Capsaicin-sensitive fibers are required for the anorexic action of systemic but not central bombesin

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Capsaicin-sensitive fibers are required for the anorexic action of systemic but not central bombesin. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1617–R1622, 1999.—Bombesin (BN) suppresses food intake in rats whether given centrally or systemically. Although the brain BN-sensitive receptors are known to be essential for the anorexic effect of systemic BN, the mode of communication between the gut and the brain remains unclear. This study assessed whether the anorexic effect of systemic BN is mediated humorally or via neural circuits. Afferent neurons were lesioned using capsaicin (50 mg/kg sc) on postnatal day 2, and responses to BN were assessed during adulthood. Capsaicin treatment decreased body weight gain significantly from postnatal age 4–7 wk. Peripheral BN (4–16 µg/kg ip) dose dependently suppressed food intake in control animals. However, this effect was completely blocked in capsaicin-treated rats. In contrast to systemic effects, feeding-suppressant effects of centrally administered BN (0.01–0.5 µg icv) were not affected by capsaicin treatment. This research suggests that peripheral BN communicates with the brain via a neuronal system(s) whose afferent arm is constituted of capsaicin-sensitive C and/or Aδ-fibers, whereas the efferent arm of this satiety- and/or anorexia-mediating circuitry is capsaicin resistant.

Satiety or meal termination occurs well before food intake by activating spinal, nonvagal neuronal circuits...
however, the characteristics and/or the identity of the neuronal pathways that convey these effects of systemically administered BN remain unknown.

Capsaicin, a neurotoxin commonly found in chili peppers, has the capacity to excite and subsequently desensitize a subset of primary sensory neurons, including both vagal and spinal fibers (22). This pharmacological deafferentation technique has the advantage over surgical vagotomy in that the efferent pathways are spared, thus circumventing the debilitating and confounding effects of efferent neural ablation. The capsaicin-lesioned fibers are thought to be peptidergic neurons of small to medium diameter and give rise to unmyelinated (C fiber) or thin-myelinated (Aδ-fiber) axons (22, 43). Thus the objective of the current study was to assess whether neonatal capsaicin exposure alters the adult feeding response to either systemically or centrally administered BN. The results revealed that neonatal capsaicin treatment completely blocked the feeding-suppressant effects of systemically but not centrally administered BN.

MATERIALS AND METHODS

Animals and Apparatus

Sprague-Dawley rats obtained from Charles River Laboratories (Rochefort, Quebec, Canada) were bred in our animal care facilities. Rat pups from several dams were pooled and culled on day 2 of life (group 1: n = 40; 20 males and 20 females; group 2: n = 16; all males) and randomly assigned to a treatment group, either saline (control) or capsaicin. They were then randomly reassigned to various dams according to their group assignment (control or capsaicin group). All pups were weaned on day 21 and subsequently group housed (3 or 4/group). Purina rat chow and tap water were available ad libitum unless indicated otherwise.

Capsaicin Treatment

On postnatal day 2, pups in the capsaicin condition (groups 1 and 2) received a single capsaicin injection (50 mg/kg sc), whereas the controls received an equal volume of (10:10:80 vol/vol/vol Tween 80:ethanol:saline). Weight gain was monitored for 6 wk after capsaicin treatment in group 1.

Experiment 1: Impact of Capsaicin Pretreatment on Body Weight Gain and Anorexia Induced by Systemic BN

Experiment 1A: Impact of capsicain treatment on body weight gain. Neonatal rats (group 1) were injected with a single dose of capsaicin (n = 20; 10 males and 10 females) or vehicle (n = 20, 10 males and 10 females). All pups were weighed twice a week until cried.

Experiment 1B: Impact of capsicain treatment on anorexia induced by systemic BN. Adult male rats (from group 2) neonatally pretreated with capsaicin or vehicle (n = 6–8/group) were systemically injected with BN (0, 4, 8, and 16 μg/kg ip), and their food intake was monitored 46–50 days of age. BN doses were administered (20–30 min before testing) in a randomized order, according to the Latin Square design. Rats were tested during the light phase (between 0900 and 1200) after having been food deprived for 18 h before testing. Food intake (Kellogg’s Froot Loops; to which they were habituated) was monitored at 30-min intervals for 3 h using electronic balances interfaced to a computerized monitoring system (31).

Experiment 2: Response to Central or Systemic BN Injection After Capsaicin Exposure

Experiment 2A: Confirmation of the loss of effects of systemically administered BN in capsaicain-exposed rats. The procedure of experiment 1 was repeated in the second, separate group of male rats (control = 11 and capsaicin pretreated = 12).

Experiment 2B: Impact of capsicain treatment on anorexic effects of centrally injected BN. Adult male rats (42–47 days of age; controls, n = 6; capsaicin treated, n = 6) were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed in a stereotaxic instrument with the skull leveled. Stainless steel guide cannulas (22 gauge) aimed at the third ventricle were implanted (on the midline, 4.5 mm posterior to bregma and 4.3 mm ventral to the skull surface), according to coordinates from Paxinos and Watson (39). The cannulas were anchored by three skull screws and acrylic dental cement. The cannulas were plugged by a removable stainless steel wire stylet.

INTRACEREBROVENTRICULAR BN INJECTIONS. Rats were entrained to a regimen of daily 4-h food (Purina Rat Chow) access for 2 wk before the experiment. Injection of BN into the third ventricle (0.1, 0.25, and 0.5 μg/iv) was given after a 7-day post surgical recovery period. The peptide doses, given in a randomized order according to the Latin Square design, were infused at a rate of 3 μl/min, and testing commenced immediately thereafter. Food intake was monitored electronically as described earlier.

Data Analysis

Repeated-measures mixed ANOVA was performed on the body weight data, with treatment effects (capsaicin vs. vehicle treated) repeated over age. The data from males and females were analyzed separately. The feeding suppressant effects of systemically administered BN were also subjected to ANOVA analysis, examining the effect of treatment (capsaicin vs. vehicle treated) repeated over time and BN dose. Post hoc comparisons used Tukey’s correction.

RESULTS

Experiment 1A: Impact of Capsaicin Pretreatment on Body Weight Gain

The ANOVA performed on the weight measurements revealed a significant treatment (capsaicin vs. vehicle treated) effect [F(1,242) = 81.629, P < 0.0001], as well as a significant interaction between the effects of treatment × age [F(11,220) = 4.97, P < 0.0001; F(11,220) = 2.78, P < 0.0001, for males and females, respectively]. The post hoc analysis revealed that the body weights of capsaicin-treated subjects were significantly lower than those of the controls between days 28 and 35 for the females and between days 28 and 42 for the males (see Fig. 1).

Experiment 1B: Impact of Capsaicin Pretreatment on Anorexic Effects of Systemic BN

The food intake data, which were analyzed by a treatment (capsaicin vs. vehicle) × BN dose (4) × time (4) mixed ANOVA, revealed a significant BN dose × capsaicin treatment interaction (F = 3.16, P < 0.0006). Analysis of the simple main effects and the subsequent post hoc analyses revealed that in control rats, sys-
Experiment 2A: Confirmation of the Loss of Response to Systemically Administered BN in Capsaicin-Exposed Rats

During the initial phase of this experiment, we reassessed the efficacy of capsaicin pretreatment in blocking the effects of systemically administered BN. In agreement with results from experiment 1, BN dose dependently suppressed food intake in the control animals but not in the capsaicin-pretreated rats (data not shown).

Experiment 2B: Impact of Capsaicin Treatment on Anorexic Effects of Centrally Injected BN

The food intake data were analyzed by a treatment (capsaicin vs. vehicle) $\times$ BN dose (4) $\times$ time (4) mixed ANOVA. Analyses of the simple main effects revealed no significant effect due to treatment [$F(1,360) = 0.084$, $P = 0.775$] but a significant effect of time [$F(3,55) = 239.49$, $P < 0.0001$] and dose [$F(3,66) = 34.12$, $P < 0.0001$] (see Fig. 2B). The effect of time appeared to be attributable to the fact that there was a time-related increase in the cumulative food intake. The main BN
dose effect appeared to be attributable to the fact that there was greater suppression of food intake with the higher doses of BN. There was no overall interaction effect $[F(23,198) = 1.193, P = 0.302]$. Post hoc analyses revealed that the feeding-suppressant effects of central BN were similar for both groups. The feeding suppressant effects were most pronounced during the initial 30-min period and were selected for illustration in Fig. 2B. At the lowest dose tested (0.1 µg icv) BN failed to significantly suppress food intake in either group. At the 0.25-µg dose BN significantly suppressed food intake in both groups ($P < 0.05$). At the highest dose tested (0.5 µg icv) BN-elicited suppression was equally pronounced in both groups (see Fig. 2B) and persisted for the 3-h test period ($P < 0.01$; data not shown).

**DISCUSSION**

Peptides of the bombesin family are thought to be one of the physiological mediators of satiety (meal termination). When administered systemically, BN (and related peptides) dose dependently suppresses food intake in a variety of species (14, 16, 24, 26). The present results clearly demonstrate that the anorectic effect of systemic BN is completely abolished in adult rats that were exposed to a single dose of capsaicin on postnatal day 2. This suggests that systemic BN requires intact capsaicin-sensitive fibers to communicate with the brain and thus argues against the notion that systemically administered BN directly affects brain functioning in an endocrine manner. From previous research, it appears that, although capsaicin treatment lesions the vagal afferent projections, this conduit of communication may not be essential for the elicitation of the BN effects. Indeed, surgical subdiaphragmatic vagotomy does not block the feeding-suppressant effects of systemically administered BN. The suggestion that systemic BN may deploy an alternate neural pathway (such as spinal afferents) for mediation of its effects is supported by the present findings and by those of Stuckey et al. (42), demonstrating that a more complete surgical neural disconnection of the gut from the brain abrogates the feeding-suppressant effects of systemically administered BN.

The present results from neonatally capsaicin-lesioned rats are not fully concordant with some earlier studies where capsaicin was administered in adulthood (28, 41). Whereas capsaicin treatment failed to affect BN-induced anorexia in one study (41), it attenuated the effects of systemically administered BN in another (28). Some of the discrepancies between these studies may be attributable to the differences in testing conditions (such as extent of food deprivation at testing, doses of BN used, etc.). However, we believe that the major contributing factor must have been the age at which the capsaicin lesion was inflicted (day 2 in our study vs. adulthood in others). Indeed, the unmyelinated primary afferent neurons are particularly sensitive to capsaicin during the initial 12 days of life but relatively resistant to such degeneration beyond the age of 14 days (21). Furthermore, it has been established that capsaicin exposure in adulthood, particularly if injected in a single dose, fails to permanently destroy all of the C fibers (20). This contrasts with the actions of capsaicin administered early in life, where the neurotoxic effects are more extensive and permanent (11, 20). Thus the subset of fibers that escape (or recover) from damage induced by adult capsaicin exposure must be important in the transmission of BN-mediated afferent signals to the brain.

Although we have built the case for capsaicin-sensitive neural pathway(s) of systemic origin in the transmission of BN effects, it could also be argued that capsaicin may damage and/or downregulate BN-sensitive circuits within the brain. This point is particularly relevant with the use of neonatal capsaicin, inasmuch as the blood-brain barrier is weaker in neonates, thus increasing the likelihood of capsaicin-elicited damage to the brain. Indeed, there have been reports of capsaicin-induced neural damage within the brains of neonatal rats exposed to capsaicin (40). There is also considerable evidence pointing to the importance of brain BN/GRP receptors in the mediation of the effects of systemically administered (32, 35) as well as endogenously released BN-like peptides (34). Thus the loss of the feeding-suppressant effects of systemic BN in neonatally capsaicin-treated rats may not be due to the loss of peripheral afferent fibers, but to the loss of central BN-sensitive neurons. If capsaicin exposure did not affect BN transmission of BN-sensitive peripheral neurons, then systemic BN may be expected to lose its potency and/or efficacy irrespective of whether the effects were mediated neurally or by direct penetration into the brain. Accordingly, we assessed the effects of BN injected directly into the brain (third ventricle) of animals neonatally exposed to capsaicin. In sharp contrast to the loss of response to peripherally administered BN, centrally administered BN maintained full potency and efficacy, despite capsaicin exposure. This suggests that capsaicin exposure did not adversely affect the brain system(s) using BN-like peptides and further supports our contention that peripheral BN must communicate with the brain through capsaicin-sensitive neural pathways. The peripheral site(s) where BN actions are initiated remain to be identified. In this regard, Kirkham et al. (25) suggested that the satiety-like effect of peripheral BN may be mediated by receptors perfused by the celiac artery because the levels of BN required to suppress food intake are much lower when infused here than when administered either intraperitoneally or by superior mesenteric infusion. Thus it remains possible that the afferent arm of the BN-responsive and capsaicin-sensitive circuit may originate from the vicinity of sites perfused by the celiac artery.

A palatable diet was selected in experiment 1, along with overnight deprivation, to ensure a strong feeding response and to maximize any differences due to the feeding-suppressive effects of BN. We previously showed that BN is effective at attenuating intake of both chow and snack food (31). On the other hand, because we used a highly palatable food, it could be argued that the capsaicin-treated rats failed to suppress intake after...
central BN administration due to an independent alteration of hedonic responsiveness to the Froot Loops rather than an interruption of BN activity. Altered responsiveness to palatable foods by capsaicin-treated rats was observed previously (7). However, in our hands, the saline-treated capsaicin-exposed rats do not demonstrate an increase in intake of palatable food, suggesting that the reward system was unaltered.

Although the major objective of the present investigation was to assess the role of capsaicin-sensitive neurons in the mediation of the anorexic or satiety effects of BN, we were also interested in evaluating the ontogenic behavioral and growth changes induced by neonatal capsaicin exposure. Thus animals were monitored for both weight and various behaviors on a weekly basis, starting from the first week of life. Consistent with earlier studies (6, 45), we noted that the capsaicin-exposed rats showed a small but significant reduction in body weight starting ~4 wk after capsaicin administration. As we did not monitor daily food intake, it is unclear whether the weight gain differences were due to altered energy intake or expenditure. However, some insights can be gleaned from activity levels, which were recorded in the present study. Sniffing (a form of exploratory activity) decreased transiently at weeks 4 and 5 (data not shown), which may be related to the decreased olfactory sensitivity (8). In addition, capsaicin treatment failed to significantly alter locomotor activity (assessed over a 24-h period) but transiently increased grooming and scratching behavior (peaking at week 5; data not shown). The latter observation is concordant with an earlier report (44). Thus the time course of the behavioral and weight gain changes do not support the idea that capsaicin-elicted weight gain changes are due to altered behavioral activity.

In summary, the present findings suggest that peripherally injected BN employs capsaicin-sensitive afferent neurons to exert its feeding-suppressant effect on the CNS. This study shows, for the first time, that in contrast to the loss of systemic effects of BN, capsaicin exposure left the central mechanisms mediating the feeding-suppressant effects of BN fully functional. Thus, whereas the afferent arm of one of the satiety circuits that is activated by BN-like peptide(s) is capsaicin sensitive, the efferent arm of that system is completely resistant to actions of this neurotoxin. Capsaicin exposure also caused a reduction in the rate of body weight gain, which appeared to be unrelated to the activity profile of the animals. Future challenges include a more precise identification of specific subpopulation of the capsaicin-sensitive afferent neurons activated by systemically injected and/or endogenously released BN-like peptides involved in meal termination.

Perspectives

One approach toward identifying the relevant neural pathways damaged by neonatal capsaicin treatment, would be to focus on the substance P (SP)-containing fibers/neurons. This suggestion is predicated on previous reports. For instance, although neonatal capsaicin exposure temporarily disrupts many neurotransmitter systems (including amino acid, biogenic amine, and peptidergic systems), the effects on SP-containing neurons of the dorsal horn of the spinal cord are not only most pronounced but seem nonreversible (9). The possibility that some of these SP neurons may be involved in the mediation of BN effects is also supported by the observation that spantide, an SP receptor antagonist, is able to block some of the actions of BN (32, 48). Previous studies reported that SP dose dependently decreased both operant responding for food, as well as free feeding in rats (19), and increased latency to consume food in food-deprived rats (5). Finally, others have suggested that SP may mediate the neonatal capsaicin-induced abrogation of CCK satiety (29). It may therefore be beneficial to explore the relationship between SP and BN-related peptides to further characterize the pathways of the gut-brain axis that mediate the satiety and/or anorexic effects.

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