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PGE₂ DEPRESSES SOLITARY TRACT-MEDIATED SYNAPTIC TRANSMISSION IN THE NTS

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Abstract

Prostaglandin E₂ (PGE₂) is a prototypical inflammatory mediator that excites and sensitizes cell bodies (Kwong and Lee, 2002; 2005) and peripheral nerve terminals (Ho et al. 2000) of primary vagal sensory neurons. Nearly all central nerve terminals of vagal afferents are in the nucleus tractus solitarius (NTS), where they operate with a high probability of release (Doyle and Andresen, 2001). We studied the effect of PGE₂ on synaptic transmission between tractus solitarius afferent nerve terminals and the second order NTS neurons in brain stem slices of Sprague Dawley rats. Whole-cell patch recording in voltage clamp mode was used to study evoked excitatory postsynaptic glutamatergic currents (evEPSCs) from NTS neurons elicited by electrical stimulation of the solitary tract (ST). In 34 neurons, bath applied PGE₂ (200 nM) decreased the evEPSC amplitude by 49 ± 5 %. In 22 neurons, however, PGE₂ had no effect. We also tested 15 NTS neurons for capsaicin sensitivity. Seven neurons generated evEPSCs that were equally unaffected by PGE₂ and capsaicin. Conversely, evEPSCs of the other 8 neurons, which were PGE₂-responsive, were abolished by 200 nM capsaicin. Furthermore, the PGE₂-induced depression of evEPSCs was associated with an increase in the paired pulse ratio and a decrease in both the frequency and amplitude of the spontaneous excitatory postsynaptic currents (sEPSCs) and TTX-independent spontaneous miniature excitatory postsynaptic currents (mEPSCs). These results suggest that PGE₂ acts both presynaptically on nerve terminals and postsynaptically on NTS neurons to reduce glutamatergic responses.

Keywords

Glutamate; AMPA receptors; Presynaptic; Postsynaptic

Many neurons in the nucleus of the tractus solitarius (NTS) receive nerve terminals whose axons arise from cell bodies of vagal primary afferent neurons located in the nodose and jugular vagal ganglia. These primary vagal afferents convey diverse sensory information from a variety of receptors located in cardiovascular, respiratory, gastrointestinal and other organs to the NTS neurons in the medulla (Contreras, 1982). Thus, the first monosynaptic contact for myelinated A-type and unmyelinated C-type axons of primary vagal afferent fibers are the second-order neurons in the NTS. These fibers reach second-order NTS neurons via the solitary tract (Doyle

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et al. 2002) where they evoke ionotropic glutamatergic and/or peptidergic synaptic potentials (Gatti et al. 1995; Aylwin et al. 1997).

For many decades, prostaglandins have been well known to be major mediators in the response to immune challenges. More recently, these lipid mediators have been implicated in physiological and pathophysiological functions in the CNS and PNS neurons (Miettinen et al. 1997; Montine et al. 1999; Liu et al. 2005). PGE₂ is a major prostanoid that is involved in a myriad CNS functions (Wolfe and Coceani, 1979; Ho et al. 1999; McCullough et al. 2004). PGE₂ is synthesized in the CNS by neurons and glia cells (Bishai and Coceani, 1992; Guay et al. 2004). Cyclooxygenase 2 (COX-2) converts free arachidonic acid to the intermediate prostaglandin H₂ (PGH₂). PGH₂ in turn is converted to PGE₂ by the enzyme PGE₂ synthase (Thoren et al. 2003). In addition to localized formation in the CNS, PGE₂ is synthesized outside the CNS and can reach NTS neurons because this area of the brain lacks a complete blood-brain barrier (Gross et al. 1990).

Functional PGE₂ receptors are thought to exist on primary vagal afferents because many distinct types of voltage-gated ionic channels and ligand-gated channels in vagal afferents are modified by exogenous PGE₂. These include: calcium-dependent potassium currents (Fowler et al. 1985), leak currents (Undem and Weinreich, 1993), hyperpolarizing-activated cationic currents (Ingram and Williams, 1996), TTX-resistant sodium currents (Gold et al. 2002; Kwong and Lee, 2005), and the ionotropic receptors TRPV1 and 5-HT₃ (Kwong and Lee, 2002). In addition, PGE₂ binding has been reported in the medial NTS (Matsumura et al. 1992), an area rich in afferent innervation. Thus, it is likely that functional PGE₂ receptors are present on vagal afferent nerve terminals in the NTS (Ek et al. 2000). In support of this possibility, Sekiyama et al. (1995) reported that the magnitude of ST evoked excitatory postsynaptic currents (evEPSCs) was facilitated by PGE₂ in three of eight NTS neurons examined. The nature of the PGE₂ receptors mediating this effect has not yet been clarified.

PGE₂ can interact with four subtypes of G-protein-mediated receptors: EP₁, EP₂, EP₃, and EP₄ (Narumiya et al. 1999). The NTS has been shown to express all four receptors (Matsumura et al. 1992; Ek et al. 2000; Nakamura et al. 2000). Likewise, EP₁₋₄ receptors are present in the cell bodies of nodose ganglia neurons (Matsumoto et al. 2005), that project their central processes to the NTS. The purpose of this work was to determine the effect of PGE₂ on synaptic transmission between ST afferents and second-order NTS neurons.

EXPERIMENTAL PROCEDURES

Slice preparation

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore. Young-Adult Sprague Dawley male rats (n = 42), 14 to 20 days old, were anesthetized with ketamine (30 mg/kg) and decapitated. The brains were rapidly removed, and 150 μm thick brainstem slices were cut at 4°C with a vibratome (VT 1000S, Leica Microsystems Inc. Nusslock, Germany). Coronal slices were kept in a holding chamber that contained artificial cerebrospinal fluid (aCSF) composed of (mM): 124 NaCl, 25 NaHCO₃, 1.2 NaH₂PO₄, 3 KCl, 2 MgSO₄, 2 CaCl₂, and 15 Glucose. The pH and Osmolarity were adjusted to 7.2 – 7.4, and 305 mOsm, respectively. The aCSF continuously bubbled with 95% O₂ - 5% CO₂. Following a 30 min recovery period at 30°C, the slices were maintained in the same chamber at room temperature (23 – 25°C). Slices were transferred to the recording chamber to study the effects of PGE₂ at 32°C.

Electrical stimulation and recording

Bipolar stimulation electrode (paired 50 μ m stainless steel wires, insulated except at the tips) was placed visually in the ST, ipsilateral to the recording site to activate primary sensory afferent fibers (Fig. 1A and 1B). The atlas of Paxinos and Watson (1998) was used for identification and nomenclature of anatomical structures. Constant-voltage pulses were delivered through a stimulus isolation unit, driven by a pulse generator (Grass S88, Astro-Med, West Warwick, RI). The ST was focally stimulated at 0.2 Hz with 10 to 50 V pulses (150 μ s duration) for a total of 10 stimuli. To eliminate the potential influence of activation of inhibitory interneurons during ST stimulation, slices were superfused with the aCSF solution containing GABA_A and glycine receptor antagonists, bicuculline (10 μ M) and strychnine (1 μ M), respectively. Since PGE₂ can affect also adenosine receptors we add to the aCSF solution the adenosine receptors antagonist, DPCPX (1 μ M).

To test whether ST-NTS responses were monosynaptic, we measured synaptic jitter. We determined the onset latencies for the evEPSCs, the time between the shock artifact and the onset of the evEPSCs. Jitter was calculated as the shock-to-shock variability in the onset latency. An onset latency variability of < 0.5 ms was taken as evidence for a putative monosynaptic response (Chen et al 2002). Paired-pulse ratio is defined as the ratio of the second evEPSC peak amplitude divided by the first evEPSC peak amplitude using a 20 ms interpulse interval.

Recordings were made at 32°C in a submersion type recording chamber perfused with aCSF at 2 ml/min. Patch electrode placement and NTS neuron selection were performed with near-infrared differential interference contrast microscopy, using a 40X (0.9 N.A.) water immersion objective attached to a fixed-stage, upright microscope (Nikon Eclipse, E600 FN, Tokyo, Japan).

Whole-cell recordings

Whole-cell voltage-clamp recordings were obtained from second-order neurons located in the caudal (Fig. 1A) and intermediate part of the NTS (Fig. 1B), with an Axopatch 200A amplifier in conjunction with pClamp 7 software (Axon Instruments, Foster City, CA). Currents were filtered at 2 – 5 KHz and digitized at 5 – 20 KHz with a DigiData 1200 interface (Axon Instruments). The resistances of the patch electrodes ranged from 5 to 8 M Ω . The intracellular recording solution contained (in mM): 144 D-gluconic acid potassium salt, 2 NaCl, 2 MgCl₂, 10 EGTA, 10 HEPES, 0.2 GTP and 3 ATP, pH 7.2 (310 mOsm). Chemicals for intracellular and extracellular solutions were obtained from Sigma (St. Louis, MO). Neurons included for study had holding currents < 100 pA with membrane potentials ranging between –60 and –65 mV for a 5 min control period, and had input resistances > 200 M Ω . Seal resistances were > 1 G Ω , and series resistance was always < 25 M Ω with 60% compensation.

Analysis of synaptic currents

To determine the action of PGE₂ on monosynaptic transmission in the NTS, the following synaptic responses were examined: (1) evEPSC peak amplitude, (2) paired-pulse ratio (PPR), (3) frequency and amplitude of spontaneous EPSCs (sEPSCs), and (4) the TTX-insensitive frequency and amplitude of miniature EPSCs (mEPSCs). For PPR measurements, ST pair stimuli were delivered at an interpulse interval of 20 ms at 0.2 Hz and the peak amplitudes of the first and second paired evEPSCs were recorded for at least 2 min before (control) and for at least 5 min after the onset of PGE₂ perfusion. For measurements of mEPSCs, slices were superfused with aCSF containing TTX (1 μ M) and the frequency and amplitude of mEPSCs recorded before (2 min) and during a five min perfusion with aCSF containing PGE₂. The frequency and the amplitude sEPSCs were analyzed by Mini Analysis Program (Synaptosoft Inc, Fort Lee, New Jersey). The baseline noise was < 8 pA. The threshold for detecting sEPSCs

varied between 8 to 15 pA, this variability did not affect the magnitude of decrease in the sEPSC amplitude induced by PGE₂. Data are expressed as mean $\pm$ S.E.M unless otherwise indicated. Differences were considered to be significant at $P < 0.05$ for Student paired t-test and $P < 0.01$ for Kolmogorov Smirnov test. The onset latency for each evEPSC was determined from 10 evEPSC delivered at 0.2 Hz. The magnitude of the stimulus artifacts was digitally reduced.

Analysis of passive and active membrane proprieties

For evaluating the neuronal excitability, 750 ms depolarizing current steps (1- and 2-times threshold current, rheobase) were applied in the current-clamp mode and the number of action potential generated were counted following each stimulus. In current clamp, input resistance was calculated by applying 450 ms hyperpolarizing current steps (50 pA) and measuring the peak steady-state amplitude of the resulting voltage transients. R_{in} was determined at resting membrane potential, -60 to -65 mV. Active membrane proprieties were evaluated by analyzing various action potential waveforms. Action potential (AP) duration was measured at a membrane potential of 0 mV. Overshoot and afterhyperpolarization (AHP) magnitudes of action potential were estimated as the peak amplitude of AP upstroke from 0 mV and down stroke from the resting membrane potential, respectively. AP threshold potential was defined as the membrane potential where the rate of depolarization starts to increase abruptly as determined by differentiation of membrane potential with respect to time (dV/dt). The minimum current needed to evoke an AP was defined as threshold current or rheobase.

Chemicals

Reagents were prepared immediately before use from freshly prepared or frozen stock solutions and dissolved in aCSF. The following chemicals were obtained from Tocris (Ellisville, MO): AP5 (D-2-amino-5-phosphonopentanoic acid), bicuculline, DPCPX (8-Cyclopentyl-1,3-dipropylxanthine), strychnine and CNQX (6-cyano-7-nitroquinoxaline). SC19220 (8-chloro-dibenz(b,f)(1,4)oxazepine-10(11H)-carboxylic acid or 2-acethylhydrazide 10(11)-carboxylic acid), AH 23848 [(4Z)-7-[(rel-1S,2S,5R)-5-((1,1'-Biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl)-4-heptenoic acid calcium salt], Sulprostone ((5Z,11 α ,13E,15R)—11,15-Dihydroxy-9-oxo-16-phenoxy-17,18,19,20-tetranorprosta-5,13-dienoic acid methane sulfonamide), Butaprost ((1R,2R,3R)-3-Hydroxy-2-[(1E,4R)-4-hydroxy-4-(1-propylcyclobutyl)-1-butenyl]-5-oxocyclopentaneheptanoic acid methyl ester), indomethacin, capsaicin, PGE₂ and TTX were purchased from Sigma (St Louis, MO).

RESULTS

The activation of cranial visceral afferent axons (and other axons) within the solitary tract (ST) can evoke short-latency, $2.3 \text{ ms} \pm 0.15$ excitatory postsynaptic currents (EPSCs) with an onset latency variability ("jitter") of $0.34 \text{ ms} \pm 0.02$, supporting the contention of a monosynaptic connection from the primary sensory afferent terminals originating from the ST to second order NTS neurons (Miles, 1986; Chen et al. 1999; Doyle et al. 2001). Only synapses revealing short latencies and low jitter, as described above, were accepted for study. In 56 neurons studied, the ST-evEPSC averaged 222.4 ± 9.9 pA. In these cells, the whole cell capacitance and membrane input resistance was 20.4 ± 1.3 pF and 335 ± 32.2 M Ω , respectively. Synaptic transmission consistently displayed synaptic depression in response to the paired-pulse stimulation of the ST. The PPR of the evEPSC averaged 0.7 ± 0.03 ($n = 56$), similar to values reported by Doyle and Andresen (2001), Sekizawa et al. (2003).

PGE₂ decreased the evEPSC amplitude

In a series of control experiments, we determined that over a period of five to ten min (in aCSF solution only) the mean evEPSC amplitude was reduced from 0 to 20 % of control (11 ± 8.0 pA; $n = 35$). Thus, we arbitrarily choose any PGE₂ effect greater than 20 % of baseline evEPSC

amplitude as a significant PGE₂-evoked response. Fifty-six second-order NTS neurons were subjected to perfusion with 100 – 200 nM PGE₂-containing aCSF for three to five min. Sixty percent of these neurons showed a PGE₂-mediated depression in the amplitude of evEPSCs synaptic transmission averaging of $49 \pm 5.0\%$ ($p < 0.01$, $n = 34$; Fig. 2B). The amplitude of evEPSCs synaptic transmission was unaffected in the remaining 22 neurons.

Because a wide range of variability existed in the input resistance (R_{in} range: 100 to 900 M Ω), we examined whether cells with different R_{in} values were related to the magnitude of depression in synaptic transmission induced by PGE₂. We divided the neurons studied in two groups: a first group with $R_{in} < 300$ M Ω and a second group with $R_{in} > 300$ M Ω . PGE₂-induced decreases in the magnitude of the evEPSCs were of similar value in both groups of neurons ($p > 0.05$). Thus, PGE₂-mediated depression of ST-evEPSCs was unrelated to changes in R_{in} in the second order NTS neurons.

Effect of PGE₂ on passive and active membrane properties

Passive and active membrane properties were examined during the peak of the PGE₂ effect on synaptic transmission. With the exception of a reduction in resting input resistance (R_{in}), none of the membrane properties examined were significantly affected by PGE₂ treatment (Table 1). The decrease in R_{in} cannot explain the depression of synaptic transmission because the percentage decrease in evEPSC peak amplitude recorded in 18 neurons did not correlate with the percentage decrease in R_{in} recorded in the same neurons ($r = 0.095$, $P = 0.73$). In addition, changes in evEPSC recorded in voltage-clamp mode should not be influenced by changes R_{in} recorded in current-clamp mode. The apparent increase in rheobase was not statistically significant due to variability in the data set.

Effects of PGE₂ on capsaicin-sensitive and capsaicin-resistant NTS neurons

Doyle et al. (2002) characterized two classes of medial nucleus tractus solitarius neurons based upon their susceptibility to blockade of afferent synaptic transmission by bath application of 200 nM capsaicin. Capsaicin-sensitive synapses had longer synaptic latencies for evEPSCs relative to capsaicin-resistant synapses suggesting that the former may be associated with slower conducting action potentials mediated by TRPV1-containing C fibers. We tested whether PGE₂-induced depression of evEPSCs was associated with capsaicin-sensitive C-fiber synapses. In 15 NTS neurons we added capsaicin (200 nM) to the superfusate at the end of the experiment. In 50 % (8/15) of these neurons 200 nM PGE₂ depressed evEPSC by $42.0 \pm 7.8\%$. Subsequent capsaicin application to the PGE₂ sensitive synapses caused a complete block of evEPSC within 10 min (Fig. 3A). By contrast, in the seven NTS neurons where PGE₂ did not produce significant depression in the amplitude of the evEPSC, capsaicin application for 10 – 20 min produced no observable change in evEPSCs (Fig. 3B). Thus, it appears that PGE₂ exerts its effects on NTS neurons whose synaptic input are likely capsaicin sensitive.

Nature of the glutamatergic receptors affected by PGE₂

Most glutamatergic responses appear to be mediated by AMPA receptors (Andresen and Yang, 1990); However Bonham and Chen (2005) indicated that second order NTS neurons also possess functional NMDA receptors. We were interested in determining which glutamatergic receptors were affected by PGE₂. Under the experimental conditions used in which extracellular Mg²⁺ and the holding membrane potential (–60 mV) suppresses NMDA receptor activity; it is unlikely that NMDA receptors would significantly contributed to the evEPSC. Nonetheless, we examined the effects of bath applying inhibitors of NMDA and AMPA receptors on evEPSCs that were depressed by PGE₂. Application of APV (100 μ M), a specific antagonist of NMDA receptors, did not measurably affect the evEPSC ($n = 4$; Fig 4). By contrast, addition of the AMPA/Kainate receptor antagonist CNQX (50 μ M) to the APV-containing solution resulted in a complete blockade of the evEPSC (4/4, Fig 4). Thus, we

conclude that PGE₂ depresses AMPA/Kainate receptor-mediated synaptic transmission in the NTS neurons.

Threshold and maximum effect of PGE₂

In the current work we did not determine concentration-response relation for PGE₂ effect on evEPSCs. However we did estimate threshold and maximum concentrations for the actions of PGE₂. In five NTS neurons 3 nM PGE₂ did not elicit a measurable change in evEPSCs waveform. Ten nM PGE₂ appears to be the minimal concentration necessary to elicit a detectable PGE₂ effect on evEPSCs ($25.6 \pm 12.7\%$ $n = 5$). On the other hand, 100 nM PGE₂ appears to produce a maximum effect on evEPSCs because a 1000 nM PGE₂ elicited no significant ($P > 0.05$ $n = 11$) increase in inhibition of evEPSC amplitude greater than that observed with 100 nM ($44.5 \pm 13.1\%$ $n = 11$). Similar results were observed when the same range of concentration of PGE₂ were applied to the same neuron ($n = 4$).

In an additional series of experiments, we investigated whether the steep concentration-response relation might be due to the presence of endogenous PGE₂. In, six neurons from control slices, we measured the effect of PGE₂ (200 nM) on evEPSCs. Then, six additional slices were incubated at least one hour in an aCSF solution containing indomethacin (3 μ M) and the effect of PGE₂ (200 nM) on evEPSCs was determined. At this concentration of indomethacin, both cyclooxygenase 1 (COX I) and cyclooxygenase 2 (COX II) enzymatic activity should be abolished (Wolfe and Coceani, 1979). The average decrease in the evoked response in control ($47.3 \pm 6.1\%$) was not significantly different ($p > 0.05$) from that of indomethacin-treated neurons ($52.2 \pm 5.5\%$), suggesting that endogenous PGE₂ does not affect the peak amplitude of the evEPSCs recorded in second order NTS neurons following ST electrical stimulation.

Presynaptic vs. postsynaptic site of PGE₂ effect

In order to evaluate whether PGE₂-induced depression of synaptic activity was due to a presynaptic effect on ST nerve terminals or at a postsynaptic site on second-order NTS neurons, we measured the PPR of the peak amplitude of the evEPSCs, the frequency and the amplitude of the spontaneous excitatory postsynaptic currents (sEPSC), and action potential-independent synaptic activity, miniature EPSCs (mEPSCs).

PGE₂ increases the paired-pulse ratio—We measured PPR of the evEPSCs before and during the peak PGE₂-induced depression of the evEPSC. In 70 % of the PGE₂-sensitive neurons (24/34) the paired pulse ratio increased by $47.9 \pm 9.5\%$ (paired t-test, $p < 0.01$, $n = 24$; Fig 5B). In the other 30 % of these neurons (10/34) five neurons showed a decrease in the PPR and five neurons showed no change in the PPR. As illustrated by the traces in Fig 5A, there was a decrease primarily in the amplitude of the first evEPSC of the paired evEPSCs. The effects of PGE₂ on PPR were maximal at approximately 3 – 5 min after the start of perfusion with PGE₂ and PPR recovered partially or completely within approximately 20 min after washout of PGE₂.

PGE₂ depressed the frequency and the amplitude of spontaneous EPSCs—PGE₂ (200 nM) decreased the frequency of the sEPSC in 20/34 PGE₂ sensitive-neurons (60 %). An example of continuous recordings of sEPSC before, during, and after perfusion with PGE₂ is shown in Fig. 6A. The cumulative probability of the sEPSC inter-event intervals was shifted to the right in the presence of PGE₂ (Fig. 6B right panel), indicating a decrease in the frequency of events (control, 3.7 ± 1.8 Hz; PGE₂ 2.2 ± 0.7 Hz $P < 0.01$ $n = 20$). In the same group of cells, the cumulative probability of the sEPSC amplitude was shifted to the left (Fig. 6B left panel), indicating a decrease in the amplitude of these events (control, 30 ± 4 pA; PGE₂ 24 ± 3 pA $P < 0.01$ $n = 20$). In the other 40 % of the PGE₂ sensitive-neurons ($n = 14$)

PGE₂ did not affect the frequency of sEPSCs (control, 1.8 ± 0.5 Hz; PGE₂ 2.1 ± 0.5 Hz $P > 0.05$). In the same group of cells, the PGE₂ also did not significantly ($p > 0.05$) affect the amplitude of sEPSCs.

PGE₂ depressed the frequency and the amplitude of spontaneous miniature EPSC (mEPSC)—In the presence of 1 μ M TTX, PGE₂ (200 nM) decreased the frequency and the amplitude of the mEPSC in 50 % of the neurons studied (4/8). An example of continuous recordings of mEPSC before and during treatment with PGE₂ is shown in Fig. 7A. The cumulative probability of the mEPSC inter-event intervals was shifted to the right in the presence of PGE₂ (Fig. 7B right panel), indicating a decrease in frequency (control, 4.4 ± 1.9 Hz; PGE₂, 2.3 ± 1.4 Hz $P < 0.05$). In the same group of cells, the cumulative probability of the mEPSC amplitude was shifted to the left (Fig. 7B left panel), indicating a decrease in the amplitude (control, 26 ± 7 pA; PGE₂, 20 ± 5 pA $P < 0.05$). Whereas in the other half of neurons studied, mEPSC amplitude and frequency (control, 3.3 ± 1.1 Hz; PGE₂, 2.9 ± 1.9 Hz $P > 0.05$) was not significantly changed after 10 min superfusion with PGE₂. These results demonstrate that in the 60 % of the neurons where the peak amplitude of the evEPSC is decreased after PGE₂ bath application, the majority of these neurons (60 – 70 %) show: (a) facilitation in the PPR, (b) decrease in the frequency of sEPSC and mEPSC, and (c) decrease in the amplitude of sEPSC and mEPSC. Such results suggest that PGE₂ can depress synaptic transmission by both pre- and postsynaptic sites-of-action at second order NTS neurons.

Effects of prostanoid receptors agonist and antagonist on PGE₂ modulation of synaptic transmission in the NTS

In order to examine the type of PGE₂ receptors underlying the depression of synaptic transmission at NTS synapses, we tested the actions of a panel of PGE₂ receptor agonist and antagonist reagents. In four neurons bath application of the EP₁ receptor antagonist (SC19220, 20 μ M for 5 min) alone did not measurably affect the peak amplitude of the control evEPSCs or control sEPSCs ($p > 0.05$). This concentration of SC19220 is reported to be five times the EC₅₀ (Rakovska and Milenov, 1984). SC19220 application also did not modify the depressive effects produced by 200 nM PGE₂ on synaptic transmission. The peak amplitude of the evEPSC decreased by $45 \pm 10\%$ ($n = 4$). The cumulative probability of the sEPSC inter-event intervals was shifted to the right in the presence of PGE₂ plus SC19220 (Fig. 8A right panel), indicating a decrease in frequency (control, 4.6 ± 1.8 Hz; PGE₂, 2.4 ± 1.2 Hz $P < 0.05$). In the same group of cells, the cumulative probability of the sEPSC amplitude was shifted to the left by PGE₂ application, indicating a decrease in the amplitude (Fig. 8A left panel). Therefore the EP₁ receptor appears not to mediate the PGE₂ effects.

Because SC19220, did not affect any of the synaptic responses measured or modify the PGE₂ actions, we simply added an EP₄ antagonist to the solution already containing the EP₁ antagonist. Thus, in additional four neurons, we tested the effects of a 5 min bath application of a mixture of EP₁ (SC19220, 20 μ M) and EP₄ (AH 23848, 30 μ M) receptor antagonists. Bath application of this mixture of antagonists alone did not measurably affect the peak amplitude of the control evEPSC nor the depression in the evEPSCs produced by bath applied 200 nM PGE₂. The cumulative probability of the sEPSC inter-event intervals was shifted to the right in the presence of PGE₂ (Fig. 8B right panel), indicating that a decrease frequency still occurred (control, 3.7 ± 0.39 Hz; PGE₂, 1.8 ± 0.3 Hz $P < 0.05$). In the same group of cells, the cumulative probability of the sEPSC amplitude was not shifted, indicating no change in the amplitude (Fig. 8B left panel). Thus, EP₄ receptors appear to regulate the magnitude of sEPSCs.

In seven neurons bath application of the EP₂ receptor agonist (Butaprost, 10 μ M for 5 min) alone did not measurably affect the peak amplitude of the control evEPSCs ($p > 0.05$). In one of these cells the subsequent application of 200 nM PGE₂ produced a 51 % decrease in the

evEPSC. In three neurons bath application of the EP₃ receptor agonist (Sulprostone, 10 μ M for 5 min) did not measurably affect the peak amplitude of the control evEPSCs ($p > 0.05$). In one of these cells, subsequent application of 200 nM PGE₂ resulted in a decreased evEPSC by 39 %. In a preliminary survey, EP₂ and EP₃ agonist produced conflicting results when tested for mimicking PGE₂-induced changes in sEPSC. Thus, we did not study the receptor pharmacology of PGE₂ actions on these synaptic potentials.

DISCUSSION

Our data shows that PGE₂ can depress the synaptic transmission between primary vagal sensory afferent fibers and second-order intermediate and caudal NTS neurons via pre- and postsynaptic sites-of-action. We base this interpretation on several observations: (1) PGE₂ altered the paired pulse ratio (PPR) in 85 % of the synapses. Changes between the magnitude of the first and second synaptic responses elicited by two presynaptic stimuli delivered at relatively short intervals (20 ms) can be quantified as the PPR. Changes in the PPR are used to test alterations in the probability of transmitter release, with an increase in the PPR being associated with a decrease in the probability of transmitter release (Thomson, 2000). We observed that, PGE₂-elicited increases in the PPR in 70 % of the synapses depressed by PGE₂. Thus, it is likely that PGE₂ has a presynaptic site-of-action. (2) At the same time that PGE₂ increased the PPR, it also inhibited the frequency of spontaneous, EPSC (sEPSCs), and the frequency of action potential-independent (miniature) EPSCs (mEPSCs). Decrease in the frequency of appearance of these currents also suggests PGE₂ can act presynaptically. (3) Finally, PGE₂ caused a decrease in the amplitudes of the sEPSCs and mEPSCs, an effect traditionally indicative of a postsynaptic site-of-action.

In brain stem preparations, sEPSC are unlikely to arise from spontaneous ST action potential activity. They could emanate from tonically firing interneurons in the brain stem circuits and/or from spontaneous mEPSCs activity. We did not directly examine, in the same cell the relative contribution of AP-dependent (sEPSCs) and AP-independent (mEPSCs) events. However, our population data obtained from measurements of spontaneous events in control solutions and in solutions containing TTX reveal similar frequency of spontaneous events: 3.7 ± 1.8 and 4.4 ± 1.9 Hz for spontaneous events before and in the presence of TTX, respectively. Thus, it appears that the majority of spontaneous activity under the conditions of our experiment appears to be attributable to mEPSCs. These results are in agreement with those reported by Kato and Shigetomi (2001) who observed in NTS neurons that sEPSC and mEPSC frequencies were similar: 4.2 ± 1.0 Hz and 3.9 ± 1.0 Hz in control solution and in the presence of TTX respectively.

ST-mediated evEPSCs are due to release of glutamate (Andresen and Yang, 1990; cf., our Fig. 4) while interneurons within the NTS can release a plethora of neurotransmitters on to second order neurons (Andresen and Kunze, 1994). It is likely that some of PGE₂'s presynaptic actions, those affecting sEPSCs, are not unique to glutamatergic synapses and involve some modification of neurosecretion common to most chemical synapses. One potential presynaptic target might be voltage-gated Ca²⁺ channels in nerve terminals because PGE₂ has been reported to decrease voltage-gated Ca²⁺ currents in both peripheral (Ikeda, 1992; Borgland et al. 2002) and central neurons (Chen and Bazan, 2005).

At the first synapse made by primary vagal sensory neurons in the NTS, PGE₂ has been reported to potentiate synaptic transmission in a subpopulation of synapses (Sekiyama et al. 1995). In three of eight synapses tested, PGE₂ (1 μ M) increased the amplitude of the evEPSC. These results would suggest that some central nerve terminals of vagal afferents may be excited by PGE₂, like some of the peripheral actions of PGE₂. However, our results indicate that the majority of central vagal synapses were depressed by PGE₂. In 34/56 NTS neurons tested, 200

nM PGE₂ produced a clear depression of the amplitude of the solitary tract-evEPSCs; the 22 neurons that did not show synaptic depression were unaffected by PGE₂. We tested whether concentrations of PGE₂ larger than 200 nM might cause synaptic potentiation. From threshold concentrations of ~ 10 nM to 1 μ M, the largest concentration tested, PGE₂ always depressed synaptic transmission at PGE₂-sensitive second-order NTS neurons. The difference between our result and those of Sekiyama et al. (1995) could be related to the small number of synapses they tested, and/or to the location of NTS neurons examined in the two studies: we studied second-order intermediate and caudal NTS neurons whereas Sekiyama et al. (1995) did not specify the location of their neurons in the NTS. Different PGE₂ receptors subtypes are known to exist (see below) and they may exert opposing effects on synaptic transmission depending upon their distribution within the NTS. In view of our observations that PGE₂ produces depression at central synapses in the NTS, we would conclude that central terminals of vagal afferents respond differently to PGE₂ than either the peripheral processes or cell body. It is important to note that not all synapses activated via solitary tract stimulation are exclusively vagal in origin; nonetheless, most evEPSCs were depressed by PGE₂.

Other primary afferent central terminals respond to PGE₂ in various manners. Nakayama et al. (2004) and Malmberg et al. (1995) showed that PGE₂ increase neurotransmitter release from the spinal cord nerve terminals of the dorsal root ganglia. By contrast Baba et al. (2001) reported only postsynaptic effects of PGE₂ with no modulation of these nerve terminals. PGE₂ can increase the calcitonin gene-related peptide release from the central nerve terminals of the trigeminal ganglia neurons (Jenkins et al. 2004). These different studies, together with our data, indicate that PGE₂ can have different modulatory actions on primary afferent central nerve terminals. Moreover the effects of PGE₂ on the cell bodies of primary afferents neurons do not always reflect the actions of PGE₂ at the central nerve terminals of these sensory neurons.

The cellular mechanism underlying the PGE₂-mediated depression of the evEPSCs remains to be elucidated. PGE₂ can alter many voltage-dependent channels and consequently affect transmitter release. Both I_{Na+} (Kwong and Lee, 2005) and I_h (Ingram and Williams, 1996) are enhanced by PGE₂ in vagal afferents, effects that might be expected to lead to an enhancement of transmitter release. PGE₂ could depress synaptic currents by inhibiting a specific class of voltage dependent calcium channel (VDCC) in presynaptic nerve terminals. In the cell bodies of the vagal afferents that project their axons into the ST, Huang et al. (2004) reported that 1 μ M PGE₂ reduced total inward Ca⁺⁺ current by ~ 30 %. By knowing which class of VDCC underlies this PGE₂-induced reduction of Ca⁺⁺ current, it could be possible to use specific blockers of VDCC and perform occlusion experiments at NTS synapses.

Because PGE₂-induced synaptic depression was manifested by both pre- and postsynaptic sites-of-actions we sought evidence whether the same or different subtypes of PGE₂ receptors might underlie the effect of PGE₂ at each of these sites. Four subtypes of PGE₂ receptors, EP₁₋₄, have been cloned and characterized (Narumiya et al. 1999). All four EP receptors are present in cell bodies of the rat vagus nerve (inferior vagal or nodose ganglion neurons; Matsumoto et al. (2005)) and presumably in vagal nerve terminals within the NTS. With the exception of EP₂ mRNA, the other EP receptors mRNAs are present in NTS neurons of the rat (Zhang and Rivest, 1999). Application of a panel of PGE₂ receptor agonist and antagonist revealed that none of the PGE₂ effects on evoked synaptic transmission could be related to the activation of specific PGE₂ receptor subtype. However, in the case of sEPSCs, it appears that EP₄ receptors regulate sEPSC amplitude. Thus, the presynaptic membrane of the primary sensory neurons and the postsynaptic membrane of second order NTS neurons examined in this study do not appear to possess a functional EP₁ – EP₄ receptors. However, it should be noted that the strength of these conclusions relies on the specificity of the reagents employed. For example, the EP₁ receptor agonist has been reported to have minimum selectivity (Hata

and Breyer, 2004). Future experiments with potent and selective PGE₂ receptor reagents should help test our conclusions.

We attempted to derive a concentration-effect relation for the actions of PGE₂ on the magnitude of the evEPSC. Using population data or recording the effects of many concentrations of PGE₂ in the same neuron. Nonetheless, we clearly could delineate a threshold concentration at about 10 nM and a maximum effect at 100 nM. Moreover, there was a significant difference between the effects of 10 nM and 100 nM PGE₂. We considered that endogenous presence of PGE₂ in the NTS could have obscured the concentration-effects relation. Thus, we tested this possibility by incubating slices with 3 – 10 μ M indomethacin. At these concentrations indomethacin acts non-selectively to inhibit both COX I and COX II (O'Banion, 1999). Indomethacin treatment did not affect the amplitude of the evEPSCs nor alter the inhibition produced by PGE₂ application. These results indicate that under non-pathological conditions the NTS may possess little endogenous levels of PGE₂. This was somewhat of a surprising finding in light of the recent observations that synaptic transmission and membrane excitability in hippocampal CA1 pyramidal neurons of the rat can be dynamically regulated by COX II activity (Chen and Bazan, 2005). Evidently, the expression of COX II may be much higher in the hippocampus than in the NTS, and/or the metabolism of this prostanoid is more efficient in the NTS.

Synaptic transmission from vagal afferents to second-order NTS neurons is characterized as highly efficient, operating with a high probability of release (Doyle and Andresen, 2001). When transmitter release occurs near maximum efficiency synaptic depression following repetitive presynaptic activity is commonly observed: This is clearly evident for solitary tract synaptic transmission *in vivo* and *in vitro* (Miles, 1986; Mifflin and Felder, 1990; Doyle and Andresen, 2001; see also references in Bonham and Chen, 2005). These synapses, like many others that have high initial probability of release, can act as low-pass filters (Abbott and Regehr, 2004; Bonham and Chen, 2005). Importantly, neuromodulators acting at presynaptic receptors that reduce the probability of transmitter release can alter the filtering characteristic of these synapses rendering them more reliable. There are many autacoids, cytokines, and neurotransmitters that can presynaptically modulating vagal neurotransmission in the NTS (Andresen and Kunze, 1994; see also references in Rogers et al. 2006). Thus, it is not too surprising that these synapses would also be regulated by lipid mediators such as PGE₂. Our results reveal that PGE₂, like GABA (Brooks et al. 1992), glutamate (Chen et al. 2002), dopamine (Kline et al. 2002), and Substance P (Sekizawa et al. 2003), also depressed synaptic transmission. By producing synaptic depression PGE₂ may convert vagal synapses from a low-pass filter function to a band-pass filter, potentially reducing spike failure in second-order NTS neurons.

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Abbreviations

aCSF	artificial cerebrospinal fluid
AHP	afterhyperpolarization
AMPA	a-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
AP	action potential
CNS	central nervous system
COX-I	Cyclooxygenase 1
COX-II	Cyclooxygenase 2
evEPSCs	evoked excitatory postsynaptic glutamatergic currents
mEPSCs	miniature excitatory postsynaptic currents
NMDA	N-Methyl-D-aspartic acid

NTS	Nucleus Tractus Solitarius
PNS	peripheral nervous system
PGE₂	Prostaglandin E ₂
PPR	paired pulse ratio
R_{in}	Input resistance
sEPSCs	spontaneous excitatory postsynaptic currents
St	Solitarius Tract
TTX	tetrodotoxin

Figure 1.

Microscopic views of 150 μm thick brain slices containing the nucleus tractus solitarius (NTS). A, slice from caudal region of the NTS limited by the AP (area postrema), the ST (solitary tract), the midline (dashed line) and the 10 (dorsal motor nucleus of the vagus) (Bregma -13.80 mm). B, Another slice obtained from the intermediate region of the NTS (Bregma -13.30 mm). The asterisk indicates position of stimulating electrode. C, an individual NTS neuron viewed with a microscope equipped with near-infrared differential interference contrast optics.

Figure 2.

Effect of PGE₂ on the amplitude of evoked EPSCs induced by electrical stimulation of the solitary tract. A, Whole-cell voltage clamp recordings of EPSCs sampled from a NTS neuron. Solitary tract (ST) stimulation, 10 V, 150 μ s; 0.2 Hz was elicited by placing a stimulating electrode on the ST ipsilateral to the recorded neuron. Each trace is an average of 10 ST-evoked EPSCs (evEPSCs). evEPSCs shown before (control), in the presence of 200 nM PGE₂ and 15 min after switching to a drug-free aCSF (washout). B, group data showing that PGE₂ significantly reduced the amplitude of the evEPSCs (* $P < 0.01$; $n = 34$). Error bars depict $\pm$ SEM.

Figure 3.

Effect of PGE₂ on the amplitude of afferent evoked EPSCs (evEPSCs) recorded in capsaicin-sensitive and capsaicin-resistant second-order NTS neurons. A, top panel, 200 nM PGE₂ decreased the evEPSC amplitude recorded in a capsaicin-sensitive neuron. Lower panel, shows five consecutive evEPSCs before (control) and following a 10 min superfusion with 200 nM capsaicin (CAP); note the evEPSCs were completely blocked by CAP treatment. B, top panel, PGE₂ (200 nM) did not affect the evEPSC amplitude in CAP-resistant neuron. Lower panel shows a train of five evEPSCs recorded from the same neuron before (control) and 10 min after superfusion with 200 nM CAP. Each trace is an average of 10 responses. The arrows in A and B shows the time of the stimulus artifact.

Figure 4. Effect of glutamate receptor antagonists on solitary tract (ST) evoked EPSCs (evEPSCs) recorded in a second order NTS neuron. evEPSC amplitude was not affected by APV (100 μ M), but it was completely blocked when CNQX (50 μ M) was added to the APV solution. Membrane potential held at -60 mV. Each trace is an average of 10 consecutive responses.

Figure 5. PGE₂ increased the paired-pulse ratio. A, Paired pulse ratio for evoked EPSCs was recorded before (control), in the presence of PGE₂ (200 nM), and 15 min following superfusion with drug free aCSF (washout). The interval between solitary tract stimulation was 20 ms. B, group data showing that PGE₂ significantly (* $P < 0.01$; $n = 24$) increased the paired-pulse ratio. Error bars depict $\pm$ SEM.

Figure 6. Effect of PGE₂ on the frequency and amplitude of spontaneous EPSCs (sEPSCs) recorded in NTS. A, recordings of sEPSCs before (CTR), and during superfusion of PGE₂ (200 nM) for 5 min. B, cumulative probability of the amplitude (left panel) and inter-event interval distributions (right panel). Solid lines depict control; dashed lines are data recorded during PGE₂ application. PGE₂ increased the inter-event interval ($p < 0.01$) and decreased the amplitude ($p < 0.01$).

Figure 7.

Effect of PGE₂ on the frequency and the amplitude of miniature EPSCs (mEPSCs). A, Control (CTR) traces of mEPSCs before and during superfusion of PGE₂ (200 nM). TTX (1 μ M) was present in control and in the PGE₂-containing aCSF. B, cumulative probability of the amplitude (left panel) and inter-event interval distributions (right panel) recorded before (solid line) and during PGE₂ superfusion (dashed line). PGE₂ increased the inter event interval ($p < 0.01$) and decreased the amplitude ($p < 0.01$).

Figure 8.

Effects of PGE₂ antagonists EP₁ and EP₂ on the PGE₂-induced changes in the frequency and the amplitude of spontaneous EPSCs (sEPSCs). A, cumulative probability of the amplitude (left panel) and inter-event interval distributions (right panel). Solid line shows the effects of an EP₁ receptor antagonist (SC19220 30 μ M) while the data portrayed by the dotted line shows the effects of PGE₂ (200 nM) plus SC19220 30 μ M. In the presence of SC19220, PGE₂ still increased the inter event interval ($p < 0.01$) and decreased the amplitude of spontaneous events ($p < 0.01$). B, cumulative probability of the amplitude (left panel) and inter-event interval distributions (right panel). Solid line shows the effects of EP₁ (SC19220 30 μ M) plus the EP₂ (AH28848 20 μ M) receptor antagonists. Dotted line shows the effects of PGE₂ (200 nM) in the presence of a mixture of SC19220 30 μ M plus AH28848 20 μ M. In the presence of this mixture, PGE₂ increased the inter event interval ($p < 0.01$) but did not affect the amplitude of spontaneous events ($p > 0.05$).

Table 1

Effect of PGE₂ on the passive and active membrane properties. Overshoot measured from 0 mV to peak depolarization of the action potential (AP). Afterhyperpolarization (AHP) measured from the resting membrane potential to the maximal membrane hyperpolarization. AP duration measured at one-half peak amplitude. The membrane input resistance shows a significant decrease (* $P < 0.05$, paired t-test) after PGE₂ treatment. The apparent increase in the rheobase after PGE₂ treatment was not significantly different ($P > 0.05$, paired t-test) owing to variability in the data set; 6 of the 10 NTS neurons showed no change. CTR denotes control; PGE₂ bath applied at 100 nM. Numbers in parenthesis are number of neurons tested.

	AP Overshoot (mV)	AP AHP (mV)	AP duration (ms)	AP Threshold (mV)	Rheobase (pA)	Number AP at 2X Rheobase	R _{in} (MΩ)
CTR	64 ± 3.3 (n = 11)	-6 ± 2.2 (n = 11)	1.1 ± 0.1 (n = 11)	-25 ± 2 (n = 11)	44 ± 15 (n = 10)	13.3 ± 2.2 (n = 12)	540 ± 46.1 (n = 12)
PGE ₂	67 ± 4.4 (n = 11)	-7 ± 2.1 (n = 11)	1.2 ± 0.1 (n = 11)	-27 ± 2 (n = 11)	82 ± 26 (n = 10)	17.9 ± 3.3 (n = 12)	346 ± 46.0* (n = 12)

* $P < 0.05$