Anesthesiology 2003; 98:521–9

Activation of Peripheral Excitatory Amino Acid Receptors Decreases the Duration of Local Anesthesia

Brian E. Cairns, Ph.D., Giulio Gambarota, Ph.D.,† Patricia S. Dunning, B.Sc., R.T.(R.),‡ Robert V. Mulkern, Ph.D.,§ Charles B. Berde, M.D., Ph.D.‖*

Background: **Postsurgical wound infiltration with the *N*-methyl-D-aspartate receptor antagonist ketamine and bupivacaine can significantly prolong the duration of local anesthesia. One possible mechanism for this effect is that increased glutamate concentrations, caused by tissue damage, sensitize nociceptive primary afferent fibers through activation of peripheral excitatory amino acid receptors.**

Methods: **The effect of intramuscular injection of hypertonic glutamate (1,000 mM), dextrose (1,400 mM), glutamate (1,000 mM) with the broad spectrum excitatory amino acid receptor antagonist kynurenate (100 mM), or isotonic saline (155 mM) on the duration of masseter muscle afferent fiber blockade after lidocaine (37 mM [1%], 10 μl) infiltration, on muscle edema formation and on muscle blood flow was examined.**

Results: **Injection of either glutamate or dextrose significantly shortened the duration of lidocaine blocks compared with isotonic saline; however, block duration was significantly shorter after glutamate than after dextrose. Injection of glutamate, but not isotonic saline, dextrose, or glutamate with kynurenate, significantly decreased the mechanical threshold of muscle afferent fibers. Injection of glutamate, dextrose, or glutamate with kynurenate produced equivalent large, long-lasting (> 60 min) edemas with high initial peak extracellular water content. Peak extracellular water decreased more rapidly when kynurenate was coinjected with glutamate. Both glutamate and dextrose significantly increased muscle blood flow for 30 min after injection. Glutamate-induced increases in blood flow were attenuated by kynurenate.**

Conclusions: **These results suggest that shortened lidocaine block durations observed after glutamate injection into the masseter muscle result from sensitization of afferent fibers as well as increases of peak extracellular water content and blood flow in masseter muscle. These effects of glutamate are mediated in part through activation of peripheral excitatory amino acid receptors.**

INFLAMMATION of craniofacial tissues often interferes with the success of local anesthesia, with reported failure rates during inflammation as high as 75%.[1–3] It is possible that sensitization of afferent fibers[4,5] as well as pharmacokinetic factors, which include tissue edema and increased blood flow, play significant roles in inflammation-related anesthetic failures. For example, it has been found that treatment of feline canine tooth pulps with histamine, a compound that is released in inflamed tissues and can sensitize afferent fibers, can dramatically shorten the duration of lidocaine blockade.[2]

Recent studies indicate potential peripheral actions of glutamate, which is found in increased concentrations in tissues after acute and chronic inflammation in both animals and humans.[6–9] Injection of glutamate into craniofacial tissues both excites and sensitizes afferent fibers through a process that involves the activation of peripheral excitatory amino acid (EAA) receptors.[10–13] Further, it has been reported that, compared with bupivacaine alone, wound infiltration with bupivacaine plus the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine can greatly increase the duration of local anesthetic block.[14] These findings suggest that elevated tissue concentrations of glutamate could activate peripheral EAA receptors to decrease the duration of local anesthetic blocks.

In the current study, to investigate the hypothesis that elevated tissue glutamate concentrations can shorten the duration of local anesthesia, the duration of lidocaine-induced blockade of masseter muscle afferent fibers was measured subsequent to injection of glutamate into the rat masseter muscle. In addition, a novel magnetic resonance imaging methodology was used to investigate the effect of glutamate injection on masseter muscle tissue extracellular water content.[15] Finally, the effect of glutamate injection on masseter muscle blood flow was determined by radiolabeled microsphere blood flow measurements.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Materials and Methods

Surgeries and procedures reported in the following sections were approved by the Children's Hospital Animal Care and Use Committee (Boston, MA).

Afferent Fiber Recordings

Adult Sprague-Dawley rats (N = 34; weight, 225–400 g) of both sexes were prepared for acute *in vivo* recording of trigeminal primary afferent fiber activity during surgical anesthesia (oxygen: 0.3–0.4 l/min; isoflurane: 2.5–3%).[10,11,13] A tracheal cannula was inserted, and rats were artificially ventilated with a rodent ventilator (mod-

* Instructor, Department of Anesthesia, § Associate Professor, Department of Radiology, ‖ Professor, Departments of Anesthesia and Pediatrics, Harvard Medical School. † Postdoctoral Fellow, Department of Radiology, University Medical Center Nijmegen, Nijmegen, The Netherlands. ‡ Laboratory Manager, Kresge Laboratory, Department of Radiology, Children's Hospital.

Received from the Departments of Anesthesia and Radiology, Harvard Medical School/Children's Hospital, Boston, Massachusetts. Submitted for publication November 15, 2001. Accepted for publication October 7, 2002. Support was provided solely from institutional and/or departmental sources.

Address reprint requests to Dr. Cairns: Children's Hospital/Harvard Medical School, 320 Longwood Avenue, Room 1180, Boston, Massachusetts 02115. Address electronic mail to: brian.cairns@TCH.harvard.edu.

el 683; Harvard Apparatus, Natick, MA). The femoral vein was catheterized to allow administration of intravenous fluids. The rat's head was then placed in a Kopf® stereotaxic frame (David Kopf Instruments, Tujunga, CA), and the skin over the dorsal surface of the skull was reflected. A trephination was made on the left side of the skull to allow a microelectrode to be lowered through the brain and into the trigeminal ganglion. In addition, an incision was made in the skin overlying the neck to expose the brainstem and upper cervical spinal cord. A C1 laminectomy was performed, and the dura overlying the brainstem–cervical spinal cord was removed to facilitate placement of a stimulating electrode in contact with caudal brainstem. On completion of all surgical procedures, the concentration of isoflurane was reduced to maintain a continued absence of reflex response to noxious toe pinch (2.5–2.8%). Heart rate, expired carbon dioxide, and core body temperature were continuously monitored throughout the experiment and kept within the physiologic range of 300–350 beats/min, 20–30 mmHg, and 36.8–37.2°C, respectively. Experiments were initiated 30–60 min after the completion of surgery.

Stimulation and Recording Techniques

Single trigeminal afferent units were recorded with parylene-coated tungsten microelectrodes (impedance 2–5 MΩ) that were slowly lowered into the trigeminal ganglion. Mechanical search stimuli were then applied *via* a blunt probe over the masseter muscle to identify trigeminal afferent fibers with muscle receptive fields. Electrical stimuli were applied to the caudal brainstem (20–100 μA, 50 μs, 0.5 Hz) or to the muscle tissue to evoke action potentials in the recorded fiber and permit the determination of antidromic–orthodromic latency. The distance between the stimulating and recording electrodes was divided by the antidromic–orthodromic latency to calculate conduction velocity (CV) for the fibers.[10,11,13]

Determination of Changes in Afferent Fiber Mechanical Thresholds

An IITC Electronic Von Frey Anesthesiometer (model 1601C or 2290; blunt polypropylene tip, diameter 0.5 mm; Woodland Hills, CA) was used to determine afferent fiber mechanical thresholds. The output of the transducer was routed through a power 1401 AD board (Cambridge Electronic Design, Cambridge, United Kingdom) for online recording of the applied stimulus and mechanical threshold, which were later analyzed offline. The baseline mechanical threshold was determined by averaging the threshold for 10 consecutive mechanical stimuli applied at 1-min intervals. After determining the baseline mechanical threshold, the needle tip of a catheter (a 27-gauge needle connected by polyethylene tubing to a Hamilton syringe, 50 μl) was carefully inserted into the masseter muscle. This catheter was used to inject either glutamate (1,000 mM, 10 μl), glutamate together with the broad-spectrum (NMDA and non-NMDA receptors) EAA receptor antagonist kynurenate (1,000 mM glutamate and 100 mM kynurenate, 10 μl), isotonic saline (155 mM, 10 μl), or dextrose (1,400 mM, 10 μl). Coinjection of kynurenate with glutamate was performed to ensure a 1:10 antagonist/agonist ratio within the injection site. All solutions were adjusted to approximate physiologic pH (7.2–7.6). All chemicals were acquired from Sigma Chemical Company (St. Louis, MO).

Thirty minutes after injection of glutamate, glutamate plus kynurenate, dextrose, or isotonic saline, mechanical stimuli were again applied with the Von Frey hair at 1-min intervals for 10 min. Then the catheter was reinserted into the muscle at the same location, and lidocaine (37 mM [1%], 10 μl; AstraZeneca, Westborough, MA) was injected. After the lidocaine injection, mechanical stimulation was resumed at 1-min intervals until the mechanical threshold of the afferent returned to baseline values. To avoid any potential confound of repeated lidocaine injection into the masseter muscle, only one afferent was examined per rat.[16]

At the end of each experiment, rats were killed with an overdose of pentobarbital (100 mg/kg administered intravenously; Nembutal, Abbott Laboratories, Chicago, IL). The brain was removed, and it was confirmed that microelectrode tracts were visible on the surface of the trigeminal ganglion. The masseter nerve was dissected free from the muscle, and a silk suture was used to estimate orthodromic conduction distance to the nearest millimeter.

Data Analysis

The activity of muscle fibers was amplified (gain: 1,000×; bandwidth: 30–1,000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2; Cambridge Electronic Design). Recorded activity was stored electronically and analyzed offline. The mean (± SD) afferent fiber mechanical threshold was calculated from data collected before injection of the various compounds into the masseter muscle and again before injection of lidocaine. Relative threshold values were calculated by dividing the measured threshold by the mean afferent fiber threshold determined before injection of the various compounds into the masseter muscle. The duration of lidocaine fiber block was the difference between the time following lidocaine injection when the threshold first increased above 2 SD of the mean prelido baseline value (*i.e.*, onset of block) and the time when it first decreased again to less than 2 SD of the mean prelido baseline value (*i.e.*, end of block; fig. 1).

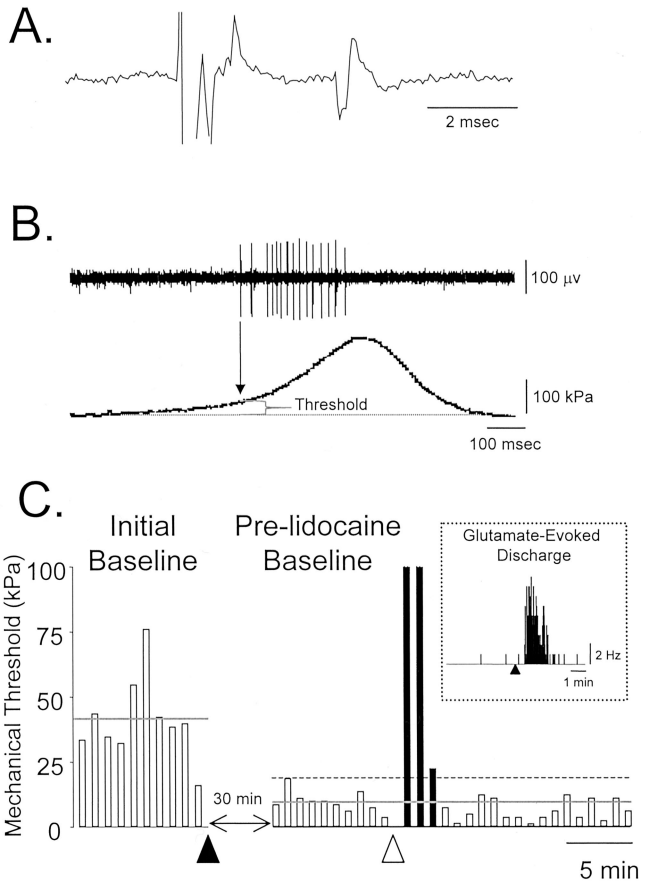

Fig. 1. The methodology used for assessment of lidocaine block duration is illustrated. (*A*) Stimulation of the caudal brainstem evoked an antidromic action potential in this masseter muscle afferent fiber. (*B*) An electronic Von Frey hair was used to determine the mechanical threshold of the afferent as indicated. (*C*) Injection of 1,000 mM glutamate (black triangle) into the afferent fiber mechanoreceptive field substantially decreased the mechanical threshold of this afferent fiber. Injection of 37 mM lidocaine into the afferent fiber mechanoreceptive field 40 min later (white triangle) significantly elevated the mechanical threshold of this afferent fiber (solid black bars) for 3 min. (*Inset*) Afferent fiber discharge evoked by injection of glutamate into the masseter muscle. Solid gray line = mean threshold; dotted line = + 2 SD.

Magnetic Resonance Imaging

To determine how solutions of glutamate, glutamate plus kynurenate, dextrose, and isotonic saline were distributed in and affected the extent of edema formation in masseter muscle tissue, T_2-weighted magnetic resonance imaging was performed on eight adult rats (weight, 250–350 g) of either sex during isoflurane (1.8–2.4%) anesthesia. In four rats, isotonic saline (10 μl) was injected into the right masseter muscle, and glutamate (10 μl) was injected into the left masseter muscle. In the other four rats, glutamate–kynurenate (10 μl) was injected into the right masseter muscle, and dextrose (10 μl) was injected into the left masseter muscle.

Magnetic resonance imaging was performed on a Bruker BioSpec (Bruker BioSpin, Rheinstetten, Germany) operating at 2 Tesla. Scans were started approximately 10 min after injection and repeated at 15-min intervals over 60 min. A volume coil was used as a transmitter to ensure homogeneous excitation, and a homemade surface coil (3 × 4 cm) was placed over the masseter muscle as a receiver. The image acquisition protocol consisted of a series of coronal multislice T_2-weighted images (2,000 ms repetition time, 40 ms echo time) of the entire muscle to obtain an estimate of the volume of edema. The in-plane spatial resolution was 0.4 × 0.4 mm, and the slice thickness was 2 mm, which provided precise measurement of the extent of edema volume. A Carr-Purcell-Meiboom-Gill imaging sequence (32 echoes, 10-ms echo spacing, slice-selective sinc pulses) was also run to measure water proton transverse relaxation times (T_2) and allow an estimate of the percentage of extracellular water.[15] The images were stored and analyzed on a personal computer station with Matlab (Mathworks, Natick, MA). On completion of magnetic resonance imaging, the anesthetic was discontinued, and the rats were permitted to recover.

Calculation of Volume and Extracellular Water Content

The volume of edematous muscle tissue was estimated by the summation of all pixels within the muscle that had signal intensities greater than the 95% confidence interval for pixel intensity within the noninjected muscle tissue. The number of pixels was multiplied by volume per pixel (0.4 × 0.4 × 2 mm = 0.32 mm^3/pixel) to yield an estimate of the edema volume.

It has previously been demonstrated that injection of saline solutions into the masseter muscle results in a biexponential T_2 signal decay.[15] Therefore, the T_2 relaxation was measured with the Carr-Purcell-Meiboom-Gill imaging sequence and the signal decay fitted to the biexponential function, $S = A_i \exp(-nTE/T_{2i}) + A_e \exp(-nTE/T_{2e})$, where n = 1,2. . .32, TE is the echo time, A_i and A_e are the amplitudes of the fast and slowly relaxing components with relaxation times T_{2i} and T_{2e}, respectively, using a Levenberg-Marquardt nonlinear least squares algorithm. The fast and slowly relaxing components are interpreted to reflect intracellular and extracellular water content in the muscle tissue, respectively.[15] By analyzing 5 × 5 pixel regions of interest throughout the edema, we identified the regions of interest with the largest amplitude of the slowly relaxing component $A_e/(A_e+A_i)$, which we report as the peak extracellular water content for that edema.[15]

Muscle Blood Flow Measurement

Adult Sprague-Dawley rats (weight, 400–500 g) were anesthetized with isoflurane (1.8–3.0% in oxygen). A tracheal cannula was inserted, and the animals were artificially ventilated. A catheter was introduced into the right carotid artery and advanced into the left ventricle to deliver radiolabeled microspheres.[17] A second cathe-

ter was inserted into the right femoral artery and used to monitor systemic blood pressure as well as to draw blood during radiolabeled microsphere injections. Temperature, mean arterial blood pressure, heart rate, and end-tidal carbon dioxide pressure were monitored throughout the experiment and maintained in the range of 36.5–37.5°C, 60 mmHg or less, 280–330 beats/min, and 20–30 mmHg, respectively. Each 1 ml of sampled blood was replaced with 2.5–3.0 ml of heparinized (4 units/ml) normal saline.

The radiolabeled microsphere technique was used to investigate the effect of injection of glutamate (1,000 mM, n = 6), dextrose (1,400 mM, n = 6), glutamate with kynurenate (100 mM, n = 5), glutamate with lidocaine (74 mM [2%], n = 6) and dextrose with lidocaine (74 mM [2%], n = 6) into the left masseter muscle on muscle blood flow. Isotonic saline was injected into the right masseter muscle as a control in each experiment. To ensure that a sufficiently large region of muscle tissue was affected by the solutions, a volume of 100 μl was injected into each masseter muscle. Solutions (10 μCi, 0.2 ml) of glass microspheres (15 ± 0.1 μm microspheres) radiolabeled with one of five isotopes (^{146}Ce, ^{51}Cr, ^{103}Ru, ^{95}Nb, ^{46}Sc) suspended in normal saline (Perkin Elmer Life Sciences, Boston, MA) were administered through the ventricular catheter. Solutions of radiolabeled microspheres were administered immediately before injection of drugs into the masseter muscle and then 5, 15, 30, and 60 min after intramuscular injections. During and after the administration of radiolabeled microspheres, blood (~1 ml, 0.6 ml/min) was taken from the femoral catheter with a syringe attached to a mechanical pump.

Calculation of Blood Flow

At the end of the experiment, animals were killed with pentobarbital (100 mg/kg Euthasol; Diamond Animal Health, Des Moines, IA), and the masseter muscles, brain, and kidneys were removed. The reference blood samples and harvested tissues were weighed and placed in 5-ml vials for analysis of radioisotope content in an auto γ counting system (Cobra II, Model D5003; Packard Instrument Company, Downers Grove, IL). The following equation was used to calculate masseter muscle blood flow:

$$\text{Muscle Blood Flow (ml/min/g)} = \frac{\text{muscle cpm}}{\text{blood cpm}} \times \frac{\text{blood removal rate (ml/min)}}{\text{wet tissue weight (g)}}$$

Statistical Analysis

A Kruskal-Wallis one-way analysis of variance on ranks and *post hoc* Dunn test were used to assess the effect of injected substances on the median relative mechanical threshold. A one-way analysis of variance and *post hoc* Student-Newman-Keuls test were used to assess the effect of injected substances on the duration of lidocaine blocks. A one-way repeated-measures analysis of variance and *post hoc* Dunnett test were used to assess temporal changes on muscle blood flow, percent extracellular water content, and edema volume. In all tests, the level of significance was set at $P < 0.05$. Mean values are reported with their SD.

Results

Lidocaine Block Duration

An example of the experimental paradigm used to assess lidocaine-induced block duration is shown in figure 1. This particular masseter muscle afferent fiber had a CV of 3.9 m/s and projected to the caudal brainstem (fig. 1A). The initial mean baseline mechanical threshold of this afferent fiber was 41.3 ± 15.7 kPa but was reduced to 9.9 ± 4.1 kPa 30 min after glutamate injection (figs. 1B and 1C). A subsequent injection of lidocaine into the afferent fiber receptive field in the masseter muscle elevated the mechanical threshold of this fiber to a level twice the SD of the mean baseline for 3 min.

Overall, injection of glutamate (n = 9 fibers; mean CV, 10.3 ± 6.7 m/s) was found to significantly decrease the mechanical threshold and shorten the duration of lidocaine block relative to isotonic saline (n = 8 fibers; mean CV, 7.8 ± 4.3 m/s; figs. 2A and 2B). Glutamate-induced sensitization and glutamate-related shortening of the duration of lidocaine block were significantly attenuated when the broad-spectrum EAA receptor antagonist kynurenate (n = 8 fibers; mean CV, 12.0 ± 7.2 m/s) was coinjected with glutamate. Hypertonic dextrose (n = 9 fibers; mean CV, 7.0 ± 3.6 m/s), which has approximately the same osmotic strength as 1,000 mM glutamate, had no effect on the mechanical threshold but did significantly shorten the duration of lidocaine block compared with isotonic saline. The duration of lidocaine block after glutamate was, however, significantly shorter than after hypertonic dextrose (fig. 2B).

Edema Volumes and Extracellular Water Content

Figure 3A shows a coronal spin-echo T_2-weighted image of the head region of a supine rat. Edemas created by the injection of drug solutions into the masseter muscle were easily identified as regions of increased signal intensity on T_2-weighted images. The T_2 relaxation decay of masseter muscle was monoexponential, whereas the T_2 relaxation within edemas exhibited a biexponential decay (fig. 3B).

Injection of glutamate, dextrose, and glutamate with kynurenate resulted in significantly greater volumes of edema than isotonic saline (fig. 4A). This result is consistent with previous findings that injection of hyper-

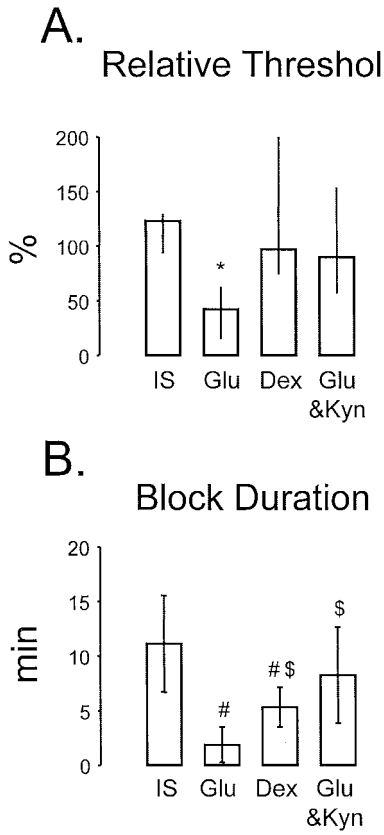

Fig. 2. (*A*) The bar graph illustrates the median relative threshold of masseter muscle afferent fibers. Lines indicate the interquartile range. The median relative threshold was significantly lowered 30 min after injection of glutamate (Glu, n = 9), but not after dextrose (Dex, n = 9) or glutamate and kynurenate (Glu & Kyn, n = 8), when compared with isotonic saline (IS, n = 8; *$P < 0.05$, Dunn test). (*B*) The bar graph indicates the mean duration of lidocaine-induced blockade of these afferent fibers. Error bars indicate SD. Preinjection of glutamate or dextrose significantly shortened lidocaine block duration compared with isotonic saline (#$P < 0.05$ compared with isotonic saline, Student-Newman-Keuls test). Preinjection of glutamate resulted in a significantly shorter duration of lidocaine block than preinjection of dextrose ($$P < 0.05$ compared with glutamate, Student-Newman-Keuls test). Glutamate-related shortening of lidocaine block duration was significantly attenuated by coinjection of kynurenate ($$P < 0.05$ compared with glutamate, Student-Newman-Keuls test).

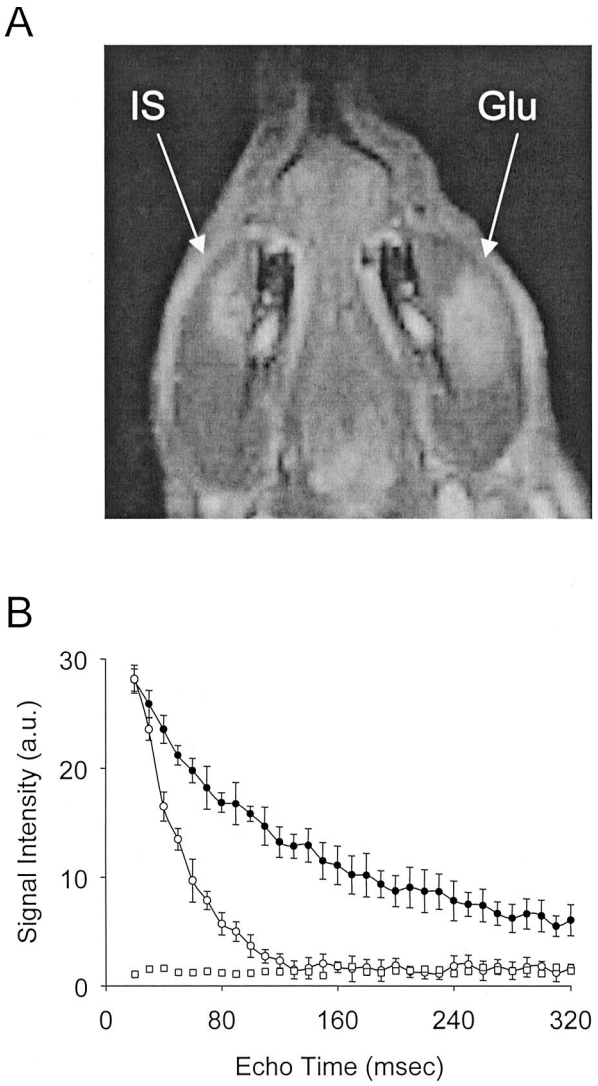

Fig. 3. (*A*) Magnetic resonance imaging shows the edemas that resulted from injection of 10 μl of isotonic saline (IS) and 10 μl of 1,000 mM glutamate (Glu) into the rat masseter muscle. Note the substantially larger edema in the muscle injected with glutamate. (*B*) The graph illustrates typical T_2 relaxation decay curves in muscle (open circle) and edema (closed circle) caused by the injection of 1,000 mM glutamate. Open squares indicate the baseline noise. The T_2 decay for muscle was monoexponential, while the T_2 decay for the glutamate edema was biexponential.

tonic solutions into the masseter muscle produces greater edema volume than isotonic solutions.[10,15] There was no significant change in the volume of edema of any of the injected solutions over the 60-min time period examined.

Changes in extracellular water content over time give an indication of the clearance of water out of the site of injection. The initial peak extracellular water content after injection of dextrose, glutamate, or glutamate with kynurenate was greater than isotonic saline. The peak extracellular water content in dextrose edemas did not change over 60 min (fig. 4B). In contrast, there were nonsignificant and significant decreases in the peak extracellular water content in glutamate and glutamate–kynurenate edemas, respectively, over the same time period.

Muscle Blood Flow

Injection of glutamate or dextrose into the masseter muscle significantly increased blood flow for a period of 30 min after injection (fig. 5). In comparison, isotonic saline injections had no effect on masseter muscle blood flow. Coinjection of glutamate with either kynurenate or 74 mM lidocaine attenuated the increase in blood flow observed when glutamate was injected alone. Coinjection of 74 mM lidocaine with dextrose also decreased dextrose-induced increased blood flow.

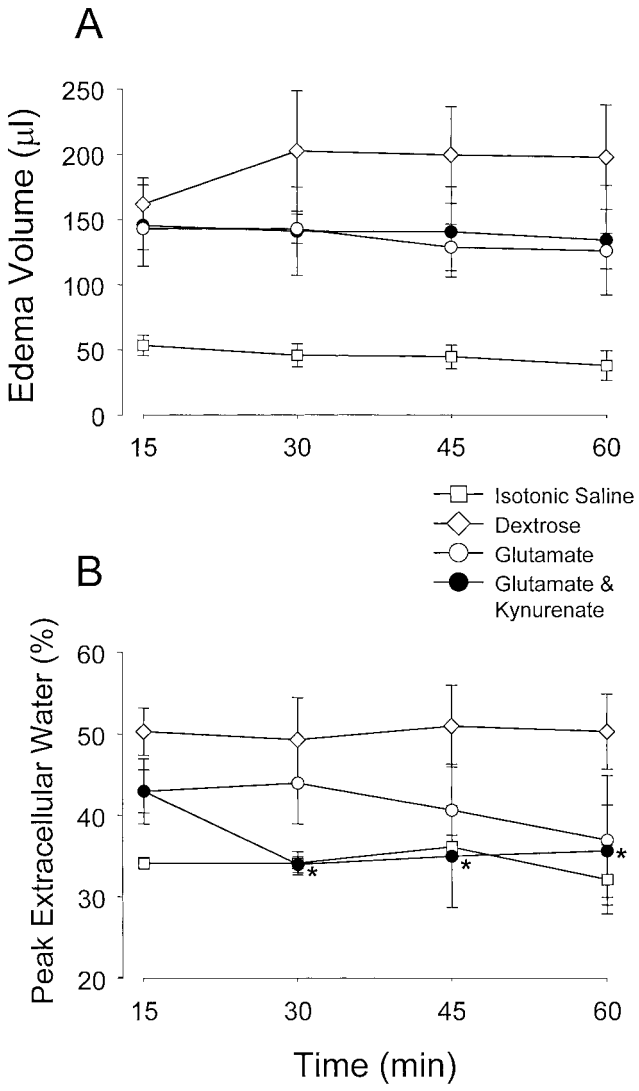

Fig. 4. The line and scatter plots show the effect of substance injection on percent volume of edema and peak extracellular water content. (*A*) There was no significant change in the volume of edema produced by injection of the various solutions into the masseter muscle over 60 min. (*B*) Coinjection of kynurenate with glutamate increased the clearance of extracellular water out of the edema 30 min after injection. Bars = SD range; **P* < 0.05, repeated-measures analysis of variance, Dunnett method.

Discussion

The findings of the current study demonstrate that increased tissue concentrations of glutamate could sensitize masseter muscle afferent fibers and shorten the duration of local anesthesia. Both glutamate-induced afferent fiber sensitization and the related decrease in the duration of local anesthesia were significantly attenuated by coinjection of the broad-spectrum EAA receptor antagonist kynurenate. Thus, a mechanism that involves peripheral sensitization through activation of peripheral EAA receptors appears to play a role in the decreased duration of local anesthesia after glutamate injection into the masseter muscle. However, injection of high concentrations of glutamate also significantly increased the peak extracellular water content in the muscle tissue and resulted in a prolonged increase in muscle blood flow. Both glutamate-related increases in extracellular water content and muscle blood flow were attenuated by kynurenate. Therefore, a pharmacokinetic mechanism that acts to redistribute the injected lidocaine away from the afferent fiber through a combination of dilution and increased muscle blood flow also appears to contribute to the shortening of local anesthetic duration by injection of glutamate into the masseter muscle.

The mechanism responsible for glutamate-induced afferent fiber sensitization is not clear. Previous *in vitro* studies have indicated that glutamate-evoked depolarization of trigeminal ganglion neurons through activation of EAA receptors lasts only a few seconds,[18,19] although considerably more prolonged trigeminal afferent activity has been observed when glutamate is injected into deep craniofacial tissues *in vivo*.[10,11,13] It is possible that even a relatively brief activation of peripheral EAA receptors, in particular the NMDA receptor subtype, significantly increases intraaxonal calcium concentrations. This effect could alter the mechanical transduction properties of masseter muscle afferents. Other compounds that are associated with afferent mechanical sensitization, such as prostaglandin E_2, serotonin, and adenosine, can increase tetrodotoxin-resistant sodium channel currents in small-diameter dorsal root ganglion neurons.[20] This effect is thought to occur through activation of a second messenger–mediated process and may in itself contribute to afferent fiber sensitization.[20] There is a fourfold to fivefold difference in the median effective concentration for lidocaine block of tetrodotoxin-sensitive and -resistant channels *in vitro*.[21,22] Thus, if alteration of tetrodotoxin-resistant sodium channels contributes to the sensitization of masseter muscle afferent fibers by glutamate, then this might explain, in part, why preinjection of glutamate shortened the duration of lidocaine afferent fiber blockade.

Glutamate injection into the masseter muscle may also result in the release of excitatory neuropeptides, such as substance P, which is known to depolarize trigeminal and dorsal root ganglion neurons[23,24] and sensitize articular (knee joint) afferents to mechanical stimuli.[25] Therefore, it is conceivable that glutamate-induced mechanical sensitization of masseter muscle afferent fibers may, in part, result from the actions of released excitatory neuropeptides such as substance P.

The large volumes of edema and increased extracellular water content in the muscle tissue that occurred after injection of glutamate most probably result from the osmotic strength of the injected solutions or a local tissue reaction. We have recently demonstrated that there is a significant relation between osmotic strength and extracellular water content in masseter muscle tissue after injection of hypertonic sodium chloride solu-

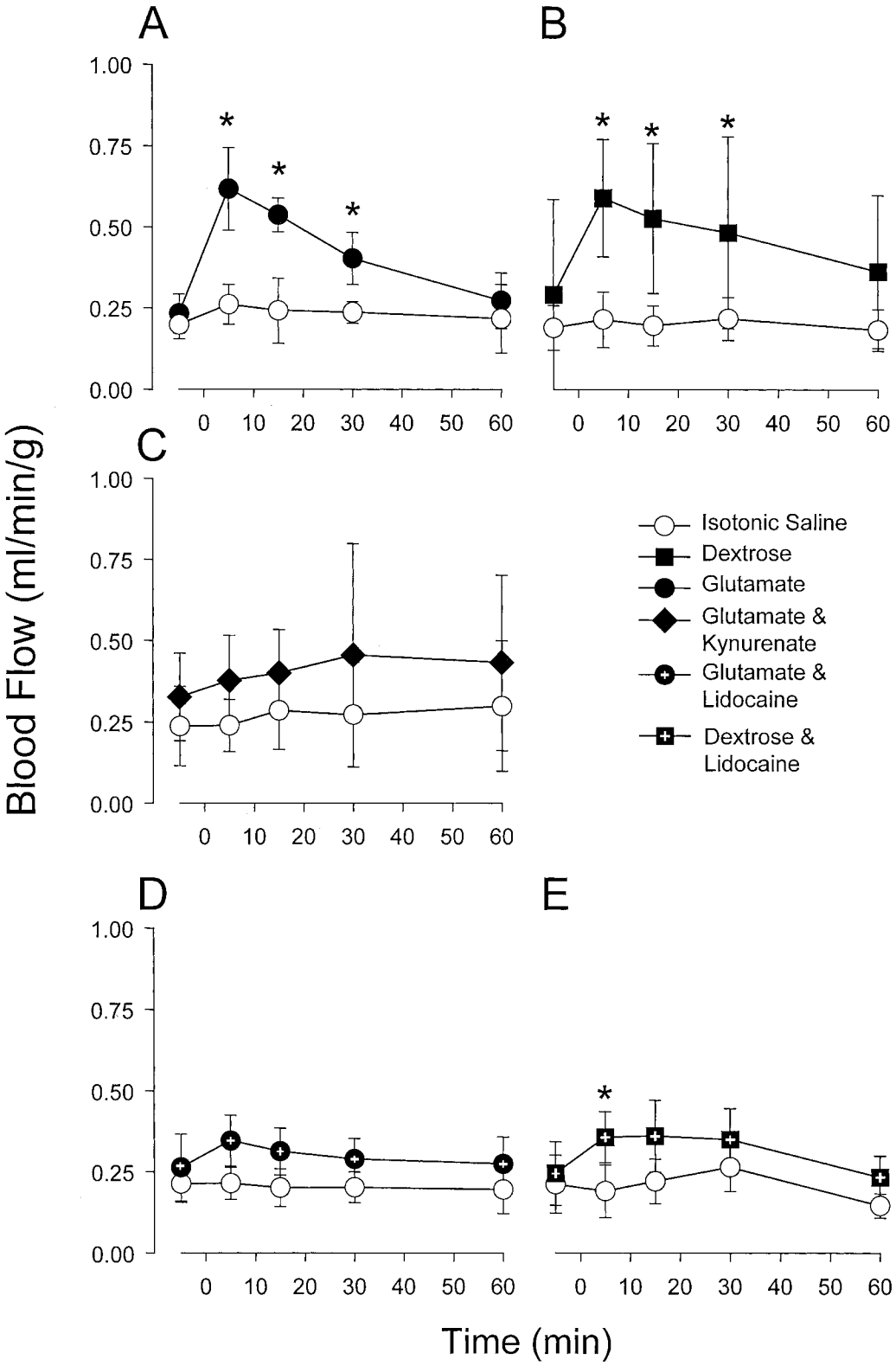

Fig. 5. The line and scatter plots show the effect of substance injection on masseter muscle blood flow. Baseline blood flow was determined approximately 5 min before injection of substances at time 0. Isotonic saline (squares), which was injected into the right masseter muscle, did not significantly affect muscle blood flow in any of the experiments. Injection of glutamate (*A*) or dextrose (*B*) into the left masseter muscle significantly increased blood flow. (*C*) Coinjection of kynurenate with glutamate into the left masseter muscle attenuated glutamate-induced increased blood flow. (*D*) Coinjection of lidocaine (74 mM) with glutamate also attenuated glutamate-induced increased blood flow. (*E*) Coinjection of lidocaine (74 mM) with dextrose into the left masseter muscle substantially decreased dextrose-induced increased blood flow. Bars = SD; *$P < 0.05$, repeated-measures analysis of variance, Dunnett method.

tions.[15] The initial volume of edema and extracellular water content in the masseter muscle after injection of either dextrose, glutamate, or glutamate with kynurenate, which all have similar osmotic strengths, were equivalent and significantly greater than isotonic saline. Thus, the initial increase in edema volume and extracellular water content after injection of dextrose, glutamate, or glutamate with kynurenate may be attributed principally to an osmotic mechanism.

On the other hand, our data also show that, while the volume of edema after injection of glutamate remains constant over 60 min, there is a gradual decrease in the peak extracellular water content. Coinjection of kynurenate with glutamate significantly increases the clearance of extracellular water from the edema. This is consistent with a report that increases in postmortem paw weight, which are thought to reflect edema water content, after subcutaneous injection of glutamate into the paw could be reduced by a non-NMDA receptor antagonist.[26] The peak extracellular water content within the edema gives an indication of the degree to which the injected lidocaine would be diluted, *i.e.*, higher extracellular water content corresponds to a greater degree of dilution.

Injection of glutamate also caused a prolonged increase in masseter muscle blood flow. Glutamate-induced increases in masseter muscle blood flow were attenuated by both coinjected lidocaine and kynurenate and appear to be mediated indirectly through excitation of afferent or efferent fibers by activation of peripheral EAA receptors. Glutamate, as well as the selective EAA receptor agonists NMDA and kainate, have been previously demonstrated to dilate cerebral arterioles, coronary arteries, and aorta.[27–30] Elevation of glutamate concentrations in vascular smooth muscle and skin has been reported to increase release of nitric oxide through activation of peripheral NMDA and non-NMDA receptors.[30–32] Inhibition of nitric oxide synthesis or loss of afferent fiber innervation prevents glutamate-induced vasodilation.[30,32] These results suggest that one mechanism whereby glutamate could increase masseter muscle blood flow is through local release of nitric oxide. In addition, activation of non-NMDA receptors on trigeminal afferent fibers releases calcitonin gene-related peptide, a potent vasodilator.[33] No matter what the underlying mechanism is, the net effect of glutamate-induced increase muscle blood flow would be to increase the clearance of injected lidocaine out of the masseter muscle tissue.

Injection of hypertonic dextrose solutions into the master muscle significantly increased extracellular water content and blood flow to the same degree as glutamate but did not sensitize masseter muscle afferent fibers. Thus, the effect of hypertonic dextrose on lidocaine block duration can be used to estimate the contribution of afferent fiber sensitization to the shortening of lidocaine block duration by glutamate. Hypertonic dextrose injections did significantly decrease lidocaine block duration, but glutamate decreased lidocaine block duration to a significantly greater extent than dextrose (fig. 2B). A comparison of the duration of lidocaine block after injection of dextrose and glutamate suggests that afferent fiber sensitization accounts for about one third of the block shortening effect of glutamate.

Inflammation of craniofacial tissues often interferes with the success of local anesthesia.[1–5] The local tissue concentration of glutamate is elevated after acute and chronic tissue inflammation in both animals and humans, which suggests that glutamate could play a role in inflammation-induced local anesthetic failure.[6–9] The physiologic relevance of the high concentrations of glutamate used in the current study may be questioned, however, since the reported concentration of glutamate during tissue inflammation[7,34] is several orders of magnitude smaller. However, release of glutamate from afferent fiber terminals is proposed to be a major source of the glutamate found in inflamed tissues,[7] and the concentration of glutamate in presynaptic vesicles may approach 200 mM.[35] Despite this high concentration, glutamate may be rapidly removed from the extracellular space by specific peripheral reuptake mechanisms.[26,36] Therefore, concentrations of glutamate measured in tissues or synovial fluid may not accurately reflect concentrations of glutamate in close proximity to the afferent fiber terminal. Indeed, concentrations of 10–20 mM glutamate activate trigeminal ganglion neurons *in vitro*,[18,19] and it has been found that high concentrations ($>$ 100 mM) are required to activate muscle afferent fibers *in vivo* or to evoke pain in human subjects.[10,11] Given these findings, the question of what constitutes a physiologically relevant concentration of glutamate remains unresolved.

Intramuscular injection of glutamate does not appear to result in tissue inflammation,[12] but does result in the formation of large edemas, increased blood flow, and sensitized muscle afferent fibers, all of which are characteristic of tissue inflammation. Glutamate-induced increases in tissue extracellular water content, blood flow, and afferent fiber sensitization are mediated through activation of peripheral EAA receptors, and the findings of the current study indicate that activation of peripheral EAA receptors can shorten the duration of local anesthesia. The current results suggest that further attention should be directed toward the clinical utility of combinations of peripheral EAA receptor antagonists and local anesthetics, particularly in situations where local anesthetics are injected into tissues where concentrations of glutamate may have become elevated, for example, during tissue inflammation.

References

1. Wong MK, Jacobsen PL: Reasons for local anesthesia failures. J Am Dent Assoc 1992; 123:69–73

2. Wallace JA, Michanowicz AE, Mundell RD, Wilson EG: A pilot study of the clinical problem of regionally anesthetizing the pulp of an acutely inflamed mandibular molar. Oral Surg Oral Med Oral Pathol 1985; 59:517–21

3. Rood JP: The organization of the inferior alveolar nerve and its relation to local analgesia. J Dent 1978; 6:305–10

4. Rood JP, Pateromichelakis S: Local anaesthetic failures due to an increase in sensory nerve impulses from inflammatory sensitization. J Dent 1982; 10:201–6

5. Rood JP, Pateromichelakis S: Inflammation and peripheral nerve sensitisation. Br J Oral Surg 1981; 19:67–72

6. Lawand NB, Willis WD, Westlund KN: Excitatory amino acid receptor involvement in peripheral nociceptive transmission in rats. Eur J Pharmacol 1997; 324:169–77

7. Lawand NB, McNearney T, Westlund KN: Amino acid release into the knee joint: Key role in nociception and inflammation. Pain 2000; 86:69–74

8. McNearney T, Speegle D, Lawand N, Lisse J, Westlund KN: Excitatory amino acid profiles of synovial fluid from patients with arthritis. J Rheumatol 2000; 27:739–45

9. Omote K, Kawamata T, Kawamata M, Namiki A: Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res 1998; 787:161–4

10. Cairns BE, Gambarota G, Svensson P, Arendt-Nielsen L, Berde CB: Glutamate-induced sensitization of rat masseter muscle fibres. Neuroscience 2002; 109:389–99

11. Cairns BE, Hu JW, Arendt-Nielsen L, Sessle BJ, Svensson P: Sex-related differences in human pain perception and rat afferent discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2001; 86:782–91

12. Cairns BE, Sessle BJ, Hu JW: Evidence that excitatory amino acid receptors within the temporomandibular joint region are involved in the reflex activation of the jaw muscles. J Neurosci 1998; 18:8056–64

13. Cairns BE, Sessle BJ, Hu JW: Characteristics of glutamate-evoked temporomandibular joint afferent activity in the rat. J Neurophysiol 2001; 85:2446–54

14. Tverskoy M, Oren M, Vaskovich M, Dashkovsky I, Kissin I: Ketamine enhances local anesthetic and analgesic effects of bupivacaine by peripheral mechanism: A study in postoperative patients. Neurosci Lett 1996; 215:5–8

15. Gambarota G, Cairns BE, Berde CB, Mulkern RV: Osmotic effects on the T2 relaxation decay of in vivo muscle. Mag Res Med 2001; 46:592–9

16. Wilder RT, Sholas MG, Berde CB: NG-nitro-L-arginine methyl ester (L-NAME) prevents tachyphylaxis to local anesthetics in a dose-dependent manner. Anesth Analg 1996; 83:1251–5

17. Taylor GA, Barnewolt CE, Adler BH, Dunning PS: Renal cortical ischemia in rabbits revealed by contrast-enhanced power Doppler sonography. AJR Am J Roentgenol 1998; 170:417–22

18. Puil E, Spigelman I: Electrophysiological responses of trigeminal root ganglion neurons in vitro. Neurosci 1988; 24:635–46

19. Pelkey KA, Marshall KC: Actions of excitatory amino acids on mesencephalic trigeminal neurons. Can J Physiol Pharmacol 1998; 76:900–8

20. Gold MS, Reichling DB, Shuster MJ, Levine JD: Hyperalgesic agents increase a tetrodotoxin-resistant Na^+ current in nociceptors. Proc Natl Acad Sci U S A 1996; 93:1108–12

21. Roy ML, Narahashi T: Differential properties of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in rat dorsal root ganglion neurons. J Neurosci 1992; 12:2104–11

22. Scholz A, Kuboyama N, Hempelmann G, Vogel W: Complex blockade of TTX-resistant Na+ currents by lidocaine and bupivacaine reduce firing frequency in DRG neurons. J Neurophysiol 1998; 79:1746–54

23. Hu HZ, Li ZW, Si JQ: Evidence for the existence of substance P autoreceptor in the membrane of rat dorsal root ganglion neurons. Neurosci 1997; 77:535–41

24. Spigelman I, Puil E: Ionic mechanism of substance P actions on neurons in trigeminal root ganglia. J Neurophysiol 1990; 64:273–81

25. Heppelmann B, Pawlak M: Sensitization of articular afferents in normal and inflamed knee joints by substance P in the rat. Neurosci Lett 1997; 223:97–100

26. Beirith A, Santos ARS, Calixto JB: Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res 2002; 924:219–28

27. Faraci FM, Breese KR, Heistad DD: Responses of cerebral arterioles to kainate. Stroke 1994; 25:2080–3

28. Ishizaka H, Kuo L: Endothelial ATP-sensitive potassium channels mediate coronary microvascular dilation to hyperosmolarity. Am J Physiol Heart Circ Physiol 1997; 273:H104-12

29. Nguyen-Duong H: Vasorelaxation induced by L-glutamate in porcine coronary arteries. J Toxicol Environ Health A 2001; 62:643–53

30. Domoki F, Perciaccante JV, Shimizu K, Puskar M, Busija DW, Bari F: N-methyl-D-aspartate-induced vasodilation is mediated by endothelium-independent nitric oxide release in piglets. Am J Physiol Heart Circ Physiol 2002; 282:H1404–9

31. Crespi F, Lazzarini C, Andreoli M, Vecchiato E: Voltammetric and functional evidence that N-methyl-D-aspartate and substance P mediate rat vascular relaxation via nitrogen monoxide release. Neurosci Lett 2000; 287:219–22

32. Omote K, Kawamata T, Kawamata M, Nakayama Y, Hazama K, Namiki A: Activation of peripheral NMDA-nitric oxide cascade in formalin test. ANESTHESIOLOGY 2000; 93:173–8

33. Jackson DL, Hargreaves KM: Activation of excitatory amino acid receptors in bovine dental pulp evokes the release of iCGRP. J Dent Res 1999; 78:54–60

34. Omote K, Kawamata T, Kawamata M, Namiki A: Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res 1998; 787:161–4

35. Riveros N, Fiedler J, Lagos N, Munoz C, Orrego F: Glutamate in rat brain cortex synaptic vesicles: Influence of the vesicle isolation procedure. Brain Res 1986; 386:405–8

36. Schmitt A, Asan E, Lesch K-P, Keugler P: A splice variant of glutamate transporter GLT1/EAAT2 expressed in neurons: Cloning and localization in the rat nervous system. Neuroscience 2002; 109:45–61