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Ethanol Potentiates Dopamine Release During Acute Hypoxia in Rat Striatum

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WANG, Y., A. L. CHIOU, C. H. JENG, S. T. YANG AND J. C. LIN. *Ethanol potentiates dopamine release during acute hypoxia in rat striatum.* PHARMACOL BIOCHEM BEHAV **66**(4) 679–685, 2000.—We, and others, have previously demonstrated that *N*-methyl-D-aspartate (NMDA) receptor is involved in hypoxia or ischemia-mediated responses. We found that the NMDA antagonist ketamine attenuates cortical nitric oxide release during cerebroischemia. It has been reported that ethanol (EtOH) antagonizes NMDA-induced responses in various systems. In the present study, the interaction of EtOH and KCl-evoked striatal dopamine release in vivo during acute hypoxia was examined. High-speed chronoamperometric recording techniques, using Nafion-coated carbon fiber electrodes, were used to evaluate extracellular dopamine (DA) concentration in the striatum of urethane-anesthetized Sprague–Dawley rats. KCl was directly applied to the striatum to evoke release of DA. These anesthetized animals were paralyzed with *d*-tubocurarine and connected to a respirator to allow controlled respiration. Systemic concentrations of oxygen were altered by changing the rate of the respirator. We previously reported that lowering the respiratory rates from 90 to 20 times/min for 5 min decreased arterial PO_2 and facilitated KCl-induced DA release in the striatum. In this study, we found that application of NMDA antagonist MK801 attenuates hypoxic DA release, suggesting that NMDA receptor is involved in this hypoxic reaction. In contrast, EtOH dose dependently enhanced KCl-evoked DA release during hypoxia. To further examine the interactions of excitatory amino acid and EtOH on DA release, glutamate was locally applied to the striatum. Glutamate-induced DA release was not affected by the systemic application of EtOH. Taken together, these data suggest that EtOH enhances DA release in vivo during short-term hypoxia, possibly through mechanisms other than excitatory amino acid pathways. © 2000 Elsevier Science Inc.

Ethanol Dopamine Hypoxia Striatum Alcohol Voltammetry

N-METHYL-D-ASPARTATE (NMDA) receptors have been suggested to participate in many hypoxia-mediated responses (3,30). Hypoxia induces aspartate release from the forebrain (18). Pretreatment with NMDA antagonist attenuates nitric oxide release from the ischemic cerebral cortex (20) and antagonizes hypoxia-elicited degenerative changes in the hippocampus and cerebral cortex (16). These data suggest that the NMDA receptor is activated during hypoxia, and its antagonists protect against insults induced by ischemia or hypoxia.

Hypoxia also alters catecholaminergic functions in various systems. The synthesis and release of catecholamines are enhanced during hypoxia in the adrenal medulla (9), carotid body (4), and sympathetic ganglia (11). Hypoxia increases catecholamine levels in the midbrain, brain stem (28), and striatum (25,31). Previous studies have indicated that increasing DA levels by exogeneous administration of DA induces oxidative stress and apoptosis in the striatum (21). It is possible that hypoxia-induced DA release may potentiate neurodegeneration in the brain through the apoptotic mechanism. Similar to hypoxia, excitatory amino acid (EAA) also modulates catecholamine turnover. NMDA induces DA and norepinephrine (NE) release in the striatum and cerebellar cortex (13,32). We have previously reported that acutely lowering arterial PaO_2 potentiated K^+-induced DA release in the striatum, which can be antagonized by a pretreatment with the NMDA antagonist ketamine, indicating that the increase of DA overflow during hypoxia may involve the activation of EAA receptors (31).

It has been known that ethanol (EtOH) alters EAA-mediated functions. EtOH inhibits glutamatergic neurotransmission in the nucleus accumbens (23), prevents NMDA-induced

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glutamate release in the striatum (6), and protects against the NMDA receptor-mediated excitotoxicity in rat primary neuronal cultures (7). In contrast to these antagonistic reactions, the interactions of EAA and EtOH may be synergistic. Glutamate was found to enhance central depressant action of EtOH (12). Glutamate uptake inhibitor potentiated, whereas NMDA receptor antagonist attenuated, EtOH-induced ascorbic acid release in striatum (35,36). These data suggest that EAA-induced responses can be differentially regulated by EtOH, which may be derived from the interactions with other receptors (12). It is not clear if EtOH synergistically or antagonistically interacts with EAA during hypoxia.

There are limitations to the study of DA function in vivo during hypoxia. Because of the low sampling rates of data collection by traditional methods, such as microdialysis, the hypoxia-induced changes in DA functions can only be detected after the hypoxic period. Furthermore, nerve tissue is very sensitive to hypoxia, and the DA response obtained after the long-term hypoxia may be confounded by effects of irreversible neuronal damage. We have previously demonstrated that DA function can be monitored every second during short-term hypoxia in vivo using chonoamperiometry (31). In this study, we used this high-speed electrochemical technique and examined the interactions of NMDA antagonist and EtOH on striatal DA release during acute hypoxia. Our data suggest that EtOH, contrary to the NMDA receptor antagonist MK801, enhances DA release during short-term hypoxia.

METHOD

Electrochemical Methods

Adult Sprague–Dawley rats were anesthetized with urethane (1.25 g/kg, IP), intubated and placed in a stereotaxic frame. Body temperature was maintained at 37°C with an isothermal pad. A portion of the skull and dura, which extended from approximately 1 mm posterior to 4 mm anterior to the bregma and from 1 to 4 mm lateral to the midline, was removed bilaterally. Remote from this site, miniature Ag/AgCl reference electrodes were inserted into the brain and cemented in place with dental acrylic.

In vivo chronoamperometric measurements of extracellular DA concentrations were performed with a microcomputer-controlled apparatus (IVEC-10, Medical Systems Corp., Greenvale, NY). The recordings were taken at rates of 10 Hz continuously during the experiment using Nafion-coated (5% solution, Aldrich Chemical Co., Milwaukee, WI) carbon fiber working electrodes. These electrodes have been shown to be highly sensitive for monoamine neurotransmitters (15). An oxidation potential of +0.55 V for 50 ms (square-wave pulses), relative to a Ag/AgCl reference electrode, was applied at a rate of 10 Hz. The resulting oxidation current was integrated during the last 80% of the pulse. The current generated during the reduction of the oxidized electroactive species was digitized in the same manner when the potential dropped back to its resting level (0 V).

The linearity and sensitivity of all electrodes used in the in vivo experiments was determined using DA standard solutions in vitro. All solutions were prepared in 0.1 M pH 7.4 phosphate-buffered saline, which also contained 250 μM ascorbic acid (AA) to mimic brain extracellular levels of this potential contaminant of the electrochemical recordings (26). Calibration curves for DA sensitivity and DA:AA selectivity ratios were determined for all electrodes prior to their use. Only electrodes exhibiting highly linear responses ($r > 0.997$) and selectivity (>500:1, compared with AA) to DA were used for the in vivo experiments. The ratios of reduction-to-oxidation currents, at the peak of the oxidation signal, were used as an index to qualitatively identify if the compound measured was DA (17,33). All in vivo signals were expressed as μM changes in DA using the in vitro calibration curves.

KCl-Induced DA Release

The release of DA was measured by the changes of extracellular DA concentration after microejection of KCl into the striatal parenchyma. KCl (70 mM, 100–200 nl) was locally applied through a multibarrel pipette (24). The working electrode and the multibarrel micropipette were mounted together with sticky wax (Kerr Inc., Sybron, CA); tips were separated by 100–150 μm. The electrode/pipette assembly

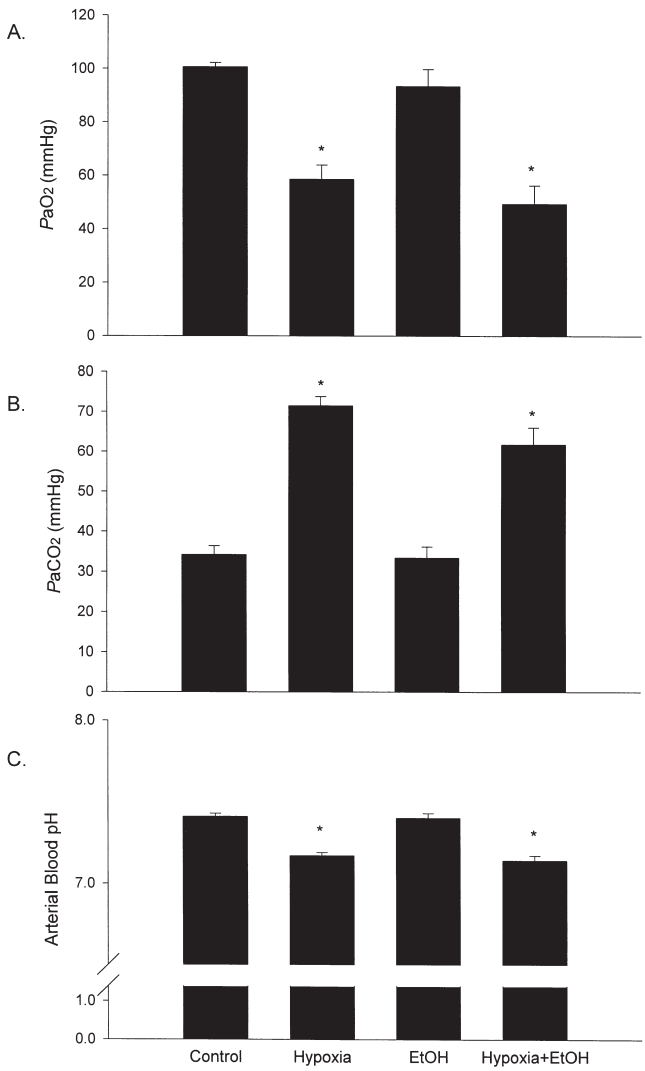

FIG. 1. Hypoxia alters arterial (A) PaO_2, (B) $PaCO_2$, and (C) pH levels. Blood gas concentrations taken 30 min after EtOH administration (1 g/kg) did not show difference to those in the control animals before hypoxic insults. Hypovententilatory hypoxia, induced by lowering the respiratory rate from 90/min (control) to 20/min for 5 min, significantly decreased the blood pH, PaO_2 concentration, and increased PCO_2 in both EtOH-treated or nontreated animals (*$p < 0.05$, one-way ANOVA and Newman–Keuls test).

was lowered into the anterior striatum (2.5 mm lateral to the midline; 1.0 mm anterior to bregma, and 4.5 mm below brain surface). Local application of drugs from the multibarrel micropipettes was performed by pressure ejection using a pneumatic pump (PPM-2, Medical Systems Corp., Great Neck, NY). The ejected volume was monitored by recording the change in the fluid meniscus in the pipette before and after ejection using a dissection microscope.

Hypoventilatory Hypoxia

After anesthetizing with urethane, animals were paralyzed with d-tubocurarine (initial dose: 0.75 mg/kg, IP, supplementary dose: 0.25 mg/kg/h, IP), and were connected to a respirator (Model 131, New England Medical Instrument, Boston, MA). Paralysis was necessary to prevent the actions of the respiratory muscles from interfering with the control of the respiratory rate by respirator. Resting respiratory rate was set to 90 times/min initially as control. Tidal volume was 1 ml/100 g body weight. Reversible hypoxia (31) was performed by lowering the respiratory rate to 20 times/min with room air for 5 min.

Blood Gas Analysis

Arterial blood (0.3–0.5 ml) was withdrawn from femoral artery through a PE tube. Blood was heparinized; blood pH, concentrations of CO_2 and O_2 were analyzed using a blood gas analyzer (ABL3, Radiometer, Copenhagen, Denmark). Blood gas values were corrected for temperature changes.

Chemicals

All drugs were dissolved in 0.9% saline. MK801 (RBI) was given at the dose of 2.5 mg/kg IP. EtOH was given at the dose of 0.5 g/kg or 1.0 g/kg IP. Dopamine, glutamate, and d-tubocurarine were purchased from Sigma Chemical Co.

Statistics

Data were expressed using the mean and standard error. Student;s *t*-test or one-way ANOVA and post hoc Newman–Keuls test were used for statistical analysis. *p*-Values less than 0.05 were considered significant.

RESULTS

Blood Gas Analysis

Twelve animals were used for the blood gas analysis. Of these, six rats were treated with 1.0 g/kg EtOH (IP). The other six rats were treated with saline. Blood gas concentrations were taken 30 min after EtOH or vehicle administration. We found that arterial PaO_2 and pH of d-tubocurarine–treated animals were not affected by systemic EtOH injection (Fig. 1, $p > 0.05$, one-way ANOVA). Lowering the respiratory rate from 90/min (control) to 20/min for 5 min significantly decreased the blood pH, PaO_2 concentration and increased $PaCO_2$ to a similar extent in animals pretreated with EtOH or vehicle (Fig. 1, $p < 0.05$, one-way ANOVA and Newman–Keuls test).

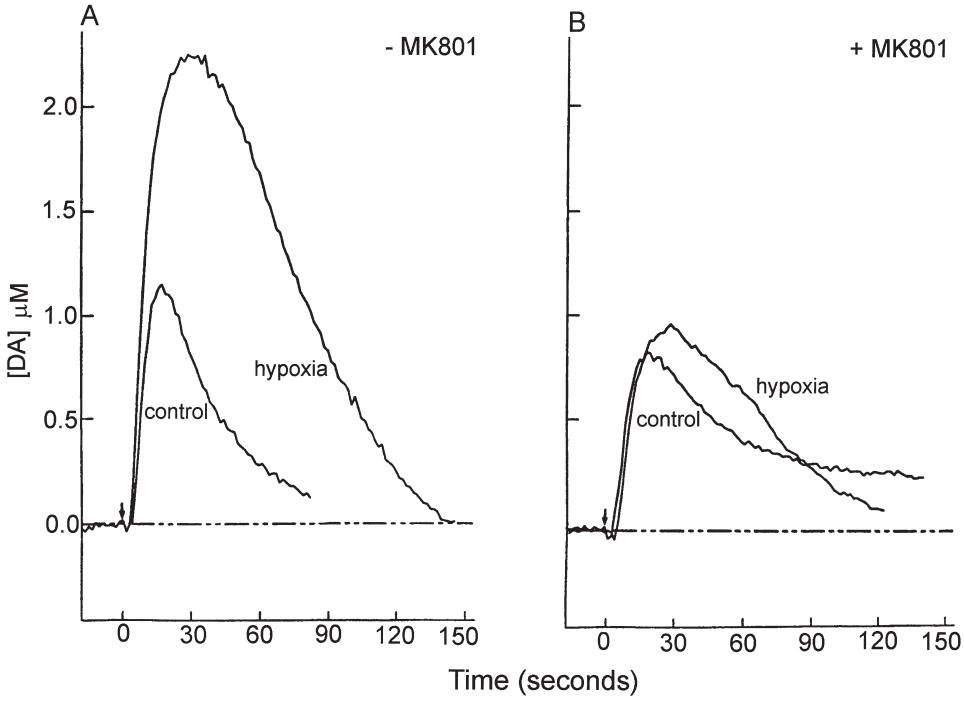

FIG. 2. Voltammetric recordings showing MK801 antagonism of the increase of K^+-induced DA release during hypoxia in two animals. (A) Local application of KCl (100 nl)-induced DA release in the striatum (lower trace). After hypoventilatory hypoxia (reducing the respirator rate to 20 strokes/min for 5 min), the peak and the duration of K^+-evoked DA release were augmented (upper trace). (B) After systemic application of MK801, hypoxia cannot increase the amplitude of K^+-evoked DA release.

TABLE 1

KCl-INDUCED DA RELEASE DURING CONTROL AND HYPOXIA WITH OR WITHOUT PRETREATMENT OF MK801

	Control	Hypoxia
Without MK801		
Peak amplitude (μM)	1.28 ± 0.19	2.13 ± 0.39*
Rise time (s)	16.0 ± 1.8	71.2 ± 34.2*
$T_{1/2}$ (s)	29.5 ± 2.5	125.5 ± 53.9*
Number of animals	7	7
With MK801		
Peak amplitude (μM)	0.86 ± 0.19	1.14 ± 0.29
Rise time (s)	16.5 ± 1.3	58.5 ± 18.4*
$T_{1/2}$ (s)	30.2 ± 3.9	84.2 ± 21.9*
Number of animals	7	7

*Significantly different from control by Student's *t*-test.

MK801 Antagonizes KCl-Induced DA Release During Hypoxia

We found that KCl-induced DA release can be altered by hypoxia. A typical voltammetric recording is demonstrated in Fig. 2A. Microinjection of 70 mM KCl (132.3 ± 5.7 nl) to the anterior striatum produced peak DA overflow averaging 1.28 ± 0.19 μM in seven rats before hypoxic insults. A same dose of KCl was given after 5 min of hypoxia. KCl-induced DA overflow was significantly increased to 2.13 ± 0.39 μM during hypoxia in the same animals (Fig. 4A). The rise time, peak DA concentration and $t_{1/2}$ of the evoked DA overflow were all prolonged by hypoxia (Table 1). These electrochemical responses were reversible, because KCl-evoked DA responses returned to the control normoxic levels after resetting the respirator to 90 times/min for 15 min.

Previous experiments have demonstrated that NMDA is involved in many hypoxia-induced responses. To investigate if hypoxia-induced DA overflow is mediated through NMDA receptors, we systemically administrated NMDA channel blocker MK801 (2.5 mg/kg IP) before hypoxia. KCl was locally applied 5 min before and 30 min after systemic MK801 application. We found that KCl-induced DA release was not significantly affected by MK801 in the anterior striatum (Figs. 2B and 4A, before MK801: 1.28 ± 0.19 μM, n 7 vs. after MK801: 0.86 ± 0.19 μM, $n = 7$, $p > 0.10$). However, DA release during hypoxia was attenuated by MK801. After injection of MK801, KCl-evoked DA release was no longer potentiated by this short-term hypoxia (Fig. 4A, 0.86 0.19 μM vs. 1.14 ± 0.29 μM, $n = 7$, $p > 0.05$, Table 1). MK801 did not alter the red/ox current ratio of electrochemical signal during hypoxia (before MK801: Red/Ox = 0.74 ± 0.08; after MK801: Red/ox = 0.84 ± 0.17). These data suggest that MK801 attenuates hypoxia-induced DA overflow.

EtOH Potentiates DA Release During Hypoxia

In contrast to the effect of MK801, EtOH potentiated the KCl-evoked DA release during hypoxia (Fig. 3) In seven rats studied for EtOH effects, we found that transient hypoxia facilitated KCl-evoked DA overflow from 1.89 ± 0.28 μM to 3.07 ± 0.52 μM ($p < 0.05$, paired *t*-test). EtOH (1.0 g/kg) significantly augmented KCl-induced DA release during hypoxia. KCl-induced DA overflow (70 mM, 131.3 ± 6.6 nl) during hypoxia was further enhanced from 3.07 ± 0.52 μM (before EtOH injection) to 11.45 ± 3.86 μM (30 min after 1.0 g/kg EtOH injection; Fig. 4B; Table 2, $p < 0.05$, one-way

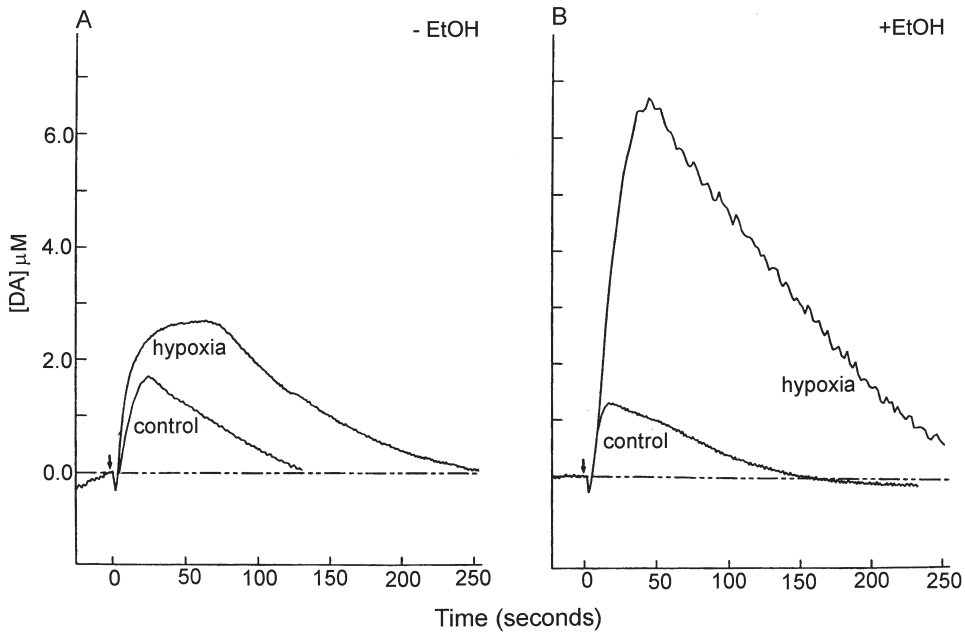

FIG. 3. EtOH potentiates the increase of K^+-induced DA release during hypoventilatory hypoxia. (A) Local application of KCl (125 nl) evoked DA release in the striatum (lower trace). After transient hypoxia for 5 min, the peak and the duration of K^+-evoked DA release were potentiated (upper trace). (B) After systemic application of EtOH (1.0 g/kg), the peak of K^+-evoked DA release was further increased by hypoxia.

ANOVA + Newman–Keuls test). The red/ox ratio of the electrochemical signals during hypoxia was not altered by the systemic administration of EtOH (before EtOH: Red/ox = 0.50 ± 0.13; after EtOH, red/ox ratio = 0.52 ± 0.08).

The interaction of EtOH and DA release during hypoxia was further normalized in all animals studied (17 rats treated with vehicle; 6 rats treated with EtOH, at a dose of 0.5 g/kg; 7 rats treated with EtOH, at a dose of 1.0 g/kg). The peak of KCl-evoked DA release during hypoxia was compared to that before hypoxia in each animal. As indicated in Fig. 5, EtOH dose dependently potentiates KCl-mediated DA release during hypoxia (Fig. 5, $p < 0.001$, one-way ANOVA, $F = 9.16$).

Glutamate-Induced DA Release

It has been suggested that EtOH interacts with excitatory amino acids in various systems. We further studied if the facilitation of hypoxic responses by EtOH is secondary to the interaction between excitatory amino acid and EtOH. In four animals studied, we found that local application of glutamate (5 mM, 162.5 ± 21.7 nl) induced DA overflow (1.64 ± 0.56 μM) in the striatum. The same dose of glutamate was given 30 min after systemic application of EtOH (1.0 g/kg, IP). We found that glutamate-evoked DA release was not significantly altered (1.71 ± 0.60 μM, $p > 0.4$, paired *t*-test) by EtOH.

TABLE 2

KCl-INDUCED DA RELEASE DURING CONTROL AND HYPOXIA WITH OR WITHOUT EtOH

	Control	Hypoxia
Without EtOH		
Peak amplitude (μM)	1.89 ± 0.28	3.07 ± 0.52*
Rise time (s)	24.8 ± 5.9	68.2 ± 12.9*
$T_{1/2}$ (s)	50.8 ± 10.3	115.3 ± 19.3*
Number of animals	7	7
With EtOH		
Peak amplitude (μM)	1.56 ± 0.31	11.45 ± 3.86*
Rise time (s)	19.7 ± 2.5	169.2 ± 47.2*
$T_{1/2}$ (s)	54.3 ± 8.4	266.0 ± 62.9*
Number of animals	7	7

*Significantly different from control by Student's *t*-test.

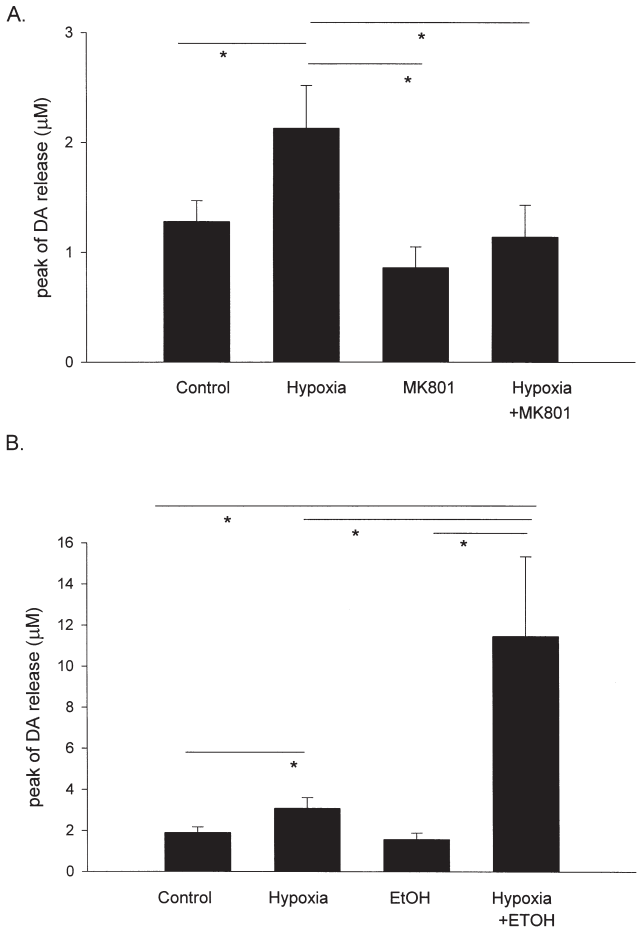

FIG. 4. Differential effects of MK801 and EtOH on DA release during hypoxia. (A) MK801 antagonized the increase of evoked DA release during hypoxia. Histograms represent KCl-induced DA release with or without hypoxia. The amplitude of KCl-induced DA release was significantly increased after hypoxic insults. MK801 (2.5 mg/kg, IP) did not alter the KCl-evoked DA release in normoxic control animals; however, it significantly attenuated DA overflow during hypoxia. (B) K^+-evoked DA release was potentiated by EtOH after hypoxia. The amplitude of KCl-induced DA release was significantly increased after hypoxia. EtOH (1.0 g/kg, IP) significantly potentiated KCl-induced DA overflow during hypoxia. *$p < 0.05$, one-way ANOVA + Newman–Keuls test.

DISCUSSION

In the present and previous studies we found that lowering the respiratory rate to 20 times/min for 5 min resulted in hypoxia, hypercapnia, acidosis, and facilitated KCl-evoked DA overflow in the striatum. We previously reported that K^+-mediated DA release in the striatum was less affected by the short-term high CO_2 air exposure, which resulted in hypercapnia and acidosis, but was greatly influenced by hypoxia. These data suggest that the increase of evoked DA release

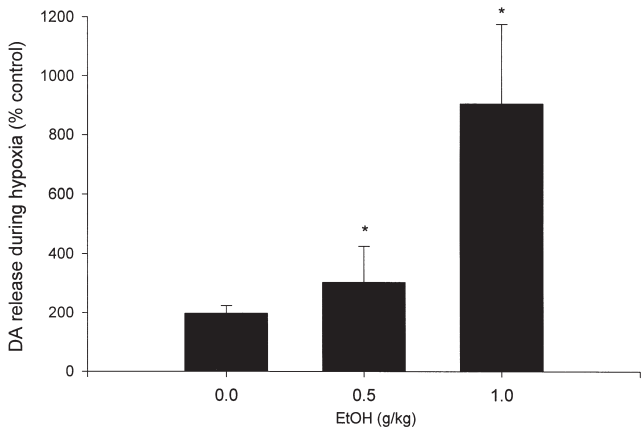

FIG. 5. EtOH dose dependently potentiates DA release during hypoxia. EtOH (0.5–1.0 g/kg) or vehicle was administered 30 min before local KCl application. KCl (100 nl) was given 15 min before and 5 min after the onset of hypoxia. KCl-evoked DA release during hypoxia was normalized by comparison to the peak DA release before hypoxic insults in each animals. Pretreatment with EtOH significantly increases DA release during hypoxia. *$p < 0.05$, one-way ANOVA + post hoc Newman–Keuls test.

during short-term hypoventilatory hypoxia is mainly mediated through the low PaO_2. Previous studies have indicated that traumatic brain injury suppressed respiratory function in EtOH-treated animals (37). Because a controlled respiration was used in our study, arterial PaO_2, $PaCO_2$, and pH were not altered after EtOH administration.

In this study, we used Nafion-coated electrochemical sensors to measure DA release during transient hypoxia. These sensors have been previously reported to have high selectivity against ascorbic acid (AA). The Nafion sensors can detect AA only when its level reaches 2 mM or increases to 100–1000 times greater than DA (14). Previous studies have indicated that hypoxia, induced by administering pure nitrogen flow for 30 s, or local administration of glutamate (100–400 nl × 10 mM) evoked AA release in rat hippocampus (5). Similarly, 10-min ischemia, induced by carotid occlusion, increased extracellular AA to a level of 50 μ striatum of the gerbil (8). In this study, a lower dosage (5 mM × 160 nl) of glutamate and less traumatic hypoxia, induced by slowing the respiratory rate to 20 times/min, were used. It is possible that AA released in our models was less than those reported earlier. Furthermore, DA signals were differentiated by measuring the red/ox current ratios during recording. DA, as measured by chronoamperometry, has a red/ox ratio of 0.51 ± 0.20 (34). No reduction current can be obtained from AA (14). We found that red/ox of KCl-induced electrochemical currents during short-term hypoxia is 0.5–0.8, suggesting that these signals were mainly composed of DA.

Increasing evidences have demonstrated that EAA is involved in hypoxic reactions. Hypoxia increases presynaptic release of EAA (18). NMDA receptor antagonists prevent hypoxia or ischemia-induced neuronal damages (20,31). We also found that MK801 antagonized evoked DA release during hypoxia, suggesting that the potentation of evoked DA release in hypoxia may be related to the activation of NMDA receptors. In contrast to the neuroprotective effects of MK801, we found that EtOH potentiated the hypoxic response in striatum. EtOH enhanced the peak of the KCl-evoked DA overflow and prolonged the rise time of extracellular DA concentration and the $t_{1/2}$ for its disappearance during hypoxia. This high and long-lasting DA release may facilitate apoptosis in striatum (21). The lack of neuroprotection by EtOH has also been reported that hypoxia-induced DNA degradation was accelerated in the presence of EtOH (2) and mortality, neurological deficits and brain edema after experimental head trauma were potentiated by EtOH in rats (29). Taken together, these data suggest that EtOH and NMDA antagonist have differential responses during hypoxia.

EtOH inhibits EAA-activated responses in various systems. EtOH attenuates glutamatergic neurotransmission in nucleus accumbens (23), prevents NMDA-induced glutamate release in the rat striatum (6), and protects against NMDA receptor-mediated excitotoxicity in rat primary neuronal cultures (7,10). On the other hand, we found that glutamate-induced DA release was not altered by systemic administration of EtOH. It has been reported that EtOH has other non-EAA reactions. The synergistic interaction between EtOH and glutamate in CNS depression was attenuated by bicuculline (12), indicating that GABAergic system is involved in this response. EtOH decreases intracellular Mg^{++} levels (1), which may facilitate EAA-induced response. NGF has been shown to protect against hypoxic/ischemic insults (19,22). However, EtOH attenuated NGF-mediated cell survival (27). It is possible that EtOH-induced increase of DA release during hypoxia is mediated through these nonexcitatory amino acid pathways.

In conclusion, our data suggest that pretreatment with EtOH potentiates DA release during short-term hypoxia. This interaction is possibly involved mechanisms other than excitatory amino acids.

ACKNOWLEDGEMENTS

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