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Cyclo(His-Pro) Potentiates the Reduction of Food Intake Induced by Amphetamine, Fenfluramine, or Serotonin

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KOW, L -M AND D W PFAFF *Cyclo(His-Pro) potentiates the reduction of food intake induced by amphetamine, fenfluramine, or serotonin* PHARMACOL BIOCHEM BEHAV **38**(2) 365–369, 1991 —Electrophysiological and pharmacological evidence suggests that cyclo(His-Pro) (cHP) could reduce food intake by modulating the actions of relevant neurotransmitters We tested this hypothesis by giving rats a combination of cHP or its analogs centrally and an anorectic, amphetamine or fenfluramine, systemically Compared to saline control, cHP at doses too low to affect food intake by itself significantly potentiated the reduction of food intake by amphetamine This potentiation is thought to be due to cHP modulation of norepinephrine (NE) action, because at the low dose used amphetamine acts mainly through NE to inhibit food intake The modulation has specific requirements for cHP structure, since it was mimicked by one but not two other analogs tested The anorectic effect of fenfluramine was also potentiated and prolonged by cHP at a dose not effective by itself Since fenfluramine is known to act by increasing brain serotonin (5-HT), the potentiation was apparently a result of an interaction between cHP and 5-HT effects To examine this interaction more directly, we administered both cHP and 5-HT centrally Again, cHP potentiated the reduction of food intake caused by 5-HT Thus the neuromodulation of feeding-relevant neurotransmitter effects, following NE and 5-HT, is probably a mechanism by which cHP reduces food intake

Cyclo(His-Pro) Amphetamine Fenfluramine Neuromodulation Serotonin (5-HT)

CYCLO(HIS-PRO), or cHP, is a putative metabolite of thyrotropin-releasing hormone (TRH). Like TRH (17, 22–24), cHP can reduce food intake (17, 18, 25). However, for cHP to do so required high doses. One reason for this could be that the structure of the cHP molecule is not optimal. To explore this possibility, attempts were made to improve the anorectic potency of cHP by modifying the structure of its molecule (13). An alternative possibility for the low cHP potency is that it achieves its anorectic effect by modulating neuronal responses to feeding-relevant neurotransmitters. Accordingly, cHP would not be effective when administered alone, but could be very potent when given in combination with an appropriate transmitter-related agent. This possibility was raised by an in vitro study on the electrophysiological actions of TRH and cHP. Although TRH could act both directly as a neurotransmitter, to stimulate neurons, and indirectly as a neuromodulator, to modify neuronal responses to other transmitters, *only* the neuromodulatory action was shared by cHP (11) Since the two peptides also shared the anorectic effect, we hypothesized that they reduce food intake by modulating the actions of feeding-relevant neurotransmitters. This hypothesis is consistent with findings by others that TRH and cHP affect other biological functions and behaviors by modulating catecholaminergic and serotonergic systems (1, 16, 21).

We tested this hypothesis by treating rats with a combination of cHP with the anorectic agents amphetamine, fenfluramine, or serotonin (5-HT). To ensure that we were dealing with modulatory actions, cHP was always administered at doses too low to affect food intake by itself. Amphetamine was used because cHP augmented the effect of amphetamine in inducing stereotypic behavior by interacting with brain catecholamines (20), and, secondly, amphetamine could inhibit food intake primarily by increasing brain catecholamines (2, 5, 7) We reasoned that the anorectic effect of amphetamine might also be potentiated by cHP. Fenfluramine is known to exert its anorectic effect primarily by increasing brain 5-HT (2, 5, 7), which, in turn, can reduce food intake (15). Since TRH potentiates the effect of 5-HT and its analogs in inducing hyperactivity (8), and since cHP shares anorectic effects with TRH, it was plausible to propose that cHP can reduce food intake by potentiating the actions of 5-HT and fenfluramine. A portion of the results has been reported in abstract form (12).

METHOD

Subjects

Adult, male Sprague-Dawley rats were used. They were housed

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individually in an air-conditioned room with lights on from 8.00 a.m. through 8.00 p.m. They had free access to water and powdered rat chow (Purina Rodent Lab Chow, No. 5001). The powdered chow was placed in a special container to prevent spillage. After arrival, the rats were given at least one week to adapt to eating powdered chow and to the new environment. The rats were then chronically implanted with a cannula, as described in detail in the preceding paper (13), for intracerebroventricular (ICV) infusion of test agents into the lateral ventricle. They were allowed to recover for one week, during which they were undisturbed

Experimental Procedures

Two kinds of procedures were followed one for experiments using powdered chow as the test diet, and the other for an experiment using milk as the test diet. In the first procedure, the rats were food-deprived daily except for 7 h from 10:00 a.m. to 5:00 p.m., during which the feeding test was carried out. At the beginning of the test, powdered chow was presented and the cumulative food consumption was measured at 1, 2, and 7 h after food presentation. Before food presentation, the subjects were handled twice; the first prepared the rat for ICV infusion and the second for intraperitoneal (IP) injection. At first, the rats were adapted to the experimental procedure and received only the handling. After 7–10 days of training, their food intake became stabilized The rats then received experimental treatments in which the first handling allowed ICV infusion of cHP, its analogs, or saline, and the second handling allowed IP injection of amphetamine, fenfluramine, or saline. The ICV infusion and the IP injection were spaced 5–8 minutes apart to allow time for the infused cHP or its analogs to act on the brain. Depending on whether amphetamine or fenfluramine was used, the interval between the IP injection and food presentation was 3 or 30 minutes, respectively.

The second experimental procedure, using milk, was the same as that described in the previous paper (13). Briefly, the rats were food-deprived for 5 h, starting at 10:00 a.m., and then subjected to a feeding test. Before the test, they were handled/ICV infused twice, spaced 10 min apart. In the first infusion cHP or saline was administered. In the second 5-HT was infused The second infusion was followed 15 min later by a 30-min feeding test, during which the rats were presented with milk At the end of the test the milk was removed for measurement and was replaced with regular food (rodent chow pellets).

At the end of the experimental treatment day, each rat was infused with angiotensin II (50 ng/5 μl/rat, ICV) to assess ICV cannula location and patency [see (13)]. Only the rats that started to drink within 2 min of angiotensin infusion were included in this report.

Test Agents

To test for neuromodulatory action, cHP (Sigma Co.) and three cHP analogs synthesized by Abbott Labs [see (13)] were used. The analogs included two (A-65171 and A-65206) that preserved the anorectic action of cHP, and one (A-65913) that reversed the anorexia. These agents, dissolved in saline, were administered through ICV infusion at 1000, 500, or 50 nmoles/10 μl/rat. The whole process included a 60-s infusion and a 30-s wait to allow for diffusion.

In order to allow for modulation, we used the doses of anorectics that induced only moderate inhibition of food intake. These doses were determined from dose-response experiments Results for d-amphetamine (Amph) and dl-fenfluramine (Fenf) are summarized in Table 1. On the basis of such results, doses of 0.4 and 1.0 mg/1 ml saline/kg body weight (b.wt.) were chosen for Amph and Fenf, respectively. For 5-HT, the dose chosen was 0.5 μmole/5 μl saline/rat, ICV.

TABLE 1

DOSE-DEPENDENT EFFECTS OF SYSTEMIC, INTRAPERITONEAL INJECTIONS OF d-AMPHETAMINE AND dl-FENFLURAMINE ON FOOD (POWDERED CHOW) INTAKE

Dose (mg/kg b wt)	Food Intake* as Mean ± SEM % at 1 h	2 h
	d-Amphetamine	
1 00	25 0 ± 7 2	74 7 ± 5 0
0 75	54.6 ± 12 7	76 5 ± 0 9†
0 50	60 9 ± 10 0	79 1 ± 6 8
0 25	73 1 ± 8 5	83 4 ± 2 4
	dl-Fenfluramine	
3 00	13.5 ± 4 9	25 0 ± 1 0
1 50	41 6 ± 6 8	60 5 ± 10 9
0 75	86.6 ± 10 8	90 8 ± 3 2
0 375	111 3 ± 9 6	117 5 ± 3 3

*Food Intake (FI) = (Postinjection FI/Preinjection control FI) × 100%, n = 4 in all cases except for the case marked †

†n = 3.

Measurement of Food Intake

Cumulative consumption of powdered chow was determined by measuring the weight difference between the beginning and the end of a test period Spillage, if any, was collected and included in the weight measurement. To normalize across rats, the chow or milk consumption was calculated as the percentage of the baseline level obtained from the last 3 days of training. Experimental and control groups were compared using the *t*-test to assess the effect of each treatment.

RESULTS

Combined Treatment With cHP and d-Amphetamine

Figure 1 shows the results of these experiments. Control administration of saline (ICV) and saline (IP injection) had no effect on food intake at any time point. Likewise, infusion of cHP at the dose of 1000 nmole/rat followed by IP injection of saline did not affect food intake either. As expected, saline infusion followed by Amph injection caused moderate reductions in food intake at 1 and 2 hours. Addition of 1000 nmoles cHP potentiated this anorectic effect of Amph at all time points. For this modulation cHP was still effective at 500 nmoles and as low as 50 nmoles (Fig. 1). None of the rats treated with saline plus Amph or cHP plus Amph showed signs of increased locomotion or other changes in behaviors, phenomena often induced by high doses of Amph. These results clearly showed that cHP at doses too low to affect food intake by itself could potentiate the anorectic effect of another agent.

Effects of cHP Analogs

In some experiments, cHP was replaced by its analogs to see if the latter also had neuromodulatory actions As shown in Table 2, analog A-65171 potentiated the effect of Amph at a dose

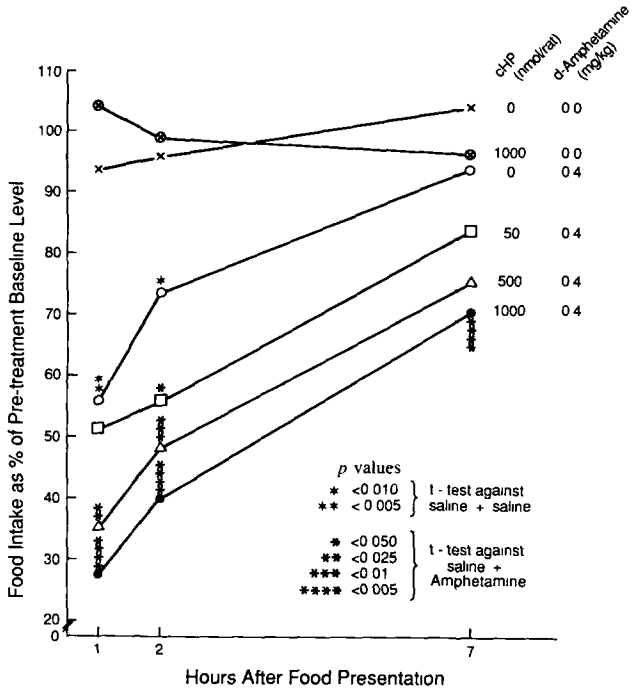

FIG 1 The effects of combining treatments with cHP and amphetamine The number of rats tested and the doses of cHP and amphetamine are listed to the right of the figure There was no statistical difference between saline plus saline and cHP plus saline groups All p values are one-tailed

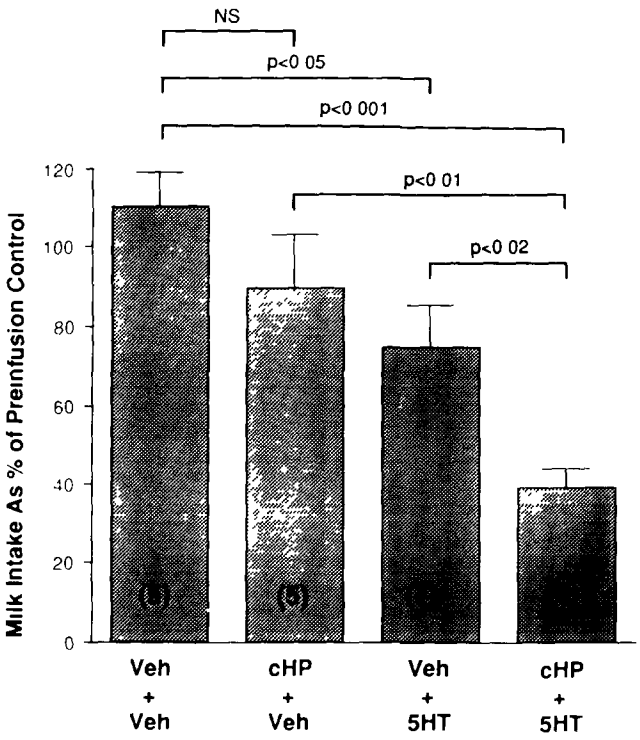

FIG 2 The effects of combining treatments with cHP and serotonin (5-HT). On the ordinate axis, 100% indicates the level of milk consumption measured during the last three training sessions, when the rats were subjected to handling only The number in parentheses indicates the number of rats tested All p values are based on t-tests and are one-tailed

of 1000 nmole/rat. (It had a nonsignificant tendency to do so at half that dose.) Another analog, A-65206, had no modulatory action. The third analog, A-65913, which by itself had a feeding-inducing effect (13), not only cancelled the anorectic effect of Amph, but even stimulated food intake for more than two hours.

Combined Treatment With cHP and dl-Fenfluramine

As shown in Table 3, comparison between the saline-Fenf and the control (saline-saline) groups shows that Fenf inhibited food intake significantly for at least two hours. This anorectic effect of Fenf, like that of Amph, was potentiated and prolonged by a preceding cHP infusion.

Combined ICV Infusion of cHP and 5-HT

As shown in Fig. 2, two infusions of saline (Veh + Veh) did not affect milk intake. Infusion of cHP (0.5 μmole/0.5 μl/rat) plus saline had a nonsignificant tendency to reduce milk intake. The central administration of 5-HT (0.5 μmole/rat) following sa-

TABLE 2

NEUROMODULATORY ACTIONS OF ICV-INFUSED cHP ANALOGS ON THE ANORECTIC EFFECT OF d-AMPHETAMINE (0 4 mg/kg b wt IP INJECTION)

Agents	Dose (nmole/ rat)	n of Rats	Food Intake[a] as Mean ± SEM % at 1 h[b]	2 h	7 h
Saline	—	10	60 8 ± 4 0	70.2 ± 3.8	93 3 ± 4 4
A-65171	1000	5	48.7 ± 4 6*	54.6 ± 6 5*	79 9 ± 16 0
A-65171	500	4	53 3 ± 12 4	62 3 ± 5 5	101 0 ± 13 9
A-65206	1000	4	65 9 ± 6 2	73 7 ± 7 2	102 6 ± 5 7
A-65913	500	6	123 9 ± 20.6†	98 5 ± 5 7†	96 7 ± 6.0

[a]Food Intake (FI) = (Postinfusion FI/Pretreatment control FI) × 100%, with 100% being the FI of rats subjected only to training handlings

[b]Time after food presentation

*p<0 05, †p<0.005, one-tailed, t-test against saline control

TABLE 3

NEUROMODULATORY ACTIONS OF cHP ON THE ANORECTIC EFFECT OF dl-FENFLURAMINE (Fenf)

cHP (nmole/ rat)	Fenf (mg/kg b wt)	n of Rats	Food Intake[a] as Mean ± SEM % at 1 h[b]	2 h	7 h
0	0[c]	6	93 3 ± 6 9	95 6 ± 5 9	103 5 ± 3 9
1000	0[c]	7	104.1 ± 3 3	93 3 ± 4 5	96 0 ± 4 7
0	1.0	9	54 1 ± 5 5*	63 5 ± 7 1*	97 5 ± 8 7
1000	1 0	10	28 3 ± 7 1†	42 4 ± 4 9†	64 7 ± 6 1†

[a]Food Intake (FI) = (Posttreatment FI/Pretreatment FI) × 100%

[b]Time after food presentation

[c]These saline + saline and cHP + saline control groups are the same as those shown in Fig 1 The FI's of these two groups are not statistically different

*$p<0$ 005, one-tailed t-test against saline + saline group, †$p<0$ 025, one-tailed t-test against saline + Fenf group

line caused a small but significant reduction in milk consumption. When the 5-HT treatment was preceded by an infusion of cHP, the inhibition of milk intake was potentiated This exaggerated reduction of food intake (down to 39% of baseline) was greater than the sum of the inhibitions (down to 64.3% of baseline) caused by cHP and 5-HT, separately. Thus HP could modulate the central 5-HT effect in potentiating its anorectic action

DISCUSSION

In the present study we have found that cHP used at doses that had no effect on food intake by themselves could potentiate the anorectic effects of amphetamine or fenfluramine. Using powdered chow as a test diet in the present experiment, we found that cHP at the dose of 1000 nmole/rat had no anorectic effect. This is different from the results of previous experiments, where the same cHP dose caused inhibition of milk intake (13) Such a difference is probably because milk intake is more vulnerable to inhibition. The main action of amphetamine is to cause the release and inhibit the reuptake of the catecholamines (2, 5, 7) norepinephrine (NE) and dopamine (DA), which mediate most amphetamine effects. Therefore, potentiation of an amphetamine effect by cHP can be due to modulation of NE and/or DA action. Indeed, cHP has been reported to increase the stereotyped behavior induced by amphetamine, apparently through a modulation of the DA system (20). In the case of the anorectic effect, however, cHP may modulate NE rather than DA· In the reduction of food intake, as the dose of amphetamine is lowered, the importance of DA decreases and that of NE increases (3). In the present study, the dose of amphetamine used was small (0.4 mg/kg b.wt.). According to a large set of results (2), the anorectic effect of amphetamine at this low dose is mediated mainly by NE. Consistent with this is the observation that amphetamine-treated rats did not show the hyperactivity which is attributable to DA [cf. (4)]. So, in the present study, the potentiation of the amphetamine effect may have resulted from the modulation of NE action by cHP. Such an interaction was also seen before in our electrophysiological study· we found that, in the ventromedial nucleus of the hypothalamus, TRH and cHP modulated NE responses in 25 of 27 NE-responsive neurons tested, primarily by potentiating excitatory responses to NE (11). The consistency of the two types of studies, the electrophysiology at the single neuron level and behavior of whole animals, suggests that neuromodulation of the action of NE could be one of the mechanisms by which cHP can affect food intake.

Another neuromodulatory effect of cHP was indicated by the interaction between cHP and fenfluramine. The anorectic effect of fenfluramine has been attributed mainly to its ability to induce a release in 5-HT (2,5). The present observation that cHP potentiated the fenfluramine effect, therefore, suggests that cHP can regulate food intake by modulating the action of 5-HT. This suggestion was supported further by the experiment in which both cHP and 5-HT were infused ICV we found that cHP potentiated the reduction of food intake caused by 5-HT (Fig. 2). In this respect, it is interesting to note that there are conditions where ICV infusions of amphetamine that induced anorexia and hypodipsia also caused release of 5-HT in addition to NE and DA (19) Therefore, cHP modulation of 5-HT could account for some of the potentiation of amphetamine action.

In the accompanying paper (13), we found that some analogs of cHP with certain structural modifications could preserve its anorectic effect Two such analogs (A-65171 and A-65206), as well as one analog (A-65913) which reversed the anorectic effect and stimulated feeding, were used in place of cHP in some experiments to see whether they also had neuromodulatory actions. The fact that A-65206 did not potentiate amphetamine, and that A-65913 still stimulated feeding indicated that the potentiation of amphetamine action by cHP was specific in that it was not common to all of its structural analogs. A-65171 did potentiate the anorectic effect of amphetamine (Table 2) But, unlike cHP which was effective even at 50 nmole/rat, analog A-65171 was effective only at 1 μmole/rat These findings reinforce the notion that there are particular requirements for the neuromodulatory action of cHP.

One major finding of the present study is that cHP could potentiate the anorectic effects of other agents at doses more than twenty times lower than that for cHP's direct action. This effectiveness of cHP at low doses suggests that cHP inhibits food intake predominantly by neuromodulatory actions.

Cyclo(His-Pro) is not the only agent which can reduce feeding by a neuromodulatory effect. For example, systemic injection of dopamine agonists at doses not effective by themselves could potentiate the anorectic effect of centrally administered neurotensin (9). This potentiation was interpreted as the result of neurotensin modulating the DA system (9). Also, it has been shown that subcutaneous injections of morphine or naloxone at low doses, in combination with low doses of 5-hydroxytryptophan, induced anorexia (6). Similarly, IP injections of low doses of cholecystokinin in combination with low doses of glucagon and/or bombesin produced anorexia that was greater than the sum of the effects of individual injections (10,14). These examples, together with

the present results, show that neuromodulation can be an important mechanism in reducing food intake.

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