsson, 1990), so CORT may act directly on these receptors in the DMH. However, the rapidity of CORT action on DA, 5-HT, and 5-HIAA concentrations in the DMH is more consistent with neuronal membrane-mediated action, and it has not been determined if the amphibian DMH contains significant numbers of membrane-associated corticosteroid receptors.

A number of dopaminergic and serotonergic neuronal systems could contribute to the CRF- or CORT-induced alterations of DA and 5-HT concentrations in the DMH. Dopaminergic cell groups innervating the DMH region include (1) the caudal dopaminergic cell group extending into the posterior hypothalamic area (A11), (2) the tuberoinfundibular dopaminergic cell group (A12), (3) the caudal incertohypothalamic dopaminergic cell group within the medial zona incerta, dorsal hypothalamic area, and medial and lateral portions of the dorsomedial hypothalamic nucleus (A13), and (4) the rostral incertohypothalamic dopaminergic cell group within the anterior periventricular hypothalamus (A14; Björklund, Lindvall, and Nobin, 1975; Chan-Palay, Zábrorszky, Köhler, Goldstein, and Palay, 1984; van den Pol, Herbst, and Powell, 1984). In fact, the proposed nonmammalian homologue of the mammalian A13 dopaminergic cell group (Lowry et al., 1996a; the paraventricular organ accompanying cells of González and Smeets, 1994) was included in the microdissection of the DMH in the present study. Potential sources of serotonergic innervation of the dorsomedial hypothalamus include the caudal linear nucleus, median raphé nucleus, and dorsal raphé nucleus (Thompson and Swanson, 1998). Finally, changes in dopaminergic metabolism may be secondary to changes in serotonergic metabolism and vice versa.

The most prominent feature of the DMH of amphibians and the target of the microdissection in the present study was the complex of bipolar, cerebrospinal fluid-contacting, and DA- and 5-HT-accumulating cells that comprise the neuronal part of the paraventricular ependymal organ of the hypothalamus (Vigh, Teichmann, and Aros, 1967; Vigh-Teichmann and Vigh, 1983; for a comprehensive list of references, see Lowry et al., 1996a). A similar group of bipolar DA- and 5-HT-accumulating neuronal cell bodies has been described within the DMH in mammals and these

![FIG. 4. Correlations between DA and 5-HT within the DMH. DA and 5-HT concentrations within the DMH were highly correlated in individual animals. (A) Correlation between DA and 5-HT concentrations in the DMH, including data from Experiments I and II. (B) Correlation between DA and 5-HT concentrations in the DMH, including data from Experiment III.](https://example.com/fig4.png)
neurons are believed to be homologous to those described in nonmammalian vertebrates (Lowry et al., 1996a). In nonmammalian and mammalian vertebrates, these hypothalamic neurons are members of the “paraneuron” class of sensory or neurosecretory cells (Fujita and Kobyashi, 1979). It has been proposed that the DA- and 5-HT-containing paraneurons in the hypothalamus have chemoreceptive or neurosecretory roles (Vigh-Teichmann and Vigh, 1983).

Coregulation of DA and 5-HT in the DMH: A Clue to Novel Regulatory Mechanisms?

A remarkable observation in the present study was the correlation between levels of DA and 5-HT within the DMH under a variety of experimental conditions; an observation consistent with synchronous release of DA and 5-HT described within the rat medial hypothalamus (Fetissov, Meguid, Chen, and Miyata, 2000). The present study points toward functionally uncharacterized DA- and 5-HT-accumulating neurons within the DMH as a potential target for rapid nongenomic effects of CORT. In rat and vole brains, the highest concentrations of CORT binding sites in synaptosomal membrane fractions are found in the hypothalamus (Towle and Sze, 1983; Orchinik, Hastings, Witt, and McEwen, 1997). Thus, the hypothalamus may be an important site for rapid, nongenomic effects of CORT in both nonmammalian and mammalian brain.

The DMH Regulates Integrated Behavioral, Autonomic, and Neuroendocrine Responses to Stress

Due to the role of the DMH in regulation of integrated behavioral, autonomic, and neuroendocrine responses to stress (for references, see Bernardis and Bellinger, 1998; Thompson and Swanson, 1998), the effects of CRF and CORT described in the present study are likely to play an important role in stress-related physiology and behavior. One of the most plausible consequences of DA and 5-HT accumulation within the DMH is feedback regulation of neuroendocrine function. The DMH is an important structure in neuroendocrine regulation (reviewed by Thompson and Swanson, 1998), and evidence suggests that DA- and 5-HT-accumulating neurons in the DMH project directly to the median eminence and intermediate pituitary in nonmammalian vertebrates (for references, see Lowry et al., 1996a). In rats, the DMH innervates the median eminence and intermediate pituitary and also provides a dense innervation of the neuroendocrine portion of the paraventricular nucleus of the hypothalamus (Merchenthaler, Hynes, Vigh, Schally, and Petrusz, 1984; ter Horst and Luiten, 1986). Consistent with these anatomical findings, recent functional studies suggest that the DMH plays an important role in corticosterone-mediated, fast-feedback regulation of the HPA axis in rats (Thrivikraman et al., 2000).

Summary

In summary, two stress-related signaling molecules site-specifically altered the tissue levels of monoamines in the dorsomedial hypothalamus. These data are significant for several reasons: (1) they point to potential sites of action for CRF and CORT in the regulation of monoaminergic metabolism in the central nervous system, (2) they will serve to focus future work on the DMH, a hypothalamic region that is likely to integrate autonomic, behavioral, and neuroendocrine responses to stress-related stimuli, and (3) they point to an acute, presumably “nongenomic,” action of corticosteroids that is likely to be mediated by membrane-associated receptors.

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