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Inhibition of Serotonin Synthesis Attenuates Inhibition of Ingestive Behavior by CCK-8

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ESFAHANI, N., I. BEDNAR, G. A. QURESHI AND P. SÖDERSTEN. *Inhibition of serotonin synthesis attenuates inhibition of ingestive behavior by CCK-8.* PHARMACOL BIOCHEM BEHAV **51**(1) 9–12, 1995. – Ingestive behavior was activated in male rats by intraoral infusion of a 1-M solution of sucrose. Injection of cholecystokinin octapeptide (CCK-8; 1.6 or 5.0 μg) inhibited ingestion of the sucrose solution and increased the concentration of 5-hydroxytryptamine (5-HT) in the paraventricular hypothalamic nuclei. The inhibitory effect of the low, but not the high, dose of CCK-8 was attenuated by depleting 5-HT in the brain with *p*-chlorophenylalanine (PCPA; 100 mg/kg for 3 days). Treatment with 5-hydroxytryptophan (20 mg/kg) increased the concentration of 5-HT in the brain of rats pretreated with either NaCl or PCPA and enhanced the inhibitory effect of CCK-8 on ingestive behavior in the PCPA-, but not NaCl-, treated rats. 5-HT may play a role in the mechanism of action of CCK-8 but additional factors must be involved.

5-Hydroxytryptamine Cholecystokinin octapeptide *p*-Chlorophenylalanine 5-Hydroxytryptophan Ingestive behavior IO intake Male rat

THERE IS now strong support for the hypothesis that release of cholecystokinin octapeptide (CCK-8) from the duodenum during a meal contributes to meal termination in the rat (18), but the brain mechanisms activated by CCK-8 are largely unknown. However, there is evidence that release of catecholamines, in part, mediates the effect of CCK-8 (1–4). In addition, systemic administration of 5-HT antagonists, especially those acting on 5-HT_{2C} receptors, attenuates the effect of CCK-8 (16,17,20). Injection of CCK-8 causes release of 5-HT in the paraventricular hypothalamic nuclei (PVN) (12) and 5-HT has long been considered involved in meal termination (5).

To investigate the possibility that release of 5-HT in the brain mediates the effect of CCK-8 on ingestive behavior, we have examined the effect of inhibiting the synthesis of 5-HT on ingestion of an intraorally (IO) administered solution of sucrose. This "IO intake test" has been developed by Grill and coworkers to specifically test consummatory ingestive behavior (i.e., the responses used to ingest food) whereas appetitive ingestive behavior (i.e., the responses used to obtain food) is ignored in this test (9). A specific test for consummatory ingestive behavior appears particularly suitable for studies on the mechanisms of action of CCK-8 because CCK-8 acts to terminate the meal (18). Moreover, because it has been suggested that 5-HT acts on the PVN to terminate carbohydrate intake specifically (13), it is possible that peripherally administered CCK-8 suppresses IO intake of sucrose via release of 5-HT in this brain area.

METHOD

Animals and IO Cannulation

Male Wistar rats (Möllegård Breeding Laboratories, Ejby, Denmark; 350 ± 12 g) were maintained individually with free access to food pellets and water in a colony room that was dark between 1200 and 2400 h. The rats were implanted with IO cannulae (9) and allowed to recover for 4 weeks.

Behavioral Testing

The pellets were removed at 0700 h each day and the rats were tested for ingestive behavior at 1300 h. The pellets were replaced after testing. The IO cannulae were connected to a peristaltic pump (Alitea XV, Ventur Alitea, Stockholm, Sweden), which delivered a 1-M solution of sucrose with a flow rate of 1 ml/min. This activates ingestive behavior, which ends by the time the rat actively rejects the sucrose solution or passively lets it drip from the side of its mouth. The infusion was then interrupted for 30 s and then started again. If a rat

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stopped ingesting the sucrose solution within 1 min of the restart of the infusion, the test ended; if not, the infusion was again interrupted for 30 s and then started. Testing was complete as soon as the rat rejected the sucrose solution within 1 min of the restart of the infusion. With very few exceptions this occurs once the rat passively rejects the sucrose solution. Active rejection of the sucrose solution did not occur under the present conditions.

The rats were tested daily for IO intake of sucrose, and intake stabilizes under these conditions within five tests (11).

Drug Treatment

Twelve rats that showed a stable intake of sucrose in the preliminary tests were randomly divided into two groups of six rats and injected with 0.9% NaCl or 100 mg/kg *p*-chlorophenylalanine (PCPA, Sigma Chemical Company, St. Louis. MO; methyl ester hydrochloride, dissolved in 2.0 ml/kg 0.9% NaCl and injected IP) at 0900 h on 3 days. Tests for sucrose intake continued during the days of PCPA injection. The rats were then injected daily with 0, 1.8, or 5.0 μg CCK-8 (Cambridge Research Biochemicals, Harston, Cambridgeshire, England; dissolved in 0.5 ml 0.9% NaCl and injected IP) 10 min before testing. The doses of CCK-8 were given in random order and selected on the basis of previous dose-response work to produce medium and maximum inhibition of ingestive behavior (2). Thereafter, the rats were given two daily injections of 20 mg/kg 5-HTP (Sigma, dissolved and injected as the PCPA) 1 h before testing and, in random order, NaCl or 1.8 μg CCK-8 10 min before testing. Thus, each rat served as its own control.

Measurement of Monoamines and Metabolites

Groups of five rats treated with PCPA or NaCl in combination with 1.8 μg CCK-8 or 5-HTP as described above were decapitated and the brains were rapidly removed, placed on dry ice, and stored at −70°C covered with foil paper. The brains were subsequently slightly thawed and 1-mm-thick slices were cut through the dorsal-ventral striatum about 1.5 mm anterior to and at the level of bregma. Samples from the dorsal striatum and the PVN were punched out using stainless steel punches, 2 mm in diameter for the striatal samples and 1 mm in diameter for the PVN samples. The samples were immediately placed on dry ice and stored at −70°C until analysis. The samples were subsequently processed as described previously (3) and the concentration of dopamine (DA), noradrenaline (NA), homovanillic acid (HVA), 3,4-dihydroxyphenyl-acetic acid (DOPAC), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) was measured by HPLC electrochemical detection as described previously (4).

Statistical Tests

The data are expressed as means ± SEM and analyzed using two-way ANOVAs for repeated measurements (21). Subsequent comparisons were made with the Newman-Keuls test (21). Conventional software for the Macintosh computer (10) was used.

RESULTS

Behavior

Treatment with PCPA reduced IO intake of sucrose, although not significantly (Fig. 1a, data from the test on the last day of PCPA treatment are shown). Treatment with CCK-8 suppressed IO intake of sucrose in rats treated with NaCl or PCPA. However, 1.8 μg CCK-8 had a smaller effect in the PCPA- than in the NaCl-treated rats. If expressed as percent of the NaCl value, control rats ingested 40.0 ± 4.7% and PCPA-treated rats ingested 79.4 ± 10.9% after treatment with 1.8 μg CCK-8 ($p < 0.01$) (Fig. 1b). Treatment with PCPA did not affect the inhibitory effect of the high dose of CCK-8 on sucrose intake (Fig. 1a,b).

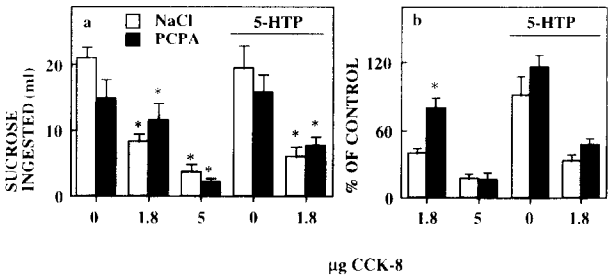

FIG. 1. (a)The mean ± SEM amount of intraorally administered sucrose ingested by male rats treated with NaCl or PCPA in combination with 5-HTP and CCK-8. Values are expressed as percent of the value of NaCl-treated controls in (b). (a)*$p < 0.05$ compared with NaCl or (b) NaCl + 1.8 μg CCK-8.

Treatment with 5-HTP reversed the effect of PCPA on the sensitivity to 1.8 μg CCK-8 (Fig. 1a,b). If expresses as percent of the NaCl + 5-HTP value, control rats ingested 32.7 ± 5.5% and PCPA-treated rats ingested 47.4 ± 5.3% after treatment with 1.8 μg CCK-8 (NS) (Fig. 1b). By itself, 5-HTP had no significant effect on the intake of sucrose in animals given either NaCl or PCPA (Fig. 1a,b).

Biochemistry

Only minor effects were noted on brain levels of DA, HVA, DOPAC, and MHPG; therefore, these are not reported.

The concentration of 5-HT and NA was unaffected by treatment with 1.8 μg CCK-8 in the dorsal striatum. Treatment with PCPA depleted 5-HT but had no effect on the level of NA (Fig. 2).

In the PVN, treatment with 1.8 μg CCK-8 markedly increased the concentration of 5-HT and also increased the level of NA. Treatment with PCPA depleted 5-HT and prevented the effect of CCK-8 but had no effect on the level of NA (Fig. 3).

The effect of NaCl, 1.8 μg CCK-8, and PCPA on the concentration of 5-HT in the PVN is duplicated in Fig. 4, which also shows the effect of 5-HTP and PCPA + 5-HTP as well as the effect of these treatments on the level of 5-HIAA. CCK-8 increased the concentration of 5-HT but had no effect on the level of 5-HIAA. 5-HTP markedly increased the level of both 5-HT and 5-HIAA and, in rats treated with PCPA, 5-HTP increased the level of 5-HT and 5-HIAA above that of the NaCl-treated rats.

DISCUSSION

A variety of pharmacological manipulations affect food intake and feeding behavior but not all of these can be related to a physiological regulator of the behavior (6). CCK-8 is such a regulator (18). Thus, treatment with CCK-8, which raises plasma levels of CCK-8 to those seen postprandially, suppresses food intake (19), and treatment with CCK_A receptor

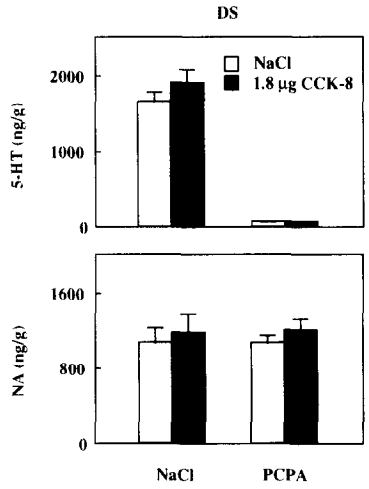

FIG. 2. The mean ± SEM concentration of 5-HT and NA in the dorsal striatum (DS) of male rats treated with NaCl or PCPA in combination with CCK-8.

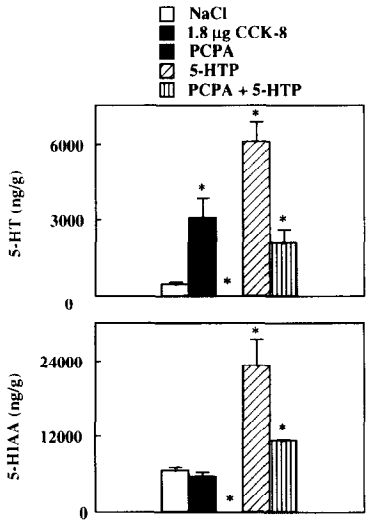

FIG. 4. The mean ± SEM concentration of 5-HT and 5-HIAA in the paraventricular nucleus of the hypothalamus (PVN) of male rats treated with NaCl, CCK-8, PCPA, 5-HTP, or PCPA in combination with 5-HTP. *$p < 0.05$ compared with NaCl-treated rats.

antagonists reverses this effect and, by itself, stimulates feeding (18).

To investigate the role of 5-HT in suppression of ingestive behavior by CCK-8, we used Grill's IO intake test (9), which specifically measures consummatory ingestive behavior. There are several reasons for this choice. First, the factors controlling meal initiation are poorly understood (22). Second, CCK-8 acts to terminate a meal and has no effect on meal initiation (18). Third, there is no specific test for appetitive ingestive behavior. Fourth, drugs affecting monoaminergic transmission can nonspecifically interfere with appetitive ingestive behavior (1–3).

We chose to deliver a solution of sucrose IO because 5-HT has been suggested to specifically inhibit intake of carbohydrates (13). IO intake of sucrose causes release of CCK-8 into the blood, treatment with CCK-8 suppresses intake, and pretreatment with a CCK_A receptor antagonist reverses the effect of CCK-8 and stimulates the intake of sucrose (3,14).

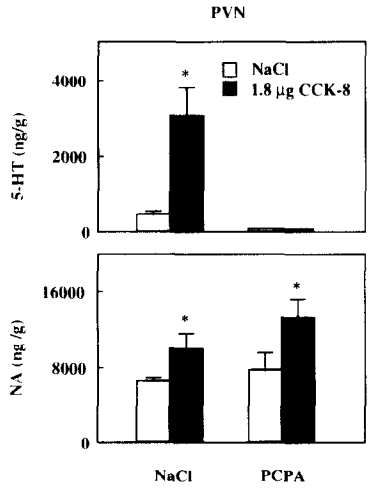

FIG. 3. The mean ± SEM concentration of 5-HT and NA in the paraventricular nucleus of the hypothalamus (PVN) of male rats treated with NaCl or PCPA in combination with CCK-8. *$p < 0.05$ compared with NaCl-treated rats.

It was hypothesized some time ago that 5-HT is involved in meal termination (5). Suppression of feeding by 5-HT may be relevant for the understanding of the mechanism of action of CCK-8 because 5-HT receptor antagonists blunt the effect of CCK-8 (16,17,20) and IP injection of CCK-8 causes release of 5-HT in the brain (12). Interestingly, administration of CCK-8 can excite the 5-HT neurons in the brain stem, which project to the PVN (7), and 5-HT is released in the PVN after injection of CCK-8 (12). It has been suggested that 5-HT acts on the PVN to suppress intake of carbohydrate and stimulate intake of protein (13).

In the present study, IP administration of 1.8 μg CCK-8 markedly increased the concentration of 5-HT in the PVN, and this effect was prevented by a treatment schedule of PCPA that depleted 5-HT from the PVN and prevented the effect of CCK-8 on PVN 5-HT levels. The inhibitory effect of this dose of CCK-8 on IO sucrose intake was attenuated by the PCPA treatment and the behavioral sensitivity to CCK-8 was restored by administration of 5-HTP in a dose that increased the concentation of 5-HT in the PVN. In the dorsal striatum, on the other hand, CCK-8 failed to affect the concentration of 5-HT, thus indicating that CCK-8 does not indiscriminately increase the level of 5-HT in any brain area.

These results and those of the microdialysis study referred to above (12) support the hypothesis that release of 5-HT in the brain in part mediates the inhibitory effect of CCK-8 on ingestion of carbohydrates. However, other mechanisms must be involved because inhibition of the synthesis of 5-HT did not affect the inhibitory effect of a higher, although not excessively high, dose of CCK-8 (5.0 μg). This dose of CCK-8 increases plasma levels of CCK-8 to those seen after a meal in the rat (19). It appears overly simplistic to assume that release of 5-HT in the PVN acts specifically to suppress carbohydrate intake. On this hypothesis inhibition of the release of 5-HT should increase carbohydrate intake, but treatment with

PCPA reduced IO intake of sucrose somewhat. In addition, treatment with 5-HTP markedly increased the level of 5-HT in the PVN but had no significant effect on the intake of sucrose in rats treated with PCPA or in control rats. However, it must be added that PCPA and 5-HTP were administered peripherally in the present study and it is known that 5-HT can act peripherally to affect the mechanisms that control feeding (8,15).

In addition to release of 5-HT, IP injection of CCK-8 causes release of DA and NA in the PVN (12). Release of DA in the PVN has no known relation to feeding (6). Here we found no effect of CCK-8 on the level of DA in the PVN. However, the concentration of NA was increased in the PVN, but not the dorsal striatum, after treatment with CCK-8. Thus, IP administration of CCK-8 inhibits IO intake of sucrose and causes release of NA in the PVN. This observation is inconsistent with the suggestion that NA acts on the PVN to stimulate carbohydrate intake (13). Clearly, the reported changes in monoamines in the PVN after peripheral administration of CCK-8 [(12), present data] and the reported data on macronutrient selection after drug or transmitter injections into the PVN (13) cannot explain the inhibitory effect of CCK-8 on consummatory ingestive behavior, measured by the ingestion of an IO-administered sucrose solutions. Elucidation of the mechanisms of action of CCK-8, therefore, awaits results of further experiments.

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