Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 464 (2003) 159–162
www.elsevier.com/locate/ejphar

Short communication

Nitroglycerin induces hyperalgesia in rats—a time-course study

Cristina Tassorelli[a,*], Rosaria Greco[a], Dechun Wang[b], Maurizio Sandrini[c], Giorgio Sandrini[a], Giuseppe Nappi[a,d]

[a] *Laboratory of Pathophysiology of Integrative Autonomic Systems, University Centre for the Study of Adaptive Disorders and Headache (UCADH), IRCCS C. Mondino Institute of Neurology, Pavia, Italy*
[b] *Shanghai Medical University, Beijing 100101, PR China*
[c] *Department of Biomedical Science, Pharmacology Section, Department of Internal Medicine, University of Modena, Modena, Italy*
[d] *Department of Clinical Neurology and Otolaryngology, University of Rome "La Sapienza", Rome, Italy*

Received 24 January 2003; accepted 31 January 2003

Abstract

Nitroglycerin is a nitric oxide (NO) donor which activates nuclei involved in nociceptive transmission following systemic administration. The effect of nitroglycerin on the nociceptive threshold was studied in rats by means of two experimental tests that explore different modalities of pain: the tail-flick test and the formalin test. Nitroglycerin induced a significant reduction in the latency of the tail flick 2 and 4 h after its administration. Similarly, formalin-induced pain-related behaviour increased significantly 2 and 4 h after nitroglycerin administration.

Keywords: Formalin test; Hyperalgesia; Migraine; Nitric oxide (NO); Pain; Tail-flick test

1. Introduction

Nitroglycerin is a highly lipophilic organic nitrate which releases nitric oxide (NO) by enzymatic and non-enzymatic reactions (Harrison and Bates, 1993). Systemic nitroglycerin activates neuronal groups in selected areas of the rat brain involved in nociception (Tassorelli and Joseph, 1995; Tassorelli et al., 2001, 1999). Several reports show that nitroglycerin induces spontaneous-like headache attacks in migraine sufferers (Iversen et al., 1989; Sicuteri et al., 1987), possibly related to sensitization phenomena, as suggested by recent neurophysiological investigations in healthy subjects (Sandrini et al., 2002).

Nitroglycerin-derived NO exerts a biological effect on neuronal activity (Ma et al., 1995). In addition, systemic nitroglycerin increases the levels of the neuronal isoform of NO synthase (NOS) in the rat medulla (Pardutz et al., 2000). Several reports suggest that NO is closely involved in the development and maintenance of hyperalgesia (Ferreira et al., 1999), which is likely to take place following the activation of Ca^{2+}-dependent NOS. To our knowledge, only one study has evaluated the effect of nitroglycerin on pain transmission in animals, yielding conflicting results (Masue et al., 1999).

In the present study, we evaluated the hyperalgesic effect of nitroglycerin by means of two experimental animal models of pain: the tail-flick test and the formalin test.

2. Materials and methods

2.1. Experimental protocol and groups

Adult male Sprague–Dawley rats (weight: 180–220 g) were housed in plastic boxes in groups of 3, with water and food provided ad libitum. They were kept on a 12-h/12-h light–dark cycle and acclimatized to the test chamber before testing.

The experimental protocol used in the present study complies with European Union guidelines for the use of experimental animals and was approved by the Ethics

* Corresponding author. IRCCS Neurological Institute C. Mondino Foundation, Via Palestro, 3, Pavia 27100, Italy. Tel.: +39-382-380221; fax: +39-382-380286.
E-mail address: cristina.tassorelli@mondino.it (C. Tassorelli).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01421-3

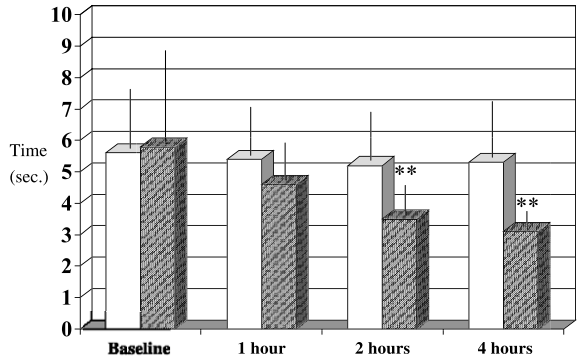

Fig. 1. Time course of the hyperalgesic response induced by nitroglycerin in the tail-flick test. Significant hyperalgesia was observed 2 and 4 h after drug administration. Data are expressed as mean ± S.D. White bar = rats treated with vehicle; hatched bar = rats treated with nitroglycerin. *$p < 0.05$ and ** $p < 0.03$ vs. controls.

Committee of the IRCCS C. Mondino Institute of Neurology.

Rats were randomly divided into groups, each comprising 5–8 animals, and treated according to the following schedule.

2.1.1. Experiment 1

Groups 1a, 1b and 1c: tail-flick latency was measured at baseline and at 1 (1a), 2 (1b) and 4 (1c) h after nitroglycerin administration.

Groups 1d, 1e and 1f: tail-flick latency was measured at baseline and at 1 (1d), 2 (1e) and 4 (1f) h after vehicle administration.

2.1.2. Experiment 2

Group 2a: i.p. injection of vehicle; formalin test performed after 1 h.

Groups 2b, 2c and 2d: i.p. injection of nitroglycerin; formalin test performed after 1 (2b), 2 (2c) and 4 h (2d), respectively.

2.2. Drugs and vehicles

Nitroglycerin (Astra, Italy), dissolved in saline, alcohol and propylene glycol, was injected i.p. at a dose of 10 mg/kg (Tassorelli and Joseph, 1995; Pardutz et al., 2000).

Formaldehyde was mixed with saline to obtain 1% formalin.

2.3. Tail-flick test

The test was performed with an Ugo Basile tail flick instrument (model 7360) that allowed automatic recording of the latency of the tail-flick response to radiant heat. The number of seconds elapsing between activation of the heat source and the rat flicking its tail away (latency) was recorded. Each evaluation was calculated as the mean of three measurements in three different parts of the tail.

2.4. Formalin test

One animal at a time was placed in a plexiglas observation chamber (10 × 20 × 24 cm) equipped with an angled mirror (45° angle) to facilitate observation of the injected paw. Animals received a 100-μl s.c. injection of 1% formalin (formaldehyde diluted in 0.9% saline) into the midplantar region of the left hindpaw. Pain-related behaviour was quantified for 1 h by counting spontaneous flinches and shakes of the injected paw: over 60-s periods for the first 5 min (min 1, 2, 3, 4 and 5) and thereafter following 4-min pauses, for 1-min periods up to the hour (Tjolsen Aberge et al., 1992). phase 1 was defined as the period from 1 to 5 min, phase 2 was defined as the period from 10 to 60 min inclusive (Hao et al., 2000).

2.5. Statistical evaluation

Analysis of variance (ANOVA) for repeated measures was used to evaluate the influence of time and treatments. Differences between groups at specific time-points were analysed by post-hoc *t*-test. A probability level of less than 5% was regarded as significant.

3. Results

3.1. Tail-flick test

Nitroglycerin induced a significant decrease in the latency of the tail flick, compared to baseline, at 2 (−30.2%) and 4 h (−35.1%); no significant difference was found between the two time points (Fig. 1). On the contrary, vehicle injection did not induce any significant change vs. baseline in tail-flick latency at any time point.

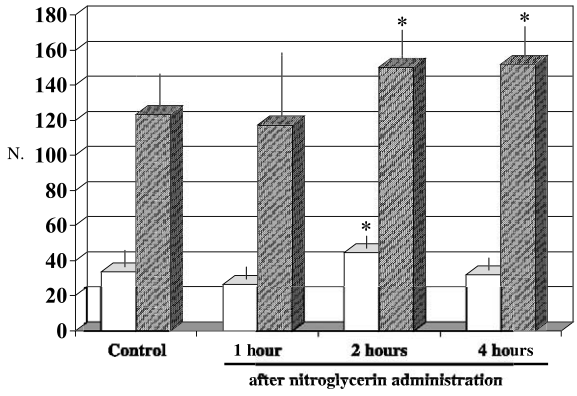

Fig. 2. Time course of nitroglycerin-induced hyperalgesia as detected in the formalin test. The formalin-induced pain-related behaviour is expressed as number of flinches and shakes during phase 1 (white bars) and phase 2 (hatched bars). Significant hyperalgesia was observed in both phases at 2 h in phase 2 and at 4 h post-nitroglycerin administration. Data are expressed as mean ± S.D. *$p < 0.03$ vs. controls.

3.2. Formalin test

When the formalin test was performed 1 h after nitroglycerin administration, no significant change was observed in either phase of the test (Fig. 2). Rats tested 2 h after nitroglycerin injection (*Group 2c*) showed a significant increase in the number of flinches and shakes during both phases of the test, while rats tested 4 h after nitroglycerin injection (*Group 2d*) showed a statistically significant increase in the magnitude of flinches and shakes only during phase 2.

4. Discussion

Several reports have suggested that NO has a hyperalgesic effect (see Hoheisel and Mense, 2000 for a review), and previous experimental studies have shown that some NO donors, e.g., 3-morphosynodiomine and sodium nitroprusside, can intensify ongoing pain (Kitto et al., 1992; Sousa and Prado, 2001). Masue et al. (1999) described thermal hyperalgesia following intrathecal administration of nitroglycerin in rats, but not following its systemic administration. In a previous report, we showed that systemic nitroglycerin induces neuronal activation in the nucleus trigeminalis caudalis (Tassorelli and Joseph, 1995), a very important structure in nociceptive transmission. More recently, our group has demonstrated that nitroglycerin increases the nociceptive RIII reflex in humans following its systemic administration (Sandrini et al., 2002).

In the present report, we provide a systematic analysis of the hyperalgesic effect of systemic nitroglycerin by evaluating its activity in different experimental models of pain over an extended period of time.

4.1. Tail-flick test

The tail-flick test reflects acute, physiological pain and results from the activation of Aδ and unmyelinated C primary afferents. In the present study, we show that nitroglycerin induces hyperalgesia 2 and 4 h after drug administration. Nitroglycerin is a lipophilic substance that easily crosses the blood–brain barrier, accumulating in the cerebral tissue (Torfgard and Ahnler, 1991), where its biological effects last several hours (Tassorelli and Joseph, 1995; Pardutz et al., 2000; Lambert et al., 2000). Nitroglycerin-derived NO can easily diffuse across cell membranes, its activity mirroring the effects of endogenous NO (Ma et al., 1995). We can therefore speculate that nitroglycerin-induced hyperalgesia may be related to a NO-mediated facilitation of nociceptive transmission in both the peripheral tissue and the spinal cord.

The failure of Masue et al. (1999) to find hyperalgesia following systemic nitroglycerin administration may be related to the much lower dose of drug used (10 μg), which might not have been able to reach the spinal cord in biologically active amounts. Another explanation may be their choice of vehicle: saline is not the most adequate solvent for nitroglycerin.

4.2. Formalin test

This test constitutes a model of inflammatory pain and consists of two distinct phases of pain-like behaviour. The first results from the activation of primary afferent fibres (C-fibre barrage), while the second is believed to reflect both inflammatory-evoked sensory activity and facilitatory processes in the spinal cord (Ito et al., 2001).

We describe a clear-cut time course for the nitroglycerin-induced changes in the pain-related response to formalin injection. No hyperalgesia was detected 1 h after nitroglycerin administration. At 2 h, significant hyperalgesia was observed, which involved both phases of the test. Finally, phase-2 hyperalgesia was detected at 4 h.

Pharmacological studies are presently ongoing in our laboratory in order to better understand the mechanisms underlying the time course of nitroglycerin-induced hyperalgesia during the formalin test and the possible involvement of differential mechanisms in the two phases. On the basis of the data presented here, we can speculate that nitroglycerin-formed NO may facilitate nociceptive transmission, both at peripheral and spinal levels. The injury to the peripheral tissue following formalin injection provokes the release of excitatory neurotransmitters, such as glutamate and substance P, from primary afferents and dorsal horn neurons (Coderre et al., 1993). The subsequent glutamate-mediated activation of NMDA receptors leads to a Ca^{2+}-influx and initiates an enzymatic cascade, which finally evokes the release of NO and prostaglandins (PGs) (Kawamata and Omote, 1999). It has been hypothesized that NO and PGs further increase glutamate release (Kawamata and Omote, 1999), leading to an ongoing activity in primary afferents, increased sensitivity of dorsal horn neurons and, finally, central sensitization (Sousa and Prado, 2001).

Nitroglycerin is immediately available in peripheral tissues, while it reaches its maximal concentration 2 h later in the brain (Torfgard and Ahnler, 1991). Therefore, the absence of nitroglycerin-induced hyperalgesia at 1 h, along with the fact that the maximal hyperalgesic effect was observed 2 h after the drug administration, seems to suggest that the most important mechanism underlying nitroglycerin-induced hyperalgesia is represented by the increased availability of nitroglycerin-derived NO at central sites (i.e., spinal cord).

Additional mechanisms may be invoked to explain the delayed appearance of nitroglycerin-induced hyperalgesia. Pardutz et al. (2000) demonstrated an increased expression of neuronal NOS in the cervical cord 4 h after the systemic administration of nitroglycerin. Activation of neuronal NOS may, in turn, interfere with ion channel activity and transmitter release, to induce spinal (and/or supraspinal) synaptic plasticity with a prolonged increase in synaptic length

(Gerber et al., 2000). Nitroglycerin-induced activation of the trigeminovascular system constitutes another possible mechanism. Reuter et al. (2002) recently demonstrated that systemic nitroglycerin infusion induces the expression of genes that are involved in inflammatory processes at meningeal level. The transcriptional events occur with a delay of some hours (2 h for nuclear factor-kappaB and 6 h for inducible NOS). Thus, it is likely that nitroglycerin-evoked inflammatory response at meningeal level may induce central sensitization, which might contribute to the intensification of nociceptive behaviour observed 2 and 4 h after its administration in the formalin test.

4.3. Conclusions

These findings provide further clues to the mechanism of the hyperalgesic effect of nitroglycerin. Given the well-known capability of the drug to induce spontaneous-like attacks in migraineurs, these results also provide interesting insight into the possible pathophysiological mechanisms underlying migraine attacks.

Acknowledgements

This work was supported by a grant from the Italian Ministry of Health (RC 2000–2001).

References

Coderre, T.J., Katz, J., Vaccarino, A.L., Melzack, R., 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 52, 259–285.

Ferreira, J., Santos, A.R., Calixto, J.B., 1999. The role of systemic spinal and supraspinal L-arginine-nitric oxide-c GMP pathway in thermal hyperalgesia caused by intrathecal injection of glutamate in mice. Neuropharmacology 38, 835–842.

Gerber, G., Young, D.-H., Hsu, C.H., Isaev, D., Randic, M., 2000. Spinal dorsal horn synaptic plasticity: involvement of group I metabotropic glutamate receptors. In: Sandkühler, J., et al. (Eds.), Nervous System Plasticity and Chronic Pain. Progress in Brain Research, vol. 129. Elsevier, Amsterdam, pp. 115–134.

Hao, S., Takahata, O., Iwasaki, H., 2000. Intrathecal endorphin-1 produces antinociceptive activities modulated by alpha 2-adrenoceptors in the rat tail flick, tail pressure and formalin tests. Life Sci. 66, 195–204.

Harrison, D.G., Bates, J.N., 1993. The nitrovasodilators: new ideas about old drugs. Circulation 87, 1461–1467.

Hoheisel, U., Mense, S., 2000. The role of spinal nitric oxide in the control of spontaneous pain following nociceptive input. In: Sandkühler, J., et al. (Eds.), Nervous System Plasticity and Chronic Pain. Progress in Brain Research, vol. 129. Elsevier, Amsterdam, pp. 163–172.

Ito, S., Okuda-Ashitaka, E., Minami, T., 2001. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci. Res. 41, 299–332.

Iversen, I.K., Olesen, J., Tfelt-Hansen, P., 1989. Intravenous nitroglycerin as an experimental model of vascular headache. Pain 38, 17–24.

Kawamata, T., Omote, K., 1999. Activation of spinal *N*-methyl-D-aspartate receptors stimulates a nitric oxide/cyclic guanosine 3,5-monophosate/glutamate release cascade in nociceptive signaling. Anesthesiology 91, 1415–1424.

Kitto, K.F., Haley, J.E., Wilcox, G.L., 1992. Involvement of nitric oxide in spinally-mediated hyperalgesia in the mouse. Neurosci. Lett. 148, 1–5.

Lambert, G.A., Donaldson, C., Boers, P.M., Zagami, A.S., 2000. Activation of trigeminovascular neurons by glyceryl trinitrate. Brain Res. 887, 203–210.

Ma, S.X., Abboud, F.M., Felder, R.B., 1995. Effects of L-arginine-derived nitric oxide synthesis on neuronal activity in nucleus tractus solitarius. Am. J. Physiol. 268, 487–491.

Masue, T., Dohi, S., Asano, T., Shimonaka, H., 1999. Spinal antinociceptive effect of epidural nonsteroidal anti-inflammatory drugs on nitric oxide-induced hyperalgesia in rats. Anesthesiology 91, 198–206.

Pardutz, A., Krizbai, I., Multon, S., Vecsei, L., Schoenen, J., 2000. Systemic nitroglycerin increases nNOS levels in rat trigeminal nucleus caudalis. NeuroReport 11, 3071–3075.

Reuter, U., Chiarugi, A., Bolay, H., Moskowitz, M.A., 2002. Nuclear factor-kappaB as a molecular target for migraine therapy. Ann. Neurol. 51, 507–516.

Sandrini, G., Tassorelli, C., Proietti Cecchini, A., Alfonsi, E., Nappi, G., 2002. Effect of nimesulide in nitric-oxide-induced hyperalgesia in humans: a neurophysiological study. Eur. J. Pharmacol. 450, 259–262.

Sicuteri, F., del Bene, E.D., Poggioni, M., Bonnazzi, A., 1987. Unmasking latent dysnociception in healthy subjects. Headache 27, 180–185.

Sousa, A.M., Prado, W.A., 2001. The dual effect of a nitric oxide donor in nociception. Brain Res. 897, 9–19.

Tassorelli, C., Joseph, S.A., 1995. Systemic nitroglycerin induces Fos immunoreactivity in brainstem and forebrain structures of the rat. Brain Res. 682, 167–178.

Tassorelli, C., Greco, R., Morocutti, A., Costa, A., Nappi, G., 2001. Nitric oxide-induced neuronal activation in the central nervous system as an animal model of migraine: mechanisms and mediators. Funct. Neurol. 16 (Suppl. 4), 69–76.

Tassorelli, C., Joseph, S.A., Buzzi, G., Nappi, G., 1999. The effect on the central nervous system of nitroglycerin-putative mechanisms and mediators. Prog. Neurobiol. 57, 606–624.

Tjolsen Aberge, O.G., Hunskaar, S., Rosland, J.H., Hole, K., 1992. The formalin test: an evaluation of the method. Pain 51, 5–17.

Torfgard, K., Ahnler, J., 1991. Tissue levels of glyceryl trinitrate and cGMP after in vivo administration in rat, and the effect of tolerance development. Can. J. Physiol. Pharm. 69, 1257–1261.