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Does Mg^{2+} deficiency induce a long-term sensitization of the central nociceptive pathways?

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Abstract

In rats, a Mg^{2+}-deficient diet, which in a few days dramatically decreased the Mg^{2+} concentration in plasma, cerebrospinal fluid (CSF) and spinal cord, was accompanied by a significant lowering of the nociceptive threshold. After reloading, the Mg^{2+} concentration was rapidly normalized in both spinal cord and CSF. In parallel, the neurological disturbances induced by Mg^{2+} deficiency vanished in less that 24 h, but the reversal of the hyperalgesia was delayed for up to 11 to 20 days. In this model, repeated doses of dizocilpine (MK-801), a non-competitive NMDA receptor antagonist, given at start of the Mg^{2+}-depleted diet, prevented hyperalgesia, suggesting the involvement of NMDA receptor channels. The delayed recovery of a normal pain threshold argues for long-term sensitization of the nociceptive pathways.

Keywords: Mg^{2+}; MK-801; Hyperalgesia; Mg^{2+}-deficiency; (Rat); Nociceptive pathway; Sensitization

1. Introduction

In earlier work, we showed that a magnesium depleted diet can produce a mechanical hyperalgesia that is dose dependently blocked by dizocilpine (MK-801), a non-competitive NMDA receptor antagonist (Begon et al., 2001; Dubray et al., 1997). Given the role of Mg^{2+} in the modulation of the NMDA receptor channel function (Wang and McDonald, 1995), we suspected that the Mg^{2+}-deficiency might have been able to lower the opening threshold of these receptors. Regarding persistent pain, many studies have reported dysregulation of the spinal nociceptive pathways involving the NMDA receptors, leading to sensitization of the spinal neurons (Coderre et al., 1993; Coderre and Katz, 1997; Woolf and Thompson, 1991). We showed that the hyperalgesia induced in neuropathic models can be reversed by either MK-801 (Begon et al., 2000; Morris, 1992) or Mg^{2+} (Begon et al., 2000; Xiao and Bennett, 1994). The aim of this study in Mg^{2+}-deficient rats was to investigate the effect of Mg^{2+} reloading on the time course of this induced hyperalgesia, and to examine the role of the NMDA receptor channels in this effect.

2. Materials and methods

2.1. Animals

The experiments were carried out on weaning Wistar rats (Charles River, IFFA-CREDO, L'Arbresle, France). The rats were housed in a temperature-controlled room (22 °C). The experiments complied with the guidelines of the Committee for Research and Ethical Issues of the I.A.S.P. (Zimmerman, 1983).

2.2. Mg^{2+}-deficiency induction

For each experiment, the rats were randomly divided into Mg^{2+}-deficient and control groups. They were fed an appropriate diet for 10 days. Food (Dubray et al., 1997) and deionized water were provided ad libitum throughout

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the experimental period. The Mg^{2+} concentration in the diet, determined by flame atomic absorption spectrometric analysis (Perkin Elmer 400, Norwack, CT), was 35 and 980 mg/kg of food, for the depleted and control groups, respectively.

2.3. Reloading of Mg^{2+}

Ten days after the start of the experiment, the animals received a single loading dose of magnesium sulfate ($MgSO_4$) (30 mg/kg, i.p.) followed by feeding with a Mg^{2+}-enriched food (980 mg/kg of food).

2.4. Test procedure and treatment protocol

The hyperalgesia was assessed using a mechanical stimulus (Randall and Selitto, 1957). Briefly, increasing mechanical pressure was exerted with an analgesimeter (Apelex type 003920, Ugo Basil, Italy) on the left hind paw until vocalization was elicited. This vocalization threshold was expressed in grams (g) corresponding to the pressure applied.

2.4.1. Assessment of the vocalization thresholds during the Mg^{2+} reloading procedure

Vocalization threshold was assessed before (day −10) and 10 days (day 0) after the start of the diet. Immediately after this last measurement, vocalization threshold was assessed on days 2, 4, 6, 10, 15, 20 and 22 after the Mg^{2+} loading dose, in both Mg^{2+}-deficient ($n=11$) and control rats ($n=7$).

2.4.2. Preemptive treatment with MK-801

The weaning rats were randomly divided into four groups. Two groups were given a Mg^{2+}-depleted diet and the two others a control diet. The rats were given blind either repeated doses of MK-801 (0.12 mg/kg, s.c., bid) or saline (2 ml/kg, s.c., bid) for 10 days. Vocalization thresholds were measured once a day for the 10 days of the first experimental period. After the last measurement, Mg^{2+} was reloaded as previously described and vocalization threshold was assessed on days 5, 7, 11, 14 and 17 after the Mg^{2+} loading dose.

2.4.3. Determination of Mg^{2+} levels

Rats were deeply anesthetized then killed by cardiac puncture. Cerebrospinal fluid (CSF), plasma and spinal cord samples were taken on day 10 of the Mg^{2+}-depleted diet, and then on days 2, 4, and 10 after Mg^{2+} reloading.

2.4.3.1. CSF extraction. After general anesthesia, CSF samples (130 μl) were withdrawn from the spinal subarachnoid space (Yaksh and Rudy,1976). They were immediately immersed in liquid nitrogen, and stored at −80 °C.

2.4.3.2. Blood samples. These were taken from the abdominal aorta.

2.4.3.3. Samples of spinal cord. The whole spinal cord was removed from lumbar to cervical areas, immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

Total Mg^{2+} concentration was measured by atomic absorption spectrometric analysis (Perkin Elmer 400).

2.5. Drugs

Dizocilpine maleate (MK-801), a non-competitive NMDA receptor antagonist (RBI, Sigma-Aldrich, France) and magnesium sulfate ($MgSO_4$) (Sigma-Aldrich) were dissolved in 0.9% NaCl (saline) on the day of the experiment.

2.6. Statistics

The data were analyzed by two-way analysis of variance (ANOVA), followed by a predicted least significant differences (PLSD) Fisher test to compare each group of rats receiving either the depleted diet with or without combined MK-801 treatment, or the normal diet + MK-801 versus the control group (normal diet + saline). Changes in Mg^{2+} concentration in plasma, CSF and spinal cord were analyzed by a two-way ANOVA, followed by a PLSD Fisher test to compare, for each time, the concentrations in the Mg^{2+}-deficient group versus those in the control group. The significance level was $P<0.05$.

3. Results

3.1. Effect of Mg^{2+} reloading on the hyperalgesia induced

After 10 days on the Mg^{2+}-depleted diet, the rats showed a dramatic decrease in vocalization thresholds (−27.0%,

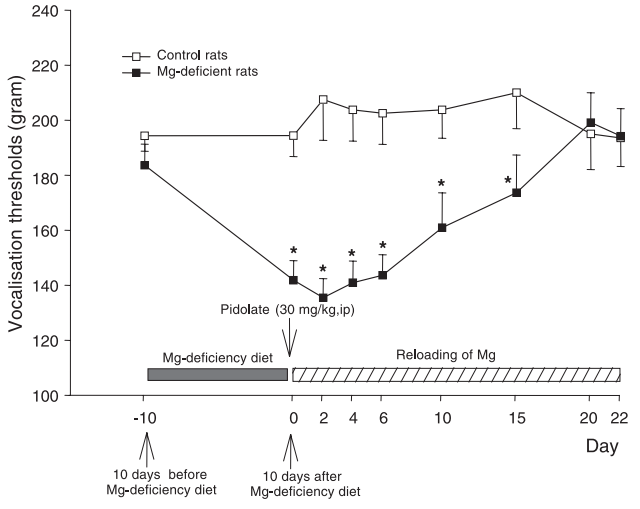

Fig. 1. Effect of a single loading dose of magnesium (30 mg/kg, i.p.) or saline (2 ml/kg, i.p.) followed by dietary reloading, on mechanical hyperalgesia in magnesium-deficient ($n=11$ per group) and control ($n=7$ per group) rats, respectively. Data are presented as means ± S.E.M. *$P<0.05$, versus the corresponding values for the control group.

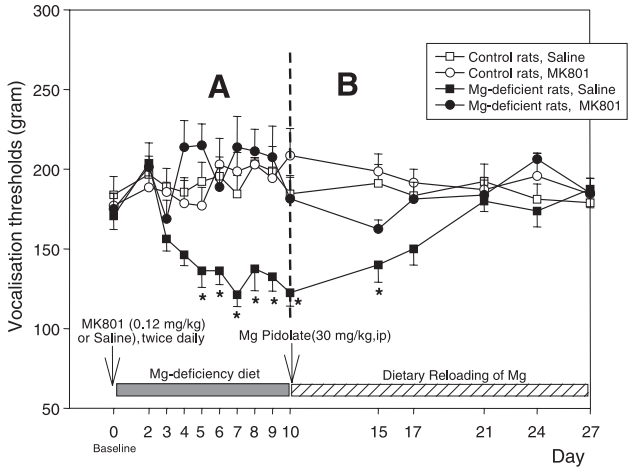

Fig. 2. (A) Effect of chronic treatment with MK-801 (0.12 mg/kg, s.c., twice daily) ($n=20$ per group) or saline (2 ml/kg, s.c., twice daily) ($n=20$ per group) on the vocalization threshold assessed by the paw pressure test, throughout the 10-day period of the magnesium-depleted diet. (B) Effect of a single loading dose of magnesium followed by dietary reloading and a normal diet given to magnesium-deficient and control rats when the treatment with MK-801 was stopped. Thus, reversibility was studied from 17 days. Data are presented as means ± S.E.M. *$P<0.05$ for MK-801-treated group versus the corresponding values of the saline-treated group for magnesium-deficient rats.

$P<0.001$) compared with the control group (194.3 ± 5.6 g). Despite complete resolution of the neurological disturbances in Mg^{2+}-depleted rats in less than 36 h, the nociceptive threshold remained lowered: −35.0%, −30.8% and −29.0%, respectively, at days 2, 4 and 6 after Mg^{2+} loading. Vocalization thresholds returned to their baseline values only 19 days after the start of the Mg^{2+}-enriched diet (199.0 ± 10.8 g) (Fig. 1).

3.2. Effect of pre-emptive treatment with MK-801

Five days after starting the on Mg^{2+}-depleted diet, the rats treated with saline showed a significant decrease (−20.14%, $P<0.01$) in vocalization thresholds. The maximum hyperalgesia was observed at day 7 (−28.9%, $P<0.001$). However, in the group given the same Mg^{2+}-depleted diet but treated with MK-801, we did not observe the development of the mechanical hyperalgesia. Neither saline nor MK-801 had any effect in normal rats.

On day 10, the injection of $MgSO_4$ followed by Mg^{2+}-reloading in the Mg^{2+}-deficient group, resulted in a gradual increase in the vocalization thresholds, which reached their control values at day 21 (180.0 ± 6.6 g). No change was observed in the Mg^{2+}-deficient rats pretreated with MK-801 (Fig. 2).

3.3. Assay of Mg^{2+} concentrations in plasma, spinal cord and CSF

3.3.1. After 10 days of the Mg^{2+}-depleted diet

The rats displayed the expected decrease in Mg^{2+} concentration in plasma (0.21 ± 0.02 vs. 0.70 ± 0.01 mmol/l in the control group, $P<0.001$) and in CSF (0.78 ± 0.07 vs. 0.94 ± 0.05 mmol/l, $P<0.05$). We also observed a significant ($P<0.001$) decrease in Mg^{2+} concentration in the spinal cord (5.49 ± 0.07 vs. 6.09 ± 0.06 mmol/kg fresh weight, $P<0.001$) (Table 1).

3.3.2. After Mg^{2+}-reloading (Table 1)

The Mg^{2+} concentration in plasma remained lower in previously Mg^{2+}-depleted animals than in the controls on day 2 (0.63 ± 0.02 vs. 0.73 ± 0.02 mmol/l, $P<0.01$) and day 4 (0.65 ± 0.03 vs. 0.76 ± 0.02 mmol/l, $P<0.05$). The return to normal values was observed only on day 10 (0.72 ± 0.01 vs. 0.74 ± 0.04 mmol/l). However, in both the spinal cord and the CSF, the Mg^{2+} concentration was normalized 48 h after Mg^{2+} reloading.

4. Discussion

Consistent with our earlier findings (Dubray et al., 1997), these experiments confirm that acute Mg^{2+} deficiency induces mechanical hyperalgesia in rats. This lowering of the nociceptive threshold can be totally reversed by Mg^{2+} reloading, attesting that even severe Mg^{2+} deficiency does not induce irreversible damage to the nociceptive pathways.

Table 1
Mg^{2+} plasma, cerebrospinal fluid and spinal cord levels in control and Mg^{2+}-deficient rats

		Day 10 of Mg-depleted diet	Days of reloading		
		0	2	4	10
Plasma (mmol/l)	Control	0.70 ± 0.01	0.73 ± 0.02	0.76 ± 0.02	0.74 ± 0.04
	Mg-deficient	0.21 ± 0.02	0.63 ± 0.02	0.65 ± 0.03	0.72 ± 0.01
		$P<0.001$	$P<0.01$	$P<0.05$	NS
CSF (mmol/l)	Control	0.94 ± 0.05	0.92 ± 0.02	0.95 ± 0.02	0.87 ± 0.02
	Mg-deficient	0.78 ± 0.07	0.95 ± 0.02	0.91 ± 0.03	0.87 ± 0.03
		$P<0.05$	NS	NS	NS
Spinal cord	Control	6.09 ± 0.06	6.04 ± 0.12	6.13 ± 0.06	5.93 ± 0.07
(mmol/kg fresh weight)	Mg-deficient	5.49 ± 0.07	5.95 ± 0.10	5.98 ± 0.07	5.94 ± 0.05
		$P<0.001$	NS	NS	NS

Data are means ± S.E.M. for 10 animals. Statistical analysis was performed with a two-way ANOVA, followed by a PLSD Fisher's test.

However, in contrast to the neurological disturbances and to the CSF and spinal cord Mg^{2+} concentrations, which were normalized 48 h after Mg^{2+} reloading, the reversal of the hyperalgesia took respectively 20 and 11 days in the first and in the second experiments. In our experiments, the time course of the Mg^{2+} concentrations was fully consistent with that reported by Chutkow (1968). The discrepancy between the time course of recovery of a normal nociceptive threshold and that of the other clinical or biological signs argues for a specific mechanism sensitizing the nociceptive pathways. Over the last decade, the pathogenesis of hyperalgesia has received major attention and much evidence has been reported supporting the role of NMDA receptors in this effect (Paoletti et al., 1995; Wang and McDonald, 1995). Considering the key role of Mg^{2+} in the functioning of the NMDA receptor channels (Traub et al., 1994), we hypothesize that this receptor channel may be directly involved in the hyperalgesia induced by Mg^{2+} depletion. Initial evidence came from our earlier results showing that, in Mg^{2+}-depleted rats, acute treatment with MK-801, a non-competitive NMDA receptor antagonist, could totally reverse the hyperalgesia (Dubray et al., 1997). In the present experiments, we found that pre-emptive treatment with repeated doses of MK-801, maintained throughout the duration of the Mg^{2+}-depleted diet, suppressed the hyperalgesia observed in Mg^{2+}-deficient rats not receiving MK-801. After this repeated treatment with MK-801 was stopped, when the rats were reloaded with Mg^{2+} we noted that the vocalization threshold remained within the normal range. If MK-801 had a direct analgesic action in the Mg^{2+}-deficient rats, then we would have expected the vocalization threshold to drop on withdrawal to watch the values recorded in Mg^{2+}-deficient rats not given pre-emptive treatment with MK-801. The difference between these two curves after Mg^{2+} reloading may be the consequence of a cascade of biochemical events in the neurons of the nociceptive pathways resulting from an over-activation of NMDA receptor channels due to the Mg^{2+} depletion, which was blocked in the animals pretreated with MK-801. Clearly, the activation of NMDA receptor channels plays a central role in the neuroplasticity of the nociceptive pathways, especially at the spinal relay (Ren and Dubner, 1999; Woolf and Thompson, 1991). Some authors have suggested (Svendsen et al., 1997) that, in several experimental models of hyperalgesia, a long-term potentiation within the dorsal horn may induce hyperalgesia and allodynia. Long-term potentiation occurring after a high-frequency conditioning stimulus was first described for the hippocampus by Bliss and Lomo (1973). Some authors showed similar long-term potentiation in dorsal horn neurons (Spillane et al., 1995; Vikman et al., 2001) or in single wide dynamic range neurons (Svendsen et al., 1997), which are believed to play an important role in the plasticity of nociceptive transmission (Rygh et al., 1999). Most authors consider that NMDA receptor channels play a key role in this process.

The time course of this Mg^{2+}-dependant hyperalgesia, together with its blockade by non-competitive NMDA receptor antagonists, argues for a central sensitization, which could represent a spinal form of long-term potentiation. Thus, the M Mg^{2+}-depleted rat may provide a new model of hyperalgesia that does not involve peripheral neuropathy, unlike mononeuropathic or diabetic models. This specificity would make it especially well suited for studying pathophysiological processes or new compounds involving the NMDA receptor channels in central nociceptive pathways.

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