Behavioral analysis of anorexia produced by hindbrain injections of AMPA receptor antagonist NBQX in rats

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Zheng, Huiyuan, Christiane Patterson, and Hans-Rudolf Berthoud. Behavioral analysis of anorexia produced by hindbrain injections of AMPA receptor antagonist NBQX in rats. J Physiol (2002) 540.1, 99–108. The caudal brain stem integrates short-term feedback signals from the oral cavity and the food-handling abdominal viscera, as well as long-term homeostatic, cognitive, and emotional signals from the forebrain, to control ingestive behavior. Glutamate, acting on various receptor subtypes, plays a prominent role in this integrative process. Fourth ventricular injection of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor blocker 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzof[1]quinoxaline-7-sulfonamide (NBQX, 0.5–5 nmol/3 μl) dose dependently suppressed intake of 15% sucrose in food-deprived and non-food-deprived rats compared with saline injection. Two consecutive paired NBQX injections (5 nmol) into the fourth ventricle did not produce conditioned taste aversion to saccharin, but LiCl did. Intraburst lick rate and lick efficiency were not affected, but burst size and number and initial lick rate were significantly decreased by NBQX. Local injection of NBQX (2 nmol) into and near the nucleus tractus solitarius also suppressed sucrose intake. These results suggest a general role for non-N-methyl-D-aspartate receptors in the transmission of positive (feedforward) signals, but do not identify the exact processing step involved, such as taste input, sensory-motor processing, or descending facilitation. More localized injections and response measures will be necessary.

ingestive behavior; nucleus tractus solitarius; glutamate; brain stem; satiation

SATIATION IS A KEY PROCESS in the overall neural control of food ingestion, in that it determines the size of a meal (43). The caudal brain stem is one of the primary recipients of important sensory information from the gastrointestinal tract relating to satiation. Vagal afferent fibers sensitive to distension of the stomach (40), nutrients (31, 41) and osmolality (30) in the small intestine, and metabolites and hormones circulating through the portal-hepatic axis (for review see Ref. 24) terminate in the dorsal vagal complex of the caudal medulla (33). This information is relayed to second- and higher-order neurons in the neunoax (11, 47). The exact neural correlate that represents or determines the behavioral outcome of satiety or end of meal is not known. Studies in decerebrate rats have shown that the basic mechanism of stopping ingestion is contained in the caudal brain stem (18), but it is well recognized that descending connections are important in the modulation of these brain stem mechanisms by diencephalic homeostatic and forebrain motivational-cognitive factors (43). It is also likely that gustatory input entering through the rostral solitary nucleus (19) and input from somatic and visceral dorsal root afferents via the spinosolitary tract (32) are integrated with vagal sensory information within the medulla. Finally, it becomes increasingly clear that humoral information is added to the mix via the area postrema (28) and its tight connections with the nucleus tractus solitarius (NTS).

As is expected for a negative-feedback loop, interruption of the vagal afferent signaling pathway at several levels results in delayed satiety and increased meal size or short-term food intake. Peripheral administration of the cholecystokinin-A receptor antagonist devazepide increases meal size (14), supposedly by blocking activation of vagal afferent fibers with terminals in the small intestine and perhaps the stomach (7). Surgical (42, 45) and chemical (12, 16) ablation of vagal afferents results in at least temporary increases in short-term food intake and meal size. Administration of the N-methyl-D-aspartate (NMDA) receptor blocker MK-801 to the fourth ventricle (10) or directly to the NTS (44) also delays satiation. Finally, surgical ablation of the area postrema and parts of the NTS results in increased intake of palatable foods (36).

The neurotransmitter released from central terminals of primary vagal afferents in the NTS is thought to be glutamate. At least a portion of vagal afferent neurons has been shown to contain glutamate immunoreactivity (38). In a microdialysis experiment, vagal afferent activation induced by cervical vagal stimulation led to an increase in interstitial glutamate concentration in the NTS (2). Released glutamate is thought to activate second-order neurons via NMDA and non-NMDA receptors. Substantial evidence for this excitatory transmission comes mainly from the cardiovascular literature (3, 4, 17), but there is one report using gastric distension as the sensory stimulus (29). More
recently, it was also shown that microinjection of the non-NMDA receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7-sulfonamide (NBQX) blocks all taste input to the rostral NTS (25).

We hypothesized that blockade of glutamatergic transmission in the NTS would also interrupt the negative-feedback loop for satiety signals and should lead to increased meal size. The observation by Treece et al. (44) that injections of the NMDA receptor antagonist MK-801 into the NTS delayed satiety and increased short-term sucrose drinking in the rat was consistent with this idea. However, further investigation of this effect demonstrated that it may involve vagal motor, rather than sensory, mechanisms, since prevention of the accelerated gastric emptying also induced by the drug blocked the increase in short-term sucrose intake (15). Thus involvement of the NMDA receptor in satiety signaling at the level of the NTS remains unclear.

In the present study, we tested the possibility that the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor might be involved in the negative-feedback loop carrying vagal satiety signals. The expectation was that local application of AMPA receptor antagonist would result in delayed satiety and increased intake. To this end, we injected the AMPA receptor antagonist NBQX into the fourth ventricle of rats trained to drink sucrose solution after 16 h of food deprivation or at dark onset without food deprivation.

Since NBQX injection decreased, rather than increased, short-term sucrose drinking and choke intake, we carried out additional experiments to determine whether the drug induces conditioned taste aversion or changes in the microstructure of licking behavior that could be indicative of more general disruption of sensory-motor processing.

MATERIALS AND METHODS

Animals

Eighty-four adult male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 280–320 g at the time of surgery were housed individually in hanging wire mesh cages in a climate-controlled room (22 ± 2°C) on a 12:12-h light-dark cycle with lights on at 0400. Food and water were available ad libitum except before tests as stated below.

Intracranial Cannula Implantation and Adaptation to Sucrose Drinking

Animals were anesthetized with ketamine, acepromazine, and xylazine (80, 1.6, and 5 mg/kg sc, respectively) and given atropine (1 mg/kg ip). For fourth ventricular injections, a 24-gauge guide cannula (Plastics One) was aimed at the fourth ventricle (2.5 mm anterior to the posterior occipital suture, on the midline, 5.0 mm below the dura), and a 30-gauge beveled injector was designed to protrude for 1.0 mm from the guide cannula. These coordinates and injector system result in fourth ventricular injections as verified by ink accumulations in the fourth ventricle, central canal, and cerebral aqueduct after terminal ink injections as determined in pilot experiments.

For direct NTS injections, a 22-gauge guide cannula was aimed to rest above the NTS at the rostrocaudal level of the area postrema (0.6 mm lateral to the midline, 5.4 mm below the skull, at the posterior occipital suture). The injector consisted of a short piece of fused silica capillary tubing (150 μm OD) glued onto 26-gauge stainless steel tubing and protruding from the guide cannula by 2.0 mm (34).

Rats were given 3–4 h of daytime access to 15% sucrose solution for 2 days before surgery. During the recovery period, a daily 30-min sucrose baseline intake was recorded within the 1st h of dark onset after 1 h of food and water deprivation and 22 h of chow and water intake, and daily body weight was also recorded. Two to 3 days before testing with NBQX began, intraventricular placement and patency of fourth ventricular cannulas were functionally confirmed by at least a doubling of plasma glucose 1 h after the injection of 5-thio-d-glucose (210 μg in 3 μl of sterile saline) (37).

Experimental Procedures

Short-term sucrose intake. In one group of rats bearing fourth ventricular cannulas (food-deprived, n = 23), food was removed at 1800 on the day before testing, and tests were conducted between 0900 and 1200 (15–18 h of food deprivation). Eleven rats were tested with the lower dose of 2 nmol of NBQX or saline and the other 12 rats with the higher dose of 5 nmol of NBQX in a counterbalanced fashion. NBQX (2 nmol of water-soluble NBQX disodium in 3 μl of sterile saline; Sigma/RBI) or saline was injected over a period of 1 min into the fourth ventricle, and after the injector was left in place for 1 min, the rats were returned to their home cage. At 5 min after termination of the injection, sucrose solution was made available, and intake was measured every 5 min to the nearest 0.1 ml for 30 min by reading the fluid level in 50-ml graduated burettes serving as drinking tubes. Spillage of fluid was not registered. Preweighed fresh chow and water were returned after sucrose testing, and the intake was determined after 24 h.

In another (naive) group of rats bearing fourth ventricular cannulas (non-food-deprived, n = 6), food and water were removed 1 h before dark onset at 1600, and 2 or 5 nmol of NBQX (both in 3 μl of sterile saline) or saline was injected into the fourth ventricle, as described above, in a counterbalanced Latin-square design. At the completion of the test, chow and water were returned to the cages, and their intake was measured after 24 h.

In a third group of rats bearing NTS cannulas (non-food-deprived, n = 14), food and water were removed 1 h before dark onset at 1600, and 2 nmol of NBQX (in 0.3 μl of sterile saline) or saline were injected in separate tests in a counterbalanced order. At 5 min after the termination of the injection, sucrose was made available, and intake was measured every 5 min for 20 min. After completion of all tests, 2% pontamine sky blue dye (0.3 μl) was injected, the animals were perfused, and the location of the injector tip was determined on frontal cryostat sections.

Chow intake. Chow intake at 30 and 60 min was measured in a new group of 11 rats. At 1600, after 16 h of food deprivation, 2 nmol (n = 9) or 5 nmol (n = 7) of NBQX (both in 3 μl of sterile saline) or saline (n = 9) was injected into the fourth ventricle. Chow intake was measured for 30 and 60 min after injection by weighing the food cup and taking spillage into consideration. Water intake was measured by weighing the water bottles before and 30 and 60 min after injection.

Water intake. Water intake was measured in another group of rats (n = 12), some of which served in the chow intake tests. At 1030–1100, after 16 h of water deprivation, 2 nmol (n = 10) or 5 nmol (n = 9) of NBQX (both in 3 μl of
sterile saline) or saline (n = 8) was injected into the fourth ventricle as described above, and water intake was measured at 5-min intervals for 30 min.

**Effectiveness of NBQX to Induce Conditioned Taste Aversion**

A new (naïve) group of rats (n = 20) was used to assess the capacity of fourth ventricular NBQX injections and intraperitoneal LiCl injections to produce conditioned taste aversion. Eight rats without cannulas served as controls with LiCl (n = 4) or saline (n = 4) injected intraperitoneally as the unconditioned stimulus (US), and 12 rats with fourth ventricular cannulas served as experimental animals with NBQX (5 nmol, n = 6) or saline (n = 6) injected into the fourth ventricle as the US. Eighteen-hour water-deprived rats were given access to saccharin solution (0.01%) for 20 min and then received the respective US on two consecutive sessions, 2 days apart. Saccharin intake was measured every 5 min. Three days after the second conditioning test, preference for saccharin over water was determined in a two-bottle choice test over a period of 24 h. Preference was determined by the following formula: [(saccharin intake)/(saccharin intake + water intake)] × 100.

**Effect of NBQX on Lick Microstructure**

A new (naïve) group of rats (n = 9) bearing fourth ventricular cannulas was trained to lick sucrose from a lickometer spout (MS-160 Davis Rig, DiLog Instruments, Gainesville, FL). During 5–6 days, partially food-deprived rats were adapted to the apparatus and trained to lick sucrose through the open shutter, until they exhibited stable licking behavior. Before tests, animals were food deprived for 15 h, and tests were carried out between 0900 and 1200. At 5 min after the end of injections, the shutter was opened and licking behavior was recorded for 5 min. Each of the nine rats was tested after 2 nmol of NBQX, 5 nmol of NBQX, or saline in a counterbalanced order, with 2–3 days between tests.

**Data Analysis and Statistical Evaluation**

The dependent variables for each experiment were subjected to separate repeated-measures ANOVAs using the program PROC MIXED in SAS (version 6.12). The P values of post hoc multiple comparisons were multiplied by an appropriate factor according to Bonferroni’s adjustment.

**RESULTS**

**Effect of Fourth Ventricular NBQX Injection on Sucrose Drinking**

Under food-deprived and non-food-deprived conditions, trained rats approached the drinking spout without delay and drank rapidly for the first 5–10 min. Saline control injections into the fourth ventricle 5 min before access to the drinking spout did not change this stable baseline behavior (Fig. 1). Injection of NBQX dose dependently decreased the amount of sucrose solution ingested. The lowest dose of 0.5 nmol did not change 30-min intake in food-deprived rats. The dose of 2 nmol was a threshold dose for both conditions. Under food deprivation conditions, it produced a significant main effect [F(2, 258) = 7.36, P < 0.001] but no significant effect at any time point [t(258) = 2.00–2.52, adjusted P = 0.07–0.28; separate ANOVA applied to group of rats receiving 2 nmol of NBQX or saline]. At dark onset, the 2-nmol dose significantly suppressed intake at 5 min [t(49) = 3.30, P = 0.032] and 10 min [t(49) = 3.4, P = 0.023]. Although with this dose rats approached the spout without delay and drank at a rate not much lower than controls for the first 5 min,
with the higher dose of NBQX (5 nmol), rats approached the spout with longer latencies and drank very little during the first 5 min. The higher dose of 5 nmol of NBQX significantly suppressed sucrose intake by 80–90% during the first half of the test [15 min, food-deprived: \( t(10) = 7.06, P < 0.01 \); dark onset: \( t(49) = 8.31, P = 0.0018 \)] and by ~50% during the second half of the test [30 min, food-deprived: \( t(10) = 4.37, P = 0.0084 \); dark onset: \( t(49) = 5.46, P = 0.0018 \)] compared with saline.

Although behavior was not systematically and continuously monitored, only slight changes were observed. After 5 nmol of NBQX, animals assumed a normal posture in a quiet corner for most of the time. When they occasionally moved to the spout, no overt changes in motor behavior such as ataxia, sluggish gait, or tremors were observed.

**Effect of Direct NTS Injection of NBQX on Sucrose Drinking**

As shown in Fig. 2, the tip of the injector was located within the NTS (n = 5), near the NTS (typically near the border between the NTS and nucleus gracilis, n = 6), or outside the medulla in the cerebellum (n = 3). An example of a tip location in the NTS and the diffusion of the injected pontamine sky blue dye is shown in Fig. 3.

![Fig. 2. Location of injector cannula tips aimed at the NTS collapsed onto 2 rostrocaudal levels (corresponding to -13.3 and -13.68 mm from bregma) of the dorsal medulla. Identification was based on injector track and darkest dye concentration obtained with terminal pontamine sky blue injection. Locations were classified as follows: within the NTS (hatched circles), near the NTS (C), and outside the medulla (not shown). ap, Area postrema; ce, central canal; cc, central; com, commissural; dm, dorsomedial; im, intermediate; med, medial subnuclei of NTS; ts, solitary tract; gr, nucleus gracilis; X, dorsal motor nucleus of vagus; 4V, 4th ventricle.](image)

The 2-nmol dose, which only marginally reduced sucrose intake when injected into the fourth ventricle, significantly suppressed sucrose intake by >80% when injected directly into the NTS [\( P(1,11) = 17.6, P = 0.0015 \); Fig. 1C]. There was still a significant suppression of intake in six animals where the injector tip was found near the NTS [20-min time point, \( t(44) = 4.68, P < 0.01 \), but not when the tip was in the cerebellum [\( t(44) = 0.39, P = \) not significant (NS); Fig. 1C].

**Effect of Fourth Ventricular NBQX on Chow Intake**

In 16-h food-deprived animals, fourth ventricular injection of 5 nmol of NBQX significantly decreased 30-min chow intake compared with saline control injection [\( t(22) = 3.87, P < 0.01 \), Bonferroni adjusted]. At 60 min, chow intake suppression by 5 nmol of NBQX was significant only if compared with the 2-nmol dose [\( t(22) = 2.89, P < 0.05 \), Bonferroni adjusted], but not with saline (Fig. 4). The lower dose of 2 nmol did not have any significant effect on chow intake at 30 or 60 min. As assessed in a different group of non-food-deprived rats, neither dose of NBQX significantly decreased 24-h chow intake (Fig. 4).

**Effect of NBQX on Water Intake**

Fourth ventricular injection of NBQX significantly and dose dependently suppressed water intake in 16-h water-deprived rats [main effect: \( F(2,13) = 52.3, P < 0.0001 \); Fig. 5]. At 5 min the suppression amounted to ~40% with 2 nmol of NBQX and ~70% with 5 nmol of NBQX.

**Fourth Ventricular NBQX Does Not Induce Conditioned Taste Aversion**

Rats that were injected intraperitoneally with LiCl immediately after drinking saccharin solution rapidly developed conditioned aversion to saccharin, in that they hardly drank any saccharin during the second...

![Fig. 3. Example of cannula tip location in NTS at the level of the rostral end of the area postrema (ap). Diffusion of pontamine sky blue dye over most of the ipsilateral dorsal NTS is shown in blue. Arrow, location of injector tip. ts, Solitary tract; X, dorsal motor nucleus of vagus.](image)
trial (Fig. 6A) and avoided saccharin completely when offered in a two-bottle choice together with plain water 2 days later (Fig. 6B). In contrast, rats that were injected with NBQX into the fourth ventricle immediately after drinking saccharin did not on average show a significant aversion to saccharin during the second conditioning trial \[main effect of treatment: F(1,10) = 0.76, P = NS\] or in the two-bottle preference test \[F(1,10) = 2.05, P = NS\], although saccharin intake and preference were 25% lower (Fig. 6). Analysis of individual data showed that two of six rats did develop a mild conditioned taste aversion to saccharin after fourth ventricular NBQX. These two rats drank almost no saccharin during the second conditioning trial, and their preferences for saccharin in the first two-bottle test were 6 and 11%, respectively, compared with 80% for the four other rats. However, in the second two-bottle test, their saccharin preference (68 and 64%) was no longer different from that of the other four rats (74%).

**Effects of Fourth Ventricular NBQX on Lick Microstructure**

Licks emitted during the first 5 min of sucrose drinking after 16 h of food deprivation were analyzed. The mean latency until the first lick was not significantly affected by either dose of NBQX compared with saline control \[F(2,8) = 1.35, P = NS; Fig. 7A\]. One rat did not approach the spout during the 5 min after 2 nmol of NBQX, resulting in the much higher mean latency for this dose. However, after the higher dose, all rats approached the spout within 20 s, again demonstrating the absence of any gross motor deficit.

Intraburst lick rate was not significantly changed by either dose of NBQX compared with saline [2 nmol: \(t(8) = 1.16, P = NS\); 5 nmol: \(t(8) = 0.96, P = NS\); Fig. 7B]. Also, lick efficiency expressed as volume per lick over the entire 5-min period was not significantly changed by the drug \[F(2,8) = 2.69, P = 0.13; Fig. 7C\].

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**Fig. 4.** Effect of 2 nmol \((n = 9)\) and 5 nmol of NBQX \((n = 7)\) and saline control injection \((n = 9)\) into the 4th ventricle on cumulative chow intake in 16-h food-deprived rats at 30 and 60 min. The effect of the same doses of NBQX and saline on 24-h chow intake was assessed in a different group of non-food-deprived rats. The higher dose of NBQX significantly suppressed chow intake at 30 min but not at 1 and 24 h. Bars that do not share the same letter (a and b) are significantly different \((P < 0.01\), based on Bonferroni-adjusted multiple comparison following ANOVA). **Fig. 5.** NBQX at 2 nmol \((n = 10)\) and 5 nmol \((n = 9)\) injected into the 4th ventricle \((4thV)\) in 16-h water-deprived rats significantly suppressed water intake compared with saline control injections \((n = 8)\). *\(P < 0.01\) based on Bonferroni-adjusted multiple comparison following ANOVA. **Fig. 6.** NBQX injection into the 4th ventricle does not produce conditioned taste aversion. A: during the 2nd trial with water deprivation-induced saccharin consumption immediately followed by 4th ventricular NBQX \((5 \text{nmol}, n = 12)\) or saline \((n = 12)\) injection or intraperitoneal injection of LiCl \((n = 8)\) or saline \((n = 8)\), LiCl, but not NBQX, was able to significantly suppress 20-min saccharin consumption. B: when given the choice between saccharin and water in consecutive 24-h 2-bottle preference tests, only rats with intraperitoneal LiCl, but not rats with 4th ventricular NBQX, injection avoided saccharin. *\(P < 0.01\) vs. intraperitoneal saline control.
DISCUSSION

In direct contrast to our hypothesis, the AMPA receptor blocker NBQX injected into the fourth ventricle or directly into the NTS potently suppressed short-term sucrose, chow, and water intake in a dose-dependent manner. Subsequent investigation of possible mechanisms responsible for this suppression revealed that fourth ventricular NBQX may produce mild conditioned taste aversion in some animals but does not delay the rats’ approach to the drinking spout and the ability to lick rapidly. Cluster size and number were significantly decreased by the higher dose, and the number of licks per minute was significantly reduced by both doses of NBQX. Although these results do not provide evidence for gross motor or lick pattern-generator disturbances responsible for reduced intake after NBQX, it cannot be ruled out that the blocker interferes with brain stem sensory-motor and taste processing and that these effects mask a satiety-delaying, intake-enhancing effect as initially hypothesized.

We became interested in the role of brain stem glutamatergic receptors, because local injection of MK-801, a channel blocker acting as a noncompetitive NMDA receptor antagonist, into the NTS delayed satiation and increased meal size (44), consistent with the idea that it inhibits transmission of satiety signals mediated by vagal afferents. Although an action on sensory transmission cannot be ruled out, it appears that most of the effect of MK-801 on food intake is due to an action on vagal motor neurons that accelerate gastric emptying (15). Because the non-NMDA receptor is responsible for the fast synaptic transmission, we hypothesized that its blockade may lead to a similar delay in satiety and increase in short-term sucrose intake.

Role of Glutamate Receptors in Sensory Signal Processing by the NTS

Whole cell patch-clamp recording from second-order neurons in the intermediate and caudal NTS revealed that stimulation of primary vagal afferents leads to a fast excitatory postsynaptic current (EPSC) that could be selectively blocked by the AMPA receptor antagonist NBQX and a slow and sustained EPSC that could be selectively blocked by the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5) in the same neuron (4), suggesting that AMPA and NMDA receptors are involved in signal transmission at this first synapse. This is consistent with the immunohistochemical localization of non-NMDA and NMDA receptors on many neurons in the NTS. Most neurons in the NTS express NMDA receptors, as shown with antibodies recognizing the NMDA type 1 and 2 receptor subunits (6, 35). Many also express the AMPA receptor glutamate type 2 and 3 receptor subunits and fewer glutamate type 1 receptor subunits (6, 23). Although AMPA receptors mediate fast excitatory transmission in a simple relay fashion, NMDA receptors, through their longer-lasting depolarization, are thought to activate many second-messenger pathways, allowing long-term plastic changes to occur, important for integrative functions. There is also evidence for metabotropic glutamate receptors in the NTS (20) that can modulate synaptic activity for even longer periods than NMDA receptors. One of the modulatory functions of NMDA and metabotropic glutamate receptors may be...
their involvement in the frequency-dependent depression of afferent signal transmission, the phenomenon of increasing depression of postsynaptic responses in second-order NTS neurons with increasing frequency of primary afferent inputs (26, 39). This may be accomplished by metabotropic (17, 26) and NMDA receptors (13) located presynaptically on vagal afferent axon terminals (1).

NMDA and non-NMDA receptors are also involved in the transmission of sensory signals to third- and higher-order neurons within the dorsal medulla or elsewhere (9, 46) and in the transmission of descending input and input from the area postrema to the NTS (5). Thus, in our in vivo model, the receptor blocker could act at any of these sites, influencing not only food intake, but also cardiopulmonary and gastrointestinal autonomic outflow. Although we did not attempt to measure it, substantial hypertension and tachycardia are very likely. These unwanted effects may have contributed to or caused the decreased food intake.

Consistent with the notion that all primary visceral afferents use glutamate and AMPA receptors for fast transmission to the second-order neurons, Li and Smith (25) showed that all taste-induced activity of rostral NTS neurons was abolished by local injection of NBQX. Therefore, we have to assume that our rats with NBQX injections into the fourth ventricle and NTS were unable to taste the sucrose solution, and this may explain why they stopped licking. Support for this explanation comes from the observation that the latency to lick sucrose after introduction of the spout was not significantly changed by NBQX. Since the first licks were not “rewarded” as usual with the sweet taste, the animals became hesitant, exhibiting long pauses interrupted with short bursts of licking. An interesting question is whether the rats decreased water intake for the same reason. Does water activate primary taste afferents, possibly mediated by aquaporin receptors? Because there is little support for such “active water tasting,” the decreased water intake may, rather, indicate a more general problem with the act of ingestion.

If the lick pattern generator per se is not affected by NBQX, could it be that the various stages of deglutition are disrupted by the drug? It has been shown that swallow initiation is not just a passive reflex but, rather, is dynamically adjusted in concert with ongoing fluid ingestion through common neural processes (21). As for the cardiopulmonary reflex pathways, NMDA and AMPA receptors are involved at almost every processing step, with AMPA receptor antagonists typically blocking fast excitatory neurotransmission and deglutition (8, 9, 22, 27). It is thus conceivable that fourth ventricular and direct NTS application of NBQX disrupted normal swallowing, leading to decreased fluid ingestion.

Finally, it is also possible that NBQX interfered with a “descending hunger signal” from forebrain/hypothalamic circuits controlling longer-term food intake, although there is no evidence for such projections to use glutamate as excitatory transmitter. This mode of action would also predict that this descending signal and thus the effect of the blocker would be greater in food-deprived animals. This was clearly not the case, and in addition it would be difficult to account for the decreased water intake if this mode of action were adopted.

The most parsimonious explanation for the NBQX-induced suppression of intake is thus a nonspecific disruptive action on taste input and/or on the sensory-motor coordination of licking and deglutition. Although the drug might have actually inhibited medullary processing of satiety signals, this effect was probably completely overshadowed by these nonspecific effects. Because many of these processing steps occur within the NTS itself or in its immediate vicinity, it is not surprising that local microinjection of the antagonist into the NTS had the same effect. As indicated by the diffusion of dye, the antagonist most likely also diffused into areas outside the NTS, although it appeared to be limited to the ipsilateral side. A systematic mapping study with small local injections may be able to dissociate a more selective effect on satiety from the more general intake-suppressing effect seen in the present study.

Perspectives

What is the neural substrate of satiety? The failure to delay satiety in the present study leaves many questions about the neural substrate of satiety unanswered. On the basis of experiments in decerebrate rats, we do know that feedback from the alimentary canal by the ingested food can stop swallowing of intraorally delivered sucrose solution (18). In other words, the indirect controls are more or less operational (43). Selective subdiaphragmatic vagal deafferentation resulted in increased meal size (42), and selective celiac branch deafferentation eliminated suppression of meal size by intestinal nutrients (45), clearly demonstrating the presence of vagal afferent satiety signals. However, here we were unable to track and isolate these signals, as they relay on second- and higher-order neurons in the medulla. One of the fundamental questions is whether satiety results from the collective activity pattern of many vagal afferents or only from a few specialized ones. Also, does the process involve integration within the NTS or within the reticular formation around the NTS, or both? Finally, if not distinguished by the fast transmission of excitatory amino acids, could peptides coreleased from vagal afferents or by second-order neurons provide a functionally specific coordinator for bringing the various substrates of oromotor behavior to a controlled halt as in meal termination?

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