Activation of NPY Receptors Suppresses Excitatory Synaptic Transmission in a Taste-Feeding Network in the Lower Brainstem

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Abstract
Consummatory responses to taste stimuli are modulated by visceral signals processed in the caudal solitary nucleus (cNST) and ventrolateral medulla. Based on decerebrate preparations, this modulation can occur through local, brainstem pathways. Among the large number of neuropeptides and neuromodulators implicated in these visceral pathways is neuropeptide Y (NPY) which is oftentimes colocalized in catecholaminergic neurons themselves implicated in glucoprivic-induced feeding and satiety. In addition to the cNST and ventrolateral medulla, noradrenergic and NPY receptors are found in circumscribed regions of the medullary reticular formation rich in pre-oromotor neurons. To test the hypothesis that NPY may act as a neuromodulator on pre-oromotor neurons, we recorded the effects of bath application of NPY and specific Y1 and Y2 agonists on currents elicited from electrical stimulation of the rostral (taste) NST in pre-hypoglossal neurons in a brainstem slice preparation. A high proportion of NST-driven responses were suppressed by NPY as well as Y1 and Y2 agonists. Based on paired pulse ratios and changes in membrane resistance, we concluded that Y1 receptors influence these neurons both pre- and post-synaptically and that Y2 receptors have a pre-synaptic locus. To test the hypothesis that NPY may act in concert with norepinephrine (NE), we examined neurons showing suppressed responses in the presence of a Y2 agonist and demonstrated a greater degree of suppression to a Y2 agonist/NE cocktail. These suppressive effects on pre-oromotoneurons may reflect a satiety pathway originating from A2 neurons in the caudal brainstem.

Keywords: oromotor, ingestion, reticular formation, norepinephrine
Introduction

Circuits controlling the consummatory behaviors of ingestion are located in the lower brainstem (7, 38, 40, 56). These circuits extend from the pons to the spinal medullary junction to encompass both sensory and motor nuclei as well as specific regions of the reticular formation (RF). One region, immediately subjacent to the nucleus of the solitary tract (NST) which includes both the intermediate subdivision of the medullary RF (IRt), and the more lateral parvocellular RF (PCRt), contains a dense constellation of pre-oromotor neurons (29, 37, 58) that provide a potent source of excitatory drive to the oromotor nuclei (59, 60). Functional inactivation of the IRt/PCRt with either the GABA agonist muscimol or glutamatergic antagonists suppresses consummatory behavior in the awake freely-moving rat (10, 11, 57).

An additional, integrative role for the IRt/PCRt is suggested by input from forebrain sites involved in homeostatic, regulatory and motor function (9, 43, 50, 61), as well as overlapping input from brainstem oro- and viscerosensory nuclei, including the rostral (gustatory) nucleus of the solitary tract (rNST), caudal (visceral) NST (cNST), the parabrachial nucleus, and ventrolateral medulla (8, 28, 31, 54, 64). Results from decerebrate preparations in which visceral signals such as gastric load (49) and glucoprivation (15, 21) modify the amount of a palatable (sweet) stimulus that is consumed, suggest that these local pathways exert a potent influence over this consummatory circuitry (reviewed in (24, 45, 46)). Although some amino acid-mediated excitatory and inhibitory inputs to IRt/PCRt pre-oromotor neurons have been identified from the rostral (gustatory) nucleus of the solitary tract (41), little is known of the neurochemical identity of other local modulatory influences on these neurons. Indirect influences on IRt/PCRt premotor neurons, however, can be inferred from brainstem/4th ventricle infusions of neuromodulators that impact feeding behavior. Brainstem infusions of opioids (32, 34), ghrelin (20), catecholamines (53), and neuropeptide Y (12, 13, 53), all modulate ingestive behavior and some or all of these responses may involve circuits complete within the brainstem.

The present study was undertaken to begin to identify neuromodulators that modify the influence of rNST (gustatory) stimulation on pre-hypoglossal neurons in the IRt/PCRt. We focused on agonists for receptors for the NPY family of peptides, in particular, Y1 and Y2 receptors, because they are well represented in the IRt/PCRt (33, 51) and because the brainstem contains sources for their endogenous ligands; i.e. NPY in the cNST and ventrolateral medulla, and PYY in the medial RF (19, 23, 26, 39, 48). Our results indeed demonstrate that
Activation of NPY receptors has a marked influence on rNST driven responses in IRt/PCRt pre-hypoglossal neurons. Both Y1 and Y2 agonists cause presynaptic inhibition and Y1 has an additional post-synaptic inhibitory effect on these responses. Because a large proportion of NPY-positive neurons in the lower brainstem co-localize with catecholamines, we also examined whether norepinephrine (NE) also modulated neurons affected by NPY. In neurons inhibited by a Y2 agonist, NE had a further suppressive effect. These inhibitory results on presumably excitatory pre-oromotor neurons are not easily related to the orexigenic effects of 4th ventricle NPY infusions, but could be explanatory for a satiety pathway originating from A2 neurons that co-localize NPY and NE.

Methods

Retrograde tracing

In order to record from identified pre-hypoglossal neurons, a retrograde tracer was injected into the hypoglossal nucleus (mXII) (41). Briefly, under deep anesthesia with a combination of ketamine and xylazine (ip: 90/30 mg/kg), Sprague Dawley rat pups (P7-P10) were placed in a stereotaxic frame and held in place with mouse ear cups. After opening the caudal medulla at the level of the area postrema, injections of rhodamine-labeled fluorescent microspheres (Invitrogen: 0.04 µm diameter, 50 to 100 nl) were made into the mXII 0.2 mm rostral to obex and 0.2 mm lateral to the midline. The depth was predetermined by observing microstimulation-induced lingual movements through a micropipette (tip diameter 20–40 µm) filled with 0.9% saline. The surgical wound was gently packed with gelfoam and the skin joined with wound clips. Pups were returned to their home cage and survived for 12–48 h to allow retrograde transport of the fluorescent tracer. All experimental protocols were approved by the Ohio State University Institutional Animal Care and Use Committee in accordance with guidelines from the National Institutes of Health.

Slice preparation

Slices containing pre-hypoglossal neurons were obtained as reported previously (41, 62). Rat pups were decapitated under deep anesthesia (33% urethane, 10 ml/kg). The brainstem was quickly extracted and placed in an ice cold, oxygenated (95% O₂, 5% CO₂) modified Kreb’s solution containing (mM): 110 choline, 25 NaHCO₃, 3 KCl, 7 MgSO₄, 1.5 NaH₂PO₄, 10 d-
Glucose, 0.5 CaCl2. The brain was blocked ~1 mm caudal to obex and rostrally at the level of the incoming VIIth nerve and glued to a ceramic block with cyanoacrylate glue for sectioning in the coronal plane on a Vibratome 1000 (Vibratome Inc, St. Louis MO). Coronal slices of the brainstem were cut with a sapphire knife at 350 μM and transferred to an incubation chamber containing an oxygenated normal Kreb’s solution (mM): 124 NaCl, 25 NaHCO3, 3 KCl, 1 MgSO4, 1.5 NaH2PO4, 10 d-glucose, 1.5 CaCl2. Sections used for recording included those from the level where NST starts to separate from the 4th ventricle to the rostral pole of the NST. Typically, four 350 μm slices were cut from this area.

**Electrophysiology**

Following 1-2 h incubation, a single slice was transferred to a custom recording chamber mounted on the stage (Siskiyou Instruments, Grants Pass, OR, USA) of an upright microscope (Nikon, E600FN). Brain slices were held in place by a custom-made gold harp fitted with nylon mesh. The chamber was continuously perfused with warmed (32°C) oxygenated normal Kreb’s solution (2 ml/min). Patch pipettes (4–6 MΩ) were formed from 1.5 mm thin wall borosilicate glass (A-M systems, Sequim, WA, USA) pulled on a Narishige PP-83 vertical Pipette puller. The pipette solution consisted of (mM): 140 potassium gluconate, 10 EGTA, 10 HEPES, 1 MgCl2, 1 CaCl2, 2 ATP, and 5 NaCl. Lucifer Yellow (0.1%) was added to the pipette solution to label recorded neurons to verify double-labeled neurons and their locations.

To evoke postsynaptic currents in IRt/PCRt neurons, a bipolar stimulating electrode (67 μm, NiCr) was placed in the rNST under brightfield optics. Single (0.20 ms, 0.1 Hz, 100-150 μA) or paired pulses (0.20 ms, 30 Hz, 100-150 μA, train rate 0.10 Hz) were applied. Under IR-DIC and epifluorescence optics, patch pipettes were guided to retrogradely labeled neurons within the RF. Patch clamp recordings were performed in whole cell configuration (Model 2400, AM Systems) by rupturing the neuronal membrane with an intact gigaohm seal (resistance ≥1 GΩ). Neurons were excluded from the study if they did not meet the following criteria: ≥1-GΩ seal, ≥100-MΩ membrane resistance, ≥40 mV action potential amplitude, and stable resting membrane potential more negative than -40 mV. Action potential properties and membrane resistance were determined under current clamp with a series of current steps: -0.2 to 0.2 nA in 0.05 nA steps. Resting membrane potential and membrane resistance were monitored periodically throughout the recordings. Responses to electrical stimulation of the rNST were typically recorded under voltage clamp near the resting membrane potential using Clampfit 9.2
software (Molecular Devices, Union City, CA). Reported values were not corrected for a junction potential of ~12 mV. In a subset of experiments, changes in spontaneous firing frequency were monitored in current clamp.

**Drug application**

All drugs were kept as a concentrated stock solution stored at -20°C, and diluted to their final concentration immediately before application. The following drugs were used: NPY (0.1-5 µM), NPY 3-36 (0.01-0.5 µM)(Sigma: St. Louis, MO, USA), a potent Y2 agonist (5, 6); D-arg-25-NPY (0.01-0.5 µM)(American Peptide Inc (Sunnyvale, CA, USA) a Y1 agonist (22); BIIE 0246 (1 µM ), a Y2 antagonist (17) and BIBO 3304 (1 µM) (Tocris Inc (Ellisville, MO, USA), a Y1 antagonist, (17, 63) and NE (2 – 100 µM) (Sigma-Aldrich, St. Louis, MO). To test the pharmacological effects on the evoked postsynaptic currents, membrane resistance and spontaneous firing frequency, drugs were applied for 1 to 10 min following a minimum of 5 min of baseline recording. Following drug application, the normal Krebs solution was reapplied. Neurons that did not show at least a partial recovery were excluded from statistical analyses.

**Data analysis**

All data were expressed as means ± SE. The amplitudes of the rNST-evoked excitatory postsynaptic currents (EPSCs) were measured following the first response to a paired pulse stimulation or following a single pulse before and after drug application. The paired pulse ratio was computed as the amplitude of the 2nd EPSC divided by the 1st EPSC. Membrane resistance was calculated as the slope of the membrane voltage against the amount of current injected. Mean spontaneous firing frequency (SFF) was measured in current clamp before and after drug application. Mean control SFF was calculated from the number of spikes occurring during a 5 min interval prior to drug perfusion; mean drug SFF was calculated as the number of spikes occurring during a 1-3 minute period following drug application. Paired t-tests or repeated measures ANOVA were used to assess drug effects (P < .05 criteria).

**Results**

The effects of bath application of NPY, D-arg-25-NPY (Y1 agonist) and NPY3-36 (Y2 agonist) on NST stimulation-induced EPSCs were studied in a total of 125 mXII-projecting IRt/PCRt
neurons. NPY suppressed rNST-evoked excitatory and inhibitory responses in a dose-dependent fashion in the subset of neurons tested with multiple concentrations (Fig. 1B). Separate repeated measures ANOVA were performed for excitatory (p<.013, N=3) and inhibitory (p < .033, N=3) responses. In a larger population of neurons tested at a concentration of 0.5 µM (N=7), the mean peak excitatory response was suppressed by nearly 60% (paired t-test: p<.008). In order to more fully determine the type and location of the receptors mediating this suppression, we examined paired pulse ratios and changes in membrane resistance to bath application of specific Y1 and Y2 agonists.

Following bath application of the Y1 receptor agonist D-arg-25-NPY (0.01-0.5 µM) or the Y2 agonist NPY3-36 (0.01 – 1.0 µM), there was a dose-dependent reduction in the peak amplitude of the NST-evoked EPSCs (Fig. 2A & Fig. 3A). At 0.5 µM, D-arg-25-NPY significantly suppressed the peak excitatory current from 59.8 pA to 33.1 pA (-44.7%) followed by recovery during washout to 53.8 pA (Fig. 2B). The difference between the control and drug conditions was statistically significant (p < .001, paired t-test, N=18). At 0.5 µM, NPY3-36 significantly suppressed the peak excitatory current from 63.5 pA to 32 pA (-49.6%) followed by recovery during washout to 55.4 pA (Fig. 3B). The difference between the control and drug condition was also significant (p <.025, N=11). Both Y1 (n=1) and Y2 (n=2) agonists also suppressed rNST-evoked inhibitory currents but the small number of inhibitory responses precluded any statistical treatment.

For both NPY agonists, there was a significant increase in the paired pulse ratio. For the 12 (out of 18) neurons showing some degree of suppression to D-arg-25-NPY, the mean paired pulse ratio increased from 0.99 during control to 1.4 following drug application (p <.004, paired t-test) (Fig. 2D). NPY3-36 increased the paired pulse ratio from 1.1 to 1.7 for the 9 neurons (out of 11) showing response suppression (p <.029) (Fig. 3D). These results imply a presynaptic inhibition of the excitatory synaptic transmission by Y1 and Y2 receptors. Furthermore, the inhibitory effects of D-arg-25-NPY or NPY3-36 on the NTS-evoked EPSCs were abolished by the NPY Y1 selective antagonist BIBO3304 (1 µM, n=4, Fig. 4A&B) or Y2 selective antagonist BIIE0246 (1 µM, n=4, Fig. 4C&D).

We further studied the site of inhibition by measuring membrane resistance. Perfusion of D-arg-25-NPY (0.5 µM) reduced the membrane resistance by 10.9% (p<.001, N=4) (Fig. 5A); bath application of NPY3-36 (0.5 µM) had no significant effect (P=0.38) (Fig. 5B). These results
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indicate that D-arg-25-NPY but not NPY3-36 also exerts a postsynaptic effect in the inhibition of IRt/PCRt neurons to rNST stimulation. The reduction in membrane resistance following D-arg-25-NPY (0.5 µM) was blocked by the Y1 selective antagonist BIBO3304 (1µM, N=4, data not shown). These findings suggest that Y1 but not Y2 receptors exist in IRt/PCRt neurons. The inhibitory effect of both NPY agonists was also apparent from a reduction in mean spontaneous firing under current clamp. Application of D-arg-25-NPY (0.5 µM) reduced the spontaneous firing frequency by 60% (p< .004, N=10), NPY 3-36 reduced activity by 42% (p<.002, N=11) (Fig. 6).

To determine if NE was effective in RF neurons responsive to an NPY agonist, we first determined that bath application of NE produced a dose-dependent decrease in rNST-induced excitatory responses from RF neurons (Fig. 7). A concentration of 2 µM NE significantly reduced rNST-induced excitatory responses by 42% from 91.2 pA to 52.67 pA (P = .012). This dose was also effective in suppressing the one inhibitory response we recorded. We then tested the effects of 2 µM NE on rNST evoked responses in pre-hypoglossal RF neurons that showed at least a 10% reduction to NPY3-36 (Fig. 8). These neurons showed a further significant decrease in the rNST evoked response compared to neurons that only received NPY3-36 (ANOVA: trial X group interaction: P= .035). The additional suppression in the evoked response to NE preceded by NPY3-36 was comparable to the reduction observed by NE alone. Specifically, the reduction seen between trial 8 (66% of baseline), to trial 10 (38% of baseline) represents a 42% reduction comparable to the mean reduction observed to 2 µM NE given by itself (Fig. 7).

Histology

Of 125 neurons identified as pre-hypoglossal and located in the reticular formation subjacent to the nucleus of the solitary tract, 26 were reconstructed (Fig. 9). We targeted neurons to be subjacent to the rostral NST, however a small proportion of them were located at an intermediate level where the NST abuts the IV ventricle (Fig 9). Most of the neurons were large multipolar neurons, oftentimes with processes extending dorsal towards or into the rNST. Neurons suppressed by Y1 and Y2 receptor agonists were intermingled with non-responsive neurons.
Discussion

We can conclude from our study that Y1 receptors influence pre-hypoglossal neurons both pre- and post-synaptically and that Y2 receptors also have a presynaptic locus. Although the source of the endogenous ligand for these receptors remains unknown, the brainstem is rich in neurons expressing NPY, as well as PYY, albeit to a much lesser extent. Both of these neuromodulators are associated with homeostatic energy regulation, suggesting that IRt/PCRt pre-oromotor neurons are themselves a site of integration.

Location of cells

Neurons in this study were located immediately ventral to the rNST. Compared with the location of pre-hypoglossal neurons described in anatomical studies, e.g. (14, 58), it is clear that our study primarily sampled the dorsal-most extent of this population, in a region similar to those reported in two previous studies (41, 62). The region immediately ventral to the rNST has the densest population of pre-hypoglossal neurons as well as the densest terminal field of afferent fibers emanating from the rNST (1, 54, 55, 59). Hence, it offers the greatest likelihood for sampling rNST/pre-hypoglossal interactions. Save for a modest propensity for pre-hypoglossal neurons innervating lingual retractor motoneurons to be somewhat dorsal compared to lingual protruder pre-motoneurons (16), a spatial specialization of IRt/PCrt pre-motoneurons has not been reported. Thus, it seems reasonable to hypothesize that the results of the present study could be extended to more ventral populations.

Pre- vs post synaptic inhibition

Excitatory input from rNST stimulation produces an inward current via non-NMDA glutamate receptors (41). In the present study, bath application of the Y1 agonist D-arg-25-NPY reduced the rNST-evoked inward current and suppressed spontaneous action potentials. Both of these effects were blocked by the specific Y1 receptor antagonist BIBO3304. An increase in the paired-pulse ratio and a decrease in membrane resistance following D-arg-25-NPY indicated both a pre- and postsynaptic location for Y1 receptors. Although Y1 receptors have not been specifically localized to pre-hypoglossal neurons in the IRt/PCRt, Y1 receptor-like immunoreactivity is well represented in this general region (33). A postsynaptic location for Y1 receptors with inhibitory effects is present in other central neurons including orexin-containing
neurons in the lateral hypothalamus (22) as well as brainstem neurons. For example, Y1 agonists post-synaptically suppressed excitatory currents in dorsal motor nucleus (DMN) neurons in response to cNST stimulation (6).

Y2 receptor mediated inhibition appeared to be pre-synaptic to IRt/PCRt pre-hypoglossal neurons, and Y2R immunoreactivity appears not only well represented in the lower brainstem (18, 42), but is clearly concentrated in the IRt/PCRt compared to either the more lateral or medial RF (see Fig. 3I) (51). Similar Y2 mediated presynaptic inhibition of glutamatergic input was also observed in orexin neurons in the lateral hypothalamus (22) as well in DMN neurons in response to cNST stimulation (4).

**Possible sources of endogenous ligand**

The endogenous ligand(s) for Y1 and Y2 receptors is(are) most likely of brainstem origin as NPY neurons in the hypothalamus do not appear to project to the brainstem reticular formation (2). Neurons expressing NPY, however are found in both the caudal NST and ventral medulla, oftentimes co-localized with catecholamines (19, 26, 39, 48) and there is extensive overlap of Y1, Y2 and α2A adrenergic receptors in the IRt/PCRt where we did our recordings (see Fig. 4e (47))(33, 51). The results of the present study demonstrating that NE acts in a parallel fashion to a Y2 agonist in suppressing rNST-induced responses is consistent with brainstem CA/NPY neurons as a (single) source for these ligands. In addition to NPY, PYY is also an endogenous ligand for Y1 and Y2 receptors, and has been localized to neurons in the medial RF (3, 23, 44). PYY immunoreactive fibers are evident in the medullary RF, including the IRt/PCRt.

**Functional significance**

NPY is a potent orexigenic peptide (52). In addition to its role in promoting food intake via hypothalamic pathways, reviewed in (65), there is growing evidence that NPY endogenous to the lower brainstem promotes food intake as well. Thus, infusions of NPY into the 4th ventricle lead to increased food consumption, similar to that observed following 3rd ventricular infusions (12, 13, 53). Likewise, glucoprivation-induced feeding appears to depend on intact brainstem catecholamine-containing NPY neurons (CA/NPY) (35, 36, 46) and glucoprivation-induced feeding can be elicited in decerebrate preparations (15, 21). Thus, a parsimonious argument
might simply hold that brainstem CA/NPY neurons augment food intake by direct action on pre-oromotor neurons. However, there are important unresolved issues with this chain of reasoning.

First, the majority of CA/NPY neurons implicated in glucoprivic-induced feeding are in the ventral medulla, somewhat removed from the 4th ventricle (reviewed in (46)). Although C1 neurons, many of which contain NPY (19, 48) traverse the pre-oromotor RF substrate en route to the cNST (8), it is not known if this projection specifically includes C1 neurons co-localizing NPY, nor is it known if this pathway actually terminates on pre-oromotor neurons. Indeed, even if this were the case, it is difficult to envision how the inhibitory effects we observed would produce an orexigenic effect. Activation of Y1 receptors is typically associated with a profound increase in food intake, e.g. (30). Thus, inhibition of (primarily) excitatory inputs from the rNST to pre-hypoglossal neurons, which are also primarily excitatory, runs counter to the expected increase in evoked activity one might expect if activation of Y1 receptors were orexigenic. Interestingly, however, this paradoxical effect parallels the influence of NPY on lateral hypothalamic neurons (22) where it was observed that Y1 agonists postsynaptically inhibit identified hypocretin/orexin neurons. On the face of it, this suppression would also be unexpected if effects were related to feeding, as hypothalamic NPY and orexin/hypocretin both increase feeding. Indeed, the authors discuss NPY’s inhibition of orexin/hypocretin neurons in the context of arousal as an alternative to feeding mechanisms. Similarly, we cannot rule out a potential role for NPY’s effect on pre-oromotor neurons that is not directly related to food intake.

Nor is this paradox resolved by the recognition that Y1 receptors also bind PYY. Like NPY, PYY induces an orexigenic response when infused into the fourth ventricle (13). Although it is likely that PYY-positive neurons, located in nucleus gigantocellularis of the medullary RF project to the IRt/PCRt (3, 23, 44), these PYY-positive neurons are in close association with melanin concentrating hormone and orexin-positive fibers, orexigenic peptides originating from the hypothalamus.

Unlike Y1 receptor activation, however, Y2 receptor activation is associated with suppressing food intake, at least in some studies, e.g. (27, 42). Because A2 neurons are strongly implicated in satiety mechanisms (reviewed in (45)), including those that survive decerebration (25), an alternative hypothesis to an orexigenic (excitatory) role for NPY on pre-hypoglossal neurons could involve the colocalization of NPY in a subset of A2 neurons (19, 48). In that case, the inhibition of pre-oromotor neurons observed in the present study could represent a brainstem
satiety pathway originating from A2 neurons in the cNST in which the release of NPY and/or NE suppresses excitatory input from the rNST. The observation that NE acts in parallel with NPY agonists supports such a pathway although it remains to be determined if such effects actually originate from A2 neurons.

Perspectives and Significance

Considerable progress has been made in identifying neural and hormonal pathways that signal metabolic need or surfeit. Ultimately, these signals must influence neurons that control the actual behavior of feeding and a number of studies suggest that the IRt/PCRt is a critical region of the medullary reticular formation for the coordination and expression of oro-motor consummatory behavior. The present paper demonstrates that activation of specific receptors for NPY or NE, neuromodulators with powerful effects on feeding behavior, also modulate the activity of pre-oromotor neurons that control lingual motoneurons. This study emphasizes the integrative capacity of brainstem circuits and suggests that these neuromodulators are likely to be but one of many in the hindbrain that mediates control of consummatory behavior.

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Disclosures:

There are no conflicts of interest.
References


Activation of NPY receptors

Figure Captions

Fig. 1 A. Stimulation of the rostral (gustatory) nucleus of the solitary tract (rNST) produces inward current during baseline condition (black). Suppression of rNST-evoked inward current following bath application of NPY (red: 0.5 µM) followed by return to baseline during washout (blue). B. Dose-dependent effect of NPY on both excitatory (solid) and inhibitory (dashed) responses. C. In a larger population of neurons, 0.5 µM NPY reduces excitatory current by nearly 60%.

Fig. 2 A. Mean suppression of rNST-evoked inward current by Y1 receptor agonist D-arg-25-NPY at 3 concentrations. B. Suppression at 0.5 µM is statistically significant (paired t-test). The suppression recovered almost completely with washout. C. Example of the increase in the paired-pulse ratio. D. The average increase in paired-pulse ratio was statistically significant for the 12 out of 18 neurons that showed suppression with D-arg-25-NPY.

Fig. 3 A. Mean suppression of rNST-evoked inward current by Y2 receptor agonist NPY3-36 at 3 concentrations. B. Suppression at 0.5 µM is significant (paired t-test) and almost completely recovers with washout. C. Example of the increase in paired-pulse ratio for one neuron: ACSF, NPY3-36, washout. D. The mean increase in paired-pulse ratio was statistically significant for the 9 /11 neurons that showed suppression with D-arg-25-NPY.

Fig. 4 A. Suppression of rNST-evoked inward current by Y1 agonist D-arg-25-NPY is blocked by inclusion of Y1 antagonist BIBO3304. B. Significant reduction of mean inward current by Y1 agonist (paired t-test: p <.028, n= 4) compared to Y1 antagonist alone or Y1 agonist together with Y1 antagonist. C. Suppression of rNST-evoked inward current by Y2 agonist NPY3-36 is blocked by inclusion of Y2 antagonist BIIE0246. B. Significant reduction of mean inward current by Y2 agonist (paired t-test: p <.05, n= 4) compared to Y2 antagonist alone or Y2 agonist together with Y2 antagonist.

Fig. 5 A. D-arg-25-NPY reduces the net change of the membrane potential induced by a series of depolarizing pulses. B. Significant reduction in membrane resistance following application of Y1 agonist (n=28). C. NPY3-36 has no discernible effect on the potential change induced by depolarizing pulses. D. No significant change in membrane resistance following application of NPY3-36.
Fig. 6 A. Reduction in spontaneous activity following infusion with Y1 agonist D-arg-25NPY is accompanied by lowered resting membrane potential. B. Significant reduction in spontaneous activity (paired t-test) following infusion with Y1 agonist returns to near normal following washout. C. Significant reduction in spontaneous activity (paired t-test) following infusion with Y2 agonist returns to near normal following washout. Decrease in spontaneous activity was not associated with a change in resting membrane potential (not shown).

Fig. 7 Response of pre-hypoglossal neurons to stimulation of the rostral nucleus of the solitary tract in the presence of norepinephrine (0 to 100 µM). The absolute value of the inhibitory current from one neuron is plotted.

Fig. 8 A. A pre-hypoglossal neuron that showed suppression of the amplitude of the rostral solitary nucleus stimulation-induced EPSC in the presence of NPY3-36, showed a further reduction following application of norepinephrine (black: baseline; red: 0.5 µM NPY3-36; blue: 0.5 µM NPY3-36 + 0.2 µM norepinephrine; green: washout). B. This reduction was significant across a population of NPY3-36 cells showing at least a mean 10% reduction following NPY3-36 (black line: n=6) compared to similar neurons continuing to receive only NPY3-36 (red line: n=5). Interestingly, in 4 neurons not showing the 10% reduction following NPY3-36 (blue line), there was no NE effect; these neurons were excluded from the ANOVA.

Fig. 9 Location of 26 neurons. Neurons with significant reductions (range: 29-100% suppression) following Y1 agonist (solid red), significant reductions (range: 22-100%) following Y2 agonist (solid blue), non-significant changes (range: 0-3%) to Y1 agonist (open red), non-significant changes (range: 0-3%) to Y2 agonist (open blue).
Fig. 1

A.

B.

C.
Fig. 2

A. Percent Response (ACSF)

B. Amplitude (pA)

C. ctrl d-arg wash

D. Paired Pulse Ratio

p < .001

n=6 n=5 n=18

ACSF D-arg (0.5 μM) Wash

ACSF (0.5 μM) Wash

p < .004

p < .004

ctrl d-arg wash ctrl d-arg wash

ACSF D-arg (0.5 μM) Wash

ACSF D-arg (0.5 μM) Wash
Fig. 3

**A.**

Percent Response (ACSF)

- n=6
- n=11
- n=5

NPY 3-36 Concentration (μM)

0.01 0.5 1.0

**B.**

Amplitude (pA)

ACSF NPY 3-36 Wash

p < .025

**C.**

ctrl NPY3-36 wash

20 pA

20 ms

**D.**

Paired Pulse Ratio

ACSF NPY 3-36 (0.5 μM) Wash

p < .03
Fig. 4

A.

D-arg

BIBO + D-arg

B.

Amplitude (pA)

p< .028

BIBO (1 μM)

D-arg (0.5 μM)

cntrl

D-arg + BIBO

BIBO

ctrl

20 ms

50 pA

50

0

150

100

p< .05

20 pA

150

100

50

0

cntrl

NPY3-36 (0.5 μM)

NPY3-36 + BIIE0246

BIIE (1 μM)

NPY3-36 + BIIE

C.

D.

Amplitude (pA)

0
Fig. 5

A. 

-68 mV  -73 mV  -66 mV
ctrl  D-arg-25 NPY  wash

p < .001

B. 

-60 mV  -61 mV  -59 mV
ctrl  NPY 3-36  wash

p = 0.73
Fig. 6

A. D-arg-25NPY (0.5 μM)

B. Frequency (spikes/sec)

C. NPY 3-36 (0.5 μM)

D. Frequency (spikes/sec)

20 mV

1 min

20 mV

1 min

p = .004

p = .002
Excitatory currents
Inhibitory currents

n=6
n=5
n=3
n=1

*  

FIG 7

0 2 5 20 100

Concentration of Norepinephrine (μM)

-150
-100
-50
0

Current (pA)

n=6  n=5
n=3  n=1
n=1
Fig. 8

A. 

B. 

Percent Baseline vs. Trial

Baseline, NPY, NE, Wash