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Opioid Receptor-Mediated Modulation of Synaptic Transmission in the Nucleus of the Solitary Tract

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Introduction

The rostral portion of the nucleus of the solitary tract (NST) plays a key role in transmitting, modulating and organizing gustatory information originating from the tongue and oral cavity. The study of synaptic processing of taste information at the first central synapse has been one focus of gustation studies. Remarkable progress has been made over the past twenty years in understanding the brainstem gustatory processing at this first central relay. This short review summarizes and discusses recent progress on the investigation of opioid-mediated modulation of taste processing in the NST. The involvement of presynaptic μ-opioid receptor mechanism and postsynaptic δ-opioid receptor mechanism on modulating synaptic transmission between the fiber terminals of the solitary tract (ST) and putative gustatory cells within the rostral NST are discussed. In addition, the intracellular signaling mechanism of δ-opioid receptor-mediated suppression of ST-evoked excitatory input to putative gustatory NST cell is discussed.

Neurotransmitters and neuropeptides in the NST: Visceral and gustatory systems

Opioids are peptides that are known to regulate food intake and modulate palatability of taste (Kelley *et al*. 2002; Levine *et al*. 1985; Morley *et al*. 1983; Parker *et al*. 1992; Rideout and Parker 1996). Numerous studies have reported that opioids increase food intake (Baker *et al*. 2004; Flood *et al*. 1987; Hadjimarkou *et al*. 2004; Levine *et al*. 1985) and opioid receptor antagonists reduce food intake and body weight in the animals (Cole *et al*. 1995; Israel *et al*. 2005; Kotz *et al*. 1995; Levine *et al*. 1991; Reid 1985) and in humans (Arbisi *et al*. 1999; Atkinson *et al*. 1985; Bertino *et al*. 1991; Fantino *et al*. 1986; Yeomans *et al*. 1997; Yeomans *et al*. 1990).

The presence of several neurotransmitters and neuropeptides, including glutamate, substance P (SP), γ-aminobutyric acid (GABA), and opioids have been indentified in the rat and hamster rostral NST (Davis 1993; Davis and Kream 1993; Kalia *et al*. 1985; Maley 1996; Maley and Panneton 1988; Sweazey 1996). Studies have demonstrated that these neurotransmitters and neuropeptides are involved in synaptic transmission in the rat and hamster rostral NST (Davis and Smith 1997; King *et al*. 1993; Li and Smith 1997; Liu *et al*. 1993; Smith and Li 1998; Wang and Bradley 1993). Studies have shown that opioids play a role in the modulation of taste responses and feeding within the gustatory region of the NST(Kotz *et al*. 2000; Kotz *et al*. 1995; Li *et al*. 2003). The involvement of opioids in the rostral NST in modulation of taste responses and feeding behavior was supported by the demonstration of the presence of MetE-immunoreactive cells and fibers (Murakami *et al*. 1987) as well as opioid receptor cells in the rat rostral NST (Lynch *et al*. 1985; Mansour *et al*. 1994; Nomura *et al*. 1996). We have reported the presence of opioid receptors in the hamster rostral NST previously (Li *et al*. 2003). The distribution of δ- and μ-opioid receptors within the rostral NST was distinguishing: μ-opioid receptors were identified in the incoming fiber terminals of the ST and the neuropil within the rostral NST whereas δ-opioid receptors were expressed on the neural somata in the rostral NST. These studies have provided convincing evidence that opioids within the rostral NST may be involved in modulation of taste processing and feeding.

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Postsynaptic δ-opioid receptor mechanism mediates opioid-induced reduction of ST-evoked excitatory postsynaptic currents (EPSCs) of PbN-projecting NST cells

We further investigated the effects of opioid receptor agonists and antagonists on synaptic responses of the rostral NST cells to electrical stimulation of the ST using whole cell recording techniques in hamster brainstem slices. Since 80% of taste-responsive neurons in the hamster project their axons to the PbN (Cho and Li 2008; Cho *et al*. 2002), we targeted rostral NST cells that project to the PbN by microinjection of fluorescent latex microspheres (FLMs) into the gustatory PbN prior to the slice preparations (Fig. 1). The EPSCs were evoked by electrical stimulation of the ST.

We first examined whether ST-evoked EPSCs of PbN-projecting rostral NST cells could be modulated by MetE. Bath application of MetE significantly reduced ST-evoked EPSCs and the MetE effect was completely eliminated in the presence of NTX, suggesting that reduction of the ST-evoked EPSCs was mediated by opioid receptors (Fig. 2).

To further test whether a pre- or postsynaptic mechanism is involved in MetE-mediated attenuation of EPSCs, we examined the paired-pulse (50 ms interstimulus intervals) depression ratio of ST-evoked EPSCs. The results showed that MetE induced reduction of both the first and second EPSCs evoked by two consecutive ST stimuli. Furthermore, the MetE-induced reduction of the first and second EPSCs was proportional, suggesting MetE modulates synaptic activity by a postsynaptic mechanism (Ennis *et al*. 2001; Kline *et al*. 2002; Miles 1986; Zucker and Regehr 2002).

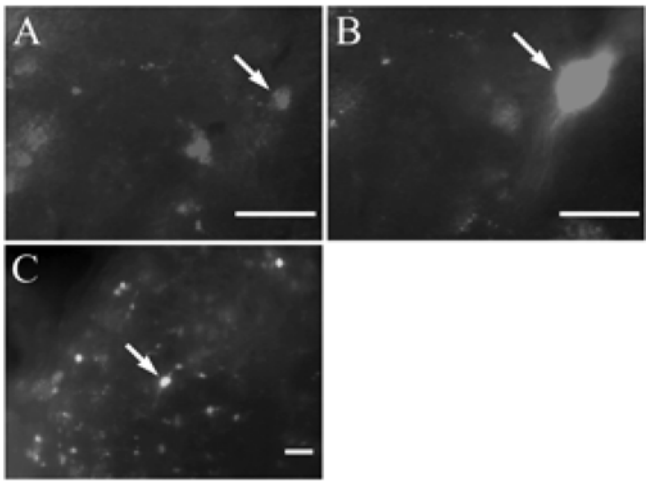

Fig. 1. Identification of parabrachial nuclei (PbN)-projecting rostral nucleus of the solitary tract (NST) neurons. *A*: photomicrograph of a rostral NST neuron identified as a PbN-projecting neuron (arrow) because it was retrogradely labeled with fluorescent latex microspheres (FLMs) following microinjection of FLMs into the gustatory PbN. *B*: high-power photograph of the same cell (arrow) identified in *A*. *C*: Low power photograph of a coronal section through the rostral central subdivision of the NST showing retrograde labeling of the NST cells following microinjection of FLMs into the gustatory PbN. The photograph was taken at the end of the recording of the cell. Scale bar = 10 μm in *A* & *B*, and 20 μm in *C*.

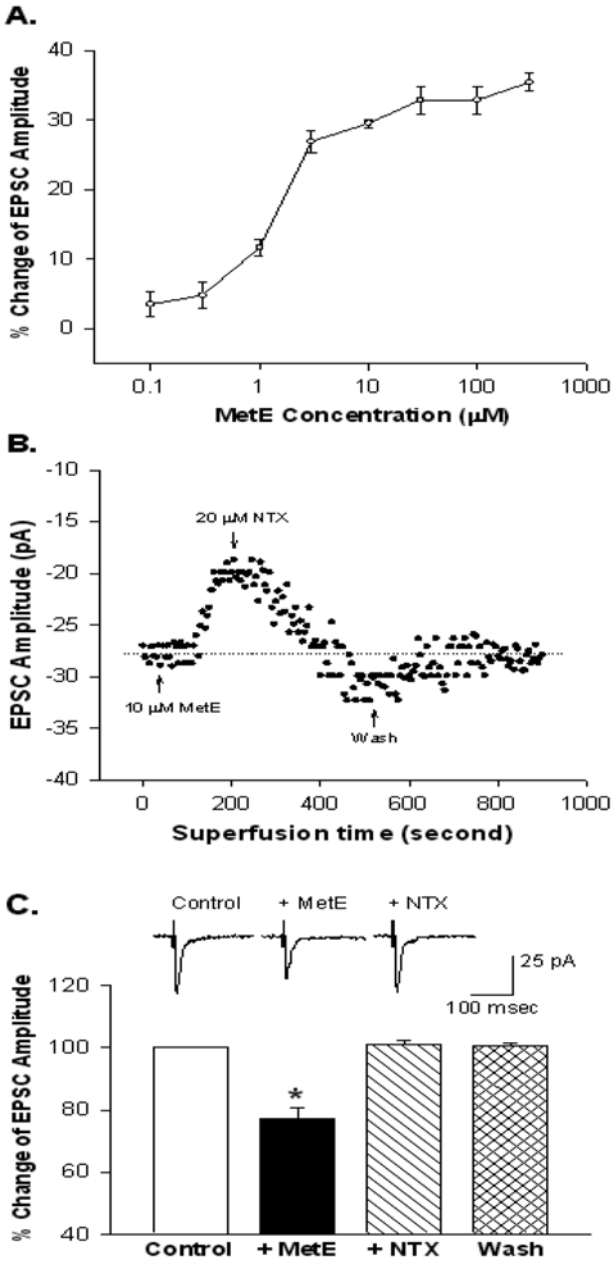

Fig. 2. Effect of opioid receptor agonist and antagonist on solitary tract (ST)-evoked excitatory postsynaptic currents (EPSCs) of PbN-projecting rostral NST neurons. *A*: concentration-response curve ($n = 4$) of met-enkephalin (MetE) on ST-evoked EPSCs. At 10 μM, MetE produced a maximum reduction of ST-evoked EPSCs. *B*: representative time course of the effect of 10 μM MetE on ST-evoked EPSCs. Naltrexone hydrochloride (NTX, 20 μM) was added to the bath at the time when the MetE effect reached a steady state and the effect of MetE was completely reversed by NTX. *C*: representative current traces show the effects of MetE and subsequent addition of NTX on ST-evoked EPSCs (*top*). Each trace on the *top* represents an average of 5 responses to ST stimulation of the same cell. The bar graph shows the average effects of MetE and NTX on ST-evoked EPSCs (*bottom*). MetE (10 μM) significantly reduced EPSCs evoked by ST stimulation ($p < 0.001$, MetE vs control groups, $n = 14$) and 20 μM NTX eliminated the effect of MetE ($p < 0.001$, MetE vs MetE + NTX groups, $n = 7$). There was no difference between control and MetE + NTX groups ($p = 0.97$). EPSCs returned to control level after washing the slices using normal media.

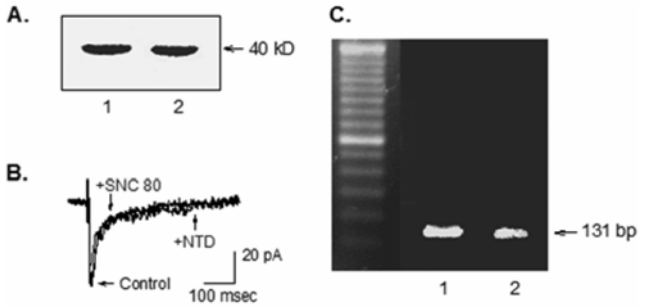

Fig. 3. Detection of δ-opioid receptor protein from the rostral NST tissue and δ-receptor protein mRNA from single PbN-projecting rostral NST cells. *A*: representative Western blot data showing the presence of δ-opioid receptor protein in tissue derived from brainstem slices containing the rostral NST (*1*) and in rostral NST tissue (*2*). The migration of a 40-kDa receptor protein was detected using rabbit polyclonal antibody (1:1000) to δ-opioid receptor. Experiments were repeated at least three times. *B*: current traces from a PbN-projecting rostral NST neuron showing that 25 μM SNC80 reduced the ST-evoked EPSC and that the SNC80 effect was blocked in the presence of 50 μM naltrindole hydrochloride (NTD) *(bottom left traces)*. *C*: after the recording, the neuron was prepared for single-cell RT-PCR. Ethydium bromide (EB)-stained agarose gel showing the RT-PCR DNA product that corresponds to δ_1-opioid receptor mRNA. The left lane is a 100-bp DNA ladder. *Lane 1* shows δ_1-opioid receptor mRNA from brainstem slices that contained the rostral NST (131 bp, 35 cycles), and *lane 2* shows δ_1-opioid receptor mRNA from the cell shown in Fig. 4B (131 bp, 70 cycles). Experiments were repeated at least three times (Zhu *et al* 2009).

It's known that there are μ- and δ-opioid receptor subtypes and MetE acts as an agonist of both μ- and δ-opioid receptor subtypes. Our tests showed that naltrindole hydrochloride (NTD), a selective δ-opioid receptor antagonist eliminated the reduction of ST-evoked EPSCs produced by MetE whereas D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) a selective μ-opioid receptor antagonist did not significantly affect the reduction of ST-evoked EPSCs induced by MetE. In addition, ST-evoked EPSCs were significantly reduced by bath application of SNC80, a highly selective δ-opioid receptor agonist. The magnitude of the reduction of the ST-evoked EPSCs by SNC80 was similar to that produced by MetE. These results suggest that MetE-induced reduction of ST-evoked EPSCs was δ-opioid receptor mediated.

There are two δ-opioid receptor subtypes; δ_1 and δ_2. We further examined which δ-opioid receptor subtype mediated the reduction of ST-evoked EPSCs. We tested the effect of 7-Benzylidenenaltrexone (BNTX), a highly selective δ_1-opioid receptor antagonist and naltriben mesylate (NTB), a highly selective δ_2-opioid receptor antagonist, on the reduction of ST-evoked EPSCs induced by SNC80. Bath application of BNTX prevented the reduction of ST-evoked EPSCs produced by SNC80 while NTB had a minimal effect on SNC80-induced reduction of ST-evoked EPSCs. Furthermore, δ-opioid receptor mediated reduction of ST-evoked EPSCs, induced by SNC80 that was not affected by the presence of NTB was completely eliminated by subsequent bath application of BNTX. These results demonstrate that the SNC80-induced reduction of ST-evoked EPSCs was δ_1-but not δ_2-opioid receptor mediated.

Expression of δ-opioid receptor proteins in the rostral NST

Our electrophysiological experiments demonstrated that postsynaptic δ_1-opioid receptors mediate SNC80-induced reduction of ST-evoked EPSCs. To scrutinize the hypothesis that δ-opioid receptor proteins are present in the rostral NST tissue, Western blot analysis was performed. The expression of δ-opioid receptor proteins was confirmed with Western blotting from both the brainstem slices that contained rostral NST and rostral NST tissue that was punched out from the brainstem slices (Fig. 3A). Immunoblotting with rabbit polyclonal δ-opioid receptor antibodies demonstrated that the corresponding δ-opioid receptor protein migrated as an approximately 40-kDa protein in both the brainstem slice tissue that contained the rostral NST and in the rostral NST tissue that was punched out from the brainstem slices.

Detection of δ-opioid receptor mRNA in PbN-projecting single rostral NST cells in which ST-evoked EPSCs were reduced by SNC80

To further demonstrate the involvement of postsynaptic δ-opioid receptors in the reduction of ST-evoked EPSCs, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) analysis to check for the presence of δ_1-opioid receptor mRNA in rostral NST tissue and in the cytoplasm harvested from single rostral NST cells in which ST-evoked EPSCs were reduced by SNC80 and the reduction was eliminated by NTD. Using oligonucleotide primers for the mouse δ_1-opioid receptor gene we detected δ_1-opioid receptor mRNA in tissue harvested from the rostral NST as well as in single PbN-projecting rostral NST cells in which the ST-evoked EPSCs were reduced by SNC80 and the SNC80 effect was eliminated by NTD (Fig. 3C).

Involvement of the phospholipase C (PLC)-inositol 1,4,5-phosphate (IP_3) pathway in δ-opioid receptor-mediated reduction of ST-evoked excitatory input to putative NST taste cells

Three opioid receptor subtypes are currently characterized by pharmacological profile; μ-, which are selectively activated by morphine, δ- by met- and leu-enkephalin, and κ- by benzomorphan opioids (Gutstein and Akil 2001). These opioid receptors are prototypical pertussis toxin (PTX) sensitive Gi/o-coupled receptors which could regulate the same spectrum of effectors, such as inhibition of adenylyl cyclase (Aicher *et al*. 2000), the N- and L-type Ca^{2+} channels (Hescheler *et al*. 1987; Johnson *et al*. 1994; Piros *et al*. 1996; Piros *et al*. 1995; Surprenant *et al*. 1990; Tallent *et al*. 1994) and PLC (Johnson *et al*. 1994; Spencer *et al*. 1997), and mobilize intracellular Ca^{2+} (Jin *et al*. 1992). The importance of opioid receptor-mediated activation of the PLC-IP_3 pathway in

achieving physiological and biological effects in pain modulation, and in various sensory systems, including visual, olfactory, and gustatory systems, has been reported.

One of the common features of G-protein coupled receptors is that the receptor signal transduction is mediated by the G-proteins. To test whether the blockage of G-protein eliminates SNC80-induced reduction of ST-evoked EPSCs, the following experiment was conducted. After confirming that SNC80 reduces ST-evoked EPSCs, anti-G protein peptide was microinjected into the cell. Intracellular administration of anti-G protein peptide almost totally eliminated SNC80-induced reduction of ST-evoked EPSCs, indicating G-proteins mediate δ_1-opioid receptor activation of putative gustatory cells within the rostral NST.

G protein coupled receptors consist of the α subunits of Gi/o (Gα subunits) and its $\beta\gamma$ dimeric partners of Gi/o (G$\beta\gamma$). Coupling of opioid receptor with G protein causes dissociation of G$\beta\gamma$ from Gα subunits. We tested whether the SNC80 effect was mediated by Gα and/G$\beta\gamma$ by intracellular administration NF023, a Gα subunit antagonist or suramin, a G$\beta\gamma$ subunit antagonist. Our results showed that both NF023 and suramin eliminated δ_1-opioid receptor responses of ST-evoked EPSCs induced by SNC80, indicating G$\beta\gamma$ subunits mediate δ-opioid receptor responses.

Opioid-induced dissociation of heterotrimeric G proteins produces diverse effects mediated by both a and $\beta\gamma$ subunits. The ability of δ-opioid receptors agonist to stimulate the formation of IP_3 and subsequent intracellular C^{2+} mobilization in NG 108-15 cells have been demonstrated (Jin *et al*. 1992; 1994; Sanchez-Blazquez *et al*. 1999; Smart and Lambert 1996). We tested whether PLC is involved in the δ-opioid receptor-mediated signaling pathway in putative gustatory NST cells. After confirming ST-evoked EPSCs were reduced by SNC80 and that the SNC80 effect was eliminated by BNTX, the cell was given U73122, a PLC inhibitor, or 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-2,5-pyrrolidinedione (U73343), a putative negative control, intracellularly. Intracellular U73122, but not U73343, eliminated SNC80-induced reduction of ST-evoked EPSCs. Furthermore, intracellular administration of +m-3M3FBS, a PLC agonist, mimicked the effect of SNC80. These data indicate that PLC is involved in the δ-opioid receptor-mediated reduction of ST-evoked EPSCs. In addition, it also indicated that the SNC80-induced activation of PLC appears to be mediated via the G$\beta\gamma$ subunits since none of the PTX-sensitive Gα-subunits can activate PLC by themselves (Rhee 2001). It has been suggested that the activation of Gi/o release G$\beta\gamma$ and in turn activate PLC (Park *et al*. 2004).

The PLC cleavages phosphatidylinositol biphosphate (PIP2) and produces IP_3. We examined the effect of IP3(1,4,5) on ST-evoked EPSCs. The ST-evoked EPSCs were reduced by intracellular administration of IP3(1,4,5) in putative gustatory cells within the NST, indicating that IP3 mediates ST-evoked EPSCs.

The main effect of IP3 is to activate IP3 receptors and release Ca^{2+} from intracellular stores (Smart and Lambert 1996). We tested whether 2-aminoethoxydiphenyl borate (2-APB), an IP3 receptor antagonist, blocks SNC80-induced reduction of ST-evoked EPSCs in PbN-projecting rostral NST cells. The results showed that the SNC80 effect was significantly eliminated by 2-APB, indicating that IP3 receptors mediate δ_1-opioid receptor responses of ST-evoked EPSCs.

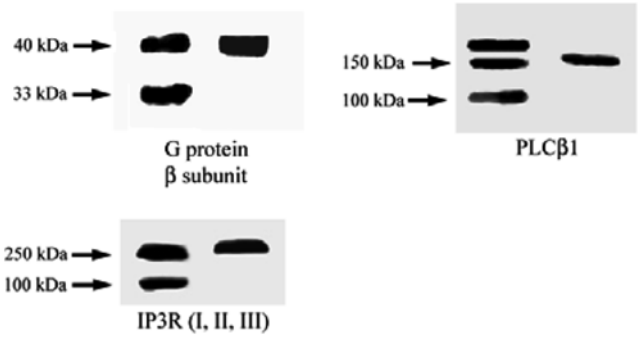

Fig. 4. Western blot analysis showing the presence of G protein β subunit, PLCβ and IP3 receptor (I, II, III) proteins in the rostral portion of the hamster NST. Rabbit polyclonal antibodies to G protein β subunit (1:1250), PLCβ1 (1:750) and IP3 receptor I/II/III (1:100) were used. The experiments were repeated at least three times.

Expression of G β, PLC β1, and IP3 receptor proteins in the rostral NST

We further examined whether Gβ, PLCβ and IP3 receptor proteins are present in the rostral NST tissue using rabbit polyclonal antibodies to Gβ, PLCβ1 and IP3 receptor I/II/III. Western blot analysis demonstrated the presence of Gβ, PLCβ and IP3R receptor proteins in the hamster rostral NST tissue (Fig. 4). These results further support our whole cell recording results that the PLC-IP3 pathway is involved in δ-opioid receptor-mediated reduction of excitatory input to putative gustatory cells within the NST.

Detection of Gβ1 and PLCβ1 mRNA in PbN-projecting single rostral NST cells in which ST-evoked EPSCs were reduced by SNC80

We also performed RT-PCR analysis to check for the presence of Gβ1 and PLCβ1 mRNA in the cytoplasm harvested from single rostral NST cells in which ST-evoked EPSCs were reduced by SNC80 and the reduction of EPSCs was eliminated by BNTX. The photomicrograph in Fig. 5 shows RT-PCR products that corresponds to Gβ1 and PLCβ1 mRNA. These results further support our hypothesis that the PLC-IP3 signaling pathway is involved in the δ-opioid receptor-mediated reduction of ST-evoked EPSCs.

μ-opioid receptor mediated modulation of ^{3}H-glutamate release from the rostral NST tissue

Studies using medullary brainstem slices have provided evidence to suggest that glutamate serves as an excitatory neurotransmitter between afferent fibers and second-order

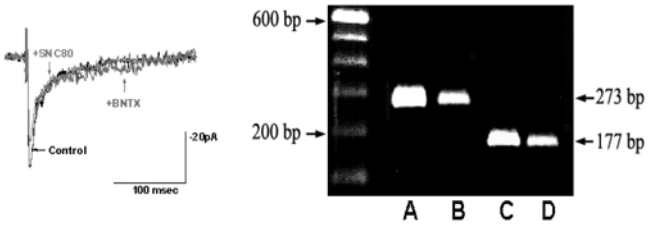

Fig. 5. Detection of G protein β1 subunit (Gβ1) and PLCβ1 protein mRNA from single PbN-projecting rostral NST cells. Current traces from a PbN-projecting rostral NST cell showing that 25 μM SNC80 reduced the ST-evoked EPSC and that the SNC80 effect was blocked in the presence of 2 μM BNTX *(left)*. After the recording, the neuron was prepared for single cell RT-PCR (*right*). The left lane is a 100-bp DNA ladder. EB-stained agarose gel showing the RT-PCR DNA products that correspond to Gβ1 and PLCβ1 mRNAs. *Lanes A* and *C* showing RT-PCR product that correspond to Gβ1 and PLCβ1 mRNAs from whole rostral NST tissue (35 cycles). *Lanes B* and *D* showing RT-PCR product that correspond to Gβ1 and PLCβ1 mRNAs from single putative gustatory NST cells in which ST-evoked EPSCs were reduced by SNC80 and that the SNC 80 effect was blocked by BNTX (70 cycles). Experiments were repeated at least three times.

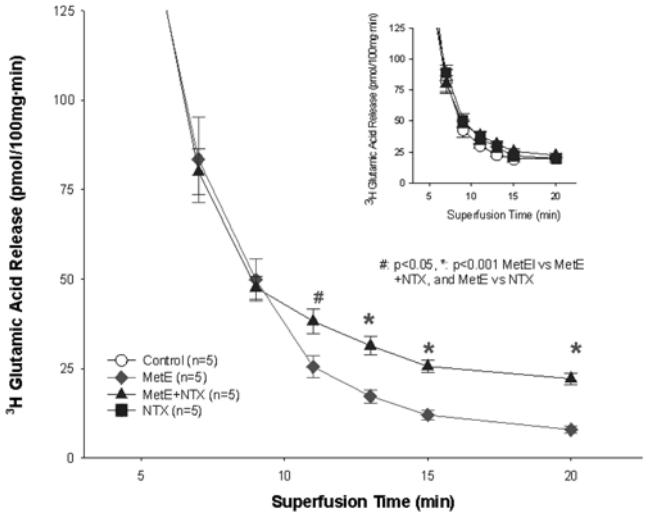

Fig. 6. The effect of MetE of on ^{3}H-glutamate efflux from the rNST tissue. 10 μM MetE (*diamond*) significantly reduced ^{3}H-glutamate efflux and the presence of 20 μM NTX (MetE + NTX, solid triangle) eliminated the reduction of ^{3}H-galutamate efflux. *Upper right*: ^{3}H-galutamate efflux in normal aCSF (*open circle*), 20 μM NTX (*solid square*) and 10 μM MetE + 20 μM NTX (*solid triangle*). # $p < 0.05$; * $p < 0.001$.

rostral NST neurons. For instance, it has been reported that responses of cells in the gustatory NST to the ST stimulation were reduced or blocked by both *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonists (Wang and Bradley 1995). We have previously reported that microinjection of kynurenic acid (KYN), a broad-spectrum excitatory amino acid antagonist, and 6-cyano-7-nitroquinoxaline-2-, 3-dione (CNQX), an AMPA/kainate receptor antagonist, into near the recorded cell, reversibly blocked responses of taste-responsive NST cells elicited by both anodal current and chemical stimulation of the anterior tongue. These data implicate glutamate as an excitatory neurotransmitter between chorda timpani (CT) gustatory fibers and taste-responsive NST cells. To investigate the influence of opioids on glutamate autorelease from the terminals of the ST, the following experiments were performed. After the rostral NST was punched out using a SS tubing (ID = 2.0 mm) from brainstem slices, the tissue (NST) was weighed and kept in a holding bath at 30°C in normal artificial cerebrospinal fluid (aCSF) saturated with 95% oxygen and 5% carbon dioxide. The tissue was then incubated in normal aCSF or aCSF with 1 mM ^{3}H-glutamic acid (final concentration) saturated by 95% oxygen and 5% carbon dioxide for 30 min at 30°C. The tissue was submerged to the superfusion solution in the superfusion chamber and maintained at 30°C with a constant flow rate of 1.0 ml/min. The amount of radioactive material (^{3}H-glutamic acid) that effluxed from each treated NST was collected at 1 min intervals while the NST was superfused with normal aCSF containing various opioid receptor agonists and antagonists. This process continued for 20 min. Each 0.2 ml superfusate collected during this period was retained and added to 15 ml of scintillation fluid. The samples were counted using a liquid scintillation spectrometer and corrected for efficiency using an automatic external standard.

Administration of MetE into superfusion bath significantly reduced ^{3}H-glutamate efflux and the reduction of ^{3}H-glutamate efflux was eliminated by NTX, indicating that opioid receptors are involved in the modulation of glutamate release from the fiber terminals of the ST (Fig. 6). MetE-induced reduction of ^{3}H-glutamate efflux was also eliminated by CTOP, indicating opioid-induced reduction of ^{3}H-glutamate efflux is μ-opioid receptor mediated. μ-opioid receptor mediated reduction of ^{3}H-glutamate efflux was further verified by the following experiments. Application of [D-Ala^2, N-MePhe^4, Gly-o]-enkephalin (DAMGO), a selective agonist for the μ-opioid receptors, reduced ^{3}H-glutamate efflux and the DAMGO effect was eliminated by the presence of CTOP (Fig. 7). In addition, bath application of neither SNC80, nor BRL-52537, a selective κ-opioid receptor agonist, affected ^{3}H-glutamate efflux, indicating neither δ- nor κ-opioid receptors are involved in ^{3}H-glutamate efflux.

Bath application of DL-*threo*-beta-benzyloxyaspartate (DL-TBOA), a glutamate re-uptake inhibitor, did not affect ^{3}H-glutamate efflux. The presence of DAMGO reduced ^{3}H-glutamate efflux but DL-TBOA did not eliminate the effect of DAMGO ^{3}H-glutamate efflux, suggesting ^{3}H-gutamate efflux is presynaptic release.

In addition, we examined whether μ-opioid receptor protein is present in the rostral NST. The detection of μ-opioid receptor protein was performed by Western blot analysis using rabbit polyclonal antibody to μ-opioid receptor. Western blot analysis showed the presence of μ-opioid receptor protein (72 kDa) in the rostral NST tissue (Fig. 8).

The result that presynaptic μ-opioid receptor mechanism

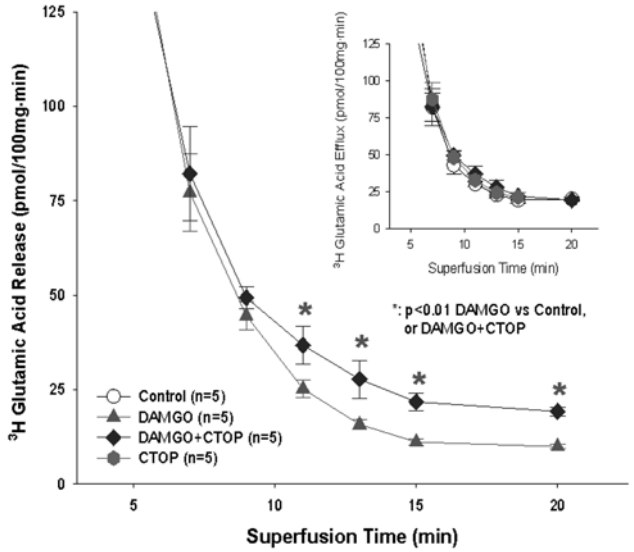

Fig. 7. The effect of 25 μM [D-Ala^2, N-MePhe^4, Gly-o]-enkephalin (DAMGO, *solid triangle*) and 2 μM D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP,) on ^{3}H-glutamate efflux from the rNST tissue. 25 μM DAMGO significantly reduced ^{3}H-glutamate efflux and the presence of 2 μM (DAMGO + CTOP, *solid diamond*) eliminated the reduction of ^{3}H-galutamate efflux. *Upper eight*: ^{3}H-galutamate efflux in normal aCSF (*open circle*), CTOP and DAMGO + CTOP. * $p < 0.001$.

plays a role in inhibitory modulation of ^{3}H-glutamate efflux is consistent with the result of our previous immunohistochemical studies. We have reported that μ-opioid receptor immunoreactivity was expressed in the fiber terminals of the ST and neuropil within the rostral NST.

Conclusions

Anatomical, immunohistochemical, electrophysiological and biochemical studies together offer convincing evidence for the involvement of opioids in the modulation of the synaptic transmission within the NST. It is suggested that glutamate serves as the primary excitatory neural transmitter between the primary gustatory fibers and taste-responsive neurons in the NST. Opioids mediate the reduction of excitatory input from the peripheral gustatory fiber terminals to second-order gustatory cells within the NST through presynaptic μ-opioid receptor and postsynaptic δ_1-opioid receptor mechanisms. The PLC-IP3 signaling pathway mediates postsynaptic δ_1-opioid receptor mechanism-induced reduction of excitatory input from the gustatory fiber terminals to gustatory cells within the NST.

Acknowledgements

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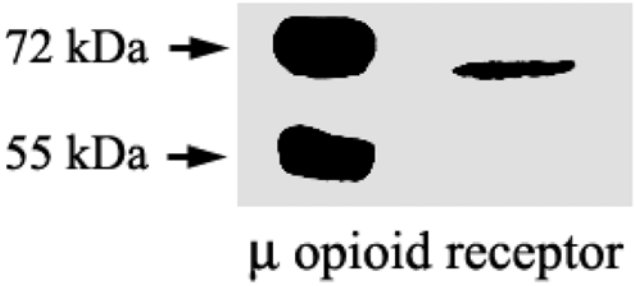

Fig. 8. Western blot analysis shows the presence of μ-opioid receptor protein in the rostral NST. Rabbit polyclonal antibody to μ-opioid receptor (1:750) was presented. Left lane is pre-stained SDS-PAGE standard, and the right lane is Western blot analysis data to μ-opioid receptor. The experiment was repeated at least three times.

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