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The role of adrenergic and cholinergic receptors on the antinociception of sildenafil in the spinal cord of rats

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ABSTRACT

The role played by spinal adrenergic and cholinergic receptors in the antinociceptive effects of intrathecal sildenafil in formalin-induced nociception was examined. Intrathecal catheters were inserted into the subarachnoid space of male Sprague-Dawley rats, and nociception was assessed using the formalin test, consisting of a subcutaneous injection of 50 μL of 5% formalin solution into the hind paw. We examined the effects of an alpha 1 adrenergic receptor antagonist (prazosin), an alpha 2 adrenergic receptor antagonist (yohimbine), a muscarinic acetylcholine receptor antagonist (atropine), and a nicotinic acetylcholine receptor antagonist (mecamylamine) on sildenafil-induced antinociception. Intrathecal sildenafil (3, 10, and 30 μg) suppressed, in a dose-dependent manner, formalin-induced flinching during phases 1 and 2 of the test. Intrathecal sildenafil (30 μg) could not show any effects against intrathecal prazosin (3 μg), yohimbine (10 μg), atropine (10 μg), and mecamylamine (10 μg) pretreatment during both phases of the formalin test. These results suggest that intrathecal sildenafil effectively attenuated the pain evoked by formalin injection. Additionally, spinal alpha 1, alpha 2, muscarinic and nicotinic receptors might play a role in sildenafil-induced antinociception.

Several lines of evidence suggest that cyclic guanosine monophosphate (cGMP) may play a pivotal role in antinociception [8,21]. Biochemically, intracellular cGMP concentrations are regulated by the action of guanylyl cyclase and the rate of degradation by cGMP-specific phosphodiesterase [3,19]. Cyclic GMP has several targets in cells, including cyclic GMP-dependent protein kinases (PKG), cyclic GMP-regulated phosphodiesterases and cyclic nucleotide-gated ion channels [15].

Sildenafil (Viagra®) is a potent and selective cGMP-specific phosphodiesterase-5-inhibitor that is widely known because of its therapeutic efficacy in erectile dysfunction; it functions by increasing intracellular cGMP levels [6,23] and has been shown to have both antinociceptive effects in the formalin test in rats, as well as affecting the writhing response induced by acetic acid and zymosan in mice [2,1], mediated through the nitric oxide (NO)-cGMP pathway [11,25]. Recent studies have suggested that the adenosine, $GABA_B$ and opioid receptors are also involved in the antinociceptive effect of intrathecal sildenafil in the formalin model [14,13,29]. Thus, sildenafil can produce an antinociceptive effect by modifying a variety of systems which may alter the nociceptive processing. Experimental data indicate that adrenergic and acetylcholine act by modulating nociceptive transmission at the spinal level, mediated through the respective receptors [18,12]. Recently it was reported that the combination of ineffective doses of adrenergic agents with sildenafil significantly inhibited the nociceptive response induced by acetic acid injection [5]. Additionally, co-administration of sildenafil and atropine was found to produce a robust anti-depressant like response in the rat forced swim test [7]. These observations suggest that the effects of the cGMP-specific phosphodiesterase inhibitor are affected by both the adrenergic and acetylcholine systems. However, the roles of spinal adrenergic and acetylcholine receptors in the antinociceptive effect of intrathecal sildenafil remain to be determined.

The purpose of the present study was to investigate the effects of intrathecal sildenafil in a formalin-induced nociception model of rat and then to determined the roles played by adrenergic and acetylcholine receptors in the effects of sildenafil at the spinal level.

All procedures were approved by the Institutional Animal Care and Use Committee at Chonnam National University. Adult male Sprague-Dawley rats weighing 250–300 g were used for all experiments. The animals were housed in groups of four, with free access to standard rat chow and tap water in a room under a 12:12 h light/dark cycle.

For drug administration, an intrathecal catheter was implanted under sevoflurane anesthesia and aseptic surgical conditions, as described previously [27]. Following catheter implantation,

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rats were individually housed. Rats showing neurological deficits postoperatively were excluded from further study and killed immediately with a volatile anesthetic overdose. At least 5 days of recovery were allowed after surgery.

The following drugs were used in this study: sildenafil, prazosin, yohimbine, atropine, and mecamylamine (Sigma Aldrich, St. Louis, MO, USA). Sildenafil was kindly provided by Pfizer Korea. Yohimbine was dissolved in distilled water and the other drugs were dissolved in saline. Intrathecal administration of these agents was performed using a hand-driven, gear-operated syringe pump. All drugs were delivered in a volume of 10 μL solution, followed by an additional 10 μL of normal saline to flush the catheter.

The formalin test was used as a nociceptive test [29]. The plantar surface of the hind paw was injected with 50 μL of 5% formalin solution subcutaneously using a 30-gauge needle. Formalin injection produces characteristic pain behavior: a rapid, brief flexion of the injected paw, which was defined as flinching. The total number of flinches was recorded over 5 min intervals. Formalin-induced flinches were observed in a characteristic biphasic response. The initial phase 1 (0–9 min) was followed by a relatively short quiescent period, which was then followed by a late phase 2 (10–60 min). Immediately following the completion of the formalin test, the rats were sacrificed by a volatile anesthetic overdose.

On the day of experiments, the rats were placed in a restraint cylinder and allowed to adapt for 15–20 min. Rats were randomly assigned to one of the drug treatment groups. Intrathecal drugs were injected 10 min before formalin injection. The control study was done with intrathecal saline or distilled water. Animals were tested only once in the formalin test. The total number of rats used was 110 with 5–7 rats per group. The researcher was blind to the drug given to experimental animals.

To investigate the antinociceptive effects of intrathecal sildenafil (3, 10, and 30 μg), the flinching response was examined for 60 min during phases 1 and 2 in the formalin test. Next, rats were pretreated with adrenergic and cholinergic receptor antagonists to determine which receptor affected sildenafil activity. There were 10 min between each of the three injections: the antagonists were administered intrathecally, followed by delivery of the intrathecal sildenafil (30 μg), and then the formalin was injected. The adrenergic and cholinergic receptor antagonists were selected on the basis of relevant receptor affinity and selectivity, and their doses were determined by preliminary experiments that were designed to identify the maximum dosage that did not affect the control formalin response or motor function. The following adrenergic and cholinergic receptor antagonists and doses were used: alpha 1 adrenergic receptor antagonist: prazosin (3 μg); alpha 2 adrenergic receptor antagonist: yohimbine (10 μg); muscarinic acetylcholine receptor antagonist: atropine (10 μg); and nicotinic acetylcholine receptor antagonist: mecamylamine (10 μg).

In order to evaluate the behavioral changes caused by sildenafil, prazosin, yohimbine, atropine, and mecamylamine, additional rats received the highest doses of agents used, and were examined after intrathecal administration. Motor function was assessed using the placing-stepping reflex and the righting reflex [28]. The former was evoked by drawing the dorsum of hind paw across the edge of the table. Normally, rats try to put their paws forward into a position for walking. The latter was evaluated by placing the rat horizontally with its back on the table; Healthy rats produce immediate, coordinated twisting of the body into an upright position. The pinna and corneal reflexes were evoked by stimulating the ear canal or cornea, respectively, with a string [28]. The healthy rats spontaneously shook their heads or blinked. Normality of behavior was judged as present or absent.

Data were expressed as mean ± SEM. In the formalin test, the time response data or the dose–response data were presented as the number of flinches or as percentage of control in each phase.

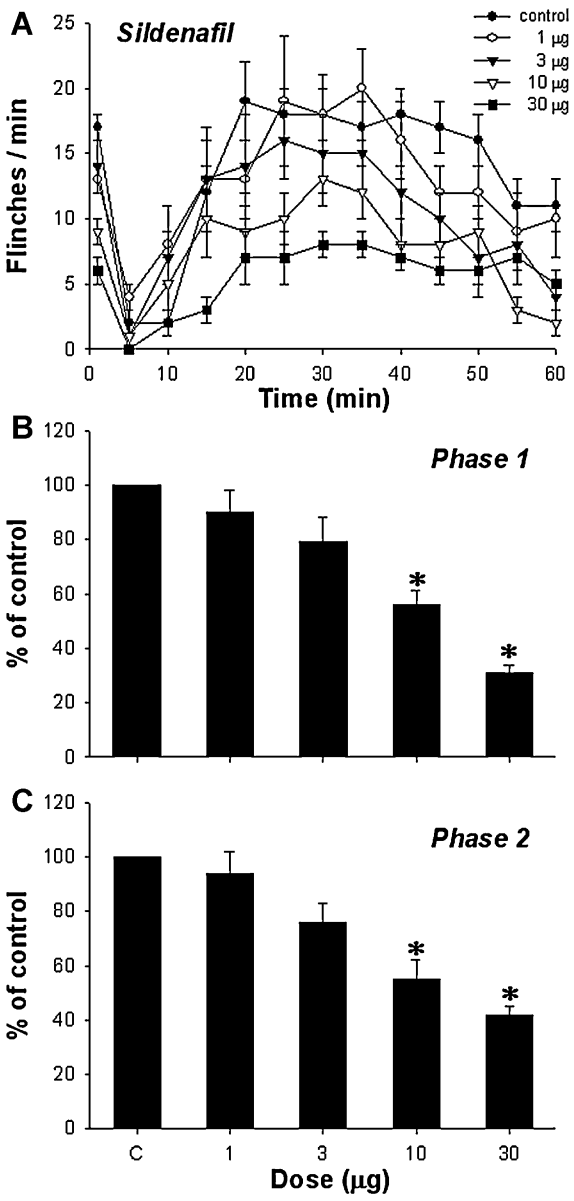

Fig. 1. Time course (A) and dose–response curves of intrathecal sildenafil on flinching during phase 1 (B) and phase 2 (C) in the formalin test. Sildenafil was administered 10 min before the formalin injection. Sildenafil produced a dose dependent suppression of flinches in phases 1 and 2 of the formalin test. Data are presented as the percentage of control. Each line represents means ± SEM of 5–7 rats. *$P < 0.05$, compared with control.

Number of flinches was converted to a percentage of control: % of control = ([sum of phase 1 or 2 flinching count with drug]/[sum of control phase 1 or 2 flinching count]) × 100. The dose–response data were analyzed using one-way analysis of variance with the Scheffe *post hoc* analysis. Comparison of antagonism for the effect of sildenafil was analyzed by the unpaired *t*-test. $P < 0.05$ was considered to be statistically significant.

Pharmacological treatment with sildenafil, prazosin, yohimbine, atropine, and mecamylamine produced normal behavior in experimental rats, as revealed by the righting and placing/stepping reflexes.

Subcutaneous formalin injection into the hind paw produced a distinct biphasic flinching response (Fig. 1). Compared to vehicle, intrathecal sildenafil reduced flinching in both phases 1 and 2 of the formalin test in a dose-dependent manner (Fig. 1).