Calcitonin gene-related peptide receptors in rat trigeminal ganglion do not control spinal trigeminal activity

Oana Covasala, Sören L. Stirm, Stephanie Albrecht, Roberto De Col, and Karl Messlinger
Institute of Physiology and Pathophysiology, University of Erlangen-Nürnberg, Erlangen, Germany

Submitted 28 February 2011; accepted in final form 23 April 2012

Calcitonin gene-related peptide receptors in rat trigeminal ganglion do not control spinal trigeminal activity. J Neurophysiol 108: 431–440, 2012. First published April 25, 2012; doi:10.1152/jn.00167.2011.—Calcitonin gene-related peptide (CGRP) and the free radical nitric oxide (NO) are regarded as key mediators in the generation of primary headaches. CGRP receptor antagonists reduce migraine pain in clinical trials and spinal trigeminal activity in animal experiments. The site of CGRP receptor inhibition causing these effects is debated. Activation and inhibition of CGRP receptors in the trigeminal ganglion may influence the activity of trigeminal afferents and hence of spinal trigeminal neurons. In anesthetized rats cranial dura mater is ineffective, trigeminal activity driven by meningeal afferent input is more likely to be controlled by CGRP receptors located centrally to the trigeminal ganglion. In conclusion, the site of CGRP receptor inhibition causing CGRP and increased after injection of olcegepant. In conclusion, olcegepant has been shown to inhibit nociceptive transmission in the cat spinal trigeminal nucleus (Storer et al. 2004). Our group has found that intravenous injection of the CGRP receptor antagonist olcegepant diminished the ongoing activity of neurons with meningeal afferent input in the spinal trigeminal nucleus (Fischer et al. 2005). Olcegepant reduced also the responses of these neurons to graded heat stimuli applied to their meningeal receptive fields. In another series of experiments the activity of spinal trigeminal neurons was increased by infusion of the NO donors sodium nitroprusside (SNP) or glyceryl trinitrate (GTN) (Koulchitsky et al. 2004, 2009). When the increasing activity plateaued after 1 h, olcegepant was injected intravenously and reduced the activity within a few minutes to the previous level (Koulchitsky et al. 2009). Recently our group has shown that an increase in c-fos-immunoreactive neurons, a marker of nociceptive activation in the rat spinal trigeminal nucleus, was reduced after inhibition of CGRP receptors with systemic administration of olcegepant. In contrast, an increase in the number of trigeminal ganglion neurons undergoing phosphorylation of extracellular receptor kinase, a fast nociceptive marker of primary afferents provoked by capsaicin injection into the facial skin, was not influenced by olcegepant preadministration (Sixt et al. 2009).

The current observations are paralleled by electrophysiological data in animal experiments. Iontophoretic administration of olcegepant has been shown to inhibit nociceptive transmission in the cat spinal trigeminal nucleus (Storer et al. 2004). Our group has found that intravenous injection of the CGRP receptor antagonist olcegepant diminished the ongoing activity of neurons with meningeal afferent input in the spinal trigeminal nucleus (Fischer et al. 2005). Olcegepant reduced also the responses of these neurons to graded heat stimuli applied to their meningeal receptive fields. In another series of experiments the activity of spinal trigeminal neurons was increased by infusion of the NO donors sodium nitroprusside (SNP) or glyceryl trinitrate (GTN) (Koulchitsky et al. 2004, 2009). When the increasing activity plateaued after 1 h, olcegepant was injected intravenously and reduced the activity within a few minutes to the previous level (Koulchitsky et al. 2009). Recently our group has shown that an increase in c-fos-immunoreactive neurons, a marker of nociceptive activation in the rat spinal trigeminal nucleus, was reduced after inhibition of CGRP receptors with systemic administration of olcegepant. In contrast, an increase in the number of trigeminal ganglion neurons undergoing phosphorylation of extracellular receptor kinase, a fast nociceptive marker of primary afferents provoked by capsaicin injection into the facial skin, was not influenced by olcegepant preadministration (Sixt et al. 2009).
All these data support the assumption that functionally active CGRP receptors are located centrally, most likely in the spinal trigeminal nucleus, where they contribute to the transmission of nociceptive afferent inputs (Meng et al. 2009; Storer et al. 2004). On the other hand, CGRP-induced relaxation of the isolated rat middle cerebral artery (Edvinsson et al. 2007) and pial arterial dilatation induced by CGRP or transcranial electrical stimulation (Petersen et al. 2004) were not significantly inhibited by luminal perfusion or infusion of olcegepant. Therefore, it has been concluded that the CGRP receptor antagonist penetrates the endothelial and blood-brain barrier poorly, so that a central action of intravenously applied olcegepant appears doubtful. This discrepancy gave rise to a new hypothesis: It seems possible that signaling by CGRP can also take place in the trigeminal ganglion, and NO could be involved in this process. We and others have found immunohistochemical evidence for functional CGRP receptors and soluble guanylate cyclase (unpublished data), the intracellular receptor of NO, in the rat trigeminal ganglion and spinal trigeminal nucleus (Lennerz et al. 2008; Efthekari and Edvinsson 2011). In cultured trigeminal ganglion neurons, NO donors have been found to increase the production and release of CGRP (Bellamy et al. 2006), while CGRP caused increased NO production and release from cultured trigeminal ganglion glial cells (Li et al. 2008). Furthermore, CGRP has been reported to cause transient inward currents and to increase intracellular Ca\(^{2+}\) levels in dorsal root ganglion neurons (Se- gond et al. 2002). These indirect findings indicate that CGRP may modify functional properties of trigeminal ganglion neurons and glial cells, thereby influencing neuronal activity of central trigeminal neurons. To clarify directly whether CGRP receptors in the rat trigeminal ganglion can modulate neuronal activity, CGRP and olcegepant were injected into the ganglion through the intraorbital canal and the activity of spinal trigeminal neurons with meningeal afferent input was recorded.

METHODS

The experiments were performed in accordance with the ethical policies of the International Association for the Study of Pain and the German laws for animal protection. The experimental protocol was reviewed by an ethics committee and approved by the local district government.

Surgical Procedures

Thirty-four male Wistar rats with body weights ranging from 250 to 350 g were used. For anesthesia they were initially placed in a closed box with an airflow of 5% isoflurane (Forene, Abbott, Wiesbaden, Germany) for ~5 min. The anesthesia was then continued through a mask imposed on the animal’s head with an isoflurane concentration of 2%. Catheters were introduced into the femoral artery and vein to record arterial pressure and infuse substances. The arterial catheter was continuously infused with saline supplemented by heparin (5 IE/ml, Heparin-Natrium 5000, Ratiopharm, Ulm, Germany) at a rate of 0.2 ml/h to avoid blood clotting. An intravenous catheter (Vasufo-T, Dispomed, Gelnhausen, Germany) used as a tracheal tube was inserted through the glottis, and the animal was artificially ventilated with room air supplemented with oxygen and 2% isoflurane. The ventilation frequency was set between 60 and 100 min\(^{-1}\) with a fixed stroke volume of 3.5 ml. To avoid an accumulation of viscous mucus in the tracheal tube, 0.05 mg/kg atropine sulfate (Braun, Melsungen, Germany) was injected intraperitoneally. The eyes of the animals were protected by an ointment (Bepanthen, Bayer, Leverkusen, Germany). Throughout the experiment the depth of anesthesia was sufficient to prevent noxious reflexes evoked by pinch stimuli or increases in blood pressure that may indicate pain. During recordings the animals were paralyzed with intravenous administration of 50 mg/kg gallamine triethiodide (Sigma, Steinheim, Germany). The arterial pressure and the expiratory CO\(_2\) were continu- ously monitored (Artema MM 200, Karl Heyer, Bad Ems, Germany); CO\(_2\) was maintained at 3–3.5%. The animals’ body temperature was held constant (36.5–37°C) by a feedback-controlled homeothermic system (TKM 0902, Förh Medical Instruments, Seeheim, Germany). After the experiments the animals were euthanized with an overdose of thiopental sodium (Trapanal, Nycomed, Konstanz, Germany).

Head Surgery

Animals were placed in a stereotaxic frame using ear bars to hold the head in a fixed position. The skin was cut in the midline from the eyes to the neck and pulled aside to expose the skull and neck muscles. With a dental drill and drops of cool isotonic saline, a cranial window of ~6 × 4 mm was made to expose the parietal dura mater. The medullary brain stem was made accessible by separating the neck muscles in the midline. The atlantooccipital ligament and the dura mater were cut to expose the dorsal medulla. A thin silicon tube (diameter 0.7 mm) connected to a 27-gauge needle (Neoject, Dispomed) was filled with fractions of 15 μl of vehicle stained with <0.01% Pontamine Sky Blue 6B (Kasei Kogyo, Tokyo, Japan) and 15 μl of a solution of olcegepant (10\(^{-5}\) M, BIBN4096BS, Boehringer Ingelheim, Biberach, Germany) or α-CGRP (10\(^{-5}\) M) separated by an air bubble of ~1 μl. The needle was introduced through the infraro- bital canal into the ipsilateral trigeminal ganglion located in the Meckel’s cave (see Fig. 1A). Access to the canal was 1 mm medial to the zygomatic process of the maxilla, from where the needle was inserted at an angle of ~10° toward the midline and ~15° downward from the plane formed by the parietal bone. The catheter with the needle was connected to a 30-μl Hamilton microsyringe (Hamilton-Bonaduz, Bonaduz, Switzerland) operated by a microinjector (Micro 4-Microsyringe Pump Controller, World Precision Instruments, Sarasota, FL).

Electrophysiological Recordings and Stimulation

Extracellular recordings were made from second-order neurons located in the spinal trigeminal nucleus caudalis with afferent input from the exposed part of the dura mater. With a microsteppe, self-made carbon fiber glass microelectrodes (impedance 0.1–5 MΩ) were inserted into the lateral brain stem 1.37–2.82 mm caudal to the obex. The recording electrode was slowly introduced through the brain stem at steps of 2.5 μm while the dura was stimulated with von Frey filaments until action potentials to the mechanical stimuli were acquired. The depth of recording sites according to the reading of the microdrive was noted. Receptive fields in the peristemeum (which covers the intact skull surrounding the cranial window), temporal muscle and neck muscles, facial skin, whiskers, and cornea were located with a fine glass rod. The extension of the meningeal receptive field in the cranial window was observed with graded von Frey filaments. The mechanical threshold in the center of the receptive field was assessed with single mechanical stimuli of 1-s duration with a custom-made combined mechano-electrostimulator. The electromag- netic driven stimulator unit has a movable metal tube (1-mm diameter) with a fine insulated silver wire placed in its lumen to form a bipolar stimulating electrode at its tip. Moving the tube caused indentations in the dura of a half-sinusoid pattern (see Fig. 1D). The applied pressure was controlled by a CED 1401 (Micro 1401mkII, Cambridge Electronic Design, Cambridge, UK) and a Spike 2 soft- ware application (Cambridge Electronic Design). With the stimulator...
tube fixed, single pulses (0.5 ms) of 0.5–7.2 mA were applied through the silver wire to the receptive field to assess the conduction latency of signals.

Recorded signals were amplified, band-pass filtered (0.5–1 kHz), and processed with CED 1401 hardware and Spike 2 software. Spike analysis was done off-line with the discharges evoked by mechanical stimulation of meningeal receptive fields as template.

Experimental Protocols

Signals, arterial pressure, expiratory CO₂ level, and body temperature were recorded online with the CED 1401 and Spike 2 software. Single half-sinusoid mechanical stimuli of 1-s duration and 1.5- to 2-fold mechanical threshold were applied to the meningeal receptive field at intervals of 1 min throughout the recordings (see Fig. 1D).
Ongoing and mechanically evoked activity were measured within a baseline period of 20 min, and then vehicle, olcegepant, or CGRP was slowly injected within 3 min into the trigeminal ganglion, followed by a second injection of another of these substances (see Fig. 1, E–G). The activity was recorded for 20 min after saline injection and for 30 min after olcegepant and CGRP.

In some experiments 0.5 ml of a solution of the NO donor GTN (250 μg/kg) in saline was infused intravenously at a rate of 8.3 μl/min prior to the recording, which started not earlier than 1 h after the end of the GTN infusion.

At the end of the experiment the x/y-position of the recording site relative to the caudal extension of the obex was noted according to the readings of the microdrives. The correct position of the injection needle was controlled by dissection of the trigeminal ganglion and inspection of the distribution of Pontamine Sky Blue (see Fig. 1C). Experiments in which the ganglion showed no blue staining were discarded. In some experiments anodal current (250 μA for 2 min) was passed through the recording electrode to localize the recording site marked by this lesion. In these cases the animal was perfused in deep anesthesia with saline followed by 4% paraformaldehyde (Roth, Karlsruhe, Germany) in phosphate-buffered saline (PBS). The trigeminal ganglion and brain stem were dissected and processed for histology. Cryostat serial sections were made to identify the extent of the solution that infiltrated the ganglion and the recording site in the spinal trigeminal nucleus.

**Chemicals**

CGRP (human α-CGRP, Calbiochem, Darmstadt, Germany) was dissolved in isotonic saline as a stock solution of 1 mM and diluted with saline (vehicle) to 10 μM immediately before use. Olcegepant (BIBN4096BS) was dissolved in acidic saline (pH 4), titrated to pH 7.0, and diluted to 1 mM for the ganglion injection. Isotonic saline of pH 7.0 was used as vehicle. GTN (1 mg/ml, Schwarz-Pharma, Monheim, Germany) was dissolved in saline (0.5 mg/ml) for intravenous infusion.

**Data Calculation and Analysis**

Discharges per minute were calculated throughout the experiment, but the injection and infusion periods were excluded. The mechanical stimulation within 1 s applied every minute caused discharges in addition to the spontaneous activity. These evoked discharges outlasted the stimulus period by 0.1 s in several cases. Therefore the ongoing activity within 58.9 s of every minute (excluding the 1.1 s of stimulation) was counted, and the mean activity within a 20-min period following each treatment was calculated. For the mechanically evoked activity the 1.1-s value of stimulus diminished by the mean activity per second of the single minute value prior to the respective stimulation was assessed, and the mean of 20 stimulations following each treatment was calculated.

Calculations were made with Excel and Statistica software 7.1 (Statsoft, Hamburg, Germany). Data were analyzed with the Wilcoxon matched-pairs test for pairwise comparison of the activity within 20-min intervals following injection of substances, if not stated otherwise. Differences are considered significant at P ≤ 0.05.

**RESULTS**

**General Properties of Spinal Trigeminal Neurons**

Recordings were made from 41 units in the right spinal trigeminal nucleus caudalis of 41 male Wistar rats with a body weight ranging from 250 to 350 g. In 14 animals GTN (250 μg/kg) was infused intravenously prior to the recordings. The units were located at a distance of 1.37–2.82 (mean 2.03) mm posterior to the obex and 0.23–1.74 (mean 0.75) mm from the midline and at a depth of 295–1,200 (median 697.5) μm from the surface of the medulla spinalis (Fig. 1B). The units showed spontaneous activity during the baseline period ranging from 5 to 2,196 impulses/min, which was not associated with any other property like location, mechanical threshold, or latency. All units had one or more mechanically sensitive receptive fields in the exposed parietal dura mater and additional sensory input from receptive fields in the facial skin, the frontal periost, the cornea, the temporal muscle, or neck muscles. The units were activated by rectangular electrical pulses (duration 1 ms) with thresholds ranging from 0.2 to 7.2 mA (mean 3.3 mA) and by half-sinusoid mechanical stimuli (thresholds ranging from 1 to 27.5 mN, mean 12.7 mN) applied to the cranial dura (Fig. 1D). The latencies after single electrical pulses close above threshold ranged from 8 to 60 ms (mean 22.52 ms), i.e., the units received afferent input from meningeal Aδ and/or C fibers assuming a distance of 25 mm from the dura mater to the caudal medulla. When the characterization of a unit was complete, the ongoing activity was recorded for at least 20 min (baseline period), while single mechanical stimuli of 1.5- to 2-fold mechanical threshold were applied.

**Responses to Vehicle Injection into Trigeminal Ganglion**

Small volumes (15 μl) of substances were slowly injected into the trigeminal ganglion within 2–3 min (intranganglionic injection). Care was taken to avoid any mechanical irritation due to movements of the inserted cannula, but in most experiments noticeable activation of spinal trigeminal neurons occurred during injection of volumes (Fig. 1, E and F). To analyze this response, we selected all units in which vehicle was injected first. The activity of the injection period was compared to the activity within the last 3 min of the baseline period. In units both without (n = 10) and with (n = 11) GTN pretreatment the injection of vehicle was accompanied by a significant increase in activity (P = 0.009 and 0.016; Fig. 2A). Because the rationale was to study possible influences of the injected substances on ongoing and mechanically induced activity, the injection period of 3 min was omitted and only the activity within a period of 20 min after the complete intranganglionic injection was analyzed for all further experiments.

In 10 experiments without GTN pretreatment the ongoing activity during a period of 20 min after the complete injection of vehicle appeared elevated but was not significantly different from the baseline (P = 0.11; Fig. 2B). In 12 experiments with GTN pretreatment the ongoing activity after injection of vehicle remained increased (P = 0.019; Fig. 2B). The mechanically evoked activity (average of 20 stimuli) did not change significantly after injection of vehicle into the trigeminal ganglion in both untreated and GTN-pretreated animals (P = 0.58 and 0.94; Fig. 2C) and was also not different between the untreated and GTN-pretreated groups (Mann-Whitney U-test, P = 0.84).

**Injection of CGRP and Olcegepant into Trigeminal Ganglion in Untreated Animals**

CGRP. In six experiments vehicle (saline) and α-CGRP (10⁻⁵ M) were injected into the trigeminal ganglion. In five experiments CGRP was injected after vehicle, and in one experiment this order was reversed. Injection of vehicle did not cause significant changes in ongoing or mechanically evoked...
activity within 20 min after injection ($P = 0.17$ and $0.46$). The ongoing activity after CGRP injection varied considerably, but no significant difference in ongoing or mechanically evoked activity between CGRP and vehicle was seen ($P = 0.60$ for both ongoing and evoked activity; Fig. 3).

**Olcegepant.** In 15 experiments vehicle and the CGRP receptor antagonist olcegepant ($10^{-3}$ M) were injected into the trigeminal ganglion. In 5 experiments olcegepant was injected after vehicle, and in 10 other experiments the injections were made in reversed order (see Fig. 1E). Neither injection of vehicle nor olcegepant administration caused significant changes in ongoing ($P = 0.23$ and $0.21$) and mechanically evoked ($P = 1.0$ and $0.91$) activity, and no difference in ongoing or mechanically evoked activity was observed between vehicle and olcegepant ($P = 0.91$ for both; Fig. 4).

**CGRP and olcegepant.** To see whether there is a direct antagonistic effect, $\alpha$-CGRP ($10^{-5}$ M) and olcegepant ($10^{-3}$ M) were injected into the trigeminal ganglion in six experiments. In three experiments CGRP was followed by injection of olcegepant, and in three other experiments the injection was in reversed order (see Fig. 1F). The ongoing activity within 20 min after injection of either substance was just significantly increased compared with baseline ($P = 0.028$ for both), but...
there was no difference between CGRP and olcegepant (P = 0.46; Fig. 5A). The mechanically evoked activity within 20 min after injection of either substance, irrespective of the order of injection, did not change compared with baseline (1st injection P = 0.92, 2nd injection P = 0.25; Fig. 5B).

Injection of CGRP and Olcegepant into Trigeminal Ganglion in Animals Pretreated with GTN

Because of the significant increase in activity remaining after vehicle injection into the trigeminal ganglion in experiments with GTN pretreatment, the ongoing activity was excluded from analysis. The mechanically evoked activity, which was not changed after vehicle injection, was further analyzed.

CGRP. In six experiments vehicle (saline) and α-CGRP (10⁻⁵ M) were injected into the trigeminal ganglion. In five experiments CGRP was injected after vehicle (see Fig. 1G), and in one experiment this order was reversed. The mechanically evoked activity did not change after injection of vehicle (P = 0.92) but decreased after CGRP injection compared with vehicle (P = 0.046; Fig. 6A).

Olcegepant. In eight experiments olcegepant (10⁻³ M) was injected into the trigeminal ganglion, in seven experiments after vehicle (see Fig. 1H). In one experiment olcegepant was injected twice; there was no difference in activity between the first and the second injection. The mechanically evoked activity did not change after injection of vehicle (P = 0.87) but increased after olcegepant injection compared with vehicle (P = 0.043; Fig. 6B).

DISCUSSION

CGRP has been recognized as an important neuronal mediator in the generation of headaches, which is evidenced by the analgesic effect of the CGRP receptor inhibitors olcegepant (BIBN4096BS) and telcagepant (MK-0974) (Ho et al. 2007; Olesen et al. 2004) in migraine and substantiated by antinociceptive effects of CGRP receptor blockade in the trigeminal system of animals (Fischer et al. 2005; Koukhitsky et al. 2009; Söxt et al. 2009; Storer et al. 2004). The sites of action of CGRP receptor antagonists are not clear, since evidence for CGRP receptor expression is found abundantly in peripheral as well as central structures (Cottrell et al. 2005; Ma et al. 2003; van Rossum et al. 1997). Regarding the course of meningeal afferents, CGRP receptors reside on different cell types in the cranial dura mater, the trigeminal ganglion, and the spinal trigeminal nucleus (Lennerz et al. 2008; Ma et al. 2003). While there is evidence that local blockade of CGRP receptors in the cranial dura mater is ineffective (Fischer et al. 2005) but
CGRP receptors controlling spinal trigeminal activity are located centrally to the trigeminal ganglion, most likely in the spinal trigeminal nucleus.

**Effects After GTN Pretreatment**

In one group of experiments the ganglion injection was made after administration of the NO donor GTN. NO donors have been shown to stimulate the expression of CGRP in cultivated trigeminal ganglion neurons (Bellamy et al. 2006) and to facilitate CGRP release from the intact trigeminal ganglion (Eberhardt et al. 2009), eventually increasing spinal trigeminal activity in animal experiments (Koulchitsky et al. 2004, 2009). In contrast to experiments without GTN pretreatment, the injection of vehicle and other volumes into the ganglion was followed by a tonic increase in activity beyond the transient peak of activity (see Fig. 2B). This may indicate that GTN not only causes activation of central trigeminal neurons (Koulchitsky et al. 2004) but can increase and prolong the response of trigeminal ganglion neurons to volume expansion. Since after GTN infusion there was no increase in mechanically evoked activity induced by stimulation of the dura mater (see Fig. 2C), the mechanism underlying the effect of volume injection is not likely to involve trigeminal fibers innervating the dura.

In contrast to our expectations, CGRP injection into the trigeminal ganglion was not followed by an increase in activity but rather the opposite was found, i.e., the mechanically evoked activity was reduced compared with vehicle injection (see Fig. 6A). It seems that under these conditions CGRP had activated an inhibitory mechanism in the trigeminal ganglion that limited the impact of primary afferent activation caused by the mechanical stimuli. Conversely, injection of olcegepant into the ganglion was followed by an increase in mechanically evoked activity compared with vehicle (see Fig. 6B). Our interpretation is that CGRP receptor inhibition in the trigeminal ganglion had neutralized the activity-limiting effect of CGRP released within the ganglion. The physiological significance of this CGRP mechanism is not clear, since increased CGRP release from the central terminals of primary afferents can induce enhanced neurotransmission (Storer et al. 2004), which may override the proposed inhibitory effect in the ganglion.

**Role of Trigeminal Primary Afferents**

The question arises as to how receptor mechanisms in the trigeminal ganglion can change the neuronal activity of second-order neurons in the trigeminal nucleus. In fact, the hypothetical mechanism outlined above can only work if the activity of neurons in the spinal trigeminal nucleus is at least partly driven by the activity of trigeminal afferents and if this afferent activity can be modulated within the trigeminal ganglion. In previous experiments our group has shown that injection of the local anesthetic lidocaine into the trigeminal ganglion dramatically reduced the activity of neurons in the spinal trigeminal nucleus, while topical application of lidocaine onto peripheral receptive fields was moderately inhibiting (Roch et al. 2007). During the preparation trigeminal afferents innervating meningeal receptive fields may have been activated, eventually sensitizing second-order neurons in the spinal trigeminal nucleus. However, only after pretreatment with the NO donor GTN did the trigeminal system appear to be sensitive to

![Mechanically evoked activity after GTN (n=6)](image)

![Mechanically evoked activity after GTN (n=8)](image)
activation and inhibition of CGRP receptors somewhere in the trigeminal ganglion. NO can induce changes in trigeminal ganglion cells that may include facilitation of CGRP release (Bellamy et al. 2006; Eberhardt et al. 2009) and glial gene expression (Li et al. 2008; Vause and Durham 2009).

Potential CGRP Signaling Within Trigeminal Ganglion

CGRP receptors potentially mediating CGRP signaling in the rat trigeminal ganglion are most likely not autoreceptors, since immunoreactivity for CGRP and the CGRP receptor proteins CLR and RAMP1 is rarely colocalized in the ganglion (Lennerz et al. 2008). Rather, functional CGRP receptors are expressed by CGRP-immunonegative neurons and glial cells (Schwann cells and satellite cells). Neurons and glial cells in sensory ganglia form functional units (Hanani 2005) and seem to communicate via gap junctions in the trigeminal ganglion (Thalakoti et al. 2007). CGRP released within the ganglion may thereby signal between different clusters of neurons and satellite cells in a paracrine manner.

The way in which inhibitory effects could be mediated by CGRP receptors is a matter of speculation. One possibility is that CGRP via intracellular mechanisms causes an increase in the open probability of K⁺ channels. Satellite glial cells are thought to stabilize the electrolyte environment by buffering extracellular K⁺ that is increased during high neuronal activity (Horio 2001). Evidence for inwardly rectifying K⁺ channels, which can observe this function in satellite cells, has been shown by immunohistochemistry (Hibino et al. 1999) and patch-clamp recordings (Cherkas et al. 2004). Thus CGRP signaling between neuron-glial clusters could contribute to stabilizing the membrane potential of primary afferents during activity. In oligodendrocytes the resting membrane potential has been found to be regulated by a protein kinase A-dependent pathway that probably controls the activity of inwardly rectifying K⁺ channels (Bolton and Butt 2006). CGRP receptors, which are known to be coupled to Gₛ proteins activating adenyl cyclase and increasing cAMP levels in different cell types (Brain and Grant 2004; Haug and Storm 2000; Takhshid et al. 2006), may be linked to such an intracellular mechanism.

In isolated dorsal root ganglion neurons CGRP receptors has been found to induce an increase in intracellular Ca²⁺ (Segond et al. 2002), which could activate Ca²⁺-sensitive K⁺ channels. Ca²⁺-activated K⁺ channels of the intermediate (IK) and small (SK) conductance type have immunohistochemically been localized in rat dorsal root ganglion neurons (Mongan et al. 2005), and the large conductance (BK) type has recently been found in the rat trigeminal ganglion to be involved in the control of CGRP release (Wulf-Johansson et al. 2010). Thus it seems possible that CGRP signaling within sensory ganglia eventually has a limiting effect on the excitatory functions of primary afferents.

Alternatively, increase in intracellular Ca²⁺ could trigger the release of inhibitory substances such as somatostatin within the trigeminal ganglion. Somatostatin, a peptide consisting of two biologically active forms with various inhibitory and antinociceptive functions (Pinter et al. 2006), is found in a subpopulation of rodent trigeminal ganglion neurons (DeLeon et al. 1994; Kummer and Heym 1986). Somatostatin receptors (sst2) have been located on small to medium-sized rat trigeminal ganglion neurons (Ichikawa et al. 2003; Takeda et al. 2007).

Somatostatin has been reported to inhibit CGRP release from dorsal root ganglion cultures (Van Op den Bosch et al. 2009) and to reduce the neuronal activity of trigeminal afferents and second-order neurons (Bereiter 1997; Takahashi et al. 2008; Takeda et al. 2007). Thus, provided that CGRP stimulates the release of somatostatin from trigeminal ganglion cells and somatostatin receptors are activated, a tonic inhibitory effect on trigeminal ganglion neurons seems plausible.

In summary, under normal conditions activation or inhibition of CGRP receptors in the trigeminal ganglion has no significant effects on the neuronal activity in the spinal trigeminal nucleus. GTN pretreatment may induce changes of CGRP receptors located on neurons or glial cells in the trigeminal ganglion, so that activation of these receptors reduces evoked primary afferent activity, thereby limiting neuronal transmission in the spinal trigeminal nucleus. Conversely, blockade of CGRP receptors in the trigeminal ganglion may unmask the effect of GTN pretreatment by inactivating the hypothetical inhibitory function of CGRP.

Possible Clinical Relevance

Given that the results of this study can be translated into the human trigeminal system, they could be relevant for the therapeutic use of CGRP receptor antagonists in migraine (Connor et al. 2009; Ho et al. 2007; Olesen et al. 2004). In our experiments without GTN pretreatment the injection of CGRP or the CGRP receptor antagonist olcegepant into the trigeminal ganglion did not change the ongoing and mechanically evoked activity of spinal trigeminal neurons. Thus, from the present data, there is no evidence that CGRP receptor antagonists act within the trigeminal ganglion to exert an antinociceptive effect. Together with the observation that local application of olcegepant onto the cranial dura mater does not change the activity of spinal trigeminal neurons (Fischer et al. 2005), a peripheral effect of CGRP receptor antagonists regulating neuronal activity appears unlikely. After GTN treatment an increase in spinal trigeminal activity has been observed (Koulchitsky et al. 2004), which was normalized after infusion of olcegepant (Koulchitsky et al. 2009). Our group has also seen upregulation of CGRP- and neuronal NOS (nNOS)-immunoreactive neurons in the trigeminal ganglion and nNOS-immunoreactive central neurons (Diederle et al. 2010). Thus we have assumed that the GTN treatment changes a naive rat into a state that may have similarities to migraine in humans. The present data indicate that after this treatment CGRP in the trigeminal ganglion has an antinociceptive rather than a nociceptive effect on mechanical stimulation of the dura mater. This probably does not abolish the therapeutic effects of CGRP receptor antagonists like olcegepant and telcagepant in migraine (Ho et al. 2007; Olesen et al. 2004), which may be based primarily on a mechanism located proximal to the trigeminal ganglion, i.e., in the central nervous system.

ACKNOWLEDGMENTS

We thank Jana Schramm and Birgit Vogler for their skilled technical assistance. Olcegepant was kindly provided by Dr. Henri Doods (Boehringer Ingelheim, Biberach, Germany).

GRANTS

The project was funded by the Deutsche Forschungsgemeinschaft (ME 995/3) and MSD Sharp & Dohme (Haar, Germany).
DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


Garry MG, Walton LP, Davis MA. Capsaicin-evoked release of immunoreactive calcitonin gene-related peptide from the spinal cord is mediated by nitric oxide but not by cyclic GMP. *Brain Res* 861: 208–219, 2000.


Haug T, Storm JF. Protein kinase A mediates the modulation of the slow Ca 2+ -dependent K + current, I ALP, by the neuropeptides CRF, VIP, and CGRP in hippocampal pyramidial neurons. *J Neurophysiol* 83: 2071–2079, 2000.


CGRP RECEPTOR EFFECTS IN TRIGEMINAL GANGLION 439

J Neurophysiol • doi:10.1152/jn.00167.2011 • www.jn.org


