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Nitric Oxide Modulation Affects the Tissue Distribution and Toxicity of Bupivacaine

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SHI, B. AND J. HEAVNER. *Nitric oxide modulation affects the tissue distribution and toxicity of bupivacaine.* PHARMACOL BIOCHEM BEHAV **66**(3) 623–629, 2000.—We have previously demonstrated that inhibition of nitric oxide synthase (NOS) alters the toxicity of local anesthetics including bupivacaine. Because significant changes in blood distribution are associated with the use of nonselective NOS inhibitors, the purpose of this study was to determine whether modification of bupivacaine toxicity by nonselective NOS inhibition is due to alteration in tissue disposition of bupivacaine. Rats were anesthetized with halothane and pretreated with either: 1) a nonselective NOS inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min, IV for 30 min); 2) a neuronal NOS inhibitor, 7-nitroindazole (7-NI, 30 mg/kg, IP); or 3) vehicle (control). Thirty minutes later, bupivacaine 2 mg/kg/min IV was infused until onset of seizures, arrhythmias, or asystole. L-NAME caused a rapid increase in plasma bupivacaine concentrations (3–4 times faster than in the other groups), which was associated with markedly lower bupivacaine doses (mg/kg) required to produce arrhythmias in L-NAME (4.2 ± 0.5) vs. control (26 ± 3, $p < 0.01$) and 7-NI groups (17 ± 3, $p < 0.01$). Myocardial bupivacaine concentrations at arrhythmia onset were slightly lower in the L-NAME group. Bupivacaine seizure doses in 7-NI and L-NAME pretreated animals were similar to control but significantly different from each other. Brain bupivacaine concentrations at seizure onset were similar among the groups. There were no significant differences between 7-NI and control groups in any parameter observed. We conclude that enhanced cardiotoxicity of bupivacaine by nonselective NOS inhibition is primarily due to rapid increases in plasma and myocardial distribution of bupivacaine. © 2000 Elsevier Science Inc.

Seizures Arrhythmias Bupivacaine Nitric oxide synthase inhibitors

NITRIC OXIDE, a neurotransmitter and endothelium-derived relaxing factor, plays an important role in the regulation of brain and cardiovascular function. The role of nitric oxide in seizure activity and cardiac arrhythmias induced by a variety of agents including anesthetics is an area of intense investigation. Both pro- and anticonvulsant actions (1,10,11,13,15,18,27,28) as well as arrhythmogenic and antiarrhythmic effects (3,20,30) of nitric oxide have been demonstrated. Differences in species studied and seizure- and arrhythmia-inducing agents may account for some of these conflicting observations (1,10,11,13,15,18). Another important factor may be inappropriate interpretation of results from the use of nonselective nitric oxide synthase (NOS) inhibitors such as N^{ω}-nitro-L-arginine methyl ester (L-NAME) (10,19). L-NAME has a profound vasopressor effect causing sustained increases in blood pressure and alteration in blood flow to different organs (8,19). Alteration in blood distribution will alter the disposition/distribution kinetics of other drugs, for example, local anesthetics administered after the administration of L-NAME (8,10,19).

We have previously demonstrated that inhibition of NOS modifies the toxicity of local anesthetics such as cocaine (7) and bupivacaine (25). The current study differs from previous studies in that in the current study the effects of L-NAME on bupivacaine toxicity and the association of toxicity with plasma, brain, and myocardial concentrations of bupivacaine at toxic end points was compared with the effects of a neuronal NOS inhibitor 7-nitroindazole (7-NI), which has minimal vasopressor (endothelial) effects (4,21). A pharmacodynamic effect is primarily considered if plasma and tissue kinetics of bupivacaine are not different among groups. Our results indicated that L-NAME dramatically modifies bupivacaine toxicity, which is associated with marked alterations in bupivacaine pharmacokinetics, while neuronal NOS inhibition by 7-NI does not alter either distribution kinetics or toxicity of bupivacaine. The results suggest that nonselective NOS inhibition by L-NAME modifies bupivacaine toxicity primarily through its pharmacokinetic action on the drug.

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METHOD

Surgical Procedures and Physiological Measurements

The experimental protocol was approved by the TTUHSC Institutional Animal Care and Use Committee in compliance with the NIH Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Female Sprague–Dawley rats 8–10 weeks of age (SASCO Animal Laboratories; Houston, TX) were anesthetized with halothane (1.75%) in oxygen. The trachea was cannulated and mechanical ventilation instituted using a rodent ventilator (Harvard Apparatus; South Natick, MA). A polyethylene catheter (PE50) was placed through the left femoral vein into the vena cava for test drug infusion, and another PE50 catheter was placed into the right femoral vein for administration of neuromuscular blocking agent. The right and left femoral arteries were cannulated for arterial pressure measurements and for blood sampling, respectively. Electrocardiogram (ECG) leads I, II, and V_1 and fronto-occipital electroencephalogram (EEG) were recorded with needle electrodes. End tidal carbon dioxide was measured using a microcapnometer (Columbus Instruments, Columbus, OH) and ventilation of the lungs adjusted so that arterial blood carbon dioxide partial pressure (PCO_2) was 31 ± 4 mmHg (NOVA STAT Profile 5 Blood Gas Analyzer, NOVA Biomedical; Waltham, MA). Rectal temperature was maintained at 37.8–38.2°C using a warming blanket. ECG, EEG, and arterial blood pressure were recorded continuously on a chart recorder (Grass Polygraph; Quincy, MA; and Hewlett Packard 7758A; Waltham, MA). After the surgical preparation, halothane concentration was decreased to 0.5% and N_2O (70%) plus O_2 was started. Doxacurium (a neuromuscular blocking agent), 0.2 mg/kg, was given intravenously (IV) as needed to prevent spontaneous ventilation.

Administration of Nitric Oxide Synthase Inhibitors and Bupivacaine

After a 30-min equilibration period, animals were pretreated as follows: 1) control group ($n = 22$): animals received peanut oil (the vehicle for 7-NI) 3 mg/kg IP plus saline (the vehicle for L-NAME) 0.05 ml/kg/min, IV; 2) 7-NI group ($n = 24$): animals in this group were given 7-nitroindazole (7-NI, Sigma Chemical Co; St. Louis, MO), 30 mg/kg (1% suspension in peanut oil) IP and saline 0.05 ml/kg/min IV. The 7-NI dose was previously shown to produce 80–85% inhibition of brain nitric oxide synthase (14); 3) L-NAME group ($n = 19$): N^{ω}-nitro-L-arginine methyl ester (L-NAME, Sigma Chemical Co; St. Louis, MO), 2 mg/kg/min IV for 30 min (60 mg/kg total), was administrated using an infusion/withdraw pump (Harvard Apparatus; South Natick, MA) and peanut oil (the vehicle for 7-NI), 3 ml/kg, was given IP. The L-NAME dose was reported to produce 70 to 90% inhibition of NOS (9,19,23).

Thirty minutes after pretreatment, rats were given bupivacaine 2 mg/kg/min (0.5% soln) IV using the infusion/withdraw pump until one of the following endpoints: arrhythmias (the first appearance of at least three consecutive abnormal beats per 10 s, accompanied by changes in arterial blood pressure), seizures (the first appearance of bursts of multiple sharp spikes of greater than 100 μV on the EEG; these were accompanied by skeletal muscle contractions, especially in the head and neck region, in animals with incomplete neuromuscular blockade), or cardiac asystole (complete disappearance of the QRS complex). The only arrhythmia we observed was atrioventricular (AV) conduction block. Only second- and third-degree AV conduction block was considered as arrhythmia. First-degree AV conduction block (prolongation of the P-R interval) and intraventricular conduction slowing (widening of the QRS complex) were not considered as arrhythmias. At each of the three toxic end points (arrhythmias, seizures, and asystole), six to nine animals from each group were killed by removing the heart 0.5 min after inspired halothane was increased to 5%.

Measurements of Bupivacaine Concentrations by HPLC

Blood (≈0.3 ml), whole brain, and heart were collected at onset of seizures, arrhythmias, and asystole for bupivacaine concentration measurements. Blood was also collected at 2.5, 5.0, 7.5, and 10.0 min after the start of bupivacaine infusion from animals that were killed at asystole. Blood was centrifuged immediately at 4°C and plasma collected. The brain and heart were removed within 1 min after the target end point and immediately rinsed with ice-cold saline, blotted, weighed, and homogenized in 5 vol (g/ml) of ice-cold 0.05 M KH_2PO_4 using a Brinkmann Polytron at setting 6 for 15 s. Plasma and the homogenates were stored at −80°C until assayed for bupivacaine concentration by high pressure liquid chromatography (HPLC) and a UV detector (25). The interday coefficient of variation using bupivacaine standard solutions was 2.5%.

Data Analysis

Data are presented as mean ± SEM. Single-factor ANOVA and Student–Newman–Keuls test were used to determine if there were significant differences among groups at each end point. The changes of plasma bupivacaine concentrations and hemodynamic parameters over time (slopes) were analyzed by MANOVA and Exact *F*-test. Statistical significance was accepted if $p = 0.05$.

RESULTS

Baseline Values and Hemodynamic Responses to L-NAME vs. 7-NI

As shown in Table 1, body weights and baseline blood gas values were similar among groups, while arterial blood pressure was significantly higher in L-NAME than in vehicle and 7-NI–treated animals. Heart rate was not significantly altered by L-NAME treatment. There was no significant difference in arterial blood pressure and heart rate between 7-NI- and vehicle-treated animals.

Changes in Plasma and Tissue Bupivacaine Concentrations and Hemodynamic Responses During Bupivacaine Infusion

During constant IV bupivacaine infusion, concentrations of bupivacaine in plasma, heart, and brain increased over time in all animals. There was no difference between the control and 7-NI groups in bupivacaine concentrations in plasma at any time point or in the rate of increase of bupivacaine concentration vs. time (Fig. 1). The rate of increase in plasma concentrations was markedly faster in the L-NAME group vs. the other two groups [both the means at the same time points, single-factor ANOVA results: 2.5 min, $F(2, 23) = 27.88$ ($p < 0.001$); 5.0 min, $F(2, 30) = 55.26$ ($p < 0.001$); 7.5 min, $F(2, 21) = 26.28$ ($p < 0.001$); 10.0 min, $F(2, 20) = 11.22$ ($p < 0.001$); (Student–Newman–Keuls test: $p < 0.001$ L-NAME vs. saline and 7-NI; and the slopes are significantly different (MANOVA results: $F(2, 19) = 25.34$ ($p < 0.001$)] (Fig. 1).

TABLE 1
BODY WEIGHTS AND BASELINE VALUES OF RATS

Groups	Body Weight*	SBP† (mmHg)	Heart Rate‡ (bpm)	pH§	PO_2# (mmHg)	PCO_2¶ (mmHg)
Control (22)	253 ± 2	123 ± 4	350 ± 8	7.47 ± 0.007	106 ± 2	31 ± 0.4
7-NI (24)	248 ± 2	134 ± 2	333 ± 5	7.46 ± 0.004	111 ± 2	31 ± 0.5
L-Name (19)	256 ± 3	157 ± 2**††	335 ± 8	7.45 ± 0.005	111 ± 3	30 ± 0.8

Blood samples were taken at 30 min after the start of administration of vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N^{ω}-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV for 30 min). SBP refers to systolic arterial blood pressure. PO_2 and PCO_2 refer to partial pressure of oxygen and carbon dioxide, respectively, in arterial blood. Values are mean ± SEM of the number of observations given in parentheses. Single-factor ANOVA results: *$F(2, 62) = 2.96$, NS: †$F(2, 62) = 45.26$ ($p < 0.001$); ‡$F(2, 62) = 1.98$, NS; §$F(2, 62) = 2.04$, NS; #$F(2, 62) = 0.76$, NS; ¶$F(2, 62) = 0.79$, NS. Student–Newman–Keuls test **$p < 0.05$ vs. Control; ††$p < 0.05$ vs. 7-NI.

As shown in Fig. 2, systolic arterial blood pressure in vehicle and 7-NI–treated animals increased slightly initially then gradually declined during bupivacaine infusion. Arterial blood pressure in L-NAME–treated animals fell sharply during bupivacaine infusion. The rate of decline in arterial blood pressure was similar in the control and 7-NI groups, but was significantly faster in the L-NAME group [MANOVA results: $F(2, 28) = 23.46$ ($p < 0.001$)]. Similarly, heart rate fell in all three groups, but at a significantly faster rate in the L-NAME group than in the other two groups [MANOVA results: $F(2, 28) = 56.68$ ($p < 0.001$)]. The dramatic decline in heart rate at 2.5 min during bupivacaine infusion was associated with marked early onset of AV conduction block in this group (on average, 2.1 min in L-NAME vs. 12.9 min in control, and 8.6 min in 7-NI groups) (Table 2).

Effects of L-NAME vs. 7-NI on Bupivacaine-Induced Seizure Activity

Bupivacaine-induced seizure activity was evaluated by three indicators: 1) cumulative doses of bupivacaine that produced seizures; 2) plasma and brain concentrations of bupivacaine at seizure onset; and 3) seizure amplitude, duration (time interval between onset of epileptiform bursts on the EEG and cessation of bursts), and number of epileptiform bursts.

Figure 3 shows representative EEG tracings of rats from the three treatment groups. Lower amplitude of seizure bursts, shorter seizure duration, and fewer numbers of epileptiform bursts were observed in L-NAME–pretreated animals than in 7-NI and vehicle-pretreated animals (Figs. 3 and 4D–F). Doses of bupivacaine that produced seizures in 7-NI and L-NAME–pretreated animals were similar to control, but were significantly different from each other (Fig. 4A). Plasma bupivacaine concentrations at seizure onset were significantly higher in L-NAME than in 7-NI and control-pretreated animals. However, brain bupivacaine concentrations at seizure onset did not differ among groups (Fig. 4B). The ratio of brain to plasma concentrations of bupivacaine at seizure onset was significantly lower in L-NAME group than in the other two groups (Fig. 4C). Animals pretreated with 7-NI had slightly (but not significantly) shorter seizure duration and fewer numbers of epileptiform bursts than control animals.

At seizure onset, heart rate was significantly lower in L-NAME than in the other two groups (150 ± 12 bpm vs. 276 ± 6 in control and 274 ± 5 in 7-NI) [single-factor ANOVA, $F(2, 51) = 82.30$ ($p < 0.001$); Student–Newman–Keuls test, $p < 0.05$]. The significant fall in heart rate at the seizure onset in the L-NAME group was due to the early occurrence of AV conduction block in this group. Seizures occurred after AV block in L-NAME–treated animals and before AV block in animals from the other two groups (Table 2). The marked fall in heart rate resulted in twofold decline in cardiac output (estimated by the rate-pressure produce = heart rate × systolic blood pressure) in the L-NAME group (20,200 ± 2400 bpm · mmHg) vs. control (40,900 ± 1800) and 7-NI (42,600 ± 1300) (single-factor ANOVA results, $F(2, 51) = 40.50$ ($p < 0.001$); Student–Newman–Keuls, $p < 0.05$) groups.

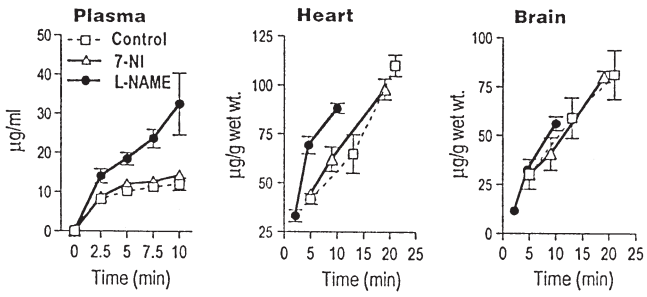

FIG. 1. Bupivacaine concentrations in plasma, heart, and brain vs. infusion time of bupivacaine (2 mg/kg/min IV) in rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N^{ω}(-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV for 30 min). Plasma concentration data were obtained from animals that were killed at asystole. Heart and brain concentration data were obtained from animals killed at seizures, arrhythmias, or asystole. Values are means ± SEM of 5 to 12 observations.

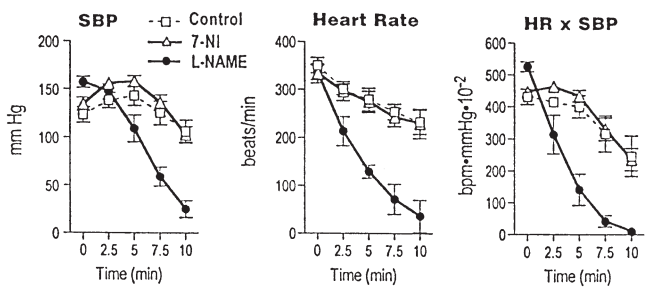

FIG. 2. Arterial systolic pressure (SBP), heart rate, and the rate-pressure product (HR × SBP) vs. infusion time of bupivacaine (2 mg/kg/min IV) in rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N^{ω}-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV for 30 min). Values are means ± SEM of 13 to 21 observations.

TABLE 2

TIME (MINUTES) AT WHICH SEIZURES AND ATRIOVENTRICULAR (AV) CONDUCTION BLOCK OCCURRED DURING BUPIVACAINE INFUSION IN RATS

Groups	Seizures*	AV Conduction Block†
Control	5.0 ± 0.2 (22)	12.9 ± 1.7 (16)
7-NI	5.4 ± 0.2 (16)	8.6 ± 1.3 (17)
L-NAME	4.6 ± 0.2 (13)	2.1 ± 0.2‡§ (19)

Animals were pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min iV). After 30 min, bupivacaine (2 mg/kg/min, IV) was infused. Data was obtained from all animals that developed seizures or AV conduction block before they were killed. Values are mean ± SEM of the number of observations given in parentheses. Single-factor ANOVA results: *$F(2, 48) = 3.08$, NS; †$F(2, 49) = 22.58$ ($p < 0.001$), ‡$p < 0.05$ vs. control; §$p < 0.05$ vs. 7-NI Student–Newman–Keuls test.

Effects of L-NAME vs. 7-NI on Bupivacaine-Induced Cardiotoxicity

Bupivacaine-induced cardiotoxicity was evaluated by: 1) cumulative doses of bupivacaine that produced arrhythmias and cardiac asystole; and 2) plasma and heart bupivacaine concentrations at onset of arrhythmias and asystole.

The only arrhythmia pattern that we observed during acute intravenous infusion of bupivacaine was AV conduction block (Fig. 5). As shown in Fig. 6, cumulative doses of bupivacaine that produced arrhythmias and asystole were markedly reduced in L-NAME–pretreated animals compared with 7-NI and vehicle pretreatment. On average, arrhythmia doses of bupivacaine in the L-NAME group (4.2 ± 0.5 mg/kg) were approximately one-sixth of the average dose that produced this end point in the control group (26 ± 3 mg/kg, $p <$ 0.01) and one-forth the arrhythmia dose in the 7-NI group (17 ± 3 mg/kg, $p < 0.01$). There were no significant differences in

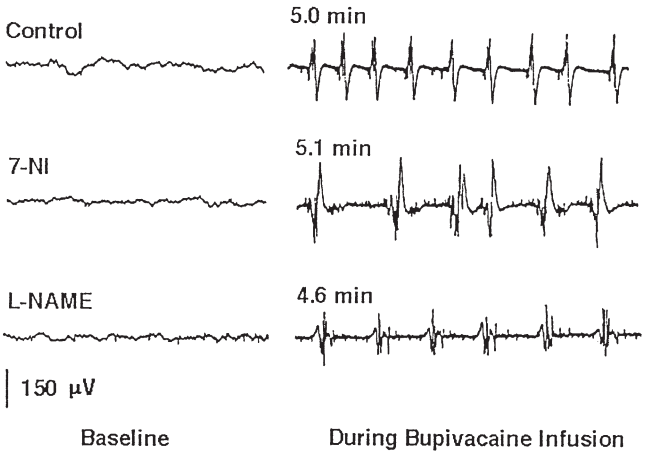

FIG. 3. Representative electroencephalogram (EEG) tracings showing fully developed seizure activity induced by bupivacaine (2 mg/kg/min IV) in rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV). Baseline tracings were recorded just before bupivacaine exposure. The time (average for the group) at which seizures started and the voltage scale are shown. The chart speed was 5 mm/s.

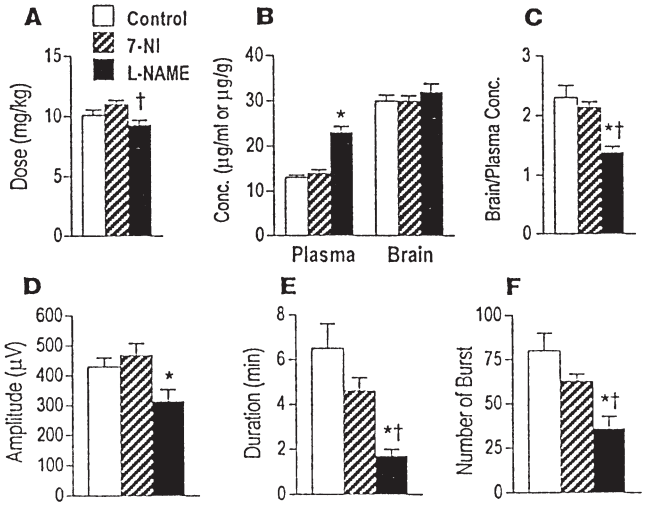

FIG. 4. Doses of bupivacaine that produced seizures (A), concentrations of bupivacaine in plasma and brain at seizure onset (B), ratios of brain to plasma concentrations of bupivacaine at seizure onset (C), seizure amplitude (D), duration (E), number of epileptiform bursts (F) of rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV). Doses and plasma concentration data were obtained from all animals that had seizures. Brain concentration data were obtained from animals killed at seizure onset. Seizure amplitude, duration and the number of epileptic bursts data were obtained from animals killed at asystole. Values are means ± SEM of 13 to 17 observations. Single-factor ANOVA results: (A) dose, $F(2, 52) = 4.36$ ($p < 0.05$); (B) plasma conc., $F(2, 17) = 36.42$ ($p < 0.001$), brain conc., $F(2, 17) = 0.89$, NS; (C) ratio, $F(2, 17) = 34.08$ ($p < 0.001$); (D) amplitude, $F(2, 19) = 5.06$ ($p < 0.005$); (E) duration, $F(2, 22) = 45.34$ ($p < 0.01$); (F) number of bursts, $F(2, 22) = 7.43$ ($p < 0.01$). Student–Newman–Keuls test *$p < 0.05$ vs. control; +$p < 0.05$ vs. 7-NI.

plasma bupivacaine concentrations at onset of arrhythmias and asystole among groups. However, myocardial bupivacaine concentrations at arrhythmia onset were significantly lower in L-NAME–pretreated animals than in animals from the other two groups. The ratio of myocardial to plasma concentrations of bupivacaine at arrhythmia onset (but not at asystole) was significantly lower in the L-NAME group than in the other two groups. There were no significant differences in plasma and myocardial concentrations of bupivacaine at onset of arrhythmias or asystole between 7-NI and control animals.

At arrhythmia onset, heart rates were similar among groups (225 ± 13 in control, 237 ± 11 in 7-NI, and 260 ± 15 bpm in L-NAME [single-factor ANOVA, $F(2, 52) = 2.11$, NS)], while systolic blood pressure was significantly higher in the L-NAME group (146 ± 5 mmHg) than in controls (91 ± 10 mmHg) [single-factor ANOVA, $F(2, 48) = 11.83$ ($p <$ 0.001); Student–Newman–Keuls test, $p < 0.05$].

DISCUSSION

The two major novel findings from this study are: 1) neuronal NOS inhibition by 7-NI does not modify either seizures or arrhythmias induced by bupivacaine because both seizure doses and brain concentrations at seizure onset were not significantly altered by 7-NI; 2) nonselective NOS inhibition by L-NAME does not alter bupivacaine seizure threshold (dose) but greatly enhances the sensitivity to bupivacaine-induced arrhythmias as evidenced by a marked (sixfold) reduction in

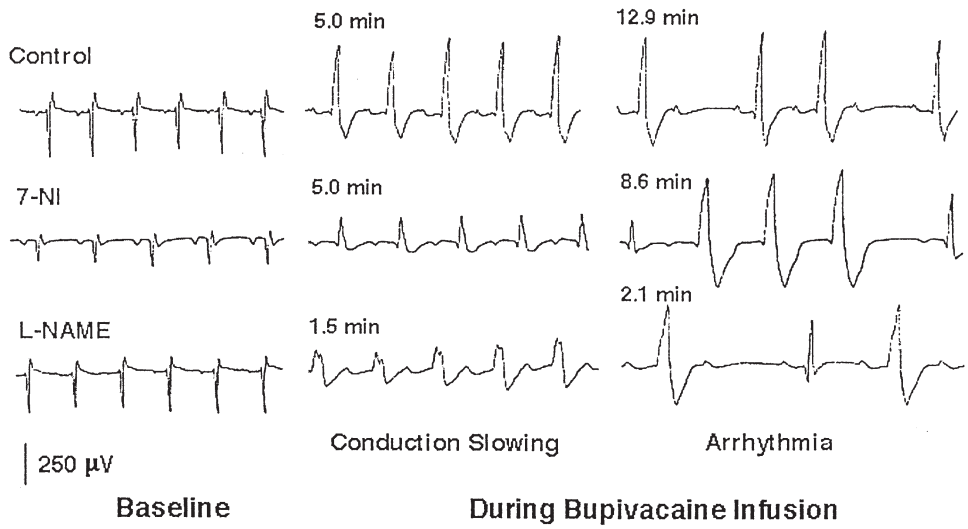

FIG. 5. Representative electrocardiogram (ECG) lead II recordings showing the pattern of arrhythmia induced by bupivacaine (2 mg/kg/min IV) in rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N^{ω}-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV for 30 min). Baseline tracings were recorded right before bupivacaine exposure. The middle portion shows the slowing of atrioventricular conduction (increased PR interval) and intraventricular conduction (widened QRS complex). The right portion of the chart shows onset of arrhythmia ($\geq 2°$ AV conduction block). The time (average for the group) at which AV conduction block started and the voltage scale are shown. Note that in the 7-NI tracing, some P waves are merged with the preceding T waves. The chart speed was 50 mm/s.

both the dose of bupivacaine that produced arrhythmia and the myocardial bupivacaine concentrations at the onset of arrhythmia after L-NAME pretreatment; and 3) L-NAME slightly lowers myocardial bupivacaine concentrations at arrhythmia onset. The results suggest that 1) nitric oxide may not play a significant role in seizure activity induced by bupivacaine, and 2) endothelial nitric oxide is apparently a mild endogenous cardioprotectant factor against bupivacaine-induced arrhythmias while neuronal nitric oxide appears not to be involved in the arrhythmogenic action of bupivacaine.

Effects of Nonselective vs. Neuronal NOS Inhibition on Bupivacaine-Induced Seizure Activity

Seizure is one of the most common and well-defined central nervous system toxic effect of local anesthetics. However, the mechanisms by which local anesthetics cause seizures remain unclear. The *N*-methyl-L-aspartate (NMDA) type of glutamate receptors has been implicated in seizure generation induced by a variety of agents including local anesthetics (10). Because local anesthetics enhance nitric oxide production (16) and nitric oxide is linked to the toxic effects of NMDA receptor activation (6,17), it is thought that nitric oxide may be involved in local anesthetic-induced seizure activity (10). However, the data published so far are conflicting regarding the role of nitric oxide in the generation of seizures. Some studies show that nitric oxide is proconvulsant (2,13,18), while others demonstrate that nitric oxide is anticonvulsant (1,15,28), or has no effect (27). As mentioned earlier, differences in species studied and seizure-inducing agents used may account for some of the discrepancies (12). Another important factor is how to interpret the results when nonselective NOS inhibitors such as L-NAME are used, as these agents significantly alter blood distribution and pharmacokinetics of other drugs (5,8,19,29,31). The rate of drug delivery to the tissues is determined by the drug concentration in the arterial blood and the tissue blood flow. Any drug that affects tissue blood flow will affect the rate of drug delivery and the total amount delivered, especially to the brain and the myocardium (19,31). We show in this study that the nonselective NOS inhibitor L-NAME significantly alters arterial blood pressure and tissue/blood distribution of bupivacaine. Because L-NAME alters tissue blood flow and redistribution, bupivacaine concentrations in tissues may not be correlated well to the concentration in the blood after L-NAME pretreatment (19). Obviously, it is the concentration at the effect sites in tissues that determines drug actions.

We found that neither L-NAME nor 7-NI altered bupivacaine doses required to produce seizures. Brain bupivacaine concentrations at seizure onset were also unaltered by L-NAME or 7-NI. Although plasma bupivacaine concentrations at seizure onset were significantly higher in the L-NAME group vs. the other two groups, brain concentrations were not different. Higher plasma but similar brain bupivacaine concentrations in the L-NAME vs. the other two groups may reflect reduced brain blood flow after L-NAME pretreatment (5,8,19). On the other hand, seizure amplitude and duration, and the number of the epileptic bursts were reduced in the L-NAME group vs. the other two groups. We attribute this to the much earlier (four to six times) onset of AV conduction block and subsequent rapid drop in arterial blood pressure in the L-NAME group vs. the other two group. A marked drop (twofold) in rate-pressure product causes reduction in brain blood flow and the decline in oxygen supply required to maintain seizures (22).

Effects of Nonselective vs. Neuronal NOS Inhibition on Bupivacaine-Induced Arrhythmias

There is evidence that both neuronal and endothelial NOS are distributed throughout the arteries and conduction system

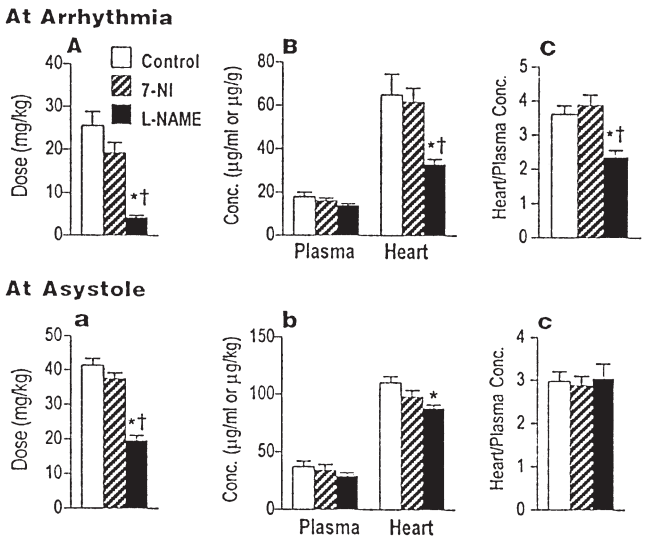

FIG. 6. Doses of bupivacaine that produced arrhythmias (A) and asystole (a), concentrations of bupivacaine in plasma and heart at onset of arrhythmia (B) and asystole (b), and ratios of myocardial to plasma concentrations of bupivacaine at onset of arrhythmia (C) and asystole (c) in rats pretreated with vehicle (control), 7-nitroindazole (7-NI, 30 mg/kg, IP), or N^{ω}-nitro-L-arginine methyl ester (L-NAME, 2 mg/kg/min IV). The only arrhythmia pattern induced by bupivacaine we observed in this study was atrioventricular conduction block. Dose data for AV conduction block were obtained from all animals that developed AV conduction block. Dose data for asystole were obtained from animals killed at asystole. Concentration data were obtained from animals killed at each specific endpoint. Values are means ± SEM of 13 to 17 observations single-factor ANOVA results: (A) dose, $F(2, 49) = 22.58$ ($p < 0.001$); (a) dose, $F(2, 22) = 37.14$ ($p < 0.001$); (B) plasma conc., $F(2, 17) = 1.75$, NS; heart conc., $F(2, 17) = 6.19$ ($p < 0.01$); (b) plasma conc., $F(2, 20) = 1.11$, NS; heart conc., $F(2, 22) = 4.76$ ($p < 0.05$); (C) ratio, $F(2, 17) = 11.5$ ($p < 0.001$); (c) ratio, $F(2, 20) = 0.06$, NS. Student–Newman–Keuls test $*p < 0.05$ vs. control; $+p < 0.05$ vs. 7-NI.

of the heart of animals and humans (24,26). Nitric oxide, which is released spontaneously under basal conditions as well as in response to physiological and pathophysiological challenges, is thought to be cardioprotectant against arrhythmias primarily through its tonic vasodilator effects (20). On the other hand, nitric oxide stimulates vagal neurotransmission causing conduction slowing and a decrease in sinus discharge rate (3). Therefore, nitric oxide may potentiate local anesthetic-induced AV conduction block. However, there are few studies about the relationship between nitric oxide and cardiac arrhythmias induced by local anesthetics (7,9,14,25). We found that the neuronal NOS inhibitor 7-NI did not alter bupivacaine doses required to produce AV conduction block and cardiac asystole, suggesting that neuronal nitric oxide synthase inhibition does not significantly modify the cardiotoxic effects of bupivacaine. Because neuronal NOS inhibition does not have effects on the cardiotoxicity of bupivacaine, the cardiac effects of nonselective NOS inhibition by L-NAME are primarily attributed to its inhibitory action on endothelial NOS. Our results show that bupivacaine doses required to produce arrhythmias were markedly reduced in the L-NAME group vs. the other two groups. This indicates that endothelial NOS inhibition increases sensitivity of rats to bupivacaine-induced arrhythmias. A pharmacokinetic mechanism is involved because there was a marked difference in plasma bupivacaine concentrations between the L-NAME group and the other two groups. We postulate that rapid increase in blood bupivacaine concentrations with unchanged heart blood flow (8) after L-NAME pretreatment produces a rapid increase in myocardial deposition of bupivacaine. This pharmacokinetic action of L-NAME appears primarily to account for early onset of arrhythmias in the L-NAME group. Our results agree with the study of Mueller who demonstrated that L-NAME–induced alterations in blood flow account for its antagonistic effects on ketamine-induced anesthesia (19). On the other hand, myocardial bupivacaine concentrations at arrhythmia onset was significantly reduced by L-NAME pretreatment, indicating increased cardiac sensitivity to the arrhythmogenic effects of bupivacaine (a pharmacodynamic action).

In conclusion: 1) nonselective nitric oxide inhibition by L-NAME does not alter bupivacaine seizure threshold but greatly enhances the sensitivity to bupivacaine-induced arrhythmia through both pharmacokinetic and pharmacodynamic mechanisms; and 2) neuronal nitric oxide inhibition by 7-NI does not significantly modify seizure activity and cardiotoxicity of bupivacaine.

The implications of this study could go beyond local anesthetic toxicity. Effects of any drug could be altered when nonselective NOS inhibitors such as L-NAME are used together with the drug, and therefore, in such a case, pharmacokinetic explanations for interactions between NOS inhibitors and the drug must be considered in interpretation of the data.

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REFERENCES

1. Boda, B.; Szente, M.: Nitric oxide synthase inhibitor facilitates focal seizures induced by aminopyridine in rats. Neurosci. Lett. 209:37–40; 1996.
2. Buisson, A.; Lakhmeche, N.; Verrecchia, C.; Plotkine, M.; Boulu, R. G.: Nitric oxide: An endogenous anticonvulsant. Neuroreport 4:444–446; 1993.
3. Elvan, A.; Rubart, M.; Zipes, D. P.: NO modulates autonomic effects on sinus discharge rate and AV nodal conduction in open-chest dogs. Am. J. Physiol. 272:H263–H271; 1997.
4. Fouyas, I. P.; Kelly, P. A.; Ritchie, I. M.; Whittle, I. R.: Cerebrovascular effects of nitric oxide manipulation in spontaneously hypersensitive rats. Br. J. Pharmacol. 121:49–56; 1997.
5. Gardiner, S. M.; Compton, A. M.; Kemp, P. A.; Bennett, T.: Regional and cardiac haemodynamic effects of N^{G}-nitro-L-arginine methyl ester in conscious, Long–Evans rats. Br. J. Pharmacol. 101:625–631; 1990.
6. Garthwaite, J.; Charles S. L.; Chess-Williams, R.: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336:385–388; 1988.
7. Heavner, J. E.; Shi, B.; Pitkänen, M.: Effects of nitric oxide synthesis inhibition with or without nitric oxide inhalation on responses to systemic cocaine administration in rats. Life Sci. 57:715–728; 1995.

8. Huang, M.; Leblanc, M. L.; Hester, R. L.: Systemic and regional hemodynamics after nitric oxide synthase inhibition: role of a neurogenic mechanism. Am. J. Physiol. 267:R84–R88; 1994.
9. Iadecola, C.; Xu, X.; Hu, J.; el-Fakahang, E. E.: Prolonged inhibition of brain nitric oxide synthase by short-term systemic administration of nitro-L-arginine methyl-ester. Neurochem. Res. 19:501–505; 1994.
10. Itzhak, Y.: Blockade of sensitization of the toxic effects of cocaine in mice by nitric oxide synthase inhibitors. Pharmacol. Toxicol. 74:162–166; 1994.
11. Karler, R.; Calder, L. D.; Chaudhry, I. A.; Turkanis, S. A.: Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci. 45:599–606; 1989.
12. Kirkby, R. D.; Carroll, D. M.; Grossman, A. B.; Subramaniam, S.: Factors determining proconvulsant and anticonvulsant effects of inhibitors of nitric oxide synthase in rodents. Epilepsy Res 24:91–100; 1996.
13. Leeuwen, R. V.; Vries, R. D.; Dzoljic, M. R.: 7-Nitro indazole, an inhibitor of neuronal nitric oxide synthase, attenuates pilocarpine-induced seizures. Eur. J. Pharmacol. 287:211–213; 1995.
14. MacKenzie, G. M.; Rose, S.; Bland-Ward, P. A.; Moore, P. K.; Jenner, P.; Marsden, C. D.: Time course of inhibition of brain nitric oxide synthase by 7-nitroindazole. Neuroreport 5:1993–1996; 1994.
15. Maggio, R.; Fumagalli, F.; Donati, E.; Barbier, P.; Racagni, G.; Corsini, G. U.; Riva, M.: Inhibition of nitric oxide synthase dramatically potentiates seizures induced by kainic acid and pilocarpine in rats. Brain Res. 679:184–187; 1995.
16. Mamiya, K.; Tomoda, M. K.; Edashige, K.; Ueda, W.; Manabe, M.: Local anesthetics enhance nitric oxide production by human peripheral neutrophils. Physiol. Chem. Phys. Med. NMR 27:111–119; 1995.
17. McFarlane, C.; Warner D. S.; Dexter, F.; Todd, M. M.: Glutamatergic antagonism: Effects on lidocaine-induced seizures in the rat. Anesth. Analg. 79:701–705; 1994.
18. Mollace, V.; Bagetta, G.; Nistico, G.: Evidence that L-arginine possesses proconvulsant effects mediated through nitric oxide. Neuroreport 2:269–272; 1991.
19. Mueller, R. A.; Hunt, R.: Antagonism of ketamine-induced anesthesia by an inhibitor of nitric oxide synthesis: A pharmacokinetic explanation. Pharmacol. Biochem. Behav. 60:15–22; 1998.
20. Pabla, R.; Curtis, M. J.: Effects of NO modulation on cardiac arrhythmias in the rat isolated heart. Circ. Res. 77:984–992; 1995.
21. Pajewski, T. N.; DiFazio, C. A.; Moscicki, J. C.; Johns, R. A.: Nitric oxide synthase inhibitors, 7-nitro indazole and nitroG-L-arginine methl ester, dose dependently reduce the threshold for isoflurane anesthesia. Anesthesiology 85:1111–1119; 1996.
22. Perlman, J. M.; Volpe, J J.: Seizures in the preterm infant: effects on cerebral blood flow velocity, intracranial pressure, and arterial blood pressure. J. Pediatr. 102:288–293; 1983.
23. Salter, M.; Duffy, C.; Hazelwood, R.: Determination of brain nitric oxide synthase inhibition in vivo: Ex vivo assays of nitric oxide synthase can give incorrect results. Neuropharmacology 34:327–334; 1995.
24. Sawada, K.; Kondo, T.; Inokuchi, T.; Aoyagi, S.: Distribution and neuropeptide content of nitric oxide synthase-containing nerve fibers in arteries and conduction system of the rat heart. Acta Anat. 160:239–247; 1997.
25. Shi, B.; Heavner, J. E.: Modification of bupivacaine toxicity by non selective vs. neuronal nitric oxide synthesis inhibition. Anesth. Analg. 84:804–809; 1997.
26. Sosunov, A. A.; Hassall, C. J.; Loesch, A.; Turmaine, M.; Burnstock, G.: Ultrastructure investigation of nitric oxide synthase-immunoreactive nerves associated with coronary blood vessels of rat and guinea pig. Cell Tissue Res. 280:575–582; 1995.
27. Stringer, J. L.; Erden, F.: In the hippocampus in vivo, nitric oxide does not appear to function as an endogenous antiepileptic agent. Exp. Brain Res. 105:391–401; 1995.
28. Theard, M. A.; Baughman, V. L.; Wang, Q.; Pelligrino, D. A.; Albrecht, R. F.: The role of nitric oxide in modulating brain activity and blood flow during seizures. Neuroreport 6:921–924; 1995.
29. Wei, H. M.; Weiss, H. R.; Sinha, A. K.; Chi, O. Z.: Effects of nitric oxide synthase inhibition on regional cerebral blood flow and vascular resistance in conscious and isoflurane-anesthetized rats. Anesth. Analg. 77:880–885; 1993.
30. Yabe, M.; Nishikawa, K.; Terai, T.; Yukioka, H.; Fujimori, M.: The effects of intrinsic nitric oxide on cardiac neural regulation in cats. Anesth. Analg. 86:1194–1200; 1998.
31. Yokoyama, M.; Hirakawa, M.; Goto, H.: Effects of vasoconstrictive agents added to lidocaine on intravenous lidocaine-induced convulsions in rats. Anesthesiology 82:574–580; 1995.