Interactive report

Activation of trigeminovascular neurons by glyceryl trinitrate

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Accepted 12 September 2000

Abstract

The effect of intra-carotid arterial infusions of glyceryl trinitrate (GTN), a substance known to precipitate headache, including migraine, upon the spontaneous activity of trigeminal neurons with craniovascular input was studied in cats. Second-order craniovascular neurons which received sensory input from the superior sagittal sinus were recorded in the trigeminal nucleus caudalis. Infusions of GTN were administered via a catheter inserted retrogradely into the common carotid artery through the lingual artery. Infusions of GTN (100 μg kg\(^{-1}\) min\(^{-1}\) in a volume of 2 ml min\(^{-1}\)) increased the mean basal discharge rate of all second-order neurons to 239±47% of control.

GTN produced a fall in mean blood pressure, but there was no correlation between this fall and the changes in discharge rate. GTN infusions sensitised neurons to the effects of electrical stimulation of the superior sagittal sinus, but not to subsequent GTN infusions. Infusions of similar volumes of vehicle did not alter the discharge rate of neurons. We conclude that GTN activates craniovascular sensory pathways at a site at, or peripheral to, the second-order neuron and that such an action may account for at least the acute-onset headache induced by GTN.

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Theme: Sensory systems

Topic: Pain modulation: pharmacology

Keywords: Glyceryl trinitrate; Trigeminal; Vascular headache

1. Introduction

Glyceryl trinitrate (GTN, nitroglycerin) was first synthesised in 1846 by Sobrero who immediately observed that it produced a ‘migraine’ when he tasted it [29]. It was used as a homeopathic remedy for headache two years later [7] and for angina and hypertension later in the nineteenth century [19]. Alfred Nobel observed that workers in dynamite factories where the active principal, nitroglycerin, was incorporated into the explosive, suffered from chronic migraine-like headaches at work and anginalike problems on their vacations [19]. The therapeutic and side effects were thought to be due to the nitrate [20], as they were shared by other organic nitrates [23], but it is likely that they are due to release of nitric oxide (NO) from the GTN donor molecules.

Glyceryl trinitrate administration leads to an immediate throbbing headache in nearly all subjects [12,19]. In susceptible subjects, GTN reliably triggers a cluster headache like pain [4]. In migraineurs, it also produces a delayed headache highly reminiscent of the spontaneous migraine headaches which these people suffer. These effects have been attributed to a selective action of GTN on extracranial and intracranial blood vessels, principally the dural arteries and large penetrating cerebral arteries, mainly through the formation of NO [22]. It is tempting to assume that the immediate headache is a consequence of the vasodilator action of GTN, but both it and the delayed headache might be due to an effect of GTN or NO on neuronal function [32]. In 1993, Olesen and co-workers demonstrated that the immediate headache induced by GTN could be ameliorated by prior subcutaneous injection of the antimigraine drug sumatriptan, which also reduced the frequency of the delayed headache [11]. In rats, intravenous administration of GTN leads to increased...
expression of c-fos, a marker for neuronal activation, in the trigeminal nucleus caudalis and several other brain nuclei [31]. This activation was suppressed by the intraperitoneal administration of the non-steroidal anti-inflammatory drug indomethacin [33], which is effective in migraine [2].

GTN-induced expression of c-fos in the caudal trigeminal nucleus does not however demonstrate unequivocally whether GTN specifically activates those neurons responsible for dural or cerebrovascular pain processing, synaptic activation is only inferred. The experiments we describe here were carried out to determine whether GTN administered into the carotid artery of cats can produce activation of neurons which also process sensory information from the dura.

2. Materials and methods

Seven male or female cats (mass 2.7±0.5 kg; mean±standard deviation) were used in these experiments. They were anesthetised with halothane 1.5%, and then with intraperitoneal injections of α-chloralose (60 mg kg⁻¹). The femoral artery and vein were cannulated to measure blood pressure and heart rate and to administer intravenous drugs and fluids respectively. Animals were intubated and ventilated with 30% oxygen in air to keep end-expiratory CO₂ in the range 3.5–4.0%. Throughout the experiment, the animal was immobilised with intermittent intravenous gallamine triethiodide, 20 mg kg⁻¹. The depth of anesthesia during immobilisation was assessed periodically during the experiment by testing for sympathetic responses (pupillary dilatation, tachycardia, raised blood pressure) to noxious stimulation, and regularly by allowing the effects of gallamine to wear off and testing for withdrawal reflexes to pinching a hindpaw. Supplementary doses of either chloralose or gallamine were given when necessary. Rectal temperature was monitored throughout the experiment with a thermostor, and was maintained at 37–38°C by means of a servo-controlled heating blanket.

The lingual artery on one side was catheterised retrogradely with a small diameter catheter (SV8 polyvinyl tubing, Critchley Electrics), advanced until its tip lay in the common carotid artery. Heparinised normal saline (50 I.U. ml⁻¹) was continuously infused through the catheter at 5 ml h⁻¹ to maintain its patency.

The cat was mounted in a David Kopf stereotaxic frame and the lower brainstem was exposed through a C1 laminectomy and occipital craniotomy. The superior sagittal sinus was exposed by making parallel incisions in the dura and the falx; a plastic sheet was then passed through the incision in the falx [16]. Current spread to the cortex was further prevented by constructing a paraffin-filled well around the craniotomy site. The superior sagittal sinus was draped over a bipolar hook electrode, insulated except for the tips. The superior sagittal sinus was stimulated with supramaximal square-wave shocks (±150 V, 250 μs duration, 0.3 Hz) with a Grass S88 stimulator.

The central tungsten wire of a glass-coated tungsten electrode [18] was used to record single unit activity in the trigeminal nucleus caudalis. The electrode was placed on the dorsal surface of the brainstem, 1.5–3 mm caudal to the obex and 2.5–4.5 mm lateral to the midline, and advanced to a depth of up to 2500 μm below the surface by means of a piezoelectric microdrive. Single unit activity was amplified, filtered and displayed on an oscilloscope. Peri-stimulus and post-stimulus histograms were compiled and used for post-experiment analysis of the latency and firing frequency of single units.

Neurons were located first by the presence of a response to the search stimulus-electrical stimulation of skin or superior sagittal sinus. Some units were tested for the presence of a cutaneous receptive field. The skin and hair of the face were examined systematically with a variety of stimuli (brush, light touch, heavy pressure and pinch), and the cells classified according to response. Low threshold mechanoreceptive (LTM) units responded to light touch or brush, and did not increase firing rate with noxious stimuli. Nociceptive specific (NS) units responded only to heavy pressure or pinch, and wide dynamic range (WDR) units responded to non-noxious stimuli, but had an increased firing rate in response to noxious stimuli [9]. Congruence of action potential shape between SSS-induced responses and RF-induced responses was assessed visually or via an averaging program to ensure that both modes of stimulation were activating the same neuron.

One to three post-stimulus histograms, each consisting of 50 successive recordings of discharges in response to stimulation, were recorded for each neuron under control conditions. Histograms were acquired in pairs, with sagittal sinus and receptive field stimuli alternating in each acquisition run. The spontaneous discharge rate of the neurons was monitored by recording peri-event histograms in which each bin recorded the number of discharges recorded over a sample time of 4 s. Control discharge rates were recorded for 15–20 min. Glyceryl trinitrate (David Bull Laboratories) 5 mg ml⁻¹ in 30% (v/v) ethanol, 30% (v/v) propylene glycol was diluted 1:100 in normal saline. Drug-free vehicle was made from ethanol (30%) and propylene glycol (30%) in distilled water, similarly diluted and infused as a control substance. Drug or vehicle was infused at a rate of 100 μg kg⁻¹ min⁻¹ or equivalent volumetric rate (2 ml kg⁻¹ min⁻¹) from a glass syringe, with a Braun Perfusor. Infusions were carried out for 15 min or until neuron discharge rates peaked and began to decline again, whichever came first.

Selected recording sites were marked by producing an electrolytic lesion with a cathodal DC current (5 μA) passed through the tungsten wire for 5–10 s. At the end of the experiment, the cat was deeply anesthetised with sodium pentobarbitone (20 mg kg⁻¹) and perfused via the aorta with 0.9% saline followed by 10% phosphate-buf-
fered formalin. The lower brainstem/upper cord was removed and stored in phosphate-buffered formalin. The brainstem was later sectioned on a freezing microtome (50 μm sections) and stained with cresyl violet. Recording sites were reconstructed from a combination of electrolytic lesions or track marks and microdrive readings.

All group data are presented as mean ± standard error (S.E.M.), except where indicated. Neurons were classified as displaying an altered discharge rate by means of the critical ratio test [24]. A variance ratio test and a two-tailed Kolmogorov–Smirnov test [28] were used to test whether the pre-GTN and post-GTN discharge rate frequency histograms could have been drawn from the same population. The variance ratio test was used to compare more than two populations of neuronal discharge rates.

All experiments described in this report were approved by this university’s Animal Care & Ethics Committee and conformed to its guidelines.

3. Results

Twenty-seven neurons in the trigeminal nucleus caudalis of 7 cats responded to electrical stimulation of the superior sagittal sinus and were chosen for further study. The latencies of the response to stimulation were in the Aδ fiber range (mean latency to earliest discharge = 10.5 ± 1.2 ms). These latencies are similar to those recorded in our previous experiments [16]. Neurons discharged 1 to 15 times after a single supramaximal shock (mean number of discharges 3.6 ± 0.6). All 27 neurons discharged in the absence of impressed electrical stimulation with a basal rate of 4.4 ± 1.0 s⁻¹ (range 0.012–23 s⁻¹). Thirteen of the 27 neurons were tested for cutaneous receptive fields and all 13 were found to have them on the face: in the first (N=5), second (N=5) or third (N=1) trigeminal divisions (2, division not decidable) and were classified as WDR (7/8) or LTM (1/8). The locations of 10 lesioned recording sites out of 12 could be found on sections of the brainstems of 6 cats. Three sites were in laminae I/II of the trigeminal nucleus caudalis (mean depth 1.4 mm) and 2 were in laminae III/IV (mean depth 2.2 mm); all of these neurons responded to GTN with accelerated discharge rates following GTN infusion. Two sites were in the neck of the nucleus caudalis (mean depth 1.7 mm); neither of the neurons recorded from these sites responded to GTN with acceleration. The other three sites were in the adjacent ventrolateral medulla or nucleus retroambigualis (mean depth 2.5 mm); 2 of the neurons recorded from these sites responded to GTN infusions with an increase in discharge rate.

Fig. 1 shows a post-stimulus histogram obtained from a neuron in the trigeminal nucleus caudalis activated with Aδ fiber latency by electrical stimulation of the superior sagittal sinus.

Infusion of GTN through the indwelling lingual artery catheter resulted in a rapid increase in the discharge rate of neurons, commencing immediately and reaching a maximum 3–4 min after the start of infusion. Discharge rates rose to about 400% of control at this peak and fell back to average about 170% of control for the remaining time of infusion. The average duration of infusions was 8.9 ± 0.9 min. The mean rate of discharge during infusion was 239 ± 47% of control over the duration of the infusion in all 32 tests. In 23 out of 32 tests (20 out of 27 neurons), this increase in rate was significant at the 0.05 level, according to the criteria of the critical ratio test.

Two neurons in the neck of the trigeminal nucleus did not respond to GTN with an accelerated discharge rate but otherwise there appeared to be no preferential location in the nucleus for neurons which fulfilled the criteria of the test, nor was there a significant difference in the pre-
infusion discharge rate between those neurons which responded \((N=23 \text{ tests})\) and those which did not \((N=9 \text{ tests})\) \((t=1.48, \text{ P NS})\). The Kolmogorov–Smirnoff test showed a significant change in population characteristics for all 32 infusions. The only neuron with LTM input was also accelerated.

A significant increase in discharge rate persisted after cessation of infusion; the mean was still 150% of control 30 min after the infusion (Fig. 3). Furthermore, in some cats the same neuron was tested more than once for its responses to GTN infusions and, in most cats, more than one neuron was tested for its response. Two to six hours separated these successive tests. The mean basal discharge rates of ‘non-naïve’ neurons recorded prior to the \(N\)th infusion were higher than the mean basal discharge rates recorded for the \((N−1)\)th infusion, but there was no difference in the percentage increase in discharge rate induced by GTN in successive infusions. However, there was some evidence that infusions of GTN sensitised neurons to the effects of electrical stimulation of the sagittal sinus: mean responses to SSS stimulation in discharges per stimulus increased according to whether the neurons had experienced one or more previous GTN infusions. These results, with the results of an analysis of variance for each measure, are shown in Table 1.

Fig. 2 shows the ongoing activity of a neuron which responded to electrical stimulation of the superior sagittal sinus and was then tested with GTN and vehicle. The neuron was spontaneously active, with a discharge rate that varied from 5 to 8 per second. Fig. 3 shows the time-averaged mean response from 32 tests in 27 neurons subjected to GTN infusions.

Infusion of GTN led to a fall in mean blood pressure, a rise in pulse pressure and a rise in heart rate in most tests, but there was a rise of mean blood pressure in five tests. Before infusion, the value of the mean arterial blood pressure was \(103±3\text{ mmHg}\) and the mean value of heart rate was \(251±5\text{ beats per minute}\). At the trough of the blood pressure fall during infusion, mean arterial blood pressure was \(92±3\text{ mmHg}\) and the mean heart rate, measured at the time of maximum changes was \(263±5\text{ beats per minute}\). The changes were significant \((t=2.89, N=30, \text{ P}<0.01 \text{ for blood pressure}; t=4.1, N=28, \text{ P}<0.0005 \text{ for heart rate}; \text{ Student’s paired} \text{ t-test})\). An example of changes in blood pressure, heart rate and expired \(\text{CO}_2\) is shown in Fig. 2. However, there was no correlation between the size of the changes in blood pressure and the size of the changes in neuronal discharge rate. Fig. 4 shows a scattergram of the percentage changes in neuronal firing rate induced by GTN plotted against the percentage changes in mean blood pressure occurring at the same time.

Infusion of the vehicle alone in 5 cats during the monitoring of 16 neurons did not produce any significant change in the discharge rates of the \(N\)th neuron (Fig. 5) nor in mean blood pressure \((t=1.07, \text{ NS})\) or heart rate \((t=0.01, \text{ NS})\). Based on average carotid flow rates (usually about \(25 \text{ ml min}^{-1}\), vehicle or GTN infusion should have produced an average ethanol concentration in the carotid blood of \(0.02\% (0.2 \text{ mg/kg})\) and, at the end of an average infusion, a body load of about \(0.002\% (0.02 \text{ mg/kg})\), not allowing for removal mechanisms.

4. Discussion

These results support the idea that glyceryl trinitrate (nitroglycerin, GTN), which produces headache in humans [34], activates the trigeminovascular sensory system in cats, and could conceivably also do so in humans. The results show that GTN increases the discharge rate of trigeminovascular second-order neurons immediately, but also that it leads to an overall increase in the discharge rate of all craniovascular neurons, beyond the time of infusion. These two effects might correlate with the immediate [12] and delayed [11] effect of GTN in producing human headache.

Experiments in animals over the last 20 years have demonstrated that there is a pool of neurons in the trigeminal nucleus caudalis and upper cervical spinal cord, which are activated by electrical, mechanical or chemical stimuli of the dural blood vessels [3,14,16,30]. Neurons in this trigeminovascular pool are potential mediators of headache in humans, because the same types of stimuli in migraineurs effectively trigger their typical headaches [5,26,27].

Neurons which responded to GTN infusions with an accelerated discharge rate were found in the trigeminal

<table>
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<th>Table 1</th>
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<td>Effects of successive infusions of GTN on ‘basal’ discharge rates of trigeminovascular neurons in the same cat prior to the (N)th infusion, on mean responses to the (N)th infusion of GTN and on mean responses to stimulation of the sagittal sinus prior to the (N)th infusion</td>
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<tr>
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<td>Resting rate ((\text{s}^{-1}))</td>
<td>2.1±0.9</td>
<td>2.6±0.9</td>
<td>4.5±1.9</td>
<td>13.1±4.5</td>
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<tr>
<td>GTN response (% of resting)</td>
<td>445±188</td>
<td>177±28</td>
<td>208±42</td>
<td>222±37</td>
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<tr>
<td>SSS response (discharges)</td>
<td>2.7±0.4</td>
<td>4.4±0.6</td>
<td>3.2±0.2</td>
<td>4.6±0.7</td>
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\(^a\) Parameters are compared across 4 infusions by an analysis of variance, with calculated F-ratio and consequent probability values shown in the two right-most columns.
Fig. 2. Top: Peri-event histogram of the same neuron shown in Fig. 1 to intravenous infusions of glyceryl trinitrate (‘GTN’) and of vehicle at a rate of 100 μg kg⁻¹ min⁻¹ (first and second bars, gray). The volume rate of both infusions was 2 ml min⁻¹. There was no change of discharge rate in response to vehicle infusion, but the rate of discharge increased to 2.5 times control at the peak of the response to GTN. Vertical axis=discharges per bin of 4 s. 2nd, 3rd and 4th panels: Blood pressure, heart rate and expired CO₂ recorded simultaneously with the cell discharge changes shown in the top panel. There was a small fall in blood pressure during the infusion, but no significant changes in heart rate or CO₂. Horizontal axis on all panels=time in s, 1 sampling bin=4 s.

nucleus caudalis itself and in the sub-adjacent ventrolateral medulla and nucleus retroambigualis. Two neurons found to be in the neck of the nucleus caudalis did not respond to GTN. GTN responsive neurons in this study were found in the same locations as fos-expressing neurons seen after GTN infusions in rats [31] and also after SSS stimulation in cats [13].

The experiments of Olesen et al. examined the headache produced by GTN [25] and demonstrated that it could be relieved by administration of the anti-migraine drug sumatriptan [11]. The same group also showed that spontaneous migraine is relieved by administration of a nitric oxide synthase inhibitor [17]. Intravenous administration of GTN to animals leads to another indication of trigeminal sensory activation, the expression of c-fos in the trigeminal nucleus caudalis [31]. It is not possible, however, to say whether neurons activated in this way had any connection with dural sensation. The usual infusion rate for GTN in human cardiovascular disease is 5–20 μg/min (approximately 0.07–0.28 μg kg⁻¹ min⁻¹) and, according to the product literature, this will produce headache in only about 2% of patients, but, under these circumstances, headache may not be a symptom to which such patients pay attention, and they may also be receiving other drugs which may prevent or ameliorate headaches. Previous studies [11] have described headache in both control subjects and migraineurs after 20 min of an infusion of 0.12 μg kg⁻¹ min⁻¹. In rats, a subcutaneous dose of 10,000 μg kg⁻¹ has been used to
induce activation of c-fos immunoreactivity [31]. The infusion rate we have used in cats, 100 μg kg⁻¹ min⁻¹, lies near the geometric mean of these amounts, but it is not easy to decide whether the three different rates represent fundamental pharmacological differences or are merely related to species differences (human vs. rat vs. cat) or route of administration.

GTN is a potent vasodilator and the headache it induces could be related to this vasodilation because both the cranial vasodilatation and the headache induced by GTN seem to be simultaneously reduced by prior treatment with sumatriptan [6], although sumatriptan is not a prophylactic agent for migraine itself. Our own observations suggest that the anti-migraine drug eletriptan can reduce or reverse the activation produced by GTN [15]. It is also possible that GTN activates the trigeminal sensory system by a neuronal mechanism [33] and it has been shown that nitric oxide is involved in a cascade of neuronal and neurovascular effects [1] which also involve calcitonin gene-related peptide [10,21] — a sensory neuromodulator implicated in migraine. Tassorelli et al. presented evidence that favours the idea that it is the neuronal, rather than the vascular, effects of GTN which underlie its ability to induce headache in humans [31]. The nitric oxide synthase inhibitor l-NAME reduces c-fos expression elicited in the trigeminal nucleus and cervical spinal cord by electrical stimulation of the superior sagittal sinus [8], which suggests that transduction of dural sensation into central neuronal activation may involve nitric oxide in some way, presumably unrelated to the vascular involvement of nitric oxide.

We calculated that the vehicle used in the GTN infusions and their controls would produce a moment-to-moment carotid blood alcohol level of 0.2 mg/ml in a carotid artery of mean flow 25 ml/min and a post-infusion whole body concentration of 0.02 mg/kg in a 2.7 kg cat. The carotid blood alcohol concentration might be expected to produce cranial vasodilatation, but this could not be monitored. Such vasodilatation and possibly a direct effect on sensory nerve endings could conceivably have activated craniovascular sensory neurons, but we saw no evidence for this. Systemically, the concentration of ethanol (≈0.002%) seems unlikely to have generated significant cardiovascular effects.

The systemic dilator effects of GTN result in a fall in blood pressure, a change which could conceivably result in changes in the discharge rate of central neurons or the ability of the system to record them. However, the blood pressure changes are relatively small and we have shown...
Fig. 5. Mean effects of infusions of glyceryl trinitrate (GTN, N=32) on the discharge rate of trigeminovascular neurons. Rates are expressed as the mean rate of discharge for the duration of the infusion, displayed as a percentage of the mean rates of discharge for 3 min prior to commencement of infusion. GTN increased the discharge rate from 4.4 to 9.0 s⁻¹, but there was no effect of the vehicle.

them to be uncorrelated with changes in recorded neuronal discharge rate. In previous experiments we have rarely seen any change in discharge rate or neuronal responses attributable to changes in systemic blood pressure.

Our experiments do not distinguish between a purely dilator effect, a neuronal activation, or even an effect on sensory modulation of central origin for GTN in this system, but they do unequivocally demonstrate sensory activation in an important component of a pathway that is related to headache. The present experiments with GTN show that neurons activated by GTN and by electrical stimulation of the dura come from the same neuronal pool, which strengthens the case for using them as a model system for the pathophysiology of headache.

Acknowledgements

This research was supported by grants from the National Health and Medical Research Council of Australia, the Migraine Trust and the Australian Brain Foundation.

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