Laboratory Investigation

Magnesium deficiency increases ketamine sensitivity in rats

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Purpose: Inhibition of the NMDA receptor likely contributes to ketamine's neurodepressive properties. Magnesium also inhibits the NMDA receptor by binding to a site associated with the ketamine-binding domain. Electrophysiological studies suggest that magnesium prevents ketamine from binding to the NMDA receptor and thereby prevents ketamine inhibition. We undertook an *in vivo* study to determine if magnesium deficiency was associated with an increased sensitivity to ketamine.

Methods: Weanling rats were maintained on a Mg^{2+}-deficient or control diet for 14 days. In Study 1, rats were anaesthetized then sacrificed and the Mg^{2+} concentrations in the brain and plasma were measured. In a second prospective study, experimental animals were rendered hypomagnesaemic and the potency of 125 $mg \cdot kg^{-1}$ *ip* ketamine was evaluated. Animals were then were fed a Mg^{2+}-containing diet and ketamine sensitivity was re-examined 14 days later.

Results: The Mg^{2+}-deficient diets rendered the rats hypomagnesaemic as indicated by the brain and plasma concentration of Mg^{2+}. In Study 2, the time to loss of righting reflex was shorter: 1.9 ± 0.3 min (n = 12) and 2.6 ± 0.2 min (n = 16, $P < 0.05$), whereas the latency to toe pinch was prolonged: 25.0 ± 5.8 min (n=12) *vs* 3.1 ± 2.1 min (n = 16, $P < 0.05$) in the Mg^{2+}-deficient compared with age-matched control animals, respectively. The hypomagnesaemic animals had a higher death rate following ketamine injection. The increased sensitivity to ketamine was no longer apparent when the animals were re-tested following replenishment of Mg^{2+}.

Conclusion: Hypomagnesaemia is associated with an increased sensitivity to ketamine.

Objectif : L'inhibition du récepteur NMDA contribue vraisemblablement aux propriétés neurodépressives de la kétamine. Le magnésium inhibe aussi le récepteur NMDA en se liant avec un site spécifique à la kétamine. Des études électrophysiologiques suggèrent que le magnésium empêche la kétamine de se lier au récepteur NMDA et prévient ainsi l'inhibition de la kétamine. Cette étude *in vivo* visait à établir si la déficience en magnésium augmentait la sensibilité à la kétamine.

Méthodes : Des rats Weanling ont été maintenus sur une diète déficiente en Mg^{++} ou sur une diète de contrôle pendant 14 jours. Au cours de la première expérience, les rats ont été anesthésiés puis sacrifiés. Les concentrations plasmatiques et cérébrales de Mg^{++} ont alors été mesurées. Dans la deuxième étude prospective, chez les animaux de laboratoire rendus hypomagnésémiques, on évaluait la puissance de 125 $mg \cdot kg^{-1}$ de kétamine *ip*. Les animaux recevaient alors une diète contenant du Mg^{++} et la sensibilité à la kétamine était réévaluée 14 jours plus tard.

Résultats : Les diètes déficientes en Mg produisaient de l'hypomagnésémie comme le révélaient les concentrations plasmatiques et cérébrales de Mg^{++}. Les rats de la deuxième étude avaient un réflexe de redressement plus court : 1,9 ± 0,3 min (n=12) *vs* 2,6 ± 0,2 min (n=16, $P < 0,05$), alors que la latence du retrait après compression de l'orteil était prolongée : 25,0 ± 5,8 min (n=12) *vs* 3,1 ± 2,1 min (n=16, $P < 0,05$) chez les rats hypomagnésémiques comparativement aux animaux de contrôle appariés pour l'âge. Les animaux hypomagnésémiques avaient un taux de mortalité plus élevé après l'injection de kétamine. L'augmentation de la sensibilité à la kétamine disparaissait quand les animaux étaient de nouveau examinés après réapprovisionnement en Mg^{++}.

Conclusion : L'hypomagnésémie augmente la sensibilité à la kétamine.

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MAGNESIUM is the fourth most abundant cation in the body and its importance in the regulation of metabolic activity is well documented.[1-5] Magnesium is a cofactor in over 300 enzyme reactions and is essential for maintaining the functional integrity of the cell membrane.[1] In humans, Mg^{2+} deficiency results from inadequate dietary intake, malabsorption, or abnormal renal excretion. It is most commonly seen in the critically ill, alcoholics, patients with burns, and those taking certain drugs (diuretics, aminoglycosides, cyclosporin and digoxin).[4] Hypomagnesaemia is associated with disorders involving the cardiac, renal, gastrointestinal, and musculoskeletal systems; it is probably the most commonly undiagnosed electrolyte abnormality in hospitalized patients.[4] Severe magnesium deficiency in humans and animals results in neurological disturbances including apathy, psychosis, hyperexcitability and seizure. Death can result from generalized convulsions.[5-7] The metabolic basis for the encephalopathy associated with hypomagnesaemia is unknown but possibly results from abnormal glutamate-mediated neurotransmission as Mg^{2+} inhibits a subtype of glutamate receptor.[3]

Glutamate is the major excitatory transmitter in the mammalian central nervous system. Glutamate activates several sub-types of receptors which are linked to ion channels.[8] These ligand-gated ion channels have been divided into NMDA (*N*-methyl-D-aspartate) and non-NMDA subtypes on the basis of their pharmacological and electrophysiological characteristics. The apparent role of NMDA receptors in a variety of neurological processes has led to intense efforts to understand their physiological properties. The effects of hypomagnesaemia on NMDA receptor function is also of interest as Mg^{2+}, at physiological concentrations, blocks the NMDA receptor.[8,9] This inhibition is essential for normal receptor function. Under resting conditions, the NMDA receptor is relatively inactive due to Mg^{2+} blockade. However, during periods of intense stimulation, Mg^{2+} is displaced from the channel pore and ions move across the pore. The resulting influx of calcium and sodium contributes to physiological processes such as learning and memory.[10] Excessive activation of the NMDA receptor is linked to disorders including stroke and epilepsy.[10]

Ketamine ((2-*o*-chlorophenyl)-2-thylaminocyclohexanone hydrochloride) also blocks the NMDA receptor and this inhibition likely contributes to ketamine's anaesthetic and analgesic properties.[11-16] Electrophysiological studies suggest that the binding sites for Mg^{2+} and ketamine reside in a similar region on the receptor.[17,18] Furthermore, Mg^{2+} can prevent ketamine from binding to the NMDA receptor.[12] Thus, we hypothesize that hypomagnesaemia would be associated with an increase in ketamine binding to the NMDA receptor. The purpose of these studies was to determine if hypomagnesaemia, induced by dietary restriction in rats, was associated with an increased sensitivity to ketamine.

Methods

The experimental protocols were approved by the Council on Animal Care at the University of Western Ontario. Twenty-one day old, weanling Wistar rats (Charles Rivers Laboratory, Quebec, Canada) were caged in an environmental vivarium under 12-hr light and dark cycles. They had free access to food and water. Rats were randomly allocated into two groups: control animals were fed a semisynthetic diet that contained Mg^{2+} (Mg^{2+} = 0.55%, Purina Basal Diet #5755, PMI Feeds, Richmond), whereas experimental animals were fed a similar diet that was deficient in Mg^{2+} (Mg^{2+} = 0.001%, Purina Mg^{2+} Deficient Diet #5865). Both diets were otherwise identical and contained casein 21%, sucrose 15%, Solka Floc 3%, RP mineral mix 5%, DL-methionine 0.15%, choline chloride 0.2%, lard 5%, corn oil 5%, dextrin 43.65%. The control diet contained RP mineral mix #10 (5%) and the Mg^{2+}-deficient diet contained RP mineral mix (5%)without Mg^{2+}.

Two series of experiments were performed. In Study 1, the purpose was to measure the Mg^{2+} concentrations in the brain and plasma of animals that had been fed control or Mg^{2+}-depleted diet for 14 days. In addition, the anaesthetic potency of ketamine was examined in weanling mice in order to estimate the sample size required for the second series of experiments. After 14 days, animals were anaesthetized with 125 $mg \cdot kg^{-1}$ *ip* ketamine (Ketalar, Park Davis). The dose of ketamine was based on published recommendations and preliminary experiments.[19,20] We observed that 100 $mg \cdot kg^{-1}$ ketamine administered to weanling rats, was not sufficient to induce anaesthesia whereas 150 $mg \cdot kg^{-1}$ ketamine was frequently lethal. Ketamine injections were administered by the same technician. Prior to injection of the drug, an "aspiration test" was performed. After the injection of ketamine, rats were gently placed on their backs. If they failed to right themselves, the time to the loss of righting reflex was noted. The anaesthetized animals were then placed on a warming blanket (38°C) and observed by a second investigator who was unaware to which group the animals belonged. Sleep time, defined as the time interval between the loss of the righting reflex and recovery of normal posture, was noted. Recovery of the toe pinch withdrawal was assessed by firmly pinching a toe on the hind limb using a rubber-shod haemostat. Times to the loss or recovery

of reflexes are standard tests used to assess the depth of anaesthesia in laboratory animals. However, the sensitivity and specificity of these tests is not certain and variability in responses does occur.[21]

Following recovery from anaesthesia, animals were decapitated. The whole brain was quickly isolated and stored at –4°C. Blood was collected for analysis of serum protein and electrolyte concentrations.

In Study 2, animals were placed on the control or Mg^{2+}-depleted diet for 14 days then anaesthetized as described above. Similar to the first protocol, 125 mg·kg^{-1} *ip* ketamine was administered and time to loss of righting reflex, sleep time, latency to withdrawal to toe pinch, and the time to crawl were determined. The animals were then allowed to fully recover from the anaesthetic. Both the Mg^{2+}-depleted and age-matched control animals were subsequently maintained on the control, magnesium-containing diet for 14 days. Ketamine (125 mg·kg^{-1} *ip*) was again injected and the anaesthetic sensitivity determined. Following the experiment, animals were sacrificed and blood collected for analysis of serum Mg^{2+}, protein, and electrolyte concentrations.

Tissue analysis

Brain Mg^{2+} concentrations were measured using the inductively coupled plasma-mass spectroscopic (ICP-MS) technique as previously described.[22] This technique provides a method of obtaining accurate, multi-element analyses of biological samples with sensitivities that surpass electrothermal atomic absorption spectroscopy or neutron activation. The sensitivity for the Mg^{2+} assay was 170 ions·sec^{-1} at a detection limit of 1.8 ng·ml^{-1}. No polyatomic ion or spectral overlap interference has been reported for Mg^{2+} at mz-1 = 24, 25 or 26.[23] On the day of analysis, brains were weighed and sections obtained for wet and dry analysis. The dry specimen was heated for one hour at 105°C, then cooled in a desiccator. The tissue was then digested with 2 ml of HNO_3 (10%), and heated for one hour. Wet samples were mixed with 1 ml HNO_3, sealed in a test tube and left to stand overnight at room temperature. These samples were then warmed at 90°C for at least six hours until digestion was complete. Digested samples were diluted to 25 ml with water and then stored at 4°C in plastic bottles. During analysis, samples were compared with standard reference material.

Statistical analysis

Results are expressed as mean ± SEM. The INSTAT Program (GraphPad Software Inc., San Diego, CA) was used to perform the statistical analysis. Differences between the groups with regard to serum and brain Mg^{2+} concentrations and serum protein and electrolyte concentrations were analysed using the Student's t test for unpaired samples. Using the method of Lachin,[24] the variable of "sleep time" was used to calculate the required sample size for Study 2. A Fisher's exact test was used to determine significant differences between groups with regard to death following the induction of anaesthesia. The time to loss of righting reflex, latency to toe pinch withdrawal, sleep time, and time to crawl were analyzed using a Mann-Whitney U test for unpaired, nonparametric data. Differences were considered significant when the P values were <0.05. An increased sensitivity to ketamine was suggested by a decrease in the time to loss of righting reflex, and an increase in sleep time, the latency to toe pinch withdrawal, and time to crawl.

Results

Study 1

Eleven rats were studied in the pilot experiment. Rats were randomly assigned to the control (n=6) or low Mg^{2+} diet (n=5). After 14 days, the Mg^{2+}-deficient rats weighed less than controls: (125.3 ± 4.8 g *vs* 151.2 ± 5.0 g, respectively P <0.05). The Mg^{2+}-deficient diet rendered the rats hypomagnesaemic (Table I). Consistent

TABLE I Plasma electrolyte and protein concentrations in control and experimental rats. Study 1: Blood samples were obtained after the animals were fed the control or Mg^{2+}-deficient diet for 14 days. Study 2: samples were obtained after the experimental and control animals were fed the same Mg^{2+}-containing diet for 14 days.

	Study 1 *plasma Mg^{2+}* *mmol L^{-1}*	*plasma Ca^{2+}* *mmol L^{-1}*	*plasma protein* *g dL^{-1}*		*Study 2* *plasma Mg^{2+}* *mmol L^{-1}*	*plasma protein* *g dL^{-1}*
Normal	1.07–1.28	2.67–3.43	4.7–8.2		1.07–1.28	4.7–8.2
Control	1.07 ± 0.06 (n=6)	2.87 ± 0.19 (n=6)	5.62 ± 0.05 (n=6)	Control	1.06 ± 0.04 (n=20)*	5.34 ± 0.10 (n=20)
Mg^{2+} Depleted	0.33 ± 0.09 (n=5)	2.84 ± 0.19 (n=5)	5.28 ± .08 (n=5)	Mg^{2+} Replenished	0.98 ± 0.04 (n=15)	5.35 ± 0.89 (n=15)

*a blood sample could not be obtained from two animals.

The normal values for the plasma concentrations of Mg^{2+}, protein and calcium are for *adult* Wistar rats (sexes combined at 19–21 wk).[47]

with a previous study, cutaneous vasodilatation and erythema were evident around the periorbital and periaural regions of the Mg^{2+}-deficient rats.[6] Serum Mg^{2+} concentrations were markedly less in the Mg^{2+}-deficient rats than in controls (Table I). The brain Mg^{2+} concentrations (wet samples) were less in Mg^{2+}-deficient rats than in the control rats (95.1 ± 1.2 μg $Mg^{2+} \cdot g^{-1}$ wet weight (n=5) *vs* 111.7 ± 6.3 μg $Mg^{2+} \cdot g^{-1}$ wet weight (n=5)) as were the total Mg^{2+} contents of the samples (95.9 ± 1.2 μg (n=5) and 111.7 ± 6.3 μg (n=5) ($P < 0.05$)). The brain Mg^{2+} concentrations (dry weights) were not different; (control 746.0 ± 24.9 μg $Mg^{2+} \cdot g^{-1}$ dry weight (n=5) and experimental animals, 742.6 ± 26.4 μg $Mg^{2+} \cdot g^{-1}$ dry weight (n=5), respectively ($P > 0.05$)). No differences were observed in the brain concentrations of copper: experimental animals 95.5 ± 2.9 $\mu g \cdot g^{-1}$ dry weight and control 83.5 ± 1.7 $\mu g \cdot g^{-1}$ dry weight, or zinc (experimental animals 651.2 ± 13.9 $\mu g \cdot g^{-1}$ dry weight, and control 617.9 ± 30.3 $\mu g \cdot g^{-1}$ dry weight). Plasma protein concentrations were also measured as proteins influence the concentration of free (ionized) Mg^{2+}.[7] Plasma protein concentrations were within the normal range and were not different between the two groups.

Ketamine (125 $mg \cdot kg^{-1}$ *ip*) induced a loss of righting reflex in four of the five Mg^{2+}-deficient rats and four of the six normal rats. One control animal died immediately following the injection of ketamine. The Mg^{2+}-deficient and control animals that failed to lose their righting reflex were re-tested 24 hr later and demonstrated a loss of righting reflex at one and three minutes, respectively. The mean times to loss of righting reflex in the experimental and control groups were 1.95 ± 0.5 min (n=5) and 2.46 ± 0.5 min (n=5), respectively.

The sleep times of animals anaesthetized on Day 1 were 87.5 ± 27.7 min (n=4) and 52.5 ± 4.3 min (n=4) whereas the latency times to toe pinch withdrawal were 61.2 ± 15.3 min (n=4) and 33.7 ± 11.2 (n=4) in Mg^{2+}-deficient and control animals, respectively. The parameter of sleep time was used to calculate sample size for Study 2. Based on these data, it was estimated that a total of 42 rats (21 rats per group) would be required for Study 2 in order to demonstrate a 36 min prolongation of sleep time in the Mg^{2+}-deficient rats compared with the control group ($\alpha = 0.25$, $\beta = 0.2$, power = 0.8). Twenty-two animals were in each group to accommodate the death of animals during the feeding time.

Study 2

Figure 1 summarizes the observations of animals enrolled in Study 2. Three Mg^{2+}-deficient rats died during the feeding period before the induction of anaesthesia, whereas none of the control animals died. These deaths were not witnessed and the cause of death is not certain. However, autopsies revealed histopathological changes suggestive of myocardial degeneration and cardiac failure. The surviving Mg^{2+}-deficient rats weighed 30.4% less than the controls: (74.0 ± 5.7 g (n = 19) *vs* 106.4 ± 2.5 g (n = 22, $P < 0.05$)). Following 125 $mg \cdot kg^{-1}$ *ip* ketamine, three of the 19 Mg^{2+}-deficient rats died during the anaesthetic (15.7%) whereas no deaths were observed in the control group ($P < 0.05$, Fisher's Exact Test, Post-Hoc analysis). The deaths were characterized by bradypnea, then apnoea with no obvious signs of neuronal hyperexcitability or seizure.

Ketamine failed to induce a loss of righting reflex in three of the 19 Mg^{2+}-deficient rats (15.8%) whereas six of 22 (27.3%) of the control animals were not induced by ketamine ($P > 0.05$, Fisher's Exact Test). The time to loss of righting reflex was 1.9 ± 0.3 min (n = 12) and 2.6 ± 0.2 min (n = 16) in the Mg^{2+}-deficient and control animals, respectively ($P < 0.05$, Mann-Whitney U test). Furthermore, the latency to toe pinch was prolonged in the Mg^{2+}-deficient animals compared with control (25.0 ± 5.8 min (n=12) *vs* 3.1 ± 2.1 min (n = 16, $P < 0.05$) (Figure 2). Surprisingly, no differences were observed in the sleep time (exper-

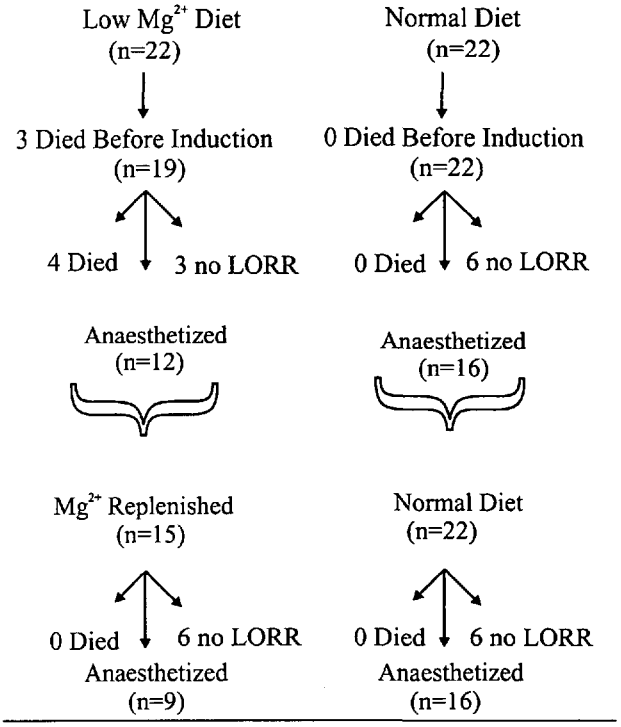

FIGURE 1 A schematic summary of the outcome of rats enrolled in Study 2. Animals that failed to loose their righting reflex following the injection of ketamine are indicated by no LORR.

imental animals 67.9 ± 6.3 (n=12) *vs* controls 67.8 ± 8.2 (n=16)) or the time to crawl (experimental animals 75.4 ± 6.3 min *vs* control 90.6 ± 9.3 min).

After two weeks of magnesium replenishment, the Mg^{2+}-deficient group still weighed 16.8% less than the control animals (160.2 ± 10.9 g (n=15) and 192.7 ± 5.1 g (n=22) ($P < 0.05$)). However, the plasma Mg^{2+} concentrations in the replenished rats were not different from those of the control group (Table I). Ketamine (125 $mg \cdot kg^{-1}$ *ip*) failed to induce a loss of righting reflex in six of the 15 replenished rats (40%), whereas six of 22 control animals (27.3%) did not loose their righting reflex following injection of ketamine. Not all of the same rats failed to loose their righting reflex following the two injections of ketamine, administered before and after Mg^{2+}-replenishment. Interestingly, the time to loss of righting reflex was longer in the Mg^{2+}-replenished rats than in control animals (4.3 ± 0.4 (n=9) and 2.5 ± 0.2 (n=16, $P < 0.05$)), (Figure 2). There was no difference in the latency to toe pinch (5.0 ± 3.3 min (n=9) *vs* 3.1 ± 2.3 min (n=16)) between the two groups. The sleep time (experimental animals 50.0 ± 9.2 min (n=9) and control 70.3 ± 11.1 min (n=16)) and time to crawl (experimental animals 81.5 ± 13 min (n=16) and control 55.0 ± 9.1 min (n=9), were not different.

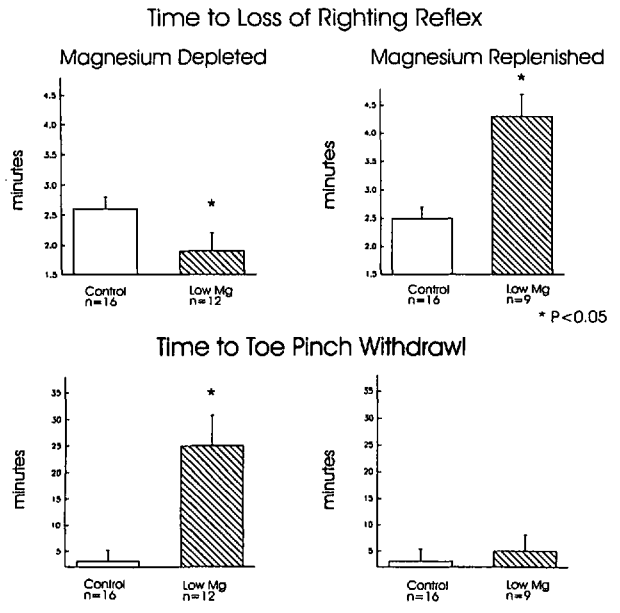

FIGURE 2 The times to loss of righting reflex and the time to toe pinch withdrawl measured before (Magnesium Depleted) and after Mg^{2+} replenishment (Magnesium Replenished) are shown. Each bar represents the mean value (SEM. $*P < 0.05$ (Mann-Whitney U test). Interestingly, the time to loss of righting reflex was *shorter* in the Mg^{2+}-depleted rats and *longer* in the Mg^{2+}-replenished rats than with control animals.

Discussion

Our results demonstrated that hypomagnesaemia in rats was associated with an increased sensitivity to ketamine as indicated by a decrease in the time to loss of righting reflex and a prolongation in the latency time to withdrawal to toe pinch. Furthermore, more deaths occurred following ketamine induction in the hypomagnesaemic group. The increased sensitivity to ketamine was no longer apparent after the Mg^{2+}-deficient animals were fed the control diet for 14 days. As discussed below, changes in the availability of drug at its site of action (pharmacokinetic effects) or the effects of the drug on the receptor (pharmacodynamic effects) could account for the increased sensitivity to ketamine.

Ketamine is rapidly absorbed following injection and its neurodepressive properties result from the effect of the parent compound on the CNS.[14,25,26] Termination of the anaesthetic effect of ketamine is due primarily to redistribution of the drug from the brain to the peripheral tissues. Ketamine is hydrolysed and methylated in the liver and the resulting breakdown products are excreted in the urine. It is likely that the enzyme systems which control the metabolism, absorption, or the volume of distribution of ketamine are dramatically altered in hypomagnesaemic animals.[1,3] Furthermore, Mg^{2+} deficiency can contribute to a variety of additional disease states or electrolyte abnormalities that could influence sensitivity to ketamine.[27]

Human and animal studies suggest NMDA receptor blockade contributes to ketamine's anaesthetic and analgesic properties.[28–30] Ketamine is referred to as an "uncompetitive antagonist" of the NMDA receptor as the rates of onset, and recovery from blockade are increased by the agonist. In contrast, a "non-competitive antagonists" binds independently of the agonist. Ketamine inhibits the NMDA receptor by two distinct mechanisms.[11,12,28,29] Similar to Mg^{2+}, ketamine occludes the channel pore in a voltage-sensitive manner.[11,12] In addition, ketamine allosterically influences receptor gating so that the receptor favours a non-conducting configuration.[28] Ketamine-induced anaesthesia in mice is antagonized by the intraperitoneal injection of NMDA, whereas the non-active isomer of NMDA, N-methyl-*L*-aspartate, fails to influence ketamine sensitivity.[30] Antagonists of the NMDA receptor, shift the ketamine dose-response curve to the left. Further, in humans there is a difference in the relative anaesthetic potency of the stereoisomers of ketamine with the S(+) isomer being 3.4 times more potent than the R(−) isomer.[14,29,31] Inhibition of the NMDA receptor is also stereoselective and the potency ratio of

the (S+) and (R–) isomers is 2:1.[32] The similarity of the potency ratios measured *in vivo* and *in vitro* suggests that inhibition of the NMDA receptor contributes to anaesthetic effects of ketamine.

Our observations are consistent with an increase sensitivity to ketamine in hypomagnesaemic animals. These data support a previous observation that hypomagnesaemic rats did not develop tolerance to repeated administrations of ketamine and had a longer sleep time compared than age-matched controls.[33] In Study 2, we detected no difference in sleep time, possibly because four Mg^{2+}-deficient rats died following the first injection of ketamine. These rats might have been a sub-population of ketamine-sensitive animals. Consistent with this suggestion, sleep time for the surviving Mg^{2+}-replete rats was shorter than in controls, following the second ketamine injection.

Increased binding of ketamine to the NMDA receptor in hypomagnesaemic rats is not consistent with a previous report which indicated that $MgCl_2$ (injected *ip*) enhanced ketamine-induced anaesthesia in mice.[30] However, large doses of $MgCl_2$ administered in the absence of ketamine can cause sedation, and it is not known if the effects of $MgCl_2$ were simply additive to those of the sedative-hypnotic effects of ketamine.[3] Blockade of the NMDA receptor by ketamine and Mg^{2+} has been extensively characterized using biochemical, electrophysiological, and molecular techniques.[12,17,28,34] The binding sites for Mg^{2+} and ketamine are likely associated with the transmembrane segment of the NMDA subunit.[17] Studies using site-directed mutagenesis suggest that the binding sites for Mg^{2+} and ketamine may overlap.[8] Such overlap could account for the ability of Mg^{2+} to protect the NMDA channel from blockade by ketamine.[12] Thus, ketamine could more readily gain access to a binding site in the presence of low extracellular concentrations of Mg^{2+}. Because the rate of dissociation of ketamine is considerably slower than that of Mg^{2+}, the channel would remain blocked for a prolonged time.

As previously described, the loss of Mg^{2+} from the central nervous system was induced in young rats by dietary restriction.[6,35] Generally, the concentration of Mg^{2+} in the CNS does not decrease until the Mg^{2+} deficit is severe. The decline in brain and cerebral spinal fluid concentrations of Mg^{2+} lags behind changes in the plasma Mg^{2+} concentration because of the active transport of Mg^{2+} across the blood-brain barrier.[3] Following Mg^{2+} replenishment, the Mg^{2+} concentration in the CNS increases rapidly (minutes to hours), in response to even small increases in the plasma concentration of Mg^{2+}. Thus, we assumed that the brain concentration of Mg^{2+} in the replete animals were within the normal range.

Magnesium is important for the synthesis, release, and post-synaptic actions of a variety of non-glutamatergic neurotransmitters.[36–38] Changes in the activity of norepinephrine, dopamine, and 5-hydroxytryptamine might also contribute to Mg^{2+}– deprivation encephalopathy.[37] However, rats fed a low Mg^{2+} diet did not demonstrate a change in the brain concentration of these monoamines.[37] Furthermore, *in vitro* evidence suggests that Mg^{2+} deficiency also reduces the production and utilization of catecholamines, and alters the presynaptic efficacy of adenosine.[36,38–41] Magnesium also influences cholinergic transmission, and ketamine is known to block nicotinic and muscarinic acetylcholine receptors.[42–45] Hence, our results do not determine causation as changes in a variety of transmitter systems might be involved. Further studies are necessary to determine if the plasma concentration of ketamine are altered in the hypomagnesaemic animals.

Clinical implications

From a clinical perspective, these data suggest that ketamine should be used with caution in hypomagnesaemic patients. Hypomagnesaemia is common in hospitalized patients (6.9 to 11 %), particularly those in the critical care environments (for review *see* Whang, 1987).[4] The disorder is characterized by muscle weakness, tetany, neurological symptoms including vertigo, irritability, aggressiveness, cardiac arrhythmias as well as ECG changes. The most potentially serious feature is epileptiform seizures. The treatment of hypomagnesaemia and the use of magnesium infusions in anaesthesia has been reviewed.[4,46]

Signs and symptoms of hypomagnesaemia usually appear when the serum Mg^{2+} concentration is <0.5 $mmol \cdot L^{-1}$[3,7]. In our study, the mean plasma concentration in Mg^{2+}-depleted rats was 0.33 $mmol \cdot L^{-1}$. In order to place our findings in a clinical context, we reviewed serum concentrations of Mg^{2+} analyzed at Sunnybrook Health Science Centre during a one year period (April 1996–1997). A total of 13,608 samples were analyzed. The serum concentration of Mg^{2+} in 455 samples was between 0.41–0.50 $mmol \cdot L^{-}$, between 0.31–0.40 $mmol \cdot L^{-1}$ in 124 samples, and in 16 samples, the concentration was <0.30 $mmol \cdot L^{-1}$. Thus, the low concentrations of Mg^{2+} measured in the experimental animals are reported for hospitalized patients.

Ketamine is extensively used in developing countries and in adverse environments such as military situations. It can be used in haemodynamically unstable patients or for brief procedures such as burn debridement. More recently, ketamine has been used in sub-anaesthetic doses for the treatment of pain. The availability of the

potent S(+) isomer of ketamine, which produces a faster recovery of psychomotor skills than does the racemic preparation, may generate renewed interest in ketamine.[31] Finally, hypomagnesaemia might be associated with an increased sensitivity to other "uncompetitive" NMDA receptor antagonists including memantine and amantadine, drug used to treat dementia and Parkinson's Disease.[34]

In summary, our results indicate that ketamine sensitivity was increased in rats rendered hypomagnesaemic by dietary deprivation of Mg^{2+}.

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