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Effect of Naloxone and Antidepressants on Hyperphagia Produced by Peptide YY

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HAGAN, M. M. AND D. E. MOSS. *Effect of naloxone and antidepressants on hyperphagia produced by peptide YY.* PHARMACOL BIOCHEM BEHAV 45(4) 941-944, 1993.—Central injection of peptide YY (PYY) elicits a powerful feeding response with a short latency in satiated rats. Because of this effect, PYY has been implicated as a neurochemical signal in bulimia nervosa. Serotonin agonists and opioid antagonists induce anorectic effects upon feeding behavior in humans and animals. Therefore, to investigate a possible interaction between PYY-induced eating and these anorexigenic agents rats were given injections of either naloxone (100 μg/3 μl, ICV, and 10 mg/kg, SC), fluoxetine (3-30 μg/3 μl and 5-10 mg/kg, IP), or clomipramine (3-30 μg/3 μl and 5-10 mg/kg, IP) prior to fourth ventricular injections of PYY (15 μg/18 μl). Central and peripheral naloxone and IP but not central injections of fluoxetine blocked PYY-induced intake. Clomipramine had no effect. This suggests that PYY-stimulated feeding may require the action of endogenous opioids and may be inhibited by serotonergic function.

Naloxone Fluoxetine Clomipramine Peptide YY Hyperphagia Bulimia nervosa PYY

PEPTIDE YY (PYY), a 36-amino acid peptide structurally similar to neuropeptide Y (NPY) and pancreatic polypeptide (PP) (26), has been implicated in the neurobiology of bulimia nervosa (12,19). For example, increased concentrations of cerebrospinal fluid (CSF) PYY have been reported in bulimics (12). In addition to the possible role of PYY in bulimia nervosa, PYY also has a potent hyperphagic effect and causes a reduced latency to eat when the peptide is injected into ventricles (3,4,19) and hypothalamic sites (23) in rats.

In contrast to PYY, 5-hydroxytryptamine (5-HT) agonists and uptake inhibitors and opioid blockers are well established as anorectic agents (14,15,29,30) and have been tested in the treatment of bulimia nervosa (2,5,11,16,17). However, little is known about the effect of such agents upon PYY-induced intake. Therefore, in this experiment the effects of fluoxetine and naloxone were tested on PYY-induced eating to determine potential underlying neurotransmitter regulation of this type of eating.

In addition, clomipramine has been tested clinically in obsessive-compulsive disorder, which has been associated with eating disorders (9,10,22). Because of this, clomipramine was also included in these experiments.

METHOD

Subjects and Surgery

Twenty-three female Sprague-Dawley rats (270-330 g) kept under standard lighting conditions (12 L : 12 D cycle, light on 0700 h) with free access to standard rat chow and water were anesthetized with sodium pentobarbital (65 mg/kg). Single cannulae were aimed at the fourth ventricle (21), −11.8 mm posterior to bregma on the midline. Depth was 7 mm from the skull surface and upper incisor bar was 3.3 mm below the interaural line. The fourth ventricle was chosen as the site of administration as PYY injected there produces hyperphagia (4) and CSF flow from that region diffuses the peptide over hindbrain structures, where PYY is predominantly concentrated (18,26,27). Rats were then allowed to recover from surgery for a minimum of 7 days and housed in group cages.

Drugs

Food intake was stimulated by ICV injection of PYY administered in 5-μg/6 μl doses every hour for a total of 15 μg PYY from three injections. Sham injections consisted of ICV physiological saline given in the same volume and procedure. Pretreatments were: naloxone (NAL), 100 μg/3 μl ICV; NAL 10 mg/kg SC; fluoxetine (FLU), 3-30 μg/3 μl ICV; FLU 5-10 mg/kg IP; clomipramine (CLOM), 3-30 μg/3 μl ICV; CLOM 5-10 mg/kg IP; or vehicle (deionized water, VEH). Substances were obtained from the following sources: porcine synthetic peptide YY (Sigma Chemical Co., St. Louis, MO); naloxone HCl (Sigma); fluoxetine HCl (Eli Lilly, Indianapolis, IN); and clomipramine (CIBA-Geigy, Summit, NJ).

Although 100 μg NAL ICV is a high dose, it was the most effective dose for blocking NPY-induced feeding (14). There-

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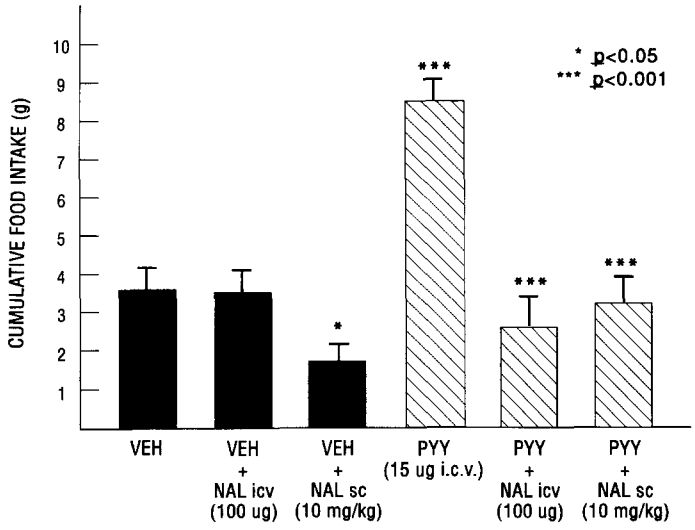

FIG. 1. Effect of 100 μg ICV and 10 mg/kg SC naloxone (NAL) on food intake (mean ± SEM) induced by fourth ventricular injections of 15 μg peptide YY (PYY). VEH, vehicle. $*p < 0.05$, $***p < 0.001$.

fore, that dose was used here to provide a comparison of the effect of NAL on PYY-induced feeding.

Procedures

Except for a 20- to 30-min period between pretreatment and PYY injections, animals had free access to food and water. Eating tests began 3 h after light onset. Immediately after pretreatment injections, animals were placed in individual cages until all animals were injected. Animals were then injected with PYY and placed back into the testing cage, at which time two preweighed Purina rat chow pellets were put in their cage. The amount of food eaten was measured at 1, 2, and 4 h. At the end of the first and second hour, animals were again injected with PYY ICV to a total dose of 15 μg. Animals were tested twice a week with a minimum of 72 h between administrations given in a counterbalanced design.

Preliminary pilot experiments with 15 μg PYY alone showed that this dose produced more eating than 5- and 10-μg injections of PYY. In these experiments, PYY was delivered in three hourly 5-μg/6 μl increments because of limits on drug solubility.

At the end of all experiments, animals were anesthetized with sodium pentobarbital and perfused intracardially with

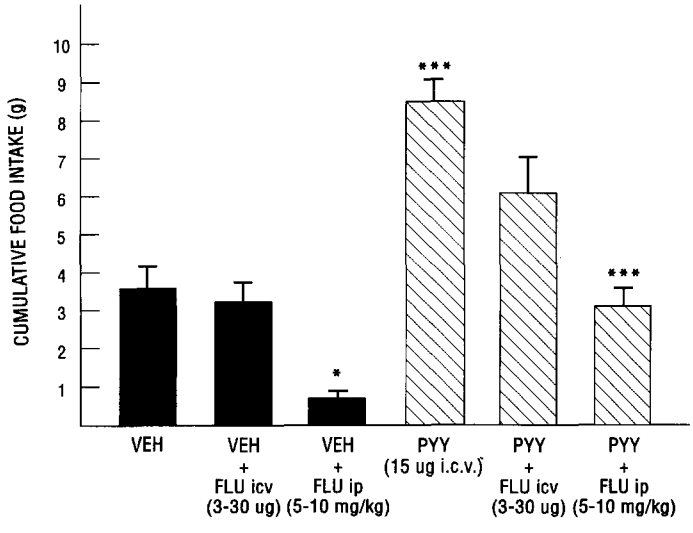

FIG. 2. Effect of 3–30 μg ICV and 5–10 mg/kg IP fluoxetine (FLU) on food intake (mean ± SEM) elicited by fourth ventricular injections of 15 μg peptide YY (PYY). VEH, vehicle. $*p < 0.05$, $***p < 0.001$.

physiological saline, followed with 10% formalin. Injection sites were determined by frozen section histology. The cannulae tracts terminated within or immediately anterior to the intended placement but were clearly within the fourth ventricle. Three animals were discarded because of improper placement. Results are expressed as mean ± SEM for 4-h cumulative food intake in grams. Data were analyzed by analysis of variance (ANOVA) using planned comparisons with probability levels adjusted to Bonferroni criteria.

RESULTS

As shown in Fig. 1, PYY induced food intake, $t(24) = 5.50, p < 0.001$, with animals eating 8.5 ± 0.6 g compared to the control condition mean of 3.6 ± 0.6 g. The incremental delivery procedure produced a steady increase in eating at every hour.

Naloxone alone given intracranially did not decrease spontaneous food intake. However, NAL SC produced a decrease in food consumption, $t(23) = 2.31, p < 0.05$, 1.7 ± 0.4 g. However, when tested for the ability to suppress PYY-induced eating NAL ICV, $t(22) = 5.90, p < 0.001$, 2.6 ± 0.8 g, and NAL SC blocked the hyperphagic effect of PYY, $t(23) = 5.64, p < 0.001$, 3.2 ± 0.7 g.

No differences in food intake were found as a function of the particular narrow range of doses tested for FLU and CLOM so the data within each drug condition were collapsed. In contrast to NAL alone, FLU alone reduced spontaneous food intake (Fig. 2) but only when administered peripherally, $t(15) = 4.19, p < 0.05$, 0.7 ± 0.2 g. FLU IP also suppressed PYY-induced intake, $t(23) = 6.87, p < 0.001$, with a mean intake of 3.1 ± 0.5 g, but FLU ICV had no significant effect on PYY-induced feeding.

Finally, as shown in Fig. 3, CLOM had no effect on food intake and failed to block PYY-induced eating when administered either centrally or peripherally.

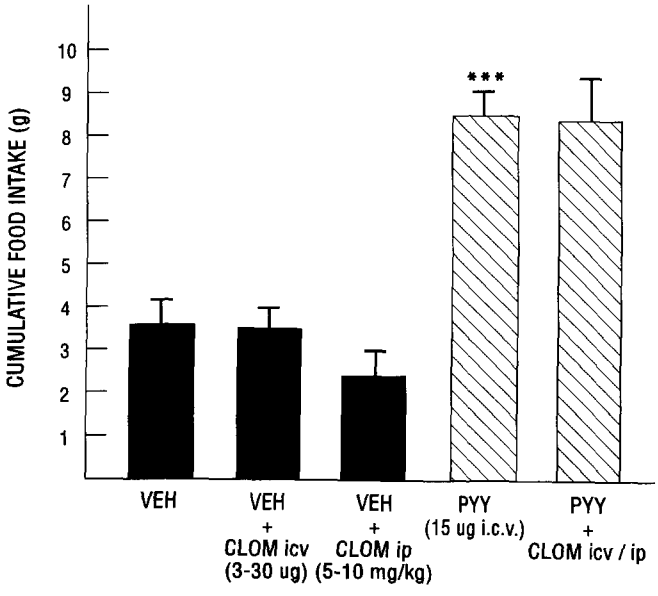

FIG. 3. Effect of 3–30 μg ICV and 5–10 mg/kg IP clomipramine (CLOM) on food intake (mean ± SEM) elicited by fourth ventricular injections of 15 μg peptide YY (PYY); routes of administration of CLOM were collapsed due to no difference in mean intakes. VEH, vehicle.

DISCUSSION

Our results confirm that PYY in the fourth ventricle produces a robust increase in food intake (4). The mechanism by which PYY causes the strong hyperphagic behavior remains unknown. In light of our data, the anorectic effects of naloxone suggest that endogenous opioids play a role in the exaggerated eating effect of PYY. Similar effects on NPY-induced feeding previously observed after these particular doses and routes of administering (14) suggest opioids may contribute to the control of eating induced by both peptides.

It is impossible to reasonably equate or compare doses of central and peripheral injections of NAL as they were used in these experiments. As cited above, however, 100 μg ICV is a dose that was effective in blocking NPY eating out of a dose range of 10–200 μg (14). The peripheral dose of 10 mg/kg NAL is a high dose and the one that produces maximum suppression of PYY-induced eating. It was, however, the highest dose tested. NAL at 10 mg/kg SC may not relate directly to the central administration and a comparison of this type is not intended. Additional data have shown, however, that NAL at as low as 1.0 mg/kg SC will significantly reduce PYY-induced eating and 0.1 mg/kg NAL SC does not (unpublished data). A similar food-suppressing effect from lower doses of NAL (1.0 and 5.0 mg/kg, SC) on NPY-stimulated feeding has been reported by Levine et al. (14).

There are other problems in comparing central vs. peripheral administrations. Peripheral injections cause NAL to act at any neuroanatomic location with NAL receptors, peripheral or central. Central injections allow better interpretation of the effects of NAL in the fourth ventricle or nearby areas. The central areas affected may or may not be similar to those affected by peripheral administrations.

Fluoxetine, a selective inhibitor of serotonin neuronal reuptake (25), disrupted the overeating effect of PYY when administered peripherally. This suggests an inhibitory role for serotonergic mechanisms in PYY-induced eating. In the present experiment, fluoxetine injected into the fourth ventricle failed to significantly reduce PYY-induced feeding. This suggests that the serotonergic neurons involved in the inhibition of this kind of eating are likely to synapse in more anterior regions rather than those in proximity to the fourth ventricle. Weiss et al. (28) recently showed that fluoxetine induces a circadian-related, nutrient-suppressive effect on carbohydrates with no effect on the consumption of protein and fat, and this effect has been localized to the paraventricular nucleus (PVN) within the medial hypothalamus.

In vitro studies with NPY have found it to be colocalized with serotonin in dorsal raphe neurons, which innervate hypothalamic nuclei, and it has been shown to reduce serotonergic metabolism (13,20), possibly explaining the way in which NPY potentiates carbohydrate ingestion. Although similar studies with PYY have not been conducted, PYY may be exerting its effects on serotonin through similar mechanisms.

In our tests with CLOM, no suppression of PYY-induced eating was observed. Although CLOM selectively inhibits 5-HT uptake in vitro, it loses that selectivity in vivo because of its metabolism to chlordesipramine, which preferentially inhibits norepinephrine uptake (1,6). Our preliminary interpretation of the absence of a CLOM effect is that norepinephrine is not involved in the regulation of PYY-induced eating. Of course, specific noradrenergic agents must be tested on PYY-induced eating to probe the existence of a functional PYY-NE interaction, if one exists.

Similar to our findings with CLOM on PYY-induced eating, Stanley et al. (24) reported no effect of a α-adrenergic

involvement in NPY-induced eating. Of course, due to extensive evidence implicating colocalization and cofunction of NE and NPY (7,8), more definitive brain mapping studies with PYY may be needed.

In summary, our results show that PYY-induced eating requires intact opioid functioning and it may be inhibited by serotonergic mechanisms. The development and testing of increasingly selective serotonin uptake inhibitors and opioid antagonists of specific affinity for subreceptor types will help elucidate the phenomenon of PYY-induced eating. If PYY mechanisms are involved in bulimia, such agents alone or in combination may prove efficacious in treating aspects of this disorder.

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