J Anesth (2009) 23:399–402
DOI 10.1007/s00540-009-0767-y

Journal of
Anesthesia

Suppression of the descending inhibitory pathway by continuous thoracic intrathecal lidocaine infusion reduces the thermal threshold of the tail-flick response in rats

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Abstract
Purpose. For the suppression of descending inhibitory pathways in animals, single-dose lidocaine blockade is reversible and causes less damage than chronic spinal cord injury, decerebration, and cold blockade of the spinal cord. However, single-dose blockade has a variable onset and is relatively short-lived. To surmount these disadvantages, we devised a continuous thoracic intrathecal lidocaine infusion and evaluated its effects in rats.
Methods. Rats were administered continuous intrathecal infusions of 0, 0.25%, 0.5%, and 1% lidocaine at 10 μl·h^{-1} following a 10-μl bolus. The effects of the continuous thoracic blockade on tail-flick (TF) latency (estimated by the percent maximum possible effect [%MPE]) and on the release of neurotransmitters in the cerebrospinal fluid (CSF) were evaluated.
Results. Continuous thoracic blockade with 0.5% and 1% lidocaine infusion reversibly shortened TF latency (%MPE, –22.0 ± 11.0 % and –21.2 ± 4.6 %, respectively, versus baseline; $P < 0.05$) during drug infusion. Compared with normal saline, thoracic intrathecal infusion of lidocaine significantly lowered norepinephrine and serotonin concentrations in the CSF at 1 h of infusion ($P = 0.02$ for both).
Conclusion. Continuous thoracic blockade by local anesthetic resulted in reversible suppression of descending inhibitory pathways for varying durations. Such blockade may provide further information regarding nociceptive transmission and the mechanisms of antinociception in animals.

Key words Descending inhibitory pathway · Lidocaine · Thoracic intrathecal injection · Tail-flick response · Rat

Introduction

Signals from peripheral nociceptors in response to noxious stimuli ascend via primary afferent neurons to secondary nociceptive neurons in the dorsal horn of the spinal cord, and sequentially project to supraspinal structures. Secondary nociceptive neurons, on the other hand, are modulated supraspinally by the descending inhibitory system. Systemically administered analgesics can induce antinociceptive effects at supraspinal, spinal, or peripheral sites, or a combination of these. The site of action of analgesics can be determined by suppressing descending inhibitory pathways. Chronic spinal cord injury [1], decerebration [2, 3], and anesthetic [4] or cold blockade of the spinal cord [5] have been used to interfere with descending transmission in animals. Lidocaine blockade of the spinal cord has advantages such as reversibility and less damage to the cord when compared with lesioning and cold blockade, although it has disadvantages such as variable onset and a relatively short duration of the peak effect with a single dose [4].

To surmount the disadvantages of single-dose lidocaine blockade of the spinal cord, we attempted continuous and reversible blockade of the descending inhibitory pathway by performing continuous infusion of lidocaine at the thoracic spinal cord in rats, and we evaluated the effect of this blockade on the thermal nociceptive response. We also investigated the influence of the continuous lidocaine blockade on the release of neurotransmitters in the cerebrospinal fluid (CSF).

Tail-flick (TF) testing using thermal radiation has been used in approximately 40% of animal studies dealing with pain measurement [6], and is sensitive to manipulations known to reduce pain in humans [7]. Although the TF response is commonly considered a spinal reflex, the response is influenced by supraspinal structures. Gebhart and Ossipov [8] have demonstrated that inhibition of the spinal nociceptive TF reflex is mediated by spinal α_2-adrenoceptors, suggesting that the reflex is modulated by descending inhibition. In this study, we used the TF test to evaluate the effect of

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Received: December 9, 2008 / Accepted: March 26, 2009

continuous blockade of the descending inhibitory pathway on the thermal nociceptive response.

Materials and methods

With the approval of the animal care and use committee of Kinki University School of Medicine, 40 male Sprague-Dawley rats weighing 340 to 420 g (10–12 weeks old) were studied. The animals were bred at the Life Science Research Institute, Kinki University School of Medicine, and were maintained under controlled conditions (temperature, 23 ± 0.5°C; humidity, 55%; 12/12-h light/dark cycle) and were fed a commercial diet of CE-2 (Clea Japan, Tokyo, Japan), with tap water available ad libitum. The experiments were performed between 1300 hours and 1700 hours under controlled conditions (temperature 23 ± 0.5°C).

Thoracic intrathecal catheterization

The thoracic subarachnoid space was cannulated with a polytetrafluoroethylene (PTFE)-lined polyethylene tube (0.3-mm external diameter and 0.11-mm internal diameter; Microspinal Catheter, Hakko, Nagano, Japan), using the method of Sakura et al. [9], with modifications. In brief, rats were anesthetized by the inhalation of 2% isoflurane in oxygen with a mask. The catheter was passed through a slit in the atlanto-occipital membrane and advanced 3 cm caudally to the level of the mid-thoracic cord. The other end of the catheter was fixed in the subcutaneous tissue to avoid dislocation. The catheter was filled with normal saline, and then the end was closed by heating. One week later, rats that exhibited any evidence of sensory or motor dysfunction were excluded from the study. In a preliminary examination, three of the animals subjected to thoracic intrathecal catheterization were killed by the intraperitoneal injection of an overdose of pentobarbital after an intrathecal infusion of 10 µl of indigo carmine. On the removal of vertebral bone, the catheter tips were found to be located between C4 and C5, and the indigo carmine dye extended into the subarachnoid space between C1 and Th2.

Upon completion of the experimental series, each animal was intrathecally administered 10 µl of 1% lidocaine. Paralytic symptoms of the forelimbs were observed, and motor and sensory responses of the hindlimbs were preserved.

Tail-flick (TF) test using radiant heat

King et al. [6] considered the decrease in TF latency that occurs in repeated testing in conscious animals to be the result of a learning effect. In this study, we performed TF testing with the animals under light anesthesia to eliminate any learning effect [10].

The Tail-Flick Unit (Model 7360; Ugo Basile, Varese, Italy) was utilized, and TF latency was measured following the method of Takasugi et al. [10]. In brief, each rat was placed in a plastic box (22 × 6.5 × 6.5 cm) that had two inlets on the front wall, for oxygen and anesthetic gases and for gas sampling, and a hole in the distal wall through which the tail protruded. Isoflurane in oxygen was introduced into the box, and the concentrations of isoflurane and oxygen in the box were continuously measured using an anesthetic gas analyzer (Capnomac Ultima; Datex, Helsinki, Finland). Under inhalational anesthesia with 1% isoflurane in oxygen, a radiant heat intensity setting of infrared radiant (IR) 20 (161.5 $mW \cdot cm^{-2}$), which resulted in a skin temperature of 65°C after 10 s, was used to obtain reliable TF latencies on repeated TF testing and stimulation of C-polymodal nociceptors [10]. Different points along the distal 5 to 6 cm of the tail were exposed, and a 10-s cutoff was used to minimize the risk of tissue damage. When a "flick" reaction occurred, an on-board sensor turned off the bulb, the second counter was stopped, and the withdrawal latency was determined to the nearest 0.1 s. There was a 10-s interval between measurements. The mean of the last five TF latencies of seven consecutive measurements was used as the representative value.

Experiment 1: influence of continuous thoracic lidocaine blockade on TF latency

The 40 rats with thoracic intrathecal catheters were randomly divided into four groups of 10 rats each; 0.25%, 0.5%, and 1% lidocaine groups and a normal saline group. Prior to each experiment, the rats were placed in the boxes and exposed to 1% isoflurane in oxygen for 20 min, and baseline TF latencies were measured. Rats in each group received a 10-µl bolus followed by 10 µl/h continuous infusion of 0.25%, 0.5%, or 1% lidocaine, or normal saline, administered by microsyringe pump (ESP-64; Eicom, Kyoto, Japan). Each animal was tested for 90 min at 15-min intervals (60 min of infusion and another 30 min after discontinuation of lidocaine). The TF latency was converted to represent the percent maximum possible effect (%MPE) according to the following formula:

$$\%\text{MPE} = [(\text{test latency}) - (\text{baseline latency}) / (\text{cutoff time}) - (\text{baseline latency})] \times 100$$

Experiment 2: effects of continuous thoracic lidocaine blockade on neurotransmitter release in the CSF

One week after execution of experiment 1, 12 of the rats used in experiment 1 were divided into two groups of 6

rats each; one group was intrathecally infused with normal saline and the other with 1% lidocaine. Infusions were performed for 60 min under the inhalation of 1% isoflurane in oxygen. Immediately after discontinuation of the infusion, 80 to 100 µl of CSF was aspirated from the cisterna magna by advancing a 27-gauge butterfly needle connected to a 1-ml syringe through the muscle layer overlying the atlanto-occipital membrane beside the catheter and into the cisternal compartment, according to the method of Takasugi et al. [11]. CSF samples were quick-frozen in liquid nitrogen and stored at –80°C until assay.

Concentrations of monoamines and amino acids related to neurotransmission in the CSF were determined by a gradient reversed-phase high-performance liquid chromatographic (HPLC) method and by an isocratic HPLC method, respectively, with a coulometric array electrochemical detector (Model 5600A; CoulArray System; ESA, Chelmsford, MA, USA). The chromatographs were quantitatively analyzed by CoulArray for Windows Data Processing Module Ver. 1.04 (ESA). CSF samples were analyzed for the levels of norepinephrine (NE), serotonin (5-HT), glutamate (Glu), and γ-aminobutyric acid (GABA).

Statistical analysis

Data are expressed as means ± SD. TF latency changes over time were compared using repeated measures analysis of variance (ANOVA), and comparisons among groups were analyzed by ANOVA followed by Dunnett's multiple comparison test or Bonferroni post-test as indicated. Differences in monoamine or amino acid concentrations among groups were determined using an unpaired *t*-test. Statistical analysis was performed using Prism 5 for Windows Ver. 5.01 (GraphPad Software, San Diego, CA, USA). The significance level was set at $P < 0.05$.

Results

Experiment 1: influence of continuous thoracic lidocaine blockade on TF latency

No significant difference was detected in baseline TF latencies among the normal saline and 0.25%, 0.5%, and 1% lidocaine groups, their values being 4.2 ± 0.7 s (estimated skin temperature [3], 46.8 ± 1.6°C), 3.8 ± 0.2 s (estimated skin temperature, 46.0 ± 0.5°C), 4.4 ± 0.7 s (estimated skin temperature, 47.2 ± 1.7°C), and 4.6 ± 0.6 s (estimated skin temperature, 47.8 ± 1.2°C), respectively. TF latency (%MPE) did not change significantly over time in the normal saline group or the 0.25% lidocaine group, whereas it decreased significantly during infusion in the 0.5% and 1% lidocaine groups when compared with baseline (–22.0 ± 11.0% and –21.2 ± 4.6% at 60 min, respectively; $P < 0.05$), and recovered to baseline after the discontinuation of lidocaine (Fig. 1).

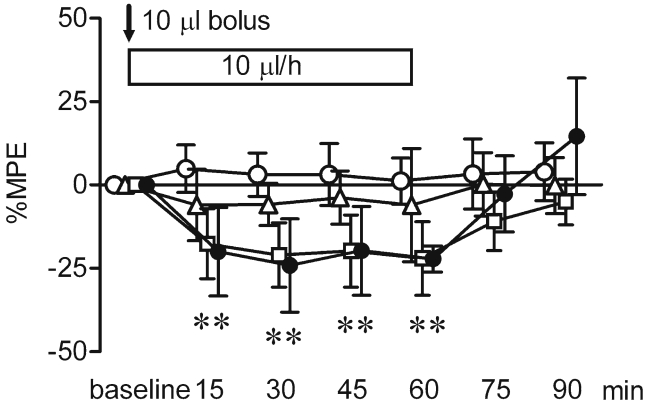

Fig. 1. Influence of continuous thoracic lidocaine blockade on tail-flick latency. Rats received a 10-µl bolus followed by 1-h of 10 µl·h^{-1} continuous infusion of 0.25% (*triangles*), 0.5% (*squares*), or 1% lidocaine (*closed circles*), or normal saline (*open circles*). Changes in tail-flick latencies were estimated by the maximum possible effect (*%MPE*). *$P < 0.05$ vs baseline values (Dunnett's multiple comparison test) and normal saline group (Bonferroni post-test)

Experiment 2: effects of continuous thoracic lidocaine blockade on neurotransmitter release in the CSF

The CSF concentrations of NE and 5-HT after 1-h thoracic intrathecal infusion were significantly lower in the lidocaine group than in the normal saline group ($P = 0.02$ for both). There was no difference between groups in the CSF concentrations of Glu or GABA (Table 1).

Discussion

This study revealed that continuous thoracic blockade with 0.5% or 1% lidocaine infusion shortened TF latency during drug infusion and reduced NE and 5-HT concentrations in the CSF.

Noradrenergic and serotonergic projections from supraspinal structures to the spinal dorsal horn constitute the descending inhibitory system, which inhibits the spinal transmission of pain signals [12]. Furthermore, supraspinal α_1-adrenoceptor-mediated noradrenergic descending projections facilitate nociceptive processing in the spinal cord [13]. Therefore, central nociceptive perception is recognized as the net result of afferent pain transmission and supraspinal modulation.

Pubols et al. [4] reported that anesthetic blockade of descending inhibitory pathways at the dorsolateral funiculus enhanced the evoked activity of dorsal horn

Table 1. Comparisons of neurotransmitter concentrations in CSF between normal saline and lidocaine groups following 1-h continuous thoracic drug infusion

	Normal saline group ($n = 6$)	Lidocaine group ($n = 6$)	P value
Norepinephrine	0.42 ± 0.19 ng·ml^{-1}	0.11 ± 0.08 ng·ml^{-1}	0.021
Serotonin	0.40 ± 0.25 ng·ml^{-1}	0.08 ± 0.05 ng·ml^{-1}	0.021
Glutamate	8.26 ± 1.78 nM	7.87 ± 3.57 nM	0.818
GABA	0.43 ± 0.17 nM	0.58 ± 0.11 nM	0.113

The P values are for the unpaired t-test
Values are means ± SD

neurons, and Jones and Gebhart [14] found that lidocaine injection into the ventrolateral funiculus produced an increase in the noxious heat-evoked response of dorsal horn neurons. In the present study, we demonstrated that the heat-evoked withdrawal response was clearly facilitated during thoracic spinal blockade by lidocaine. Taken together, these findings suggest that, although a noradrenergic-supraspinal α_1-adrenoceptor pathway mediates the pronociceptive effect, descending spinal projections mainly play a role in the inhibition of nociceptive transmission.

Thermosensitive ion channels that are expressed in primary sensory neurons belong to the transient receptor potential (TRP) superfamily. Aδ fibers exhibit TRPV2-containing channels with a thermal activation threshold of more than 52°C but do not express TRPV1-containing channels with a threshold of more than 43°C, while C fibers exclusively exhibit TRPV1- containing channels [7, 8]. In the setting of the TF testing in the present study, the radiant heat would have produced a TF response with a latency of approximately 3–6 s, which equates to an estimated skin temperature of 44°C –50°C [10]. Therefore, our findings suggest that thoracic blockade by lidocaine facilitates the ascending transmission mediated by C-polymodal nociceptors [15, 16].

Gebhart and Ossipov [8] demonstrated that inhibition of the spinal nociceptive TF reflex was mediated by spinal α_2-adrenoceptors, and we confirmed that the reduction of NE and 5-HT concentrations in the CSF following continuous thoracic blockade was associated with facilitation of the TF response. Our interpretations of these data are that the lidocaine thoracic blockade suppresses noradrenergic and serotonergic descending inhibitory pathways, resulting in the facilitation of ascending transmission mediated by C-fibers.

The present study reveals that continuous thoracic blockade by local anesthetics can provide reversible suppression of descending inhibitory pathways of varying duration and with less damage to the spinal cord than lesioning. These properties of continuous thoracic blockade should enable researchers to determine whether drugs have spinal or supraspinal effects and may provide further information regarding nociceptive transmission and the mechanisms of antinociception in animals.

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