How can an inert gas counterbalance a NMDA-induced glutamate release?

Nicolas Vallee,1,2 Jean-Claude Rostain,2 and Jean-Jacques Risso1

1Institut de Médecine Navale du Service de Santé des Armées, IRBA Toulon, Department of Marine and Underwater Research, UMR-MD2, Toulon Cedex 9; and 2Université de la Méditerranée, Laboratoire de Physiologie et Physiopathologie en Conditions d’Oxygénation Extrêmes, UMR-MD2, Institut Jean-Roche, Faculté de Médecine Nord, Marseille Cedex 20, France

Submitted 30 January 2009; accepted in final form 19 August 2009


Previous neurochemical studies performed in rats have revealed a decrease of striatal dopamine and glutamate induced by inert gas narcosis. We sought to establish the hypothetical role of glutamate and its main receptor, the N-methyl-D-aspartate (NMDA) receptor, in this syndrome. We aimed to counteract the nitrogen narcosis-induced glutamate and dopamine decreases by stimulating the NMDA receptor in the striatum. We used bilateral retrodialysis on awake rats, submitted to nitrogen under pressure (3 MPa). Continuous infusion of 2 mM of NMDA under normobaric conditions (0.01 MPa) (n = 8) significantly increased extracellular average levels of glutamate, aspartate, glutamine, and asparagine by 241.8%, 292.5%, 108.3%, and 195.3%, respectively. The same infusion conducted under nitrogen at 3 MPa (n = 6) revealed significant lower levels of these amino acids (n = 8/6, P > 0.001). In opposition, the NMDA-induced effects on dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels were statistically not affected by the nitrogen at 3 MPa exposure (n = 8/6, P > 0.05). Dopamine was increased by >240% on average. HVA was decreased (down to 40%), and there was no change in DOPAC levels, in both conditions. Results highlight that the NMDA receptor is not directly affected by nitrogen under pressure as indicated by the elevation in NMDA-induced dopamine release under hyperbaric nitrogen. On the other hand, the NMDA-evoked glutamate increase is counteracted by nitrogen narcosis. No improvement in motor and locomotor disturbances was observed with high striatal concentration in dopamine. Further experiments have to be done to specify why the striatal glutamate pathways, in association with the inhibition of its metabolism, only are affected by nitrogen narcosis in this study.

Address for reprint requests and other correspondence: N. Vallee, Institut de Médecine Navale du Service de Santé des Armées, IRBA Toulon, Dept. of Marine and Underwater Research, UMR-MD2, BP 20548, 83049 Toulon Cedex 9, France (e-mail: n.vallee@imnssa.net).

http://www.jap.org 8750-7587/09 $8.00 Copyright © 2009 the American Physiological Society
conducted to reveal changes in behavioral signs, including the disruption of the motor and locomotor coordination and the sedative effect induced by the nitrogen at 3 MPa, or some stereotyped activities.

Surgery
Anesthesia was induced by halothane (5% with O2) (Halothane, Belamont) and then deeply prolonged with pentobarbital sodium (30 mg/kg ip) (Sanofi Santé Animal) and ketamine (0.40 mg/kg im; Imalgène 500, Laboratoire Rhône-Mérieux). Rats were stereotactically implanted with two intracerebral guides (CMA/12 guide cannulae; Phymep) in each striatum (from interaural: anterior 10.0 mm, lateral 2.8 mm, height 6.4 mm) according to the atlas of Paxinos and Watson (36).

Microdialysis in Hyperbaric Conditions

The microdialysis equipment consisted of microinjector pumps (CMA/102; Phymep) customized to support high pressure, a Rattern cage (Bioanalytical Systems,) to prevent fluid line tangling, (CMA/102; Phymep) customized to support high pressure, a Microdialysis in Hyperbaric Conditions and Watson (36).

Concerning retrodialysis, two syringes perfused the rat brain, and then deeply prolonged with pentobarbital sodium (30 mg/kg ip) (Sanofi Santé Animal) and ketamine (0,40 mg/kg im; Belamont) and then deeply prolonged with pentobarbital.

Apparatus and Chromatography

Amino acid analyses. Microdialysis sample content was analyzed by high-performance liquid chromatography (CMA/260 Degasser, Kontron Instrument HPLC pump 422, HPLC pump 420, HPLC Autosampler 465) coupled with fluorometric detection (CMA/280 Fluorescence Detector). HPLC was carried out with a reverse-phase C18 column (3 μm, 200 × 3 mm, Phymep) stabilized at 25°C under gradient conditions. Eluent A consists of a sodium acetate buffer (0.01 M sodium acetate, 15% MeOH, 2 mM triethanolamine, 0.3 mM EDTA, pH 9.3), and eluent B was eluent A with the alcohol content adjusted to 45% (0.01 M sodium acetate, 45% MeOH, 2 mM triethanolamine, 0.3 mM EDTA, pH 9.3). Measurement of amino acid concentrations needed a precolumn derivatization (fixation of a fluorophore to the sample to produce fluorescent derivatives) with an ortho-phthalaldehyde-dihydrine (OPA) reagent (37 MOpH, OPA, 128 mM 2-mercaptoethanol, 25% methanol, water, 24 mM borax, pH 10) mixed online and added to the dialysate (vol/vol). Glutamate, glutamine, aspartate, and asparagine were analyzed.

Catecholamine determination. Dopamine extracellular levels in brain dialysates were measured with HPLC coupled to an electrochemical detector (Decade II, Antec, Leyden, The Netherlands) working at 720 mV and 20 μA, an automatic injector (Triathlon, Spark), and an AZUR software integrator. The pump (Shimadzu, LC-1 OAD VP) delivered the mobile phase at 0.37 ml/min into a reverse-phase C18 column (3.2 × 200 mm ID, particle size 3 μm, Phymep) warmed at 30°C. The mobile phase consisted, for 1,000 ml, of 5 g of citric acid, 15 g of dipotassium hydrogen orthophosphate (K2HPO4), 0.13 g EDTA, 3.5 ml of triethylenamine (Sigma, St-Quentin Fallavier, France), and 1.7 mg of heptan-sulfonic acid in 1.1 of HPLC-grade water (Sigma; Carlo Erba, Italy) completed by 200 ml of methanol (Merck) and 30 ml of tetrahydrofurane (Merck). Final pH was adjusted to 4.2 with acetic acid.

DOPAC and HVA were measured with HPLC coupled to an amperometric detector (Eldec 105, Precision Instrument). The glassy carbon electrode was at +650 mV against an Ag/AgCl reference electrode. A 1-μl sample was injected into a reverse-phase C18 column (3.2 × 200 mm ID, particle size 3 μm, Phymep). The same mobile phase was delivered at 0.3 ml/min by a Bio-Teck Kontron 525 pump.

Statistical Analysis

For each subject, baseline was determined using the four samples preceding gas pressure exposure. Samples were next expressed as a percentage of baseline, taken as the 100% value (baseline). Data for the whole group were noted using median value and the 25th to 75th percentiles. Nonparametric statistical tests were used as the number of animals was small. First, baseline groups were compared with the compression and exposure stages for each experiment type and each molecule, with a Kruskal-Wallis test followed by a Dunn test. Then the effects of NMDA infusion were compared under nitrogen exposure and in atmospheric conditions for each molecule development, and at each time point (Mann-Whitney test).

Exposure during compression time (from 0.01 MPa to 3 MPa) was differentiated from the stay at maximal pressure period (3 MPa).

RESULTS
No rat died because of the surgery. Twenty of 35 of the rats previously surgically implanted presented a complete sequence of
Behavioral Observations

The behavioral signs observed in rats submitted to NMDA infusion at atmospheric pressure differed from those exposed to NMDA and to nitrogen under pressure.

Under atmospheric conditions and during the microdialysis control period, the rats of the different groups did not show any behavioral alterations. Under atmospheric conditions and during the NMDA infusion, rats (n = 8) showed motor and locomotor hyperactivity due to the hyperexcitability of the central nervous system. There was no alteration of the righting reflex.

Under nitrogen at 3 MPa, without (n = 6) or with NMDA infusion (n = 6), motor and locomotor disturbances, uncoordinated movements, ataxia and some stereotyped activities were visually observed. The animals staggered before falling and lying on one side. Nitrogen at 3 MPa impaired an animal’s ability to right itself. No sign of hyperexcitability stage occurred.

Neurochemical Study

In rat striatum, during the control period, the medium concentrations of glutamate, aspartate, glutamine, and asparagine were, respectively, 1.87 ± 0.85, 0.33 ± 0.16, 109.87 ± 40.21, 0.54 ± 0.09 μM. Concerning monoamines, the medium concentration of dopamine was 8.19 ± 6.94 nM, and those of DOPAC and HVA were, respectively, 0.61 ± 0.57 and 0.43 ± 0.62 μM.

The NMDA infusion under atmospheric conditions (0.01 MPa) significantly increased striatal levels of the four amino acids studied here: glutamate, aspartate, glutamine, and asparagine (Table 1). The same infusion conducted under nitrogen at 3 MPa revealed significant lower levels of these amino acids (Table 2). In opposition, the NMDA-induced effects on monoamine levels were statistically not affected by the nitrogen at 3 MPa exposure (Table 3). More details on each molecule follow.

Extracellular Glutamate Levels

Under atmospheric conditions (0.01 MPa), and compared with baseline, NMDA infusion increased extracellular glutamate levels from the beginning to the end of the experiment (Table 1 and Fig. 1A). The increase of glutamate averaged 241.8%, compared with baseline, and the maximum value was 717.6%.

Under nitrogen at 3 MPa, and compared with baseline, extracellular glutamate levels decreased from the compression stage to the end of the 3 MPa period (Table 1 and Fig. 1A). The average decrease in glutamate reached 10.2% during the compression stage, and 23.6% during the maximal nitrogen pressure exposure (Fig. 1A).

When both conditions were combined, NMDA retrodialysis and high pressure of nitrogen, extracellular glutamate levels remained unchanged from the compression stage to the end of the 3 MPa period (Table 1 and Fig. 1A).

Glutamate levels were significantly higher when comparing values recorded during NMDA infusion in the atmospheric pressure group to those recorded during nitrogen exposure with NMDA, or to those recorded during nitrogen exposure alone (Table 2). The intergroup comparisons (Table 2) highlighted no significant difference between the nitrogen exposure group and the nitrogen at 3 MPa plus NMDA group.

**Table 1. Intragroup comparison of striatal amino acid baselines against their developments under nitrogen at 3 MPa (n = 6), or under atmospheric pressure with NMDA retrodialysis (n = 8), or under nitrogen exposure with NMDA retrodialysis (n = 6)**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Baseline</th>
<th>Nitrogen at 3 MPa</th>
<th>NMDA at 0.01 MPa</th>
<th>Nitrogen at 3 MPa + NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control at 0.01 MPa Median (1st, 3rd quartile)</td>
<td>Comp</td>
<td>Stay at 3 MPa Median (1st, 3rd quartile)</td>
<td>Comp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P value</td>
<td>Post hoc test</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>99.7 (95.9, 109.0)</td>
<td>0.006 ■ &lt;0.001 ■■■■■■■■■</td>
<td>241.8 (435.3, 747.2)</td>
<td>0.297 ■</td>
</tr>
<tr>
<td>Glutamine</td>
<td>100.5 (95.5, 110.1)</td>
<td>&lt;0.001 ■■■■■■■■■ &lt;0.001 ■■■■■■■■■</td>
<td>108.3 (104.0, 115.9)</td>
<td>0.251 ■</td>
</tr>
<tr>
<td>Aspartate</td>
<td>102.2 (88.2, 110.8)</td>
<td>0.866 ■ 0.427 0.019 ■■■■■■■■■</td>
<td>292.5 (195.9, 530.4)</td>
<td>0.253 ■</td>
</tr>
<tr>
<td>Asparagine</td>
<td>99.1 (93.6, 108.5)</td>
<td>0.742 ■ 0.019 ■■■■■■■■■ 0.011 ■■■■■■■■■</td>
<td>195.3 (140.1, 289.6)</td>
<td>0.122 ■</td>
</tr>
</tbody>
</table>

Baselines and periods at maximal pressure [but not the compression (Comp) stage] were described using median values, with the 25th and 75th percentiles in parentheses. Each baseline was compared with compression stage (≤3 MPa) and compared with a maximum pressure period (3 MPa) using a Kruskal-Wallis test (P values were noted, α = 5%), followed by a post hoc test transcribed by squares. Each square represents a string of samples (median value) that have to be compared with the baseline samples, arranged in chronological order: 2 squares for series of samples available during the compression stage (or similar); 9 squares for 9 series of samples available during the stay at the maximal pressure (or similar). Dark squares indicate a significant difference, as pointed out by a post hoc Dunn test (α = 5%), between samples at the corresponding time point and its baseline. White squares indicate no significant difference between the string of samples at the corresponding time point and the baseline. NMDA, N-methyl-D-aspartate. Boldface P values are significant.
Table 2. Intergroup comparison of amino acid developments, under nitrogen at 3 MPa (n = 6) and under atmospheric pressure with NMDA retrodialysis (n = 8), and under nitrogen exposure with NMDA retrodialysis (n = 6)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Nitrogen vs. NMDA</th>
<th>Nitrogen + NMDA vs. Nitrogen</th>
<th>Nitrogen + NMDA vs. NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Comp 3 MPa P value</td>
<td>Comp 3 MPa P value</td>
<td>Comp 3 MPa P value</td>
</tr>
<tr>
<td>Glutamate</td>
<td>&lt;0.001</td>
<td>0.624</td>
<td>0.462</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt;0.001</td>
<td>0.734</td>
<td>1.000</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.041</td>
<td>0.521</td>
<td>0.946</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.047</td>
<td>0.910</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The compression stages (≤3 MPa) and the maximal pressure period (3 MPa) of all groups were compared against each other, using a Kruskal-Wallis test (α = 5%). P values were noted. Boldface P values are significant.

Extracellular Glutamine Levels

NMDA infusion significantly increased extracellular glutamine levels, under atmospheric conditions, in the second part of the experiment (Table 1 and Fig. 1B). Glutamine levels increased up to 108.3%, on average, compared with baseline, and the maximum value was 172.5%.

Under nitrogen at 3 MPa, and compared with baseline, extracellular glutamine levels decreased from the compression stage to the end of the 3 MPa period (Table 1 and Fig. 1B). Decrease in glutamine averaged 17.6% during the compression stage, and 37.3% during the maximal nitrogen pressure period (Fig. 1B).

Under nitrogen at 3 MPa with NMDA retrodialysis, and compared with baseline, no change in extracellular glutamine was recorded (Table 1 and Fig. 1B).

Glutamine level was significantly lower when comparing values recorded during nitrogen exposure only to those recorded during NMDA infusion whatever the pressure (Fig. 1B and Table 2). No significant difference was found between the nitrogen at 3 MPa plus NMDA group and NMDA infusion in atmospheric pressure group (Table 2).

Extracellular Asparagine Levels

Nitrogen + NMDA infusion increased extracellular asparagine levels from the beginning to the end of the experiment (Table 1), with a maximum up to 467% (Fig. 1C). Under nitrogen at 3 MPa, with or without NMDA, aspartate levels remained unchanged compared with the baseline (Table 1 and Fig. 1C). Actually, the nitrogen exposure groups have shown no significant difference between them (Table 2). We recorded significantly higher levels of aspartate in the NMDA infusion in the atmospheric pressure group than in the both nitrogen at 3 MPa groups (Table 2).

Extracellular Dopamine Levels

The NMDA infusion under atmospheric conditions increased extracellular dopamine levels from the beginning to the end of the experiment (Table 3 and Fig. 2A). Dopamine levels reached a medium value of 242.9%. The maximum value was 991.5%.

Table 3. Comparison of striatal monoamine baselines with their developments under atmospheric pressure with NMDA retrodialysis (n = 8), and under nitrogen exposure with NMDA retrodialysis (n = 6)

<table>
<thead>
<tr>
<th>Monoamine</th>
<th>NMDA at 0.01 MPa</th>
<th>Nitrogen at 3 MPa + NMDA</th>
<th>Nitrogen + NMDA vs. NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>0.01 MPa</td>
<td>Median (1st, 3rd quartile)</td>
</tr>
<tr>
<td>Control at 0.01 MPa</td>
<td>Median (1st, 3rd quartile)</td>
<td>P value</td>
<td>242.9 (116.4, 546.4)</td>
</tr>
<tr>
<td>Dopamine</td>
<td>100.0 (81.8, 116.5)</td>
<td>88.7 (68.9, 103.4)</td>
<td>0.502</td>
</tr>
<tr>
<td>DOPAC</td>
<td>99.4 (77.5, 110.7)</td>
<td>38.8 (31.1, 46.5)</td>
<td>0.013</td>
</tr>
<tr>
<td>HVA</td>
<td>99.9 (86.6, 110.8)</td>
<td>38.8 (31.1, 46.5)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Baselines and maximal pressure periods (but not the compression stage) were described using median values, with 25th and 75th percentiles in parentheses. Each baseline was compared with its compression stage (≤3 MPa) and compared with the maximal pressure period (3 MPa) using a Kruskal-Wallis test (P values were noted, α = 5%), followed by a post hoc test transcribed by squares. Each square represents a string of samples (~median value) that have to be compared with the baseline samples, arranged in chronological order. Dark squares indicate a significant difference, as pointed out by a post hoc Dunn test (α = 5%), between samples at the corresponding time point and its baseline. White squares indicate no significant difference between the string of samples at the corresponding time point and the baseline. Boldface P value are significant.
The same infusion conducted under 3 MPa of nitrogen increased extracellular dopamine levels up to an average value of 276.4%, during the maximal pressure period (Table 3 and Fig. 2A). The dopamine peak corresponded to 858.2%

The comparison between these two developments did not reveal significant differences.

Extracellular DOPAC Levels

Neither under atmospheric conditions nor nitrogen at 3 MPa did NMDA infusion induce changes in extracellular DOPAC levels compared with its baseline (Table 3 and Fig. 2B). The comparison between these two developments did not show significant differences, which leads to the conclusion that the same pattern can be observed between the control experiment and the nitrogen exposure group (Table 3).

Extracellular HVA Levels

The NMDA infusion, applied under atmospheric conditions, significantly decreased (61.2% on average) striatal HVA levels between the control period and the remainder of protocol compared with baseline (Table 3). HVA levels were decreased from the start, by 43.7% in the first 20 min, to the end, by 62.3% in the last minutes (Fig. 2B).

Under nitrogen at 3 MPa with NMDA retrodialysis, the development was the same as under atmospheric conditions, as no significant differences were observed in the comparison of these two developments (Table 3). A significant decrease occurred, under nitrogen at 3 MPa with the NMDA retrodialysis, from the time of compression (−57.2%) to the end of the maximal nitrogen pressure period (−67.0%) (Fig. 2B).

DISCUSSION

Levels of dopamine and glutamate recorded in the striatum under pressure of nitrogen are in accordance with our previous laboratory studies (43). Under nitrogen narcosis, extracellular glutamate, glutamine, and asparagine levels in the striatum were recorded, and no change in aspartate development was observed (43). Balon et al. (3), Dedieu et al. (17), and Lavoute et al. (24) have reported a decrease in striatal dopamine level. Our present results complete those of previous studies and confirm the action of NMDA on its glutamatergic receptor.

Under atmospheric conditions, NMDA-Receptor stimulation by NMDA retrodialysis in the striatum induces increases in dopamine and glutamate in this structure. This is a good evidence for interactions between these transmitters. NMDA retrodialysis also involves an increase in glutamine, aspartate and asparagine levels, and a decrease in DOPAC and HVA levels under atmospheric conditions.

The interactions between glutamate and dopamine disappear under nitrogen narcosis. Indeed nitrogen at 3 MPa did not change the increase of dopamine induce by striatal NMDA infusion but suppressed the increase of glutamate, glutamine, aspartate, and asparagine and the decrease of DOPAC levels.
Nitrogen has no effect on the NMDA-induced decrease in HVA levels. First these results indicate that NMDA receptors remain functional under nitrogen narcosis, as NMDA infusion significantly potentiates dopamine increase. Second, they show that there is no more NMDA-induced interaction between glutamate and dopamine under nitrogen pressure. The activation of the NMDA receptor in the striatum is not sufficient to increase extracellular concentration of glutamate under nitrogen exposure. Balon et al. (3) and Lavoute et al. (26) have also reported the inversion of striatal dopamine release by NMDA injection in the substantia nigra pars compacta; they also conclude that NMDA-Receptor was not affected by nitrogen at pressure. Hence, the failure, under nitrogen narcosis, in the NMDA-NMDA interaction is shown by the gray area, which is followed by the maximal pressure period, 3 MPa (or similar). Dotted lines with dashes mark experiments using NMDA retrodialysis in the striatum under atmospheric pressure. Solid lines mark experiments using NMDA retrodialysis under nitrogen at 3 MPa. Intergroup comparison show no significant change in development of monoamine levels (n = 6) nitrogen-oxygen pressure atmospheres on levels of extracellular monoamines [A: dopamine; B: dihydrophenylacetic acid (DOPAC) and homovanillic acid (HVA)] in rat striata. The ordinate of each graph shows the level of monoamine expressed as the percentage of the baseline level, which is the mean of the 4 consecutive values observed immediately before the beginning of the compression/infusion of NMDA (2 mM). The time of compression (or similar) is shown by the gray area, which is followed by the maximal pressure period, 3 MPa (or similar). Dotted lines with dashes mark experiments using NMDA retrodialysis under nitrogen at 3 MPa. Intergroup comparison show no significant change in development of monoamine levels (n = 0.05, Kruskal-Wallis test) between the NMDA-nitrogen group and the NMDA-atmospheric group. Each point is the median; the 25–75th percentiles are not represented to avoid confusion. For individual time point, there was no significant change (α = 0.05, Dunn test) between the NMDA-nitrogen group and the NMDA-atmospheric group at the corresponding time point.

**NMDA and Basal Ganglia Pathways**

The dichotomy between glutamate and dopamine developments points to the conclusion that the feedback/forward pathway controls in basal ganglia (20, 31–33, 39, 40) are disrupted under nitrogen pressure. 

**Dopamine pathway.** Dopamine cells follow the same developments after NMDA stimulation, whether under nitrogen exposure or not. Dopamine cells seem to be resistant to nitrogen narcosis, whereas glutamergic cells are affected directly or indirectly. Dopamine axon terminals in the striatum are relatively insensitive to a variety of glutamate agonists (6, 15, 29, 44). The striatal action of NMDA on nigrostriatal dopaminergic neurons is not more clearly established (2, 8, 9, 11, 12, 23, 27, 29). Under atmospheric conditions, Marti et al. (28) suggested that NMDA-induced dopamine increase appears to be a result of the activation of the striatonigral pathway. Indeed, in this study, this means that GABAergic cells of the striatonigral pathway are thus not directly inhibited by nitrogen under pressure. Lavoute’s findings (26) support these suggestions.

**Glutamate pathway.** Glutamate level, which is decreased under nitrogen narcosis, cannot be restored by the activation of striatal NMDA receptor.

According to Marti et al. (28), NMDA-evoked striatal glutamate release is mediated by the activation of striatofugal GABAergic neurons and requires dopamine receptor activation. The same team also claims that striatal glutamate release evoked by NMDA is a result of the disinhibition of thalamostriatal and corticostriatal glutamatergic projections. Indeed, the striatal glutamate decrease described in our work would plead in favor of a disturbance at the level of thalamostriatal and corticostriatal glutamatergic projections, but not in GABAergic striatofugal cell disturbances as we can still observe a striatal dopamine increase related to these cells (see above).

This does not exclude that NMDA stimulation induces striatal dopamine increase, and dopamine remains necessary to modulate striatal glutamate release evoked by NMDA (10).

**Metabolite Interpretation**

According to Dedieu et al. (17), DOPAC and HVA metabolisms should be enhanced by nitrogen exposure, and dopamine should be decreased. DOPAC is generated from dopamine within dopaminergic nerve terminals by monoamine oxidase (MAO). HVA is produced from extracellular dopamine and DOPAC by catechol-O-methyltransferase (COMT) (18). Although there is an increase in NMDA-induced dopamine, no change was observed in its degradation into DOPAC. The HVA decrease could indicate a downregulation of the COMT induced by the NMDA infusion. Indeed, the dopamine increase, in part, could result from a nondegradation of itself.

Glutamine and asparagine are the metabolic precursors of glutamate (16, 21) and aspartate (22, 30, 35), respectively. They can be considered as an indicator of their metabolism. Here it was demonstrated that NMDA infusion was effective to increase glutamate, aspartate, and their metabolites levels under atmospheric conditions. However, this method failed to increase them under nitrogen exposure. Indeed, concomitant failures in effluxes of glutamine and asparagine suggest that the decrease of glutamate and aspartate can be of metabolic origin (33). Moreover, the release of aspartate, the cotransmitter of glutamate, is also thought to be of metabolic origin (20, 33). Hence, the failure, under nitrogen narcosis, in the NMDA-induced aspartate increase can be attributed to the effect of the gas on metabolic mechanisms.

The neurochemical study performed on metabolites indicates that nitrogen under pressure does not affect the vesicular neurotransmitter release alone. Indeed the neurotransmitter...
synthesis, uptake, or degradation should also be affected by nitrogen under pressure.

Behavior and Neurotransmission

These different neurochemical changes highlighted with NMDA, whether under nitrogen pressure or not, could partly explain the motor and locomotor behavior disruptions under nitrogen narcosis. The increase of motor and locomotor activity that occurs with the NMDA infusion under atmospheric condition can be attributed to the increase of dopamine and glutamate levels. Hence, the decreased activity observed under nitrogen pressure, even with NMDA receptor stimulation, seems to be related to the decrease in glutamate (and aspartate) level, as dopamine level was shown to be largely enhanced with NMDA. Nevertheless, decreased locomotor activity, sedation, and motor disturbances under nitrogen-oxygen pressure, previously reported by Balon et al. (3), can be attributed to a decrease of dopamine levels (4). It confirms that both dopamine and glutamate are keys in nitrogen narcosis behavior.

Conclusion

In conclusion, the viability of the NMDA receptor is not directly affected by nitrogen under pressure. The NMDA-evoked glutamate increase is counteracted by nitrogen narcosis. On the other hand, the NMDA-induced dopamine increase is not affected by the high pressure of nitrogen. Nevertheless, no improvement in motor and locomotor disturbances was observed with high striatal concentration in dopamine. Using the retrodialysis technique in nitrogen narcosis, we show for the first time dissociation between glutamate and dopamine pathways of basal ganglia. Indeed, the study of both dopamine and glutamate at the same time should be considered systematically to better improve the neurochemical disorders of nitrogen narcosis. Moreover, troubles of the glutamatergic release are also coupled to disturbances in glutamate aspartate and asparagine levels in the striatum. Subsequent experiments have to be done to specify what properties make the striatal glutamate pathways sensitive to nitrogen under pressure.

ACKNOWLEDGMENTS

We thank the following people, all from UMR-MD2 laboratories, for valuable contributions to this work: Myriam Nicolas and Boualem Zouani, laboratory technicians; Patrick Ledrut and Olivier Moreau, mechanics; and Bruno Schmid, research and development technician.

GRANTS

This study was supported by Grant No. 01/0809 from the Délégation Générale pour l’Armement, Paris, France.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


