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NMDA Receptor-blocker Ketamine Protects During Acute Carbon Monoxide Poisoning, While Calcium Channel-blocker Verapamil Does Not

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Key words: blood pressure; carbon monoxide; cerebral edema; dose–response; glucose; heart rate; hypothermia; ketamine; lactate; neurological index; verapamil.

Levine-prepared, female, Sprague-Dawley rats were used to investigate the possible protective effects of the NMDA receptor-blocker anesthetic ketamine and the Ca^{2+} channel-blocker verapamil (0.4 mg kg^{-1} 'low dose', and 1.0 mg kg^{-1} 'high dose') in rats during acute 2400 ppm carbon monoxide (CO) poisoning. Blood glucose and lactate concentrations, heart rate, mean arterial blood pressure (BP), body temperature (BT), neurological function and cerebral cortical water content were measured. In most cases glucose increased after 45 min and then fell to initial values after 90 min. Lactate concentration increased sharply during CO exposure in the saline and in the low- and high-dose verapamil groups, while the lactate increase in rats given ketamine at 40 mg kg^{-1} was significantly lower than with saline. Lactate was also significantly lower in these rats after 90 min than in the high-dose verapamil group. Lactate was normal in all four groups after 2 and 4 h of air recovery. Ketamine significantly lowered the heart rate prior to CO exposure, and the heart rate remained significantly below values for the saline and for the low- and high-dose verapamil groups throughout CO exposure. The BP decreased in all groups during CO exposure, and the BP recovery which took place in all four groups was significantly more rapid in the ketamine group. Recovery from CO-induced hypothermia was similar in the ketamine and saline groups, whereas rewarming tended to occur more slowly and less completely in the two verapamil-treated groups. There were no significant differences in neurological function among the four groups, as assessed after 4 h of recovery. However, cerebral edema was significantly reduced by treatment with 40 mg kg^{-1} ketamine as compared with saline. Verapamil at neither the low nor the high doses was of significant benefit in this regard. No rat in the 40 mg kg^{-1} ketamine group died during CO exposure, whereas all deaths in the other groups took place during CO exposure. The use of higher and lower doses of ketamine suggest 40–80 mg kg^{-1} as most effective in suppressing lactate production; 40 mg kg^{-1} ketamine may be optimal with regard to survival. The results suggest that ketamine is beneficial, when administered before and during acute severe CO poisoning, in reducing blood lactate and cerebral edema and in improving BP recovery and survival. Verapamil, in contrast, appears to provide no benefits in these respects.

INTRODUCTION

Hypotension, hypothermia, altered blood glucose, increased blood lactate concentration, eventual cerebral cortical edema and increased neurological deficit and death are some of the responses to acute severe CO intoxication that we have demonstrated.[1,2] In the Levine-prepared rat (unilateral carotid-jugular occlusion), mortality increases from near zero with 1500 ppm CO exposure for 90 min to approximately 50% at 3000 ppm.

Neurological deficit has been shown to correlate directly with cerebral edema measured both gravimetrically[2] and by NMR imaging techniques.[3] Penney and his collaborators[1,4,5] found that mortality and post-CO neurological deficit are increased in rats incurring more extreme cases of hyper- or hypoglycemia during CO exposure. Thus, the glucose level changes in a complex manner during and after CO poisoning, and appears to be an important determinant of brain damage and survival. Whether this is related to lactate production and resultant acidosis remains controversial.

Ketamine is a dissociative anesthetic which is also an *N*-methyl-D-aspartate (NMDA) receptor-blocker.[6–9] Hoffman *et al.*,[10] using ketamine-treated rats which had undergone common carotid ligation and hemorrhagic hypotension, demonstrated improved neurological outcome and decreased plasma catecholamines and glucose. Others,[11] using the isolated rat optic nerve preparation, report that ketamine is able to protect CNS white matter as well as gray matter from anoxic injury. Aside from its anesthetic effect, ketamine also blunts severe hypotension,[12] a condition common to acute CO poisoning and an action which may exacerbate the damage produced by CO. Moderation of the hypotension could by itself theoretically confer protection.

Studies suggest that Ca^{2+} flux into the neuron may be a critical factor during CNS ischemia/hypoxia. According to a review,[13] such Ca^{2+} shifts occur mainly

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at excitatory pre-synaptic sites and seem to precede structural ischemic cell alteration in post-synaptic areas. Prophylactic treatment and post-ischemic intervention with various substances which block Ca^{2+} entry through slow channels may reduce delayed brain injury by preventing cytosolic Ca^{2+} overload. Verapamil is reported to be beneficial in animal models subjected to hypoxia[14] and carotid ligation,[15] and in ischemic spinal cord preparations.[16,17]

Only a few recent studies have begun the examination of therapeutic approaches to minimizing cognitive and neurological dysfunction and mortality during acute CO poisoning.[18–20] A goal of this study was to examine separately the possible protective actions of the anesthetic and NMDA receptor-blocker ketamine and the calcium channel-blocking agent verapamil during acute CO poisoning in a rat model. Because ketamine showed several protective characteristics in the first experiment, a second goal was then to explore its actions on a dose-related basis.

METHODS

Animal treatment

Female Sprague-Dawley rats 100–140 days of age, obtained from Charles River Breeding Laboratories (Wilmington, MA), were used. Two days prior to CO exposure, a modified Levine (21) preparation was produced under ketamine (Ketaset, 80 mg kg^{-1})/zylazine (Rompun, 4.8 mg kg^{-1}) anesthesia, as previously described.[2] In it, catheters made of PE-50 polyethylene tubing were inserted into both the jugular vein and the common carotid artery, toward the heart. The catheters were threaded under the skin to the nape of the neck and tied in place. The external lengths of the catheters were plugged with Amphenol pins (no. 220-P02-100) and coiled up with masking tape when not in use. This procedure effectively occludes the major blood vessels to one side of the brain, placing it at increased hypoxic/ischemic risk, while providing ports for blood withdrawal, injection and the monitoring of vital functions. The rats were allowed to recover under close observation. They were given the analgesic Buprenex (0.3 mg kg^{-1}) on the day of surgery and the day after. Operated rats were no different behaviorally from unoperated controls. The animal protocol was approved by an institutional review (Animal Investigation) committee and conformed to NIH guidelines for animal use.

The rats were confined in plastic restrainers and exposed to 2400 ppm CO for 90 min inside a large transparent plastic bag. The CO exposure apparatus and protocol have been described in detail previously.[2,4,5] In the first experiment, rats received verapamil at two different dose levels (0.4 and 1.0 mg kg^{-1}) and ketamine at a single dose level (40 mg kg^{-1}) or normal saline, all in a maximal volume of 0.5 ml. In the second experiment, rats received ketamine at four different dose levels (20, 40, 60 and 80 mg kg^{-1}) or normal saline, again all in a maximal volume of 0.5 ml. These agents were infused through the jugular catheter using a Harvard Apparatus Co. infusion/withdrawal pump (model 940). Two-thirds of each solution was given during a 15-min period prior to CO exposure and one-third over the 90-min period of CO exposure. In most cases, two rats from different groups were exposed to CO simultaneously. Following CO exposure, the rats were transferred to room air for recovery.

Measurements and data analysis

Rectal body temperature (BT) was monitored with Yellow Springs Instrument Co. tele-thermometers (model 43TD), using YSI 400 series probes. Blood pressure (BP) was monitored with Statham P23id pressure transducers and recorded on a Gould model 2400 chart recorder. Heart rate was derived from the BP record. Whole blood drawn from the carotid catheter was assayed for glucose and lactate using a Yellow Springs Instrument Co. 2300 STAT glucose/L-lactate analyzer. Measurements were made at −15, 0 (CO exposure begun), 45, 90 (CO exposure ends), 210 and 330 min (2 and 4 h of room air recovery).

The neurological index (NI) was assessed before treatment and at 330 min on a scale from 6 to 30 on the basis of behavior: general appearance, posture, shuffle (walking on front legs only), circling, activity and the ability to hang on to a metal screen.[2,4,5] A normal rat scores 6, whereas a rat with severe abnormalities in terms of appearance, posture and motor ability scores up to 30. Immediately following final measurement of NI, rats were sacrificed with i.v. ketamine (50 mg kg^{-1})·KCl (0.5 ml, 10% solution). The cerebral cortices were removed onto moist filter paper, separated into six sections by region (right front, middle, and rear, left front, middle and rear), weighed and dried at 60°C for the determination of water content.

Data analysis and graphic display were carried out on a Macintosh microcomputer. Most values are means ± SEM. Student's *t*-test was used for statistical analysis. Differences that resulted in $P \leq 0.05$ were considered significant.

RESULTS

In the first experiment in which rats received low-dose verapamil (0.4 mg kg^{-1}), high-dose verapamil (1.0 mg kg^{-1}), ketamine (40 mg kg^{-1}) or saline, acute CO exposure resulted in increases in blood glucose concentration to values of 123–152 mg dl^{-1} in all groups after 45 min. Initial glucose values were 83–91 mg dl^{-1}. This was followed by a decrease to or below the initial values after 90 min. Glucose increased modestly after 2 h of air recovery (104–114 mg dl^{-1}) and fell once again to initial values after 4 h. No significant differences were detected among the four treatment groups at any time point (data not shown).

Blood lactate concentration increased sharply during CO exposure in the saline group, from an initial value of 13.3 mg dl^{-1} to 116.0 mg dl^{-1} after 45 min (Fig. 1). Lactate remained high at 90 min. The response of the low-dose verapamil group was nearly identical, while lactate tended to be higher (non-significant) during CO exposure in the high-dose verapamil group. Lactate

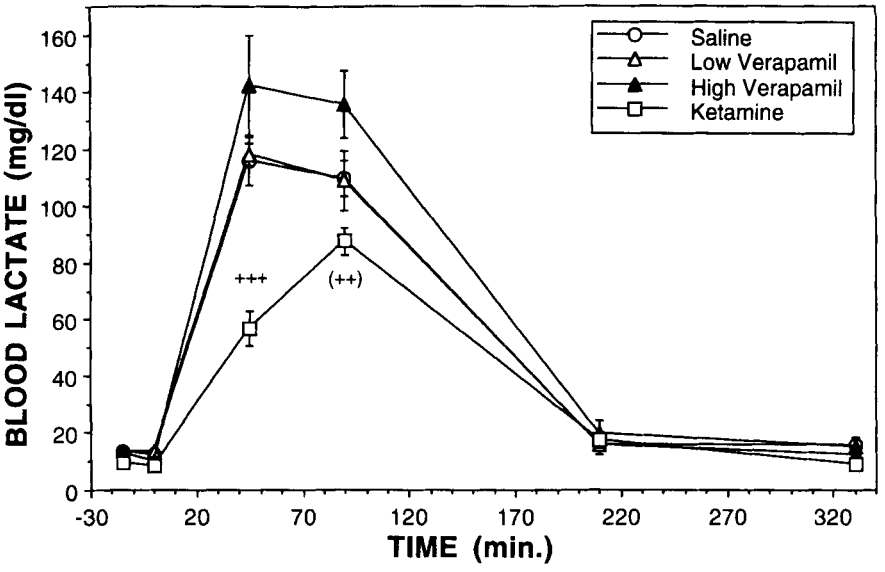

Figure 1. Blood lactate concentration of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Saline, 0.4–0.5 ml; low-dose verapamil, 0.4 mg kg^{-1} in 0.4–0.5 ml; high-dose verapamil, 1.0 mg kg^{-1} in 0.4–0.5 ml; ketamine, 40 mg kg^{-1} in 0.4–0.5 ml. Vertical bars represent 2 SEM. The numbers of animals in each group were initially 10 (ketamine) or 11 (saline, low and high verapamil) and decreased to 6 (high verapamil), 7 (low verapamil) or 8 (saline and ketamine) during CO exposure. Compared to saline value at same time: $^{+++}P < 0.001$. Compared to the high-dose verapamil group: $^{++}P < 0.01$.

concentration increased more slowly and was significantly lower in the ketamine group after 45 min than with saline, and was significantly lower than in the high-dose verapamil group after 90 min. There were no differences in blood lactate between the four treatment groups after 2 and 4 h of air recovery.

Ketamine significantly lowered heart rate prior to CO exposure (Fig. 2). Heart rate in the ketamine group remained significantly below values for the saline and the low- and high-dose verapamil groups at three of the six monitoring points during CO exposure.

Mean arterial BP decreased in all groups during CO exposure (Fig. 3). There was an inexplicible transient increase in BP after 15 min of CO exposure in the ketamine group. Recovery from hypotension took place in all four groups following CO exposure, but was more rapid in the ketamine group; significant differences were apparent 30 and 60 min after termination of CO exposure.

Body temperature decreased in all four groups during CO exposure (Fig. 4). Recovery from hypothermia of the ketamine group was similar to that of the saline group, while the process tended to occur more slowly and less completely (non-significant) in the two verapamil-treated groups.

Survival rate was highest in the ketamine and low-dose verapamil groups (76–78%), and lowest in the high verapamil group (60%). Seventy-two per cent of

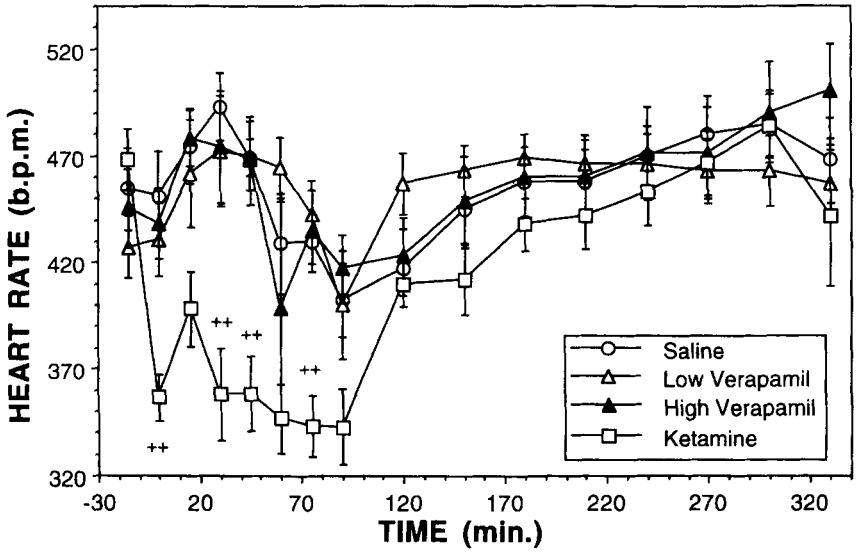

Figure 2. Heart rate of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Treatment, numbers of animals and other symbols and abbreviations are as in Fig. 1. Compared to saline value at same time: $^{++}P < 0.01$.

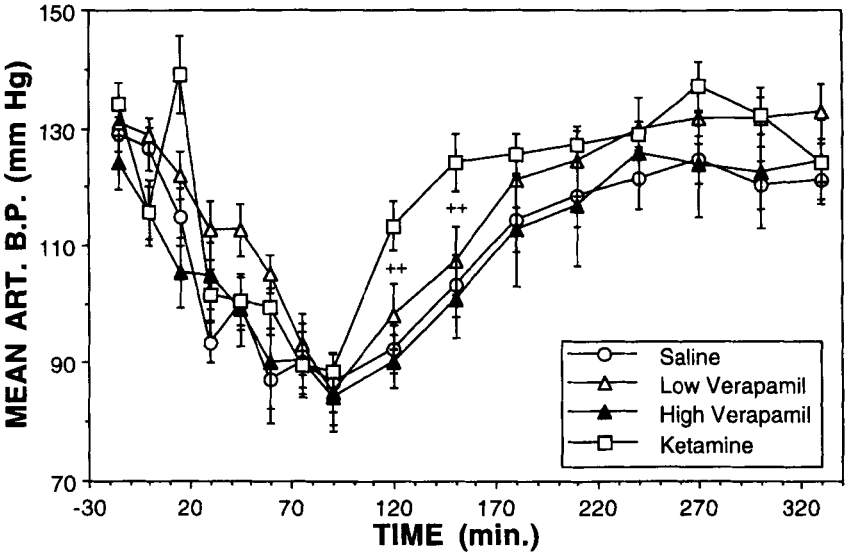

Figure 3. Mean arterial blood pressure of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Treatment, number of animals, symbols and abbreviations are as in Fig. 1.

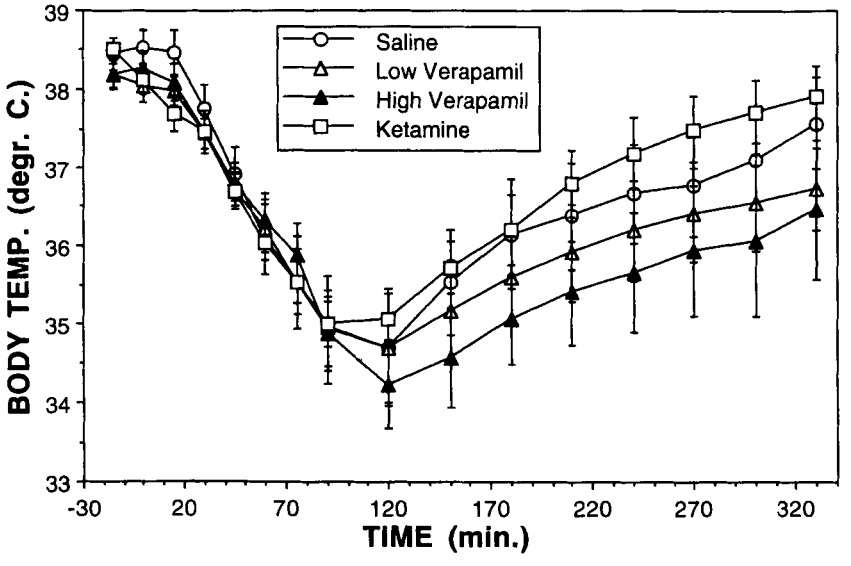

Figure 4. Rectal temperature of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Treatment, numbers of animals, symbols and abbreviations are as in Fig. 1.

the saline group survived. None of the differences were statistically significant. Deaths occurred only after CO exposure ended in the ketamine group, while death occurred during CO exposure in the other three groups, and death was particularly early in the high-dose verapamil and saline groups (data not shown).

No significant difference in NI was observed among the four groups after 4 h of recovery (Fig. 5, top). Behavioral impairment was modest in all cases, involving NI values of 10–13.7, compared to approximately seven prior to CO exposure.

Left to right cortical water content difference was significantly reduced by treatment with ketamine as compared with saline alone (Fig. 5, bottom). Although verapamil at both the low and the high doses also tended to reduce cortical edema, the differences were not statistically significant.

In the second experiment in which rats received ketamine at four different dose levels (20, 40, 60 and 80 mg kg^{-1}), or saline only, acute CO exposure resulted in increases in blood glucose concentration. Initial glucose values were 77–100 mg dl^{-1}. This was followed by a decrease to or below initial values after 90 min. No significant differences were detected among the five treatment groups at any time point (data not shown).

Blood lactate concentration increased sharply during CO exposure, from initial values of 8.6–13.8 mg dl^{-1} (Fig. 6). Lactate remained high at 90 min in most of the groups. Ketamine at a dose of 20 mg kg^{-1} significantly decreased lactate after 45 min as compared with the saline group. Ketamine at doses of 40, 60 and 80 mg kg^{-1} further lowered lactate concentration at 45 min; i.e. lactate in these three groups was significantly lower than that of the saline group, and was also significantly lower than that of the rats receiving ketamine at 20 mg kg^{-1}. Lactate of the 40 and 60 mg kg^{-1} ketamine groups was also significantly lower than that of the saline group at 90 min. There

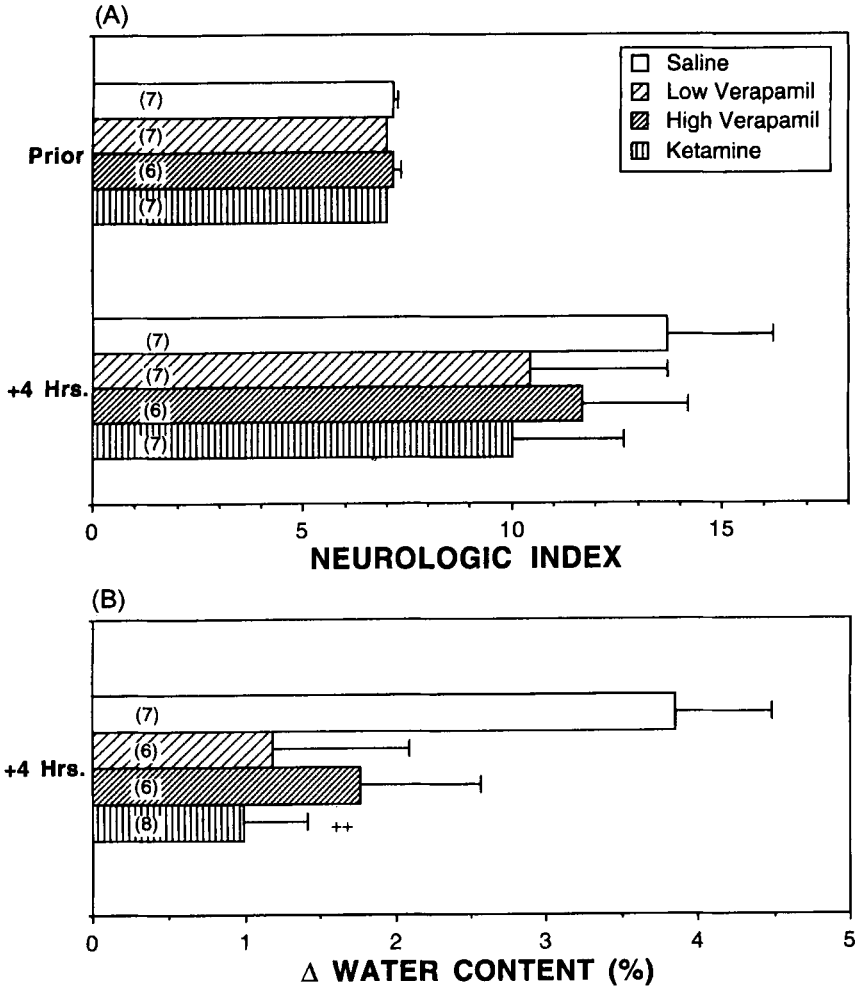

Figure 5. Neurological index (A) and cerebral cortical water content difference (right to left) (B) of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Treatment, symbols and abbreviations are as in Fig. 1. Numbers of animals are given in parentheses.

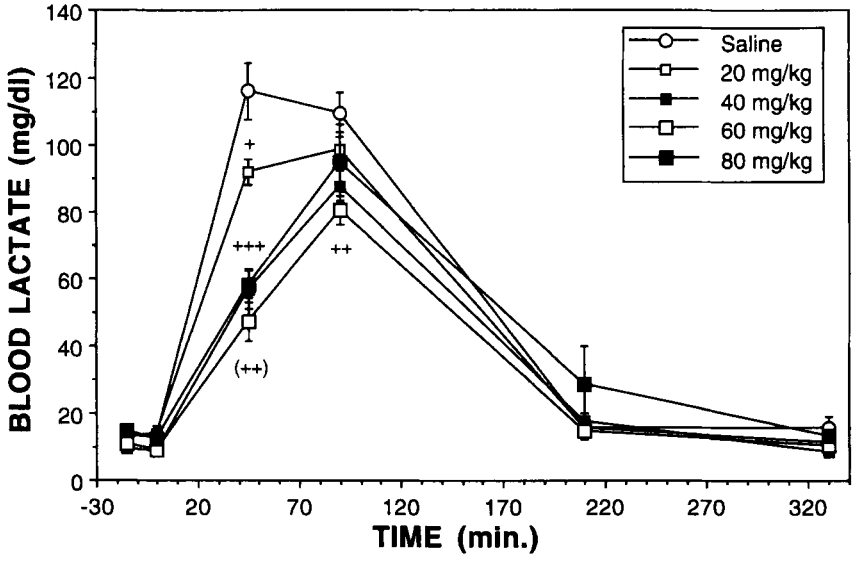

Figure 6. Blood lactate concentration of female Levine-prepared rats treated with 2400 ppm carbon monoxide for 90 min and allowed to recover in room air for 240 min. Saline, 0.4–0.5 ml; ketamine, 20, 40, 60 and 80 mg kg^{-1} in 0.4–0.5 ml. Treatment, symbols and abbreviations are as in Fig. 1. The numbers of animals in each group were initially 8 (60 mg kg^{-1} ketamine), 10 (20 mg kg^{-1} ketamine), 11 (saline and 80 mg kg^{-1} ketamine) or 12 (40 mg kg^{-1} ketamine) and decreased to 4 (80 mg kg^{-1} ketamine), 5 (20 mg kg^{-1} ketamine), 6 (60 mg kg^{-1} ketamine), 8 (saline) or 9 (40 mg kg^{-1} ketamine) during CO exposure. Compared to saline value at same time: $^{+}P < 0.05$ and $^{++}P < 0.001$. Compared to 20 mg kg^{-1} ketamine group: $^{\leftrightarrow}P < 0.01$.

were no differences in blood lactate between the five treatment groups after 2 and 4 h of air recovery.

As stated above, ketamine significantly lowered heart rate prior to CO exposure. In this case, the depression of heart rate became greater as the ketamine dose level was increased, e.g. 26 bpm at 20 mg kg^{-1} and 118 at 80 mg kg^{-1}. This pattern of dose-related bradycardia resulting from ketamine continued during CO exposure (data not shown).

Mean arterial BP decreased in all five groups during CO exposure. The presence of ketamine at various dose levels had no significant influence on the degree of hypotension achieved. Similar degrees of BP recovery took place in all five groups following CO exposure (data not shown).

Body temperature decreased in all four groups during CO exposure. Recovery from hypothermia of the rats receiving 20 and 40 mg kg^{-1} ketamine doses and those receiving saline tended to be faster than those receiving ketamine at doses of 60 and 80 mg kg^{-1}. There were no significant differences (data not shown).

Survival rate was highest in the rats receiving ketamine at 40 and 60 mg kg^{-1} and in those receiving saline (72–75%), and lower in rats receiving ketamine at 20 mg kg^{-1} (50%) and 80 mg kg^{-1} (36%). Deaths when they occurred in the 40 mg kg^{-1} ketamine group took place after the termination of CO exposure, while deaths in the saline group and the other three ketamine groups took place during CO exposure (data not shown).

No significant difference in NI was observed among the five groups after 4 h of recovery. The tendency for rats to remain sleeping during most or all of the recovery period in those groups receiving 60 and 80 mg kg^{-1} ketamine presented a problem in the assessment of NI.

As stated above, left to right cortical water content difference was significantly reduced by treatment with 40 mg kg^{-1} ketamine as compared with saline. The difference was not significant when 20 mg kg^{-1} ketamine was used. Too few animals were available at the 60 and 80 mg kg^{-1} ketamine dose levels to provide useable data in this regard.

DISCUSSION

The hypotension, hypothermia, cerebral edema and neurological deficit observed in the present study are the usual responses to acute severe CO poisoning in the Levine-prepared rat model,[1,2] as are the increases in blood glucose[2,5] and lactate.[22,23] Sokal and his collaborators have reported similar changes in blood glucose and lactate in other animal models and in man during CO exposure.[24] While previous studies from this laboratory have attempted to characterize the response of the Levine-rat model to acute severe CO poisoning,[25] this is our first to explore possible approaches to protection. The efficacy of the NMDA receptor-blocker ketamine and the Ca^{2+} channel-blocker verapamil were tested in our standard protocol, involving a 90-min exposure of conscious instrumented rats to 2400 ppm CO. To our knowledge this is the first study to report a beneficial effect of ketamine during acute CO poisoning.

Ketamine is a dissociative anesthetic known to be an NMDA receptor-blocker.[6–9] Hoffman *et al.*,[10] using ketamine-treated rats which had undergone common carotid ligation and hemorrhagic hypotension, demonstrated improved neurological outcome and decreased plasma catecholamines and glucose. Others,[11] using the isolated rat optic nerve preparation, report that ketamine is able to protect CNS white matter as well as gray matter from anoxic injury. MK-801, another NMDA receptor-antagonist, has been shown to protect from the delayed amnesia produced in mice by acute CO exposure.[19] Aside from its anesthetic effect, ketamine also blunts severe hypotension,[12] a condition common to acute CO poisoning and an action which may exacerbate the damage produced by CO. The moderation of the hypotension should by itself theoretically confer protection by increasing cerebral perfusion.

The dose of ketamine used in the first experiment was 50% of the usual anesthetic dose. It occurred to us that a calming action of ketamine may have contributed to the decreased rate of lactate build-up, since rats often struggle at various stages of CO exposure. If this were the case, the body temperature of the ketamine-treated rats should have decreased more rapidly than that of the saline controls because of decreased heat generation. This was clearly not the case. This line of reasoning also speaks against the involvement of a major metabolic depressant action of ketamine, a characteristic common to many anesthetics, which might have been, but apparently was not, involved in conferring protection from CO poisoning.

Benefit derived from ketamine apparently did not derive from maintenance of BP either. With the exception of the initial 15 min of CO exposure, BP of the ketamine-treated rats declined in a fashion similar to that of the control and the verapamil-treated rats. Bradycardia was the only striking hemodynamic effect that we observed with ketamine treatment during CO exposure. It is unclear whether the bradycardia was involved in limiting lactate production, cerebral edema, etc.

The doses of ketamine used in the second experiment were half of that used in the first experiment, as well as 50% and 100% above. Ketamine dose levels of 40–80 mg kg^{-1} were clearly the most effective in terms of the suppression of lactate production, whereas 20 mg kg^{-1} was only partially effective. While recovery of body temperature was not different from the controls at 20 and 40 mg kg^{-1}, body temperature tended to remain depressed at the higher ketamine dose levels. The latter phenomenon is likely to be related to the extended period of unconsciousness (sleeping?) experienced by the rats given the highest doses of ketamine.

This is the first study to examine the possible protective effect of Ca^{2+} channel-blockers in acute CO poisoning. The results fail to show a beneficial effect of verapamil, and in fact suggest that verapamil at the higher dose used (1.0 mg kg^{-1}) is detrimental. Current research suggests that Ca^{2+} flux into the neuron may be a critical factor during CNS ischemia/hypoxia. Such Ca^{2+} shifts evidently occur mainly at excitatory presynaptic sites and precede structural ischemic cell alteration in post-synaptic areas.[13] Prophylactic treatment

and post-ischemic intervention with various substances which block Ca^{2+} entry through slow channels may reduce delayed brain injury by preventing cytosolic Ca^{2+} overload.

It was reported by Cartheuser *et al.*[26] that hyperpnea persisted and heart rate remained stable for a longer period in spontaneously breathing, lightly anesthetized rats receiving verapamil prior to progressive hypoxia. This, however, was not the case in anoxia or ischemia. It was suggested that benefit was due to improved cerebral oxygen delivery. Other hemodynamic and metabolic parameters were not measured, however. In a follow-up study of various Ca^{2+} channel-blockers,[14] verapamil, gallopamil and nimodipine were found to increase significantly the tolerance of severe progressive hypoxia in rats breathing spontaneously during light anesthesia. Fendiline and bepridil showed slight benefit, while cinnarizine, diltiazem and flunarizine slightly reduced tolerance times. At protective doses, verapamil, gallopamil and nimodipine had no cardiac depressor effects but increased ventilatory drive, the mechanism that may have been responsible for their beneficial action. In the present study, verapamil exerted no cardiac chronotropic action; however, we did not monitor ventilation rate.

Verapamil was also reported to prevent cerebral acidosis during moderate hypoxia and hypotension in rats with carotid ligation, but it reportedly[15] does not abolish the increase in glucose metabolic rate in the caudate-putamen. Flunarizine increased survival time during hypobaric hypoxia in mice, although its effect was most pronounced in complete cerebral ischemia (i.e. decapitation).[27] Although we did not examine cerebral acidosis in the CO-exposed rats, verapamil in our hands did not significantly lower cerebral edema below that of saline controls.

In canine studies, four of five dogs treated with verapamil ($0.4\ mg\ kg^{-1}$) before and after thoracic aorta occlusion were able to walk post-operatively, while control dogs suffered dense paraplegias.[16] Verapamil ($0.4\ mg\ kg^{-1}$) maintained cerebral autoregulation during reperfusion and dampened hyperperfusion of the distal spinal cord during reperfusion, following proximal-descending thoracic–aortic cross-clamping in beagles.[17]

Although the studies reviewed above claim protection from verapamil in various animal models of hypoxia and ischemia, we have failed to show any protective effect for this drug during acute CO poisoning. This may be related to the fact that carboxyhemoglobinemia produces neither pure hypoxia nor pure ischemia. Under ideal conditions (i.e. without unilateral carotid/jugular occlusion), cerebral blood flow increases many-fold with CO exposure[28] until the point at which cardiac function is compromised, at the same time that blood oxygen-carrying capacity is decreasing. This condition is identical to neither hypoxia nor ischemia, but combines aspects of both. The Levine preparation simply serves to further limit blood flow to one side of the brain, giving the rat a more human-like response to CO.

In summary, the results of this study with the Levine-prepared rat indicate that the use of ketamine is potentially protective when administered before and during severe acute CO poisoning. Ketamine reduces the elevation of blood lactate, improves blood pressure recovery and limits cerebral edema. Ketamine may also reduce mortality during CO poisoning. The optimal dose of ketamine for benefit in terms of these parameters appears to be $40–60\ mg\ kg^{-1}$. Verapamil, in contrast, whether at a relatively low or a high dose, provides no benefits in terms of lactate elevation, blood pressure recovery, cerebral edema or survival. Indeed, verapamil appears to slow and render incomplete the recovery of body temperature.

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