

Epinephrine Prevents Muscle Blood Flow Increases after Perineural Injection of Tetrodotoxin

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Background: The local anesthetic properties of tetrodotoxin, a potent naturally occurring sodium channel blocker, have been recently reexamined. It was found that sciatic nerve block duration could be greatly increased and systemic toxicity greatly decreased if epinephrine was injected with tetrodotoxin. The mechanism that underlies epinephrine-mediated prolongation of tetrodotoxin nerve blocks is not known, but indirect evidence suggests that epinephrine may slow the clearance of tetrodotoxin from the site of injection. The authors hypothesized that tetrodotoxin causes vasodilatation at its injection site, which accelerates its systemic uptake, and that this vasodilatation is attenuated by coinjected epinephrine.

Methods: The radiolabeled microsphere technique was used to measure tissue blood flow in anesthetized rats before and after perisciotic injection of tetrodotoxin alone and in combination with epinephrine.

Results: Tetrodotoxin, in a dose of 0.1 ml of a 60 μ M solution, significantly increased blood flow in perisciotic muscle at the injected side compared with simultaneous contralateral control and ipsilateral preinjection baseline. Tetrodotoxin did not increase blood flow in the sciatic nerve. Coinjection of epinephrine with tetrodotoxin prevented tetrodotoxin-induced increases in perisciotic muscle blood flow over time and did not alter sciatic nerve blood flow. Arterial blood pressure was maintained with this dose of tetrodotoxin, although brain blood flow decreased. Coinjection of epinephrine with tetrodotoxin prevented decreases in brain blood flow. Higher doses of tetrodotoxin produced hypotension.

Conclusions: Vasoconstriction in the perisciotic muscles by epinephrine may contribute to the prolongation of tetrodotoxin-induced sciatic nerve blocks and the reduction of systemic toxicity of tetrodotoxin.

TETRODOTOXIN, a highly specific sodium channel blocker, can produce nerve blocks with a relative lack of local neurotoxicity.¹ In addition, tetrodotoxin does not cause seizures, myocardial depression, or arrhythmias,² which can be associated with the use of the standard amino-amide and amino-ester local anesthetic agents. Despite these promising local anesthetic properties, tetrodotoxin has not found its way into clinical use because of its systemic toxicity, which results in hypoten-

sion as well as muscular weakness that can lead to diaphragmatic paresis and respiratory failure. However, recent studies in a rat sciatic nerve block model indicate that the addition of epinephrine to tetrodotoxin prolongs nerve block duration by more than 13-fold compared with blocks performed with tetrodotoxin alone. In contrast, epinephrine rarely prolongs nerve block by conventional local anesthetics by more than twofold. Coinjected epinephrine also markedly reduced the systemic toxicity of tetrodotoxin (fourfold increased median lethal dose) and improved its therapeutic index.^{3,4}

Local vasoconstriction is the presumed mechanism responsible for prolongation of local anesthetic block by epinephrine,⁵⁻⁸ although direct measurements of regional blood flow do not consistently demonstrate significant reductions in local blood flow by epinephrine.^{9,10} Although systemically administered tetrodotoxin causes hypotension, which is proposed to result from an alteration in vascular tone,^{2,11-13} the effects of perineural injection of tetrodotoxin, alone or combined with epinephrine, on regional blood flows have not been investigated. In the current study, we employed the radiolabeled microsphere technique to test the hypothesis that tetrodotoxin causes vasodilatation in the sciatic nerve or in adjacent skeletal muscle around the injection site and that coinjection of epinephrine attenuates tetrodotoxin-induced vasodilatation in these tissues. We also examined some systemic hemodynamic consequences of tetrodotoxin injection.

Materials and Methods

Experimental Preparation

The Boston Children's Hospital Animal Care and Use Committee approved all surgeries and procedures. Adult male Sprague-Dawley rats (400-500 g; Charles River Laboratories, Wilmington, MA) were prepared for *in vivo* measurement of blood flow with modifications of a technique described previously.⁹ Briefly, rats were anesthetized with isoflurane (2.5% in 100% oxygen) and instrumented with two vascular catheters. One of these catheters (internal/external diameter 0.56/1.0 mm) was placed in the left ventricle *via* the right carotid artery (microsphere infusion catheter) and systolic and diastolic pressures were monitored (Model 76; Hewlett-Packard, Palo Alto, CA). The other catheter (internal/external diameter 0.58/0.965 mm) was placed in the right axillary artery (reference withdrawal catheter) and arterial pressure was monitored. Tracheas were cannulated and rats were mechanically ventilated (Rodent ventilator SAR-830/P, Ardmore, PA).

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Blood gas analysis was performed during each experiment after steady-state conditions were achieved before nerve block injections, and end-tidal carbon dioxide was repeatedly measured for ventilation adjustment. Ventilation was adjusted to keep end-tidal carbon dioxide in a range between 25–30 mmHg. This was based on pilot experiments conducted over a broader range of ventilator settings indicating that this would result in arterial carbon dioxide partial pressures of roughly 33–42 mmHg, thereby minimizing effects of hypocapnia or hypercapnia on brain blood flow. In these pilot experiments, there was a good correlation (Pearson correlation coefficient, $r = 0.841$) between end-tidal carbon dioxide partial pressure and arterial carbon dioxide partial pressures (data not shown).

Throughout the experiment, rectal temperature was maintained in the range of 36.5°–37.5°C.

For the regional blood flow experiments with perisciatic injection of tetrodotoxin 60 μM , 0.1 ml, with or without epinephrine, inspired isoflurane concentration was maintained at 2.5% in oxygen. Inspired concentrations were delivered using a Fluotec MK III (Engler Engineering Corp., Hialeah, FL) and calibrated using Datex (Datex-Ohmeda, Inc., Madison, WI) agent analyzers.

Experiments examining systemic hemodynamic effects of tetrodotoxin at higher doses (120 μM , 0.1 ml, with or without epinephrine) were performed under two conditions: first with constant inspired isoflurane concentration at 2.5% and second with variable inspired isoflurane concentrations titrated to maintain arterial blood pressure in a physiologic range after perisciatic tetrodotoxin injection. Removed blood samples were replaced by heparinized (4 U/ml) normal saline in a 3:1 ratio used to flush the arterial lines.

Perisciatic Nerve Injection Paradigm

Perisciatic injections were made on the left side with a 25-gauge 1-inch needle attached to a 1-ml syringe that was inserted percutaneously from the posterior aspect of the upper leg and advanced anterolaterally toward the greater trochanter. On bony contact, the needle was withdrawn 1 mm and then 0.1 ml of solution was injected.

The effects of tetrodotoxin with or without epinephrine on local and systemic blood flow was investigated by perisciatic nerve injection of 0.1 ml in three groups of animals ($n = 6$ for each group) receiving either normal saline, tetrodotoxin 60 μM , or tetrodotoxin 60 μM with epinephrine 55 μM , respectively.

As noted in the previous section, additional groups of animals ($n = 6$ for each group) received perisciatic injection of a higher dose of tetrodotoxin, namely 0.1 ml of a 120 μM solution with or without epinephrine 55 μM , to investigate effects of these doses on mean arterial blood pressure, brain blood flow, and rectus abdominus muscle blood flow. Fluorescein dye (Isothiocyanate-dex-

tran, MW = 2,000,000; Sigma-Aldrich, St. Louis, MO) 0.5 mg in 0.1 ml solution) was added to all nerve block injectates to allow identification of perisciatic injection sites upon dissection. A previous study has shown no effect on local blood flow with this dose of fluorescein.⁹

Blood Flow Determination

The radiolabeled microsphere technique^{14,15} was employed in the current study to measure regional blood flow. The technique employs sequential injections into the left ventricle of suspensions of glass microspheres (15 \pm 0.1 μm in diameter) labeled with one of five isotopes: ¹⁴⁶CE, ⁵¹Cr, ¹⁰³Ru, ⁹⁵Nb, and ⁴⁶Sc (Perkin Elmer Life Sciences, Boston, MA). As each isotope has a distinct gamma emission spectrum, blood flow can be examined at five different time points. Solutions of one of the different isotopes (10 μCi , 0.2 ml) were administered through the left ventricular catheter at baseline (5 min before perisciatic injection) and then 5, 15, 30, and 60 min after sciatic block injections. Beginning 10 s before the microsphere injection and continuing for 75 s, a reference blood sample (\sim 1 ml) was withdrawn from the arterial catheter at a rate of 0.6 ml/min using a Harvard Apparatus pump (Harvard Apparatus, Inc., Holliston, MA). At the completion of the experiment, the animals were sacrificed with injection of pentobarbital 100 mg/kg through the intracardiac catheter. Then, the fluorescein-labeled segments of muscle adjacent to the sciatic nerve (including the piriformis, deep portions of the gluteus, and origins of the biceps femoris or adductor muscles), and sciatic nerve, as well as brain and rectus abdominus muscle, were harvested. Rectus abdominus served as a second distant control site to assess systemic effects on muscle blood flow. The reference blood samples and harvested tissues and organs were weighed and placed in 5-ml vials for analysis of radioisotope activity in an automatic γ -counting system (Cobra, Model D5003; Packard Instrument Co., Downers Grove, IL).

Tissue blood flows were calculated with the following equation:

$$\text{Tissue blood flow at time points (ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}) = \text{tissue counts per min/blood counts per min} \times \text{blood withdrawal flow rate/wet tissue weight}$$

Statistical Analysis of Data

Regional blood flow values were analyzed using analyses of variance for repeated measures. *Post hoc* comparisons were performed using Bonferroni/Dunn tests in two ways:

1. At each tissue site (sciatic nerve, perisciatic muscle, brain, rectus abdominus), regional blood flows at repeated time points after nerve injection were compared with the prenerve-injection baseline regional blood flows in the same tissue site, and

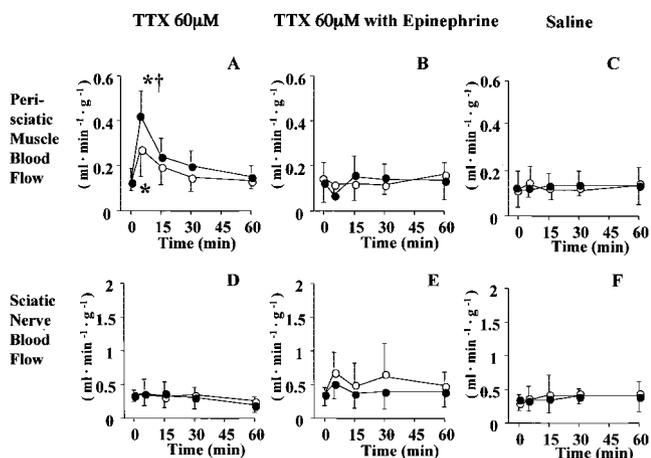


Fig. 1. Effects of left perisciatic injection of 0.1 ml of: tetrodotoxin (TTX) 60 μM (A), tetrodotoxin 60 μM plus epinephrine 55 μM (1:100,000) (B), or saline (C) on blood flow in perisciatic muscle and tetrodotoxin 60 μM (D), tetrodotoxin 60 μM plus epinephrine 55 μM (1:100,000) (E), or saline (F) in sciatic nerve. The plots show blood flows in left (drug-injection, ●) and right (contralateral control, ○) tissues. Compared with baseline, tetrodotoxin significantly increased perisciatic muscle blood flow bilaterally (A), but the increases in flow were significantly greater on the ipsilateral (left) side compared with contralateral side at 5 min postinjection. Coinjection of epinephrine with tetrodotoxin prevented tetrodotoxin-induced increase in bilateral perisciatic muscle blood flow (B). Saline injection produced no changes in perisciatic muscle blood flow (C). Neither tetrodotoxin alone (D), tetrodotoxin coinjected with epinephrine (E), nor saline control (F) altered sciatic nerve blood flow significantly at any time point. $n = 6$ animals for each condition. Data are expressed as mean \pm SD. * $P < 0.05$ comparing same-site measurements at that time point *versus* measurements at time 0 (preinjection). † $P < 0.05$ comparing between-site (ipsilateral *versus* contralateral control) at the same time point.

2. At each time point, regional blood flows for sciatic nerve and perisciatic muscle were compared between the injected (left) side and the corresponding tissue site in the control (right) side.

Measurements of arterial blood pressure were compared with preinjection baseline values in the same tissue using analysis of variance for repeated measures, followed by Bonferroni/Dunn tests. Data points and error bars are expressed as mean \pm SD. The significance level was set at $P < 0.05$.

Results

Effect of Perisciatic Injection of Tetrodotoxin, 60 μM , 0.1 ml, With and Without Epinephrine, 55 μM (1:100,000), on Blood Flow in Perisciatic Muscle and in Sciatic Nerves

Compared with preinjection baseline, perisciatic muscle blood flow (all values expressed as $\text{ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$, mean \pm SD) on the injected (left) side increased 3.7-fold at 5 min after sciatic nerve injection of tetrodotoxin 60 μM , 0.1 ml (fig. 1 A; 0.11 ± 0.035 preinjection, 0.42 ± 0.12 at 5 min postinjection, $P < 0.05$). Contralateral

(right) perisciatic muscle blood flow increased by 2.3-fold relative to preinjection baseline at 5 min postinjection (fig. 1 A; 0.12 ± 0.033 preinjection, 0.28 ± 0.12 at 5 min postinjection, $P < 0.05$). At 5 min postinjection, perisciatic muscle blood flow was significantly greater on the injected side compared with the contralateral side (fig. 1 A; 0.42 ± 0.12 on the injected side, 0.28 ± 0.12 on the contralateral side, $P < 0.05$).

Coinjection of epinephrine 55 μM (1:100,000) with tetrodotoxin 60 μM , 0.1 ml prevented tetrodotoxin induced increases in ipsilateral and contralateral perisciatic muscle blood flow (fig. 1 B). At 5 min postinjection, mean ipsilateral perisciatic muscle flow in the tetrodotoxin-plus epinephrine group (0.07 ± 0.038) was sixfold lower than that seen at 5 min postinjection in the tetrodotoxin-alone group (0.42 ± 0.12 ; $P < 0.05$). Contralateral perisciatic muscle flow at 5 min postinjection in the tetrodotoxin-plus epinephrine group (0.12 ± 0.06) was 2.3-fold lower than that seen at 5 min postinjection in the tetrodotoxin-alone group (0.28 ± 0.12 ; $P < 0.05$).

Control animals receiving perisciatic injection of saline 0.9%, 0.1 ml, showed no changes in ipsilateral or contralateral perisciatic muscle blood flow (fig. 1 C).

In contrast to findings in perisciatic muscle, tetrodotoxin did not significantly alter ipsilateral or contralateral sciatic nerve blood flow (fig. 1 D; ipsilateral 0.34 ± 0.076 preinjection, 0.37 ± 0.21 at 5 min postinjection; contralateral 0.34 ± 0.095 preinjection and 0.35 ± 0.16 at 5 min postinjection, $P > 0.05$ for each comparison).

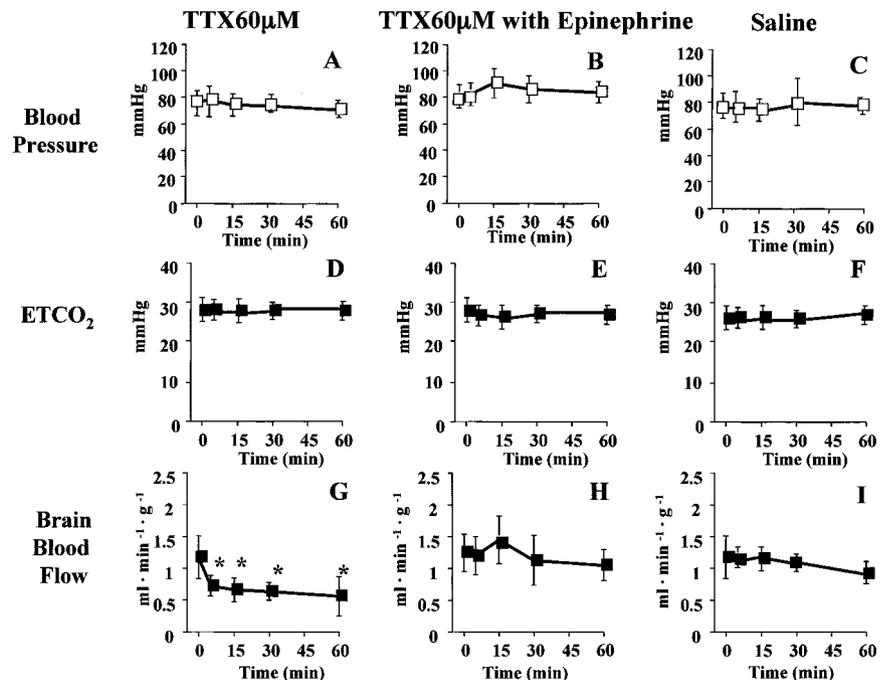
Coinjection of epinephrine with tetrodotoxin did not significantly alter sciatic nerve blood flow (fig. 1 E; ipsilateral 0.35 ± 0.16 preinjection and 0.52 ± 0.23 at 5 min postinjection; contralateral 0.37 ± 0.16 preinjection and 0.70 ± 0.34 at 5 min postinjection; $P > 0.05$ for each comparison).

Rats receiving control perisciatic injections of 0.9% saline, 0.1 ml, showed no changes in ipsilateral or contralateral regional blood flow in sciatic nerves (fig. 1 F).

Systemic Hemodynamic Effects of Tetrodotoxin, 60 μM , 0.1 ml, With and Without Epinephrine

Perisciatic injection of tetrodotoxin 60 μM , 0.1 ml, with or without epinephrine 55 μM (1:100,000) did not alter mean arterial blood pressure in animals maintained at a constant inspired isoflurane concentration of 2.5% (fig. 2 A and 2 B). For the tetrodotoxin alone condition, group blood pressures (mean \pm SD) at times 0, 5, 15, 30 and 60 min were 77 ± 12 , 78 ± 14 , 74 ± 8 , 73 ± 5 , and 71 ± 6 mmHg, respectively (fig. 2 A). For the tetrodotoxin with epinephrine condition, group mean arterial blood pressures at these times were 81 ± 11 , 82 ± 10 , 91 ± 13 , 86 ± 13 , and 84 ± 8 mmHg, respectively (fig. 2 B). These blood pressures were not significantly different from those of control animals receiving perisciatic injections of saline 0.9%, 0.1 ml (fig. 2 C). Heart rates remained between 240–340 beats/min (data not shown).

Fig. 2. Systemic effects of perisciatic injection of 0.1 ml of either tetrodotoxin (TTX) 60 μM , tetrodotoxin 60 μM plus epinephrine 55 μM (1:100,000), or saline. Data are shown from the same groups of animals described in Fig. 1. Mean arterial blood pressure measurements from groups receiving injections of tetrodotoxin 60 μM (A), tetrodotoxin 60 μM plus epinephrine 55 μM (1:100,000) (B), or saline (C) are shown. End-tidal carbon dioxide (ETCO₂) measurements from groups receiving injections of tetrodotoxin 60 μM (D), tetrodotoxin 60 μM plus epinephrine 55 μM (1:100,000) (E), or saline (F) are shown. There were no statistically significant changes in blood pressure or end-tidal carbon dioxide over time within each treatment group, nor were there between-group differences in blood pressure or end-tidal carbon dioxide at each time point. Tetrodotoxin 60 μM produced significant reductions in brain blood flow at all time points postinjection (G). The tetrodotoxin 60 μM plus epinephrine 55 μM (H) and saline (I) groups showed no significant changes in brain blood flow over time. * $P < 0.05$ comparing same-site measurements at that time point versus measurements at time 0 (preinjection).



End-tidal carbon dioxide partial pressure was maintained constant throughout the experiments (fig. 2 D, E, and F), and did not differ significantly at any time point between groups receiving tetrodotoxin (fig. 2 D), tetrodotoxin + epinephrine (fig. 2 E), or saline (fig. 2 F).

Rats receiving tetrodotoxin 60 μM , 0.1 ml showed significant decreases in brain blood flow (fig. 2 G). Coinjection of epinephrine 55 μM (1:100,000) with tetrodotoxin 60 μM , 0.1 ml prevented tetrodotoxin-induced decreases in brain blood flow (fig. 2 H). Controls receiving saline injections showed no significant changes in brain blood flow (fig. 2 I).

Rats receiving tetrodotoxin 60 μM , 0.1 ml showed significant increases in rectus abdominus muscle blood flow, with values increasing from $0.047 \pm 0.019 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ preinjection to a maximum of $0.094 \pm 0.034 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ at 15 min postinjection. Rats receiving tetrodotoxin 60 μM , 0.1 ml coinjected with epinephrine 55 μM (1:100,000) and rats receiving saline perisciatic injections showed no significant increases in rectus abdominus blood flow at any time point.

Systemic Hemodynamic Effects of Tetrodotoxin, 120 μM , 0.1 ml, With and Without Epinephrine 55 μM (1:100,000)

Perisciatic injection of tetrodotoxin 120 μM , 0.1 ml produced more marked systemic hemodynamic effects. In animals maintained at a constant inspired isoflurane concentration of 2.5%, perisciatic injection of tetrodotoxin 120 μM , 0.1 ml, mean arterial blood pressure decreased significantly ($P < 0.05$) from $82 \pm 11 \text{ mmHg}$ preinjection to $43 \pm 2 \text{ mmHg}$ at 5 min and blood pres-

ures remained low, with mean values ranging from 43–47 mmHg throughout the 60 min measurement period.

In additional groups of animals receiving perisciatic injection of tetrodotoxin 120 μM , 0.1 ml, the inspired isoflurane concentration was reduced to 1.7–2.2% after sciatic injection in an attempt to maintain blood pressure within the physiologic range. With blood pressures maintained unchanged from preinjection baseline, these animals also showed marked reduction in brain blood flows and significant increase in rectus abdominus muscle blood flows. Coinjection of epinephrine 55 μM with tetrodotoxin 120 μM , 0.1 ml, significantly delayed the onset of tetrodotoxin-induced decreases in brain blood flow and increases in rectus abdominus blood flow over the first 15–30 min, respectively, but did not prevent these changes from occurring at later time points.

Discussion

Local Effects of Tetrodotoxin With and Without Epinephrine

The results of the current study indicate that perisciatic nerve injection of tetrodotoxin does not increase blood flow in the sciatic nerve but significantly increases local blood flow in the perisciatic muscles at the injection site. Increases in local blood flow were more prolonged and of greater magnitude in ipsilateral, compared with contralateral, perisciatic muscles.

It may seem at first glance counterintuitive that epinephrine can prolong the duration of tetrodotoxin block from 1 h to more than 13 h³ without producing signifi-

cant changes in sciatic nerve blood flow. Epinephrine is rapidly metabolized *in vivo*. It would be improbable that it could continue to produce local vasoconstriction in either nerve or muscle for more than 10 h after an intramuscular injection; previous studies suggest that epinephrine vasoconstricts tissues for less than 1 h.^{6,7,9,10} Based on these considerations, on compartment models analogous to those described by Sinnott *et al.*,¹⁶ and on the observed effect of epinephrine on regional blood flow in surrounding muscle but not in nerve, we interpret these observations as follows. Epinephrine, by its vasoconstriction in the surrounding muscle, prolongs tetrodotoxin blockade predominantly by slowing initial systemic uptake of tetrodotoxin, thereby maintaining a concentration gradient from the injection site into nerve, facilitating initial entry of tetrodotoxin into nerve in the first 20–30 min after injection (fig. 3A). This action seems more plausible than an action involving delayed removal of tetrodotoxin from nerve over the subsequent 2–12 h (fig. 3B).

Systemic Effects of Tetrodotoxin, With and Without Epinephrine

Previous studies reported that epinephrine increased the median lethal dose for tetrodotoxin in rats by roughly fourfold in spontaneously ventilating rats. Systemic toxicity of tetrodotoxin is presumed, based on studies in larger animals, to reflect a combination of respiratory depression, associated with paralysis of the muscles of respiration, and hypotension, attributable predominantly to vasodilatation. Our experiments were all performed in anesthetized rats receiving controlled ventilation. It is plausible that tetrodotoxin exerts systemic toxicity through a combination of hemodynamic and respiratory effects in spontaneously ventilating animals; the current study paradigm does not provide information regarding respiratory consequences of systemic tetrodotoxin uptake.

Blood pressure remained stable after injection of tetrodotoxin 60 μM 0.1 ml, (~ 4.5 $\mu\text{g}/\text{kg}$; fig. 2), with or without epinephrine. At this dose of tetrodotoxin, coinjection of epinephrine prevented reductions in brain blood flow.

Perisciatic nerve injection of 0.1 ml, 120 μM tetrodotoxin is equivalent to a systemic tetrodotoxin dose of ~ 9 $\mu\text{g}/\text{kg}$, which is very near the median lethal dose of 13 $\mu\text{g}/\text{kg}$ for tetrodotoxin in spontaneously ventilating rats.^{3,4} In rats maintained at a constant inspired isoflurane concentration of 2.5%, this dose of tetrodotoxin produced marked hypotension. In rats receiving this dose of tetrodotoxin, when the isoflurane concentration was reduced, blood pressure could be maintained but brain blood flow was decreased. Epinephrine briefly delayed, but did not prevent, these reductions in brain blood flow.

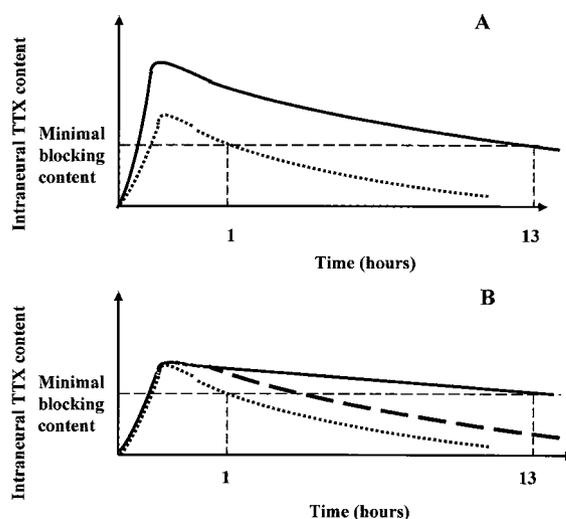


Fig. 3. Heuristic diagram of possible pharmacokinetic mechanisms for the prolongation of tetrodotoxin (TTX) block by epinephrine. (A) Vasoconstriction of perisciatic muscle. The solid curve depicts idealized intraneural content after blocks using tetrodotoxin plus epinephrine, whereas the dotted curve depicts idealized intraneural content after blocks using tetrodotoxin alone. Epinephrine, by its vasoconstriction in the surrounding perisciatic muscle, would be predicted to slow initial systemic uptake of tetrodotoxin from injection sites, thereby maintaining a concentration gradient between surrounding tissues and the nerve, facilitating initial entry of tetrodotoxin into nerve in the first 20–40 min after injection, and producing a higher early peak intraneural tetrodotoxin content. This mechanism is consistent with the observed effect of epinephrine on surrounding muscle blood flow, as shown in Fig. 1, and can explain marked prolongation of nerve blockade by an action of epinephrine confined to the first hour after injection. (B) Decreased sciatic nerve blood flow. The dotted curve depicts idealized intraneural content after blocks using tetrodotoxin alone. Epinephrine-induced decreases in sciatic nerve blood flow would not be expected, based on compartment models, to significantly alter early peak intraneural tetrodotoxin content, but rather to delay clearance of tetrodotoxin from the nerve. The dashed line depicts idealized intraneural tetrodotoxin content after a block with tetrodotoxin plus epinephrine, under the assumption that epinephrine reduces sciatic nerve blood flow for up to 1 h (contrary to experiment), with little or no effect on surrounding muscle blood flow (contrary to experiment). The short duration of the actions of epinephrine in this case cannot account for the 13-fold prolongation of tetrodotoxin blockade by epinephrine. The solid line depicts idealized intraneural content after a block with tetrodotoxin plus epinephrine under the assumption (contrary to experiment) that epinephrine could reduce sciatic blood flow for up to 10 h with little or no effect on surrounding muscle blood flow. This mechanism is implausible based on the known short duration of the actions of epinephrine in tissues and on the observed lack of effect of epinephrine on sciatic blood flow, as shown in Fig. 1.

In previous studies using isoflurane-anesthetized rats¹⁷ or dogs,¹⁸ brain blood flow remained relatively constant over a mean arterial blood pressure range of 60–100 mmHg. Previous studies have shown that systemic administration of tetrodotoxin decreases blood pressure, which may result from a direct relaxant effect on vascular smooth muscle^{11,19} or an indirect effect mediated through blockade of sympathetic efferents and vasomotor reflex pathways.^{11,20} Voltage gated sodium channels

are found in vascular smooth muscle cells,²¹⁻²⁵ which suggests that the activation of sodium channels may be involved in contraction²³ and regulation of Ca²⁺ homeostasis²⁵ in vascular smooth muscle.

It remains unclear whether the observed reductions in brain blood flow in our studies reflect direct actions of tetrodotoxin on cerebral metabolism or cerebrovascular regulation or whether they reflect relative shunting of cardiac output from the brain to the periphery, as the result of either direct effects of tetrodotoxin on peripheral vascular smooth muscle or tetrodotoxin-induced blockade of sympathetic efferents²⁰ (as brain blood flow is much less dependent on sympathetic regulation than most peripheral tissues). The latter interpretation is more consistent with the view that tetrodotoxin crosses the blood-brain barrier very poorly.

The significance of brain blood flow relative to systemic toxicity of tetrodotoxin is of potential clinical interest. One of the major systemic toxicities of tetrodotoxin is profound hypotension, along with muscle weakness and respiratory failure. Decreased blood flow to brain and to brainstem respiratory centers with hypotension could impair respiratory drive and respiratory reflexes. Those central impairments may exacerbate weakness as a result of peripheral actions of tetrodotoxin on phrenic and intercostal nerve conduction, neuromuscular junctions, and directly on skeletal muscle in the diaphragm and intercostal muscles.

The predominant action of epinephrine appears to be delayed systemic distribution of tetrodotoxin by a local vasoconstrictive mechanism, although it is plausible that some combination of local and systemic actions of epinephrine may contribute to the positive effects of coinjected epinephrine on tetrodotoxin-induced systemic toxicity. To a small extent, the cerebrovascular effects of epinephrine may be from isoflurane-induced changes in blood-brain barrier permeability.²⁶ In a recent study on cerebrovascular effects, epinephrine was shown to increase mean arterial pressure and cerebral blood flow more in isoflurane-anesthetized sheep than in awake sheep.²⁶ In the presence of an equivalent effect on mean arterial pressure, the exaggerated cerebrovascular effects of epinephrine under anesthesia appear to be centrally mediated isoflurane-dependent changes in blood-brain barrier permeability, thereby causing a direct influence on the cerebral vasculature.²⁶

Conclusions

Further interpretation of the pharmacokinetic and pharmacodynamic effects of epinephrine in prolonging tetrodotoxin blockade requires measurement of tetrodotoxin tissue uptake and distribution analogous to the studies of lidocaine and other local anesthetics by Strichartz *et al.*¹⁶ Our efforts to carry out these experiments

have thus far been confounded by radiochemical impurities or exchange of radiolabel in commercial preparations of radiolabeled tetrodotoxin. Methods are under investigation to circumvent this technical problem.

It may be that our ability to measure small changes in sciatic nerve blood flow was impeded by its small size, as we estimate that only approximately 100 microspheres were contained in each sciatic nerve sample, substantially less than the 400 or more microspheres that were imbedded in all other tissue samples harvested. We believe that we have improved on both the precision and accuracy of this model compared with a previous study,⁹ and the reproducibility and comparatively small standard deviations in sciatic blood flow measurements in the current study support this assertion. Nevertheless, because of the limitations of small numbers of microspheres in each sample, our results cannot exclude the possibility that we may have missed relatively small changes in sciatic nerve blood flow after injection of tetrodotoxin with or without epinephrine and that these small changes in blood flow might influence nerve block durations.

In conclusion, we observed that epinephrine attenuates tetrodotoxin-induced increases in muscle blood flow at the injection site, which may partly contribute to the prolongation of tetrodotoxin-induced nerve blocks and to the attenuation of tetrodotoxin-induced systemic toxicity. Further pharmacokinetic/pharmacodynamic studies may clarify the mechanisms of prolongation of tetrodotoxin block by epinephrine. Combinations of tetrodotoxin (or other site 1 toxins) with epinephrine may have a future clinical utility in providing prolonged nerve blockade with a reduced risk of local neurotoxicity¹ compared with some alternative approaches.

References

1. Sakura S, Bollen AW, Ciriales R, Drasner K: Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels. *Anesth Analg* 1995; 81:338-46
2. Kao CY: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmacol Rev* 1966; 18:997-1049
3. Kohane DS, Yieh J, Lu NT, Langer R, Strichartz GR, Berde CB: A re-examination of tetrodotoxin for prolonged duration local anesthesia. *ANESTHESIOLOGY* 1998; 89:119-31
4. Kohane DS, Lu NT, Cairns BE, Berde CB: Effects of adrenergic agonists and antagonists on tetrodotoxin-induced nerve block. *Reg Anesth Pain Med* 2001; 26:239-45
5. Bernards CM, Kopacz DJ: Effect of epinephrine on lidocaine clearance in vivo: A microdialysis study in humans. *ANESTHESIOLOGY* 1999; 91:962-8
6. Selander D, Mansson LG, Karlsson L, Svanvik J: Adrenergic vasoconstriction in peripheral nerves of the rabbit. *ANESTHESIOLOGY* 1985; 62:6-10
7. Myers RR, Heckman HM: Effects of local anesthesia on nerve blood flow: Studies using lidocaine with and without epinephrine. *ANESTHESIOLOGY* 1989; 71:757-62
8. Fink BR, Aasheim GM, Levy BA: Neural pharmacokinetics of epinephrine. *ANESTHESIOLOGY* 1978; 48:263-6
9. Palmer GM, Cairns BE, Berkes SL, Dunning PS, Taylor GA, Berde CB: The effects of lidocaine and adrenergic agonists on rat sciatic nerve and skeletal muscle blood flow in vivo. *Anesth Analg* 2002; 95:1080-6
10. Partridge BL: The effects of local anesthetics and epinephrine on rat sciatic nerve blood flow. *ANESTHESIOLOGY* 1991; 75:243-50
11. Kao CY, Nagasawa J, Spiegelstein MY, Cha YN: Vasodilatory effects of tetrodotoxin in the cat. *J Pharmacol Exp Ther* 1971; 178:110-21

12. Kao CY: Pharmacology of tetrodotoxin and saxitoxin. *Federation Proc* 1972; 31:1117-23
13. Li MK: Action of puffer fish poison. *Nature* 1963; 23:791
14. Prinzen FW, Bassingthwaite JB: Blood flow distributions by microsphere deposition methods. *Cardiovasc Res* 2000; 45:13-21
15. Tabrizchi R, Pugsley MK: Methods of blood flow measurement in the arterial circulatory system. *J Pharmacol Toxicol Methods* 2000; 44:375-84
16. Sinnott CJ, Cogswell IL, Johnson A, Strichartz GR: On the mechanism by which epinephrine potentiates lidocaine's peripheral nerve block. *ANESTHESIOLOGY* 2003; 98:181-8
17. Hoffman WE, Edelman G, Kochs E, Werner C, Segil L, Albrecht RF: Cerebral autoregulation in awake versus isoflurane-anesthetized rats. *Anesth Analg* 1991; 73:753-7
18. Gelman S, Fowler KC, Smith LR: Regional blood flow during isoflurane and halothane anesthesia. *Anesth Analg* 1984; 63:557-65
19. Lipsius MR, Siegman MJ, Kao CY: Direct relaxant actions of procaine and tetrodotoxin on vascular smooth muscle. *J Pharmacol Exp Ther* 1968; 164:60-74
20. Feinstein MB, Paimre M: Mechanism of cardiovascular action of tetrodotoxin in the cat: Block of conduction in peripheral sympathetic fibers. *Circ Res* 1968; 23:553-65
21. Sturek M, Hermsmeyer K: Calcium and sodium channels in spontaneously contracting vascular muscle cells. *Science* 1986; 233:475-8
22. Okabe K, Kitamura K, Kuriyama H: The existence of a highly tetrodotoxin sensitive Na channel in freshly dispersed smooth muscle cells of the rabbit main pulmonary artery. *Pflugers Arch* 1988; 411:423-8
23. Shinjoh M, Nakaki T, Otsuka Y, Sasakawa N, Kato R: Vascular smooth muscle contraction induced by Na⁺ channel activators, veratridine and batrachotoxin. *Eur J Pharmacol* 1991; 205:199-202
24. Cox RH, Zhou Z, Tulenko TN: Voltage-gated sodium channels in human aortic smooth muscle cells. *J Vasc Res* 1998; 35:310-7
25. Boccara G, Choby C, Frapier JM, Quignard JF, Nargeot J, Dayanithi G, Richard S: Regulation of Ca²⁺ homeostasis by atypical Na⁺ currents in cultured human coronary myocytes. *Circ Res* 1999; 85:606-13
26. Myburgh JA, Upton RN, Grant C, Martinez A: The cerebrovascular effects of adrenaline, noradrenaline and dopamine infusions under propofol and isoflurane anaesthesia in sheep. *Anaesth Intensive Care* 2002; 30:725-33