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RAPID COMMUNICATION

Muscimol Injections Into the Median Raphe Nucleus Increase Serum ACTH and Corticosterone Concentrations Via a Nonserotonergic Mechanism

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PARIS, J. M., S. A. LORENS, J. M. LEE, H. MITSUSHIO, J. C. RITCHIE AND C. B. NEMEROFF. *Muscimol injections into the median raphe nucleus increase serum ACTH and corticosterone concentrations via a nonserotonergic mechanism.* PHARMACOL BIOCHEM BEHAV **39**(3) 765–768, 1991.—Midbrain raphe serotonin (5-HT) neurons can influence the pituitary-adrenal axis. The midbrain raphe nuclei also contain a number of non-5-HT neurons, including gamma-aminobutyric acid (GABA) interneurons which can modulate 5-HT neuronal activity. We investigated the effects of intraraphe injections of the $GABA_A$ agonist, muscimol, on serum adrenocorticotropin hormone (ACTH) and corticosterone concentrations. Rats were infused with muscimol (0, 25, 50, and 100 ng in 0.5 μl saline) into the median raphe nucleus (MR). The animals were killed 30 min later, and trunk blood was collected for measurement of serum concentrations of ACTH and corticosterone by radioimmunoassay. Muscimol dose dependently increased plasma concentrations of these two pituitary-adrenal hormones. In order to determine the role of MR 5-HT neurons in these effects, separate groups of implanted animals were infused with either the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) or ascorbic acid vehicle into the MR. Two weeks later, the animals were infused with muscimol (100 ng in 0.5 μl) and sacrificed as above. Treatment with 5,7-DHT, which markedly reduced hippocampal concentrations of 5-HT (−83%) and 5-HIAA (−73%), did not block intra-MR muscimol-induced elevations in ACTH and corticosterone. Thus, 5-HT neurons within the MR apparently do not mediate the increased activity of the pituitary-adrenal axis produced by stimulation of MR $GABA_A$ receptors.

Median raphe nucleus GABA Muscimol ACTH Corticosterone Serotonin

THE serotonin- (5-hydroxytryptamine; 5-HT) containing neurons of the midbrain dorsal and median raphe nuclei (DR and MR, respectively) are involved in regulating the secretion of a number of hormones including β-endorphin, corticosterone, prolactin, vasopressin and renin (1, 3, 5, 7, 12, 16, 30). Intrinsic gamma-aminobutyric acid (GABA)-containing interneurons can regulate the activity of midbrain 5-HT cells (8, 18, 20, 21). Biochemical and electrophysiological studies have shown that GABA provides inhibitory control over 5-HT neurons (8, 20, 21), while microinjections of the $GABA_A$ agonist, muscimol, into the midbrain raphe induce a generalized behavioral stimulation in rats which is characterized by hyperlocomotion, hyperphagia, and hyperdipsia (11,26). However, these effects of muscimol do not appear to be mediated by 5-HT neurons (10, 24, 32).

In addition, preliminary evidence from our laboratory has indicated that muscimol injections into the MR may induce an aversive or anxiogenic state as measured by a conditioned place-preference paradigm (13). Since most aversive and stressful conditions stimulate the pituitary-adrenal axis (6), it was of interest to ascertain whether intraraphe infusions of muscimol produced similar effects. Therefore, the objectives of this study were to determine: 1) whether muscimol infusions into the MR stimulate secretion of adrenocorticotrophic hormone (ACTH) and corticosterone; and 2) to what extent do 5-HT neurons mediate this phenomenon. The latter objective was accomplished through the use of discrete chemical lesions of MR 5-HT neurons in-

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duced by the selective 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT).

METHOD

Male Sprague-Dawley rats (Sasco-King, Orange, WI), housed under standard laboratory conditions (275–325 g), were used. Food and water were available ad lib. Methods for cannula implantation and 5,7-DHT lesions have been detailed previously (24,25). For the lesion experiment, implanted rats were treated with the norepinephrine (NE) and dopamine (DA) reuptake inhibitor, nomifensine maleate (15 mg/kg, IP), 30–40 min prior to neurotoxin or vehicle infusions. Under ether anesthesia, the animals then received an infusion of 5,7-DHT creatinine sulfate (Sigma, St. Louis, MO; 8.0 μg in 2.0 μl of 0.1% ascorbate in 0.9% saline, as the base; 0.2 μl/min) or vehicle (2.0 μl) directly through the cannula. The animals were allowed to recover for a period of two weeks and were handled daily for five days prior to MR infusion to accustom them to the injection procedure (24,25). On the test day, they were removed from their home cage and infused with either vehicle or muscimol (25, 50 or 100 ng) in 0.5 μl saline vehicle and then returned to their home cage. Thirty min following drug infusion, each animal was individually carried to an adjacent room where it was decapitated. All animals were sacrificed between 0900 and 1400 h. Trunk blood was collected, serum harvested and stored frozen for subsequent determination of ACTH and corticosterone concentrations by sensitive and specific radioimmunoassay methods (22). The brain stems were placed in formalin for histological analysis. Two weeks following 5,7-DHT lesions, each animal was injected with vehicle or muscimol (100 ng/0.5 μl). Thirty minutes later, each animal was decapitated and trunk blood collected and processed as noted above. The hippocampus and striatum were dissected, and tissue concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined by high performance liquid chromatography (HPLC) with electrochemical detection (24,25) The data were analyzed by analysis of variance (ANOVA) and post hoc comparisons made with Duncan's multiple range test(31). Data were considered to be significant when the probability (p) was less than or equal to 0.05.

RESULTS

Histological analysis revealed that, of the 74 rats studied, 12 had to be eliminated from further analysis because their MR cannula placements were greater than 1.0 mm lateral or ventrolateral to the midline; data from 4 other rats were excluded due to technical errors during the in vitro assays. MR cannula placement and histological damage from an intra-MR 5,7-DHT lesion have been described in an earlier publication (18). As we have previously reported, the 5,7-DHT injections produced cell loss (presumably 5-HT neurons) with minimal glial scarring and no cavitation in the region surrounding the cannula tip (24).

Figure 1A shows the dose-dependent effects of intra-MR muscimol infusions on serum ACTH and corticosterone concentrations. Both 50 and 100 ng infusions of muscimol significantly ($p<0.05$) increased ACTH and corticosterone. ACTH levels were increased 211% and 122%, while corticosterone was increased 292% and 350% by 50 and 100 ng muscimol, respectively. Although 25 ng of muscimol increased serum concentrations of both substances, these values did not attain statistical significance ($p>0.05$).

The effects of MR 5,7-DHT lesions on the enhancement of ACTH and corticosterone secretion produced by intra-MR muscimol infusions are shown in Fig. 1B. There were significant muscimol, $F(1,21)=14.61$, $p<0.001$ and $F(1,22)=33.7$, $p<0.001$, respectively, but not lesion effects ($p>0.5$) on both ACTH and corticosterone serum levels. Muscimol (100 ng) increased ACTH by 83% and 106% (compared to vehicle-injected rats) in control and lesion groups, respectively, while corticosterone was increased by 338 and 256% in these same groups. Thus, MR 5,7-DHT lesions were unable to block the muscimol-induced stimulation of the pituitary-adrenal axis.

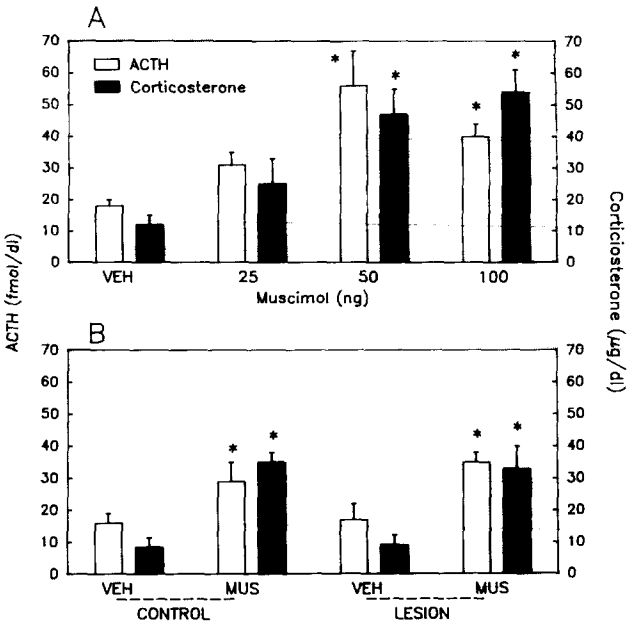

FIG. 1. (A) The effects of various doses of intra-MR muscimol (25, 50 and 100 ng in 0.5 μl 0.9% saline vehicle) and vehicle on serum concentrations of ACTH and corticosterone; (B) the effects of intra-MR 5,7-DHT (Lesion) or ascorbic acid vehicle (Control) treatment on muscimol (25, 50, 100 ng 0.5 μl saline vehicle) induced increases in ACTH and corticosterone; all data represent mean ± SEM, N = 5–8 rats/group; *significant difference from corresponding VEH group, $p<0.05$.

Intra-MR 5,7-DHT lesions failed to block muscimol's effects even though hippocampal 5-HT and 5-HIAA levels were significantly reduced by 80% and 73%, respectively ($p<0.05$; Table 1). In addition, there were small yet statistically significant reductions in striatal levels of these amines (Table 1) although striatal levels of DA were unaffected by MR lesions (data not shown). These effects are consistent with results obtained previously with MR 5,7-DHT lesions (24,25).

DISCUSSION

The $GABA_A$ agonist, muscimol, dose dependently increased serum concentrations of ACTH and corticosterone following its injection into the rat MR. Furthermore, lesions of 5-HT neurons within the MR were unable to block these stimulatory effects of muscimol.

A number of studies have indicated that injections of muscimol into the DR or MR induce locomotor hyperactivity, hyperphagia and hyperdipsia (11,26). Muscimol's effects are potentiated by administration of benzodiazepines and inhibited by the $GABA_A$ antagonist, bicuculline (27). Interestingly, although stimulation of $GABA_A$ receptors within the midbrain raphe inhibits 5-HT neuronal activity (8) and alters 5-HT metabolism in the forebrain (20,21), lesions of 5-HT neurons made with 5,7-DHT are unable to block the behavioral effects of muscimol (10, 24, 32).

TABLE 1

REGIONAL CONCENTRATIONS OF 5-HT AND 5-HIAA FOLLOWING INTRA-MR INFUSIONS OF MUSCIMOL IN CONTROL AND MR 5,7-DHT-TREATED RATS

	5-HT	5-HIAA
Hippocampus		
Control/Veh	664 ± 29	310 ± 23
Control/Muscimol	662 ± 33	255 ± 9
Lesion/Veh	132 ± 29(−80%)*	83 ± 14(−73%)*
Lesion/Muscimol	110 ± 16(−83%)*	68 ± 13(−73%)*
Striatum		
Control/Veh	896 ± 22	373 ± 20
Control/Muscimol	962 ± 49	417 ± 26
Lesion/Veh	756 ± 51(−16%)*	262 ± 29(−30%)*
Lesion/Muscimol	707 ± 52(−27%)*	263 ± 29(−37%)*

Data represent mean ± SEM of concentrations of 5-HT and 5-HIAA in the hippocampi and striata of rats infused with muscimol (100 ng/0.5 μl) into the MR; N = 6–8/group; numbers in parentheses are percent change from corresponding Control values; *significant difference ($p<0.05$) from corresponding Control group.

Preliminary studies in our laboratory have indicated that muscimol injections into the MR might be inducing an aversive or angiogenic state as measured in a conditioned place preference paradigm (13). Since novelty or an aversive environment will increase serum concentrations of corticosterone (6), we wanted to test whether or not microinjections of muscimol were producing a similar hormonal state within the animal. Our finding that intra-MR muscimol stimulates ACTH and corticosterone secretion is in contrast to the work of Miguez and colleagues (17) who found that intracerebroventricular infusions of GABA *decreased* serum corticosteroid levels. It is likely that infusion of drug into the ventricles affects many brain regions simultaneously, whereas the effects following intra-MR infusions represent an effect relatively specific to this nucleus. The anatomical specificity of this effect is supported by the finding that intra-*DR* infusions of glutamate do not alter plasma ACTH concentrations (2).

The fact that 5,7-DHT lesions did not attenuate the muscimol-induced increases in corticosteroid is interesting, particularly in light of evidence that 5-HT1A and 5-HT2 receptor agonists alter corticosteroid secretion (1, 5, 7, 12, 16). Additional pharmacological evidence has also pointed toward a direct role for serotonin on corticotropin-releasing factor (CRF) secretion (4, 19, 22, 23). This is supported by anatomical evidence for 5-HT innervation of CRF perikarya in the paraventricular nucleus of the hypothalamus (15). Although there is abundant evidence that 5-HT neurons are involved in corticosteroid secretion, the present results indicate that the ascending serotonergic projections from the MR do not appear to be critical in mediating muscimol-induced neuroendocrine effects. This is supported by the fact that muscimol has been demonstrated to *inhibit* the functional activity of 5-HT neurons (8, 9, 20, 21). Accordingly, if 5-HT plays a role in activating the hypothalamic-pituitary-adrenal axis, then by inhibiting 5-HT neuronal activity, GABA may work in modulating the serotonergic "tone" of this system. Alternatively, the present results suggest that, similar to its effects on locomotor activity, eating and drinking, a GABA-*non*-5-HT circuit may mediate muscimol's effects on ACTH and corticosterone secretion. One intriguing possibility is that GABAergic interneurons may synapse directly upon CRF-containing neurons in the MR which in turn project to CRF-containing neurons in the hypothalamus (14, 28, 29). Further studies are necessary to confirm this hypothesis.

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