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Dual effect of local application of nitric oxide donors in a model of incision pain in rats

Wiliam A. Prado*, Viviane F. Schiavon, Fernando Q. Cunha

Department of Pharmacology, Faculty of Medicine of Ribeirão Preto-USP, Av. Bandeirantes 3900, CEP 14049-900, Ribeirão Preto, São Paolo, Brazil

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Abstract

The effects of local application of a cream containing nitric oxide (NO) donors, *S*-nitroso-*N*-acetylpenicillamine (SNAP) or isosorbide dinitrate were studied in a rat model of incision pain. An incision was made in the plantar aspect of a hind paw and the cream was applied inside the surgical wound. SNAP (1–10%) or isosorbide (2.5–5%) reduced the incision allodynia as measured with von Frey filaments. Higher concentrations produced a smaller or no effect, but SNAP (30%) intensified the allodynia. Allodynia was also intensified by SNAP (5% or 30%) in rats pretreated with intraplantar 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 4 μg), a guanylate cyclase inhibitor. The effect of isosorbide (5%) was prevented by ODQ. The cream containing SNAP released 10- to 20-fold more nitrite than did isosorbide from a macrophage culture. We conclude that local application of drugs generating a low NO concentration reduces incision pain through activation of guanylate cyclase. Drugs generating high NO concentrations, however, intensify pain via a guanylate cyclase-independent mechanism.

Keywords: Pain; Incision pain; Allodynia; SNAP (*S*-nitroso-*N*-acetylpenicillamine); Isosorbide; Nitric oxide (NO); ODQ (1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one)

1. Introduction

Nitric oxide (NO) is a highly diffusible and short-lived gaseous molecule known to be involved in many physiological functions including vasodilatation, macrophage cytotoxicity, neurotoxicity and plasticity in the central nervous system (Schuman and Madison, 1994). NO is produced from L-arginine by calcium-dependent constitutive NO synthase (NOS) isoforms, including neuronal NOS (nNOS) and endothelial (eNOS), or the calcium-independent inducible NOS (iNOS) that requires activation by endotoxin or cytokinins (Moncada, 1997). The NO formed activates soluble guanylate cyclase, which produces an increase in cyclic GMP (cGMP) levels (Meller and Gebhart, 1993).

Evidence has accumulated for the involvement of NO also in nociceptive processing, both peripheral and central (Meller and Gebhart, 1993). Most of the data available for the participation of NO in nociception in the central nervous system are based on the antinociceptive effects of NOS inhibitors, as demonstrated in models of mechanical or thermal phasic (Przewlocki et al., 1993; Przewlocka et al., 1994; Inoue et al., 1997) or chemical persistent pain (Haley et al., 1992; Moore et al., 1992; Babbedge et al., 1993; Malmberg and Yaksh, 1993; Hao and Xu, 1996; Machelska et al., 1997) in rodents, and interpreted as indicative of a pronociceptive action of NO. However, the antinociceptive efficacy of NOS inhibitors was not confirmed in some experiments also using rodent models of mechanical or thermal phasic (Meller et al., 1992; Zhuo et al., 1993; Iwamoto and Marion, 1994; Xu et al., 1995; Machelska et al., 1997; Aley et al., 1998) or chemical persistent pain (Goettl and Larson, 1996). Consistent with the notion of a central pronociceptive effect of NO, several experiments have shown the hyperalgesic property of intrathecal NO donors such as 3-morpholinosydnonimine (SIN-1) (Przewlocki et al., 1993), *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) (Przewlocka et al., 1994) and triamine 18, $H_2NCH_2CH_2N[N(O)NO]\text{-}CH_2CH_2NH_3^+$ (NOC-18) (Inoue et al., 1997) in rat models of phasic pain. However, Sousa and Prado (2001) have shown that low intrathecal doses of SIN-1 reduced, while high doses had no effect on, or increased, the mechanical allodynia induced by chronic

* Corresponding author. Tel.: +55-21-16-602-3038; fax: +55-21-16-633-2301.

E-mail address: wadprado@fmrp.usp.br (W.A. Prado).

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sciatic nerve ligature in rats. In contrast, intrathecal SIN-1 was only antinociceptive in the rat tail flick test. Therefore, different pain models and different doses of drugs that interfere with the NO-cGMP pathway may be the reasons for the discrepant results found in the literature.

The literature is also controversial regarding the effects of NO at the periphery. Intraplantar NOS inhibitors were found ineffective in the rat paw pressure (Aley et al., 1998) or the formalin tests (Granado-Soto et al., 1997; Aguirre-Bañuelos and Granados-Soto, 1999). However, Duarte and Ferreira (2000) found a NOS inhibitor very potent against hyperalgesia induced by intrplantar carrageenin in the rat paw pressure test. Intraplantar (Duarte et al., 1990; Kawabata et al., 1992b; Nakamura et al., 1996) or subcutaneous (Kawabata et al., 1992a) administration of the NO precursor, L-arginine, reduces the hyperalgesia evoked by carrageenan in the rat paw pressure test. However, hyperalgesia induced by intraplantar L-arginine has already been reported for the same test (Aley et al., 1998). Low doses of intraplantar L-arginine increase the first phase, while high doses reduce the second phase of the mouse response to intraplantar formalin (Kawabata et al., 1994). On the other hand, L-arginine has been reported to alleviate some types of chronic pain in patients (Takagi et al., 1990; Harima et al., 1991).

Peripheral administration of NO donors to laboratory animals or to humans also produced discrepant results. Intraplantar SIN-1 is hyperalgesic in the rat paw pressure test (Aley et al., 1998), whereas intraplantar sodium nitroprusside (200–500 μg) (Duarte et al., 1990), SIN-1 (50–100 μg) (Ferreira et al., 1991) or SNAP (50–200 μg) (Cunha et al., 1999) reduces the prostaglandin E_2-induced hyperalgesia in the rat paw pressure test. In human volunteers, NO evokes pain following intradermal (Holthusen and Arndt, 1994) or intravenous administration (Kindgen-Milles and Arndt, 1996). In contrast, glyceryl trinitrate reduces the pain evoked by vein sclerotherapy in the legs (Berrazueta et al., 1994) and controls pain in women with severe dysmenorrhea (Pittrof et al., 1996). Transdermal nitroglycerin was useful for the management of shoulder pain due to supraspinatus tendinitis (Berrazueta et al., 1995) and as a coadjuvant in opiate therapy for cancer pain control (Lauretti et al., 1999a).

Explanations for the discrepant results on the involvement of NO in nociceptive processing include drug specificity, dose, route of administration, distribution and pharmacokinetics, as well as local conditions associated with the primary disorders that follow tissue injury (Luo and Cizkova, 2000). Different pain models produce different pain states and, therefore, may reveal different effects for drugs that interfere with NO production.

The present study examined the effects of local application of a hydrophobic sterile cream containing various concentrations of NO donors (isosorbide dinitrate or SNAP) in a rat model of model of persistent postoperative pain (Brennan et al., 1996). We show that local application of drugs generating low NO concentration reduces, whereas a high NO concentration intensifies, incision pain.

2. Material and methods

2.1. Subjects

Male Wistar rats (160–180 g) were used. They were housed individually in cages with food and water available ad libitum, and kept on a 12-h light/dark cycle, with the dark cycle beginning at 07:00 h. All tests were held in the morning. The study was conducted in accordance with the IASP guideline on the use of laboratory animals (Zimmermann, 1983).

2.2. Surgery

Each animal was anaesthetised with 1.5% halothane in oxygen via a loose-fitting, cone-shaped mask. The plantar aspect of the right hind paw was prepared in a sterile manner with a 10% povidone–iodine solution. A 1-cm longitudinal incision was made with a surgical blade through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel. The plantaris muscle was elevated, but its origin and insertion were left intact. After hemostasis, 100 mg of drug-free hydrophobic sterile cream (placebo) or hydrophobic sterile cream containing an NO donor in variable concentrations was applied gently along the surface of the plantaris muscle, and the skin was apposed with two 5–0 nylon sutures. The cream was a mixture of vaseline (95%) with unsaturated fatty acids (5%). After these procedures, the animal was allowed to recover in the home cage for a period of 2 h.

In three different groups of rats the incision was made in both hind paws. In the first group (control), placebo was applied to one hind paw and mechanical threshold (see below) was measured in the same paw. Cream containing a NO donor was applied to one hind paw in the remaining groups, and mechanical thresholds were measured in the treated (second group) and untreated (third group) hind paw.

2.3. Nociceptive testing of mechanical threshold

Tactile threshold was measured with von Frey filaments (Stoelting). Rats were placed in an elevated clear plastic cage with a nylon mesh bottom, which allowed easy access to the paw plantar surface. Before each test, the animals remained in the cage for approximately 15 min to allow behavioural accommodation. The area tested was the mid-plantar right hind paw and the tactile thresholds were recorded immediately before and 2, 6 and 24 h after surgery. The paw was touched with one of a series of 18 von Frey filaments with logarithmically incremental stiffness (0.0045–125.892 g, lower and upper limit of the test, respectively). Each filament was applied from underneath the nylon mesh floor, through the mesh, vertically to the plantar surface with sufficient force to bend the filament a little. A single trial consisted of six applications of a particular filament, applied once every 3–4 s. Testing was initiated with the 1.48 g filament in the middle of the series. A response was defined as withdrawal of the stimulated paw. In the absence of a response to a

particular filament, the next stronger filament was used; in the case of a response, the next weaker filament was presented. The up–down method was used to record the threshold (Chaplan et al., 1994).

2.4. Culture of macrophages

Murine peritoneal macrophages (Mϕs) were harvested from C57Bl/6 mice (bred and maintained at the School of Medicine, University of São Paulo, Ribeirão Preto, Brazil) injected intraperitoneally 3 days earlier with 2 ml of a sterile thioglycolate solution (3% w/v in phosphate buffered saline). The cells were plated at 1×10^6 cells/1.0 ml in RPMI 1640 plus 10% heat-inactivated foetal calf serum, 100 U/ml penicillin and streptomycin (100 μg/ml) in 24-well flat-bottomed plates (Nunc, Roskilde, Denmark), and incubated overnight at 37 °C in an atmosphere of 5% CO_2. The culture medium was then discarded and the macrophage mono-

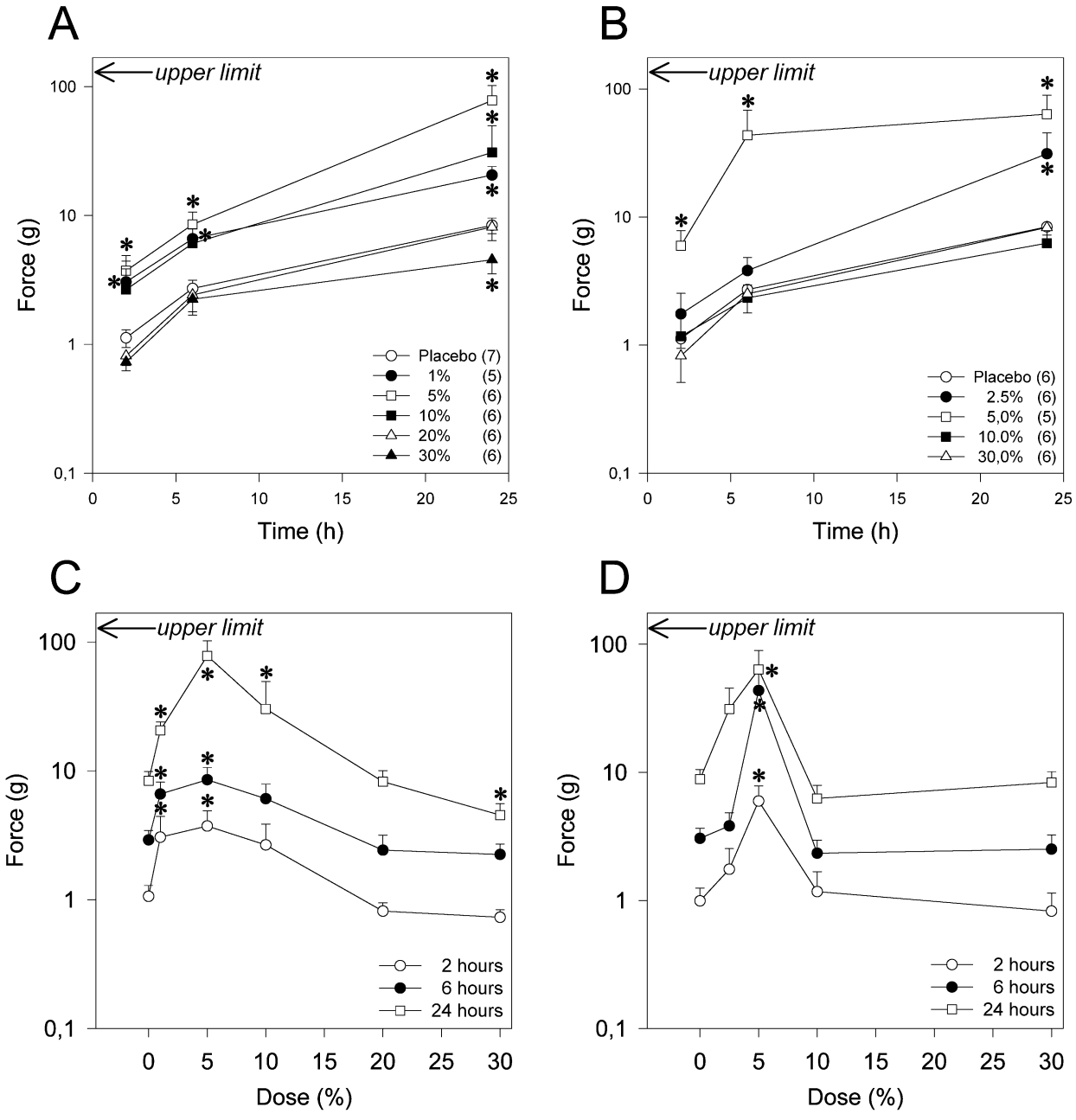

Fig. 1. Changes produced by local application of a drug-free cream (Placebo) or cream containing SNAP or isosorbide dinitrate on the paw withdrawal thresholds to tactile stimuli in a rat model of incision pain. (A) Effects of SNAP (1–30%). (B) Effects of isosorbide (2.5–30%). (C and D) Dose–response curves for the effects of SNAP or isosorbide at the different observation times, respectively. Thresholds of the injured paw in response to tactile stimuli are given as force (g) on a logarithmic scale. The time (in h) after cream application is given on the abscissa. The upper limit of the test (125.892 g) is indicated in each graph. Points are means (± S.E.M.). The number of rats in each group is given in parenthesis. (*) $P < 0.05$ compared to the placebo group.

layers were washed with Hank's medium and 1.0 ml fresh RPMI was added to the cultures. A cream (100 mg) containing isosorbide or SNAP at concentrations of 5%, 10% and 30% (w/w) was added to the macrophage cultures and the nitrite concentration in the supernatants was determined 24 h later. The nitrite concentration following the cream containing isosorbide (10%) or SNAP (10%) was also determined at 6, 12, 48 and 72 h as well.

2.5. Nitrite measurement

Nitrite (NO_2^-) concentration in the supernatants was determined by the Griess reaction as described elsewhere (Green et al., 1982). Briefly, Griess reagent (NEED) (0.1% w/v) plus sulphanilamide (1% w/v in H_3PO_4 5% v/v) at room temperature (100 μl) was incubated with an equal volume of supernatant. The absorbance at 540 nm was measured and NO_2^- was calculated from a standard curve of 1–200 μM $NaNO_2$ and the result are expressed as μg of NO_2^- formed.

2.6. Drugs

Isosorbide dinitrate was purchased from GreenPharm of Brazil; *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) was from RBI (Natick, MA, USA); 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) was from Tocris Cookson, MO, USA. ODQ was diluted in 4% dimethylsulfoxide (DMSO) and administered by the intraplantar route 30 min before incision of the same paw.

2.7. Statistical analysis

The results were plotted as medians with corresponding confidence limits (95%) and presented in graphs as means (± S.E.M.). The experimental groups were compared by the non-parametric Kruskall–Wallis test and differences in the data at each time point were compared by the two-tailed Mann–Whitney test. The level of significance was set at $P<0.05$. In vitro results were also expressed as means ± S.E.M. and statistical significance ($P<0.05$) was assessed by analysis of variance (ANOVA) followed by Bonferroni's *t*-test.

3. Results

3.1. The effects of local SNAP or isosorbide on incision tactile allodynia

The time course of the effects of local application of placebo or a cream containing SNAP or isosorbide dinitrate on the tactile threshold of the incised foot of rats is shown in Fig. 1A and B, respectively. The mean threshold (± S.E.M.) in the incised foot pretreated with placebo decreased significantly from the cut-off (125.892 g) before surgery to 1.12 ± 0.18, 2.72 ± 0.48 and 8.39 ± 1.15 g at 2, 6 and 24 h, respectively ($t_{320} \geq 133.35$; $P<0.001$; Dunnett's test). These results did not differ significantly from those obtained with a group of rats submitted to similar procedures except for the local application of placebo (not shown in figures).

Rats treated with a cream containing SNAP (1% or 5%) showed a dose-dependent increase in the withdrawal threshold, which was significantly different from that of the

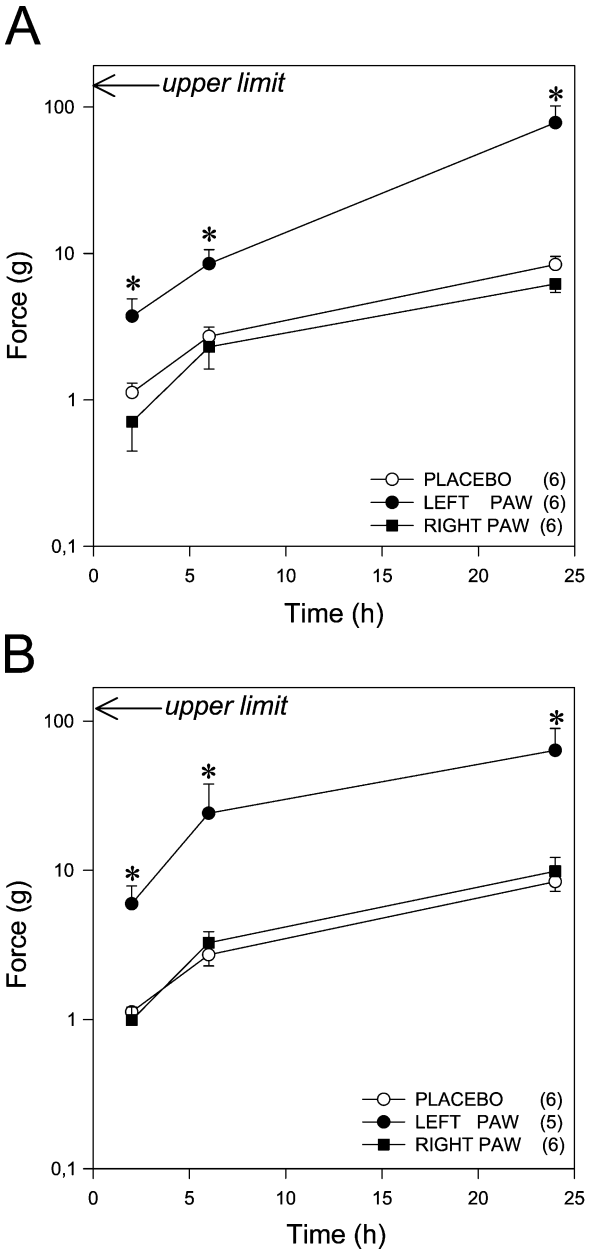

Fig. 2. Changes produced by local application of a drug-free cream (Placebo) or a cream containing 5% SNAP (A) or 5% isosorbide dinitrate (B) on the paw withdrawal thresholds to tactile stimuli in a rat model of incision pain. Incisions were made in both hind paws and the cream was applied to the left paw only. Thresholds, timing, upper limit of the test, points, and number of rats in each group are as in Fig. 1. (*) $P<0.05$ compared to the placebo group.

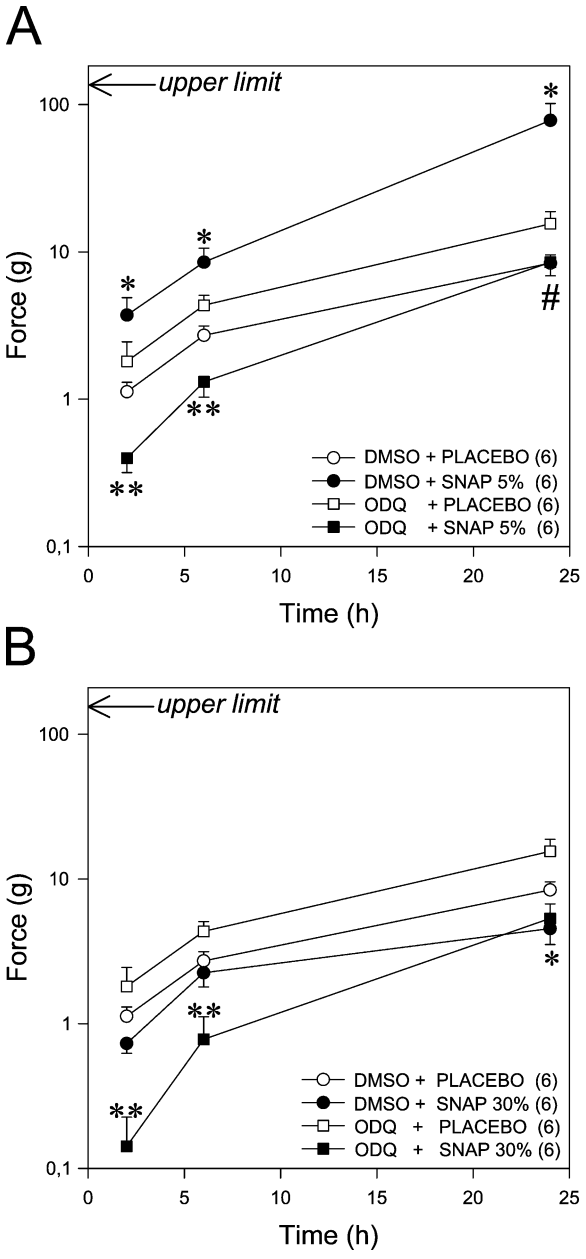

Fig. 3. Changes produced by intraplantar application of the soluble guanylate cyclase inhibitor, 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 4 μg), on the effects of local application of a drug-free cream (Placebo) or a cream containing 5% (A) or 30% (B) SNAP in a rat model of incision pain. ODQ was administered 30 min before surgery. Thresholds, timing, upper limit of the test, points, and number of rats in each group are as in Fig. 1. (*) $P<0.05$ compared to control (DMSO + placebo) group. (**) $P<0.05$ compared to the control and DMSO + SNAP groups. (#) $P<0.05$ compared to the DMSO + SNAP group.

placebo group throughout the period of observation. SNAP (10%) was still effective to reduce the tactile allodynia but the effect was significantly different from that in the placebo group at 24 h only. Higher concentrations (20% and 30%) produced effects that did not differ from those obtained with the placebo group, but the threshold was significantly lower than that in control rats 24 h after 30% SNAP.

Rats treated with a cream containing 2.5% isosorbide had thresholds different from those of the placebo group throughout the period of observation, but a significant difference was obtained at 24 h only. Rats treated with 5% isosorbide were significantly different from the placebo rats throughout the period of observation. Higher concentrations of isosorbide (10% and 30%) produced changes in the thresholds that did not differ significantly from those of

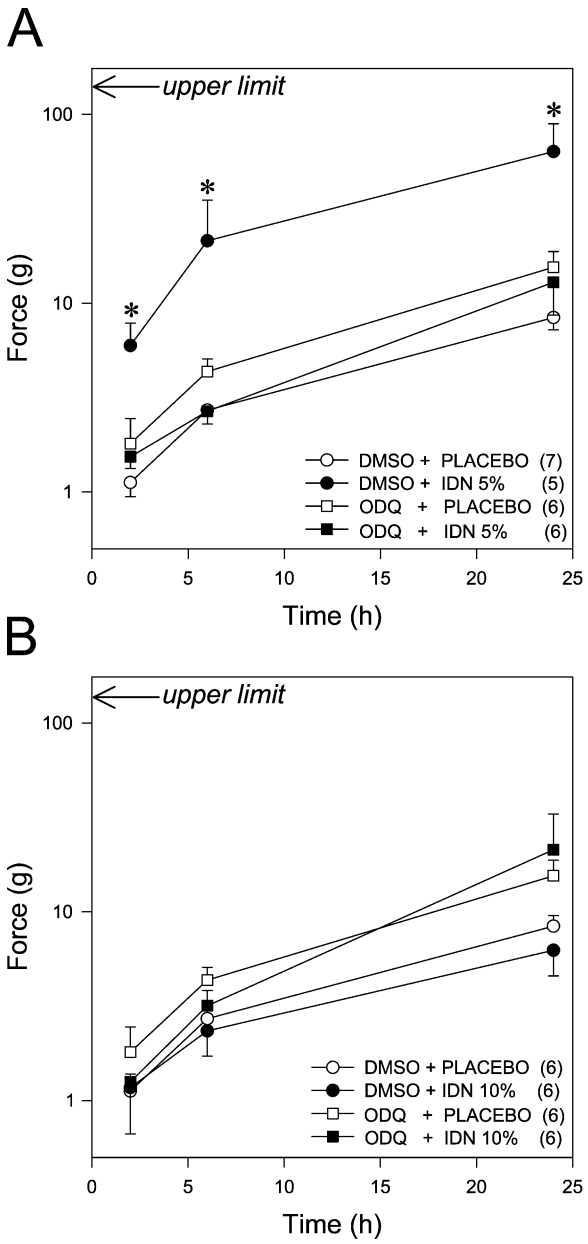

Fig. 4. Changes produced by intraplantar application of the soluble guanylate cyclase inhibitor, 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 4 μg), on the effects of local application of a drug-free cream (Placebo) or a cream containing 5% (A) or 10% (B) isosorbide dinitrate (IDN) in a rat model of incision pain. ODQ was administered 30 min before surgery. Thresholds, timing, upper limit of the test, points, and number of rats in each group are as in Fig. 1. (*) $P<0.05$ compared to the control and DMSO + IDN groups.

the placebo group. The curves in Figs. 1A and B were significantly different ($H_{17}=84.34$ and $H_{14}=63.28$, respectively; $P<0.001$). The corresponding dose–response curves obtained for SNAP and isosorbide at the different times of observation are shown in Fig. 1C and D, respectively. Lower concentrations of SNAP (0.1%) or isosorbide (1%) produced no effect (not shown in the figures).

Rats incised in both hind paws had a significantly lower tactile threshold than did placebo-treated rats when the test was done with 5% SNAP- (Fig. 2A) or 5% isosorbide- (Fig. 2B) treated paws. In contrast, the thresholds in the contralateral (non-treated) paws did not differ significantly from those of placebo-treated rats. The curves in Fig. 2A and B were significantly different ($H_8=45.78$ and $H_8=44.49$, respectively, $P<0.001$).

3.2. Changes produced by intraplantar ODQ in the effects of local SNAP or isosorbide on incisional tactile allodynia

The time course of the changes produced by previous intraplantar administration of ODQ (4 μg) in the effects of local application of a drug-free cream (placebo), or a cream containing SNAP or isosorbide dinitrate on the tactile threshold of the incised foot of rats is shown in Figs. 3 and 4, respectively. In this experiment, DMSO (4%) or ODQ was applied by the intraplantar route 30 min before surgery.

SNAP (5%) significantly reduced the incision allodynia in DMSO-treated rats throughout the period of observation, but intensified the allodynia in rats treated 30 min earlier with ODQ (Fig. 3A). The effects of SNAP in ODQ-treated rats were significantly different from those in the remaining groups at 2 and 6 h after surgery and significantly different from those in DMSO-treated rats at 24 h after surgery.

SNAP (30%) did not significantly change the incision allodynia in DMSO-treated rats throughout the period of observation, but intensified the allodynia in rats treated 30 min earlier with ODQ (Fig. 3B). The effects of SNAP in ODQ-treated rats were significantly different from those in the saline-treated groups of rats at 2 and 6 h after surgery. The curves in Fig. 3A and B were significantly different ($H_{10}=55.42$ and $H_{11}=63.76$, respectively, $P<0.001$).

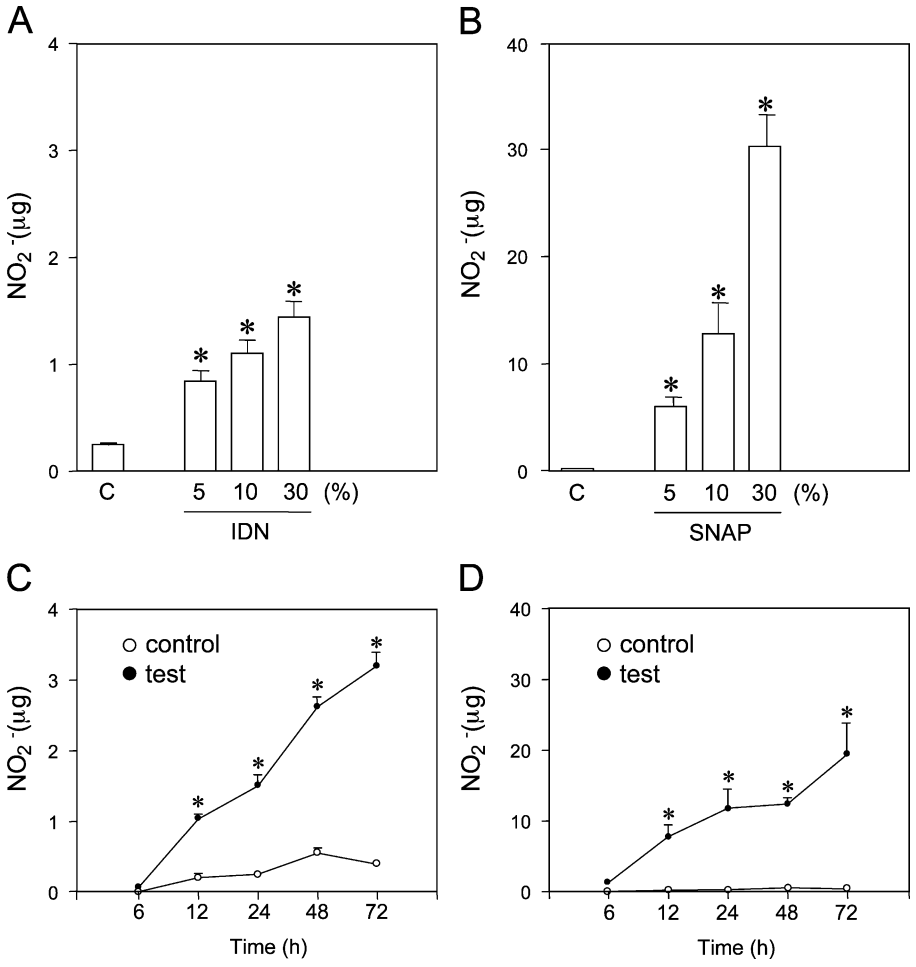

Fig. 5. Concentration-dependent release of nitrite (NO_2^-) obtained by adding a drug-free cream (C) or a cream containing isosorbide dinitrate (IDN) (in A) or SNAP (in B) into the supernatant of a murine macrophage culture. The kinetics of nitrite production following 10% IDN or 10% SNAP are shown in (C) and (D), respectively. (*) $P<0.05$ compared to control.

Isosorbide (5%) significantly reduced the incision allodynia in DMSO-treated rats throughout the period of observation, the effect being completely abolished in rats treated 30 min earlier with ODQ (Fig. 4A).

The incision allodynia in either DMSO- or ODQ-treated rats was not significantly changed by isosorbide (10%) throughout the period of observation (Fig. 4B). The curves in Fig. 4A and B were significantly different ($H_{11} = 55.39$ and $H_{11} = 59.59$, respectively, $P < 0.001$).

The incision allodynia obtained in rats treated with placebo was not significantly changed by the previous intraplantar administration of ODQ (4 or 8 μg) or DMSO (not shown in the figures).

3.3. In vitro study

The cream containing isosorbide (Fig. 5A) or SNAP (Fig. 5B) produced a concentration-dependent release of NO, as determined from the concentration of nitrite found in the culture supernatant. However, at each NO donor concentration the amount of nitrite produced by SNAP was 10- to 20-fold greater than that produced by isosorbide. The kinetics of nitrite production following isosorbide or SNAP (10%) are shown in Fig. 5C and D, respectively. A slow production of nitrite was found in both cases. A significant difference from the control (medium without NO donor) was detected 12 h after the addition of an NO donor to the medium and persisted for up to 72 h. Again, the production of nitrite by SNAP was about 10-fold higher than that produced by isosorbide at all time points analysed. The kinetics of nitrite production following SNAP in macrophage-containing medium were similar to those for medium without macrophages, whereas isosorbide generated nitrite only in the presence of macrophages (not shown in the figures).

4. Discussion

The present experiments demonstrated that local application of a cream containing SNAP (1% and 5%) or isosorbide (5%) significantly reduced the incision tactile allodynia in rats. The effect was observed 2 h after drug administration and persisted for at least 24 h. Higher concentrations of NO donors, however, produced a smaller or no effect. In the case of the cream containing 30% SNAP, a significant pro-allodynic effect was also demonstrated 24 h after drug administration. These results somewhat support the recent demonstration that intrathecal administration of SIN-1, another NO donor, also induces a dual effect in a model of neuropathic pain, i.e., low doses of SIN-1 reduce, while high doses intensify or have no effect on the tactile allodynia produced by chronic sciatic ligature in rats (Sousa and Prado, 2001). However, the incision allodynia was significantly reduced by the local application of SNAP or isosorbide in one hind paw but remained unchanged in the contralateral hind paw. Therefore, the anti-allodynic effects of local application of NO donors are unlikely to be due to drug diffusion to the central nervous system.

Activation of soluble guanylate cyclase is the best-documented action of NO (Vincent, 1994). At the dose utilised here, ODQ can act as a specific inhibitor of soluble guanylate cyclase. The tactile allodynia caused by surgery was not changed significantly by the previous intraplantar administration of ODQ at doses shown earlier to increase the hyperalgesia induced by carrageenan in the rat paw pressure test (Cunha et al., 1999). Thus, the ongoing incision allodynia in these animals does not depend on the activation of guanylate cyclase. However, the local application of 5% or 30% SNAP in ODQ-treated rats produced a further decrease in the tactile threshold, an effect that was significantly different from that observed in the placebo group for up to 6 h after surgery. The previous administration of ODQ fully inhibited the anti-allodynic effect of 5% isosorbide but did not modify the inefficacy of 10% isosorbide. We may then suspect that activation of soluble guanylate cyclase is involved in the anti-allodynic effects of NO donors, but not in the pro-allodynic effect of high concentrations of SNAP. The pro-allodynic effect of SNAP (5%) in ODQ-treated rats is surprising but may indicate that when the local production of cGMP is impaired even low amounts of NO facilitate peripheral nociceptive processing. The anti-allodynic effect of the cream containing SNAP is consistent with the recent demonstration that intraplantar injection of SNAP significantly reduces the intraplantar prostaglandin E_2-induced hyperalgesia in the rat paw pressure test, an effect also shown to be significantly attenuated by intraplantar injection of ODQ (Cunha et al., 1999).

An important difference between the two NO donors used here was revealed in an in vitro study showing that the cream containing SNAP generated 10- to 20-fold more nitrite than the cream containing isosorbide. In addition, the cream containing SNAP generates 10-fold more nitrite than does isosorbide at all observation times. The amount of nitrite is not an ideal index of NO formation (Feelish, 1998), but the kinetics of nitrite production fit well with the kinetics of NO release. We may therefore suspect that the pro-allodynic effect obtained with SNAP but not with isosorbide is due to the property of SNAP to release NO in concentrations much higher than those released by isosorbide. Apparently, there is a poor correlation between the in vitro and the in vivo studies, since both NO donors at similar concentrations reduced allodynia. The minimum NO concentrations needed for this effect cannot be predicted from the present experiments. However, cream containing 5-fold more isosorbide than SNAP was required to produce a significant anti-allodynic effect. We may then suspect that antinociception is the main property of the NO-induced stimulation of guanylate cyclase at the periphery also. On the other hand, the pronociceptive effect of NO donors depends on the drugs property to release large amounts of NO in the medium, which may substitute for the anti-

allodynic effect of small amounts of NO via a guanylate cyclase-independent pathway. The involvement of other pathways, rather than of the NO-cGMP pathway, in NO-induced hyperalgesia had been suggested by others (Luo and Cizkova, 2000). We did not succeed in our attempts to dissolve higher amounts of isosorbide in the cream and, therefore, cannot exclude the possibility that more concentrated isosorbide would also intensify the incisional allodynia.

An in vitro study also revealed that the cream containing isosorbide but not SNAP required the presence of cells in the medium, indicating that for NO release to occur the nitrate requires enzymatic bioactivation. Depending on the reaction conditions, isosorbide generates NO, and NO_2^- but not NO^- or NO^+, whereas SNAP generates NO, NO_2^-, NO^- and NO^+, but not NO_2^+ (Feelish, 1998). A possibility thus remains that the proallodynic effect of high doses of SNAP is due to NO-related species and not to NO itself.

In summary, the present study revealed that a cream containing NO donors such as SNAP or isosorbide reduces incision tactile allodynia in rats, a model of postoperative pain (Brennan et al., 1996). The local use of a NO donor may thus be useful for clinical postoperative pain management. Transdermal nitroglycerine alone, another NO donor, had no postoperative analgesic efficacy, but prolonged the analgesic effect of spinal sufentanil (Lauretti et al., 1999b) or neostigmine (Lauretti et al., 2000). The lack of analgesic efficacy for nitroglycerine itself may be due to the low drug concentration achieved in the incision region following transdermal application. Further experiments are required to investigate the clinical usefulness of a cream containing a NO donor applied directly inside the surgical wound as an alternative for postoperative incision pain control.

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