Effect of Excitatory Amino Acid Receptor Blocker MK-801 on Overall, Neurologic, and Morphologic Outcome after Prolonged Cardiac Arrest in Dogs

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Excitatory amino acids accumulating in the brain during ischemia may cause selective neuronal damage postischemia. This hypothesis was tested in a series of studies using MK-801, an N-methyl-D-aspartate (NMDA) receptor blocker, in a reproducible outcome model of prolonged cardiac arrest in dogs. After normothermic ventricular fibrillation cardiac arrest, the dogs were resuscitated with closed-chest femoral veno-arterial cardiopulmonary bypass. At 4 h they were separated from bypass, ventilation was controlled for 20 h, and intensive care was continued to 96 h. In Study I, ventricular fibrillation cardiac arrest (no-flow) was 17 min; starting immediately with reperfusion, MK-801 1200 mg/kg (n = 5) or an equal volume of placebo (n = 5) was infused over 12 h in blinded, randomized fashion. In Study II, the duration of the no-flow period was reduced to 15 min, and MK-801 2400 mg·kg\(^{-1}\) (n = 4) or placebo (n = 4) was infused. In Study III, no-flow lasted for 15 min, and MK-801 2400 mg/kg was started 30 min before ventricular fibrillation (n = 4); comparison was with Study II controls. In all three studies, MK-801 plasma concentrations peaked at >50 ng/ml and were 15–30 ng/ml over 12 h. All 22 dogs of experiments within protocol survived with severe brain damage. MK-801 delayed return of pupillary reactivity, EEG activity, consciousness, and respiration, necessitating longer periods of controlled ventilation. Neurologic deficit scores, overall performance categories, and brain and heart morphologic damage scores at 96 h did not differ between placebo and MK-801 pretreatment or post-treatment groups. These negative outcome results after prolonged cardiac arrest do not negate the hyperexcitability hypothesis of selective vulnerability, but suggest the existence of additional mechanisms of secondary brain damage. (Key words: Brain, ischemia; resuscitation; MK-801; NMDA receptor blocker. Heart, arrest; resuscitation. Pharmacology, anticonvulsants: MK-801. Resuscitation.)

NEUROLOGIC RECOVERY after cardiac arrest (temporary complete global brain ischemia) depends on the duration of ischemia, details of resuscitation, and occurrence and management of secondary derangements—the postresuscitation syndrome.\(^1,2,3\) After cardiac arrest (no-flow) of 10 min or longer, in spite of arterial normotension, there is global cerebral hypoperfusion.\(^4,5\) Mechanisms underlying the selective vulnerability of neurons in the hippocampus CA1 layer, neocortex, striatum, and cerebellar cortex to temporary global ischemia,\(^6\) epilepsy,\(^7,8\) or hypoglycemia\(^9\) are not clear.

Excitatory neurotransmitters (e.g., glutamate, aspartate) have been shown to accumulate to neurotoxic concentrations during ischemia.\(^10–12\) The excitotoxic hypothesis\(^11,12\) suggests that excessive release of glutamate during ischemia results in selective hyperexcitation after ischemia. This causes overactivity at NMDA receptors, which leads to postsynaptic ionic fluxes and opening of calcium channels, causing neuronal damage.\(^13–17\)

The anticonvulsant agent, 5-methyl-10,11-dihydro-5H-dibenzo (a, d) cyclohepten-5,10-imine (dizocilpine maleate, i.e., MK-801, Merck, Sharp & Dohme, West Point, PA), is a potent, noncompetitive antagonist of the NMDA subtype of glutamate receptors.\(^18–21\) The physiologic effects and neuronal protection by MK-801 seem similar to those reported for the dissociative anesthetics phencyclidine (PCP) and ketamine.\(^22–25\)

MK-801 has recently been reported to protect against neuronal degeneration in animal models of (incomplete) focal ischemia,\(^26–28\) neonatal hypoxia,\(^29\) and hippocampal ischemic damage in the gerbil.\(^30,31\) Hippocampal neurons in (incomplete) forebrain ischemia of rats were protected by MK-801 in three studies,\(^20,25,32\) but no hippocampal protection was found in another similar rat model\(^33\) (see Discussion). Beneficial effects appeared more likely when the drug was given before (protection) than after the insult (resuscitation), and when MK-801 plasma levels were at least 15–20 ng/ml.

At the time of this study (February 1988), no report was available on a study of the effects on outcome of MK-801 when given for protection or resuscitation after cardiac arrest. Therefore, the objective of this study\(^34\) was to determine with certainty, in a narrowly controlled and reproducible cardiac arrest outcome model in dogs,\(^22,25–30\)
whether or not MK-801 protects against or resuscitates from ischemic-anoxic encephalopathy.

Methods

This project was approved by the Animal Care and Use Committee of the University of Pittsburgh School of Medicine. We used 30 custom-bred male hunting dogs, from the same breeding colony, aged 10 (8–12) months and weighing 22 (18–25) kg. Three dogs were used for preliminary experiments to rule out major side effects that could offset a beneficial cerebral effect. Of the 27 definitive experiments, five were eliminated because of experimental errors (see exclusions below). The 22 that followed protocol represented Study I (n = 10) with ventricular fibrillation (VF) cardiac arrest (no-flow) of 17 min and postarrest MK-801 or placebo; Study II (n = 8) with VF 15 min and postarrest large-dose MK-801 or placebo; and Study III (n = 4) with VF 15 min and pre plus post-arrest MK-801.

General Protocol

Preparation. In all dogs of all three studies, anesthesia was induced with diazepam 0.5 mg/kg plus fentanyl 5.0 μg/kg iv, followed by N₂O:O₂ 66:33% plus halothane via face mask, until tracheal intubation and mechanical IPPV. Tidal volumes were 15–20 ml/kg at a frequency adjusted to keep the end-tidal PCO₂ at 30–35 mmHg (4–5%). Hydration was with Ringer’s solution iv, 5 ml · kg⁻¹ · h⁻¹. A gastric tube and a bladder catheter were inserted.

Monitoring. Continuously monitored were: electrocardiogram (ECG), heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), end tidal PCO₂, core temperature in the pulmonary artery (Tpa), and electroencephalogram (EEG). Intermittently monitored were: PaO₂, PaCO₂, pH, base excess (BE), blood glucose, serum electrolytes, hematocrit, hemoglobin, and activated clotting time (ACT) during cardiopulmonary bypass. Controlled pre- and postarrest were: Tpa at 38.0 ± 0.5°C; MAP at 100 ± 15 mmHg; CVP and PAOP at 5–15 mmHg; cardiac output at >50% baseline; PaCO₂ at 30–35 mmHg; PaO₂ at >100 mmHg (to 24 h); BE at ±7 mEq/l, and blood glucose at 90–175 mg/dl prearrest.

Insult. After prearrest baseline measurements, each dog was paralyzed with pancuronium and the lungs were ventilated with O₂ 100% for 1 min, then room air for 4 min. This reduced the effect of anesthesia in a standardized manner. Then VF cardiac arrest was induced by external transthoracic electric shock and IPPV was stopped. EEG activity ceased within 15 s.

Resuscitation. After normothermic VF (no-flow) for 15 or 17 min, reperfusion was with total cardiopulmonary bypass for 3–5 min. This was followed by defibrillation and continued partial cardiopulmonary bypass (assisted circulation) for 4 h. The closed-chest cardiopulmonary bypass method used was venoarterial pumping (by Biomedicus centrifugal nonocclusive self-regulating pump) from a thin-walled multi-hole venae cavae catheter (inserted prearrest via the right external jugular vein) via a membrane oxygenator (Sci-Med Corporation, Minneapolis, MN) (which also served as filter and bubble trap), through a flow meter, into a short femoral artery cannula. The circuit was primed with dextran 40 in isotonic saline, plus Ringer’s solution 50:50. This decreased the hematocrit transiently from 45 ± 4% prearrest to 25 ± 3% for 4 h postarrest. Hematocrit was restored to 40 ± 3% thereafter. Cardiopulmonary bypass was with heparinization for 4 h (ACT > 4.5 min). To control MAP at 100 mmHg during and after bypass, we used epinephrine before defibrillation and norepinephrine thereafter. During total bypass we controlled blood flow at >100 ml/kg, and decreased flow rates from 1 to 4 h of partial bypass. Immediately after defibrillation, moderate hypertension of MAP > 140 mmHg was induced for 5–10 min to enhance reperfusion. PICO₂ = 100% was used to 2 h.

Intensive Care. From 2–20 h, N₂O:O₂ 50:50% was used to provide analgesia and pancuronium to insure paralysis. Fentanyl 100 μg/dog iv, maximally every 4 h, was to be given only for sustained hypertension (MAP > 130 mmHg) and mydriasis (Fentanyl was rarely needed). Pulmonary care included control of blood gases, humidification of inhaled gases, and intermittent endotracheal suction, sighing, and position change. After IPPV to 20 h, the pancuronium effect was reversed with neostigmine 1 mg plus atropine 0.4 mg iv and a standardized attempt at weaning from IPPV was made. The endotracheal tube was removed when carinal and upper airway reflexes were active and cardiovascular-pulmonary variables were stable. After tracheal extubation, O₂ was administered by face mask. If PaO₂ tended to be <80 mmHg or PaCO₂ > 40 mmHg during spontaneous breathing, the trachea was reintubated and IPPV continued. From 20–72 h, seizures, opisthotonus, severe running movements, and exhaustive hyperventilation were controlled with diazepam 0.1 mg/kg iv as needed. Dextrose 5% in NaCl 0.45% was given only from 6–96 h. After 24 h, oral fluids and food were given when possible. Intensive care to 96 h was standard in all animals.

STUDY I—MK-801 AFTER VF CARDIAC ARREST OF 17 MIN

Ten dogs were subjected to VF cardiac arrest of 17 min (table 1). The objective was to test the effect of MK-
Table I. Study I—MK-801 Standard Dose* after VF Cardiac Arrest of 17 Min in Dogs (Randomized Concurrent Controls with Placebo)

<table>
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<tr>
<th>Exp. No.</th>
<th>Dog No.†</th>
<th>Pupil Light Reflex Return (min)</th>
<th>EEG Return (min)</th>
<th>IPPV (b)</th>
<th>Survived (b)</th>
<th>Best 24–96 h</th>
<th>Final at 96 h</th>
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<td>60 ± 47</td>
<td>72 ± 24</td>
<td>150 ± 54</td>
<td>70 ± 32</td>
<td>55 ± 11</td>
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* MK-801 300 μg/kg iv 0–5 min postarrest, plus 75 μg·kg⁻¹·h⁻¹ for 12 h iv.
† Dog numbers in chronologic sequence of experiments.

801 in a dose assumed to be therapeutic, infused over 12 h postarrest, on overall and cerebral recovery and outcome to 96 h. VF 17 min was selected first because previously, using the same model, VF 20 min had resulted in survival with neurologic dysfunction,[46] whereas VF 15 min[46] had resulted in neurologic recovery in dogs that were mildly hypotensive (35–36°C) during arrest.[1,39,41]

Control group (n = 5) and MK-801 group (n = 5) received the same standard therapy. The drug infusion, from vials containing either MK-801 or placebo (prepared by Merck Sharp & Dohme) was started immediately with reperfusion. A bolus containing MK-801, 300 μg/kg (solution 150 μg/ml), or an equal amount of placebo was infused into the arterial cannula of the bypass circuit (2 ml/kg) from resuscitation time 0–5 min. This was followed by a maintenance infusion of 75 μg·kg⁻¹·h⁻¹ (0.5 ml·kg⁻¹·h⁻¹) over 12 h, into the CVP catheter. Investigators and evaluators were unaware of the solution infused.

For MK-801 plasma concentrations, arterial blood samples were obtained as early as possible (control baseline) and postarrest at 5 min (end of bolus infusion), and 1, 2, 4, 6, 9, 12, 24, 48, and 96 h. Arterial blood was drawn into heparin-moistened syringes, then immediately centrifuged at +4°C and frozen at −20°C. Samples were analyzed within 20 days by one of the authors (T.F.G.), using radioimmunoassay. The preparation involved solvent extraction with N-acetylation. Metabolites were removed or not reactive. The sensitivity of the evaluation was 15 pg/ml and the interassay variation was 5–7% (mean of triplicates).

Study II—Large-Dose MK-801 After VF Cardiac Arrest of 15 Min

Since Study I showed no improvement in outcome after MK-801 (see Results), we reasoned that the arrest of 17 min may have been too severe and the MK-801 maintenance plasma levels of >15 ng/ml may have been insufficient for therapeutic efficacy. Therefore, we designed Study II with the same protocol but an arrest of 15 min (eight dogs), and twice the dose of MK-801. Four dogs received 600 μg/kg iv during the first 5 min of reperfusion, followed by 150 μg·kg⁻¹·h⁻¹ for 12 h postarrest and four dogs received placebo. (An exception was dog #12, which received an initial bolus of only 300 μg/kg.)

The bolus was given iv instead of ia, as in Study I, to avoid possible toxic tissue concentrations.

Study III—Large-Dose MK-801 Before and After VF Cardiac Arrest of 15 Min

Since Study II also showed no improvement in outcome after large-dose MK-801 postarrest (see Results), we designed Study III to evaluate the effect on outcome of an MK-801 iv infusion started 30 min before arrest. We used the same dose as in Study II and the same 12-h infusion as in Studies I and II. The rationale for pretreatment was that excitatory neurotransmitters accumulate during ischemia. Four dogs received MK-801 600 μg/kg over 5 min, starting 30 min before induction of VF, followed by 150 μg·kg⁻¹·h⁻¹ for 12 h. The infusion was continued throughout, except for the 15-min arrest period, when it was stopped. Results were compared with those of the
four placebo dogs in Study II, which was performed 2 weeks earlier (tables 2 and 3).

**OUTCOME EVALUATION**

**Early neurologic evaluation.** Starting with reperfusion, constriction of dilated pupils and return of pupillary light reflex were recorded, as well as return times for any EEG activity, sustained EEG activity, and presence or absence of EEG convulsions.

**Neurologic Deficit (ND) Scoring.** We used our canine modification of the ND scoring system, which we originally developed for monkeys. It reflects the cerebral dysfunction of the animals. The total ND score (0% = normal; 100% = brain death) consists of five components: 1) the maximal (worst) ND score comprises 20% each for reduced consciousness; 2) abnormal breathing; 3) abnormal cranial nerve function; 4) abnormal motor and sensory function; and 5) abnormal behavior. Dogs with ND scores < 15% had essentially normal cerebral function, those with ND < 25% appeared awake; those with 25—40% were severely neurologically damaged but arousable; and those with scores > 40% were comatose and may have had running movements, spasticity, opisthotonus, and respiratory hypertension. Those with ND 80—100% were brain dead.3

**Overall Performance Categorization (OPC).** This principal clinical outcome measure reflects performance capability including disabilities of cerebral plus extracerebral origin and therefore of organ systems malfunction. This categorization is an adaptation of the Glasgow Head Injury Outcome Scale 1—5 for monkeys and dogs. OPC 1 = normal, fully aware, walking; OPC 2 = moderate disability, aware, sitting, feeding self; OPC 3 = severe disability, stupor, cannot feed self, cannot sit, purposeful reacting to pain; OPC 4 = coma or vegetative state, no

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<th>IPPV (d)</th>
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* MK-801 600 μg/kg iv 0—5 min postarrest (except dog 12, 300 μg/kg) plus 150 μg·kg⁻¹·h⁻¹ for 12 h iv.† Dog numbers in chronologic sequence of experiments.‡ Group differences: all with P > 0.05, NS.

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* MK-801 600 μg/kg 30—25 min before cardiac arrest, continued with 150 μg·kg⁻¹·h⁻¹ for 12 h.† Dog numbers in chronologic sequence of experiments.‡ Group differences: all with P > 0.05, NS.
purposive reaction to pain; and OPC 5 = brain death or death.\textsuperscript{3}

ND scores and OPCs were determined every 8 h between 24 and 96 h. No central nervous system depressants were given after 72 h so as not to influence final evaluation of OPC and ND. The final evaluation at 96 h was also made by an observer not involved in life support. NDs and OPCs reported were the consensus of > three observers. Interobserver differences in the past have been about ±5% for NDs and ±0% for OPCs.\textsuperscript{2,3,5,40}

Morphologic examination.\textsuperscript{3,5,44} At 96 h, anesthesia and tracheal intubation were reestablished in the same way as for preparation (see above). A cisternal puncture was made for measurement of brain cytotoxic enzymes (CK, LD, and ASAT) in the cerebral spinal fluid (CSF).\textsuperscript{57}

The chest was then opened, the thoracic aorta clamped, and brain and heart perfused with buffered paraformaldehyde 3% (pH 7.3) under 100 mmHg pressure, unless venous return was clear. Each perfused brain was sliced into 3-mm thick coronal sections that were examined for gross lesions. After usual processing, six to ten sections of each brain, stained with hematoxylin-eosin, were examined by one author (A.R.) who did not know the treatment given. Evaluation was with 40–400 × magnification of 16 anatomic areas that were evaluated for the severity and extent of ischemic neuronal changes, infarcts, and edema. The extent of infarction or edema, and the number of neurons with ischemic changes in each particular area, were scored subjectively based on the cumulative experience of the pathologist, as relatively minimal (1+), mild (2+), moderate (3+), severe (4+), or no involvement (0). Ischemic neurons were shrunken, angular, and hyperchromic. The above severity number was not multiplied for edema, by two for ischemic neuronal changes, and by four for infarction (necrosis of neurons, glia, and vasculature). The total score for each brain (both sides) was the sum of all multiplied severity scores for all 16 anatomic areas.

Morphologic damage of the heart was evaluated by quantifying macroscopically ischemic lesions (necrotic, infarcted, pale, or hemorrhagic) as present of total subepicardial plus subendocardial surface area.\textsuperscript{44}

Exclusions. Experiments that did not follow protocol because of deviation from the prescribed limits of physiologic variables and deaths due to primary extracerebral complications were to be excluded from outcome comparisons.\textsuperscript{3,5,41} Instances of death before 96 h initiated by brain damage (brain death) in spite of life support according to protocol were to be included. Postischemic exclusion criteria had detailed limits for severe or prolonged hypotension, hypertension, acidemia, hypoxemia, hypercarbia, hyperthermia, hyperthermia, prearrest hyperglycemia, uremia, sepsis, or other rare extracerebral complications.\textsuperscript{35}

Data Analysis

Mean values and standard deviations of prearrest and postarrest data were compared between placebo and MK-801 groups. Key outcome variables (OPC, ND, HD) were compared within each study. Since some dogs deteriorated after initial improvement, OPC and ND were recorded separately as “best OPC and ND” between 24 and 96 h, and “final OPC and ND” at 96 h. Differences between groups in continuously observed data were examined with the Mann-Whitney test. For ND%, we also used the Mann-Whitney test. OPCs were evaluated with the Fisher exact test, which was also used for group comparison of EEG and IPPV results.\textsuperscript{45}

Results

In Studies I, II, and III, experiments according to protocol showed no difference between MK-801 and placebo groups in: 1) immediate prearrest variables, including MAP, PaO\textsubscript{2}, PaCO\textsubscript{2}, pH\textsubscript{1}, hematocrit, cardiac output, Tpa (36.8–38.8°C), and blood glucose (120–250 mg/dl); 2) epinephrine, defibrillation energy, norepinephrine, and NaHCO\textsubscript{3} requirements; and 3) postarrest hematocrit and blood glucose. Resuscitation with bypass achieved good venous return and flows with the same reperfusion-pressure pattern in all dogs; spontaneous heart beat was restored in all dogs within 5 min of starting bypass. During and after partial bypass, vasopressor were rarely needed in either group. At 4 h, all dogs could be weaned from bypass without complications.

Study I—MK-801 After VF Cardiac Arrest of 17 Min

All ten dogs were within protocol (table 1, fig. 1); nine survived to 96 h; placebo group dog #4 (table 1) died at 58 h of cardiogenic shock with pulmonary edema. Return times for pupillary constriction, light reflex, and EEG activity were all longer in the MK-801 group (table 1). After bypass, cardiac output and other cardiovascular-pulmonary variables did not significantly deviate from prearrest baseline values, with no difference between groups. Urine flow returned at 1–3 h of CPB, with no group difference. The average duration of IPPV required beyond the prescribed 24 h was longer and the number of unweanable dogs was higher in the MK-801 group (NS) (table 1). Four of five MK-801 dogs versus only one of five placebo dogs required IPPV beyond 24 h. At 96 h, all four surviving placebo dogs were breathing spontaneously adequately, while three of the five MK-801 dogs still required IPPV. Pharyngeal, laryngeal, and carinal reflexes recovered slower in the MK-801 group, requiring delays in extubation even after weaning from IPPV.

There was no difference in outcome between the groups (table 1, fig. 1). Immediately after weaning from
NO EFFECT OF MK-801 ON OUTCOME AFTER PROLONGED CARDIAC ARREST

<table>
<thead>
<tr>
<th>BEST OPC 24–96h</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
</tr>
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<tbody>
<tr>
<td>5 Brain Death</td>
<td>PLACEBO</td>
<td>MK-801</td>
<td>PLACEBO</td>
</tr>
<tr>
<td>4 Coma</td>
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<td></td>
<td>MK-801 POST ARREST</td>
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<tr>
<td>3 Severe Disability</td>
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<td>2 Moderate Disability</td>
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<tr>
<td>1 Normal</td>
<td></td>
<td></td>
<td>MK-801 PRE &amp; POST</td>
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</tbody>
</table>

Fig. 1. Best overall performance categories (OPC 1–5) between 24 and 96 h in MK-801 versus placebo control dogs in Studies I, II, and III. Each symbol represents one dog: Studies I and II with blinded concurrent placebo controls, Study III with unblinded controls (same as Study II) immediately preceding.

IPPV, ND scores in both groups decreased to 30–60% and remained within that range until 96 h. There were three dogs in each group that showed slight secondary deterioration of ND scores between 24–48 h and 96 h. Overall performance categories (OPC) paralleled ND scores. None of the ten dogs reached good cerebral outcome (OPC 1 or 2) and none developed brain death (OPC 5) (fig. 1).

Brain cytosolic enzyme levels in the cisternal CSF at 96 h were low and showed no significant difference between groups. CSF-CK levels (normal < 7 U/l) were 6 ± 6 (1–16) U/l in the MK-801 group versus 7 ± 4 (3–13) U/l in the placebo group (NS).57

There were no pathologic findings on gross examinations of vital organs' surfaces and their cut surfaces, except for morphologic changes of the hearts of all dogs in both groups. All heart lesions were pale necroses without hemorrhages, similar to those described previously.58,44 All lesions were in the right ventricle, none in the left ventricle and septum. The total ischemic-necrotic area in the placebo group was 1.0 ± 0.8% (0.2–2.6%), whereas it was higher in the MK-801 group, namely 3.7 ± 4.3% (0.1–10.0%) (NS). No coronary thrombi were found. There was no visceral congestion, ascites, intestinal necrosis, or pulmonary consolidation in either group.

In the brain, total histologic damage (HD) scores were 107 ± 22 (80–144) in the placebo group; and 104 ± 31 (64–152) in the MK-801 group (NS) (fig. 2A). The distribution of ischemic neuronal lesions throughout the brains of both groups were similar. Almost all lesions were ischemic neuronal changes. There was no edema. Minimal to moderate infarctions were in three of five placebo dogs in the striatum, and in one of five MK-801 dogs in the neocortex and the striatum. Average scores given for the hippocampus were 12 ± 2.5 (8–16) in the placebo group, and 14 ± 1.9 (12–16) in the MK-801 group (NS). None of the placebo or MK-801 dogs had an entirely clean, lesion-free hippocampus.

MK-801 plasma concentrations were zero before arrest in all dogs. At the end of the bolus infusion at resuscitation time 5 min they were 58 ± 14 (42–75) ng/ml. Between 1 and 12 h of infusion, mean MK-801 plasma concentrations were 15–25 ng/ml. At 24 h they were 1–3 ng/ml, and at 48 and 96 h essentially zero. MK-801 CSF concentrations at 96 h were essentially zero (<0.04 ng/ml).

STUDY II—LARGE DOSE MK-801 AFTER VF CARDIAC ARREST OF 15 MIN

Of the ten dogs entered, one dog receiving MK-801 and one receiving placebo were excluded because of death due to respiratory care errors. Thus, data from four
MK-801 and four placebo dogs were analyzed (table 2, fig. 1).

Return times for pupillary constriction, light reflex, and EEG activity, were again longer in the MK-801 group (NS) (table 2). IPPV was required beyond the prescribed 24 h in two of four placebo dogs because of increased alveolar-arterial $P_{A}$ gradient (suspected pulmonary edema), and in two of four MK-801 dogs because of coma, areflexia, and hypoventilation (table 2). There again was no group difference in ND, OPC, and HD (table 2, fig. 1). Brain cytosolic enzyme levels in the CSF (n = 3) and gross necropsy findings were as in Study I. Myocardial damage, as in Study I, concentrated around the right ventricle. The total ischemic-necrotic area in the placebo group was 1.2 ± 2.0% and in the MK-801 group 2.7 ± 1.7 (NS).

Total HD scores were 92 ± 12 (76–108) in the placebo group and 92 ± 23 (68–130) in the MK-801 group (NS) (fig. 2B). Again, lesions were almost exclusively ischemic neuronal changes, without infarcts and without edema. The total scores of the hippocampal lesions were 11 ± 1.7 (8–12) in the placebo group, and 12 ± 0 in the MK-801 group (NS).

MK-801 plasma concentrations were zero before arrest. At the end of the bolus infusion at resuscitation time 5 min they were 350 ± 140 (203–531) ng/ml. Between 1 and 12 h, mean MK-801 plasma concentrations were 27–45 ng/ml. At 24 h they were 1.4–4.9 ng/ml, which did not correlate with inability to be separated from IPPV. MK-801 CSF concentrations at 96 h (n = 2) were essentially zero (<0.2 ng/ml).

**Study III—Large-Dose MK-801 Before and After VF Cardiac Arrest of 15 Min**

Of the eight dogs entered, four were excluded: one dog asphyxiated from an error in airway care; one needed IPPV for 56 h and died after an accidental disconnection; one died from IPPV-induced pneumothorax at 40 h; and one developed diffuse hemorrhagic diathesis (disseminated intravascular coagulation) during bypass. Thus, data from four MK-801 dogs were analyzed (table 3, fig. 1) and compared with the four placebo dogs of Study II.

There again was no group difference in return times for pupillary constriction, light reflex, and EEG activity, nor in ND, OPC, and HD (tables 2, 3; fig. 1). IPPV beyond 24 h was required in three of four MK-801 dogs versus two of four Study II placebo dogs. Brain cytosolic enzyme levels in the CSF and autopsy findings were similar to those in Study I. Myocardial damage areas were in the Study II control group 1.2 ± 2.0% and in the Study III MK-801 group 1.0 ± 1.2% (NS). Lesions again were predominantly in the right ventricle outflow tract.

In the brain, HD scores were similar as in Studies I and II (fig. 2C). Total HD scores were 142 ± 11 (126–142)
HISTOPATHOLOGIC CHANGES – STUDY II
96h after VFCA 15min

in the MK-801 group, as compared with the Study II controls with HD 92 ± 12 (76-108) (NS). One of the Study III dogs with MK-801 developed moderate infarctions in the neocortex. Otherwise, the lesions again were almost exclusively ischemic neuronal changes and no edema. HD scores in the hippocampus in the MK-801 group of Study

HISTOPATHOLOGIC CHANGES – STUDY III
96h after VFCA 15min

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III were 15 ± 2 (12–16) as compared with the Study II controls with a score of 11 ± 2 (8–12) (NS).

MK-801 plasma concentrations were zero before infusion. At the end of the bolus infusion at 25 min prearrest, MK-801 plasma concentrations were 543 ± 11 (551–551) ng/dl—higher peaks than with the same dose postarrest in Study II. Immediately prearrest, MK-801 plasma concentrations were 81 ± 16 (47–94) ng/dl. From 5 min postarrest to 12 h, mean MK-801 plasma concentrations ranged from 36–54 ng/dl. MK-801 plasma concentrations at 24 h (all < 4.7 ng/ml) did not correlate with ability to wean from IPPV. MK-801 CSF level at 96 h in the one dog studied was essentially zero (0.05 ng/ml).

**Discussion**

In this reproducible dog model of prolonged cardiac arrest, an infusion of MK-801, whether started after or before the insult, failed to alter neurologic, overall, and histologic outcome. Lack of a statistically significant group difference can be due to small numbers. A larger sample size would be required if mean ND scores and other outcome variables (OPC, HD) would have shown a tendency toward benefit from MK-801. This was not the case. In all three studies OPCs, NDs and HDs were similar or worse in the MK-801 groups than in the control groups. Postarrest perfusion failure and free radical triggered necrotizing cascades might offset a beneficial effect of MK-801. The role of neurotransmitters may include more than glutamate receptors.26–28

Our requirements for animal outcome models to permit convincing evaluation of cerebral resuscitation potentials include: 1) prompt restoration of spontaneous circulation (without additional low flow); 2) cerebral reperfusion without “trickle flow” areas (CBF < 10 ml/100 g·min−1); to allow the agent to reach the neurons; 3) all control experiments within protocol should achieve survival with neurologic deficit; and 4) the insult should be sufficiently moderate to allow mitigation of brain damage by a known effective therapy, such as hypothermia. Our model seems to have met requirements 1 and 2 with cardiopulmonary bypass, 3 because of consistent survival with OPC 3–4 outcomes, and 4 because VP 15-min outcomes were improved in previous studies by mild hypothermia.23,29,40 In recent studies without MK-801, after normothermic VC no-flow of 15, 17, or 20 min and cardiopulmonary bypass, we have achieved survival to 96 h with OPC 3 or 4 (none with OPC 1, 2, or 5) in 16 of 17 dogs studied.23,29,40 In previous studies without MK-801, after VC no-flow of 15 min, we achieved OPC 1 and 2 in six dogs whose Tpa was accidentally slightly lower (35.5–36.9°C) at the start of VC, as compared with OPC 3 or 4 in five dogs with 37–38°C.39,40 In the present study, all dogs had Tpa > 37°C and all achieved only OPC 3 or 4. VF of 10 or 12.5 min at Tpa 37.5°C was followed in some previous experiments by OPC 1 or 2.40 Therefore, the VF times of 15 and 17 min used in this study seems to be not too long for revealing a beneficial effect. Even a subclinical beneficial effect of MK-801 should have been evident in the histologic findings, particularly in the hippocampus, which was not the case (fig. 2A–C).

MK-801 seems to readily pass the blood-brain barrier.‡‡ We reached and exceeded therapeutic plasma concentrations of MK-801 of 15–20 ng/ml.25–29,32 Near-zero CSF levels of MK-801 and normal brain cytosolic enzyme levels at 96 h are explained by the late sampling to avoid needle trauma that could influence ND or OPC evaluations. Loss of brain enzymes into the CSF peaks at 48 h postarrest; levels then decrease rapidly.37 At 96 h they were not known before.

Could cerebral hypoperfusion have prevented MK-801 from reaching ischemic neurons? This is unlikely for the following reasons. Cerebral perfusion failure postarrest consists of initial multifocal “no-reflow”,46 (which does occur with normotensive or hypotensive reperfusion), brief global hyperemia, and then delayed and prolonged global4 and multifocal hypoperfusion.46,47 We conducted noninvasive multifocal local cerebral blood flow studies (1CBF) with Xenon-enhanced CT starting 10 min postarrest.47 The protracted “trickle flow” areas seen with normotension and normal Hct were abolished with hypertensive hemodilution.48 The cardiopulmonary bypass model of the present MK-801 study induced hypertensive hemodilution and no microinfarcts. MK-801 seems to increase global CBF in normal dogs, without changing CMRO2, but not to change global CBF or CMRO2 after ischemia.49 In focal ischemia, the ability of MK-801 pretreatment to reduce brain infarct size in models of permanent or temporary middle cerebral artery occlusion could be explained by its antiepileptic effect. Even if without seizures, MK-801 does not alter CMRO2.

In one pilot experiment before this study, we gave MK-801 to a normal unanesthetized spontaneous breathing dog. MK-801 600 µg/kg iv over 5 min resulted at 10 min in sedation and bradypnea. The pupils dilated but retained a light reflex. Breathing became shallow and he became comatose, cyanotic, and hypoxicemic. O2 inhalation reversed the cyanosis. At 60 min, he was still comatose (MK-801 plasma level 87 ng/ml), at 90 min he showed opisthotons and running movements, at 150 min he began to wake up, and at 48 h he appeared normal. A second

‡‡ Shearman G: Personal communication.
pilot experiment was to rule out brain damage caused by MK-801 when administered before and after VF arrest of 5 min, which in previous experiments was survived without causing brain damage.\textsuperscript{2,35,39} Otherwise, the protocol of Study III was used. The dog was weaned from bypass at 4 h and from IPPV at 24 h, regained consciousness, walked at 28 h, and recovered completely. Brain histologic damage scores were zero (normal brain). Could cardiopulmonary bypass \textit{per se} have caused brain damage? In a third pilot experiment with VF of only 30 s and bypass of 4 h, neurologic recovery was complete; there was no histologic brain damage.\textsuperscript{39} ND in patients with open-heart surgery under bypass is probably the result of low perfusion pressure, thrombemboli, or bubble emboli. In this study, MK-801 delayed the early postarrest recovery of EEG activity, pupillary light reflex, airway reflexes, and adequate spontaneous breathing. Although three of the nine placebo-treated dogs with arrest also needed IPPV beyond the prescribed 24 h, none needed it to 96 h. In contrast, ten of the 13 MK-801-treated dogs needed IPPV beyond 24 h, and seven even to 96 h. Because of this respiratory depressant effect, clinical trials of MK-801 in conscious, not ventilated patients (e.g., with focal ischemia), should include intensive care.

In studies that showed benefit of MK-801 or other drugs with possible cerebral resuscitation effect, subtle differences in physiologic variables that are known to influence outcome after global ischemia (e.g., temperature, glucose, perfusion pressure, pH) may have effects erroneously attributed to the drug.\textsuperscript{2,39,41,60,61} Studies in gerbils are flawed by the inability to monitor cardiovascular pulmonary parameters and by the gerbils tendency to convulse. We have documented that mild hypothermia (34–36 °C), induced before\textsuperscript{2,39} or after\textsuperscript{41,52} the onset of VF arrest, mitigates neurologic deficit.\textsuperscript{2,39,41} Hossmann showed the same in cats for recovery of EEG activity.\textsuperscript{51} Mild cerebral hypothermia is readily induced by CNS depressants. The focal brain ischemia studies\textsuperscript{26–29,33} and global ischemia rat studies with positive effects of MK-801\textsuperscript{25,52} need re-evaluation with accurate control of brain or core temperature. Our dog cardiac arrest study\textsuperscript{54} and the rat global ischemia study by Block and Pulsinelli\textsuperscript{55} were accurately controlled for temperature; they found no benefit.

Recently, Perkins \textit{et al.}\textsuperscript{54} found that in dogs after ascending aorta occlusion of 11 min, MK-801 postarrest did not improve outcome. Hippocampal neurons were not protected. Their model, however, was not reproducible, as three of nine placebo-treated dogs and 5 of 9 MK-801 treated dogs achieved complete neurologic recovery. Lanier, \textit{et al.}\textsuperscript{55} also found no difference in outcome after MK-801, using our monkey neck tourniquet model.\textsuperscript{43} Unfortunately, their negative results are also not convincing, since neurologic function scores in both groups varied between 40\% (disabled) and 100\% (normal).

\textbf{We conclude} that sustained plasma concentrations of MK-801 (which apparently can mitigate the damage produced by focal or incomplete ischemia), initiated pre- or postarrest, do not mitigate neurologic dysfunction or histologic brain damage (not even in the hippocampus) after VF cardiac arrest in dogs. This negative outcome result does not negate the hyperexcitability hypothesis of selective vulnerability. It suggests the existence of additional mechanisms of secondary brain damage. MK-801 can cause prolonged CNS depression, including hypventilation. Further studies in controlled outcome models with ICBF monitoring, might be necessary, to evaluate MK-801 treatment after shorter arrests in combination with other neurotransmitter blockers, and as one component of tailored multifaceted etiology-specific treatment protocols.

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