Integration of cornea and cardiorespiratory afferents in the nucleus of the solitary tract of the rat

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Boscan, Pedro, and Julian F. R. Paton. Integration of cornea and cardiorespiratory afferents in the nucleus of the solitary tract of the rat. Am J Physiol Heart Circ Physiol 282:H1278–H1287, 2002—We determined the activity of neurons within the nucleus of the solitary tract (NTS) after stimulation of the cornea and assessed whether this input affected the processing of baroreceptor and peripheral chemoreceptor inputs. In an in situ, unanesthetized decerebrate working heart-brain stem preparation of the rat, noxious mechanical or electrical stimulation was applied to the cornea, and extracellular single unit recordings were made from NTS neurons. Cornea nociceptor stimulation evoked bradycardia, and extracellular single unit recordings were made from NTS neurons. Cornea nociceptor stimulation evoked bradycardia and an increase in the cycle length of the phrenic nerve discharge. Of 90 NTS neurons with ongoing activity, corneal stimulation excited 51 and depressed 39. There was a high degree of convergence to these NTS neurons from either baroreceptors or chemoreceptors. The excitatory synaptic response in 12 of 19 baroreceptive and 10 of 15 chemoreceptive neurons was attenuated significantly during concomitant electrical stimulation of the cornea and assessed whether this input affected the processing of baroreceptor and peripheral chemoreceptor inputs. In an in situ, unanesthetized decerebrate working heart-brain stem preparation of the rat, noxious mechanical or electrical stimulation was applied to the cornea, and extracellular single unit recordings were made from NTS neurons. Cornea nociceptor stimulation evoked bradycardia, and extracellular single unit recordings were made from NTS neurons. Cornea nociceptor stimulation evoked bradycardia and an increase in the cycle length of the phrenic nerve discharge. Of 90 NTS neurons with ongoing activity, corneal stimulation excited 51 and depressed 39. There was a high degree of convergence to these NTS neurons from either baroreceptors or chemoreceptors. The excitatory synaptic response in 12 of 19 baroreceptive and 10 of 15 chemoreceptive neurons was attenuated significantly during concomitant electrical stimulation of the cornea. This inhibition was GABA_A receptor mediated, being blocked by pressure ejection of bicuculline. Thus the NTS integrates information from corneal receptors, some of which converges onto neurons mediating reflexes from baroreceptors and chemoreceptors to inhibit these inputs.

heart rate; chemoreflex; baroreflex; nociception; oculocardiac reflex; oculocardiac reflex

THE OCULOCARDIAC REFLEX (OCR) is a trigeminovagal reflex that can produce potent bradycardia and cardiac arrhythmias such as nodal rhythm, ectopic beats, ventricular fibrillation, or asystole. The OCR may be induced by applying pressure on the eyeball or caused by an orbital hematoma, ocular trauma, eye pain, and traction of an extraocular muscle during treatment of poor alignment of the visual axis (strabismus). The OCR is most often induced during strabismus surgery in children but also occasionally during retinal surgery. Hypoventilation and increased partial pressures of CO_2_ significantly increase the incidence of bradycardia during strabismus surgery (5). Transient cardiac arrests may occur every 1 in 2,200 patients (14), and atropine has been given as a preventive drug, but this remains controversial because it does not always prevent the OCR-evoked arrhythmia (32).

Located in the dorsomedial aspect of the caudal medulla, the nucleus of the solitary tract (NTS) receives and integrates a vast number of visceral afferents important for regulating multiple autonomic functions, including the cardiovascular and respiratory systems (37, 42). In addition to receiving cardiorespiratory afferents, such as those from baroreceptors and peripheral chemoreceptors (e.g., 11, 15, 26, 41), the NTS also receives inputs from the ventral region of lamina I and II of the caudointermediate region of the trigeminal nucleus (30, 34). This part of the trigeminal nucleus receives afferents from both ocular nociceptors (3, 27, 33) and afferents that mediate the diving response (17, 34). Consistent with the idea that the NTS may be important for integrating these trigeminal inputs is the finding that thermal or chemical nociceptive stimulation of the cornea induced c-fos immunoreactivity in the NTS of rats (31). However, the functional significance of a corneal afferent input to the NTS remains unknown.

In a recent study (7), we reported that stimulation of corneal nociceptors produced apnea, bradycardia, and hypertension in an unanesthetized decerebrate rat preparation. This pattern of response was common to all types of stimuli applied to the surface of the cornea, including chemical, mechanical, and electrical, and similar to that observed during ophthalmic surgery in humans (5, 14, 32), which can lead to cardiac arrest (12, 23, 24). Furthermore, the cardiac component of the corneal-evoked response was mediated, in part, by the NTS (7). We showed that inactivation of the NTS reduced the corneal-evoked bradycardia by ~50% (7). Thus part of the trigeminal input to the NTS may convey information from corneal nociceptors important for mediating the reflex cardiac response.

In the present study, we demonstrate that activation of corneal afferents can affect the activity of NTS neurons. Furthermore, some of these inputs converge onto neurons activated by baroreceptor and peripheral chemoreceptor afferents. We suggest that the NTS is a...
site of integration for corneal nociceptors and reflexes maintaining cardiorespiratory homeostasis.

METHODS

Setting Up a Working Heart-Brain Stem Preparation

Because of the depressant effects of anesthesia, we chose to employ an unanesthetized decerebrate and arterially perfused in situ working heart-brain stem preparation (WHBP) (36). This preparation has good mechanical stability of the brain stem for long-term NTS neuronal recording and preserves trigeminal inputs (18). Following the United Kingdom Home Office guidelines for animal experimentation, Sprague-Dawley rats (75–150 g, both male and female) were anesthetized deeply with halothane, and, in the absence of withdrawal responses after a pinch to a paw or the tail, they were decerebrated precociliarily, cerebellsetomized, and transected subdiaphragmatically. The upper half of the animal was made viable by arterial perfusion via a double-lumen catheter inserted into the descending aorta. The main lumen (16 gauge) was used for perfusing a Ringer solution containing 1.25% Ficoll as an oncotic agent, gassed with carbogen (95% O₂–5% CO₂), and warmed to 31°C. The smaller lumen (18 gauge) was connected to a transducer for measurement of aortic perfusion pressure (PP). A phrenic nerve was isolated in the thorax and cut at the level of the diaphragm, and its spontaneous activity was recorded via a glass suction electrode. Phrenic nerve activity (PNA) was passed via a headstage (Neurolog 201) to trigger transistor time constant). The respiratory motor pattern consisted of an ed, and hence preparation viability. An electrocardiogram was also recorded, and the R-wave was discriminated using a window discriminator (Neurolog 201) to trigger transistor transistor logic (TTL) pulsesc. The interval between these was used to derive instantaneous heart rate (HR).

Stimulation Methods

Corneal nociceptors. The cornea was stimulated using either one of three stimulation methods. Method 1 consisted of a mechanical pneumatic prodder with a tip diameter of 0.5 mm held in a three-dimensional micromanipulator. The stimulus was applied for 1.5 s. The prodder was aimed at the central area of the cornea of the right eye. The strength of the stimulus applied was predetermined at the start of the experiment by adjusting the pressure (0.4–0.7 bar) and hence force needed to evoke a cardiorespiratory reflex response. This range of air pressure evoked a linear force at the tip of the prodder from 9.7 ± 0.7 to 25.5 ± 1.9 g. The stimulus strength was set 0.1 bar above the threshold to elicit a cardiorespiratory reflex and was maintained constant throughout the duration of the experiment. Method 2 was as follows. Because the pneumatic prodder induced mechanical instability of the brain stem during single unit recording, the ipsilateral cornea surface was stimulated electrically (10–40 V, 0.5–1 ms, 0.3–16 Hz). Pulses were applied via a column of agar (2% made in saline). The agar was supported in a tube that was held in a micromanipulator. At one end of the tube, the agar protruded and was in direct contact with the cornea. This ensured that the cornea was kept moist. A silver wire was inserted through the opposite end of the tube into the agar but did not come into direct contact with the surface of the cornea. Method 3 was as follows. Additionally, during single unit recording, we stimulated the contralateral cornea with blunt forceps. This caused far less mechanical instability compared with the prodder. Because the applied force could not be accurately controlled for repeated trials, we did not quantify neuronal responses. However, the stimulus did allow us to test whether neurons also received inputs from the contralateral cornea and whether this was transduced by mechanoreceptors.

Arterial baroreceptors. The pressure within the ipsilateral carotid sinus was elevated. This was achieved by increasing the flow rate of freshly gassed perfusate through this region via a second double-lumen catheter inserted into the left common carotid artery in the cranial direction. The tip of this catheter was positioned at the bifurcation of the common carotid artery. Perfusion was driven through the main lumen (22 gauge), whereas the smaller lumen (23 gauge) was used to measure common carotid artery pressure being close to the carotid sinus. The induced increase in carotid sinus pressure was between 10 and 40 mmHg. In between stimulation trials, the carotid sinus was perfused continuously with perfusate taken from the main supply to the preparation via a parallel circuit. Connected to this circuit was a three-way stopcock and 1-ml syringe, which was used to draw up fresh perfusate before injecting it as a bolus into the carotid sinus to raise pressure.

Peripheral chemoreceptors. Sodium cyanide (CN) solution (0.03%) was injected directly into the descending aorta. The exact dose used was determined at the start of every experiment. A range of CN doses were given (7–21 μg), and the dose chosen was suprathreshold but submaximal. Once selected, the dose of CN did not change throughout the course of the experiment. On the basis of previous evidence (13, 19), the dose range of CN used in the present study was relatively selective for stimulating arterial chemoreceptors. Furthermore, in the WHBP, the CN-evoked chemoreceptor reflex response was abolished by denervating the vagi and carotid sinus nerves (37). The WHBP permits repeated CN stimulation of peripheral chemoreceptors, which give consistent reflex responses and does not cause toxic side effects due to the large circulating volume of 200 ml.

Combined stimulation protocol. In some experiments, we combined electrical stimulation of the cornea with either arterial baroreceptor or peripheral chemoreceptor activation. In both cases, electrical stimulation of the cornea (10–40 V, 0.5–1 ms, 0.3–16 Hz) started before and finished after baro- and chemoreceptor stimulation (2–5 s). The total time of cornea stimulation was 5–12 s. The intensity of baro- and chemoreceptor stimulation was as described above.

Extracellular Recording and Drug Ejection

One barrel of a two-barreled microelectrode assembly contained sodium chloride (1.5 M), giving a resistance of 15–35 MΩ. The second barrel contained bicuculline methiodide (100 μM). The electrode was driven in 1- to 3-μm steps using a piezoelectric inchworm stepper motor (Burleigh), which provided positional feedback. Single unit recordings were made between 300 and 700 μm below the dorsal medullary surface at the level of the nucleus tractus solitarius. Below 700 μm, respiratory cells were found consistently, and this was taken as the ventral extent of the NTS. The second barrel of the microelectrode assembly was connected to a pressure line. Pressure was applied from a pressure source. The latter consisted of a custom-built device that permitted precise monitoring and control of the applied pressure to the barrel. Similar to ionophoretic application, the drug volume ejected was unknown and depended on the applied pressure and resistance of the microelectrode. In the present study, we
used ejection pressures that caused no obvious changes in the signal-to-noise ratio. Because the WHBP has a well-perfused brain stem, drugs washed out efficiently (2–5 min).

Data Analysis

All data were relayed via a 1401 CED interface to a computer running Spike 2 software (CED) with custom-written scripts for data acquisition and on- and off-line analysis. Baseline HR, PP, and PNA cycle length were all measured. The absolute peak value of the reflex response in HR was compared between mechanical and electrical stimulation of a cornea. PNA cycle length was defined as the time interval between the onset of two consecutive bursts. This was averaged over at least three control cycles and compared with that evoked during stimulation of the cornea. The data expressed include control values and the response [i.e., change (Δ)] values.

For single unit neuronal activity, the peak frequency of the firing response evoked by stimulation of peripheral chemoreceptors and baroreceptors was measured. This peak activity was compared with ongoing discharge measured before the stimulus over the same duration as the evoked response. Synaptic responses evoked from electrical stimulation of the cornea were measured as the frequency of action potentials that occurred during 900 ms after each single pulse. This was compared with the frequency of ongoing activity during the same length of time immediately before the onset of stimulation. An increase in neuronal firing >30% was used as the threshold to indicate excitation after stimulation of the cornea, baroreceptors, and chemoreceptors. In contrast, when afferents were stimulated and evoked a reduction in the ongoing activity, >30% was considered an inhibitory input. With the use of a two-tailed Student’s t-test applied to the raw data, the significance of effects was assessed. The data expressed include control values and the Δ-values.

All values are means ± SE; n is the number of preparations for the systemic results and the number of cells for the single unit recordings. Differences were taken as significant at the 95% confidence limit.

RESULTS
Cardiorespiratory Response Evoked From Corneal Nociceptors

In 10 WHBP, baseline HR, PP, and PNA cycle length were 300 ± 6 beats/min, 84 ± 1.5 mmHg, and 3.14 ± 0.2 s, respectively. Mechanical noxious stimulation of the right cornea using a pneumatic prodder evoked a bradycardia and an increase in PNA cycle length of −90 ± 8 beats/min and 1.3 ± 0.2 s, respectively (P < 0.01, n = 10; Fig. 1). In these same WHBP, electrical stimulation of the left cornea (40 V, 0.5 ms, 16 Hz; 20 pulses in total) evoked a similar bradycardia and PNA response to that observed during mechanical noxious stimulation (−102 ± 9 beats/min and 1.1 ± 0.2 s, respectively, P < 0.01, n = 10; Fig. 1). With both types of stimuli, small increases in PP were evoked but not significant, reflecting the low vascular resistance in the WHBP.

These data indicate that electrical stimulation of the cornea evokes a qualitatively similar pattern of cardiorespiratory reflex response to that observed with mechanical noxious stimulation. For this reason, we assumed that the response to electrical stimulation could be associated with corneal nociception and employed this method of stimulation during NTS single unit recording. In the latter studies, baseline cardiorespiratory variables were not different to those reported above.

Corneal Receptor Activation of NTS Neurons

Two types of evoked synaptic response. One hundred sixty-nine single units were recorded extracellularly from the NTS. These had an ongoing activity of 2.9 ± 0.3 Hz. Of the 169 neurons, 90 responded to corneal afferent stimulation: 51 were synaptically excited after electrical stimulation of the ipsilateral cornea, as shown by an increase in spiking activity from 3.0 ± 0.3
to 5.2 ± 0.3 Hz (Fig. 2A; P < 0.01). In contrast, 39 of 169 NTS neurons tested decreased their baseline firing rate after electrical stimulation of the ipsilateral cornea (from 3.3 ± 0.3 to 1.8 ± 0.2 Hz; Fig. 2B, P < 0.01). The remaining 79 NTS neurons showed no measurable response (from 2.7 ± 0.3 to 3.1 ± 0.3 Hz). Note that there was no difference in the basal firing rates of these three groups of neurons (P = 0.42).

Convergence of ipsi- and contralateral corneal afferents to the NTS. Despite our attempts, it was not possible to maintain a single unit NTS recording during mechanical prodding of the contralateral cornea. However, stimulation of the contralateral cornea using blunt forceps was effective and produced far less mechanical disturbance. Thirty-six of fifty-one neurons orthodromically excited by electrical stimulation of the ipsilateral cornea (see above) were tested. Of these 36 neurons, 26 were also excited by mechanical stimulation of the contralateral cornea; the remainder did not respond. In addition, of the 39 neurons that decreased their ongoing firing during electrical stimulation of the ipsilateral cornea (see above), 23 were tested, of which 16 neurons were also depressed by mechanical activation of the contralateral cornea. The 7 remaining cells failed to respond. These results indicate a high correlation (~70%) in the pattern of response during electrical and mechanical stimulation and a high degree of convergence from ipsi- and contralateral corneal nociceptors to NTS neurons (Fig. 2).

Latency of excitatory synaptic response evoked from the cornea. The onset latency of excitatory synaptic responses evoked after electrical stimulation of the ipsilateral cornea was measured. This ranged between 50 and 140 ms with a mean of 82.1 ± 5 ms, and different neurons displayed contrasting patterns of evoked synaptic response, including single and multiple evoked action potentials. In the latter, frequencies ranged between 2 and 14 Hz and could last up to 1 s poststimulus (Fig. 3).

Patterns of Corneal Receptor Convergence to Cardiorespiratory NTS Neurons

Baroreceptors. A total of 45 cells were characterized physiologically as baroreceptive because an increase in ongoing activity from 2.02 ± 0.3 to 8.2 ± 1.8 Hz (P < 0.01; Fig. 4B) was observed during an elevation in pressure within the ipsilateral carotid sinus. We tested for a convergent input from the ipsilateral cornea in all 45 baroreceptive neurons. Of these 45, 13 cells showed an excitatory synaptic response, as portrayed by an increase in firing frequency from 2.3 ± 0.5 to 5.2 ± 0.6 Hz...
Hz ($P < 0.01$). In contrast, the ongoing activity of 14 neurons was decreased from $3.2 \pm 0.5$ to $1.5 \pm 0.3$ Hz ($P < 0.05$). The remainder failed to respond (control activity: $2.2 \pm 0.7$ vs. $2.3 \pm 0.7$ Hz during corneal stimulation, $n = 18$, $P = 0.9$).

Peripheral chemoreceptors. NTS neurons were characterized as chemoreceptive if they responded to aortic injection of CN (see METHODS). All NTS chemoreceptive neurons tested displayed ongoing activity ($1.9 \pm 0.6$ Hz, $n = 53$), which was not correlated with the central inspiratory activity recorded from the phrenic nerve. After CN injection, the ongoing activity increased to $9.1 \pm 1.6$ Hz ($P < 0.01$; Fig. 5B). The following responses in chemoreceptive neurons were observed during electrical stimulation of the ipsilateral cornea: 1) an increase in activity (from $2.8 \pm 0.4$ to $5.4 \pm 0.4$ Hz, $n = 25$, $P < 0.01$); 2) a decrease in ongoing activity from $3.6 \pm 0.4$ to $2.0 \pm 0.4$ Hz ($n = 10$, $P < 0.05$); and 3) no effect (i.e., control $2.8 \pm 0.5$ vs. $3.1 \pm 0.5$ Hz, $n = 18$, $P = 0.7$).

Inhibitory Effect of Corneal Stimulation on Convergent Excitatory Synaptic Inputs from Cardiorespiratory Receptors to NTS Neurons

Baroreceptors. We assessed whether the afferent input evoked from corneal afferents could affect the evoked excitatory synaptic response produced during baroreceptor stimulation in 19 of 45 baroreceptive cells. These 19 neurons had an ongoing activity of $2.0 \pm 0.4$ Hz that was either attenuated or not affected by stimulation of the ipsilateral cornea alone (from $2.3 \pm 0.6$ to $0.9 \pm 0.5$ Hz, $n = 7$, $P = 0.02$; and from $2.7 \pm 0.9$ to $2.9 \pm 0.9$ Hz, $n = 12$, $P = 0.4$, respectively). In 12 of 19 neurons (5 attenuated and 7 not affected), the baroreceptive-induced synaptic response of $7.7 \pm 0.9$ Hz was attenuated to $3.8 \pm 0.8$ Hz ($P < 0.01$; Fig. 4) during concomitant corneal afferent activation. In the remaining seven cells, the baroreceptor-evoked activity was not modified by corneal stimulation.

Peripheral chemoreceptors. We tested if the afferent input evoked from corneal receptors could affect the excitatory synaptic response elicited from peripheral chemoreceptors in 15 of 53 chemoreceptive NTS neurons. The ongoing activity of these 15 neurons was either attenuated or not affected when the ipsilateral cornea was stimulated alone (from $2.7 \pm 0.4$ to $1.6 \pm 0.4$ Hz, $n = 7$, $P = 0.04$; and from $3.1 \pm 0.9$ to $3.6 \pm 1$ Hz, $n = 8$, $P = 0.66$, respectively). Simultaneous corneal stimulation attenuated the chemoreceptor-evoked excitatory synaptic response from $8.4 \pm 1.5$ to $4.0 \pm 0.9$ Hz (Fig. 5; $P < 0.01$) in 10 of 15 cells studied (5 attenuated and 5 not affected). The ongoing activity in these 10 cells was $2.7 \pm 0.9$ Hz. The chemoreceptor-evoked activity was not modified in the remaining five cells.

Role of GABA<sub>A</sub> Receptors in the Corneal-Evoked Inhibitory Modulation of Convergent Cardiorespiratory Inputs to NTS Neurons

Baroreceptors. The corneal receptor-evoked depression of the baroreceptor-mediated excitatory synaptic
response was tested after ejection of bicuculline (100 μM) in five neurons. In all cases, ejection of bicuculline prevented the corneal-evoked inhibition of the baroreceptor-evoked synaptic response: in all five neurons, stimulation of the carotid sinus baroreceptors alone elicited an increase in activity from 2.3 ± 0.4 to 7.3 ± 0.9 Hz (P < 0.01). Simultaneous stimulation of corneal receptors depressed the baroreceptor-evoked response to 4.7 ± 1.2 Hz (P < 0.05). The latter was reversed to 10.7 ± 2.5 Hz (Figs. 4 and 6; P < 0.01) after ejection of bicuculline. This activity level was not different from control (i.e., 7.3 ± 0.9 Hz, P = 0.2). Interestingly, bicuculline itself at the dose used affected neither control basal activity (2.3 ± 0.5 Hz) nor the baroreceptor-evoked response (7.8 ± 1.6 Hz; Figs. 4D and 6).

Peripheral chemoreceptors. In five neurons in which corneal stimulation attenuated the chemoreceptor-evoked excitatory synaptic response, we ejected bicuculline (100 μM). In all cases, the GABA_A antagonist prevented the corneal-evoked attenuation of the chemoreceptor-induced response. Stimulation of the peripheral chemoreceptors alone evoked an excitatory synaptic response of 9.7 ± 2.2 Hz from a baseline of 1.4 ± 0.6 Hz (P < 0.01). However, this excitatory response was reduced 4.1 ± 1.5 Hz with concomitant stimulation of the cornea (Figs. 5 and 6; P < 0.01). After ejection of bicuculline, simultaneous stimulation of both receptor types produced a response of 12.4 ± 1.1 Hz, which was not different to when chemoreceptors were activated alone (i.e., 9.7 ± 2.2 Hz, P = 0.06). Bicuculline at the dose used affected neither baseline activity (1.6 ± 0.6 Hz) nor the chemoreceptor-evoked synaptic response (12 ± 2.3 Hz; Figs. 5D and 6).

DISCUSSION

In this study, we report for the first time that NTS neurons can be modulated synaptically from corneal sensory afferents. Mechanical and electrical stimulation evoked two types of synaptic response: excitatory and inhibitory. It appears that there is a high degree of convergence from both ipsilateral and contralateral corneal nociceptors to NTS neurons. Furthermore, corneal inputs can converge onto neurons activated by cardiorespiratory afferents. Stimulation of corneal afferents can depress the excitatory synaptic response evoked from baroreceptors and chemoreceptors via activation of GABA_A receptors.

Consideration of Methods Employed

Modality of corneal afferents stimulated. In the present study, we stimulated the cornea mechanically.

Fig. 4. Electrical stimulation of corneal afferents reduces the baroreceptor-induced excitatory synaptic response of an NTS neuron. A: NTS neuronal response to electrical stimulation of the cornea showed a slight reduction in its ongoing activity (22 V, 1 Hz, 0.5 ms); B: excitatory synaptic response after stimulation of the baroreceptors by elevating pressure within the ipsilateral carotid sinus; C: attenuation of the baroreceptor-evoked response by concomitant electrical stimulation of the ipsilateral cornea using the same stimulation variables as in A; D: after pressure ejection of bicuculline (to block GABA_A receptors), both the ongoing activity and the baroreceptor-evoked response were unaffected. E: bicuculline prevented the corneal afferent-induced attenuation of the baroreceptor-evoked response. CSP, carotid sinus pressure.
and electrically. Our electrical stimulation parameters were suitable to include activation of both A-δ and C fibers, which are present in the cornea and mediate nociceptive inputs (10, 43). Both mechanical and electrical stimulation elicited a bradycardia and an increased expiratory interval. This pattern of response was similar to that previously reported by us during corneal stimulation with bradykinin and capsaicin (7). Because of the similarity in the capsaicin/bradykinin-induced cardiorespiratory reflex response with that evoked by both mechanical and electrical stimuli, we suggest that the latter results from activation of corneal afferents that include nociceptors.

The working heart-brain stem preparation. The WHBP is an unanesthetized decerebrate in situ preparation that offers some advantages over existing in vivo models to study cornea nociceptive pathways and their modulation of cardiovascular afferent integration in the brain stem of the rat (or mouse). Although descending influences from the midbrain and higher centers are lost due to decerebration, sympathetic-parasympathetic modulation of the heart is well preserved. In addition, reflexes controlling the cardiopulmonary system in the WHBP are fully viable, including those mediated via the trigeminal system (18). The absence of anesthesia in the WHBP is a distinct advantage for studying cardiorespiratory reflexes because they are not only robust but can be stimulated repeatedly with controlled stimuli and persist for hours. The absence of inflating lungs and minimal pulsatility in the arterial system enhances mechanical stabilization of the brain stem to assist long-term cellular recording of physiologically characterized neurons and neuropharmacological intervention. With this stability, simultaneous studies at the systemic and cellular level are possible. On the other hand, the WHBP is not without drawbacks, including loss of the higher centers of the brain, thereby oversimplifying the remaining circuitry. The lack of arterial pulsation may lead to a difference in the pattern of ongoing activity of NTS neurons and in their response to baroreceptor stimulation. Furthermore, the absence of adrenal glands and hypothalamus compromise the endocrine component of cardiovascular responses.

Pressure ejection technique. The single unit recording pressure ejection technique was used during this study to apply drugs to single NTS neurons. The technique proved to be effective but has its limitations. The volume ejected, dose, and specific site of action remain unknown. With pressure ejection, the volume delivered is too small to be measured and depends on the applied pressure and resistance of the microelectrode. The
pressure ejection used in this study either caused no effect or a transient, minimal change in the signal-to-noise ratio of spikes. In the latter cases, the signal-to-noise ratio returned to control after drug delivery. This implies an absence or minimal mechanical disturbance to the neuron under study. Another potential problem is drug leakage. We used relatively high-resistance electrodes to minimize this. Indeed, based on our findings, leakage is unlikely or ineffective because cornea-evoked inhibition of baro- and chemoreceptor synaptic excitatory responses were only prevented when bicuculline was ejected with positive pressure. Furthermore, this effect was completely reversible, supporting an absence of significant leakage.

**Corneal-Evoked Cardiorespiratory Responses**

The cardiorespiratory responses mediated via the trigeminal system such as those evoked during a diving response, facial noxious cooling, and electrical stimulation of trigeminal nerves or the trigeminal nucleus all consist of a bradycardia and apnoea (25, 39). This is comparable with that evoked from the cornea, as described in the present study, and is similar to the so-called OCR observed during both ophthalmic surgery (5, 12, 14, 23, 32) and ophthalmic subconjunctival injections in conscious humans (23).

In contrast to the present study, Bereiter et al. (4) described a tachycardia after corneal stimulation with mustard oil in the anesthetized cat. Our preliminary data also indicate a tachycardia can be evoked by applying 8–10% CO₂ directly to the cornea (unpublished observations). A plausible explanation for a tachycardia response may be related to a different subpopulation of corneal nociceptors. In support of this, Chen et al. (9) compared capsaicin and CO₂ stimulation of the feline cornea and demonstrated that more than one population of nociceptive sensory fibers exist. Furthermore, corneal stimulation with CO₂ showed at least four different subpopulations of sensory neurons in the caudointermedial trigeminal nucleus of rats (22). Thus it is feasible that different stimuli activate distinct corneal receptors connected by specific afferent pathways that relay to separate parts of the trigeminal nucleus. The latter may project to different central sites, which ultimately determines the polarity of the cardiac response. To substantiate this claim, electrical stimulation of tooth pulp in rats evoked a tachycardia (1, 2). Dental nociceptors are known to synapse and express c-fos immunoreactivity in laminae I and II of the caudointermedial trigeminal nucleus but dorsal to the area known to receive corneal afferents (1, 2).

**Plausible Central Pathway from the Cornea to the NTS**

Corneal nociceptive sensory afferents project directly to the ventral area of laminae I and II of the caudal and intermediate regions of the trigeminal nucleus (3, 22, 27, 33). This region expresses c-fos immunoreactivity when corneal nociceptors are stimulated (4, 31), indicating an evoked increase in neuronal activity in this nucleus.

Laminae I and II of the caudal and intermediate regions of the trigeminal nucleus project to many brain stem nuclei, including the parabrachial, Kolliker fuse, ventrolateral medulla, nucleus ambiguus, and NTS (8, 26, 34, 38). All the latter can exert powerful influences on the cardiovascular and respiratory systems. Regarding the NTS, we (7) previously reported that blockade of synaptic transmission within this nucleus, with either cobalt chloride or isoguvacine (a GABA_A agonist), attenuated the cornea-induced bradycardia by ~50%. However, based on the latency of excitatory synaptic inputs recorded in the NTS after electrical stimulation of the cornea (50–140 ms), it appears that there may be both relatively direct and indirect routes. Indeed, the NTS may receive multiple inputs from more than one brain stem nuclei involved in the processing of corneal nociceptive information.

Interestingly, the region of the trigeminal nucleus innervated by cornea inputs exhibited c-fos after activation of afferents mediating the diving response. This region was found to be crucial for the expression of the cardiorespiratory response that accompanies a dive.
Furthermore, the diving response evokes a comparable cardiorespiratory reflex response to corneal nociceptors stimulation in animals in vivo (35, 28, 13) and in situ WHBP of rats (18) and humans (20, 21). Thus understanding the corneal nociceptive pathways may help to understand the mechanisms of the diving response and vice versa.

Patterns of Convergence of Corneal and Cardiorespiratory Afferents in the NTS

The NTS plays a pivotal role in cardiovascular homeostasis. Two homeostatic reflexes mediated through the NTS originate from baroreceptors and peripheral chemoreceptors. When stimulated, both receptors evoke a reflex bradycardia (42). This is similar to that evoked from the cornea. In the present study, we described that many corneal-receptive NTS neurons received convergent excitatory input from baroreceptors (~29%) and peripheral chemoreceptors (~47%). On the basis of the possibility that NTS neurons may be organized in respect to their output targets, this excitatory convergence may be to a subpopulation of NTS neurons projecting to cardiac vagal motoneurons. This was supported by our previous finding that the reflex bradycardia produced by baro-, chemo-, and corneal receptors was attenuated by inactivating the same region of the NTS (7).

In addition to excitatory convergence, many NTS neurons were depressed by stimulating the cornea. In some of these cells, the convergent excitatory synaptic response evoked by baroreceptor and peripheral chemoreceptor stimulation was attenuated during concomitant activation of corneal nociceptors. We showed that this depression was mediated by an inhibitory GABAergic mechanism. The question remains as to the physiological significance of this interaction.

Hypothetical Explanation for Corneal-Induced Depression of Baro- and Chemoreceptor-Evoked Synaptic Responses in the NTS

It is possible that the corneal-induced reflex response takes precedence over homeostatic reflexes. In this case, corneal afferents may need to suppress components of the baroreceptor and chemoreceptor reflexes at the level of the NTS. For example, corneal nociceptors may prevent the baroreceptor reflex-mediated sympathoinhibition, thereby allowing manifestation of a pressor effect. Likewise, the peripheral chemoreceptor-mediated tachypnea may be prevented by corneal afferents, allowing a reflex apnea to be expressed. These theoretical interactions could explain the corneal afferent-induced inhibition of the excitatory synaptic responses evoked by baroreceptors or peripheral chemoreceptors. This type of interaction is similar to the inhibition of baroreceptor reflex-evoked responses in the NTS by somatic noxious stimulation of the limbs (6, 29). Recently, we showed that substance P appears to be a neurotransmitter released in the NTS after stimulation of forelimb somatic afferents. Although we do not know the transmitter(s) responsible for driving the GABAergic neurons in the NTS during stimulation of corneal nociceptors, substance P-containing fibers project from the trigeminal nucleus to the NTS (40, 44). Hitherto, a possible neurotransmitter from the trigeminal nucleus to the NTS could include substance P.

In conclusion, the NTS may play an active role in the cardiorespiratory reflex response during activation of corneal nociceptors (7). Furthermore, corneal sensory inputs converge with baroreceptor and peripheral chemoreceptor circuits within the NTS. Some of this convergence was antagonistic, resulting in a corneal nociceptor-mediated inhibition of signaling evoked by baroreceptor and chemoreceptor afferents. We show that the latter is caused by corneal receptor-mediated activation of a GABAergic mechanism within the NTS. We propose that this interaction is essential for ensuring the expression of the corneal nociceptor reflex-bradycardia, pressor response, and respiratory depression even in the presence of high baroreceptor or peripheral chemoreceptor afferent drive.

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REFERENCES


