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Local perfusion of corticosterone in the rat medial hypothalamus potentiates D-fenfluramine-induced elevations of extracellular 5-HT concentrations

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ABSTRACT

The dorsomedial hypothalamus (DMH) plays an important role in coordinating physiological and behavioral responses to stress-related stimuli. In vertebrates, DMH serotonin (5-HT) concentrations increase rapidly in response to acute stressors or corticosterone (CORT). Recent studies suggest that CORT inhibits postsynaptic clearance of 5-HT from the extracellular fluid in the DMH by blocking organic cation transporter 3 (OCT3), a polyspecific CORT-sensitive transport protein. Because OCTs are low-affinity, high-capacity transporters, we hypothesized that CORT effects on extracellular 5-HT are most pronounced in the presence of elevated 5-HT release. We predicted that local application of CORT into the DMH would potentiate the effects of D-fenfluramine, a 5-HT-releasing agent, on extracellular 5-HT. These experiments were conducted using *in vivo* microdialysis in freely-moving male Sprague–Dawley rats implanted with a microdialysis probe into the medial hypothalamus (MH), which includes the DMH. In Experiment 1, rats simultaneously received intraperitoneal (i.p.) injections of 1 mg/kg D-fenfluramine or saline and either 200 ng/mL CORT or dilute ethanol (EtOH) vehicle delivered to the MH by reverse-dialysis for 40 min. In Experiment 2, 5 μM D-fenfluramine and either 200 ng/mL CORT or EtOH vehicle were concurrently delivered to the MH for 40 min using reverse-dialysis. CORT potentiated the increases in extracellular 5-HT concentrations induced by either i.p. or intra-MH administration of D-fenfluramine. Furthermore, CORT and D-fenfluramine interacted to alter home cage behaviors. Our results support the hypothesis that CORT inhibition of OCT3-mediated 5-HT clearance from the extracellular fluid contributes to stress-induced increases in extracellular 5-HT and 5-HT signaling.

Introduction

The dorsomedial hypothalamus (DMH) plays an important role in coordinating neuroendocrine, autonomic, and behavioral responses to stress-related stimuli. Stimulation of the DMH induces a cascade of physiological and behavioral responses similar to responses elicited by emotional or exteroceptive stressors, including activation of the hypothalamic–pituitary–adrenal (HPA) axis, tachycardia, hypertension, behavioral arousal and anxiety-like behavior (DiMicco et al., 2002). Conversely, inhibition of the DMH inhibits these responses following exposure of rats to stress-related stimuli (DiMicco et al., 2002). The control of HPA axis activity by the DMH is complex and appears to involve excitatory and inhibitory projections to the paraventricular nucleus of the hypothalamus (PVN), enabling the DMH to stimulate or inhibit the HPA axis, possibly through local γ-aminobutyric acid (GABA)-ergic and glutamatergic synaptic circuits in the region of the PVN (Herman et al., 2002). In contrast, DMH regulation of stress-induced tachycardia and hypertension involves descending projections to the raphe pallidus and rostroventrolateral medulla in the brainstem (DiMicco et al., 2002). The mechanisms through which the DMH regulates behavioral arousal and anxiety-like responses remain largely unknown, although projections to the noradrenergic locus coeruleus seem to be involved (Aston-Jones et al., 2001).

A number of studies in mammalian and nonmammalian vertebrates have found that stress, stress-related neuropeptides such as corticotropin-releasing factor (CRF), or the stress hormone corticosterone (CORT), increase tissue concentrations of monoamines selectively in the DMH. For example, exposing rats to restraint or

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immobilization stress increases tissue concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, the major metabolite of 5-HT) in the DMH (Culman et al., 1980; Shekhar et al., 1994; Lowry et al., 2003). Similarly, i.p. injection of CORT rapidly (within 20 min) increases tissue concentrations of 5-HT and 5-HIAA in the DMH of rats (Losada, 1988) and roughskin newts, *Taricha granulosa* (Lowry et al., 2001). Since measurements of extracellular 5-HT concentrations in the DMH have not been performed in rats exposed to acute stress or CORT, it is not clear if the previously observed stress- and CORT-induced increases in tissue concentrations of 5-HT in the DMH represent changes in synthesis, storage, release, or clearance of the neurotransmitter.

Interestingly, stress- or CORT-induced increases in concentrations of 5-HT in the DMH are highly correlated with increases in other monoamines, including DA and NE (Lowry et al., 2001, 2003). One mechanism that could account for the co-regulation of 5-HT, DA, and NE concentrations in the DMH is transport and clearance of monoamines in the DMH by CORT-sensitive organic cation transporters (OCTs). OCTs are a family of low-affinity, high-capacity and bidirectional transporters of organic cations including 5-HT, DA, NE, and histamine (for review, see Koepsell et al., 2003). OCTs include OCT1, OCT2, and OCT3, also known as the extraneuronal monoamine transporter (EMT). Both OCT2 and OCT3 are expressed in brain, but OCT3-immunoreactive cells appear to be expressed most abundantly in hypothalamic regions such as the DMH (Gasser et al., 2006, 2009; Amphoux et al., 2006; Vialou et al., 2008). Organic cation transporter 3 mRNA expression (Amphoux et al., 2006) and OCT3-immunoreactive cells (Gasser et al., 2009) have been described also in the rat midbrain raphe complex. If OCT3 is expressed in serotonergic neurons, it could regulate extracellular 5-HT concentrations through presynaptic mechanisms, in addition to the proposed postsynaptic mechanisms.

Organic cation transporter 3 mRNA expression, OCT3 BREAK IN "immuno-reactivity". and OCT3-mediated uptake have been characterized in the DMH (Gasser et al., 2006, 2009; Vialou et al., 2008). OCT3-immunoreactive astrocytes and ependymal cells are abundant in the ependymal lining of the third ventricle adjacent to the DMH (Gasser et al., 2006, 2009; Vialou et al., 2008). OCT3-mediated uptake of [^{3}H]-histamine, a prototypic organic cation for OCT-mediated uptake studies, in freshly prepared DMH minces that include the ependymal lining is inhibited by CORT with an IC_{50} of approximately 30 nM (Gasser et al., 2006) and, therefore, offers a plausible hypothetical mechanism underlying the rapid stress- and CORT-induced increases in monoamine concentrations in the DMH. Evidence that OCTs participate in 5-HT clearance in the CNS is provided by findings that local perfusion of the OCT inhibitor decynium 22 (D-22) into the MH, an area encompassing the DMH, dose-dependently increases extracellular 5-HT concentrations in freely moving rats (Feng et al., 2005). A role for OCT3 in regulation of monoaminergic signaling may not be limited to the DMH as monoamine content and turnover in a number of brain regions are altered in OCT3-deficient mice (Vialou et al., 2008), and blockade of OCT-mediated transport using D-22 modifies the rate of hippocampal 5-HT clearance (Baganz et al., 2008).

Organic cation transporter regulation of monoaminergic signaling may have important implications for stress-related behavior. Organic cation transporter 3-deficient mice have altered anxiety-related behavior, while blockade of organic cation transporters using D-22 has antidepressant-like behavioral effects in mice with reduced expression of the 5-HT transporter (SERT; Baganz et al., 2008).

In this study, we further test the hypothesis that OCTs regulate the clearance of hypothalamic 5-HT in a stress-sensitive manner. Since OCTs function as low-affinity, high-capacity monoamine transporters, we predicted that blockade of OCT3 in the MH would enhance extracellular 5-HT concentrations under conditions of increased extracellular 5-HT concentrations. This type of scenario might occur in some stressful situations, following treatment with selective 5-HT reuptake inhibitors, or in individuals with low expression of the high-affinity, low capacity SERT, such as carriers of the short (s) variant of the SERT-linked polymorphic region (Hariri and Holmes, 2006). In this study, we measured extracellular 5-HT concentration after delivering CORT into the DMH using reverse-dialysis, in combination with systemic or local administration of submaximal doses of D-fenfluramine, a 5-HT-releasing agent. In addition, we analyzed home cage behavior in order to identify potential interactions between CORT and D-fenfluramine in the regulation of behavior.

Materials and methods

Animals

Adult male Sprague–Dawley rats (University of South Dakota Laboratory Animal Services, Vermillion, SD, USA) were maintained in a temperature-controlled environment with a reversed 12L:12D photoperiod (lights off, 10:00 A.M.). Food and water were available *ad libitum*. All experiments were approved by the University of South Dakota Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drug treatments

For i.p. injections, D-fenfluramine (Sigma-Aldrich, St. Louis, MO) was dissolved in saline vehicle and administered in a concentration of 1 mg/kg body weight (solution concentration: 1 mg/mL). This dose of D-fenfluramine has been shown to increase hypothalamic extracellular 5-HT concentrations by approximately 250% above baseline levels (Tao et al., 2002). For reverse-dialysis perfusions, D-fenfluramine was prepared as a 5 μM concentration in aCSF. Based on pilot dose-response experiments (data not shown), this concentration of D-fenfluramine increases extracellular 5-HT to approximately 200% over baseline levels. Previous work has shown that intra-hypothalamic reverse-dialysis of 100 μM D-fenfluramine can increase extracellular 5-HT 3000% (Tao et al., 2002); consequently, the 5 μM concentration used in this study was submaximal. Corticosterone (Sigma-Aldrich) was dissolved in 100% EtOH and serially diluted in aCSF to a final concentration of 200 ng/mL. Control perfusions for CORT consisted of the dilute EtOH vehicle (V, 0.02% EtOH in aCSF).

Surgical procedures

Stereotaxic surgeries were performed aseptically under xylazine–ketamine anesthesia (Ketoset 50 mg/kg i.p., Fort Dodge Labs Inc., Fort Dodge, IA; xylazine, 10 mg/kg i.p., Vedco, Inc., St. Joseph, MO). Deeply anesthetized rats were mounted in a stereotaxic frame in the flat-skull position and implanted with a unilateral guide cannula (20-G cut to project approximately 4 mm dorsal to the DMH; Plastics One, Inc. Roanoke, VA), directed towards the DMH. The coordinates for placement of the microdialysis probe were, relative to bregma: AP −3.3 mm; ML ±0.7 mm, DV −8.6 mm from the cortical surface (Paxinos and Watson, 1986). The pedestal was fixed to the skull with a combination of glass ionomer cement (GC Corp., Alsip, IL) and cranioplastic acrylic (Plastics One Inc.) using anchoring screws for support. After surgery, the rats were housed individually and allowed to recover for 3 to 5 days before undergoing further experimental procedures.

Microdialysis procedures and analysis of extracellular serotonin concentrations

The night before experimentation, rats were lightly anesthetized with a ketamine–xylazine mixture and implanted with a concentric