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Establishment of an Animal Model of Epidural Anesthesia and Sedative Tail-Flick Test for Evaluating Local Anesthetic Effects in Rats

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Abstract: The tail-flick (TF) test is the most frequently used method to measure pain levels and assess the effects of anesthesia. In this study, we performed the TF test in rats sedated via an indwelling epidural catheter and then examined the effectiveness of this method in evaluating the local anesthetic effects. First, an epidural catheter was inserted into the epidural space, and anesthetic [lidocaine (L) or lidocaine including adrenaline (AL)] or normal saline (NS) was administered. Under sedation, we measured the dose for disappearance of the TF response, time to TF response recovery, onset and regression of local anesthesia, as well as the effect of an added agent on its continuation. The time course of TF latency (% maximum possible effect) in the NS group did not change during the experiment. In the AL group, TF latency increased significantly more than baseline during the 30-min period after injection. This was also significantly higher than the latency in the NS group and the L group. In the L group, the TF latency increased significantly above baseline for 20 min after injection and was significantly higher than that in the NS group. Due to the fact that we were able to detect the effect of local anesthesia onset and regression, as well as the local anesthesia continuation action of an additive agent, in rats sedated via an indwelled epidural catheter, we consider our method to be an improvement over conventional methods.

Key words: epidural rat model, evaluating local anesthetic effect, indwelling epidural catheter, isoflurane, tail-flick test

Introduction

Various methods are used to examine the effect of local anesthetics, which differ in their clinical effects and durations [16, 26]. Evaluating the effectiveness of local anesthetics necessitates the ability to precisely detect the amount of pain relief achieved following infringement stimulation *in vivo*. Infringement stimulation involves various methods, including pinprick and forceps pinch, but these types of methods are not always constant in intensity [3, 7, 31]. Furthermore, they may damage the skin of the animal and possibly lead to increased difficulty in evaluating the effect of the anesthesia. The tail-flick (TF) test is the most frequently used perception test for measuring the severity of acute or chronic pain. It uses radiant heat to both measure the latency of the

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response to thermal noxious stimuli and assess the pain threshold and effect of anesthesia. Radiant heat is a type of constant infringement stimulation that does not damage the skin when a proper cut-off time is used [4, 11].

Drug administration in the TF test is mainly via spinal injection, intravenous administration in tails occluded by a tourniquet, oral administration, subcutaneous injection or infiltration anesthesia [5, 9, 17, 18 ,20–23, 27]. Spinal anesthesia via injection is the most frequently used TF test for measuring the anesthesia effect [5, 18, 20, 22, 27]. Addition of adrenaline to intrathecally administered lidocaine is a common practice, but it leads to neurotoxicity through some unknown mechanism. However, toxicity leading to functional impairment or histologic damage may be related to a vasoconstrictive effect upon anesthetic exposure [8]. Adrenaline is commonly added to lidocaine solutions to prolong the duration of anesthesia. Therefore, as it is necessary to assess the effects of adrenaline in order to properly evaluate the effects of the local anesthetic, spinal anesthesia via injection should consequently be avoided. The subcutaneous veins on the tail can be easily cannulated for intravenous administration in tails occluded by a tourniquet, although this should be avoided whenever possible because sensory neural function decreases during ischemia [21].

Furthermore, oral administration, subcutaneous injection and infiltration anesthesia are methods that have imprecise administration sites and dosages and, from an anatomical point of view, lead to imprecise effectiveness. In order to effectively compare anesthesia effects *in vivo*, we must remove the anatomical factor. Furthermore, due to a learning effect for pain and the difficulty of prolonged physical restraint, it is difficult to accurately measure escape responses in TF tests in conscious rats. We thus hypothesized that a TF test in rats sedated by anesthetic administered locally via an indwelling epidural catheter would provide an injection site and dose precise enough to evaluate the *in vivo* effects.

In order to test this hypothesis, we examined the effectiveness of evaluating local anesthetic effect using a TF test on rats sedated via an indwelling epidural catheter in the epidural space. Two experiments were used to evaluate the feasibility of this model. In the first experiment, we evaluated the dose of local anesthesia for disappearance of the TF response and time for TF recovery. Lidocaine was administered continuously into the epidural space, and the dose of local anesthesia that extended the TF latency was determined. We also determined the measurement time necessary for detection of both the onset and regression of the anesthesia effect. In the second experiment, we administered doses of lidocaine, adrenalin containing lidocaine, and saline equivalent to those in the first experiment and measured both their TF latency and the detectability of their effects.

Finally, we discussed the feasibility of this model.

Materials and Methods

Animals

With the approval of the Animal Care and Use Committee of the Kinki University Faculty of Medicine, this study used male Sprague-Dawley rats (*Rattus norvegicus*) (Japan SLC, Inc., Shizuoka, Japan) weighing 340–420 g (10–12 weeks old, n=55 total for experiments 1 and 2). The rats were maintained under controlled conditions (temperature, 23 ± 0.5°C; humidity, 55%; 12/12 h light/dark cycle) and fed *ad libitum* a commercial diet (CE-2, CLEA Japan, Inc., Tokyo, Japan) and tap water. The experiments were performed between 12:00 and 17:00 under controlled conditions (temperature 23 ± 0.5°C). All procedures were conducted in the Life Science Research Institute, Kinki University Faculty of Medicine.

The epidural space was cannulated with a Teflon-lined polyethylene tube (0.3 mm outer diameter and 0.11 mm inner diameter; Microspinal Catheter, Hakko Co., Ltd., Nagano, Japan) using a method modified from those of Sakura *et al.* and Jensen and Yaksh [12, 25]. In brief, rats were anesthetized by inhalation of 2% isoflurane (Forane, ABBOTT Japan Co., Ltd., Tokyo, Japan) in oxygen with a mask. The epidural space was exposed under a stereoscopic microscope without cerebrospinal fluid leakage. The catheter was inserted through a slit in the atlantooccipital membrane and extended 11 cm, the distance necessary to arrive at the lumbar vertebrae (L5–L6) based on a preliminary experiment with a dissected rat. The other end of the catheter was fixed in the subcutaneous tissue to avoid dislocation (Fig. 1). One week later, rats were examined for evidence of sensory or motor dysfunction.

Tail-flick test

A tail-flick measuring device (Model 7360, Ugo Basile, Varese, Italy) was used that included a stimulator powered by an infrared light source (8 V, 50 W; Halogen

"Bellaphot", Mod. 64607, OSRAM), the intensity (max, 20 W) of which could be adjusted from 10–99 in 1-digit increments.

Rats were placed in a plastic box (22 × 6.5 × 6.5 cm), and 1.0% isoflurane concentrations were administered. The inspired gas concentration was then measured in the rats. The ventral surface of the distal 5–6 cm of the tail was placed over a 0.5-cm hole in an aluminum box, and an infrared radiant (IR) bulb was placed beneath the hole (Fig. 2). The tail was exposed to the IR bulb at a distance of 5-mm in each trial, and a 10-s cutoff was used to minimize the risk of tissue damage. Before each of these experiments, experimental rats were placed in the boxes and exposed to the gas for 20 min. Based on a previous study, the minimum alveolar concentration, the concentration evoked by a noxious stimulus (ED_{50}) that blocks movement, decreases by 42% with every 10°C drop in temperature [6]. Therefore, in order to avoid onset of hypothermia, a thermocouple was used to measure rectal temperature of anesthetized rats, and a thermal plate connected to a feedback warmer beneath the box maintained the temperature at a constant 36–38°C.

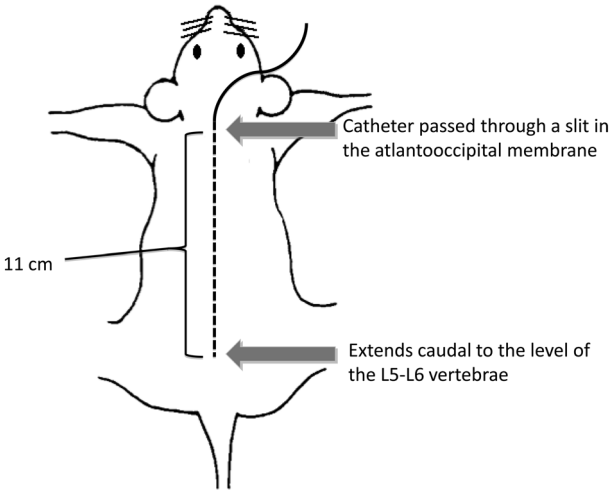

Fig. 1. Schematic of catheter insertion. A catheter was inserted through a slit in the atlantooccipital membrane and extended 11 cm to a level caudal to lumbar vertebrae L4–L6. The other end of the catheter was fixed in the subcutaneous tissue to avoid dislocation.

Experiment 1: Evaluation of the dose of local anesthetic for disappearance of tail-flick response and time for tail-flick recovery

Ten rats implanted with epidural catheters were evaluated. TF latency was measured at baseline and continuously after starting injection of 2% lidocaine (200 μl/h). Following epidural administration, each rat was tested at 10-min intervals. Continuous administration was stopped when the TF response disappeared, and interval testing was stopped when the TF response recovered. Disappearance of the TF response (reaching the cut-off time), time of stopping administration (dose for disappearance of TF response) and time of TF response recovery were measured (Fig. 3). If the TF response had not disappeared after 30 min, the dose was increased by 50 μl every 10 min until disappearance was achieved.

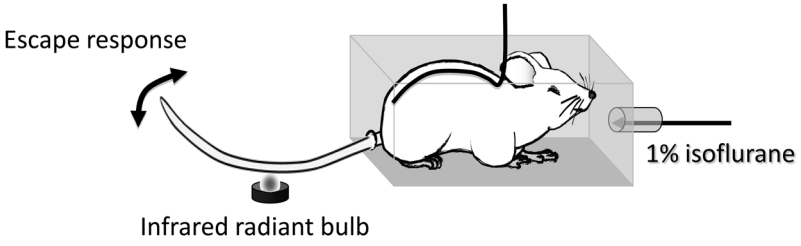

Fig. 2. Tail-flick device. The rats were placed in a plastic box (22 × 6.5 × 6.5 cm). The upper panel pivoted horizontally to enable placement of the rat inside. Two holes in the front of the box functioned as inlets for oxygen and anesthetic gases and facilitated gas sampling. The distal wall had a hole through which the tail protruded. The side and bottom surfaces of the box were black, and the roof was transparent. The box was covered with a cloth during testing. Inspiratory concentrations of 1.0% isoflurane were used. Rats were then tested for inspired gas concentration. The ventral surface of the distal 5–6 cm of the tail was placed over a 0.5-cm hole in an aluminum box, and an infrared radiant bulb was placed beneath the hole.

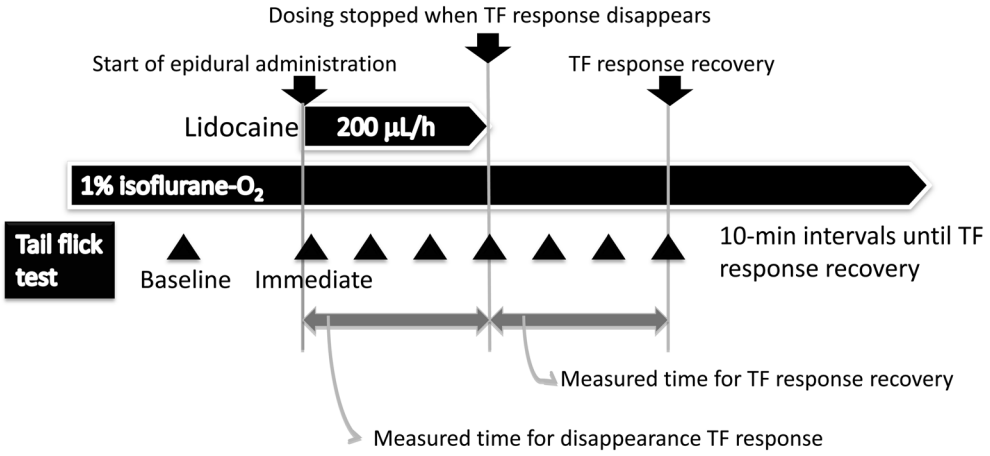

Fig. 3. Dosing for disappearance of the tail-flick (TF) response and time for TF response recovery. Measured TF latency of the baseline and starting of continuous injection of lidocaine (200 μl/h). Following the start of epidural administration, each rat was tested at 10-min intervals. Continuous injection was stopped when the TF response disappeared. The 10-min interval test was stopped when the TF response recovered.

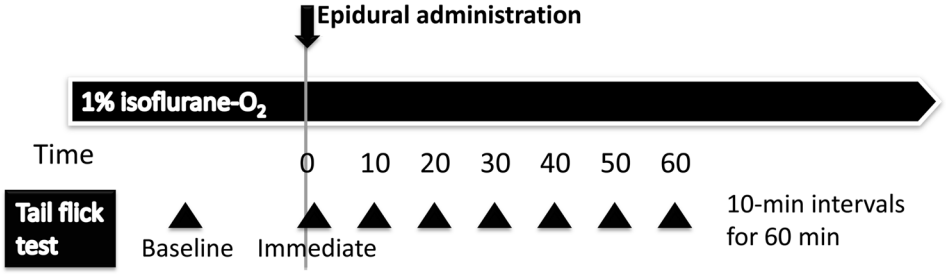

Fig. 4. Experimental schedule. Rats in the NS, L, and AL groups received an epidural injection of 30 μl of normal saline, lidocaine, or adrenaline accompanied by lidocaine, respectively. Following epidural injection, each rat was tested at 10-min intervals for 60 min.

Experiment 2: Evaluation of detectability of various local anesthesia effects

Forty-five rats implanted with epidural catheters were randomly divided into three groups (n=15 each) as follows: NS (normal saline), L (2% lidocaine; Xylocaine Injection Polyamp, Astra Zeneca K.K., Osaka, Japan); and AL (2% lidocaine, 1:80,000 adrenaline; Xylocaine Cartridge for Dental Use, Dentsply-Sankin K.K., Tokyo, Japan). TF latency was converted to represent the maximum possible effect (MPE) according to the following formula:

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

Following epidural injection of 30 μl drugs, each rat was tested at 10-min intervals for 60 min (Fig. 4). The TF latency was then evaluated as %MPE.

Upon completion of both experimental series, all catheterized rats were killed by an epidural injection of 30 μl indigo carmine followed by injection of a fatal dose of pentobarbital into the peritoneum. The location of the catheter tip and distribution of the dye in the epidural space were determined after removal of the vertebrae.

Statistical analysis

TF latency changes over time were compared using repeated measures analysis of variance (ANOVA), and comparisons between groups were analyzed by ANOVA, followed by either a post-hoc Dunnett's multiple comparison test or Tukey's multiple comparison test as appropriate. Statistical analysis was performed using Prism 5 for Windows Ver. 5.01 (GraphPad Software Inc., San Diego, CA, USA). The significance level was set at $P<0.05$.

Table 1. Dose for disappearance of TF response and time for recovery

Subject ID (n=10)	Disappearance of TF response (min)	Injected dose (μl)	TF response recovery time (min)	Comments
1	10	33	40	
2	10	33	20	
3	20	66	30	
4	10	33	30	
5	10	33	40	
6	10	33	40	
7	10	33	20	
8	10	33	30	
9	30	150	30	50 μl bolus at 30 min
10	10	33	30	
Mean	13 (10–30)	37 (33–66)[a)]	30 (20–40)	

[a)]Subject 9 data excluded because of bolus administration.

Results

Position of the epidural catheter

Upon completion of the experimental series, the rats were sacrificed. In all rats, the tip of the catheter was placed caudal to the lumbar vertebrae at the level of L4–L6.

Experiment 1: Evaluation of the dose of local anesthesia for the disappearance of the tail-flick response and the time for tail-flick recovery

The time to reach the cut-off was measured in 10 rats. In eight rats, the time was 10 s (dose of 33 μl lidocaine), and in one rat it was 20 s (66 μl). One rat did not reach the cut-off time within 30 min of continuous administration. This rat reached the cut-off time following administration of 50 μl bolus. The average TF response recovery time after discontinuing injection was 30 min (20–40 min) (Table 1).

Experiment 2: Evaluation of detectability of the effects of various local anesthetics

There were no significant differences in baseline TF latency between the NS, AL, and L groups. The time course of TF latency (%MPE) in the NS group did not change during the experiment. In the AL group, TF latency increased significantly more than the baseline (P<0.05) during the 30-min period after injection. This was also significantly higher than the latency in the NS group (P<0.05) and L group (P<0.05). In the L group, the TF latency increased significantly more than the baseline (P<0.05) for 20 min after injection and was significantly higher than that in the NS group (P<0.05) (Fig. 5).

Discussion

In the first experiment, we showed disappearance of the TF response following continuous lidocaine injection into the epidural space, and TF response recovery was seen 30 min after discontinuing anesthetic. Rat 9 did not show disappearance of TF latency with continuous injection, but immediately showed disappearance with bolus injection. Therefore, it appears that the anesthetic did not arrive by a catheter obstructed, and that the obstruction was removed by bolus injection. Alternatively, it is possible that the anesthetic was not delivered due to a loose connection between the catheter and syringe and that the reconnection for bolus injection was connected closely. The TF response reversibly disappeared following 30 μl of lidocaine, and the response recovery was observable within 60 min. In the second experiment, we administered 30 μl normal saline, lidocaine, and adrenaline accompanied by lidocaine, and tested each rat at 10-min intervals for 60 min. TF latency in the NS group did not change, and the addition of adrenaline prolonged the TF latency of lidocaine. Therefore, this model allowed detection of both the onset and regression of local anesthesia, as well as the effect of adrenaline on its continuation action. Furthermore, oral administration, subcutaneous injection, and infiltration anesthesia are methods that have imprecise administration sites and dosages, and from an anatomical point of view with respect to nerve reachability, lead to imprecise effectiveness.

In contrast, epidural administration is a precise method due to insertion of a catheter near the nerve tract. In this study, all animals were catheterized in the lumbar epidural space. Catheters were inserted along a caudal

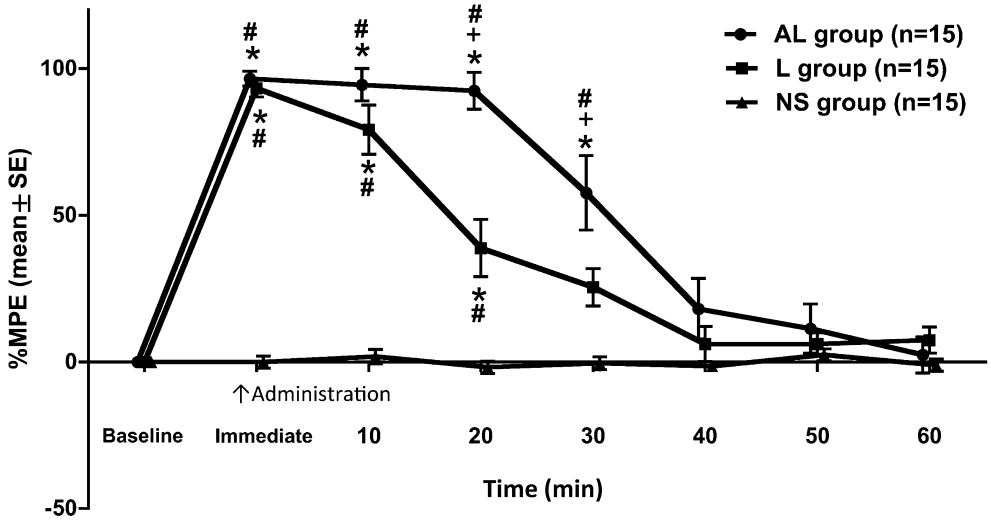

Fig. 5. Change in tail-flick latency after dosing. **P*<0.05 compared with the control (NS) group. ^{+}P<0.05 compared with the L group. $^{\#}P$<0.05 compared with the NS group. Circles, squares, and triangles show the TF latency from before administration to 60 min after administration in the AL group, L group, and NS group, respectively. In the AL group, TF latency immediately after injection increased significantly for 30 min compared with the baseline (*P*<0.05), was significantly higher than that in the NS group (*P*<0.05), and increased significantly for 20–30 min compared with the L group (*P*<0.05). In the L group, the TF latency immediately after injection increased significantly for 20 min compared with the baseline (*P*<0.05) and was significantly higher than that in the NS group (*P*<0.05).

course, and the tips were placed caudal to the lumbar vertebrae at the level of L4–L6. A previously reported method involved removal of surrounding muscles and lumbar vertebrae and then insertion of a catheter [19]. Vertebral arch exposure and excision may produce neurologic injury, because the vertebral arch approaches posterior branches of the spinal nerves. In contrast, in our model, insertion through a slit in the atlantooccipital membrane involved a small incision, no excision of bone, and no touching of the spinal cord posterior root. Therefore, the catheter insertion method in our model is less invasive. The fact that this method of dosing already has clinical applications [2, 13] indicates that dosing into the epidural space via an indwelling catheter is effective for evaluating the clinical effect of local anesthetics. Epidural anesthesia blocks the spinal root domain in the epidural space, which includes loose connective tissue, lipids, and the batson venous plexus [10]. The continuance effect time can be extended with addition of a vasopressor, which allows the local anesthetic to be retained longer. Therefore, epidural anesthesia is a model for blocking anesthesia, because it has similar effects on the nerve and surrounding tissue. Furthermore, epidural anesthesia provides an administration site and a precise dose via an indwelling catheter. We considered administration with an indwelling epidural as suitable for evaluating the effect of local anesthetics. Therefore, epidural anesthesia was used to create an animal model of clinically applied local anesthesia.

In conscious animals, TF latency decreases with repetition of stimulation [14]. Learning inevitably occurs from cues and contributes to the TF latency, and this learning effect is controlled by the sedative. However, a normal dose of sedative results in TF latency increasing from baseline within 60 min of administration [28]. In this study, the TF test was performed in sedated animals, and a difference in TF latency did not appear during the measurement time of 60 min. Moreover, TF latency remained constant, and dispersion of the TF latency was more suppressed than the reported TF latency in conscious control animals [9, 24]. The learning effect was controlled by use of a sedative (1% isoflurane), which does not affect TF latency[28]. In contrast, bath-applied isoflurane (1.5%, which is higher than what we used in our model) diminishes dorsal root-evoked polysynaptic, but not monosynaptic, excitatory postsynaptic currents. Isoflurane prolongs the decay phase of evoked and miniature gamma-aminobutyric acid type A receptor-medi-

ated inhibitory postsynaptic currents and increases the amplitude of the muscimol-induced current [30]. This may be a possible mechanism for the antinociceptive effect of isoflurane in the spinal cord. However, 1.5% isoflurane increases TF latency, but 1% isoflurane (same as our model) does not affect TF latency in comparison with no anesthesia [28]. Therefore, 1% isoflurane has no action in the spinal cord. In addition, TF latency was tested for 60 min under 1% isoflurane and showed no change. Therefore, TF latency measurement under a constant concentration of isoflurane is reliable. The thermal stimulus threshold for the activation of C polymodal nociceptors is about 5°C lower than that of Aδ polymodal nociceptors [28, 29]. The transient receptor potential (TRP) contains thermosensitive ion channels, which are expressed in primary sensory neurons. Aδ fibers exhibit properties that may be explained by TRPV2-containing channels (thermal activation threshold >52°C) but do not express TRPV1 (>43°C), whereas C fibers exclusively exhibit TRPV1 [1, 15]. A radiant heat intensity of 161.5 mW/cm^2 results in a constant skin temperature of 44–50°C and stimulates C polymodal nociceptors, whereas 1% inhaled isoflurane excludes the influence of supraspinal structures and provides a reliable TF latency for repeated TF testing [28]. This pain stimulation method for TF testing was applied in the present study.

Other studies, which have typically evaluated the clinical effects of local anesthetic by measuring the escape response for infringement stimulation *in vivo*, suffered from the learning effect as well as imprecise pain stimulation and administration methods. In our model, the radiant heat (method of pain stimulation) intensity (161.5 mW/cm^2) was constant, dispersion of the TF latency was inhibited by suppression of the learning effect, and administration with an epidural catheter allowed a precise injection site and dose. Our model allowed us to detect the effect of local anesthesia onset and regression, as well as the local anesthesia continuation action of an additive agent, all of which are improvements over conventional methods. In conclusion, our model is effective for evaluating the effects of local anesthesia.

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