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RAPID COMMUNICATION

The Effects of Acute Cocaine Administration on the DOI-Induced Head-Twitch Response in Reserpinized Mice

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DARMANI, N. A. *The effects of acute cocaine administration on the DOI-induced head-twitch response in reserpinized mice.* PHARMACOL BIOCHEM BEHAV **49**(1) 229–232, 1994. – Previously it was shown that acute cocaine administration dose dependently reduces the 5-HT_2-receptor-mediated DOI [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane]-induced head-twitch response (HTR) in mice via indirect stimulation of the inhibitory adrenergic α_2- and serotonergic 5-HT_{1A} receptors. In addition, the inhibitory capacity of cocaine was enhanced fourfold in mice with 5-HT_2-receptor supersensitivity induced by a single injection of DOI 48 h prior to experimentation. The aim of the present investigation was to determine the inhibitory capacity of cocaine in reserpinized mice. A single injection of reserpine 48 h prior to DOI administration caused supersensitivity in the DOI-induced HTR. Two reserpine injections did not further enhance this supersensitivity effect. Once reserpinized 5-HT_2-receptor supersensitive animals were less responsive to the inhibitory effects of cocaine on the DOI-induced behavior than were the mice, as reported previously, that were made supersensitive by DOI pretreatment. The inhibitory capacity of cocaine was further attenuated when mice were reserpinized twice prior to determination of its effects on the DOI-induced behavior. Taken together with previously published data, the present investigation lends further support for the importance of endogenous levels of 5-HT and norepinephrine on the ability of cocaine to attenuate the DOI-induced HTR.

Head-twitch response DOI Reserpine Cocaine

IT IS well accepted that activation of dopaminergic mechanisms (via inhibition of dopamine uptake) primarily mediate the locomotor, reinforcing, and discriminative stimulus effects of cocaine [reviews: (4,20,30)]. Recently, a plethora of diverse evidence has emerged that strongly indicates that the stimulant also affects serotonergic neuronal systems and that the alterations may modify the dopaminergic components of cocaine's actions. Supporting evidence for cocaine's potent effects on the functional activity of central serotonergic systems include: electrophysiological (5,28); self-administration (3,30); neuroendocrine (22); determination of brain serotonin concentration, turnover, and uptake studies (2,13,29). Serotonin can specifically bind and functionally stimulate at least eight different serotonergic receptors (5-HT_{1A-1E}, 5-HT_2, 5-HT_3, and 5-HT_4) (33). Furthermore, other novel serotonergic receptors (such as 5-$HT_{5A,B}$) have been discovered and their pharmacological properties are under investigation (24).

Many investigators have utilized serotonergically induced behaviors in rodents as models for activation of specific serotonergic receptor sites [review: (15)]. One such model for activation of 5-HT_2 receptors is the head-twitch response (HTR) in mice that is produced by selective (e.g., DOI) and nonselective (e.g., 5-MeO DMT) 5-HT_2 agonists (8,10) and is dose dependently attenuated by the selective and nonselective 5-HT_2 receptor antagonists (10,15). Darmani et al. (11) has shown that acute cocaine pretreatment attenuates the DOI-induced HTR in a dose-dependent manner. Because acute cocaine administration can also increase synaptic concentrations of norepinephrine and serotonin via inhibition of their uptake [review: (31)], it was suggested that the stimulant reduces the HTR frequency indirectly by stimulating the inhibitory serotonergic 5-HT_{1A} and postsynaptic adrenergic α_2 receptors (11). Inhibition of HTR by costimulation of either serotonergic 5-HT_{1A} (1,10) or adrenergic α_2 receptors (16,17) is a well established phenomenon. The dopaminergic system does not modulate the serotonergically induced HTR (14,16). Relative to normal animals (11), 5-HT_2 receptor-supersensitive mice are four times more sensitive to the inhibitory effect of cocaine

(7). This enhancement of cocaine's inhibitory effect is reflected by augmentation in the sensitivity of both inhibitory receptors in attenuating the DOI-induced HTR in 5-HT_2 receptor-supersensitive mice. The monoamine depleting agent, reserpine, also produces monoamine receptor supersensitivity (6,18,32). The purpose of the present study was to further investigate the role of endogenous serotonin and norepinephrine for the discussed inhibitory components of acute cocaine administration on the DOI-induced HTR in monoamine depleted (reserpinized) mice.

METHOD

Animals and Drugs

Albino ICR mice were bred in the animal facilities of the Kirksville College of Osteopathic Medicine. Male mice (22–25 g) were used throughout the study. Animals were housed in groups of five on a 12-h L : 12-h D cycle at a room temperature of 22 ± 1°C with ad lib supply of food and water. All experiments were performed between 0800 and 1700 h. Reserpine was obtained from Sigma (St. Louis, MO) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI HCl) from Research Biochemicals, Inc. (Natick, MA). Cocaine HCl was obtained from the National Institute on Drug Abuse. DOI and cocaine were dissolved in distilled water, whereas reserpine was suspended in distilled water following sonication. All drugs were given intraperitoneally at a volume of 10 ml/kg.

Experimental Protocols

Previous studies have shown that once-daily reserpine treatment for either 1, 2, or 3 days enhances the functional sensitivity of both catecholaminergic and serotonergic receptors when animals are tested with corresponding agonists 24–120 h after the last reserpine injection (6,21,32). For the initial study in the present investigation, mice were treated with either a single intraperitoneal injection of reserpine (2 mg/kg, $n = 50$) or distilled water ($n = 8$). The reserpine-treated mice were then divided into five groups. Forty-eight hours later each water-treated mouse was allowed 20 min to acclimate in a plastic holding cage (40 × 25 × 16 cm) lined with wood chippings. Immediately after a second injection of distilled water, each mouse was transferred to the observation cage (40 × 25 × 16 cm) and 10 min later received DOI (2.5 mg/kg). The DOI-induced HTR was counted cumulatively at 4-min intervals for the next 20 min. Mean score (±SEM) for every 4-min time period was calculated. In a protocol similar to the water-pretreated control mice, the reserpine-treated groups received either a single injection of distilled water (group 1) or a single injection of varying doses of cocaine [0.5 (group 2), 2.5 (group 3), 10 (group 4) or 20 (group 5) mg/kg, $n = 7$–12] 10 min prior to DOI (2.5 mg/kg) administration. The induced HTR was scored for 20 min as described above.

Because a single injection of reserpine may not totally abolish the neuronal monoamine storage capacity (21), a second group of mice were treated twice prior to determination of the effects of cocaine on the DOI-induced HTR. Thus, mice were treated with either distilled water ($n = 5$) or reserpine (2 mg/kg, $n = 30$) twice, once at zero time and again 24 h later. Twenty-four hours following this treatment protocol, the twice-vehicle-treated control group received an injection of distilled water 10 min prior to DOI (2.5 mg/kg) administration. The induced HTR was counted for the next 20 min as described previously. The twice-reserpine-treated mice were divided into five groups. They received either an injection of distilled water (group 1) or a single injection of varying doses of cocaine [0.5 (group 2), 2.5 (group 3), 10 (group 4), or 20 (group 5) mg/kg) 10 min prior to an intraperitoneal injection of DOI (2.5 mg/kg). The DOI-induced HTR was scored for the next 20 min as described earlier.

Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) and post hoc analysis by either Dunnett's *t*-test or Scheffe's *F*-test. A p value of <0.05 was necessary to achieve statistical significance. The 50% inhibition dose (ID_{50}) was computed by InPlot.

RESULTS

Previously, Darmani et al. (11) had shown that acute cocaine pretreatment dose dependently attenuated the DOI-induced HTR in normosensitive mice with an ID_{50} of 11.8 (6.4–21.4) mg/kg. In the present study, once-reserpine-pretreated mice exhibited a greater frequency of HTR (49% increase, $p < 0.05$) relative to vehicle-exposed control group in the 20-min observation period in response to a 2.5 mg/kg dose of DOI administered 48 h following the initial pretreatment procedure (Fig. 1A). This reserpine-induced supersensitivity in DOI-induced response was dose- and time-dependently reduced by cocaine [ID_{50} = 8.5 (4.1–17.4) mg/kg] when the stimulant was administered 10 min prior to DOI administration (Fig. 1A,B). At 0.5 mg/kg dose, cocaine had no significant effect but larger doses of the stimulant (2.5, 10, or 20 mg/kg) significantly reduced the total cumulative DOI-induced HTR score in these mice by 34, 56, and 66%, respectively ($p < 0.05$) (Fig. 1A). The 2.5 mg/kg dose of cocaine produced significant attenuation in the second observation interval (i.e., 4–8 min), whereas larger doses of the stimulant was effective within the first observation period ($p < 0.05$) (Fig. 1B). These effects persisted throughout the experiment.

When mice were treated twice with reserpine (i.e., 48 and 24 h prior to DOI administration) a similar supersensitivity (47% increase, $p < 0.05$) was observed in DOI-induced HTR score relative to the vehicle-pretreated control group (Fig. 2A). However, the ability of cocaine to reduce the DOI-induced HTR in these mice was significantly reduced relative to reserpine once-pretreated group. Only the 20 mg/kg dose of cocaine significantly ($p < 0.05$) attenuated the total DOI-induced HTR score by 56% in the twice-reserpinized mice [ID_{50} = 19.9 (5.7–70) mg/kg] (Fig. 2A). This inhibition was apparent within the first time interval ($p < 0.05$) (Fig. 2B).

DISCUSSION

This study demonstrates that a single administration of reserpine is sufficient to induce supersensitivity in the functional activity of DOI-induced 5-HT_2 receptor-mediated HTR in mice. Further enhancement in HTR supersensitively was not evident when animals were pretreated twice with reserpine. The present data is in agreement with previous reports that demonstrate such supersensitivity to other 5-HT agonists (6, 21,32).

Previously it was shown that acute cocaine administration dose-dependently reduced the DOI-induced HTR [ID_{50} = 11.8 (6.4–21.4) mg/kg] in normosensitive mice (11). This inhibition is suggested to be due to cocaine-induced increases in concentrations of norepinephrine and serotonin via inhibition of their uptake and, therefore, indirect stimulation of the re-

spective inhibitory adrenergic α_2-and serotonergic 5-HT_{1A} receptors. When 5-HT_2 receptor function was made selectively supersensitive (9), the ability of cocaine to reduce the DOI-mediated behavior was enhanced by approximately four times [ID_{50} = 3.3 (1.6-6.7) mg/kg] (7). In the present study, when mice were injected once with reserpine, cocaine attenuated the DOI-induced HTR with an ID_{50} dose of 8.5 (4.1-17.4) mg/kg. The inability of lower doses of cocaine to attenuate the HTR frequency in once-reserpinized 5-HT_2 receptor-supersensitive mice suggests that partial depletion of the inhibitory endogenous monoamines (i.e., norepinephrine and serotonin) has occurred. Indeed, a single injection of reserpine is reported to reduce 5-HT levels by 75-90% (21). Such reserpine treatment attenuates the ability of intravenously administered cocaine to suppress the firing of A10 dopamine neurons by reducing the

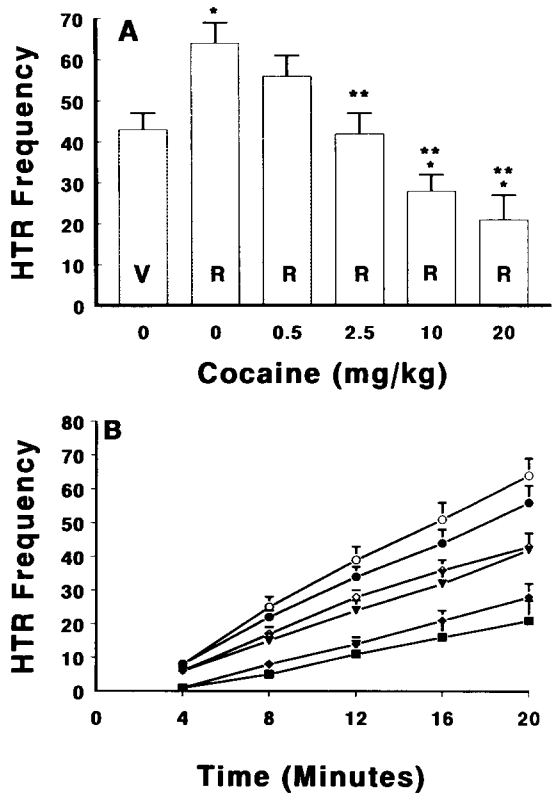

FIG. 1. (A) Dose-dependent inhibitory effect of cocaine on the head-twitch frequency (mean ± SEM) induced by 2.5 mg/kg DOI in once-reserpinized (R) mice. A single injection of reserpine (2 mg/kg) or vehicle (V) was administered to different groups of mice 48 h prior to testing procedure. The cited doses of cocaine were administered 10 min prior to DOI injection and the total DOI-induced HTR frequency was counted for 20 min. *Significant difference between vehicle (V) pretreated control and reserpinized mice ($p < 0.05$). **Significant difference between the 0 (water control) and the cited doses of cocaine ($p < 0.05$) in reserpinized mice. (B) Dose- and time-response inhibition of DOI-induced HTR (mean ± SEM) by the cited doses of cocaine recorded at 4-min intervals following administration of DOI in the above once reserpine pretreatment schedule. [(Cocaine doses administered in reserpinized mice: 0 = 0; ● = 0.5; ▼ = 2.5; ◆ = 10; ■ = 20 mg/kg) (vehicle pretreated group receiving water prior to DOI administration = ◇)]. The 2.5 mg/kg cocaine dose produced significant attenuation from the second observation interval, whereas larger doses of the stimulant were significantly effective within first observation period ($p < 0.05$).

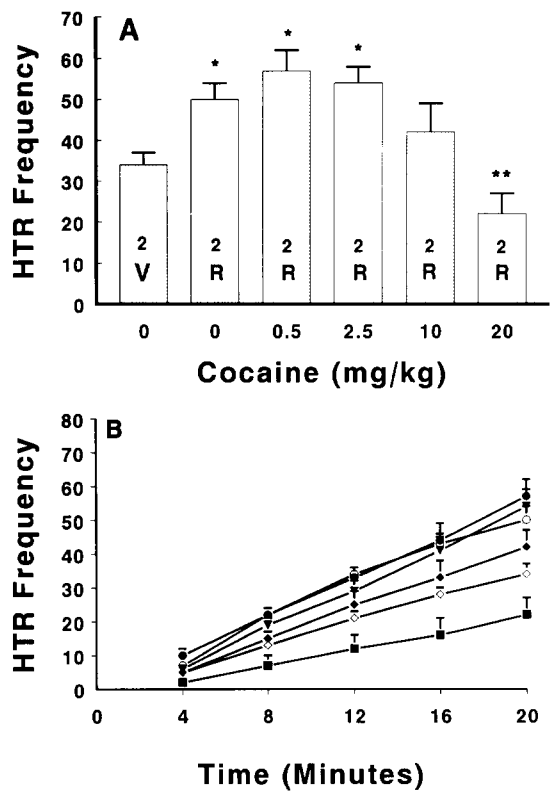

FIG. 2. As for Fig. 1 except animals were reserpinized twice (2R) prior to testing protocol. For further details see Method and Result sections.

endogenous levels of dopamine (12). Following a two-time reserpine injection schedule, the ability of cocaine to reduce the DOI-induced HTR score was further attenuated [ID_{50} = 19.9 (5.7-70) mg/kg] and only the 20 mg/kg dose of the stimulant had a significant effect. Thus, the present data suggests that repeated reserpine administration appears to further reduce endogenous levels of the inhibitory monoamines. Indeed, a recent study supports this notion (27). Moreover, the latter report also concludes that repeated reserpinization uncouples the synergistic interaction between dopamine D_1 and D_2 receptors in the production of dopamine-mediated behavioral stereotypy by depleting brain dopamine levels by 98%.

In summary, it appears that central levels of norepinephrine and 5-HT are of paramount importance in the cocaine-induced attenuation of 5-HT_2-receptor mediated head-twitch response. Thus, relative to normosensitive and selectively made 5-HT_2 receptor-supersensitive mice, twice- reserpinized 5-HT_2 receptor-supersensitive mice require greater amounts of cocaine to attenuate the DOI-induced HTR. At such high doses, cocaine may attenuate the induced behavior either via inhibition of uptake of the cited inhibitory monoamines because reserpine does not affect monoamine synthetic capacity (23,26) or via other central neurotransmitters such as benzodiazepine (25) or GABA (19) receptor systems that can modulate the 5-HT-induced HTR frequency (16).

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