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Manipulation of Central GABAergic and Dopaminergic Systems Alters Stress Responding in the Rat

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HAWKINS M. F., A. A. BAUMEISTER, R. H. LARUE, S. M. UZELAC, L. T. FOUNTAIN AND A. C. HINDELANG. *Manipulation of central GABAergic and dopaminergic systems alters stress responding in the rat.* PHARMACOL BIOCHEM BEHAV **66**(3) 667–670, 2000.—Activation of central $GABA_A$ systems with muscimol has been shown to facilitate stress responding and GABA is known to modulate central dopaminergic activity. To evaluate the possibility that this effect of muscimol may depend upon a dopamine mechanism we have tested the effect of intracerebroventricular coadministration of muscimol and the selective D_1 antagonist SCH 23390 on behaviors evoked by tail pinch stress. When injected by themselves muscimol (1.75 nmol) facilitated stress-evoked oral behavior while SCH 23390 (6–600 nmol) produced a dose-related suppression of oral behavior. Coadministration of muscimol and doses of SCH 23390 selected for producing no (6 and 30 nmol), or marginal (60 nmol), effects on stress responding resulted in a dose-related reversal of the increase in orality seen with muscimol alone. The results are consistent with the notion that stressful stimuli activate central $GABA_A$ systems which, in turn, enhance dopaminergic neurotransmission. © 2000 Elsevier Science Inc.

Muscimol SCH 23390 Stress Tail pinch Stereotypy GABA Dopamine

STRESSORS elicit behavioral responses such as eating, vocalization, gnawing, and other stereotypies from animals. Evidence suggests that central dopaminergic and GABAergic systems are involved in the stress response. Stressors are known, for example, to strongly activate central dopamine pathways (4,5,11,12), to stimulate the release of GABA (14), and to alter the density of dopamine (3) and GABA receptor sites (2,6,9,16). It has also been demonstrated that both naturally occurring stereotypy and stereotypy evoked by stress or pharmacological manipulation are augmented by activation of central dopamine and GABA systems (1,13,19,20).

GABA and dopamine systems interact with one another and, as GABA is recognized as an inhibitory neurotransmitter in the CNS, initially it was believed that the role of GABA in regard to dopamine was an inhibitory one (18). It is now realized that the relationship between these two systems is more complex, and that GABA can exert both agonistic and antagonistic effects on dopamine neurotransmission. It has been found, for example, that activation of $GABA_A$ receptors stimulates dopaminergic activity while $GABA_B$ activation inhibits it (7,13).

We have reported previously that microinjections of the $GABA_A$ agonist muscimol into the lateral cerebral ventricle or the ventral midbrain potentiate behavioral responses evoked by tail-pinch stress (10). The purpose of the studies reported here was to investigate the possibility that enhanced stress responding produced by muscimol might involve a dopamine-dependent step and might, therefore, be attenuated by concurrent administration of a selective dopamine antagonist.

METHOD

Subjects

Male Sprague–Dawley rats were obtained from the Division of Laboratory Animal Medicine, School of Veterinary Medicine, Louisiana State University. They were housed individually in suspended metal cages in a temperature-con-

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trolled vivarium (22°C). Lab chow and water were constantly available, and lights were cycled on a 12:12-h photoperiod (on 0700 h).

Surgery and Histology

At approximately 300 g of body weight animals were randomly assigned to one of three experimental groups (described below), and were implanted bilaterally with permanently indwelling stainless steel guide cannulae (22 gauge) targeted at the lateral cerebral ventricles. Coordinates were 0.0 mm anterior to bregma, ±1.6 mm lateral from the midline, and −3.0 mm from the dura (17). Surgery was accomplished with the aid of a small animal stereotaxic instrument under xylazine (5 mg/kg, IM) and Ketamine HCl(90 mg/kg, IM) anesthesia. Animals were permitted 5 days of postoperative recovery before the beginning of experimental testing.

Upon completion of testing animals were sacrificed with an overdose of anesthetic and were given bilateral intracerebroventricular injections of India ink (3.0 μl/side). The presence of ink in the fourth ventricle was taken as confirmation of cannula placement. Data reported below are for the 32 animals with confirmed implants.

Procedure

Experiments were performed on three groups of animals. A repeated-measures protocol was employed in which each animal served as its own control by receiving vehicle injections and all levels of the drug(s). The order of presentation of drug doses across days was randomized for each animal, and animals were tested every other day to permit one drug-free day between subsequent injections. The low SCH group ($n = 11$) received intraventricular injections of saline control and a total of 6, 30, and 60 nmol (half of the dose infused into each ventricle) of the selective D_1 antagonist SCH 23390 [R(+)-7-Chloro-8-hydroxy-3-methyl-l-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; Research Biochemicals International] (SCH). The high SCH group ($n = 9$) received intraventricular injections of saline control and a total of 60, 300, and 600 nmol of SCH. The third group (muscimol + SCH, $n = 12$) received concurrent intraventricular injections of 1.75 nmol (half per ventricle) of the selective $GABA_A$ agonist muscimol (5-Aminomethyl-3-hydroxyisoxazole, Sigma Chemical Co.) and the low doses of SCH listed above. The following five combinations of muscimol, SCH, and vehicle control were used for this group: saline + saline, muscimol + saline, muscimol + 6 nmol SCH, muscimol + 30 nmol SCH, and muscimol + 60 nmol SCH.

Drugs were reconstituted with sterile 0.9% saline from frozen, lyophilized aliquots on the day of injection. Microinjection was via 30 gauge injector needles that extended 1.0 mm below the ventral tip of the guide cannula. Injector needles were connected to 10-μl syringes mounted in a Sage infusion pump that delivered the injectant to the two ventricles simultaneously at a rate of 0.5 μl/min. A total volume of 1.0 μl/side was used. Injectors were left in place for one additional minute after injection to permit diffusion from the injection site.

On the day of experimentation an animal was taken from its home cage and was restrained by hand while the injection took place. Immediately afterward the animal was placed in a suspended stainless steel cage, and its tail was passed through the wire mesh floor of the cage. Tail pinch was achieved with a modified pair of hemostatic forceps that were padded with latex tubing. These were clamped at a premarked position 5.0 cm from the distal tip of the tail, and were left in place for 4 min during which behavioral observations were made. This procedure was repeated at three recording intervals: immediately after injection (0 min), and 30 and 60 min after injection.

Lab chow was continuously available in the testing cage so that gnawing could be measured by weighing (to the nearest 0.01 g) particles of chow that fell through the wire floor of the cage during the 4 min of tail pinch. Duration (to nearest 0.1 s) of total oral behavior was also recorded. Total oral behavior was defined as any visible orofacial movement. For the muscimol + SCH group, total oral behavior was partitioned into orality directed at food (e.g., eating, gnawing, licking, chewing) and orality not directed at food (e.g., licking and biting of self or cage, teeth chattering) by recording the duration of orality not directed at food and subtracting this from the duration of total oral behavior.

Statistics

Data were analyzed by analysis of variance for repeated measures followed by the Student–Newman–Keuls procedure. Pearson product-moment correlation coefficients were also calculated.

RESULTS

Effects of SCH 23390

Intraventricular microinjection of SCH 23390 produced significant and dose-related reductions in stress-induced gnawing and oral behavior. The highest dose of SCH (600 nmol) resulted in a virtually complete elimination of gnawing (mean = 0.03 g) and oral stereotypy (mean = 2.23 s), which was evident immediately after injection and which persisted across the three recording intervals.

Effects on gnawing. The three high doses of SCH suppressed gnawing by 87% (saline, mean = 7.39 g; SCH, mean = 0.97 g). This reduction was significant, $F(2, 16) = 4.502$, $p = 0.028$, and the effects of the three doses did not vary across the three recording intervals or differ from one another (data not shown). The low doses of SCH did not significantly reduce gnawing behavior (see Fig. 1A).

Effects on duration of oral behavior. As with gnawing, the three high doses of SCH significantly reduced the duration of stress-induced oral behavior by 73% (saline, mean = 118.98 sec; SCH, mean = 32.03 s; data not shown), $F(3, 24) = 18.046$, $p < 0.001$. The effects of the three doses did not differ among themselves, but a main effect for recording interval was found, $F(2, 16) = 4.553$, $p = 0.027$, in which average duration of orality was significantly lower at 30 min (37.36 s) than at 0 min (60.68 s) or 60 min (63.25 s).

A dose-related reduction in duration of orality was also found with the low doses of SCH, $F(3, 30) = 5.236$, $p = 0.005$. This effect is displayed in Fig. 1B. Post hoc analysis revealed that while the amount of orality displayed after the 6- and 30-nmol doses did not differ from saline or from one another, the 60-nmol dose decreased it significantly relative to saline and to the 6 nmol dose. A significant interaction between recording interval and dose also occurred with the lower doses, $F(6, 60) = 2.263$, $p = 0.049$. This interaction was attributable largely (five of six comparisons) to differences between the 60-nmol dose and the other means.

Effects of Coadministration of SCH 23390 and Muscimol

Drug treatment did not significantly alter stress-evoked gnawing, but did affect the duration of oral behavior. Figure 2 displays seconds of total oral behavior (A), seconds of orality

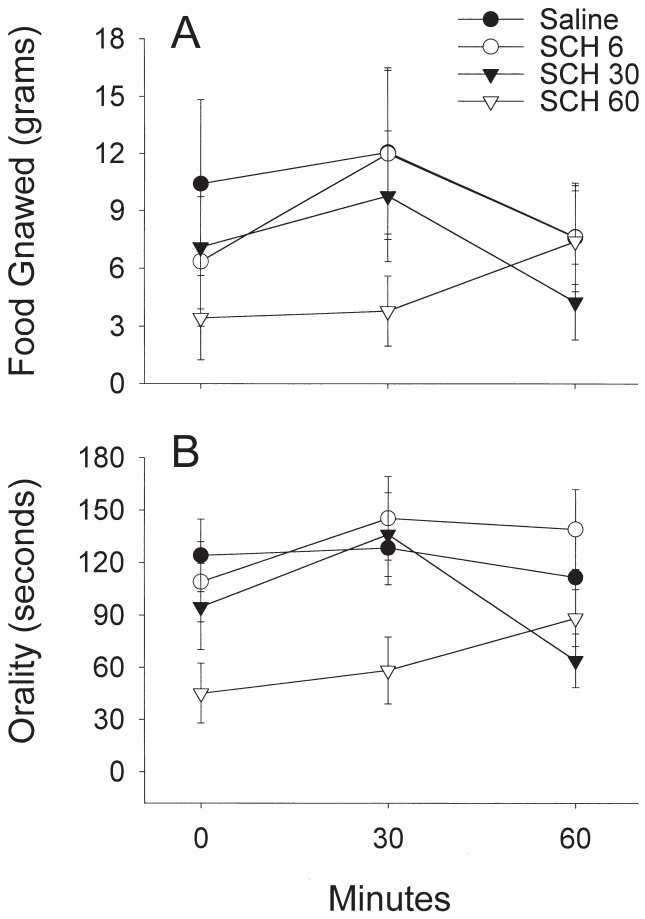

FIG. 1. Mean (±SE) number of grams of food gnawed (A) and seconds of orality observed (B) at 0, 30, and 60 min following completion of intracerebroventricular microinjection of saline control or 6 nmol (SCH 6), 30 nmol (SCH 30), or 60 nmol (SCH 60) of SCH 23390.

directed at food (B), and seconds of orality without food (C) collapsed across the recording intervals.

Effects on total oral behavior. The number of seconds of total oral behavior elicited by stress was altered by drug dose (Fig. 2A), $F(4, 44) = 3.493$; $p = 0.015$. When muscimol was administered without SCH, the number of seconds of orality increased significantly to approximately double the saline–saline control value (88 vs. 171 s, respectively). This elevation was reversed in a dose-dependent way by concurrent administration of SCH. While 6 nmol of SCH had no effect on the muscimol-induced increase in orality, the orality displayed after coadministration of muscimol and the 30- and 60-nmol doses of SCH was statistically indistinguishable from the saline–saline control condition.

A main effect of recording interval was also found, $F(2, 22) = 5.488$, $p = 0.012$. From 0 to 60 min postinjection total orality increased 30% from 113 s to 147 s.

Effects on orality without food. Orality, which occurred in the absence of food, was not affected by drug administration (Fig. 2C). Although a significant interaction between recording interval and drug dose was observed, $F(8, 88) = 2.388$, $p = 0.022$, post hoc analysis revealed that this was attributable to a

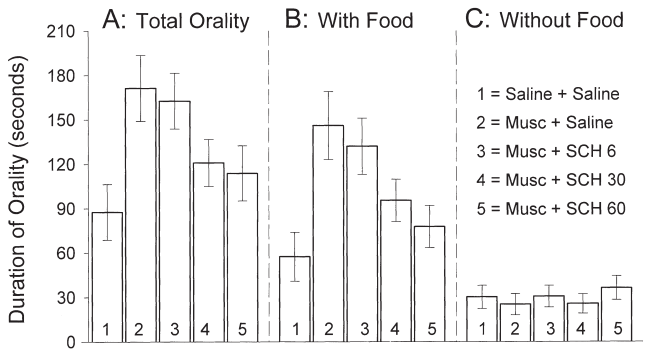

FIG. 2. Mean (±SE) duration of total oral behavior (A), orality directed at food (B), and orality without food (C) following intracerebroventricular microinjection of saline control (Saline + Saline), 1.75 nmol of muscimol (Musc + Saline), and coadministration of 1.75 nmol of muscimol and 6, 30, and 60 nmol of SCH 23390. Data are collapsed across the three recording intervals.

single increase in stereotypy from 0 to 60 min following coadministration of muscimol and 60 nmol of SCH.

Effects on orality with food. The effects of drug dose on total oral behavior were due to orality which occurred while the animals were holding food. A significant correlation existed between scores for total orality and orality with food ($r = 0.91$, $p < 0.001$). Oral behavior with food is shown in Fig. 2B. Under this condition there was a significant effect of drug dose, $F(4, 44) = 4.646$, $p = 0.003$, which paralleled the effects seen with total orality. Orality was significantly increased relative to saline control following muscimol injection, and this increase was reversed by concomitant administration of 30- and 60-nmol doses of SCH. The 6 nmol dose of SCH had no effect on elevated orality, and orality following this dose was significantly higher than was seen following the 60-nmol dose.

The effects of muscimol and SCH on oral behavior demonstrate that, while gnawing was not statistically affected by drug dose, significant changes were occurring in orality directed at food as a consequence of drug administration. Correlational analysis also revealed that gnawing scores were significantly correlated with both total orality ($r = 0.59$, $p < 0.001$) and with orality with food ($r = 0.69$, $p < 0.001$).

As was seen with duration of total oral behavior, oral behavior in the presence of food varied with recording interval, $F(2, 22) = 3.508$, $p = 0.048$. From 0- to 60-min duration of oral behavior directed at food increased 30% (87 to 113 s).

DISCUSSION

Our results demonstrated that activation of $GABA_A$ receptors with muscimol potentiates stereotyped oral behavior directed at food in response to stress. These findings confirm our previous report of enhancement of stress-evoked responding by muscimol (10), and are consistent with other findings of selective activation of oral behavior by $GABA_A$ agonists (19,20).

We also observed that antagonism of D_1 dopamine receptors with SCH 23390 produced a dose-related inhibition of stress-evoked behavior. Whether this effect with higher doses (60–600 nmol) represents a specific effect upon stress responding or a generalized suppression of behavior is debatable, and must be interpreted with caution. It is known that SCH 23390 can induce catalepsy (19) and, as reported here,

the 600-nmol dose of SCH resulted in a virtually complete elimination of gnawing and orality. Unlike the higher doses, lower doses of SCH (6–60 nmol) had marginal, or no, effect on stress responding. Gnawing was not affected by these doses and duration of orality was decreased by the 60-nmol dose only.

When the low doses of SCH were coadministered with muscimol a dose-dependent blockade of the muscimol effect was observed. Following muscimol injection without SCH coadministration, both duration of total oral behavior and duration of oral behavior in the presence of food increased an average of 125%. Although coadministration of 6 nmol of SCH had no effect on the heightened responding produced by $GABA_A$ activation, the 30- and 60-nmol doses of SCH reversed both measures of stress to control values. The fact that SCH suppressed GABA-enhanced stress responding at doses that produced no (30 nmol), or marginal (60 nmol), effects on behavior by themselves suggests the possibility that muscimol and SCH 23390 are exerting their effects through a common mechanism.

These results are consistent with the notion that stressful stimuli activate central $GABA_A$ systems which, in turn, enhance dopaminergic neurotransmission. This proposition is congruent with other research regarding GABA/dopamine interactions in the A9 and A10 dopamine systems. Such research has led to the conclusion that $GABA_B$ receptors exist on dopamine perikarya in these areas and that, when these receptors are activated by intrinsic GABA interneurons or by GABA afferents from other areas, they tonically inhibit dopaminergic activity via an increase in potassium conductance. In addition, the GABAergic interneurons intrinsic to the A9 and A10 areas are postulated to contain $GABA_A$ receptors which, when activated, inhibit these interneurons and, thereby, disinhibit the dopamine neurons with which the interneurons make synaptic contact (8,13,15). As a result, $GABA_B$ agonists would be expected to decrease dopamine activity in these systems, and $GABA_A$ agonists would enhance dopamine function. The stress-enhancing effect of muscimol, therefore, may be attributable to the well-established facilitation of responding seen with activation of dopamine systems.

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