

Potentiation of reflex blinks by glyceryl trinitrate in the rat: A novel model for migraine.

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❶ INTRODUCTION

Migraineurs are often highly sensitive to nitric oxide donor compounds, such as glyceryl trinitrate (GTN). In many sensitive individuals, GTN causes severe headache developing over the course of hours into full migraine. In animal studies, GTN increases neuronal activity in the trigeminovascular pathway and elevates neurotransmitter levels in the brainstem.

Many migraineurs also display abnormalities in blink reflexes, the processing of which is known to involve brainstem circuits. Reflex blinks can be elicited in rodents by electrical stimulation of the supraorbital nerve or by electrical or physical stimulation of the corneal surface and/or palpebral areas. We investigated the effect of GTN, in the anaesthetised rat, on reflex blinks evoked by air puff stimulation of the corneal surface and palpebral margin, to determine whether such reflexes may prove useful as the basis for a novel animal model to evaluate potential anti-migraine therapeutic agents.

❷ METHODS

Male Sprague-Dawley rats (300-375 g, n=23) were anaesthetised with urethane (1.25 g/kg i.p); the trachea, and left femoral blood vessels were cannulated to facilitate maintenance of clear airway, recording of arterial blood pressure and intravenous administration of pharmacological agents. Reflex blinks were evoked by brief air puffs directed onto the surface of the cornea and the upper palpebral margin. Where necessary, the cornea was kept moist by the occaisional application of artificial tear fluid. Electromyographic activity of the *orbicularis oculi* muscle (*OO*EMG) was recorded via a silver ball electrode resting lightly on the surface of the upper eyelid. Good electrical contact was ensured by gentle close clipping of the eyelid fur and application of electrode gel between the skin and the silver ball.

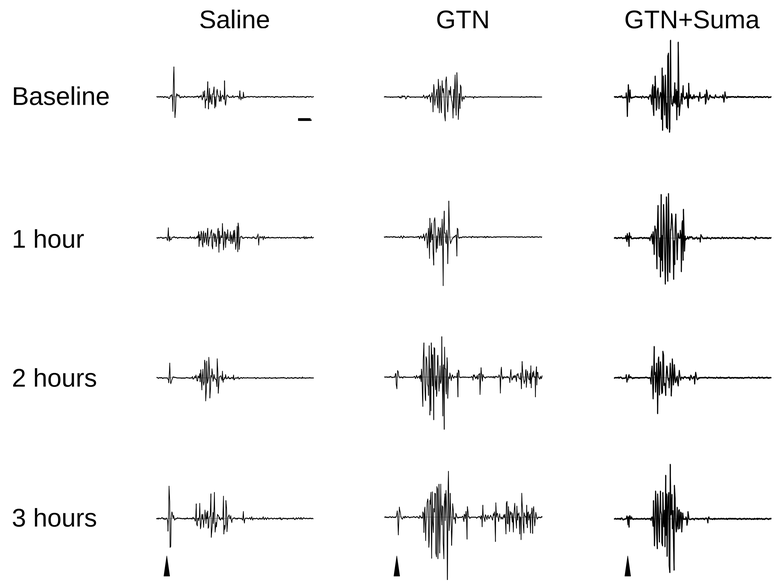
A single puff stimulus was delivered once every 15 minutes using a custom-built air jet apparatus with the jet nozzle positioned 5-6 mm from the surface of the cornea. The evoked EMG activity, recorded on a computer using Spike 2 software (CED, Cambridge, UK), was rectified and averaged in 1 hour epochs. After 1 hour of baseline recording, either 0.9% saline, GTN (50 μg/kg/hour) or GTN plus sumatriptan (300 μg/kg) was infused over 30 minutes. Infusion volume was 2 ml/kg in all cases. Stimulation at the rate of 4 puffs per hour was continued for at least 3 hours post-infusion. The magnitude (area under curve of mean rectified EMG) of the combined R1+R2 *OO*EMG response at each hour post-infusion was compared to baseline.

1.



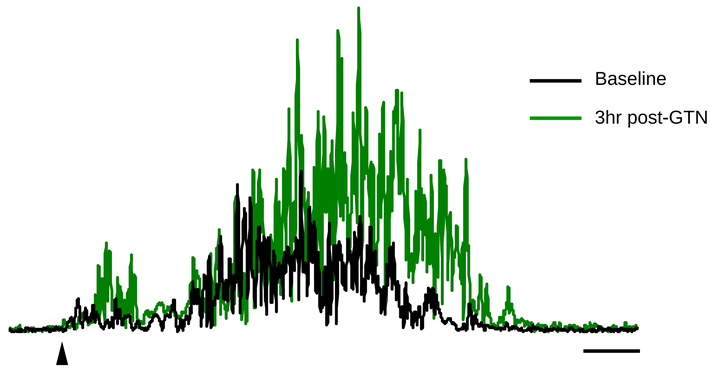
The photograph illustrates the experimental set-up with ball-ended silver recording wires resting on the skin surface. The indifferent electrode was placed 10-12 mm away from the recording site over the *OO*. The air puffer nozzle is positioned perpendicular to the surface of the cornea at a distance of approximately 5 mm. Illustrated animal is Agouti rat.

2. Raw EMG activity in *orbicularis oculi (OO)* muscle.



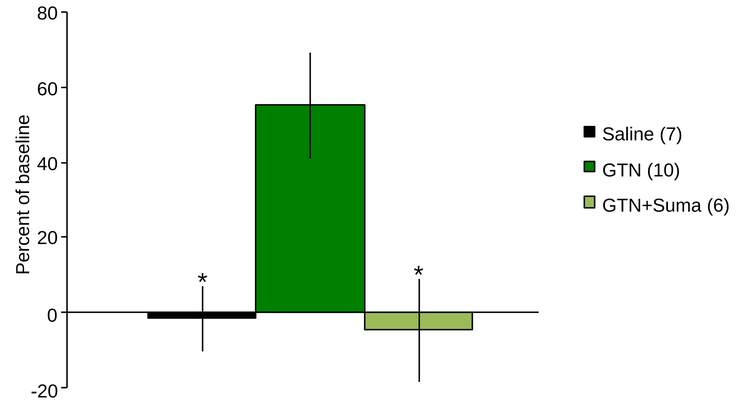
Raw EMG traces showing activity in the ipsilateral suprapalpebral orbicularis oculi muscle evoked by air puffs. Each trace represents a single sweep recorded during each of the four 1 hour epochs - baseline up to 3 hours post-treatment. Following GTN infusion, there is a progressive increase in the intensity of the reflex EMG response. Co-infusion of sumatriptan with GTN inhibits this effect. By comparison, the EMG remains stable after saline infusion. Arrows indicate timing of stimulus; scale bar, 10 ms.

3. Effect of GTN on rectified *OO* EMG.



In this example, the combined R1 and R2 components of the blink reflex (mean of rectified signals, 4/hr) are potentiated at 3 hours following GTN infusion. Arrow indicates timing of stimulus; scale bar, 10 ms)

4. GTN Potentiation of *OO*EMG inhibited by sumatriptan.



Histogram comparing the effects of saline, GTN and GTN plus sumatriptan on the magnitude of the evoked orbicularis oculi EMG at 3 hours post-infusion. The * marker denotes significant difference compared to GTN (P<0.01, 1-way ANOVA with Holm-Sidak *post-hoc* test for multiple pairwise comparisons). Bracketed values, n for each group.

5. Table of data for saline, GTN and GTN plus sumatriptan.

Treatment	% Change from baseline post-treatment		
	1 Hr	2 Hr	3 Hr
Saline (7)	-4.9 ± 8.5	+2.3 ± 11.7	-1.6 ± 8.7 *
GTN (10)	+6.5 ± 13.5	+41.9 ± 16.6	+55.3 ± 14.2
GTN+Sumatriptan (6)	+11.2 ± 21.0	-5.0 ± 19.5	-4.6 ± 13.6 *

The tabulated data demonstrate how the effect of GTN increases up to 3 hours post-infusion. * Denotes significant difference with respect to GTN, as in Figure 4.

❸ SUMMARY

- Silver wire electrodes resting on the skin surface provide a reliable and completely non-invasive method of recording EMG activity from the OO muscle.
- Corneal air puff reliably evokes reflexes blinks in the anaesthetised rat
- GTN infusion significantly potentiates the intensity of air puff-evoked reflex blinks.
- The antimigraine drug, sumatriptan, inhibits the potentiation of reflex blinks.
- The preparation may prove a useful novel electrophysiological model for evaluation of candidate antimigraine compounds in the laboratory.

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