

## Effect of Lidoflazine on Cerebral Blood Flow and Neurologic Outcome When Administered After Complete Cerebral Ischemia in Dogs

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This study tested the hypothesis that lidoflazine, a calcium entry blocking drug, will improve post-ischemic neurologic outcome when administered after a period of complete cerebral ischemia, and that the improvement in outcome is related to an increase in post-ischemic cerebral blood flow (CBF). Complete cerebral ischemia was produced in 31 dogs by temporary ligation of the aorta and venae cavae. In six dogs, 10 min of complete cerebral ischemia was followed by an infusion of lidoflazine 1.0 mg · kg<sup>-1</sup> iv over 10 min. CBF and cerebral metabolic rate for oxygen (CMRO<sub>2</sub>) were measured pre-ischemia, and for 90 min post-ischemia. The results from these six dogs were compared with results previously obtained from eight untreated dogs under similar, but not identical, conditions. The CBF measured post-ischemia in the dogs administered lidoflazine did not differ from the CBF measured post-ischemia in the untreated dogs. Both groups showed an initial hyperemia post-ischemia, followed by significant decreases in CBF from control values by 35 min post-ischemia. The post-ischemic CMRO<sub>2</sub> also did not differ between lidoflazine treated and untreated groups. In 25 dogs, 11 min of complete cerebral ischemia was followed by an iv infusion of either lidoflazine 1.0 mg · kg<sup>-1</sup> or saline placebo. The same iv infusions were repeated at 8 and 16 h post-ischemia. Seven dogs were excluded from data analysis for failure to meet pre-established protocol criteria. Neurologic injury was evaluated in the remaining dogs at 48 h post-ischemia by an observer blinded to the treatment groups. Only a suggestion of improved neurologic outcome was noted in the nine dogs having received lidoflazine, when compared to the nine dogs having received saline placebo ( $P = 0.077$ ). If lidoflazine administered post-ischemia in the dog does improve neurologic outcome, then it must be by a mechanism which is independent of restitution of post-ischemic CBF. (Key words: Brain: blood flow; ischemia; metabolism; oxygen consumption. Heart: cardiac arrest. Pharmacology: lidoflazine.)

THE OCCURRENCE OF NEUROLOGIC DEFICITS following successful resuscitation from prolonged cardiac arrest remains a significant dilemma in modern medicine. Many studies have been designed to evaluate the effects of various agents or interventions in attenuating neurologic deficits following cerebral ischemia. Recently, much interest has been concentrated on the calcium entry blockers

for treating both cardiac and cerebral ischemia. A prolonged decrease in cerebral blood flow (CBF) following global ischemia (delayed post-ischemic hypoperfusion state) has been well documented,<sup>1-3</sup> and has been postulated to contribute to the severity of the neurologic deficit.<sup>3,4</sup> Studies of nimodipine, a calcium entry blocker, have shown amelioration of the delayed post-ischemic hypoperfusion state in dogs<sup>5</sup> and cats<sup>6</sup> when treated pre-ischemia, and in dogs when treated post-ischemia.<sup>7,8</sup> Improvement in neurologic outcome has been demonstrated in dogs when treated pre-ischemia with nimodipine.<sup>5</sup> Similar beneficial results have been obtained in primates treated with nimodipine following 17 min of complete cerebral ischemia.<sup>4</sup> It has been hypothesized that the improvement in post-ischemic neurologic outcome may be related to the abolition or attenuation of the delayed post-ischemic hypoperfusion state. However, this hypothesis has recently been questioned by a study showing improved post-ischemic cerebral histopathology without improvement in post-ischemic CBF<sup>9</sup> in rats receiving flunarizine either pre- or post-ischemia.

Lidoflazine, another calcium entry blocker, has been effective in the treatment of patients with ischemic heart disease,<sup>10</sup> and has been shown to have cardioprotective effects in dogs<sup>11</sup> and humans<sup>12</sup> during cardiac ischemia. The effects of lidoflazine as a cerebral protective agent have also been investigated, but the reported results have been contradictory. Lidoflazine given to dogs post-ischemia has been reported to either ameliorate<sup>13</sup> or to have no effect<sup>14</sup> on the delayed post-ischemic hypoperfusion state. Neurologic outcome in dogs treated with lidoflazine following ischemia has been reported as either improved<sup>15,16</sup> or not affected.<sup>17</sup>

To test the hypothesis that calcium entry blockers produce improvements in post-ischemic neurologic outcome and post-ischemic CBF restitution, the current study was designed to evaluate the effects of lidoflazine on post-ischemic CBF, cerebral metabolic rate for oxygen (CMRO<sub>2</sub>), and neurologic outcome in an established canine model of complete cerebral ischemia. To facilitate direct comparisons between the effects of lidoflazine and

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Received from the Department of Anesthesiology, Mayo Clinic, Mayo Medical School, Rochester, Minnesota. Accepted for publication September 30, 1986. Supported in part by research grant NS-07507 from the National Institutes of Health, Public Health Service. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, October, 1986.

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¶ Vaagenes P, Cantadore R, Safar P, Alexander H: Effect of lidoflazine on neurologic outcome after cardiac arrest. (Abstract) ANESTHESIOLOGY 59:A100, 1983.

nimodipine, our methods closely approximate the methods used by Steen *et al.*<sup>5,7</sup> to evaluate nimodipine.

### Methods

Thirty-one unmedicated fasting mongrel dogs, weighing 9.5–17.0 kg, were studied. The protocol was approved by the Institutional Animal Care and Use Committee.

#### CEREBRAL BLOOD FLOW AND METABOLISM

Six dogs were used to study the effects of lidoflazine on post-ischemic CBF and CMRO<sub>2</sub>. Anesthesia for the pre-ischemic surgical preparation was induced and maintained with 1% halothane in 60–70% nitrous oxide and oxygen. Succinylcholine 40 mg iv was given to facilitate tracheal intubation followed by a continuous infusion of 150 mg · h<sup>-1</sup>. Ventilation was controlled using a Harvard® pump set to deliver a tidal volume of 15–20 ml · kg<sup>-1</sup> and a rate adjusted to maintain PaCO<sub>2</sub> between 35–40 mmHg. Cannulae were surgically inserted into a femoral artery for blood pressure monitoring (zero reference—base of skull) and blood sampling and into a femoral vein for fluid and drug administration. A thoracotomy was performed through the right fourth intercostal space, and umbilical tapes were placed around the ascending aorta, inferior vena cava, and the superior vena cava above the azygos vein. The sagittal sinus was exposed, isolated, and, after heparinization (300–400 units · kg<sup>-1</sup> iv), cannulated as previously described.<sup>17</sup> This technique allows direct measurement of CBF, by a square-wave electromagnetic flow meter<sup>18</sup> (EP 300 API, Carolina Medical Electronics), from the anterior, superior, and lateral portions of both cerebral hemispheres representing approximately 54% of the total brain weight.<sup>17,19</sup> Intracranial pressure (ICP) was measured using an epidural fiberoptic device (LADD Research Industries, Inc., Burlington, VT), and, thereafter, the cranium was rigidly closed with Surgicel® (Johnson & Johnson) and Super Line® adhesive (Rawn Company). Body temperature, measured by esophageal and parietal epidural thermistors, was maintained between 36.9–37.1° C using heating lamps and blankets. The electrocardiogram (ECG) was monitored using needle electrodes. A four-lead, two-channel bifrontal and biparietal electroencephalogram (EEG) was recorded intermittently from electrodes glued to the skull. Arterial and sagittal sinus blood gases were determined by electrodes at 37° C (Instrumentation Laboratory 1303). End-tidal halothane and CO<sub>2</sub> were monitored with a mass spectrometer (Perkin-Elmer 1100 Medical Gas Analyzer). Blood oxygen contents were calculated from measurement of oxyhemoglobin concentrations (CO-oximeter, IL 282) and oxygen tensions.<sup>20</sup> CMRO<sub>2</sub> was calculated as the product of CBF and the arterial-sagittal sinus blood oxygen content difference. Blood glucose was determined by a membrane-

bound enzyme technique (Yellowsprings Instruments Model 23A Glucose Analyser). The dogs' ears were packed with cotton, and their eyes were covered.

Following surgical preparation, halothane was discontinued. When the end-tidal halothane concentration was less than 0.1% for 15 min, pre-ischemia control measurements of physiologic and cerebral variables were obtained three to five times over a 15–30 min period. Complete cerebral ischemia for 10 min duration was achieved by using previously described methods.<sup>1,21,\*\*</sup> Briefly, umbilical tapes around the ascending aorta and venae cavae were simultaneously occluded, confining the cardiac output to the coronary and pulmonary circulation. Occlusion was verified by mean arterial pressure (MAP) of zero, rapid onset of an isoelectric EEG, cessation of flow from the sagittal sinus, and no increase in the ICP. The sagittal sinus cannula was occluded to avoid aspiration of air. Coincident with the EEG becoming isoelectric, nitrogen was substituted for nitrous oxide in the inspired gas. After exactly 10 min, the tape around the aorta was released, the tapes around the venae cavae having been released 15–20 s earlier.

Immediate post-ischemic treatment consisted of sodium bicarbonate 20 mEq iv, normal saline 100–300 ml iv, and transient hyperventilation. All dogs had a MAP greater than 60 mmHg within 1 min post-ischemia. Two minutes post-ischemia, lidoflazine 1.0 mg · kg<sup>-1</sup> in a volume of 6.4 ml was administered iv over a 10-min period. The lidoflazine diluent consisted of the following: 10.0 g ethanol 99%, 1.0 g acetic acid, 15.0 g glycerol, and water to 100 ml. For a period of 90 min post-ischemia, arterial blood gases, MAP, heart rate, temperature, blood glucose, ECG, EEG, CBF, and CMRO<sub>2</sub> were intermittently measured. Following the study period, all dogs were killed, and the brains were removed for weighing so that total CBF and CMRO<sub>2</sub> could be expressed as a function of brain weight.

#### NEUROLOGIC OUTCOME STUDIES

In the 25 dogs used to evaluate neurologic recovery following complete cerebral ischemia, 70% nitrous oxide and oxygen was inspired. Succinylcholine 40 mg was given iv to facilitate tracheal intubation. Ventilation was controlled as in the previously described dogs. Cannulae were inserted percutaneously into a femoral artery for blood pressure monitoring and blood sampling, and into a peripheral vein for fluid and drug administration. After infiltration of the chest wall with 20 ml of procaine 0.5%, a thoracotomy was performed, and umbilical tapes were placed as described above. An ECG and a four-lead, two-

\*\* Brockman SK, Jude JR: The tolerance of the dog brain to total arrest of circulation. *Bulletin of The Johns Hopkins Hospital* 106:74–80, 1960.

channel bifrontal and biparietal EEG were recorded using needle electrodes. Arterial blood gases, end-tidal CO<sub>2</sub>, and blood glucose were monitored. Esophageal temperature was maintained at 36.5–37.5° C using heat lamps and blankets. All dogs received penicillin 600,000 units i.m. and streptomycin in 500 mg i.m. pre-ischemia.

Following surgical preparation, control measurements of arterial blood gases, MAP, heart rate, temperature, and blood glucose were obtained. The inspired gas was changed to 100% oxygen 5 min prior to ischemia, and to room air 20 min post-ischemia (provided PaO<sub>2</sub> > 60 mmHg). Complete cerebral ischemia was achieved as described above; however, in this series, ischemia was maintained for 11 min rather than 10 min. This period of ischemia in pilot studies produced control animals that survived to 48 h post-ischemia, but were uniformly injured neurologically. Immediately post-ischemia, all dogs received sodium bicarbonate 20 mEq iv, saline 50 ml iv, and transient hyperventilation. Epinephrine 25–50 µg boluses iv were given as needed to obtain a MAP > 60 mmHg within 1 min post-ischemia. Metabolic acidosis was treated with additional sodium bicarbonate. Two minutes post-ischemia, ten dogs received lidoflazine 1.0 mg · kg<sup>-1</sup> iv (same diluent as previously described) infused over 10 min in a volume of 6.4 ml, and 15 dogs received saline placebo 6.4 ml iv infused over 10 min. At 8 and 16 h post-ischemia, the same respective iv infusions of lidoflazine or placebo were repeated.<sup>16</sup> The thoracotomy was closed, and residual air was aspirated through a chest tube which was later removed. Arterial blood gases were measured at 5 and 20 min post-ischemia, and as needed thereafter. Mechanical ventilation was continued until spontaneous ventilation maintained the PaCO<sub>2</sub> < 45 mmHg. The dogs were then extubated and observed. Arterial blood gases were measured at 15–30 min post-extubation, and dogs were returned to their cages, provided PaCO<sub>2</sub> < 45 mmHg and PaO<sub>2</sub> > 60 mmHg. At 24 h post-ischemia, dogs received water *po ad lib* and/or an iv infusion of dextrose 5% in lactated Ringer's solution (200 ml) as determined by physical exam.

Forty-eight hours post-ischemia, dogs were evaluated neurologically and assigned to one of four categories<sup>21</sup> by an observer blinded to the treatment groups. Grade 1 (no damage) dogs ate and behaved normally with coordinated movements. Grade 2 (moderate damage) dogs could stand alone, but were ataxic or exhibited partial to complete blindness. Grade 3 (severe damage) dogs could not stand alone or were comatose. Grade 4 (dead) dogs died within 48 h post-ischemia. The surviving dogs were killed after the 48 h evaluation, and at necropsy the thorax was examined to assess the presence of pulmonary injury which may have affected the neurologic outcome.

Results of the CBF and CMRO<sub>2</sub> study were compared with the results of a recent study performed in our lab-

oratory by Stangland *et al.*<sup>22</sup> using similar, but not identical, pre-ischemic conditions. Stangland *et al.* substituted N<sub>2</sub> 60–70% for N<sub>2</sub>O 60–70% at 5 min pre-ischemia. Anesthesia was maintained with bupivacaine 0.25%, infiltrated into wound edges. Pre-ischemia control measurements of CBF and CMRO<sub>2</sub> were obtained with dogs receiving N<sub>2</sub> 60–70% in O<sub>2</sub>, as opposed to N<sub>2</sub>O 60–70% in O<sub>2</sub> in the current study. Statistical comparisons of cerebral and physiologic variables between lidoflazine treated and untreated dogs<sup>22</sup> were performed using Student's *t* test for unpaired data. Comparisons between pre- and post-ischemic variables were examined by one-way analysis of variance. Post-ischemic variables showing significant differences were compared to pre-ischemic control values, using the paired *t* test with the Bonferroni correction for multiple comparisons.<sup>23</sup> Statistical comparison of neurological outcome (1 *versus* 2–4 and 1,2 *versus* 3,4) between lidoflazine-treated and placebo-treated dogs was made using the Fisher exact test.<sup>24</sup> A difference of *P* < 0.05 or its Bonferroni corrected equivalent was considered significant. Data are presented as mean ± SEM.

## Results

In the animals in whom CBF and CMRO<sub>2</sub> were measured, selected values of MAP, blood gases, ICP, and blood glucose are presented in Table 1. There were no significant differences in blood gases between the lidoflazine-treated and the previously reported<sup>22</sup> untreated dogs during the pre- or post-ischemic periods. PaO<sub>2</sub> and PssO<sub>2</sub> were not statistically analyzed because of differences in the inspired F<sub>I</sub>O<sub>2</sub> between lidoflazine and untreated dogs. In both lidoflazine and untreated dogs, the PaCO<sub>2</sub> showed a significant increase, and the pH showed a significant decrease from control values in the immediate post-ischemic period. Both values returned to baseline by 15 min post-ischemia. In the lidoflazine-treated dogs, beginning at 3 min post-ischemia and for the duration of the study, the MAP remained significantly less than the control MAP, and was significantly less than the MAP for the untreated dogs, only at 3, 30, and 60 min post-ischemia. The lowest MAP recorded, 68 ± 9 mmHg, was in the lidoflazine-treated dogs at 2 min post-ischemia. The decrease in MAP in the lidoflazine-treated dogs appeared to coincide temporally with the infusion of lidoflazine.

CBF data for the lidoflazine-treated group and the untreated dogs of Stangland *et al.*<sup>22</sup> are presented in figure 1. CBF in the lidoflazine-treated dogs decreased significantly from control CBF at 3 min post-ischemia and, thereafter, increased, reaching a peak at 7 min post-ischemia. Infusion of lidoflazine, beginning at 2 min post-ischemia, coincided with the initial decrease in CBF noted in the lidoflazine-treated dogs. The CBF in the untreated

TABLE 1. Selected Values of Mean Arterial Pressure (MAP), Intracranial Pressure (ICP), Arterial Blood Gases, Sagittal Sinus PO<sub>2</sub>, and Blood Glucose in Untreated\* and Lidoflazine-treated Dogs Pre- and Post-ischemia (mean ± SEM)

State	n	MAP (mmHg)	ICP (mmHg)	PaO <sub>2</sub> (mmHg)	PaCO <sub>2</sub> (mmHg)	pH	Buffer Base (mEq·l <sup>-1</sup> )	PssO <sub>2</sub> (mmHg)	Glucose (mg·dl <sup>-1</sup> )
Control									
Untreated	8	113 ± 7	6 ± 1	147 ± 5	38 ± 1	7.32 ± 0.01	40 ± 0	42 ± 1	107 ± 8
Lidoflazine	6	123 ± 8	4 ± 2	131 ± 5	39 ± 1	7.32 ± 0.01	40 ± 1	60 ± 3	132 ± 16
1 Min post-ischemia									
Untreated	8	114 ± 10	24 ± 5†	319 ± 57‡	57 ± 3†	7.22 ± 0.02†	42 ± 2	67 ± 5†	186 ± 30
Lidoflazine	6	82 ± 13	10 ± 5	87 ± 8†	60 ± 3†	7.19 ± 0.03†	40 ± 1	46 ± 4†	300 ± 27††
30 Min post-ischemia									
Untreated	8	105 ± 6	1 ± 0†	128 ± 4§	39 ± 1§	7.34 ± 0.01§	42 ± 1§	34 ± 1§	151 ± 16§
Lidoflazine	6	76 ± 6††	1 ± 1	105 ± 10	40 ± 1	7.32 ± 0.01	41 ± 1	42 ± 2†	219 ± 21††
90 Min post-ischemia									
Untreated	8	99 ± 10	1 ± 1†	129 ± 6	38 ± 1	7.30 ± 0.01	39 ± 1	27 ± 2†	148 ± 17
Lidoflazine	6	81 ± 12†	1 ± 0	133 ± 6	40 ± 1	7.30 ± 0.02	40 ± 1	27 ± 2†	163 ± 32

\* Data for untreated dogs from Stangland *et al.*<sup>22</sup> PaO<sub>2</sub>, PssO<sub>2</sub> not statistically analyzed with unpaired *t* tests because of differences in experimental conditions.

† Significantly different from control by paired *t* test with Bonferroni correction (*P* < 0.05/Bonferroni correction considered significant).

‡ FiO<sub>2</sub> = 1.0.

§ 35 min sample.

†† Significantly different from untreated by unpaired *t* test (*P* < 0.05 considered significant).

dogs increased during the first 3 min post-ischemia. By 35 min post-ischemia, the CBF had decreased to flows significantly less than pre-ischemic values in both groups, and remained so for the duration of the study. There were no significant differences in CBF between the lidoflazine and untreated groups at any time during the study, except during the control period.

CMRO<sub>2</sub> values for both the lidoflazine and untreated groups are presented in figure 2. There were no significant differences between groups at any time, except for the lidoflazine-treated dogs having a significantly higher control CMRO<sub>2</sub> than the untreated dogs. Both groups had a decrease in CMRO<sub>2</sub> by 15 min post-ischemia, followed by a gradual increase. Values for lidoflazine-treated dogs remained significantly below control values from 15 min post-ischemia until the end of the study.

In both the lidoflazine and untreated groups, the ICP showed a significant increase 1–3 min post-ischemia, followed by a return to control values or less by 15 min post-ischemia. The untreated dogs had a significantly greater ICP at 3 min post-ischemia than the lidoflazine-treated dogs. There were no significant differences in blood glucose between groups pre-ischemia; however, both groups had significant increases in blood glucose 1–3 min post-ischemia. The lidoflazine-treated group had significantly greater blood glucose values than the untreated group at 1 and 30 min post-ischemia. Following aortic occlusion, the EEG became isoelectric within 57 ± 14 s in the untreated dogs and 31 ± 3 s in the lidoflazine-treated dogs (*P* < 0.1). The EEG demonstrated return of activity (spindles) post-ischemia at 16 ± 4 min in the untreated dogs and 18 ± 3 min in the lidoflazine-treated dogs (*P* < 0.5).

In the neurologic outcome study, seven dogs were excluded from data analysis prior to final neurologic eval-

uation by the blinded observer for failure to meet pre-established protocol criteria. One dog from either treatment group was excluded when found to have a pneumothorax at 8 h post-ischemia. An additional five dogs from the placebo-treated group were excluded for failure to maintain a PaO<sub>2</sub> > 60 mmHg post-ischemia. Of

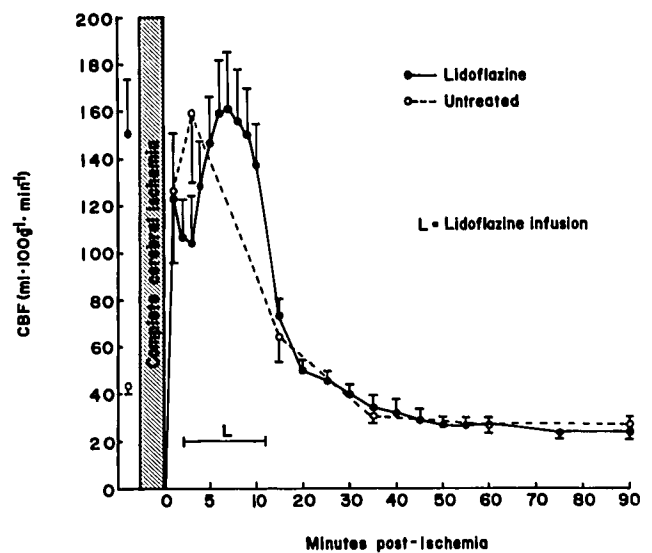


FIG. 1. CBF values (mean ± SEM) pre-ischemia and after 10 min of complete cerebral ischemia for lidoflazine-treated and previously reported<sup>22</sup> untreated dogs. Pre-ischemia, there were significant differences in CBF secondary to similar, but not identical, experimental conditions. CBF in the lidoflazine-treated dogs showed a brief initial decrease post-ischemia; this was followed by a transient period of hyperemia seen in both groups. Subsequently, there was a period of prolonged hypoperfusion in both groups. Post-ischemia, there were no significant differences between the two groups. Note the change in time scale after 10 min.

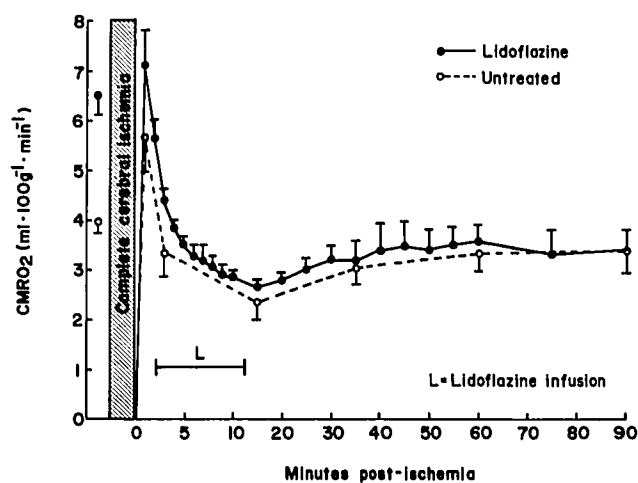


FIG. 2.  $CMRO_2$  values (mean  $\pm$  SEM) pre-ischemia and after 10 min of complete cerebral ischemia for lidoflazine-treated and untreated dogs. Pre-ischemia there were significant differences in  $CMRO_2$  secondary to similar, but not identical, experimental conditions. Post-ischemia, there were no significant differences between the two groups. Note the change in time scale after 10 min.

## Discussion

In experimental animals following a prolonged period of complete cerebral ischemia, there is a 10–15 min period of reactive hyperemia followed by a prolonged period of hypoperfusion.<sup>1–3</sup> This post-ischemic hypoperfusion state has also been reported in humans,<sup>25</sup> and may be a factor contributing to the ultimate neurologic deficit.<sup>3,4</sup> Accordingly, attenuation of post-ischemic hypoperfusion may improve neurologic outcome following an ischemic episode.<sup>3–5,7,15</sup> Nimodipine, a calcium entry blocker, administered post-ischemia significantly increased post-ischemic CBF in dogs,<sup>7</sup> and significantly improved neurologic outcome in primates.<sup>4</sup> The current study was undertaken to see if lidoflazine, another calcium entry blocker, might have effects similar to nimodipine on post-ischemic CBF and neurologic outcome in the dog. In contrast to results of the nimodipine study,<sup>7</sup> lidoflazine, administered iv post-ischemia, had no effect on CBF and only a suggestion of improvement in neurologic outcome.

The comparison of historical controls to the six dogs used in the current CBF and  $CMRO_2$  study deserves comment. The model used in this experiment has been validated<sup>18–20</sup> and described<sup>1,5,7,8,22</sup> in many previous studies. Due to the reproducibility of the delayed post-ischemic hypoperfusion state with this model, new data has often been compared to historical controls. The study by Stangland *et al.*<sup>22</sup> employed some minor changes in the model by altering the type of anesthesia administered and the time sequence of  $N_2O$  and  $N_2$  administration. Stangland *et al.* infiltrated the chest wall with bupivacaine 0.25% and obtained control CBF and  $CMRO_2$  values with dogs breathing  $N_2$  60–70% in  $O_2$ . The current study differed in that no local anesthesia was used, and control CBF and  $CMRO_2$  values were obtained with the dogs breathing  $N_2O$  60–70% in  $O_2$ . Nitrous oxide 60% has been shown to significantly increase both CBF and  $CMRO_2$  in this model.<sup>26</sup> The CBF and  $CMRO_2$  results found by Stangland *et al.* have pointed out the reproducibility of this model in reliably producing the delayed post-ischemic hypoperfusion state, despite minor changes in the protocol, and wide variability in pre-ischemic CBF and  $CMRO_2$ . This is consistent with the findings of previous studies<sup>1,8,22</sup> from our laboratory, in which post-ischemic CBF in untreated dogs did not differ, despite large differences in pre-ischemic CBF.

Our CBF results are in agreement with some other reports. Dean *et al.*,<sup>14</sup> using a radiolabelled microsphere technique, measured CBF before and after 12 min of complete cerebral ischemia in dogs. Ischemia was produced by cross-clamping the aorta. As in our study, lidoflazine  $1.0 \text{ mg} \cdot \text{kg}^{-1}$  iv given post-ischemia was found to have no effect on post-ischemic CBF when compared

the 18 remaining dogs, there was no statistically significant difference in neurologic outcome between lidoflazine- and placebo-treated dogs when evaluated at 48 h post-ischemia (Table 2). Six of the nine lidoflazine-treated dogs were judged grade 1 (normal), whereas seven of the nine placebo-treated dogs were judged grades 2–4 (abnormal). Comparison of grade 1 (normal) to grades 2–4 (abnormal) achieved a level of  $P = 0.077$ . When comparing grades 1–2 to grades 3–4, a level of  $P = 0.5$  was achieved. There were no significant differences in MAP, blood glucose, or arterial blood gases between the groups, either pre- or post-ischemia (Table 3). Following aortic occlusion, the EEG became isoelectric within  $53 \pm 6$  s in the placebo-treated dogs and  $48 \pm 4$  s in the lidoflazine-treated dogs ( $P < 0.5$ ). In the immediate post-ischemic period, one dog from the lidoflazine-treated group and one from the placebo-treated group required iv boluses of epinephrine totalling, 100 and 300  $\mu\text{g}$ , respectively, to obtain MAP  $> 60$  mmHg within 1 min post-ischemia.

TABLE 2. Grade of Neurologic Damage at 48 h Post-ischemia\*

	Grade of Neurologic Damage				
	n	1 (None)	2 (Moderate)	3 (Severe)	4 (Dead)
Placebo	9	2	3	3	1
Lidoflazine	9	6	—	2	1

\* By Fisher exact test, comparison of grade 1 (normal) to grades 2–4 (abnormal) between lidoflazine- and placebo-treated groups achieved a level of  $P = 0.077$ .

TABLE 3. Mean Arterial Pressure (MAP), Arterial Blood Gases, and Blood Glucose at Control, 5 Min Post-ischemia, and at the Time of Extubation (Mean  $\pm$  SEM) in the Neurologic Outcome Groups.

State	n	MAP (mmHg)	PaO <sub>2</sub> (mmHg)	PaCO <sub>2</sub> (mmHg)	pH	Buffer Base (mEq · l <sup>-1</sup> )	Glucose (mg · dl <sup>-1</sup> )
Control							
Placebo	9	153 $\pm$ 5	412 $\pm$ 14*	37 $\pm$ 1	7.34 $\pm$ 0.01	41 $\pm$ 1	93 $\pm$ 9
Lidoflazine	9	153 $\pm$ 3	395 $\pm$ 14*	37 $\pm$ 1	7.34 $\pm$ 0.01	40 $\pm$ 1	91 $\pm$ 4
5 Min post-ischemia							
Placebo	9	141 $\pm$ 10	419 $\pm$ 44*	40 $\pm$ 2	7.33 $\pm$ 0.03	41 $\pm$ 1	175 $\pm$ 21
Lidoflazine	9	132 $\pm$ 9	405 $\pm$ 30*	43 $\pm$ 2	7.24 $\pm$ 0.02	38 $\pm$ 1	159 $\pm$ 11
Extubation							
Placebo	9	>70	83 $\pm$ 4†	37 $\pm$ 2	7.40 $\pm$ 0.02	44 $\pm$ 1	—
Lidoflazine	9	>70	83 $\pm$ 4†	35 $\pm$ 1	7.39 $\pm$ 0.02	42 $\pm$ 0	—

\* FiO<sub>2</sub> = 1.0.

† FiO<sub>2</sub> = 0.21.

to untreated dogs. Vaagenes *et al.*<sup>16</sup> in preliminary studies used a hydrogen clearance method to measure CBF in a few of their dogs at 8 and 16 h post-ischemia (following 10 min of ventricular fibrillation) and found no difference in CBF between lidoflazine-treated (1.0 mg · kg<sup>-1</sup> iv) and saline-treated dogs.

Our results conflict with those of White *et al.*<sup>13</sup> In that study, dogs were anesthetized with ketamine and cardiopulmonary bypass was begun. Regional CBF was measured using a thermal washout technique.<sup>27</sup> Ischemia was induced by stopping the bypass pump for 20 min. Lidoflazine 1.0 mg · kg<sup>-1</sup> was infused iv over 15 min post-ischemia. CBF was 75% of control at 20 and 90 min post-ischemia in lidoflazine-treated dogs, whereas untreated dogs had CBF values of 55% and 5% of control at 20 and 90 min, respectively. However, the findings of White *et al.*<sup>13</sup> must be questioned in light of a recent study which failed to validate their thermal washout technique for the measurement of CBF.††

On balance, the existing data strongly support the conclusion that lidoflazine, unlike nimodipine, has no effect on the delayed post-ischemic hypoperfusion state.

Our lidoflazine-treated dogs did not have statistically significantly improved neurologic outcomes (at the *P* < 0.05 level) at 48 h post-ischemia when compared to placebo-treated dogs. However, the suggestion of improved outcome in our study (*P* = 0.077) may be consistent with two other reports. Winegar *et al.*<sup>15</sup> subjected dogs to 15 min of cardiac arrest. Following resuscitation (requiring 6–10 min), dogs received lidoflazine 1.0 mg · kg<sup>-1</sup> iv and intensive care treatment for the next 12 h. Lidoflazine-treated dogs showed significantly better neurologic scores when compared to placebo-treated dogs. Vaagenes *et al.*<sup>16</sup> evaluated neurologic status in dogs subjected to 10 min of ventricular fibrillation followed by

lidoflazine 1.0 mg · kg<sup>-1</sup> iv immediately post-ischemia and repeated at 8 and 16 h post-ischemia. Dogs received intensive care therapy for the next 96 h. Lidoflazine-treated dogs scored significantly better neurologically than placebo-treated dogs. By contrast, in a similar protocol which differed by using an asphyxial model of cardiac arrest, Vaagenes *et al.*<sup>1</sup> found no difference in the neurologic outcome of lidoflazine-treated *versus* placebo-treated dogs. They postulated that asphyxial arrest imposes a more severe insult to the animals which may offset any beneficial effects of the lidoflazine treatment.

On balance, the combined data do not permit any conclusion to be drawn as to the usefulness of lidoflazine in improving neurologic outcome when administered post-ischemia.

In contrast to the current study, Steen *et al.*,<sup>5,7</sup> using the same model we have used, found that nimodipine iv given pre- or post-ischemia to dogs resulted in a significant increase in CBF during the post-ischemic period. Similarly, Milde *et al.*,<sup>8</sup> again in the same model, have shown that nimodipine administered iv at either 15, 30, or 60 min post-ischemia will attenuate or reverse the post-ischemic hypoperfusion state. In neurologic outcome studies in dogs, Steen *et al.* found that nimodipine administered iv pre-ischemia was associated with significant improvement in neurologic outcome,<sup>5</sup> whereas post-ischemic treatment with nimodipine iv was associated with equivocal neurologic outcome.<sup>7</sup> A subsequent study by Steen *et al.*,<sup>4</sup> using a more sensitive primate model, showed significant improvement in neurologic outcome following 17 min of complete cerebral ischemia and treatment with nimodipine. Taken together, these studies would suggest that the amelioration of post-ischemic cerebral hypoperfusion by nimodipine may contribute to the subsequent neurologic improvement observed post-ischemia in treated animals.

On the other hand, Deshpande and Wieloch,<sup>9</sup> using a rat model of forebrain ischemia, studied the post-ischemic

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effects of the calcium entry blocker, flunarizine, on CBF and cerebral histopathology. At 60 min post-ischemia, they found no significant differences in local CBF (measured using a  $^{14}\text{C}$ -iodoantipyrine technique), following a 9-min episode of forebrain ischemia in rats treated with flunarizine when compared to placebo-treated rats. At 7 days post-ischemia, histopathologic evaluation revealed significantly decreased numbers of irreversibly damaged neurons in the flunarizine-treated rats when compared to placebo-treated rats. This study, in contrast to the studies of nimodipine, would suggest that amelioration of post-ischemic CBF is not necessary for the subsequent improvement in cerebral histopathology noted in flunarizine-treated rats.

The mechanism(s) by which the calcium entry blockers might be effective in ameliorating post-ischemic neurologic injury remains uncertain. Siesjö<sup>3,28</sup> and White *et al.*<sup>29</sup> have reviewed the possible mechanisms which may explain the role of calcium ( $\text{Ca}^{++}$ ) in relation to cellular injury. To summarize, prolonged ischemia leads to depletion of cellular ATP stores, with the loss of normal cell membrane ionic gradients. As membrane potential decreases, voltage-dependent  $\text{Ca}^{++}$  gates open, with resultant massive intracellular influx of  $\text{Ca}^{++}$ . Increasing intracellular concentrations of  $\text{Ca}^{++}$  activate the enzyme phospholipase  $\text{A}_2$ , with subsequent release of free fatty acids. With recirculation and reoxygenation to previously ischemic cells, free fatty acids are oxidized, with the formation of prostaglandins, thromboxane  $\text{A}_2$ , and, possibly, free radicals. Thromboxane  $\text{A}_2$  has potential vasoconstrictor activity. Free radical formation can lead to fragmentation of membrane-bound fatty acids and protein cross-linkages with enzyme inactivation. Calcium may also have other adverse effects. Increased intracellular  $\text{Ca}^{++}$  tends to increase membrane ionic conductances so as to sustain and enhance further  $\text{Ca}^{++}$  leakage into cells. Along with  $\text{Ca}^{++}$ , there is an increase in membrane permeability to other ions, such as sodium and chloride, which are followed by water, leading to cellular swelling and brain edema. In addition, increased membrane permeability may divert respiratory energy from ATP production to futile  $\text{Ca}^{++}$  cycling. Thus, there are many events in which  $\text{Ca}^{++}$  may participate which hypothetically lead to cell injury. The calcium entry blockers may act to inhibit or attenuate some of the described events. While the nimodipine-induced changes in post-ischemia CBF are significant and readily measurable, it may be that other, less easily identifiable events are more important contributors to the improved neurologic outcome.

In summary, lidoflazine treatment following a period of complete cerebral ischemia had no effect on the delayed post-ischemic hypoperfusion state. However, while not statistically significant, there was a suggestion of improved neurologic outcome following a period of complete ce-

rebral ischemia in dogs administered lidoflazine post-ischemia. As was true in the case of nimodipine, we believe that confirmation (or denial) of this suggestion will require further studies in a more sensitive and relevant primate model.

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