Regional effect of naltrexone in the nucleus of the solitary tract in blockade of NPY-induced feeding

C. M. KOTZ,1,2 M. J. GLASS,3 A. S. LEVINE,1,2,3 AND C. J. BILLINGTON2,4
1Departments of Food Science and Nutrition, 2Psychiatry, and 3Medicine, University of Minnesota, Saint Paul 55108; and 4Minnesota Obesity Center, Veterans Affairs Medical Center, Minneapolis, Minnesota 55417

Kotz, C. M., M. J. Glass, A. S. Levine, and C. J. Billington. Regional effect of naltrexone in the nucleus of the solitary tract in blockade of NPY-induced feeding. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R499–R503, 2000.—Naltrexone (NLTX) in the nucleus of the solitary tract (NTS) decreases feeding induced by neuropeptide Y (NPY) in the paraventricular nucleus (PVN). We sought to determine the NTS region most sensitive to NLTX blockade of PVN NPY-induced feeding. Male Sprague-Dawley rats were fitted with two cannulas; one in the PVN and one in a hindbrain region: caudal, medial, or rostral NTS or 1 mm outside the NTS. Animals received NLTX (0, 1, 3, 10, and 30 µg in 0.3 µl) into the hindbrain region just prior to PVN NPY (0.5 µg, 0.3 µl) or artificial cerebrospinal fluid (0.3 µl). Food intake was measured at 2 h following injection. PVN NPY stimulated feeding, and NLTX in the medial NTS significantly decreased NPY-induced feeding at 2 h, whereas administration of NLTX in the other hindbrain regions did not significantly influence PVN NPY induced feeding. These data suggest that opioid receptors in the medial NTS are most responsive to feeding signals originating in the PVN after NPY stimulation.

feeding behavior; hypothalamic paraventricular nucleus; opioid receptor; brain mapping

ADMINISTRATION OF NALTREXONE (NLTX, opioid receptor antagonist) into the nucleus of the solitary tract (NTS) decreases feeding induced by neuropeptide Y (NPY) injected into the paraventricular nucleus (PVN), indicating that feeding produced by PVN NPY relies on functional opioid receptor pathways within the NTS (10, 11). However, the region of the NTS where opioid blockade is important is not clearly defined.

Defining the areas of the NTS most relevant to PVN NPY-induced feeding is important because the NTS is a large, complex neural center containing a diverse population of neurotransmitters and receptors, and there are intra-NTS neural connections in addition to connections between the NTS and several other brain regions. Functionally, the NTS is involved in several processes and integrates sensory, autonomic, and motor events serving to maintain homeostasis (1). The connectivity of the NTS with other feeding-related neural centers reflects an important role in ingestive behavior. The NTS receives primary afferent fibers from gustatory and visceral receptors and relays this information to ventral forebrain areas involved in modulating autonomic and limbic processes, including the PVN and the medial amygdala, and, in turn, receives efferent connections from these areas. The NTS is the first relay in ascending taste pathways and processes sensory and motor information to and from the gastrointestinal tract. Furthermore, lesions of the NTS and surrounding area postrema (3, 5, 17) or destruction of its fiber connections with the PVN (8) have been shown to affect feeding behavior. Opioid peptide immunoreactive neurons (7), preproopiodl mRNA (2), opioid receptor mRNA (12), and opioid receptor binding have been demonstrated in the NTS, and administration of mu opioid receptor antagonist in the NTS increases feeding (9).

The NTS can be segregated along structural-functional lines, including rostral, intermediate, and caudal regions. The rostral NTS receives primary afferents from taste receptors on the tongue and epiglottis (1, 18). Separating the rostral and caudal NTS at the level of the obex is the intermediate region that contains nuclei relaying gastrointestinal, respiratory, and cardiovascular information (1). The caudal NTS contains nuclei that process respiratory and cardiovascular information (1).

In the present study, we sought to define the most important NTS region for NLTX blockade of PVN NPY-induced feeding by targeting four different regions: three within the NTS and one just outside the NTS. We also decreased injection volume and sought to determine specificity of the NLTX blockade by administering a range of NLTX doses.

METHODS

Animals. Male Sprague-Dawley rats (Harlan, Madison, WI) weighing 250–350 g were individually housed in conventional hanging cages with a 12:12-h light-dark photoperiod (lights on at 0700) in a temperature-controlled room (21–22°C). Teklad lab chow and water were allowed ad libitum, except where noted.

Cannulation. Rats were anesthetized with Nembutal (40 mg/kg) and fitted with two 26-gauge stainless steel guide cannulas (Plastics One, Austin, TX): one placed just above the PVN and one placed just above an NTS subregion: caudal (cNTS; at the rostral extent of the area postrema), medial (mNTS; at the rostral extent of the intercalated nucleus), and rostral (rNTS; at the rostral extent of the nucleus ambiguus).
The NTS regions targeted are 1 mm apart in the caudal-to-rostral direction. Another set of animals was fitted with a PVN cannula and a cannula 1 mm ventromedial to and outside of the rNTS (xNTS). Stereotaxic coordinates were determined from the rat brain atlas by Paxinos and Watson (15) and are listed in Table 1. The injector extended 1 mm beyond the end of the guide cannula. For all cannulations, the incisor bar was set at 3.3 mm below the ear bars. Regions targeted and representative schematics of correctly placed PVN and NTS cannulas are illustrated in Fig. 1. At least 7 days elapsed following surgery before experimental trials.

Verification of cannula placement. After both experiments, brains were dissected out and stored in a 10% formaldehyde solution for later placement verification by histological examination. Schematics of coronal sections demonstrating the injection occurred outside a 0.25-mm radius from the targeted site. Several rats pulled off their cannulas during the course of the feeding studies. These rats were also excluded from the final analysis: rNTS, three rats excluded; mNTS, two rats excluded; and xNTS, zero rats excluded. Cannula placement was deemed incorrect when the injection extended 1 mm beyond the end of the guide cannula. All injections took place between 1000 and 1200.

Table 1. Stereotaxic coordinates for specific brain regions targeted

<table>
<thead>
<tr>
<th>Region</th>
<th>Lateral</th>
<th>Anterior/Posterior</th>
<th>Dorsal/Ventral</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVN</td>
<td>−0.5</td>
<td>−1.9</td>
<td>−7.3</td>
</tr>
<tr>
<td>rNTS</td>
<td>−2.0</td>
<td>−2.6</td>
<td>−7.2</td>
</tr>
<tr>
<td>mNTS</td>
<td>−1.4</td>
<td>−3.7</td>
<td>−6.9</td>
</tr>
<tr>
<td>dNTS</td>
<td>−0.8</td>
<td>−4.5</td>
<td>−7.0</td>
</tr>
<tr>
<td>xNTS</td>
<td>−0.6</td>
<td>−2.6</td>
<td>−7.9</td>
</tr>
</tbody>
</table>

Reference landmarks for lateral and anterior/posterior paraventricular nucleus (PVN) and nucleus of the solitary tract (NTS) coordinates are based on bregma and the interaural line, respectively. All dorsal/ventral measurements are made from the level of the skull surface. The injector extended 1 mm beyond the end of the guide cannula. rNTS, rostral nucleus of the solitary tract; mNTS, medial nucleus of the solitary tract; dNTS, caudal nucleus of the solitary tract; xNTS, region 1 mm outside the rostral nucleus of the solitary tract.

Fig. 1. Schematic illustrating hindbrain regions targeted. Letters indicate specific regions: A, rostral nucleus of solitary tract (NTS; rNTS); B, area outside of NTS (xNTS); C, medial NTS (mNTS); D, caudal NTS (cNTS). Numbers on lower right of each schematic indicate stereotaxic coordinate for anterior/posterior measurement (mm from bregma). Some of adjacent brain areas are labeled as follows: 4V, fourth ventricle; 10, dorsal motor nucleus of vagus; 12, hypoglossal nucleus; AP, area postrema; Cu, cuneate nuclei; DMSp5, dorsal paragigantocellular nucleus; ECu = external cuneate nucleus; FVe, F cell group, vestibular; Gi, gigantocellular reticular nucleus; Gr, gracile nuclei; In, intercalated nuclei; IRt, intermediate reticular nucleus; MdD, medullary reticular field, dorsal; MdV, medullary reticular field, ventral; MVe, medial vestibular nucleus; MVeV, medial vestibular nucleus, ventral; PCr, parvocellular reticular nucleus; PrB, nucleus of Probst's bundle; PrH, prepositus hypoglossal nucleus; Ro, rostral nucleus; Sp5c, spinal trigeminal nucleus, caudal; SpVe, spinal vestibular nucleus; X, nucleus X; InM, intermediate nucleus of medulla.

The same lot of laboratory chow was used for all of the experimental trials.

Experimental design and statistical analysis. Each rat received each treatment once on separate days, with at least 48 h between treatments to allow drug clearance from the CNS and for normal feeding patterns to be reestablished. Treatments were randomly assigned and given in a counterbalanced design, such that each treatment was represented equally (to the greatest extent possible with uneven animal
numbers) on each injection day. The treatments are listed in Table 2. All data were first analyzed by repeated-measures two-factor ANOVA including one within factor (NTS region) and one between factor (NLTX dose) to determine regional effects. Data were then analyzed separately by one-factor ANOVA for each NTS region targeted. After the determination that NPY significantly stimulated feeding in all animals and that NLTX alone in the NTS subregions had no significant effect on feeding, the saline only and NLTX only groups were deleted from the analysis such that only those animals receiving NPY were included. Post hoc testing for significant ANOVAs was performed using paired t-tests.

To determine whether repeated injections resulted in decreased responsiveness to PVN NPY and NTS NLTX, feeding data resulting from NPY treatment only and NLTX treatment only were analyzed by one-factor ANOVA (day = independent variable, 2-h food intake = dependent variables). These analyses revealed no significant differences in 2-h feeding response to NPY and NLTX over time (NPY: $P = 0.10$, respectively; NLTX: $P = 0.69$, respectively). Because animals may become conditioned to either eat or not eat following injection of various compounds, the effect of day on food intake for all saline-treated animals was analyzed by one-factor ANOVA (day = independent variable, 2-h food intake = dependent variables). This analysis revealed no significant differences in 2-h food intake in the control animals due to treatment day ($P = 0.46$, respectively).

**RESULTS**

At 2 h postinjection, PVN NPY significantly stimulated food intake and there was a significant effect of region of NLTX administration on PVN NPY-stimulated feeding ($F(3,25) = 2.99$, $P = 0.05$). PVN NPY-induced feeding was significantly decreased following NLTX administration into the mNTS ($F(4,32) = 3.13$, $P = 0.03$; Fig. 2C) but not into the rNTS ($F(4,24) = 2.25$, $P = 0.09$; Fig. 2A), cNTS ($F(4,28) = 1.15$, $P = 0.35$; Fig. 2D), or xNTS ($F(4,20) = 0.47$, $P = 0.75$; Fig. 2B).

Post hoc paired t-test analysis of the data generated with mNTS injections indicates that the feeding response of rats injected with 10 µg NLTX was significantly different from that of rats treated with NPY alone, NPY + 1 µg NLTX, and 3 µg NLTX ($P = 0.0006$, $P = 0.0289$, and $P = 0.0212$, respectively). Although 30 µg NLTX did not significantly reduce PVN NPY-induced feeding ($P = 0.1455$), the effects of 10 µg and 30 µg NLTX on PVN NPY-induced feeding were not significantly different from each other ($P = 0.29$).

**Table 2. List of treatments given to each rat**

<table>
<thead>
<tr>
<th>NTS Subregion</th>
<th>PVN</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF (0.3 µl)</td>
<td>aCSF (0.3 µl)</td>
</tr>
<tr>
<td>aCSF (0.3 µl)</td>
<td>NPY (0.5 µg)</td>
</tr>
<tr>
<td>NLTX (1 µg)</td>
<td>NPY (0.5 µg)</td>
</tr>
<tr>
<td>NLTX (3 µg)</td>
<td>NPY (0.5 µg)</td>
</tr>
<tr>
<td>NLTX (10 µg)</td>
<td>NPY (0.5 µg)</td>
</tr>
<tr>
<td>NLTX (30 µg)</td>
<td>NPY (0.5 µg)</td>
</tr>
<tr>
<td>NLTX (30 µg)</td>
<td>aCSF (0.3 µl)</td>
</tr>
</tbody>
</table>

*Treatments were given in a counterbalanced order. NTS subregion injection was made first followed by the PVN injection 60 s later. See Fig. 1 for specific NTS regions targeted. aCSF, artificial cerebrospinal fluid; NPY, neuropeptide Y; NLTX, naltrexone.***

**DISCUSSION**

These data suggest that opioid receptors in the mNTS are most responsive to feeding signals originating in the PVN after NPY stimulation. NLTX given in the rNTS, cNTS, or xNTS failed to significantly reduce food intake elicited by NPY injected into the PVN. Lesion studies have indicated that the mNTS is important in ingestive behavior (6). Previously, we reported that NLTX administered into the rNTS blocks PVN NPY-induced feeding (10, 11). However, in the current study, the rNTS NLTX injections did not significantly decrease PVN NPY-induced feeding, whereas injections of NLTX into the next-closest site, the mNTS, significantly decreased PVN NPY-induced feeding. There are several plausible reasons for the discrepancy between these studies. First, in the current study, it appears that NLTX in the rNTS did decrease PVN NPY-induced feeding, but the difference in food intake did not reach significance. Second, our initial studies targeting the rNTS employed injection volumes of 1 µl and a dose range of 5–50 µg NLTX, whereas, in the current studies, we employed a 0.3-µl injection volume and a dose range of 1–30 µg NLTX. It is possible that the results attributed to the rNTS region actually represent blockade of opioid receptors in the mNTS or that the high dose of NLTX in the previous studies was sufficient to allow nonspecific receptor binding. Receptors for other anorectic neuropeptides such as calcitonin and neurotensin have been demonstrated in the rNTS (14, 19). Additionally, calcitonin, bombesin, and neurotensin have anorectic effects when injected into the rNTS (4). Thus it is possible that in the previous studies NLTX was binding nonspecifically to non-opioid receptors.

On the basis of the notion that larger injection volumes distribute injectate over a larger area, we reduced the injection volume in the current study. However, the theory that larger volumes distribute further has not been empirically demonstrated for active neurotransmitters. Rather, the work of Nicholson (13) indicates that distribution is highly susceptible to receptor-mediated uptake of the injected compound. With high-uptake constants (greater receptor affinity and/or increased receptor density), there will be very limited distribution. Although uptake constants for most compounds are not readily available, if one assumes even minor uptake, the distribution of most compounds (including NLTX) is limited to a 0.5-mm distance from the injection site. The regions we selected were ~1 mm apart, and thus the possibility that enough NLTX diffused from the injected site to the next site is remote. The most definitive proof that the results observed are not due to diffusion of NLTX lies within the data itself. There are definite differences in response at sites that are separated 1 mm from each other (Fig. 2). If NLTX were easily diffusing from one site to another, one would expect the responses due to NLTX injection within each site to be very similar. Several nuclei lie adjacent to the mNTS. These include the cuneate, vestibular, reticular, intercalated, preposit-
tus hypoglossal, and spinal trigeminal nuclei (15). Although NLTX action at these sites cannot be ruled out, the possibility of having sufficient NLTX diffusion to these sites seems remote based on the diffusion estimates discussed above and knowledge of the role of these areas in ingestive behavior is limited.

One interpretation of these data is that feeding signals arising from stimulation of NPY receptors in the PVN result in opioid release within the mNTS, and blockade of mNTS opioid receptors prevents binding of these released opioids. However, the origin of the presumed opioid release in the mNTS is unknown. Cell bodies containing preproopioid mRNA are distributed throughout the brain and thus could arise from several regions. On the basis of known connectivity of the NTS with other brain regions, these cell bodies could be located in the PVN, within mNTS interneurons, or within intermediate sites between the PVN and NTS, such as the parabrachial nucleus. However, PVN fibers are known to project to brain stem regions, and disruption of these fibers alters food intake (8). Thus it is possible that the present results represent actions along a direct PVN-NTS path. Evidence against this interpretation is the failure of peripheral naloxone to modify c-Fos patterns in the NTS after PVN stimulation by NPY (16), although peripheral naloxone admin-
istration is different from the specific site of NLTX stimulation in the current study. Similarly, the behavioral mechanisms underlying the anorectic effect of NLTX remain unclear. Whether it reflects the effects of opioid-receptor blockade on sensory, autonomic, and/or motor processes that are mediated within the mNTS is uncertain.

The segregation of the NTS into three subregions, while serving as a simplifying scheme for localization of injection sites, does not take into account the complexity of neural organization within each respective area. Many subnuclei lie within each region, some of which span the rostral, intermediate, and caudal boundaries employed in the present study. The exception is the medial NTS region targeted, which is a specific subnucleus and may explain the sensitivity of the mNTS to NLTX injections. Future experiments with injections directed at specific other NTS subnuclei would have to be conducted to identify in more precise detail the particular subnuclei involved in opioid-regulated food intake within the NTS. However, the present experiment provides useful information, which circumscribes the likely areas for future investigations.

In summary, the present results suggest that opioid receptors in the mNTS are most responsive to feeding signals originating in the PVN after NPY stimulation. Further studies employing specific neuroanatomic injections and/or lesions in conjunction with behavioral measures will help elucidate whether the PVN NPY-mNTS opioid functional connection described here follows a direct monosynaptic pathway or a more complex multisynaptic pathway.

We thank Mary Mullet and Jennifer Lockie for expert technical assistance with the histology and food intake measurements and Dr. James Pomonis for preparation of the histology schematics.

Supported by the Dept. of Veterans Affairs, National Institute of Drug Abuse DA-03999, MN Obesity Center National Institute of Diabetes and Digestive and Kidney Diseases DK-50456.

Address for reprint requests and other correspondence: C. Billing-

Received 26 October 1998; accepted in final form 7 September 1999.

REFERENCES


