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The Expression of Behavioral Sensitization to Amphetamine: Role of CCK_A Receptors

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DESOUSA, N. J., G. R. WUNDERLICH, C. DE CABO AND F. J. VACCARINO. *The expression of behavioral sensitization to amphetamine: Role of CCK_A receptors*. PHARMACOL BIOCHEM BEHAV **62**(1) 31–37, 1999.—These studies investigated whether endogenous activation of CCK_A receptors mediates the expression of amphetamine (AMP)-induced locomotor activity. In Experiment 1, locomotor activity was assessed in rats pretreated with the CCK_A antagonist devazepide (0.001, 0.01, and 0.1 mg/kg) and subsequently injected with AMP (1.5 mg/kg). In Experiment 2, rats were administered AMP (1.5 mg/kg) once daily for 7 days. Following a 10-day withdrawal, locomotor activity was assessed following treatment with devazepide (0.001, 0.01, and 0.1 mg/kg) and AMP (0.75 mg/kg). In both studies, rats were classified as low (LR) or high (HR) responders based upon a median split of their locomotor response to a novel environment. Results from Experiment 1 showed that AMP potentiated the expression of locomotor activity, and this effect was most pronounced in HR rats. However, devazepide did not affect AMP-induced locomotion. Results from Experiment 2 demonstrated that chronic AMP pretreatment augmented the locomotor response to subsequent AMP challenge, and this effect was most pronounced in the HR group. Further, this augmented response was blocked by devazepide in HR rats. These findings constitute the first demonstration that endogenous CCK_A receptor activation is an important substrate mediating AMP-induced locomotor activity in animals with a previous history of AMP treatment. © 1998 Elsevier Science Inc.

Locomotor activity Cholecystokinin CCK_A receptors Devazepide Dopamine Amphetamine Sensitization Individual differences

CHOLECYSTOKININ (CCK), a 33-amino acid peptide identified 3 decades ago (12), is found throughout the mammalian peripheral and central nervous system. Although CCK exists in several forms, the principal active type is CCK_8 (2). CCK is synthesized de novo (6), released via a calcium-dependent mechanism (5), and is active at both CCK_A and CCK_B receptor subtypes (18). Evidence indicates that CCK may act either as a neurotransmitter or neuromodulator in several brain regions (29).

CCK has been shown to coexist with dopamine (DA) in a subset of mesencephalic neurons that terminate in nucleus accumbens (NAcc) (8,9,16). Neurochemical and electrophysiological studies suggest that CCK_A receptors mediate DA agonist-like effects in this region. For example, NAcc CCK_A receptor activation potentiates K^+-stimulated endogenous DA release (17,30) as well as increasing extracellular NAcc concentrations of DA and its metabolites, DOPAC and HVA (13,31). Consistent with these findings, behavioral studies have demonstrated that exogenous activation of NAcc CCK_A receptors potentiates mesolimbic DA-dependent behaviors, such as hyperlocomotion induced by DA or the DA agonist amphetamine (AMP) (3,4,28).

Despite these positive findings with exogenous CCK, however, studies addressing the role of endogenous CCK_A receptor activation in DA-mediated behaviors such as unconditioned locomotor activity have generated primarily negative results. For example, Crawley reported that NAcc and VTA microinjections of the CCK_A antagonist devazepide did not block the expression of baseline locomotor activity in either an illuminated or darkened environment (3). In addition, in-

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fusions of devazepide into the NAcc and VTA did not affect DA-induced locomotor activity. Similarly, Higgins and colleagues reported no effect of systemic devazepide on locomotion induced by a low dose of AMP (7). These results appear to suggest that endogenous activation of CCK_A receptors does not contribute to the expression of unconditioned locomotor activity. However, it is also possible that the lack of behavioral effects seen following CCK_A receptor blockade result from the use of behavioral paradigms that fail to induce sufficient levels of endogenous CCK release.

Several studies indicate that CCK release may occur only under specific conditions. For example, electrophysiological data suggest that colocalized CCK may be released in the NAcc only during high levels of neuronal activity (1,8). In parallel, in vivo microdialysis studies show that CCK is coreleased with DA in the NAcc following systemic administration of a moderately high dose (1.5 mg/kg) of AMP (11). In light of these results, it was hypothesized that CCK_A receptor blockade should be maximally effective following experimental manipulations that correspond with high levels of DA release. The present experiments utilized acute and chronic AMP treatments to investigate this hypothesis.

In the first experiment, the effect of devazepide on AMP-induced locomotor activity was examined. The AMP dose utilized in this study was higher than that of Higgins et al. (7), and consistent with that shown to release endogenous stores of DA and CCK (11). In vivo (19,23,32) and in vitro (14,15,21) studies suggest that under certain conditions mesolimbic DA released following stimulant challenge is augmented in animals that have previously been administered stimulants. Further, chronic systemic AMP treatment results in increased gene expression in those mesolimbic cells expressing mRNA for both CCK and the DA synthesis enzyme tyrosine hydroxylase (11). Thus, Experiment 2 was designed to investigate the role of endogenous CCK in the expression of the sensitized locomotor response to AMP. In both experiments, individual differences in locomotor responsivity to a novel environment was also included as a variable, as previous research has shown this measure to be positively correlated with individual differences in mesolimbic DA activity (10,20).

METHOD

This research was conducted with due regard for the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University of Toronto policy.

Subjects

Male Wistar rats (obtained from Charles River, Canada), weighing approximately 250 to 275 g upon arrival, were housed individually in suspended wire mesh cages (Experiment 1) or clear Plexiglas cages (Experiment 2) in a temperature-controlled (21 ± 1°C) colony room. Rats were maintained on a 12 L:12 D cycle (lights on at 0700 h). Food and water were available in the home cages ad lib and all behavioral testing was carried out during the animals' light cycle.

Drugs

Devazepide [3*S*(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide; $C_{25}H_{20}N_4O_2$] was generously provided by Merck Sharpe and Dohme, Harlow, UK. Devazepide (also known as L-364,718 or MK329) is a competitive antagonist with high selective affinity for the CCK_A receptor (i.e., IC_{50} = 0.2 nM at rat pancreatic CCK_A receptor vs. 31.7 nM at mouse cortical CCK_B receptor). For Experiment 1, solutions of devazepide were prepared to the appropriate concentration in a vehicle of 95% saline (0.9% NaCl), 2.5% ethanol, and 2.5% propylene glycol and stored frozen (−20°C). Solutions were thawed, stirred, and administered IP in a volume of 2 ml/kg body weight on test days. For Experiment 2, devazepide was first suspended in Tween 80 (0.1%) then dissolved in saline and stored frozen (−20°C). On test days, devazepide and vehicle were thawed, stirred, and administered IP in a volume of 1 ml/kg body weight.

AMP was obtained from the Bureau of Dangerous Drugs, Ottawa, Canada. AMP was dissolved in physiological saline (0.9% NaCl). Following preparation, AMP was refrigerated (4°C) in covered glassware for a maximum of 5 days AMP solution or vehicle was administered IP in a volume of 1 ml/kg body weight.

Apparatus

All locomotor activity testing was conducted in a dimly lit room containing a bank of 16 separate locomotor cages. The cages (34 × 33 cm) were constructed of metal sides, wire mesh floors and front, and Plexiglas top and back. Each cage was outfitted with two horizontal infrared emitters with opposing detectors, positioned 3 cm above the floor and 11 cm apart on the side walls dividing the cages into thirds. The cages were interfaced with a microcomputer running in-house software, which measured crossovers (defined as the consecutive interruption of front and back beams).

Procedure

Experiment 1. Rats (n = 16) were habituated for a period of 2.5 h prior to testing. During the test phase, animals were removed from their home cage and placed in the locomotor apparatus for a habituation period of 30 min, during which time their baseline locomotion was measured. Following this period, animals were injected (IP) with devazepide or vehicle and placed back into their locomotor cages for another period of 30 min. Animals were then removed from their test chamber and administered (IP) either AMP (1.5 mg/kg) or saline, and returned to their locomotor cages. Locomotor activity was then recorded for 60 min.

Rats were injected in a counterbalanced order with one of five treatments. Treatment 1 consisted of a vehicle treatment followed by saline. A second treatment consisted of vehicle followed by AMP. Treatments 3 to 5 consisted of devazepide injections (0.001, 0.01, or 0.1 mg/kg), again followed by AMP. Testing was separated by a period of at least 48 h.

Experiment 2. The experimental protocol consisted of three distinct phases. In the first phase, rats (n = 68) were injected (IP) with either saline or AMP (1.5 mg/kg) once per day for 7 consecutive days in their home cage. In the second phase, animals were given a 10-day drug-free withdrawal period. In the third phase, animals were placed in the locomotor boxes for a 60-min habituation period, during which time their baseline locomotor activity was measured. After 60 min, animals were injected (IP) with devazepide or vehicle and placed back into their locomotor cages for a further 30-min period. After the 30-min period, all rats received an AMP injection (0.75 mg/kg, IP) and their activity was measured for a further 60 min.

Animals were randomly assigned to five separate groups. A control group (n = 16) received saline pretreatment during the first phase and in the third phase received a vehicle injec-

tion prior to the AMP challenge. A second control group (n = 16) received AMP pretreatment during the first phase, and in the third phase received a vehicle injection prior to the AMP challenge. Each of the remaining three groups (n = 12 for each) received AMP pretreatment in the first phase and in the third phase received devazepide (0.001, 0.01, or 0.1 mg/kg) prior to the AMP challenge. In both Experiments 1 and 2 the total number of crossovers, summed at 10-min intervals, in the 60 min following AMP challenge was used for statistical analysis.

Statistical Analyses

In Experiment 1, the total number of cage crossovers, summed across six 10-min intervals, was analyzed using a two-way (treatment × time) repeated-measures analysis of variance (ANOVA). As the main question addressed in Experiment 1 was the effect of devazepide on the locomotor response to an acute AMP challenge, it was of interest to examine whether there was any evidence of AMP sensitization resulting from the use of a repeated measures design. To address this issue, a one-way ANOVA was used to analyze the effect of the number of preceding AMP injections on the subsequent locomotor response to AMP. In Experiment 2, a mixed two-way (treatment × time) ANOVA was used to analyze the total number of cage crossovers. Where appropriate, post hoc comparisons were conducted using the least significant difference (LSD) test. In addition to analyzing the data from all animals, separate analyses were also conducted for low responder (LR) and high responder (HR) animals based upon a median split within each group of each animal's total locomotor activity during their initial habituation period. For all analyses, the accepted level of significance was $p < 0.05$.

RESULTS

Experiment 1

Locomotor activity was assessed using crossover totals, summed over 10-min intervals, across the 6O-min test session. Figure 1a shows the effect of devazepide on AMP-induced activity (mean ± SEM crossovers) summed over 60 min for all animals tested. A median split was performed based on the total number of crossovers during the initial habituation period. This split yielded two separate groups of animals: LRs and HRs to a novel environment. The effect of devazepide on AMP-induced activity (mean ± SEM) in LRs and HRs are shown in Fig. 1b and c, respectively. As can be seen in Fig. 1a, b, and c, AMP treatment significantly increased locomotor activity relative to saline treatment, and this effect was most pronounced in the HR group. Additional analyses of these data revealed that, relative to saline treatment, AMP produced an increase in percentage of baseline locomotion that was 35.1% greater in the HR than in LR animals (data not shown). As depicted in Fig. 1a and b, amphetamine-induced locomotor activity was not attenuated by devazepide in either LR or HR animals. No evidence of AMP sensitization was found, as previous exposure to AMP did not affect subsequent AMP-induced locomotion.

All animals. Statistical analyses support this description of the data. A one-way ANOVA of the 10-min crossover totals revealed no significant effect of the number of previous AMP injections on subsequent AMP-induced locomotion. The interaction of number of previous AMP injections by time was also nonsignificant. A two-way repeated-measures ANOVA (AMP × time) of the 10-min crossover totals for all rats showed significant main effects for AMP, $F(1, 30) = 34.99$, $p < 0.000002$, and time, $F(5, 150) = 2.75$, $p < 0.02$, as well as a significant interaction, $F(5, 150) = 8.57$, $p < 0.000001$. Post hoc analyses using the LSD t-test demonstrated that AMP-induced crossover locomotion was significantly ($p < 0.00001$) greater than saline crossover locomotion across each 10-min interval tested. A second two-way repeated-measures ANOVA (devazepide × time) of crossover totals revealed significant

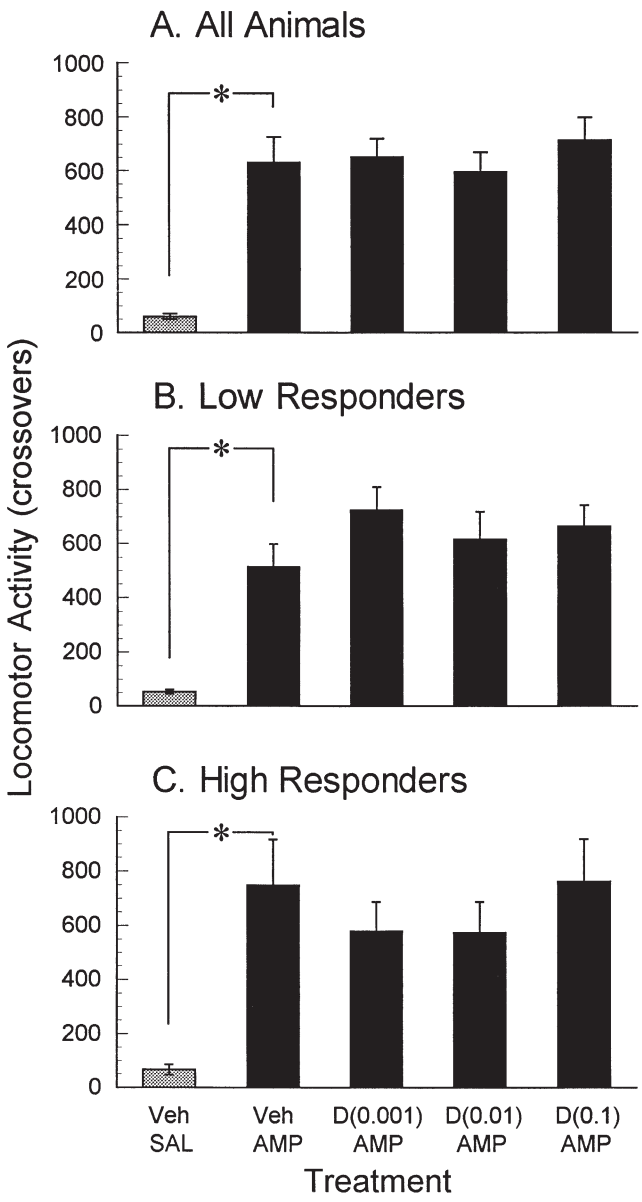

FIG. 1. The mean (± SEM) number of crossovers during the 60-min period following injection (IP) of either vehicle and saline (Veh SAL), vehicle and 1.5 mg/kg amphetamine (Veh AMP), or devazepide (0.001, 0.01, and 0.1 mg/kg) and 1.5 mg/kg amphetamine [D(0.001) AMP, D(0.01) AMP, and D(0.1) AMP]. (A) Results from all animals. (B) Results from animals demonstrating low locomotor responsivity to a novel environment (low responders). (C) Results from animals demonstrating high locomotor responsivity to a novel environment (high responders). *Denotes significantly different at $p < 0.05$.

main effects for time, $F(5, 300) = 35.43$, $p < 0.000001$; however, the main effect of devazepide and the interaction failed to reach statistical significance.

Low responders. For the LR group, a two-way repeated-measures ANOVA (AMP × time) of crossover totals demonstrated a significant main effect of AMP, $F(1, 14) = 29.79$, $p < 0.0001$, and a significant interaction, $F(5, 70) = 3.27$, $p < 0.01$. The main effect of time did not reach significance. AMP-induced crossover locomotion was significantly ($p < 0.05$) greater than saline across each 10-min interval tested, as shown by the LSD post hoc *t*-test. A second two-way repeated-measures ANOVA (devazepide × time) of crossover totals revealed a significant main effect only for time, $F(5, 140) = 17.16$, $p < 0.000001$. Both the main effect of devazepide and the interaction were not statistically significant.

High responders. Two-way repeated-measures ANOVA (AMP × time) of crossover totals for HR rats demonstrated significant main effects for AMP, $F(1, 14) = 16.07$, $p < 0.01$, and time, $F(5, 70) = 2.91$, $p < 0.05$, as well as a significant interaction, $F(5, 70) = 5.39$, $p < 0.001$. Analysis of AMP-induced crossover locomotion using the LSD post hoc *t*-test revealed that it was significantly ($p < 0.001$) higher than saline across each 10-min interval tested. A second two-way repeated-measures ANOVA (devazepide × time) of crossover totals showed a significant main effect for time, $F(5, 140) = 18.15$, $p < 0.000001$. The main effect of devazepide and the interaction did not reach statistical significance.

Experiment 2

As in Experiment 1, locomotor activity was analyzed using crossover totals, summed over 10-min intervals, across the 60-min test session. Figure 2a shows the effect of devazepide on the sensitized locomotor response (mean ± SEM crossovers) to AMP summed over 60 min for all animals tested. As in Experiment 1, animals were separated into either LR or HR groups based on a median split of the total number of crossovers during the initial habituation session. The effect of devazepide on the sensitized locomotor response (mean ± SEM crossovers) to AMP in LRs and HRs is shown in Fig. 2b and c, respectively. As shown in Fig. 2a, rats chronically treated with AMP showed a sensitized locomotor response to a subsequent injection of AMP. There was a general tendency for devazepide to attenuate the expression of this sensitized AMP response. Separate analysis of LR and HR animals (Fig. 2b and c) revealed that the sensitized behavioral response to AMP was most pronounced in the HR group. Additional examination of these results showed that, relative to saline treatment, AMP produced an increase in percentage of baseline locomotion that was 22.1% greater in the HR than in LR animals (data not shown). As depicted in Fig. 2b and c, the amphetamine-sensitized locomotor effect was blocked by devazepide treatment only in the HR group.

All animals. Statistical analyses support this description of the data. A two-way mixed ANOVA (sensitization × time) of the 10-min crossover totals for all rats revealed significant main effects for sensitization, $F(1, 30) = 6.25$, $p < 0.05$, and time, $F(5, 150) = 13.12$, $p < 0.000001$, as well as a significant interaction, $F(5, 150) = 7.45$, $p < 0.00001$. Post hoc analyses using the LSD *t*-test demonstrated that AMP-induced crossover locomotion was significantly ($p < 0.05$) greater for AMP- than saline-pretreated animals at all time intervals except the first (20, 30, 40, 50, and 60 min). A second two-way mixed ANOVA (devazepide × time) of crossover totals showed signif-

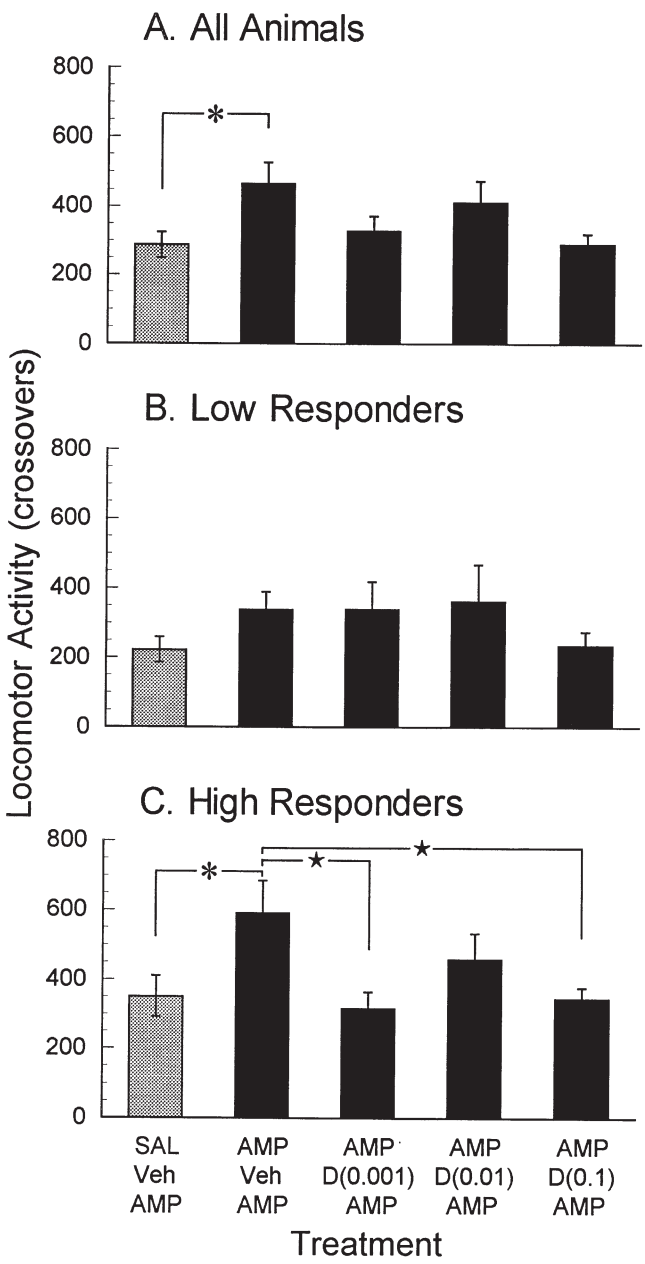

FIG. 2. The mean (± SEM) number of crossovers during the 60-min period following experimental treatment. Two groups were pretreated (IP) once daily for 7 days with either saline (SAL Veh AMP) or 1.5 mg/kg amphetamine (AMP Veh AMP), and tested 10 days later following administration (IP) of vehicle and 0.75 mg/kg amphetamine. Three additional groups were similarly pretreated with 1.5 mg/kg amphetamine, and then tested following administration of devazepide (0.001, 0.01, 0.1 mg/kg) and 0.75 mg/kg amphetamine [AMP D(0.001) AMP, AMP D(0.01) AMP, AMP D(0.1) AMP]. (A) Results from all animals. (B) Results from animals demonstrating low locomotor responsivity to a novel environment (low responders). (C) Results from animals demonstrating high locomotor responsivity to a novel environment (high responders). * and ★ denote significantly different at $p < 0.05$.

icant main effects for time, $F(5, 240) = 30.11$, $p < 0.000001$. The main effect of devazepide approached, but failed to reach, statistical significance, $F(3, 48) = 2.32$, $p = 0.087$. The interaction of devazepide and time was not significant.

Low responders. A two-way mixed ANOVA (sensitization × time) of crossover totals for the LR group showed a significant main effect of time, $F(5, 70) = 10.79$, $p < 0.000001$, but not sensitization. However, the sensitization by time interaction was significant, $F(5, 70) = 4.65$, $p < 0.001$, and LSD post hoc analysis revealed that AMP-induced crossover activity was significantly ($p < 0.05$) higher for AMP- than saline-pretreated rats across several time intervals (20, 30, 40, and 50 min). A second mixed ANOVA (devazepide × time) of crossover totals demonstrated a significant main effect only for time, $F(5, 110) = 12.96$, $p < 0.000001$. The main effect of devazepide and the interaction did not reach statistical significance.

High responders. Two-way repeated-measures ANOVA (sensitization × time) of crossover totals for HR rats demonstrated significant main effects for sensitization, $F(1, 14) = 4.74$, $p < 0.05$, and time, $F(5, 70) = 5.51$, $p < 0.001$, as well as a significant interaction, $F(5, 70) = 3.64$, $p < 0.01$. LSD post hoc analysis revealed that AMP-induced crossover locomotion was significantly ($p < 0.05$) higher in AMP- than saline-pretreated animals at several time points (20, 30, 40, and 50 min). A final mixed ANOVA (devazepide × time) of crossover totals showed significant main effects for devazepide, $F(3, 22) = 3.24$, $p < 0.05$, and time, $F(5, 110) = 17.10$, $p < 0.000001$. The devazepide by time interaction was not significant.

DISCUSSION

Previous research suggests that if colocalized mesolimbic CCK contributes to locomotion, it may do so only during conditions of elevated mesolimbic DA activity (1,8). To this end, both acute and chronic AMP manipulations were chosen as a model for activation of mesolimbic DA release. In Experiment 1, an acute AMP model that has previously been shown to release endogenous stores of mesolimbic CCK was used. Results showed that while AMP significantly potentiated locomotor activity, devazepide failed to block this effect. As an added component, animals were split into LRs and HRs to a novel environment based on previous research suggesting individual differences in this measure are positively correlated with individual differences in mesolimbic DA activity (10,20). Separate examination of animals divided into LR and HR groups showed a more pronounced AMP response in HR animals. However, devazepide treatment did not affect AMP-induced activity in either group. Thus, these data suggest that during presumed high levels of mesolimbic activity produced by acute AMP challenge, coexisting CCK does not contribute to the expression of locomotor activity through a CCK_A receptor mechanism.

The results of Experiment 1 in the present study are in general agreement with previous results in which CCK_A antagonists are without effect on the locomotor activation produced by acute AMP challenge. For example, systemic injection of devazepide does not affect AMP-induced hyperactivity in animals with high or low AMP responsivity (7). Similarly, Crawley demonstrated that devazepide has no effect on lit-environment, dark-environment, or DA-induced locomotion when injected onto either mesolimbic cell bodies or their NAcc terminals (3).

In Experiment 2, animals pretreated chronically with AMP demonstrated an augmented locomotor response to a subsequent AMP challenge following 10 days of withdrawal compared with saline-pretreated controls. This finding is consistent with a large body of literature showing that chronic pretreatment with psychostimulants results in a sensitized behavioral response to stimulant administration (14,22,24). Although not reaching statistical significance, administration of devazepide prior to the AMP challenge resulted in a strong trend towards an attenuation of the sensitized AMP response. Designation of animals as either LR or HR based upon a median split of the total locomotor activity during the initial habituation period revealed a heightened AMP sensitization response in HR animals. Further, devazepide strongly attenuated the locomotor response to AMP challenge in HR animals, while having no effect among LR animals. This finding suggests that endogenous CCK_A receptor activation is an important mechanism underlying individual differences in the locomotor response to AMP in animals with a history of chronic stimulant treatment.

Given the systemic nature of the devazepide and amphetamine treatments, the present studies do not allow us to determine the critical site(s) of action involved in mediating the reported effects. CCK receptors are found in abundance throughout several brain regions, as well as in the alimentary tract (12). Although it is possible that systemically administered devazepide may be acting via peripheral mechanisms to inhibit AMP sensitization, it is more likely that devazepide is acting via CCK_A receptors located within the NAcc. Such an explanation is tenable, given research suggesting that the expression of behavioral sensitization to AMP is associated with increased release of NAcc DA (14,22,23) and that NAcc CCK_A receptors potentiate DA transmission and DA-mediated behaviors (3,27,28). Consistent with this idea, preliminary data from our laboratory suggest that AMP sensitization is blocked by direct microinfusion into the NAcc of the selective CCK_A antagonist PD-140,548 (34). However, it remains possible that additional sites contribute to the blockade of AMP sensitization induced by systemically administered devazepide.

There are several interpretations that may account for the present results. In Experiment 1, the dose of AMP employed was 1.5 mg/kg. In Experiment 2, animals were first pretreated with 1.5 mg/kg and then tested following a lengthy withdrawal period with a dose of 0.75 mg/kg. This lower dose was used to reduce the likelihood of AMP-induced stereotypy in AMP-sensitized animals. If the blockade of AMP-induced activity by devazepide in Experiment 2 is related to the use of a lower AMP test dose than that used in Experiment 1, it could simply be argued that CCK modulates locomotion produced by low, but not moderate, doses of AMP. This hypothesis is at odds, however, with previous reports showing that in nonsensitized animals devazepide does not block locomotion induced by low doses of AMP (7).

A second interpretation is that devazepide attenuates the behavioral response to AMP only in AMP-sensitized HR animals because a) the total amount of DA released is augmented in these animals, and b) colocalized CCK is preferentially released under these conditions. Although this remains an attractive hypothesis, previous research calls into question the latter premise by showing that an acute AMP challenge of 1.5 mg/kg is sufficient to induce release of both DA and CCK (11). Further, the present results show that AMP-induced locomotor activity was greater following the acute manipulation of Experiment 1 than following the chronic manipulation of Experiment 2, as the challenge dose of AMP employed was lower in Experiment 2. Although DA release was not mea-

sured in the present set of studies, previous research has shown that AMP-induced DA release correlates with locomotor activity within the dose ranges employed here (25). These data indirectly suggest that AMP-induced DA activity may actually have been greater following the acute manipulation in Experiment 1 than following the chronic manipulation in Experiment 2. The fact that devazepide was effective in Experiment 2, but not in Experiment 1, suggests that increased DA release alone may not be the critical variable involved in activating CCK release in the present study.

Another explanation for the present results is that the contribution of endogenous CCK in the expression of locomotor activity is somehow modified by a regime of chronic AMP administration such as that employed in Experiment 2. Consistent with this CCK plasticity hypothesis, chronic AMP administration alters the number of CCK binding sites in numerous brain regions (26) as well as CCK mRNA levels in the substantia nigra pars compacta and ventral tegmental area (11,33). As such, it is possible that chronic AMP treatment results in sensitized mesolimbic CCK_A mechanisms either at the transmitter or receptor level. This upregulation of CCK_A mechanisms would presumably act to further facilitate DA activity and DA-dependent behaviors.

In summary, the present studies provide evidence that endogenous CCK_A receptor activation is a critical mechanism underlying individual differences in the locomotor response to AMP in AMP-sensitized, but not drug-naïve, animals. In general, these data may have implications regarding the role of CCK_A mechanisms in mediating the neurobiological effects of chronic psychostimulant intake, and suggest a useful paradigm for the investigation of the functional significance of colocalized neuropeptides.

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