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Sildenafil increases diclofenac antinociception in the formalin test

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Abstract

The antinociceptive activity of an inhibitor of phosphodiesterase 5, alone or combined with diclofenac, was assessed in the formalin test. Local administration of diclofenac produced a significant antinociception in both phases of the formalin test in female Wistar rats. In contrast, 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [3,4-d]pyrimidin-5-yl)phenylsulfonyl]-4-methyl piperazine (sildenafil, an inhibitor of phosphodiesterase 5) produced significant antinociception, only during the second phase of the formalin test. Non-effective doses of sildenafil (25–100 μg/paw) significantly increased the antinociceptive effect of an inactive dose of diclofenac (25 μg) in both phases of the test. The antinociception produced by the drugs alone or the combination was due to a local action, as its administration in the contralateral paw was ineffective. Since sildenafil is a potent and selective inhibitor of phosphodiesterase 5, our results suggest that this drug produced its antinociceptive activity, and increased that of diclofenac, probably through the inhibition of cyclic GMP degradation. © 2001 Published by Elsevier Science B.V.

Keywords: Diclofenac; Sildenafil; cGMP; Synergism; Antinociception

1. Introduction

It has been proposed that cyclic GMP is involved in antinociception. This proposal is based on the observation that local administration of L-arginine produces antinociception in rats with carrageenin-induced hyperalgesia, the effect being blocked by nitric oxide (NO) synthesis inhibitors and methylene blue (a soluble guanylyl cyclase inhibitor) (Duarte et al., 1990). In prostaglandin- and carrageenin-induced hyperalgesia, the local administration of opiates or non-enzymatic NO donors also produces antinociception. While pretreatment of the rats' paws with methylene blue inhibits the action of morphine and the NO donor, the NO synthase inhibitor only inhibited opiate analgesia (Ferreira et al., 1991). Moreover, the intraplantar injection of dibutyryl-cyclic GMP caused antinociception (Ferreira and Nakamura, 1979).

Indirect observations also demonstrate that cyclic GMP is involved in antinociception. Recent evidence indicates that antinociception produced by dipyrone, diclofenac, ketorolac, nimesulide and meloxicam can be diminished by either NO synthase or guanylyl cyclase inhibitors, such as N^G-L-nitro-arginine methyl ester (L-NAME) and 1*H*-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), respectively (Tonussi and Ferreira, 1994; Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996; Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto, 2000). Thus, this evidence suggests the participation of the NO–cyclic GMP pathway in the antinociception of these non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, there are also data to support the possibility that the same mechanism participates in morphine-induced antinociception (Ferreira et al., 1991; Granados-Soto et al., 1997; Aguirre-Bañuelos and Granados-Soto, 1999).

Intracellular cyclic GMP concentrations are regulated by the action of guanylyl cyclases and by the rate of degradation by cyclic GMP-specific phosphodiesterases

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(Beavo, 1995; Pyne et al., 1996). NO activates soluble guanylyl cyclase, which in turn catalyzes the formation of cyclic GMP from GTP, whereas cyclic GMP-specific phosphodiesterases catalyze the hydrolysis of cyclic GMP to GMP, ending signal transduction (Pyne et al., 1996). Phosphodiesterase 5, phosphodiesterase 6 and phosphodiesterase 9 are specific for cyclic GMP; however, the phosphodiesterase 5 isoenzyme seems to be the most relevant enzyme for cyclic GMP inactivation in cells (Beavo, 1995; Pyne et al., 1996).

Sildenafil (Viagra®) is a novel inhibitor of the cyclic GMP-specific phosphodiesterase 5 (Terrett et al., 1996), which has been shown to be effective in the clinical management of erectile dysfunction (Boolell et al., 1996). Since sildenafil increases the intracellular concentrations of cyclic GMP, we hypothesized that sildenafil will indeed produce antinociception and/or increase that produced by morphine. Recently, we have reported that sildenafil itself produced antinociception and increased that produced by morphine (Mixcotal-Zecuatl et al., 2000). The purpose of the present study was to investigate the peripheral effect of sildenafil (a specific inhibitor of phosphodiesterase 5) on the diclofenac-induced antinociception in the formalin test.

2. Material and methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 120–160 g) from our own breeding facilities were used in this study. Female animals were used based on our observation that formalin injection produces the same pattern of flinching in either sex (unpublished observation). The stage of the estrous cycle was not determined in our experiments. The rats had free access to food and drinking water before the experiment. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (IASP, 1983). Additionally, the study was approved by the local Animal Care Committee.

2.2. Measurement of antinociceptive activity

Antinociception was assessed with the formalin test. Rats were placed in an open Plexiglas observation chamber for 30 min, to allow them to accommodate to their surroundings, then they were removed for formalin administration. The rats were injected with 50 μl of dilute formalin (1%), s.c., into the dorsal surface of the right hind paw (intraplantar, i.pl.), using a 30-gauge needle. The animal was then returned to the chamber for observation. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. The rats were observed for nociceptive behavior immediately after formalin injection. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection (Aguirre-Bañuelos and Granados-Soto, 2000). Flinching was readily discriminated and was characterized as rapid and brief withdrawal or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). At the end of the experiment the rats were killed in a CO_2 chamber.

2.3. Drugs

Diclofenac sodium was obtained from Merck Mexico (Mexico City). Sildenafil citrate (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1-*H*-pyrazolo [3,4-d]pyrimidin-5-yl)phenyl sulfonyl]-4-methyl-piperazine) was a gift of Laboratorios Proquigama (Mexico City).

2.4. Study design

The rats received saline and increasing doses of either sildenafil (25, 50, 100 and 200 μg, i.pl.) or diclofenac (25, 50, 100 and 200 μg, i.pl.) 20 min before formalin injection. To determine if sildenafil was able to increase the effect of diclofenac, increasing doses of sildenafil were coadministered with a non-effective dose of diclofenac (25 μg). To assess if the effect was due to a local action, formalin was administered in one paw and the tested drug in the contralateral paw. Rats in all groups were tested for any possible behavioral side effects, such as reduction of righting, stepping, corneal and pinna reflex and catalepsy.

2.5. Data analysis and statistics

All results are presented as means ± S.E.M. for six to eight animals per group. Curves were made for number of flinches against time. The area-under-the-number-of-flinches-against-time curves (AUC) for both the first and second phase was calculated according to trapezoidal rule. Analysis of variance followed by Tukey's test was used to test the significance of differences between treatments. A $P < 0.05$ was considered significant.

3. Results

3.1. Antinociceptive effect of diclofenac and sildenafil alone

Ipsilateral, but not contralateral, diclofenac sodium pretreatment produced antinociception in both first and second phase of the formalin test (Fig. 1). During the first

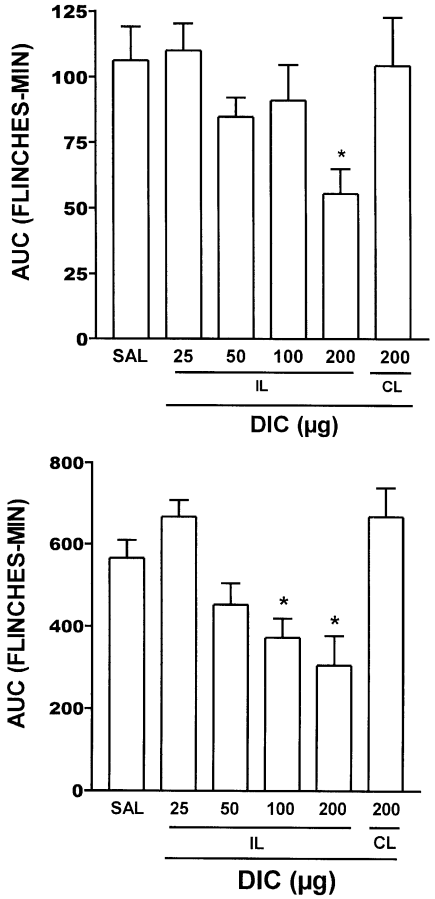

Fig. 1. Local antinociceptive effect of diclofenac during the first (top) and second (bottom) phases of the formalin test. Rats were pretreated with an i.pl. injection of saline or diclofenac into either the right (ipsilateral, IL) or left (contralateral, CL) paw, before formalin injection. Data are expressed as the area-under-the-number-of-flinches-against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from saline ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

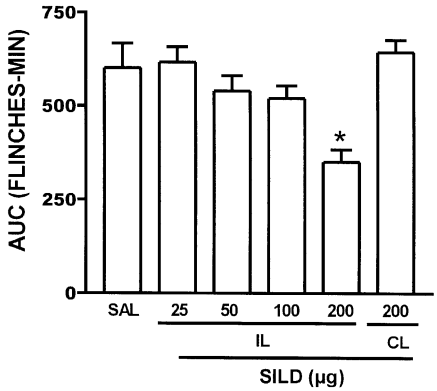

Fig. 2. Local antinociceptive effect of sildenafil during the second phase of the formalin test. Rats were pretreated with an i.pl. injection of saline or sildenafil into either the right (ipsilateral, IL) or left (contralateral, CL) paw, before formalin injection. Data are expressed as the area-under-the-number-of-flinches-against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from saline ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

phase, the antinociceptive effect was only evident at the highest dose tested (200 μg/paw). In contrast, during the second phase, diclofenac produced antinociception at lower doses (50–200 μg/paw). In the same way, ipsilateral, but not contralateral, administration of sildenafil only reduced flinching behavior during the second phase of the formalin test (Fig. 2). No side effects were observed in either control or treated group.

3.2. Antinociception of the combination diclofenac–sildenafil

In order to test the effect of sildenafil on diclofenac-induced antinociception, a non-effective dose of diclofenac (25 μg, i.pl.) was combined with increasing and non-effective doses of sildenafil (25, 50 and 100 μg, i.pl.). Doses were selected based on their lack of significant effect in the formalin test. The local coadministration of sildenafil and diclofenac produced a significant reduction in the number of flinches during both phase 1 (Fig. 3) and phase 2 (Fig. 4), compared to the effect of either drug alone ($P < 0.05$). During phase 1, only the highest dose of sildenafil (100 μg/paw) was able to significantly increase the diclofenac-induced antinociception. However, lower doses of sildenafil (25–50 μg/paw) produced significant antinociception during the second phase of the test. The

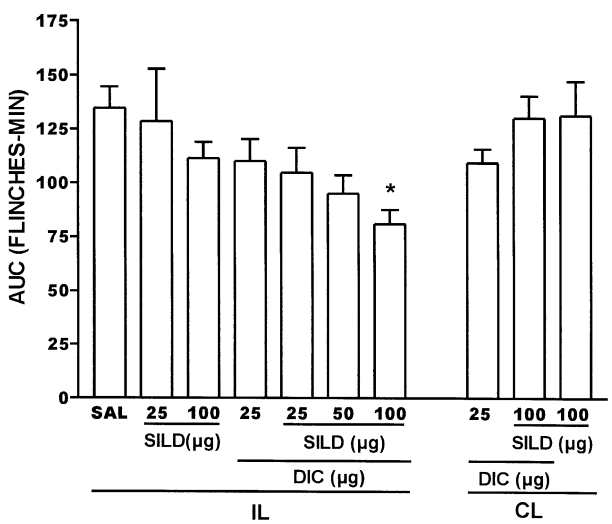

Fig. 3. Peripheral antinociceptive effect of the diclofenac–sildenafil combination during the first phase of the formalin test. Bars corresponding to diclofenac alone were taken from the data on Fig. 1. Rats were pretreated with saline or the diclofenac–sildenafil combination (DIC25–SIL 25, 100 μg) into either the right (ipsilateral, IL) or left (contralateral, CL) paw, before formalin injection. Data are expressed as the area-under-the-number-of-flinches-against time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from saline and either drug alone ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

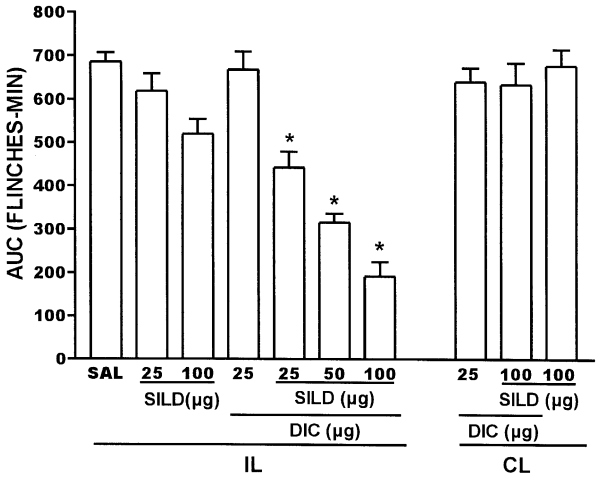

Fig. 4. Peripheral antinociceptive effect of the diclofenac–sildenafil combination during the second phase of the formalin test. Bars corresponding to sildenafil or diclofenac alone were taken from the data on Figs. 1 and 2. Rats were pretreated with saline or the diclofenac–sildenafil combination (DIC25–SIL 25, 100 μg) into either the right (ipsilateral, IL) or left (contralateral, CL) paw, before formalin injection. Data are expressed as the area-under-the-number-of-flinches-against-time curve (AUC). Bars are the means ± S.E.M. for six to eight animals. * Significantly different from saline and either drug alone ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

antinociceptive action of the combination was due to a local effect, as administration of the individual drugs or their combination to the contralateral paw did not produce any effect (Figs. 3 and 4). No side effects were observed with the combination.

4. Discussion

4.1. Antinociceptive action of diclofenac

Diclofenac is known to cause antinociception after systemic administration in several models of pain in animals (Takashima et al., 1972; Menassé et al., 1978; Noguchi et al., 1984; Scholer et al., 1986; López-Muñoz et al., 1996; Torres-López et al., 1997; Reyes-García et al., 1999), as well as in clinical pain in humans (Stacher et al., 1986; for a review see Todd and Sorkin, 1988). In our study, we observed that the ipsilateral, but not contralateral, administration of diclofenac produced dose-related antinociception in both phases of the formalin test. These results demonstrate the peripheral antinociceptive efficacy of diclofenac, supporting a significant participation of peripheral pronociceptive prostaglandins in the antinociceptive effect of this drug in the formalin test.

Our observation confirms the findings of Tonussi and Ferreira (1994) regarding peripheral antinociceptive effect of diclofenac in the rat knee joint incapacitation and the rat paw hyperalgesia tests. However, the fact that diclofenac was able to block nociception during phase 1 of the formalin test suggests that this drug could have an opioid-like effect on this model. It is well known that peripheral or intrathecal opiate drugs administered before phase 1 can suppress, in a dose-dependent fashion, the first and second phase responses (Malmberg and Yaksh, 1992). In contrast, NSAIDs are only able to suppress the second, but not the first, response in the formalin test. There is evidence that diclofenac can directly block the inflammatory sensitization, in addition to its inhibitory actions on prostaglandin synthesis (Tonussi and Ferreira, 1994; López-Muñoz et al., 1996). It has been suggested that this property of diclofenac is due to the activation of a NO–cyclic GMP pathway in the periphery. In addition, there are reports that morphine can indeed activate the same pathway to produce its antinociceptive effect (Ferreira et al., 1991; Duarte et al., 1992; Granados-Soto et al., 1997; Aguirre-Bañuelos and Granados-Soto, 1999). Therefore, it is likely that the morphine-like antinociceptive effect of diclofenac on the first phase could be due to the activation of the NO–cyclic GMP pathway, leading to direct blockade of inflammatory sensitization.

In addition to the effect on the first phase, diclofenac diminished in a dose-dependent manner the formalin-induced nociception during second phase. Only few studies have assessed the local (peripheral) action of NSAIDs. In the present work, we demonstrated that diclofenac is able to produce its antinociceptive effect by acting locally, as its administration in the contralateral paw was ineffective. Therefore, these results indicate that peripheral prostaglandins play an important role in nociception during phase 2. In addition, the data indicate that peripherally administered NSAIDs could be useful to relieve pain locally at the inflammation site. The report that topical administration of diclofenac gel blocks acute nociception in mice (Sengupta et al., 1998) is in line with this suggestion.

4.2. Antinociceptive action of sildenafil

We have previously reported that sildenafil (an inhibitor of phosphodiesterase 5) is itself able to produce antinociception and also increases morphine-induced antinociception (Mixcotal-Zecuatl et al., 2000). In the present study, we confirmed our previous observation that sildenafil is able to produce peripheral antinociception in the formalin test. The fact that sildenafil is a potent, selective and reversible phosphodiesterase 5 inhibitor (Terrett et al., 1996; Moreland et al., 1999) that blocks cyclic GMP hydrolysis ($K_i = 3$ nM), suggests that sildenafil could produce its antinociceptive effect through the increase in intracellular cyclic GMP levels. As also reported previously, we now found that low doses of sildenafil (25–100 μg/paw) did not produce antinociception in the formalin test. In contrast, a higher dose (200 μg/paw) of the phosphodiesterase 5 inhibitor significantly decreased the

number of flinches during the second, but not the first, phase of the formalin test. The antinociceptive effect of sildenafil was due to a local action, as the contralateral administration of the drug was ineffective in reducing flinching. Our results suggest a significant participation of peripheral phosphodiesterase 5 in the second phase of the formalin test. In addition, these data confirm our hypothesis that the inhibition of phosphodiesterase 5, and therefore the accumulation of cyclic GMP, is enough to produce antinociception.

4.3. Potentiation of diclofenac antinociception by sildenafil

Non-effective doses of sildenafil (25–100 μg/paw) increased the activity of a non-effective dose of diclofenac (25 μg/paw). The effect of the combination was due to a local action, as the contralateral administration of the diclofenac–sildenafil combination did not produce any effect. This confirms previous observations by our group of the ability of sildenafil to increase the antinociceptive effect of morphine in the formalin test (Mixcotal-Zecuatl et al., 2000). In addition, the results agree with previous reports on the action of selective (1-[3-chloroanilino]-4-phenylphthalazine, MY5445) and non-selective (caffeine) phosphodiesterase inhibitors to increase the antinociception produced by some NSAIDs and morphine (Ferreira et al., 1991; Duarte et al., 1992; López-Muñoz et al., 1996; Aguirre-Bañuelos et al., 1999; Mixcotal-Zecuatl et al., 2000). However, the present results demonstrate for the first time that an interaction between sildenafil and diclofenac occurs at the local level, and hence involves a peripheral mechanism of action. Since sildenafil is a selective inhibitor of phosphodiesterase 5, its administration results in a cyclic GMP level increase (Terrett et al., 1996; Moreland et al., 1999). Therefore, it is tempting to suggest that this increase in cyclic GMP levels produces the observed synergy between sildenafil and diclofenac. In addition, there is also evidence that diclofenac can directly activate the NO–cyclic GMP pathway in the periphery (see above; Tonussi and Ferreira, 1994; López-Muñoz et al., 1996).

In summary, local administration of diclofenac and sildenafil produced peripheral antinociception in the formalin test. In addition, sildenafil increased the action of diclofenac, probably through the inhibition of the cyclic GMP degradation.

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