Peptides that Regulate Food Intake

Norepinephrine is not required for reduction of feeding induced by cholecystokinin

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Cannon, C. Matson, and R. D. Palmiter. Norepinephrine is not required for reduction of feeding induced by cholecystokinin. Am J Physiol Regul Integr Comp Physiol 284: R1384–R1388, 2003; 10.1152/ajpregu.00689.2002.—CCK octapeptide (CCK-8) is released by the gut in response to a meal and acts via CCK_{A} receptors on vagal afferents to induce satiety. However, the central neural pathways by which peripheral CCK-8 affects feeding are poorly understood. In the present study, we tested the hypothesis that norepinephrine (NE) is necessary for satiety induced by peripheral CCK-8 by using mice lacking dopamine β-hydroxylase (Dhb^{-/-}) mice are as responsive as their normal littermates to the satiating effects of CCK-8 as their normal littermates.

METHODS

All experiments were conducted in accordance with protocols approved by the University of Washington Animal Care Committee. Mice congenitally deficient in dopamine β-hydroxylase (Dbh^{-/-}) were produced as previously described (52). Heterozygous littermates were used as controls. Mice were housed in Plexiglas cages with cob bedding and a cotton nestlet until a temperature-controlled room with a 12:12-h light/dark cycle. They were maintained on pelleted mouse breeder diet (Lab diet 5015, Test Diet, Richmond, IN) that has a slightly higher fat content (25% of calories from fat) than standard mouse diet. CCK-8 (Sigma, St. Louis, MO) was dissolved in sterile PBS at room temperature and administered by intraperitoneal injection in a volume of 0.01 ml/g body wt. Doses were counterbalanced.

Experiment 1. Six Dbh^{-/-} and eight heterozygous (Dbh^{+/-}) male mice were used. Mice were housed by 10.220.33.4 on August 13, 2017 http://ajpregu.physiology.org/ Downloaded from
individually and because Dbh⁻/⁻ mice are cold sensitive (53), all cages were arranged so that 50% of the cage floor was over a heating pad. A thermometer left in an empty cage with the tip over the heated section registered 26°C. Most mice chose to make their nests on the heated side and, rather than sleeping curled, were often found sprawled belly down on a spot of Plexiglas cleared of shavings. This was especially true of the knockouts.

Mice were each given three doses (4, 8, and 16 μg/kg body wt) of CCK-8, and PBS was given as control. These doses were chosen based on prior experience with CCK in mice, as well as reports within the literature that suggest mice are less sensitive to a given dose of CCK than are rats (30). We attempted to train the mice to drink sucrose at a regular time each day, but the mice showed sporadic interest after 2 wk, so the mice were fasted before being tested with CCK to motivate them to consume. Metabolic rate in mice is sensitive to ambient temperature, and we found that mice kept on the heating pads had very mild hyperphagia after a 24-h fast. However, significant hyperphagia was observed after 48 h without food. The level of hyperphagia produced by this protocol was comparable to that observed in mice of this size after a 24-h fast at ambient room temperature, and the mice were not observed to be abnormally voracious. At the start of the 48-h fast, food was removed in the late afternoon and mice were given a clean cage with fresh bedding. Because mice become significantly hyperphagic on days when fresh chow is added to the cage, food removed from each cage was individually bagged and returned to the same cage at the end of the fast. On testing days, mice were weighed in the late afternoon and then injected with CCK-8 or PBS. Chow was immediately returned to the overhead hopper. Chow was removed and weighed at 0.5, 1, 24, and 48 h after return of the food. Body weight was measured daily.

Data were analyzed for the 0.5- and 1-h intervals separately from the 24- and 48-h intervals. Each set was analyzed by three-way repeated-measures ANOVA using Statistica. The factors used in the three-way analysis were dose, time, and genotype.

**Experiment 2.** In experiment 1, mice responded to all doses of CCK with significant hypophagia, so the present experiment was conducted to include lower doses that may have a less pronounced effect on intake. Thirteen DbH-deficient (Dbh⁻/⁻, mean body weight 23.83 ± 1.48 g) and twelve heterozygous (Dbh⁺/−, 19.34 ± 0.70 g) female mice were used. Mice were housed in groups of four or five in cages containing two or three Dbh⁻/⁻ and two Dbh⁺/−. After a fast of 24 h, mice were removed from the home cage, weighed, and injected. Immediately after injection, each mouse was transferred to a familiar Plexiglas testing cage containing three pellets of weighed chow, cob bedding, and ad libitum access to water. Mice were observed during the testing period for behavioral signs of nausea (elongation of the body, gaping, raising the tail, and lowering the belly to the floor), ataxia, sedation, and anxiety (locomotion within the cage, avoidance of the front of the cage). Mice did not exhibit any of the above signs and spent the majority of time eating, drinking, grooming, or exploring the cage. Chow was removed and weighed at 0.5 and 1 h, when mice were returned to their home cage. Mice were given ad libitum access to chow for 3 or more days between tests. Data were analyzed as described for experiment 1.

**RESULTS**

**Experiment 1.** CCK reduced deprivation-induced cumulative food intake in a dose-dependent manner in
both Dbh<sup>−/−</sup> and Dbh<sup>+/+</sup> littermates within the first hour ($F_{1,12} = 59.07, P < 0.01$; Fig. 1, A and B). There was a trend toward greater reduction of feeding in the Dbh<sup>−/−</sup> mice, although this did not reach significance ($F_{1,12} = 1.29, P = 0.28$). Despite smaller body size (Table 1), Dbh<sup>−/−</sup> mice were equally capable of hyperphagia after a fast. Mice continued to eat throughout the hour, so that there was also a significant effect of time ($F_{1,12} = 69.00, P < 0.01$), although the interaction between time and genotype was not significant ($F_{1,12} = 2.51, P = 0.14$).

At 24 and 48 h after CCK, there was no significant effect of dose ($F_{1,12} = 0.77, P = 0.52$) or time ($F_{1,12} = 0.36, P = 0.56$) on food intake. There was a significant effect of genotype ($F_{1,12} = 10.23, P < 0.01$), with the smaller Dbh<sup>−/−</sup> mice eating less than Dbh<sup>+/+</sup> littermates. It was previously reported that Dbh<sup>−/−</sup> mice have elevated metabolic rate and decreased feed efficiency (53). In other words, under basal conditions, Dbh<sup>−/−</sup> mice eat less than Dbh<sup>+/+</sup> littermates when total intake is compared, but when intake is normalized to body weight, Dbh<sup>−/−</sup> mice eat more per gram body weight than Dbh<sup>+/+</sup> littermates.

There was no significant effect of dose on body weight (data not shown). When body weight values were averaged across the four treatment conditions, Dbh<sup>+/+</sup> and Dbh<sup>−/−</sup> mice lost 10% of the prefast body weight after 48 h and regained weight similarly (Table 1).

**Experiment 2.** CCK reduced food intake in a dose-dependent manner in both Dbh<sup>−/−</sup> and Dbh<sup>+/+</sup> littermates within the first hour ($F_{1,23} = 407.41, P < 0.01$) (Fig. 2, A and B). There was a significant effect of genotype on food intake ($F_{1,23} = 21.43, P < 0.01$); Dbh<sup>−/−</sup> mice had greater hyperphagia in response to a fast. As in the previous experiment, there was no significant interaction between genotype and dose of CCK ($F_{1,23} = 1.01, P = 0.41$); CCK attenuated intake similarly in both Dbh<sup>−/−</sup> and Dbh<sup>+/+</sup> mice.

**DISCUSSION**

The present data contradict the hypothesis that NE is necessary for the satiating effects of CCK. Central NE was first implicated in the initiation of feeding by Grossman (15, 16), who determined that microgram quantities of NE, introduced into the hypothalamus, rapidly elicited feeding in satiated rats. Grossman's observations have been supported by subsequent findings (6, 7, 21, 23). However, the entire NE content of the rat brain, 6 nmol (39), is much less than the smallest dose effective at eliciting feeding. Of this total brain NE, the estimated size of the “functional” compartment of readily releasable NE is only 20–35% (13, 14, 50). In addition, the smallest doses necessary to initiate feeding in satiated animals have been reported to elicit signs of discomfort, such as vocalization and ataxia (44).

Further studies refined the putative role of NE based on adrenergic receptor subtype ($\alpha$ vs. $\beta$) and location (medial vs. lateral hypothalamus) (21–23). Although high doses of agonists were used, these studies also demonstrated the effect of adrenergic antagonists on deprivation-induced feeding. An $\alpha$-adrenergic antagonist, phentolamine, reduced deprivation-induced feeding when injected into the VMH. On the other hand, a $\beta$-adrenergic antagonist, propranolol, actually enhanced intake in deprived rats when injected into the medial or lateral hypothalamus. Leibowitz (21) proposed that endogenous NE reduces feeding when it acts at $\beta$-adrenergic receptors within the lateral hypothalamus (LH), but enhances feeding at $\alpha$-adrenergic receptors within the ventromedial hypothalamus (VMH). In addition, endogenous NE release in the preoptic and anterior hypothalamus may play an im-

![Fig. 2. Low doses of CCK-8 reduced the deprivation-induced intake of female Dbh<sup>−/−</sup> mice. Intake of mice (cumulative grams ± SE) was measured 30 min (A) and 1 h (B) after a 24-h fast. CCK-8 reduced food intake in a dose-dependent manner in both Dbh<sup>−/−</sup> mice and Dbh<sup>+/+</sup> controls within the first hour ($F_{1,23} = 407.41, P < 0.01$). Dbh<sup>−/−</sup> littermates were used as controls; they have normal levels of NE and Epi.](http://ajpregu.physiology.org/)
portant role in sustaining a meal, once initiated. Depriva-
tion-induced feeding and lever pressing for food is accom-
panied by an elevated release of endogenous NE in
this area (26, 32, 34), and doses of NE closer to the
physiological range (1.48, 0.74, or 0.37 nmol) in these
areas potentiated spontaneously initiated feeding by
>200% (44, 46).

Feeding induced by intrahypothalamic NE (2.5 μg) is
attenuated by systemic CCK-8 (37). An increased re-
lease of NE has been documented in the whole hypo-
thalamus during either feeding or treatment with per-
ipheral, satiating doses of CCK (17, 18). Because the
effect of NE on feeding is site specific, these results are
difficult to interpret. Because CCK-8 inhibits feeding,
if NE plays a role in CCK-8-induced satiety, one might
expect that CCK would cause increased NE release in
the LH, where NE acts to reduce intake. One might
also expect diminished release in the VMH and/or
anterior hypothalamus, where NE can stimulate feed-
ing or increase meal size and duration, respectively.
However, in microdialysis experiments, Myers and
McCaleb (36, 37) found just the opposite: peripheral
CCK-8 in sated rats increased NE release in the VMH
and preoptic area and diminished release in the LH.
The authors concluded that “endogenous CCK inter-
acts functionally with a pathway of noradrenergic neu-
rons responsible for initiating satiety, or with those
neurons that activate a feeding response.” However,
these results contradict the noradrenergic hypothesis
of CCK-induced satiety as detailed above.

The chronic loss of NE did not have an appreciable
effect on responding to CCK. However, the present
data cannot rule out the possibility that NE and/or
epinephrine may play a marginal role in the response
to CCK. In addition, it is possible that compensation
for the chronic lack of NE has occurred. For example,
transient interference with NE signaling could affect
CCK action but with chronic interference, compensa-
tory changes could result in normal responses. Lack of
NE during fetal development is lethal (52), and
mothers must be treated with the synthetic precursor
L-threo-3,4-dihydroxyphenylserine (DOPS) for Ddh−/−
mice to be born. DOPS is converted by the enzyme
aromatic acid decarboxylase to NE directly, thus by-
passing DBH. In the present study, Ddbh−/− mice were
never given DOPS after birth. It is possible that the
Ddbh−/− mice developed, in the absence of postnatal
NE, some compensatory processes not present in nor-
mal mice. In addition, because DA is the precursor of
NE, Ddbh−/− mice release DA rather than NE from their
noradrenergic terminals. This “ectopic dopamine” may
contribute to the unusual phenotype of these mice,
although it is not clear at this time that it does. Most
phenotypic characteristics of Ddbh−/− mice can be res-
cued by treatment with DOPS that restores NE levels
to some degree while presumably leaving ectopic dopa-
mine intact (52, 53). In this instance, there is no phe-
notype to reverse and it is difficult to assess whether
compensation for chronic NE deficiency has occurred.
However, barring the possibility of compensation for
chronic loss of NE, we conclude on the basis of the
present results and the literature cited here (17, 18, 31,
32, 37), that NE is not important for CCK-induced
satiety.

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