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Centrally Administered CCK-8 Suppresses Activity in Mice by a "Peripheral-Type" CCK Receptor

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BRITTON, D. R., L. YAHIRO, M. J. CULLEN, J. F. KERWIN, JR., H. KOPECKA AND A. M. NADZAN. *Centrally administered CCK-8 suppresses activity in mice by a "peripheral-type" CCK receptor.* PHARMACOL BIOCHEM BEHAV **34**(4) 779–783, 1989.—Cholecystokinin octapeptide (CCK-8) administered either systemically (IP) or centrally (ICV) suppresses several types of behavior in mice including exploratory locomotion, rearing and grooming. At doses equimolar to those active for CCK-8, neither desulfated CCK-8 (CCK-8-DS), nor the protected C-terminus tetrapeptide fragment, BOC-CCK-4, is behaviorally active when administered either centrally or systemically. A potent and selective antagonist to the peripheral type (Type A) CCK receptor, A-65186, when given systemically, blocked the effects of systemically administered CCK-8, but failed to block the effects of ICV administered CCK-8. Central administration of A-65186 blocked the effects of ICV administered CCK-8. These results demonstrate that administration of exogenous CCK-8 to mice can suppress exploratory locomotion by acting either centrally or peripherally and that in either case the demonstrated behavioral effects are mediated via a "peripheral" type (Type A) CCK receptor.

CCK CCK-8 Cholecystokinin A-65186 Mice Locomotion Type A CCK receptors

CHOLECYSTOKININ octapeptide (CCK-8), in addition to its presence and functional significance in the gastrointestinal system, is widely distributed in the brain of a number of species including mice (11), rats (9,34), monkeys (2) and man (30). In addition, receptors for the peptide are widely distributed in brain (13, 15, 24, 32), as well as in peripheral organs (15) of a variety of species. These findings have suggested a functional role for CCK peptides in the CNS as well as in the periphery.

The characteristics of binding in the various tissues indicate the existence of at least two distinct types of binding sites. These have been referred to as Type A and Type B for the predominant forms in the periphery and in the cerebral cortex respectively (24). The Type A site binds CCK-8, CCK-DS and Boc-CCK-4 with affinity constants of 0.5, 164 and 3170 nM respectively (23), while affinities for the Type B site are 5.1, 267 and 25.0 nM respectively for CCK-8, CCK-DS and Boc-CCK-4. Recent evidence indicates that, although Type B receptors predominate in the CNS, a smaller, but identifiable population of the Type A sites also exist (13).

Several laboratories have reported a complex of behavioral suppression following the administration of CCK-8. Systemically administered CCK-8 suppresses both locomotor activity and appetite in several species including rats and mice (1, 3, 26, 35). The pharmacology of this effect has been identified as being mediated via the Type A receptor on the basis of its sensitivity to blockade by the antagonist L-364,718 (22).

The physiological and pharmacological significance of the CCK system in the CNS is not as well defined as that in the periphery. The distribution of Type A and Type B receptors appears to vary across species (13,32). Even within the same species, studies conducted with rats have produced inconsistent results with some reports of locomotor suppression following ICV administration (16,19), while others have failed to find such suppression (18,20). Those reports which have confirmed effects of CCK following ICV administration have not provided a consistent picture of the nature of the receptor subtype involved.

The recent availability of high affinity CCK antagonists such as L-364,718 (3) has added significantly to the arsenal available to pharmacologists to identify the physiological roles of CCK peptides.

We have used a newly identified D-glutamate derived antagonist of the Type A CCK receptor, A-65186 (N-3-quinolinoyl-R-Glu-N,N-alpha-di-N-pentylamine, molecular weight = 455, Fig. 1) (Kerwin *et al.*, in press) to characterize the behavioral effects of CCK peptides following peripheral or central administration in the mouse. A-65186 has been shown to bind to the Type A pancreatic CCK receptor with high affinity ($K_i = 5.4$ nM) and selectivity (~670-fold) compared to its affinity for the Type B site in the guinea pig cerebral cortex ($K_i = 3600$ nM). The antagonist nature of its interaction at the Type A receptor is defined on the basis of its ability to inhibit CCK-8-stimulated amylase release ($IC_{50} = 14.5$ nM) (Kerwin *et al.*, in press).

METHOD

Animals

Male Swiss CD-1 mice (Charles River) (22–27 g) are brought

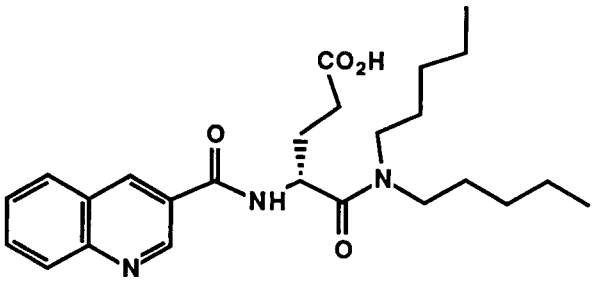

FIG. 1. Structure of A-65186.

to the test room in their home cages (20 animals per cage) at about 10:00 hr on the morning of the test. They are provided ample food (Purina Lab Chow) and water until the time of their injections.

Injections

For IP injections a volume of 10.0 ml/kg is given with a 26 ga, ⅜ inch needle. ICV injections are given by a free-hand method similar to that previously described (12). Animals are placed on a slightly elevated metal grid such that their bodies are supported by the grid bars while their legs extend through the grid. They are restrained by the thumb and forefinger at the level of the shoulders thus immobilizing their heads. Injections are made with 30 ga needle with a "stop" consisting of a piece of tygon tubing to limit penetration of the needle to 5.0 mm below the surface of the skin. The needle is inserted at a midline point equidistant from each eye and an equal distance posterior from the level of the eyes such that the injection site and the two eyes form an equilateral triangle. The needle is inserted perpendicular to the plane of the skull and the injection volume (5 μl) expelled smoothly over a period of approximately 1 sec. Independent studies show good dye distribution throughout the ventricular system in over 90% of the mice. Test compounds are prepared so that the observers are unaware of the treatment the animals are receiving. Animals which received both ICV and IP injections received the ICV injection first followed immediately by the IP injection regardless of the drug being injected.

Behavioral Observations

Mice are placed individually in clear plastic cages. Each cage measures $19 \times 26 \times 15$ cm in height and contains a 60-tube polypropylene test tube rack (NALGENE #5970–0020) placed on end in the center of the cage to enhance exploratory activity. Ten to 20 such units are placed on two levels of shelving to allow an observer to watch all cages from a sitting position. The observations take place between 12:00 and 17:00 hr. Immediately after injections mice are placed in the cages and allowed a 15-min recovery period prior to the beginning of the observations. Observations consist of sampling each animal's behavior at 30-sec intervals for the 30-min duration of the test. Each sampling consists of recording the activity category for the animals at that instant when the observer turns his or her attention to that cage. If the instantaneous glance at the animal is sufficient to be certain of the appropriate category of on-going behavior, the observer may continue watching that animal an additional 1–3 seconds to clarify the nature of the on-going activity. The activity category is entered via a keyboard into a data collection program on a personal computer. This allows a single observer to record data from up to 20 animals per session.

Behavior is classified into one of several categories depending on the position and activity of the animal. Locomotion as reported here consists of either floor locomotion or active climbing on the rack.

Data Analysis

Differences among groups are analyzed by Newman-Keuls analysis and a probability level of $p<0.05$ accepted as significant. Typically each group consists of ten animals.

In our experience a small proportion of the control ICV injected animals show obvious motoric abnormalities following injections. Approximately 5% show reduced activity and an occasional animal (less than 1%) shows abnormally elevated levels of activity with almost constant locomotion. Given that these are relatively infrequent occurrences and that it is difficult to attribute these effects to trauma resulting from the injections per se, we make no attempt to normalize the data by deleting such "outliers" after the test period begins. The few animals which fail to show normal recovery within the first few minutes following injections are readily identified during the 15-min adaptation period prior to formally gathering data. These animals are excluded from the test. In other studies (manuscript in preparation) we have determined that the procedure of ICV injections does not significantly alter behavior on any of a number of parameters including locomotion compared to animals merely restrained for an equivalent period or animals which had the ICV needle inserted without any injection. Although animals were used in this test only once, they have been kept in the facility for up to several weeks following such procedure with no observable ill effects.

RESULTS

The effects of IP administration of CCK-8 are shown in Fig. 2A. There was significant suppression of locomotor activity at 10.0 ($p<0.05$) and 100.0 ($p<0.01$) nmol/kg, $F(3,36)=9.64$. Neither CCK-8-DS nor Boc-CCK-4 were active at the doses indicated in Fig. 2B and 2C.

The Type A CCK receptor antagonist, A-65186, at doses of 1,000 and 10,000 nmol/kg, IP fully blocked the suppressive effects of 100 nmol/kg, IP CCK-8, $F(4,55)=2.75$, with approximately 50% reversal occurring at a dose equimolar to that of CCK-8 (Fig. 3).

The results of ICV administration were similar to those seen with IP administration. CCK-8 produced an approximately 50% suppression of locomotion at a dose of 3.0 nmol and a greater suppression at 10 nmol per mouse, $F(4,40)=4.1$, $p<0.01$. Both BOC-CCK-4 and CCK-8-DS were inactive at doses up to 10.0 and 5.7 nmol per mouse respectively (Fig. 4).

The suppressive effect of ICV administered CCK-8 occurred at approximately the same total dose as that which produced effects when administered IP. This effect was also reversed by ICV co-administration of A-65186 (Fig. 5A), $F(4,55)=2.75$. One explanation for this would be that the ICV administered CCK-8 was passing into the peripheral circulation and initiating its effects by acting on Type A CCK receptors located outside of the CNS.

In order to determine if the ICV administered CCK-8 was indeed acting within the domain of the CNS, some animals were given ICV CCK-8 followed immediately by peripherally administered A-65186. The results of this approach are shown in Fig. 5B. Under these conditions of administration, CCK-8 significantly suppressed activity in all groups, $F(5,50)=3.99$, with no evidence of blockade of the CCK-8 effects by the peripherally administered A-65186.

In no case have we observed an effect of A-65186 alone on locomotor activity. The effects of A-65186 at 10,000 nmol/kg

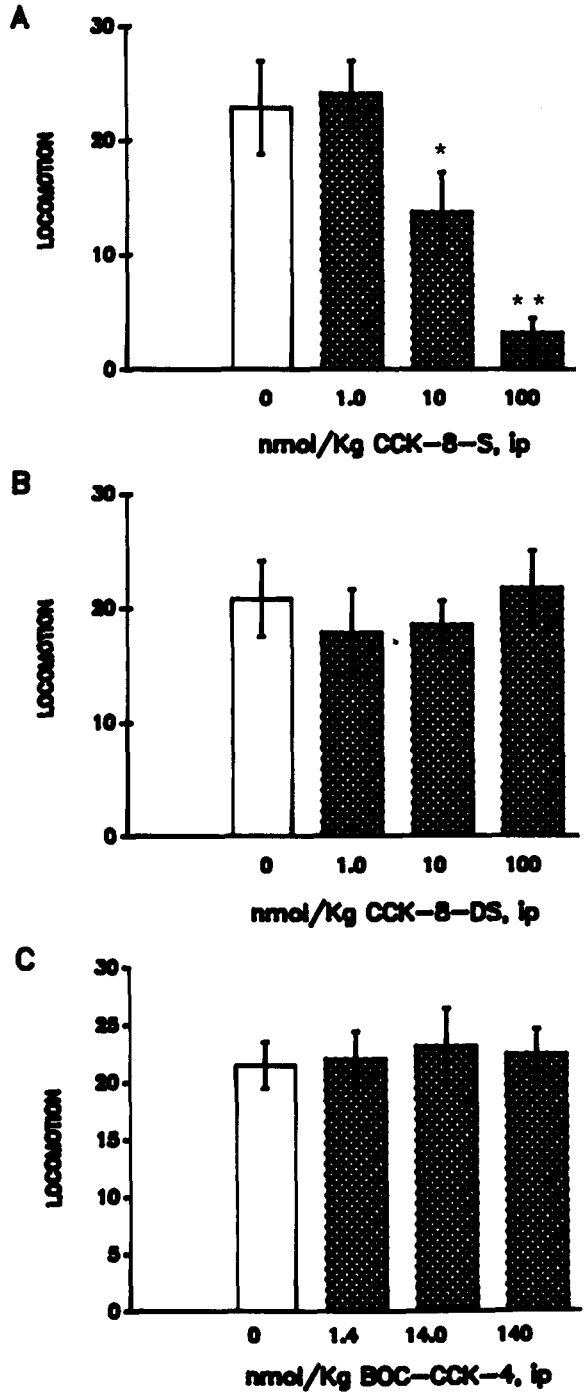

FIG. 2. Effects of IP administered CCK-related peptides on locomotor activity. CCK-8 (2A), CCK-8-DS (2B), Boc-CCK-4 (2C). Significant vs. control, $*p<0.05$, $**p<0.01$.

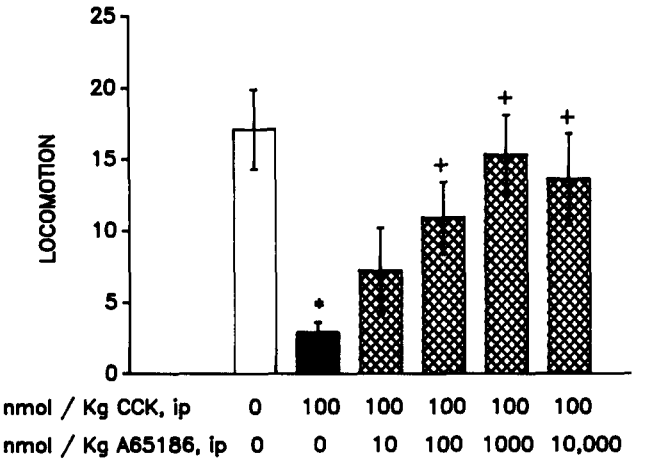

FIG. 3. Effects of IP administered A-65186 on locomotor effects of IP administered CCK-8. *Significant ($p<0.05$) vs. control. $^+$Significant ($p<0.05$) vs. CCK-8 alone.

administered IP are shown in Fig. 5B. In other studies when A-65186 was given ICV alone no effects on locomotor activity were observed at doses up to 30 nmol (data not shown).

DISCUSSION

There is now good evidence for the ability of proglumide and benzotript as well as the more potent antagonist, L-364,718, to block the suppressive effects of peripherally administered CCK-8 on exploratory activity in mice (8,22). The present data demonstrate the ability of the Type A CCK receptor antagonist A-65186 to suppress the peripherally behavioral actions of CCK-8.

Several studies with rats suggest the behavioral effects (25,33), as well as some component of the gastric (29) effects following peripheral administration of CCK-8 are mediated via vagal afferent projections to the nucleus of the solitary tract (5,28), the paraventricular nucleus of the hypothalamus (10) and the lateral amygdala.

Behavioral effects of CCK-8 injected into specific brain regions in the rat have suggested considerable site dependence. In the posterior portion of the nucleus accumbens CCK-8 can potentiate the locomotor stimulating actions of dopamine with which it is co-localized (14), while injections into the anterior portion of the accumbens actually suppress the DA effects (7). Injections into the striatum have been reported to be without effect (7,21). The presumably more global distribution which accompanies ICV administration has not typically been associated with identifiable behavioral effects in the rat.

There have been fewer studies of central administration of CCK-8 in the mouse. Because of the species differences in receptor type and distribution, we were interested to determine if a more consistent CNS effect could be identified in this species than has been reported for the rat.

These data provide evidence that, in the mouse, CCK-8 can act centrally to alter locomotor activity and that those centrally mediated behavioral effects are mediated by a Type A receptor just as are the peripheral actions. There is good evidence that centrally administered peptides can be actively transported across the blood-brain barrier and escape into the general circulation [see (27) for review]. Given the acute method of ICV administration in this study we were also conscious of the possibility that a compromised blood-brain barrier could allow an additional avenue by which ICV administered peptides could escape into the periphery. The fact that the ICV effects were not blocked by even high doses of systemically administered A-65186 strongly argues against an interpretation that the centrally administered CCK-8 was diffusing into the general circulation and acting peripherally.

The fact that administration of A-65186 alone failed to alter locomotor responsiveness in the present test fails to support a

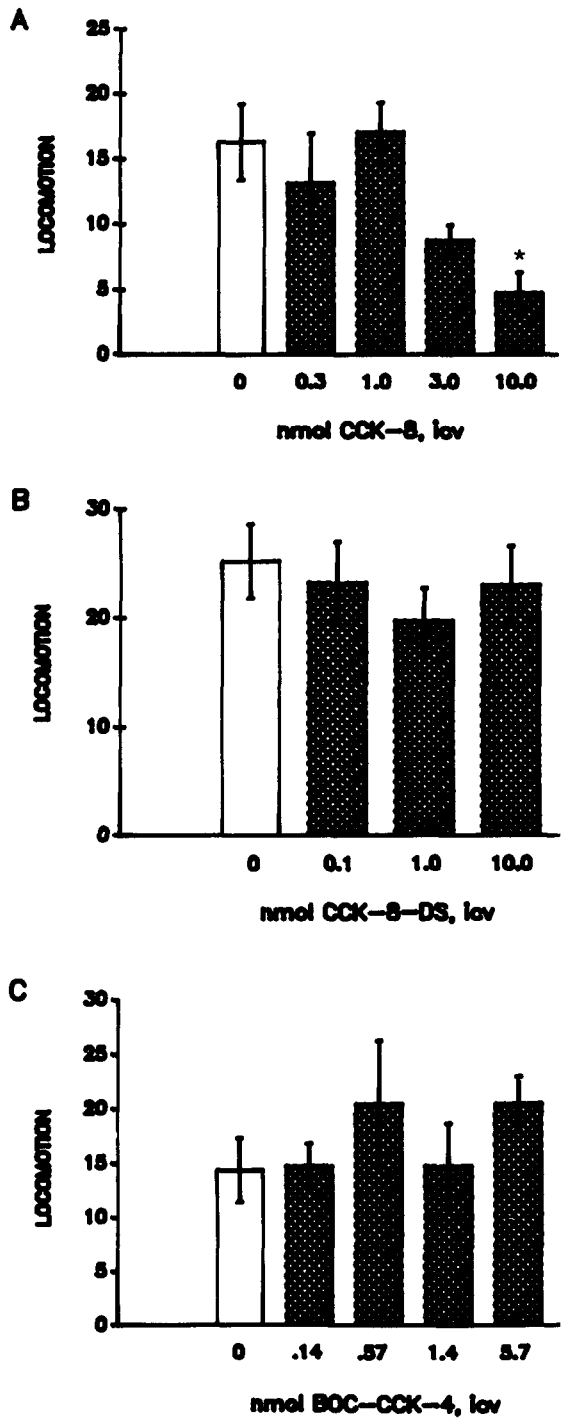

FIG. 4. Effects of ICV administered CCK-8 (4A), CCK-8-DS (4B) and Boc-CCK-4 (4C) on locomotor activity. *Significant vs. control ($p<0.05$).

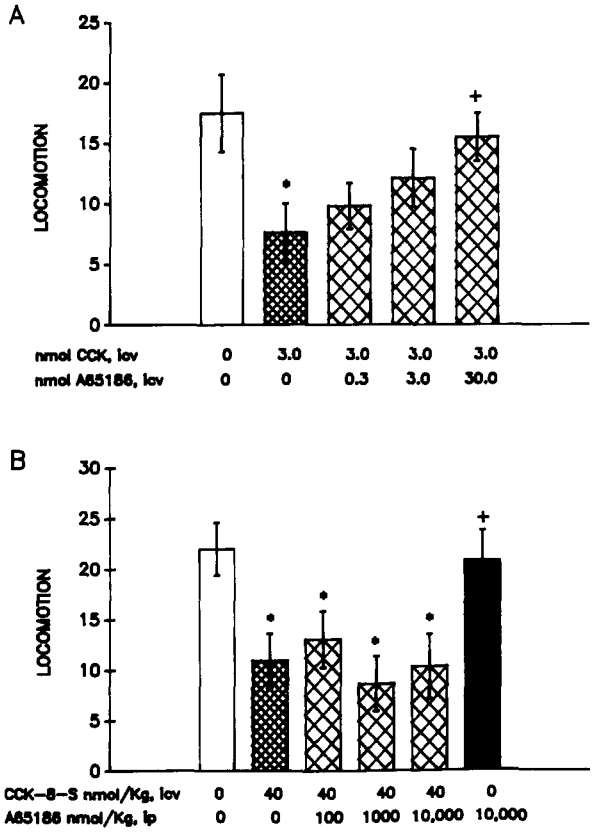

FIG. 5. Effects of A-65186 administered either ICV (5A) or IP (5B) on ICV CCK-8-induced locomotor suppression. (3.0 nmol/animal, ICV = 120 nmol/kg; 40 nmol/kg = 1.0 nmol/animal.) *Significant ($p<0.05$) vs. control. $^{+}$Significant ($p<0.05$) vs. indicated dose of ICV CCK-8.

physiological role for endogenous CCK as a modulator of locomotor activity under these test conditions. We cannot rule out, however, the possibility that endogenous CCK may participate in the regulation of locomotor activity under other conditions.

The findings reported here are qualitatively similar for both IP and ICV effects in terms of the behavioral consequences and the receptor type involved. It remains to be determined if there are similar underlying mechanisms or CNS pathways involved in the actions of CCK-8.

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