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Lack of the nitric oxide-cyclic GMP-potassium channel pathway for the antinociceptive effect of intrathecal zaprinast in a rat formalin test

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Abstract

Zaprinast is a phosphodiesterase inhibitor that is active in various models of pain when administered locally. In addition, the antinociception of zaprinast is involved in the nitric oxide (NO)-cGMP pathway. However, the effect of zaprinast administered spinally has not been examined. Therefore, this study examined the effect of zaprinast on the formalin-induced nociception at the spinal level. Next, the role of the NO-cGMP-potassium channel pathway on the effect of zaprinast was further clarified. Catheters were inserted into the intrathecal space of male Sprague-Dawley (SD) rats. Pain was induced by applying 50 μl of a 5% formalin solution to the hindpaw. The change in the zaprinast-induced effect was examined after an intrathecal pretreatment with a NO synthase inhibitor (L-NMMA), a guanylyl cyclase inhibitor (ODQ) or a potassium channel blocker (glibenclamide). Zaprinast produced an antinociceptive effect during phase 1 and phase 2 in the formalin test. Intrathecal L-NMMA, ODQ and glibenclamide did not reverse the antinociception of zaprinast in either phase of the formalin test. These results suggest that zaprinast is effective against both acute pain and the facilitated pain state at the spinal level. However, the NO-sensitive cGMP-potassium channel pathway is not contributable to the antinociceptive mechanism of zaprinast in the spinal cord.

Keywords: Antinociception; Formalin test; Nitric oxide-cGMP-potassium channel pathway; Spinal cord; Sprague-Dawley rat; Zaprinast

It has been suggested that cyclic guanosine monophosphate (cGMP) is involved in antinociception [2,9,12,13,15,19]. Guanylyl cyclase catalyzes the formation of cyclic GMP from GTP, leading to cGMP synthesis, whereas cyclic GMP-specific phosphodiesterase catalyzes the hydrolysis of cGMP to GMP, which stops the signal transduction [20]. Therefore, the intracellular cGMP concentration is regulated by the action of guanylyl cyclase as well as by its rate of degradation by cGMP-specific phosphodiesterase [3,20]. In addition, guanylyl cyclase is activated by nitric oxide (NO) [6]. Both the NO-cGMP signaling pathway and the potassium channels participate in the antinociceptive mechanism [7,8,10–15,17–19,21,25]. Furthermore, it has been suggested that the potassium channels are opened by the activation of the L-arginine-NO-cGMP pathway [14,17].

The aforementioned findings suggest that the inhibition of cGMP-specific phosphodiesterase can increase the level of cGMP level, which can lead to the antinociception. And this effect may be mediated via the L-arginine-NO-cGMP-potassium channel pathway.

The formalin test is an experimental pain model that causes acute pain and the facilitated pain which is prominent considering the decreased level of the afferent input.

The objectives of the present study were to investigate the effect of zaprinast, which is a inhibitor of cGMP-specific phosphodiesterase 5, 6 and 9, on formalin-induced nociception in a rat at the spinal level, and to determine the sequential

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participation of NO and cGMP synthesis followed by potassium channel opening relative to the effect of intrathecal zaprinast.

The Institutional Animal Care Committee, Research Institute of Medical Science, Chonnam National University approved all of the experimental procedures. A polyethylene-10 tube was inserted into the subarachnoid space of male Sprague-Dawley rats (250–300 g) through a slit that had been made in the atlantooccipital membrane under enflurane anesthesia [24]. Any rats with neurological deficit after surgery were excluded and euthanized immediately with an overdose of volatile anesthetics. At least 5 days of recovery after surgery were allowed before commencing the behavioral study.

The following drugs were used in this study: zaprinast (Tocris Cookson Ltd., UK), N^G-monomethyl-L-arginine acetate (L-NMMA, Sigma, St. Louis, USA), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ, Sigma) and glibenclamide (Sigma). Zaprinst, ODQ and glibenclamide were dissolved in dimethylsulfoxide (DMSO), and the L-NMMA was dissolved in normal saline. All the drugs were delivered as a 10 μl solution.

The formalin test was used as the nociceptive model. A subcutaneous injection of formalin (5%, 50 μl) into the plantar surface of the rat hind paw produces specific pain behavior (flinching response) that is readily discriminated and characterized as being rapid with the brief withdrawal or flexing of the injected paw. Such pain behavior is quantified by periodically counting the number of flinches of the injected paw after the injection. The number of flinches was counted for 1 min periods for 1 and 5 min and at 5 min intervals from 10 to 60 min. The formalin-induced flinching behavior is biphasic. The initial acute phase (0–9 min) is followed by a relatively short quiescent period. This is then followed by a prolonged tonic response (10–60 min). At the end of the experiment, the rats were euthanized using volatile anesthetics.

The effect of intrathecal zaprinst (10, 30 and 100 μg) pretreatment, administered 10 min before the formalin injection, was examined. Moreover, zaprinast was delivered intrathecally 9 min after the formalin injection in order to examine the effect of the zaprinast posttreatment (100 μg).

The rats were pretreated with L-NMMA (NO synthase inhibitor), ODQ (guanylyl cyclase inhibitor) or glibenclamide (potassium channel blocker) in order to determine how the L-arginine NO-cGMP-potassium channel pathway participates in the antinociceptive effect of zaprinast. These three drugs were administered intrathecally 10 min before delivery of intrathecal zaprinast (100 μg). The formalin was injected 10 min later. The maximum doses of L-NMMA (10 μg), ODQ (4 μg) and glibenclamide (3 μg) were selected based on their lack of any significant effect on the controlling the formalin response from the pilot experiments.

Data are expressed as mean ± standard error of mean (S.E.M.). In the formalin test, the time response data or the dose–response data are shown either as the number of flinches or the sum of the number of flinches in two phases.

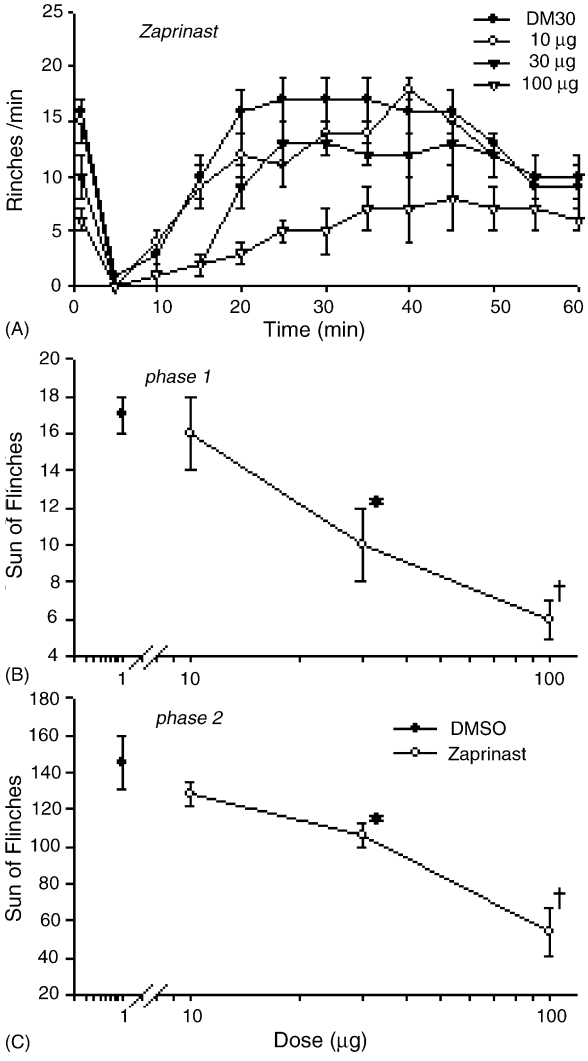

Fig. 1. Time course and dose–response curves of intrathecal zaprinast on flinching in the formalin test. Zaprinast was administered 10 min before the f (formalin) injection (A). Data are presented as either the number of flinches or the sum of flinches. Zaprinast produced a dose-dependent inhibition of flinches in phase 1 (B) and phase 2 (C) of the formalin test. Each line represents mean ± S.E.M. of 5–8 rats. Compared with the control, $^{*}P < 0.01$, $^{\dagger}P < 0.001$.

The dose–response data were analyzed by one-way analysis of variance with Scheffe for the post hoc test. The comparison of the zaprinast pretreatment and posttreatment, and the antagonism for the effect of zaprinast were analyzed using a *t*-test, with $P < 0.05$ being considered statistically significant.

The subcutaneous injection of formalin into the hindpaw of the rat caused a biphasic flinching response of the injected paw. Intrathecal zaprinast, administered 10 min prior to the formalin injection, resulted in the dose-dependent inhibition of the flinching response during phase 1 and phase 2 in the formalin test (Fig. 1). Posttreatment with intrathecal zaprinast reduced the flinching behavior during phase 2 (Fig. 2).

Intrathecal L-NMMA (NO synthase inhibitor), ODQ (guanylyl cyclase inhibitor) and glibenclamide (potassium channel blocker) alone did not affect the controlling the