Video Article

Intracerebroventricular Treatment with Resiniferatoxin and Pain Tests in Mice

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Abstract

The transient receptor potential vanilloid type 1 (TRPV1), a thermosensitive cation channel, is known to trigger pain in the peripheral nerves. In addition to its peripheral function, its involvement in brain functions has also been suggested. Resiniferatoxin (RTX), an ultrapotent TRPV1 agonist, has been known to induce long-term desensitization of TRPV1, and this desensitization has been an alternative approach for investigating the physiological relevance of TRPV1-expressing cells. Here we describe a protocol for intracerebroventricular (i.c.v.) treatment with RTX in mice. Procedures are described for testing nociception to peripheral TRPV1 stimulation (RTX test) and mechanical stimulation (tail pressure test) then follow. Although the nociceptive responses of mice that had been administered RTX i.c.v. were comparable to those of the control groups, RTX-i.c.v.-administered mice were insensitive to the analgesic effect of acetaminophen, suggesting that i.c.v. RTX treatment can induce supraspinal-selective TRPV1 desensitization. This mouse model can be used as a convenient experimental system for studying the role of TRPV1 in brain/supraspinal function. These techniques can also be applied to studies of the central actions of other drugs.

Video Link

The video component of this article can be found at https://www.jove.com/video/57570/

Introduction

Animals receive various physical and chemical stimuli from their environment through sensors on the peripheral nerves. The transient receptor potential vanilloid type 1 (TRPV1) is one of the thermosensitive, nonselective cation channels that act as heat sensors[1,2], and activation and/or modulation of TRPV1 is known to be a key step for nociception in both normal and inflammatory contexts[3]. Although the overall expression pattern is controversial, expression of TRPV1 has also been suggested in supraspinal regions, being involved in various brain activities (including nociception[4], thermoregulation[5], anxiety[6], attention deficit hyperactivity disorder[7], and epilepsy[8]). Moreover, it has recently been suggested that acetaminophen, a widely used painkiller, mediates the activation of central TRPV1 to elicit its analgesic action[9,10].

Administration of excess TRPV1 agonist including capsaicin and resiniferatoxin (RTX) to animals leads to the death of TRPV1-positive neurons and long-lasting desensitization to TRPV1 agonists[11,12]. Combined with the local application (intrathecal[13,14], intracisternal[15,16,17], and intraganglional[18]), this chemical ablation approach has provided an alternative way to investigate the physiological functions of TRPV1. We have recently reported that intracerebroventricular (i.c.v.) injection of RTX inhibits the analgesic effect of acetaminophen in mice, suggesting supraspinal-selective TRPV1 desensitization[19]. In this manuscript, we present the precise protocol for i.c.v. injection and subsequent pain tests.

Direct injection of drugs into the ventricles of the brain makes it possible to study their central effects while minimalizing any peripheral effects. The i.c.v. injection procedure presented here is a modification of the method reported by Haley and McCormick[20]. This method is simple involving insertion of an injection needle into the lateral ventricles through the coronal suture and does not require any special equipment or surgical procedures for cannulation.

Peripheral local application of TRPV1 agonists evokes a burning pain sensation and neurogenic inflammation. Mice that are systemically treated with RTX, and TRPV1-KO mice, are insensitive to this stimulation[13]. We have performed intraplantar injection of RTX (RTX test) to confirm the preservation of peripheral TRPV1 in RTX-i.c.v. mice. This method is a modification of the conventional formalin test[21].

It has been reported that mice systemically treated with RTX and TRPV1-KO mice show a normal threshold to mechanical stimuli[11,13,22]. Here we present a procedure for the tail pressure test for testing changes in the analgesic effect of acetaminophen.

All of these procedures are orthodox and versatile, and can be applied to studies of other drugs.

Protocol

All of the experimental protocols used here were approved by the Animal Care and Use Committee of Musashino University. Male ddY mice (SLC, Shizuoka, Japan) were kept for at least 7 days under a 12-h light/dark cycle before experiments with water and food ad libitum. 5- or 6-week-old mice were used for the experiments.

1. Preparation of Drugs

1. **RTX**
   NOTE: Alcoholic RTX solution can cause severe skin burns and eye damage. Make sure to use rubber gloves and glasses for protection when handling. This stock solution can be used for 6 months.
   1. Add 500 µL of ethanol to 1 mg of RTX.
   2. Add 500 µL of polyoxyethylene (20) sorbitan monooleate to the solution above and vortex well.
   3. Add 4 mL of physiological saline to the mixture and vortex well.
   4. Aliquot 40 µL of the solution into 1.5-mL screw cap tubes, and store them at -40 °C.

2. **Acetaminophen**
   1. Add 20% w/v propyleneglycol solution to acetaminophen at a concentration of 30 mg/mL, and dissolve with a sonicator. Since acetaminophen may precipitate at room temperature several hours after dissolution, prepare just before use or keep the solution warm until use.

2. Subcutaneous or Intracerebroventricular Injection of RTX

1. Thaw the stocked solution prepared in 1.1. above and dilute it to 20 µg/mL in saline or artificial cerebrospinal fluid (ACSF) consisting of (in mM): 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 11 glucose, 1.3 $MgSO_4$, 2.5 $CaCl_2$ equilibrated with 95% O_2 and 5% CO_2 (pH 7.2).
2. Anesthetize mice with pentobarbital sodium salt (60 mg/kg, intraperitoneally), and check for loss of the righting reflex.
3. For s.c. treatment, inject RTX (20 µg/mL) into the back of the neck at a volume of 0.1 mL/10 g body weight. For the control group, inject the vehicle (10% ethanol, 10% polyoxyethylene (20) sorbitan monooleate and 80% saline) in the same way.
4. For i.c.v. treatment, inject 5 µL of RTX (20 µg/mL) into the right lateral ventricle. For the control group, inject the vehicle (10% ethanol, 10% polyoxyethylene (20) sorbitan monooleate and 80% ACSF) in the same way.
   1. Pass a disposable 27-G needle through a metal tube (0.8 mm I.D.) to expose the 3.0-3.5 mm tip of the needle (**Figure 1A**).
   2. Disinfect the head of mouse with 70% alcohol, and hold the squamosal bones of the mouse firmly with the fingers (**Figure 1B**).
      NOTE: Pay attention to the positions of the squamosal protrusions, since these protrusions will serve as landmarks for injection.
   3. Move the needle laterally on the scalp, and find the sagittal suture as the needle tip is hooked on the suture.
   4. Move the tip about 1 mm to the right, then move the tip rostrally, and find the coronal suture as with 2.4.3. (**Figure 1B**).
   5. Insert the needle slowly and vertically, inject the RTX solution in about 10 seconds, and hold it for about 10 seconds.
   6. Withdraw the needle slowly, and return the mouse to its home cage. Bleeding is usually minimal or absent. If major bleeding occurs, use of another mouse should be considered.

5. Assign the pretreated mice as subjects for the RTX test or the tail pressure test (Step 3 and 4, respectively).

3. RTX Test

NOTE: Testing is performed between 10:00 AM and 5:00 PM. The testing room is maintained at 200 lux and 24-26 °C.

1. One week after pretreatments with RTX (Step 2.), transfer mice to the testing room at least 60 min prior to starting the test.
2. Weigh and place each mouse individually in a plexiglass cage (29.5 × 17.5 × 13.5 cm^3 height) at least 30 min prior to starting the test in order to allow it to acclimate to the environment.
   NOTE: The order of tests should be counterbalanced across pretreatment groups.
3. Administer acetaminophen (300 mg/kg) to the mouse intraperitoneally 20 min before the test.
4. Hold the mouse loosely in a small cloth bag, and insert a 30-gauge needle into the heel of the right hind paw. Advance the needle subcutaneously to near the walking pads, and inject 20 µL of RTX solution (0.05 µg/mL).
5. Measure the period of licking/biting behavior in theglabrous region of the affected paw in each 5-min block.

4. Tail Pressure Test

NOTE: A Randall-Selitto-type pressure meter is used to assess the threshold for acute mechanical nociception. Testing is performed between 10:00 AM and 5:00 PM. The testing room is maintained at 200 lux and 24-26 °C.

1. One week after pretreatments with RTX (Step 2.), transfer mice to the testing room, and weigh and place each mouse individually in a plexiglass cage.
2. Mark the spots at 1.5 and 2.5 cm from the base of the tail.
3. Hold the mouse loosely in a small cloth bag, and apply pressure to the spots with a blunt probe.
   NOTE: A cutoff pressure of 250 g is imposed to avoid tissue damage.

4. Determine the pressure required to elicit escape behavior (tail whisking, twisting, and squeaking), and calculate the nociceptive threshold by averaging the pressure determined at the two spots.
5. Repeat steps 4.3. to 4.4. every 15 min.
6. After obtaining the baseline, administer acetaminophen (300 mg/kg) to the mouse intraperitoneally. After administration, repeat steps 4.3. and 4.4 every 15 min.

Representative Results

The i.c.v.-treated mice show no apparent abnormalities in their appearance, spontaneous activities, body weight[19] and core body temperature (Vehicle-treated group, 38.4 ± 0.3 °C, n = 6; RTX-treated group, 38.7 ± 0.2 °C, n = 6).

Figure 2A-B show the responsiveness of s.c.- or i.c.v.-treated mice to the intraplantar injection of RTX. The licking/biting behavior of vehicle-treated mice was remarkable in the first 10 min[19]. Although the s.c.-pretreated mice did not show licking/biting behavior at all, the i.c.v.-pretreated mice normally responded to the plantar injection of RTX. Moreover, as shown in **Figure 2B**, intraperitoneal administration of acetaminophen (300 mg/kg) reduced the licking/biting behavior of vehicle-i.c.v.-treated mice but not that of RTX-i.c.v.-treated mice.

Figure 2C shows the analgesic effects of acetaminophen (300 mg/kg) in the tail pressure test. Acetaminophen reduced the nociceptive response of vehicle-pretreated mice in both tests, but the analgesic effects of acetaminophen were inhibited in mice that were pretreated i.c.v. with RTX.

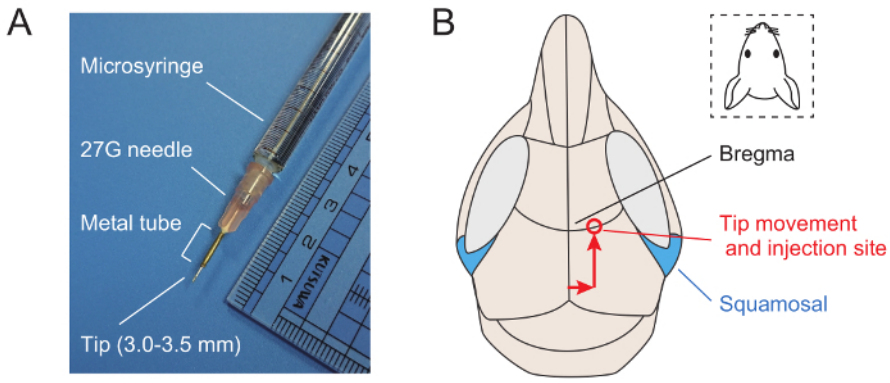

Figure 1: Photographic and schematic views of i.c.v. injection. (A) Needle used for i.c.v. injection. **(B)** Schema of the mouse skull and the movement of the needle tip. Squamosal bones are shown in blue. Please click here to view a larger version of this figure.

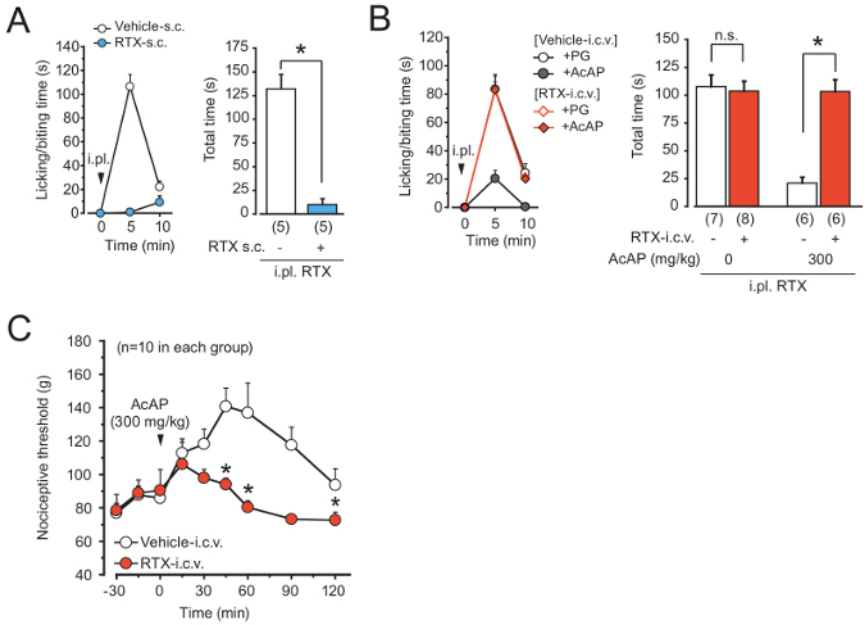

Figure 2: Nociceptive responses of mice that were pretreated s.c. or i.c.v. with RTX. (A) Time course (left panel) and total time of licking/biting behavior (right panel) of s.c.-pretreated mice. RTX was injected into the plantar area at time zero (indicated by arrow head). **(B)** Time course (left panel) and total time of licking/biting behavior (right panel) of i.c.v.-pretreated mice. Either acetaminophen (300 mg/kg) or its vehicle (20% propyleneglycol) was administered intraperitoneally 20 min before intraplantar injection of RTX (indicated by arrow head). **(C)** Mechanical pain threshold in the tail of i.c.v.-pretreated mice and the analgesic effect of acetaminophen.All data were expressed as mean ± SEM. The number of mice in each group is shown in parentheses. The two-tailed Mann-Whitney U-test was used to compare the data for two groups. Differences at $P<0.05$ were considered to be significant. AcAP, acetaminophen; PG, propyleneglycol; n.s., not significant; i.pl., intraplantar injection. These figures have been modified from Fukushima et al[19]. Please click here to view a larger version of this figure.

Discussion

The most critical step in these experiments is the success of the i.c.v. injection. The i.c.v. injection technique used here is quite simple but requires some practice. Prior to experiments, practice with dyes (e.g. 0.5% trypan blue in saline) is recommended. If the injection is performed correctly, a needle mark should be evident on the coronal suture and the injected dye should be present in the contralateral ventricle and the third ventricle. Moreover, forcible insertion should be avoided during injection. If the needle tip is correctly placed on the coronal suture, the needle should penetrate the skull smoothly.

This i.c.v. technique can also be applied to awake, non-anesthetized mice, and we have reported the acute central effects of drugs examined using this technique[23,24]. Although the present procedure is advantageous in that no special equipment for cannulation is necessary, the i.c.v. injection can be performed only once. If repeated administration of drugs is required, cannulation is necessary.

The RTX test presented here is an easy-to-use approach for assessing the function of peripheral TRPV1[3,19]. Nociceptive behavior can be observed most prominently at a dose of 1-10 ng RTX and inhibited by co-injection of capsazepine, a TRPV1 antagonist[19,25]. In the formalin test some groups video-tape the experiments, but the post-hoc observation is often difficult because mice tend to cover the affected paw with the head and body. Therefore, experimenters in our laboratory observe and measure the licking/biting behavior directly. In this scenario, care should be taken not to disturb the mice. In addition, in pain tests, it is very important to sufficiently calm the mouse. Excessively strong gripping and a noisy environment could produce stress-induced analgesia and delay the nociceptive response.

Mice that are i.c.v.-pretreated with RTX show a normal nociceptive response in the RTX test and the tail pressure test. However, these mice are insensitive to the analgesic effects of acetaminophen, which has been suggested to mediate central TRPV1[9,10]. These results suggest that supraspinal-selective TRPV1 desensitization can be induced in RTX-i.c.v. mice. Although TRPV1 desensitization has been performed with local application of agonists[13,14,15,16,17,18], supraspinal-selective desensitization has not yet been achieved. The RTX-i.c.v. injection protocols presented here will provide a convenient experimental model for studying the role of TRPV1 in supraspinal function.

Disclosures

The authors have no conflicts of interest to declare

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