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Opposite Actions of CCK-8 on Amphetamine-Induced Hyperlocomotion and Stereotypy Following Intracerebroventricular and Intra-accumbens Injections in Rats

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WEISS, F., D. J. TANZER AND A. ETTENBERG. *Opposite actions of CCK-8 on amphetamine-induced hyperlocomotion and stereotypy following intracerebroventricular and intra-accumbens injections in rats.* PHARMACOL BIOCHEM BEHAV 30(2) 309–317, 1988.—Cholecystokinin-octapeptide (CCK-8) has recently been found to coexist with dopamine (DA) in a subpopulation of midbrain DA neurons. The present study investigated the functional nature of this coexistence by testing the effects of intracerebroventricular (ICV) and intra-nucleus accumbens (NAS) applications of CCK-8 in two behavioral assays of DA function (i.e., stimulant-induced hyperlocomotion and stereotypy). Rats were injected with 1 or 3 mg/kg of d-amphetamine sulfate (AMP) 15 minutes prior to ICV (2 μg) or intra-NAS (20 ng, 200 ng, or 2 μg) injections of CCK-8 or haloperidol (HAL; 5 μg). ICV administered CCK-8 was found to antagonize the locomotor stimulatory effects of the low AMP dose, while the same peptide treatment markedly potentiated the stereotypy produced by the high dose of AMP. Similar results were obtained when CCK-8 was microinjected directly into the NAS, with the strongest effects observed following the smallest (i.e., 20 μg) dose. These results suggest that both locomotor-antagonizing and stereotypy-potentiating effects of central CCK application depend on CCK-DA interactions in the nucleus accumbens.

d-Amphetamine Cholecystokinin-octapeptide Haloperidol Intracerebroventricular Nucleus accumbens Dopamine Hyperlocomotion Stereotypy

IT is well established that cholecystokinin octapeptide (CCK-8) exists within a subpopulation of mesolimbic dopaminergic (DA) neurons [16, 17, 41] where it is thought to serve neurotransmitter or neuromodulator functions [12, 15, 40, 45, 46, 49]. Although the precise functional significance of this DA-CCK coexistence is still unknown, electrophysiological [47,49] and biochemical findings [45,46] suggest that, at the synaptic level, CCK and DA are functionally opposed to each other. This view is supported by animal behavioral studies in which centrally administered CCK exerted DA antagonist-like or neuroleptic-like effects on several DA-dependent behaviors. For example, CCK-8 has been found to delay acquisition and facilitate extinction of active and passive avoidance behavior and to produce dose-dependent reductions in responding for intracranial self-stimulation (ICSS) [13, 14, 20, 21, 43, 44]. In addition to its own actions, CCK-8 has also been reported to potentiate some of the behavioral effects of the neuroleptic agent, haloperidol [29].

Although there is considerable evidence that CCK exerts neuroleptic-like effects on operant responding, its actions on stimulant-induced behaviors (such as hyperlocomotion or stereotypy) remains unclear. Unlike typical DA antagonists which effectively reverse *both* the locomotor and stereotyped behaviors induced by DA agonist drugs (such as amphetamine or apomorphine), CCK-8 appears to effectively antagonize only the stimulant-induced locomotor hyperactivity ([18, 29–32, 44]; but see [7]) while its effects on stereotyped behaviors have been, at best, inconsistent [9, 30, 31, 44]. For example, like others (e.g., [18, 30, 31, 37]) our own preliminary investigations have found intracerebroventricular CCK-8 to reverse d-amphetamine-stimulated hyperlocomotion, but not stereotypy. In fact, the peptide caused a slight, but reliable *enhancement* of stereotyped behaviors

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[11]. This finding suggested that CCK may interact differently with the DA mechanisms involved in the generation of stereotypy and locomotor hyperactivity. Therefore, the present series of experiments was devised to more closely examine the nature of CCK effects on *d*-amphetamine-induced motor behaviors. Experiment 1 is a replication and extension of our preliminary findings. Confirmation of these results was deemed necessary in view of the surprising and "paradoxical" facilitatory and inhibitory action of CCK. Experiment 2 compares CCK effects on hyperlocomotion and stereotypy following intra-accumbens injection to those of the DA receptor antagonist haloperidol.

GENERAL METHOD

Animals

The subjects were 163 male Sprague-Dawley rats (Charles River Co.) weighing 250–450 g at the time of surgery. The animals were individually housed in a temperature-controlled (22°C) vivarium on a 12-hr light-dark schedule and received ad lib access to food and water.

Drugs

d-Amphetamine sulfate (AMP) was dissolved in 0.9% saline at a concentration of either 1 mg or 3 mg/ml (weight of free base) Haloperidol was prepared in a vehicle solution of 0.002 M lactic acid (5 μg/μl) and CCK-8 sulfate ester (Peninsula Labs.) was dissolved in 0.9% saline at concentrations of 20 ng, 200 ng and 2 μg/μl.

Measurement of Stereotypy and Locomotor Behavior

Locomotor activity was measured by individually placing animals into one of 16 identical activity cages, each 25×40×20 cm and equipped with two infrared photocells positioned on their long axes. The entire activity apparatus was located inside a small sound attenuated room equipped with a one-way mirror for behavioral observations. Photocell "interruptions" served as a measure of locomotor activity and were recorded on a bank of digital counters located in a separate room.

Stereotypy behaviors were rated according to a 0–4 point scale (adapted from Ernst [10]) by a trained observer who was unaware of the treatment conditions of the animals. During 10 sec observations, stereotypy scores were defined as follows: (0)=no abnormal behavior, (1)=continuous locomotion during the 10 sec interval, (2)=repetitive sniffing with locomotion restricted to a small portion of the test chamber, (3)=repetitive head or limb movements with animal confined to one location in the cage, (4)=repetitive and continuous licking or gnawing of wire cage floor or walls. Locomotor counts and stereotypy ratings were taken at 5 minute intervals over a 60 min (Experiment 1) or 90 min (Experiment 2) period.

Histology

After completion of the experiment each animal was administered an overdose of sodium pentobarbital and perfused with 50 ml of 0.9% saline followed by 50 ml of formalin. Brains were removed and stored in 10% formalin. Cannulae placements within the lateral ventricle or nucleus accumbens were subsequently confirmed from 50 μ frozen cresyl violet-stained sections. Data from animals whose cannulae placements could not be confirmed were excluded from the analysis. The exclusions were made without knowledge of the animals experimental treatment.

Data Analysis

Stereotypy scores and Locomotor counts were each analyzed by a Two-Factor (Drugs × Time) Analysis of Variance (ANOVA). After confirmation of significant differences in the split-plot ANOVA the data were collapsed across "Time" and significant differences between individual means were then identified using Newman-Keuls post hoc tests.

EXPERIMENT 1

Surgery

Seventy-six rats were anesthetized by an intraperitoneal (IP) injection of sodium pentobarbital (50 mg/kg) and stereotaxically implanted with a chronic indwelling stainless steel guide cannula (outer diameter 0.7 mm; Plastic Products Co.) aimed at either the right or left lateral ventricle. With the incisor bar of the stereotaxic instrument at 3.3 mm below the interaural line, the coordinates were 0.8 mm posterior to bregma, 1.3 mm lateral to midline and 3.4 mm ventral to the skull surface (Paxinos and Watson [31]). A recovery period of 7 days after surgery was given before testing began.

Intracerebroventricular Injection

The animals were injected with d-amphetamine sulfate (AMP) or saline (VEH). Fifteen minutes after AMP treatment each rat received an intracerebroventricular (ICV) injection of either saline (VEH; 1 μl) or CCK-8 (2 μg) The ICV injection was administered through an internal cannula (outer diameter 0.4 mm; Plastic Products Co.) which was inserted with its tip extending 1 mm below the guide cannula. The internal cannula was connected via PE 20 tubing to a Hamilton microsyringe driven by a syringe pump (Razel Scientific Instruments). Each 1 μl injection was completed over a 40 sec period, however, the cannula was left in place for an additional 30 seconds to allow for diffusion away from the cannula tip. The CCK dose was chosen on the basis of our own pilot data, and published reports by others demonstrating that centrally administered doses of approximately 2 μg effectively antagonize drug-induced hyperlocomotion [18,37]. There were four independent treatment groups in the locomotor activity test corresponding to IP pretreatment with AMP (1 mg/kg) or saline (VEH) followed by ICV injection of CCK-8 (CCK) or saline (VEH). Only two independent treatment conditions were used in the stereotypy study [i.e., AMP (3 mg/kg; IP) followed by CCK or saline (VEH)] since stereotyped behavior, unlike locomotion, does not occur spontaneously in nondrugged animals. Each subject was tested in only one condition and testing commenced immediately following completion of the ICV injections.

RESULTS

Locomotor Behavior

The low dose of AMP (1 mg/kg) produced a marked increase in spontaneous locomotor activity (mean±S.E.M. total activity counts=1202±260) compared to saline control animals (416±62). As shown in Fig. 1, this stimulant effect was effectively reversed by ICV application of a 2 μg/rat dose of CCK-8. A Two-Factor ANOVA confirmed that there were statistically reliable effects between Groups, $F(3,29)=$

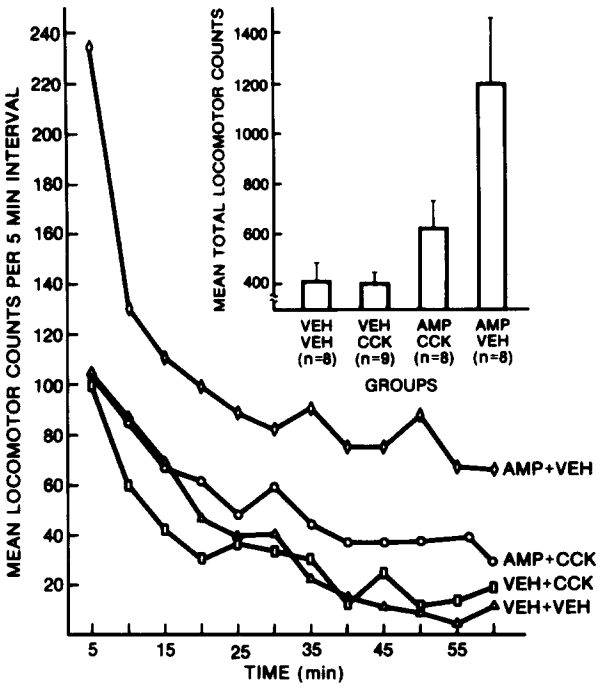

FIG. 1. Mean locomotor counts for *d*-amphetamine (AMP; 1 mg/kg) or saline-pretreated rats after ICV injection of CCK-8 (2 μg) or its vehicle. CCK-8 completely antagonized the hyperlocomotion produced by AMP. Inset: Mean ±S.E.M. locomotor counts over the entire 60 min test session.

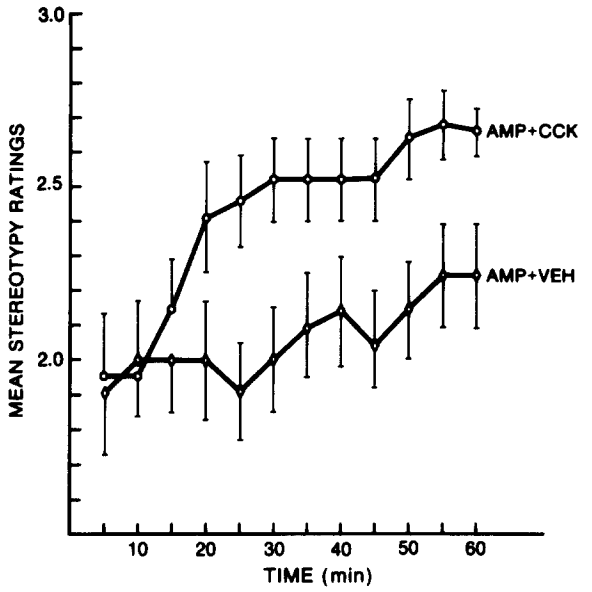

FIG. 2. Mean stereotypy ratings in rats pretreated with 3.0 mg/kg of *d*-amphetamine (AMP) followed by an ICV injection of either CCK-8 (2 μg) or its vehicle. The error bars represent standard errors of the mean. CCK-8 potentiated the stereotyped behavior throughout the entire test session.

7.1, $p<0.001$, over Time, $F(11,319)=37.8$, $p<0.001$, and for the Group × Time interaction, $F(33,319)=1.8$, $p<0.005$. The main effect for Groups is undoubtedly attributable to the performance of the AMP+VEH group. The relative decline in locomotor activity over time was evident across all conditions and accounts for the significant "Time effect." The Group × Time interaction resulted from the greater differences in locomotor counts between AMP+VEH animals and all other groups during the first 5 min of the session compared to later time points—thus the groups behaved differently over the course of the session. Post hoc Newman-Keuls tests confirmed that the "Group" effects (see panel insert in Fig. 1) were attributable to the AMP+VEH condition which was significantly different from all other treatment groups ($p<0.01$) No differences were found among VEH+VEH, VEH+CCK, and AMP+CCK treatments.

Stereotypy Test

The high dose of AMP (3 mg/kg) generated moderate stereotyped behaviors (Mean rating score ±S.E.M.: 2.0±0.12). ICV application of CCK-8 resulted in a slight, but statistically reliable potentiation of stereotyped activity (2.4±0.11), $F(1,41)=3.9$, $p<0.05$. The time course of this effect is shown in Fig. 2. It is evident that in both treatment conditions stereotyped activity increased slightly throughout the course of the test session yielding a significant effect for Time, $F(11,451)=8.1$, $p<0.001$. Note also that the stereotypy enhancing effect of CCK-8 appeared relatively late (i.e., 20 min after CCK-8 infusion), but persisted throughout the entire 60 min session. This "late" potentiation is reflected in a statistically significant Drugs × Time interaction, $F(11,451)=2.6$, $p<0.005$.

DISCUSSION

The results of Experiment 1 indicate that ICV injected CCK-8 can both facilitate and inhibit different AMP-stimulated motor behaviors. While we recognize the interpretational problems associated with ICV CCK administration ([6] for review) as well as the limitations of testing only a single dose, the two opposing behavioral actions of the same treatment are nonetheless interesting. For example, in contrast to the stereotypy results observed by us with ICV application, intra-caudate CCK injections typically do not influence stereotyped behaviors [7,44] while peripheral peptide administration is frequently associated with antistereotypic effects (e.g., [5, 50, 51]). These findings imply that the observed *enhancement* of stereotyped behavior is neither peripherally mediated nor the result of a peptide action on striatal DA mechanisms, but may depend on CCK-DA interactions in the nucleus accumbens. Consequently, it remains to be determined (a) whether our ICV findings indeed reflect central CCK actions and (b) whether both the locomotor-antagonizing and stereotypy potentiating properties may be subserved by a selective interaction with mesolimbic DA transmission in the NAS.

EXPERIMENT 2

Surgery

Eighty-seven rats were anesthetized with sodium pentobarbital (60 mg/kg; IP) and stereotaxically implanted with bilateral chronic indwelling stainless steel guide cannulae (outer diameter 0.7 mm; Plastic Products Co.) aimed at the medial nucleus accumbens (NAS). With the incisor bar of the stereotaxic instrument at 3.3 mm below the interaural line, the coordinates were 1.9 mm anterior to bregma, 2.0 mm lateral and 6.0 mm ventral to the skull surface (Paxinos

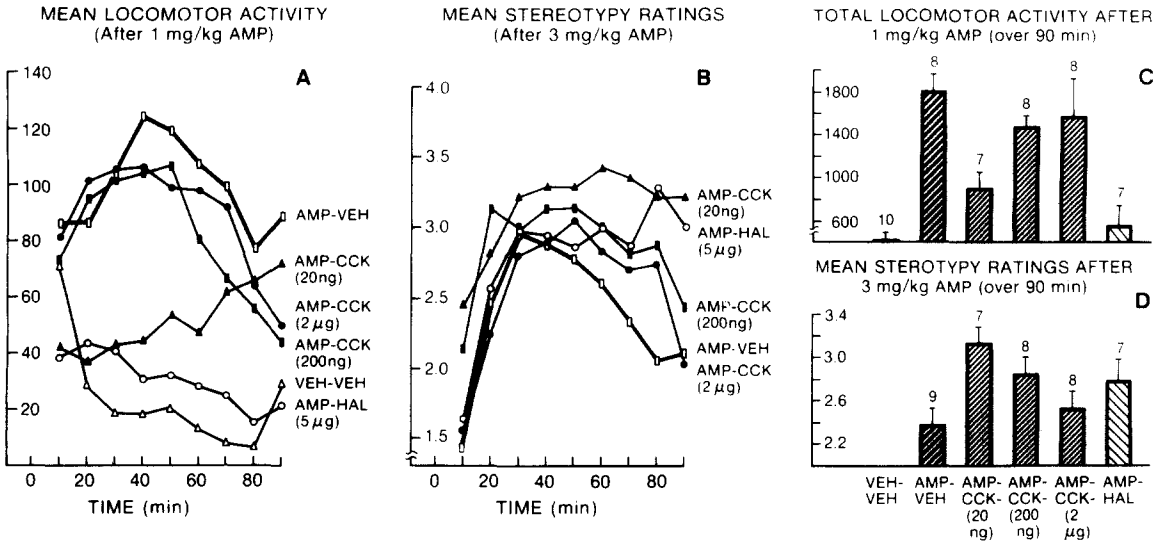

FIG. 3 (A) Mean number of locomotor counts per 10 min interval for *d*-amphetamine- (AMP; 1 mg/kg) or vehicle- (VEH) pretreated rats after intra-NAS microinjection of haloperidol (HAL), CCK-8 (20 ng, 200 ng or 2 μg) or their vehicle solutions (VEH). HAL and CCK-8 (20 ng) completely antagonized the hyperlocomotion produced by AMP. (B) Mean stereotypy rating per 10 min interval in rats pretreated with 3.0 mg/kg of AMP followed by bilateral intra-NAS microinjections of HAL, CCK-8 or VEH. (C) Mean ±S.E.M. total locomotor counts over the entire 90 min test session. (D) Overall Mean±S.E.M. stereotypy rating scores for each group during the test. Note that the treatment conditions listed in panel D pertain also to panel C. No VEH-VEH group was included in the stereotypy test.

and Watson [31]). A recovery period of at least 7 days post-surgery was provided before testing began.

Intracerebral Injections

The animals were injected subcutaneously (SC) with *d*-amphetamine sulfate [(AMP) 1 or 3 mg/kg] or saline [(VEH) 1.0 ml/kg], 15 minutes prior to bilateral intra-accumbens (NAS) microinjections of either vehicle solutions ([VEH] saline or lactic acid), haloperidol [(HAL) 5 μg], or CCK-8 (20 ng, 200 ng or 2 μg). The injection volume for the SC injections was held constant at 1.0 ml per kg of body-weight and the NAS microinjections were administered using the same procedures described for ICV injections in Experiment 1.

Treatment Conditions

There were six independent treatment groups in the *locomotor activity test* corresponding to SC pretreatment with *d*-amphetamine (1 mg/kg) or saline (VEH) followed by intra-NAS microinjection of either CCK-8, haloperidol or vehicle solutions (VEH); i.e., AMP-CCK (20 ng), AMP-CCK (200 ng), AMP-CCK (2 μg), AMP-HAL (5 μg), AMP-VEH and VEH-VEH groups. Five independent treatment conditions were used in the *stereotypy test: d*-amphetamine (3 mg/kg; SC) followed by haloperidol, one of three doses of CCK or VEH; i.e., AMP-HAL (5 μg); AMP-CCK (20 ng), AMP-CCK (200 ng), AMP-CCK (2 μg) and AMP-VEH groups. The precise procedures for both locomotor and stereotypy testing were as described in the General Method section. Testing for locomotor activity and stereotypy began immediately following intra-NAS microinjections. The duration of the test session was 30 minutes longer than in Experiment 1 to permit observation of the cessation of CCK effects.

RESULTS

Locomotor Activity

Amphetamine pretreatment produced a marked increase in locomotor activity (Mean total ±S.E.M. locomotor counts: 1787.8±176.1) as compared to nonAMP-treated control animals (421.9±68.2). Haloperidol microinjections effectively reversed this AMP-induced hyperlocomotion (552.3±171.3). CCK-8 administration antagonized hyperlocomotion in a dose-dependent manner. Maximum antagonist effects were obtained with the smallest (20 ng) dose while the highest CCK-8 dose (2 μg) produced only negligible reductions in locomotor behavior. In fact, the locomotor activity of the 20 ng treatment group was comparable to that of the HAL group during the first half of the 90 min session. Unlike HAL, however, the CCK effects had dissipated by the end of the 90 min session. No differences were found between the lactic acid and saline control animals and the data from these two control groups were therefore pooled. The mean locomotor data for each group during the 90 min test session are illustrated in Fig. 3 (panels A and C).

Differences among treatment groups were confirmed by Two-Way ANOVA; $F(5,40)=7.4, p<0.0001$. In addition, the ANOVA revealed a significant effect for "Time" reflecting the relative decline in locomotor activity over the course of the test session; $F(5,440)=2.2, p<0.01$. It is also evident that the time course of locomotor effects of the two CCK-8 groups that showed only weak response attenuation (i.e., the 200 ng and 2 μg groups) parallels that of the AMP-VEH group, peaking between 30 and 50 min after peptide administration. Presumably, this reflects the time course of amphetamine's behavioral effects. Conversly, locomotor counts of the VEH-VEH, HAL, and 20 ng CCK groups decreased during the same time interval—these differences in group effects within the test session produced a significant

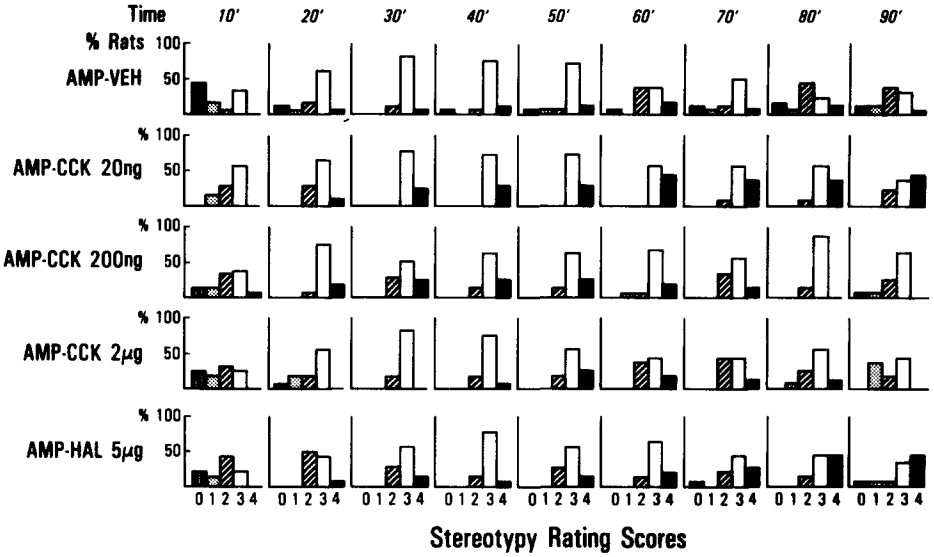

FIG. 4 Distribution of stereotypy rating scores (i.e., the percentage of ratings given a score of 0,1,2,3, or 4) by drug group and per 10 min interval. All animals were pretreated with *d*-amphetamine (AMP; 3 mg/kg) before intra-NAS microinjection of HAL, CCK-8 or VEH.

"Drug × Time" interaction; $F(55,440)=1.4$, $p<0.02$. Also, it is interesting to note that the locomotor attenuating action of both HAL and the 20 ng CCK-8 dose is already evident at the outset of the test session. Newman-Keuls post hoc tests on the "Groups" factor collapsed across "Time" revealed that only the AMP-HAL ($p<0.01$) and AMP-CCK (20 ng) ($p<0.05$) conditions were significantly different from AMP-VEH control animals. No differences were found among either AMP-HAL, VEH-VEH, and AMP-CCK (20 ng), or between AMP-CCK (200 ng) and AMP-CCK (2 μg) groups. However, the difference in activity between AMP-CCK (20 ng) and AMP-CCK (2 μg) animals approached statistical significance at the $0.1>p>0.05$ level. Thus, although CCK effects appear to have been dose-dependent, only the 20 ng dose resulted in a statistically reliable attenuation of AMP-induced hyperlocomotion.

The reductions in locomotor activity observed in the HAL and 20 ng CCK groups were not a function of increased stereotyped activity. Behavioral observations of the animals during the test session did not reveal any reliable stereotypic behaviors in any experimental condition. The Mean ratings over the 90 min session for individual treatment conditions were as follows: VEH-VEH =0.1; AMP-VEH=1.3; AMP-CCK (2 μg)=1.2; AMP-CCK (200 ng)=1.2; AMP-CCK (20 ng)=1.7 and AMP-HAL=0.5 (where a "1" rating reflects "continuous locomotor activity"). The reductions in locomotion were, therefore, not a secondary effect of some drug-induced potentiation of AMP actions that induced stereotypy instead of hyperlocomotion

Stereotypy Test

Pretreatment with the 3 mg/kg dose of AMP generated moderate stereotyped activity (Mean rating score ±S.E.M. of the AMP-VEH group=2.4±0.15) which was potentiated by all doses of CCK-8. As in the locomotor test, the magnitude of CCK effects was dose-dependent with maximum effects observed at the smallest (20 ng) dose (Mean rating score ±S.E.M.=3.14±0.15). Intra-accumbens injections of HAL in AMP-treated animals similarly resulted in higher rating scores compared to AMP-VEH-treated rats. The stereotypy data are represented in Figs. 3 and 4. Figure 3 shows the mean stereotypy ratings for each treatment group per 10 minute interval (Fig. 3, panel B) and averaged across the entire 90 min session (Fig. 3, panel D). In Fig. 4, the distribution of stereotypy rating scores (0–4) is expressed in terms of percentages of the total number of ratings for each treatment group obtained per 10 min interval. It is obvious that the enhancement of stereotyped behavior was strongest in AMP-CCK (20 ng) and AMP-HAL animals. In contrast to CCK which produced increases in stereotypy as early as 5 min post-injection (interval not shown), HAL effects did not exceed control levels (AMP-VEH) until the second half of the test session. However, both treatments appeared to produce "long-term" effects. As shown in Figs. 3 and 4, the activity of control rats began to decline at about 40 min post-injection while the "intensity" of stereotyped behavior in AMP-CCK (20 ng) and AMP-HAL animals remained elevated until the end of the 90 min session. Figure 4 illustrates that all drug treatments resulted in a marked shift to the right in the distribution of rating scores which was particularly prominent in the AMP-CCK (20 ng) and AMP-HAL conditions. It also appears that in the three CCK groups the frequency of "low" stereotypy rating scores ("1" and "2") decreased while "high" rating scores ("3" and especially "4") increased as a function of CCK dose. Thus, the relative frequency of rating scores of "4" (licking/gnawing stereotypies) is highest in the AMP-CCK (20 ng) group.

A Two-Way ANOVA confirmed that there were significant differences among drug conditions; $F(4,34)=3.05$, $p<0.02$. In addition, the ANOVA yielded a significant effect for "Time," reflecting the shifts in stereotyped behaviors over time in all treatment groups; $F(17,578)=13.2$, $p<0.001$. There was no significant "Groups × Time" interaction. The reliable "Group" differences were further examined by post hoc Newman-Keuls analyses which revealed reliable differ-

ences only between the AMP-VEH and AMP-CCK (20 ng) groups ($p<0.05$). A marginal difference was found between the AMP-CCK (20 ng) and AMP-CCK (2 μg) groups ($0.1>p>0.05$). No significant differences were found between other treatment conditions. Thus, as with the locomotor test, only the 20 ng dose produced a statistically reliable effect.

In summary then, CCK-8 produced dose-dependent effects on both AMP-stimulated hyperlocomotion and stereotypy. While CCK (at the 20 ng dose) effectively antagonized locomotor activity, the same CCK dose produced a reliable potentiation in stereotyped behaviors (i.e., a shift toward higher rating scores reflecting more confined stereotyped response categories). A similar pattern was observed in neuroleptic HAL-treated rats.

GENERAL DISCUSSION

The present series of experiments sought to further define the putative DA modulatory function of CCK using two well established behavioral assays of central DA function. The results and their implications can be summarized as follows. (1) Centrally administered CCK-8 effectively antagonizes the locomotor hyperactivity stimulated by a low dose of AMP, but potentiates stereotyped behavior induced by a high AMP dose. (2) The reversal of AMP-stimulated hyperlocomotion seems to be specific to a DA antagonist action of CCK in the nucleus accumbens (the brain region presumed to mediate the locomotor effects of DA agonist drugs (e.g., [4, 8, 22]). In contrast, the CCK-induced enhancement of AMP-stimulated stereotypy seems to be independent of a direct peptide action in the dorsal striatum (the brain region presumed to mediate these behavioral effects of AMP [3, 5, 24, 34]) since it was observed with intra-NAS as well as ICV administration. (3) Intracerebroventricular and discrete accumbens CCK microinjections produce almost indentical effects on AMP-stimulated motor behaviors. Therefore, the behavioral effects of ICV administered CCK can at least partly, if not wholly, be explained by an action on central DA substrates via central CCK receptors. In view of the finding that CCK can pass rapidly from the CSF into the peripheral circulation [32], it has been argued that the locomotor attenuation of microgram doses of ICV injected CCK may not reflect a direct interaction with central DA, but rather a state of "behavioral sedation" via an action at peripheral CCK receptors [6]. This explanation is, however, incompatible with the *increases* in behavior (i.e., stereotypy) observed following the 2 μg ICV peptide dose. Similarly, the locomotor activity results cannot easily be accounted for by some general performance attenuating (i.e., sedative or aversive) action of ICV CCK since the peptide had no effect on the behavior of non-amphetamine treated animals (i.e., the VEH+VEH and VEH+CCK groups performed equivalently; see Fig. 1).

The reversal of AMP-stimulated hyperlocomotion following intra-NAS microinjection provides further support for the hypothesis that DA and CCK may be functionally opposed to each other in the nucleus accumbens. Although the locomotor attenuation following ICV peptide administration was only suggestive of central DA antagonist activity, the results obtained after intra-NAS application confirmed that these CCK effects were specific to mesolimbic DA function. This finding is consistent with earlier reports in which low nanogram doses of intra-NAS injected CCK-8 attenuated brain stimulation reward [43], spontaneous as well as apomorphine-stimulated locomotor activity [21, 44], and exerted neuroleptic-like actions on avoidance responding [13]. The magnitude of CCK effects (at the 20 ng dose) was comparable to that of the neuroleptic HAL during the first 30 min of the locomotor activity test (Fig. 3, panel A). In contrast to the 20 ng dose, the effects of the larger CCK doses (in both behavioral assays) were not statistically reliable. This dose-response relationship presumably represents the descending limb of a U-shaped D-R function. Similar dose-response characteristics have been obtained in other behavioral as well as neurophysiological work [7, 9, 28, 45, 46].

While the locomotor attenuating effects of CCK are in agreement with the literature, some inconsistencies remain with other work in which the peptide was shown to potentiate hyperlocomotion following combined injection of DA and CCK into the NAS [7]. Peak facilitatory effects in this research were observed with CCK doses in the picogram and low nanogram range. These findings have, therefore, been interpreted as evidence for a facilitatory role of CCK on mesolimbic DA transmission at "physiological" doses. The neuroleptic-like actions of the peptide (e.g., [18,37]), on the other hand, have been attributed to "pharmacological" effects [6,17] (e.g., the possibility of depolarization block in A 10 DA neurons induced by the microgram CCK doses used in these studies [40]). However, our results do not support this hypothesis. According to this view, CCK should have most effectively attentuated hyperlocomotion at the highest intra-NAS dose (2 μg), whereas little attenuation or an enhancement of locomotor activity should have been observed with the 200 ng and 20 ng CCK doses. This was clearly not the case, and in fact, our dose-response curve points in the opposite direction. The apparent discrepancies between locomotor facilitatory and inhibitory actions of CCK cannot, therefore, be accounted for simply as a function of dose (see [43] for related discussion). It has been argued on the basis of immunohistochemical findings that CCK may modulate DA activity differently in the caudal dorsomedial NAS [i.e., the region of CCK-DA coexistence (e.g., [17]) than in the rostral NAS (i.e., the area where independent DA and CCK terminals appear to predominate (e.g., [17])] [6]. The injection sites in the present work were, in fact, located more rostrally (see also [44]), than those reported to produce DA facilitatory effects [7]. The issue remains unresolved, however, because (a) both DA-facilitatory [7] and DA-inhibitory effects [21] have been reported after CCK injections into the caudal NAS (i.e., the region of coexistence) and (b) recent immunohistochemical evidence suggests that the innervation of the NAS by midbrain neurons containing both CCK and DA may be more extensive than previously thought [39].

The same peptide treatment which effectively antagonized hyperlocomotion (i.e., identical injection sites and doses) produced a marked dose-dependent potentiation of stereotypy. In both behavioral assays the same CCK doses had comparable, albeit opposing, behavioral potencies. Since the stereotypy potentiating action of ICV CCK was also observed following intra-NAS injection, these results imply the nucleus accumbens as the site of action for CCK's stereotypy (as well as locomotor) effects. It is of interest to examine whether these results can indeed be accounted for by a CCK-induced facilitation of DA transmission in the NAS as others have suggested [7]. One might, for example, speculate that CCK has "bifunctional" agonist/antagonist properties which will, depending on the pertinent stimulation conditions, result in facilitation or attenuation of DA transmission in the NAS. Put simply, CCK might antagonize

mesolimbic DA activity stimulated by low AMP doses (antagonism of hyperlocomotion), but enhance the actions of higher stimulant doses (potentiation of stereotypy).

However, this interpretation is not supported by the data or by related evidence. *First,* in vivo CCK effects on DA release indicate that such stimulation-dependent interactions proceed in the opposite direction. Thus, CCK increased basal, but inhibited evoked ^{3}[H]DA release from the NAS [46]. *Second,* in the present work, the DA antagonist, haloperidol, like CCK, shifted the distribution of stereotypy ratings towards scores of "4" following injection directly into the NAS. *Third,* while "hyperlocomotion" and "stereotyped sniffing" can be elicited following discrete stimulation of mesolimbic DA, these behaviors are qualitatively different from the "head and limb" and "licking/gnawing" stereotypies associated with DA function in the dorsal striatum and pallidum [3, 5, 19, 23, 24, 34]. It is of interest then, that the CCK-induced potentiation in stereotypy we observed following accumbens injection was clearly characterized by both a *decrease* in (accumbens-mediated) "sniffing" and "continuous locomotor activity" (rating scores "1" and "2") and a profound *increase* in (caudate-mediated) "licking" and "biting" (rating score "4"; see Fig. 4). Thus, it would seem unreasonable to conclude—on the basis of existing data—that the facilitation of stereotyped behaviors was the result of (CCK-induced) increased DA activity in the NAS.

How then can one account for the stereotypy results? While we cannot, as yet, offer any firm explanation, several possibilities exist. First, the increased stereotypy may be related to diffusion of small amounts of NAS-injected drugs to sites in the dorsal striatum or globus pallidus associated with stereotyped behaviors. Low (i.e., "presynaptic") doses of several neuroleptics (including HAL) are known to produce a "paradoxical" potentiation of drug-stimulated locomotor and stereotyped behaviors (e.g., [26,36]). Consequently, diffusion of presynaptic concentrations of HAL into the dorsal striatum might explain the observed increases in amphetamine-induced stereotypy. While our results do not completely rule out the possibility that the HAL effects may be attributable to a presynaptic action at striatal DA terminals following diffusion, they do not support such a conclusion in the case of CCK. Thus, HAL effects had a late onset (i.e., 40 min post-injection; Fig. 3, panel B) which could be interpreted to reflect the diffusion time into the caudate. In contrast, the stereotypy effects of CCK (particularly at the 20 ng dose) effects were evident immediately after injection and, therefore, appear site-specific. Moreover, although very little is known about possible pre- vs. postsynaptic concentration effects in the case of CCK, the peptide caused a marked potentiation in stereotypy at the 2 μg ICV dose (i.e., a dose which cannot be considered "presynaptic"). Alternatively, CCK's stereotypy effects may have been produced not by diffusion of CCK-8 itself, but of a cleavage product such as CCK-4 into the dorsal striatum. In fact, CCK-4 has been reported to have amphetamine-like effects after ICV administration [42] and to stimulate locomotor activity when microinjected into the NAS [20]. The possibility that the present stereotypy results were caused by diffusion of the tetrapeptide metabolite of CCK-8 into the dorsal striatum is, however, again difficult to reconcile with the fact that the potentiation occurred immediately following injection. The conclusion that the peptide's stereotypy effects were not mediated by a direct action at (either pre- or postsynaptic) striatal receptors is further supported by the failure of intracaudate administered CCK to influence striatal DA function in several behavioral paradigms [7, 44, 48].

It would seem then that CCK's stereotypy effects are not easily explained in terms of either a facilitation of DA transmission in the NAS or a direct (i.e., diffusion-related) interaction with striatal DA substrates. The opposing actions of CCK on stimulant-induced hyperlocomotion and stereotypy are, therefore, perhaps best explained in terms of Lyon and Robbins' [27,35] hypothesis concerning amphetamine effects and the functional organization of the striatum in controlling motor behavior [35]. AMP-stimulated stereotyped behaviors, according to this view, are the result of an increased repetition rate of all motor activities which will eventually result in a "competition" among various behaviors such that behavioral sequences will become more and more incomplete. Based on evidence implicating mesolimbic DA in the locomotor effects of AMP, this hypothesis predicts the occurrence of more confined stereotyped responses (repetitive head movements, gnawing/licking) following pharmacological blockade (or lesion) of DA transmission in the NAS. Since orofacial and head and limb stereotypies are thought to compete behaviorally with the locomotor effects of AMP such treatments, by eliminating competing locomotor activity, would facilitate the development of confined stereotypies (for supportive evidence see [22, 25, 38]. In this "response competition" view, the potentiation of stereotypy after intra-NAS microinjections of CCK-8 would be expected if the peptide produced a selective antagonism of DA activity in the NAS. The decrease in mesolimbic DA activity would presumably release caudate-mediated behaviors from competing locomotor activity and hence allow for the occurrence of more confined stereotyped behavioral responses [35]. This interpretation of our results appears to be supported by the "symmetrical" dose-response relationships that we observed for the peptide's two opposing effects. Figure 3 (panels C and D) shows that the more strongly CCK antagonized "accumbens-mediated" hyperlocomotion, the more it potentiated "caudate-mediated" stereotypy. This account seems also supported by the results obtained with haloperidol. Although the potentiation in stereotypy produced by HAL was not statistically reliable (when expressed in terms of mean rating scores), HAL animals displayed considerably more "licking/biting" stereotypies than their respective controls (see Fig. 4). Nonetheless, the HAL data must be viewed with caution because of the possibility that the drug had "presynaptic" effects following diffusion into the dorsal striatum.

To summarize, the present results demonstrate that CCK-8 exerts DA antagonist effects on AMP-induced hyperlocomotion and hence provide further support for the view that DA and CCK are functionally opposed in the NAS. The "paradoxical" potentiation of stereotyped behavior by intra-NAS injected CCK-8 involved striatal-mediated behaviors, and can, therefore, not readily be attributed to a facilitation of mesolimbic DA transmission. In accordance with the "response competition hypothesis" of striatal motor function, it is possible that both the locomotor-antagonizing and stereotypy-potentiating properties of CCK-8 are subserved by a singular antagonist action on mesolimbic DA transmission.

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