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# B vitamins induce an antinociceptive effect in the acetic acid and formaldehyde models of nociception in mice

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**Abstract**

The effect of some B vitamins in chemical and thermal models of nociception in mice was investigated. The association thiamine/pyridoxine/cyanocobalamin (TPC, 20–200 mg/kg, i.p. or per os), thiamine, pyridoxine (50–200 mg/kg, i.p.) or riboflavin (3–100 mg/kg, i.p) induced an antinociceptive effect, not changed by naloxone (10 mg/kg, i.p.), in the acetic acid writhing model. Treatment for 7 days with thiamine/pyridoxine/cyanocobalamin (100 or 200 mg/kg, i.p.), thiamine (50 or 100 mg/kg) or pyridoxine (50 or 100 mg/kg) or acute treatment with riboflavin (6 or 12 mg/kg, i.p) inhibited the nociceptive response induced by formaldehyde. The B vitamins did not inhibit the nociceptive response in the hot-plate model. Both 7-day thiamine/pyridoxine/cyanocobalamin (100 mg/kg, i.p.) or acute riboflavin (25 or 50 mg/kg, i.p.) treatment partially reduced formaldehyde-induced hindpaw oedema. The B vitamins antinociceptive effect may involve inhibition of the synthesis and/or action of inflammatory mediators since it was not observed in the hot-plate model, was not reversed by naloxone, only the second phase of the formaldehyde-induced nociceptive response was inhibited, and formaldehyde-induced hindpaw oedema was reduced. © 2001 Published by Elsevier Science B.V.

*Keywords:* B vitamin; Thiamine; Riboflavin; Pyridoxine; Nociception; Pain

## 1. Introduction

B vitamin deficiency, particularly that of thiamine, pyridoxine, riboflavin and cyanocobalamin, may result in pathological conditions including convulsions, carpal tunnel syndrome and chronic pain, indicating that these substances are essential to the normal function of the nervous system (Claus et al., 1984; Schaeffer, 1987; Marcus and Coulston, 1996). Not surprisingly, B vitamins have been used mainly in the treatment and prophylaxis of disorders resulting from their deficiency.

However, B vitamins have been recently evaluated as useful drugs to treat pathological conditions, particularly painful disorders, not necessarily associated with their deficiency. Supplying pyridoxine and thiamine can relieve the pain associated with neuropathic disorders, carpal tunnel syndrome and premenstrual tension (Jorg et al., 1988; Bernstein, 1990; Bernstein and Dinesen, 1993; Wyatt et al., 1999), while riboflavin may be effective in migraine prophylaxis (Schoenen et al., 1998). It has also been demonstrated that the analgesic effect of nonsteroidal anti-inflammatory drugs is increased in patients simultaneously treated with B vitamins (Brüggemann et al., 1990; Kuhlwein et al., 1990).

However, there are fewer studies carried out in experimental animals with the aim of characterising the antinociceptive effect induced by B vitamins and also the mechanisms possible involved. It has been demonstrated that the association thiamine/pyridoxine/cyanocobalamin induces an antinociceptive effect in the hot plate and benzoquinone-induced abdominal constrictions tests with mice and rats (Bartoszyk and Wild, 1990; Zimmermann et al., 1990). The increased effect of nonsteroidal antiinflammatory drugs in rats treated with pyridoxine has also been demonstrated in the carrageenan-induced hyperalgesia model (Zimmermann et al., 1990). Regarding riboflavin, there is no report of the effect of this B vitamin on an experimental nociceptive model.

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The antinociceptive effect induced by B vitamins may be dependent on their action in the central nervous system (CNS). The increased activity of dorsal horn (Zimmermann et al., 1990; Fu et al., 1988) and thalamus (Jurna et al., 1990) neurons induced by electrical stimulation of C fibers or noxious skin heating is reduced by pyridoxine or the association thiamine/pyridoxine/cyanocobalamin. These effects may be related to changes in the synthesis of neurotransmitters that have an important role in the modulation of the nociceptive response in the CNS. Pyridoxine deficiency is associated with a reduced content of 5-hydroxytryptamine and other neurotransmitters in the hypothalamus (Dakshinamurti et al., 1987; Paulose et al., 1988), whereas treatment with this vitamin increases 5-hydroxytryptamine synthesis in the brain (Hartvig et al., 1995).

The B vitamin antinociceptive effect may also involve inhibition of the synthesis of inflammatory mediators that could activate or sensitise primary afferent fibers, although this hypothesis has not yet been investigated directly. An effect has been observed in animal models of nociception associated with inflammation (Kasdan and Janes, 1987; Bartoszyk and Wild, 1990; Zimmermann et al., 1990). Moreover, Brüggemann et al. (1990) and Kuhlwein et al. (1990) showed that B vitamins reduced the pain associated with inflammatory conditions in humans.

In spite of the clinical evidence indicating the analgesic effect of some B vitamins, there are fewer studies showing their effect on different aspects of the inflammatory response, including nociception and oedema. The present study investigated the effect of some B vitamins on the nociceptive response in various experimental models, in order to determine if there is pharmacological support for their clinical use as analgesics or adjuvants of antiinflammatory drugs.

## 2. Materials and methods

### 2.1. Experimental animals

Male Swiss mice, weighing between 25 and 35 g on the experimental day, were used. The experiments were carried out at an ambient temperature of 27°C, which corresponds to the thermoneutral zone for rodents. Food and water were available ad libitum. All protocols were approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais.

### 2.2. Procedures

#### 2.2.1. Evaluation of the motor activity

To investigate if the treatments could influence the motor activity of the animals and consequently impair the assessment of the nociceptive behaviour in the experimental models, the motor activity of the animals was evaluated in a rota-rod apparatus, according to a procedure proposed by Vaz et al. (1996), which is a modification of the method originally described by Dunham and Miya (1957). The day before the experiment, the animals were trained on the apparatus. On the experiment day, the animals were placed on a rota-rod (14 rpm) and the time they remained on the apparatus was determined. The cut-off time was set at 1 min. After determination of the baseline values, the animals were treated with the B vitamins and 1 h later they were again tested in the apparatus.

#### 2.2.2. Acetic acid-induced abdominal constrictions

The nociceptive response was evaluated after the intraperitoneal (i.p.) injection of acetic acid according to the model described by Vaz et al. (1996), which is a modification of the model originally described by Koster et al. (1959). Acetic acid (0.6%, 10 ml/kg) was injected intraperitoneally and the number of abdominal constrictions associated with total stretching of the hind limbs was counted over a period of 20 min.

#### 2.2.3. Formaldehyde-induced nociceptive response

The nociceptive response was evaluated after the subcutaneous (s.c.) injection of formaldehyde according to the model described by Vaz et al. (1996), which represents a modification of the original model described by Hunskaar and Hole (1987) for mice. The nociceptive stimulus consisted of a subcutaneous injection of formaldehyde (0.92%, 20 μl, diluted in saline) into the dorsum of the right hindpaw. The time the animals spent licking the injected hindpaw was determined between 0 and 5 min (first phase) and 15 and 30 min (second phase) after the injection of formaldehyde. In some protocols, hindpaw oedema was evaluated 4 h after injection of formaldehyde. After killing the animals by decapitation, both hindpaws were cut at the knee joint and weighed in an analytical balance. Oedema was defined as the weight difference between the injected and the contralateral non-injected hindpaw.

#### 2.2.4. Hot-plate model

The nociceptive response was evaluated in the hot plate model as described by Eddy and Leimbach (1953), a modification of the original method of Woolfe and MacDonald (1944). The animals were placed on a heated (56°C) metal plate and the latency for jumping or licking the paws was determined.

### 2.3. Drugs

Thiamine hydrochloride ($B_1$), riboflavin ($B_2$), pyridoxine hydrochloride ($B_6$), cyanocobalamin ($B_{12}$), morphine hydrochloride (Sigma, USA), naloxone hydrochloride (RBI, USA), acetic acid and formaldehyde (Merck, Brazil) were used. Thiamine, pyridoxine and cyanocobalamin were chosen among the B vitamins because they represent the vitamins most frequently encountered in the medications available for clinical use. Riboflavin, although less fre-

quently encountered in the B vitamins associations, was used as there is one report showing that it may be useful in migraine prophylaxis. Acetic acid solution was prepared in distilled water. All the other solutions were prepared in saline immediately before the experiments. Associations of thiamine, pyridoxine and cyanocobalamin were used in a ratio of 20:20:1. This means that a dose of 100 mg/kg of thiamine/pyridoxine/cyanocobalamin indicates 100 mg/kg of thiamine, 100 mg/kg of pyridoxine and 5 mg/kg of cyanocobalamin. This ratio is similar to that present in some medications available for clinical use. The association thiamine/pyridoxine/cyanocobalamin and riboflavin were administered intraperitoneally at doses ranging from 20 to 200 mg/kg, except in one protocol in which the association thiamine/pyridoxine/cyanocobalamin was administered per os at doses of 500 and 1000 mg/kg. When a 7-day treatment was used, which we defined as chronic, the highest dose of the association thiamine/pyridoxine/cyanocobalamin was 100 mg/kg. Isolated vitamins were always administered intraperitoneally at one or more of the following doses: Thiamine (50, 100 and 200 mg/kg), pyridoxine (50, 100 and 200 mg/kg), cyanocobalamin (2.5, 5 e 10 mg/kg) and riboflavin (3, 6, 12, 25, 50 and 100 mg/kg). An acute (−1 h) regimen of administration was always used unless stated otherwise in the figures.

### 2.4. Statistical analysis

The results were analysed by one-way analysis of variance. Bonferrroni's post hoc test was used when the main effect was significant and statistical significance was inferred at $P < 0.05$ level.

## 3. Results

### 3.1. Effect of acute and chronic B vitamins treatment on the motor activity of the animals

Table 1 shows that the i.p. injection of the association thiamine/pyridoxine/cyanocobalamin (200 mg/kg) or riboflavin (12 or 25 mg/kg) did not reduce the time the animals spent on the rota-rod when this was counted 1 h later. Similarly, the treatment with thiamine/pyridoxine/cyanocobalamin (100 mg/kg) for 7 days, the last dose being injected 1 h before the test, did not reduce the locomotor activity of the animals.

Table 1
Effect of B vitamins on the performance of mice in the rota-rod test
The time the animals spent on the apparatus was measured before any treatment. Animals were treated with thiamine/pyridoxine/cyanocobalamin (TPC) 200 mg/kg (thiamine 200 mg/kg, pyridoxine 200 mg/kg and cyanocobalamin 5 mg/kg, i.p.) or riboflavin (12 mg/kg, i.p.) and tested on the apparatus 1 h later. Another group was treated with thiamine/pyridoxine/cyanocobalamin 100 mg/kg (thiamine 100 mg/kg, pyridoxine 100 mg/kg, cyanocobalamin 2.5 mg/kg, i.p.) for 7 days and was tested on the apparatus 1 h after the last injection. Cut-off time was defined as 1 min. Data are presented as means ± S.E.M.

| Experimental group | Time spent on the apparatus | |
|---|---|---|
| | Before treatment | 1 h after (last) treatment |
| | *Acute treatment* | |
| Saline 4 ml/kg, i.p. ($n = 5$) | 53 ± 3 | 60 ± 0 |
| TPC 200 mg/kg, i.p. ($n = 5$) | 57 ± 7 | 60 ± 0 |
| Riboflavin 12 mg/kg, i.p. ($n = 6$) | 58 ± 2 | 60 ± 0 |
| | *7-Day treatment* | |
| Saline 4 ml/kg, i.p. ($n = 5$) | 60 ± 0 | 60 ± 0 |
| TPC 100 mg/kg, i.p. ($n = 5$) | 59 ± 1 | 60 ± 0 |

### 3.2. Effect of acute B vitamin treatment on the nociceptive response induced by i.p. injection of acetic acid

Fig. 1A shows that the i.p. injection (−1 h) of thiamine/pyridoxine/cyanocobalamin dose-dependently reduced the number of abdominal constrictions induced by acetic acid. The highest dose (200 mg/kg) inhibited the nociceptive response by 78%. Per os administration (−1 h) of thiamine/pyridoxine/cyanocobalamin also induced a significant reduction of the number of acetic acid-induced abdominal constrictions (Fig. 1B). In the next set of experiments, the effect of the isolated vitamins was investigated. I.p. injection (−1 h) of thiamine (Fig. 2A) or pyridoxine (Fig. 2B) significantly reduced the nociceptive response induced by acetic acid, although a dose–response relationship was not observed. The nociceptive response induced by acetic acid was dose-dependently inhibited by previous (−1 h) treatment with riboflavin (Fig. 2D), even with doses as low as 3 mg/kg. However, the nociceptive response induced by acetic acid was not inhibited by previous treatment with cyanocobalamin (Fig. 2C), at doses that ranged between 2.5 and 10 mg/kg, the same doses of this vitamin present in the association thiamine/pyridoxine/cyanocobalamin.

### 3.3. Effect of acute and chronic B vitamin treatment on the nociceptive response induced by subcutaneous injection of formaldehyde into the hindpaw

Fig. 3A shows that the acute i.p. injection (−1 h) of thiamine/pyridoxine/cyanocobalamin did not change the first phase of the formaldehyde-induced nociceptive response, but partially reduced the second phase. The reduction induced by the highest dose (200 mg/kg) was 35%, which did not reach statistical significance. However, when the animals were treated with thiamine/pyridoxine/cyanocobalamin (50 or 100 mg/kg) for 7 days, the last dose being injected 1 h before the test, significant inhibition (55% and 52%, respectively) of the nociceptive response was observed (Fig. 3B). Next, the effect of isolated thiamine, pyridoxine or riboflavin on formaldehyde-

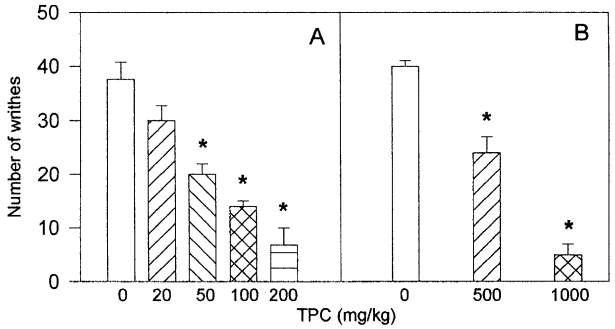


Fig. 1. Effect of B vitamins on the nociceptive response in the acetic acid-induced constriction model. Intraperitoneal (A) or per os (B) administration of thiamine/pyridoxine/cyanocobalamin (TPC) was carried out 1 h before the injection of acetic acid (0.6%, 10 ml/kg, i.p.). Thiamine/pyridoxine/cyanocobalamin doses were given as indicated. The number of writhes was determined for a period of 20 min after the injection of acetic acid. Means ± S.E.M. for groups of 5–6 mice are shown. * $P < 0.05$.

induced nociception was investigated. I.p. injection of thiamine (Fig. 4A), pyridoxine (Fig. 4B), at the doses of 50 or 100 mg/kg, for 7 days, the last dose being injected 1 h before the test, also significantly reduced the nociceptive response induced by formaldehyde. Reduction of the second phase of the formaldehyde-induced nociceptive response was also observed after acute treatment with riboflavin (6 or 12 mg/kg, i.p.; Fig. 4C). An important aspect related to riboflavin is that its antinociceptive effect was observed after a single injection and also with smaller doses than those used for thiamine and pyridoxine.

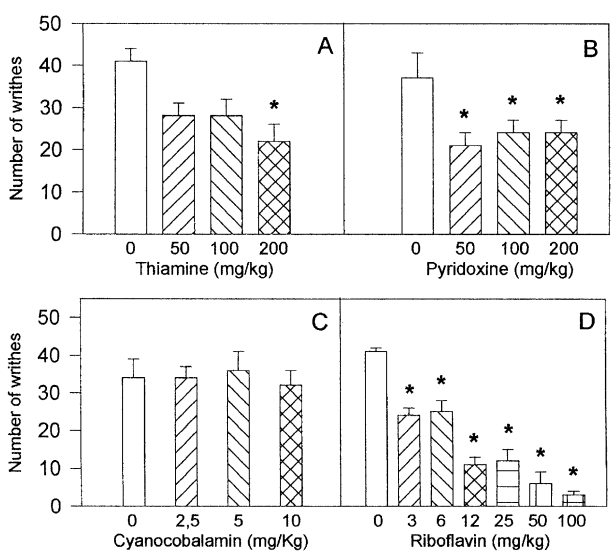


Fig. 2. Effect of B vitamins on the nociceptive response in the acetic acid-induced constriction model. Intraperitoneal injection of thiamine (A), pyridoxine (B), cyanocobalamin (C) or riboflavin (D) was carried out 1 h before the injection of acetic acid (0.6%, 10 ml/kg, i.p.). Doses were given as indicated. The number of writhes was determined for a period of 20 min after the injection of acetic acid. Means ± S.E.M. for groups of 5–9 mice are shown. * $P < 0.05$.

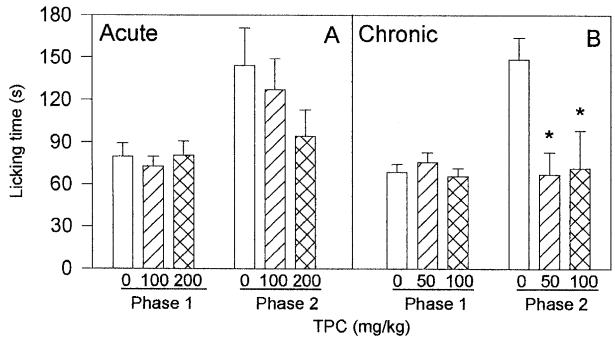


Fig. 3. Effect of B vitamins on the nociceptive response induced by subcutaneous injection of formaldehyde into the hindpaw. In panel (A), mice were treated with the association thiamine/pyridoxine/cyanocobalamin (TPC, i.p., doses indicated) 1 h before the subcutaneous injection of formaldehyde (0.92%, 20 μl). In panel (B), the animals were treated with the association thiamine/pyridoxine/cyanocobalamin (i.p., doses indicated) for 7 days and formaldehyde was injected 1 h after the last thiamine/pyridoxine/cyanocobalamin dose. Licking time was determined 0–5 min (phase 1) and 15–30 min (phase 2) after formaldehyde injection. Means ± S.E.M. for groups of eight mice are shown. * $P < 0.05$.

### *3.4. Effect of acute and chronic B vitamin treatment on the nociceptive response in the hot-plate model*

One hour after treatment of the mice with the association thiamine/pyridoxine/cyanocobalamin (100 or 200 mg/kg, i.p.), the latency to jumping or licking the hindpaws on a hot plate was determined. Fig. 5A shows that the latency was not altered by treatment with thiamine/pyridoxine/cyanocobalamin. Similarly, treatment with thiamine/pyridoxine/cyanocobalamin (50 or 100 mg/kg, i.p.) for 7 days, the last dose being injected 1 h before the test, did not change the latency to the nociceptive response in the hot plate model (Fig. 5B). Similarly, no antinociceptive effect was observed in this model after acute riboflavin treatment (12 or 25 mg/kg, i.p., −1 h).

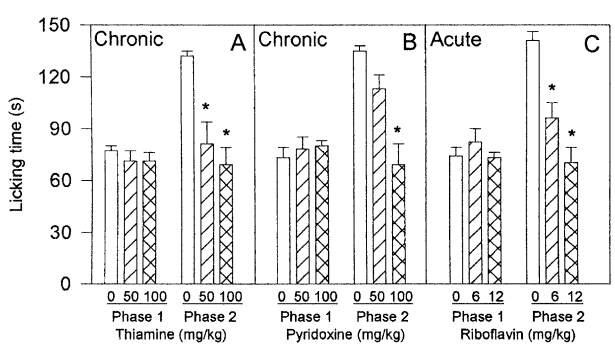


Fig. 4. Effect of thiamine, pyridoxine or riboflavin on the nociceptive response induced by subcutaneous injection of formaldehyde into the hindpaw. Mice were treated with thiamine (A) or pyridoxine (B) for 7 days and formaldehyde (0.92%, 20 μl) was injected 1 h after the last B vitamin dose. Riboflavin (C), on the other hand, was injected as a single dose, 1 h before the injection of formaldehyde. Licking time was determined 0–5 min (phase 1) and 15–30 min (phase 2) after formaldehyde injection. Means ± S.E.M. for groups of 5–6 mice are shown. * $P < 0.05$.

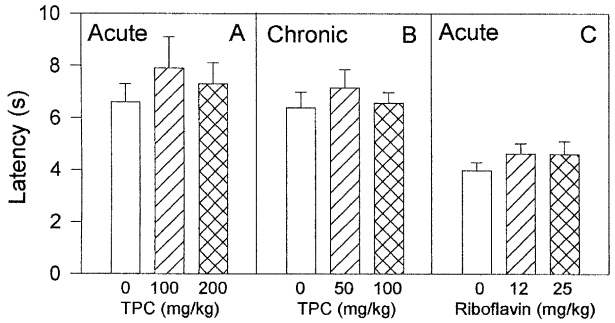


Fig. 5. Effect of B vitamins on the nociceptive response in the hot-plate model. In panel (A), mice were treated with the association thiamine/pyridoxine/cyanocobalamin (TPC, i.p., doses indicated) 1 h before being placed on the hot plate (56°C). In panel (B), the animals were treated with the association thiamine/pyridoxine/cyanocobalamin (i.p., doses indicated) for 7 days and placed on the hot plate 1 h after the last thiamine/pyridoxine/cyanocobalamin dose. In panel (C), mice were treated with riboflavin (i.p., doses indicated) 1 h before being placed on the hot plate. Latency to jumping or licking the paws was determined. Means ± S.E.M. for groups of 6–7 mice are shown. * $P < 0.05$.

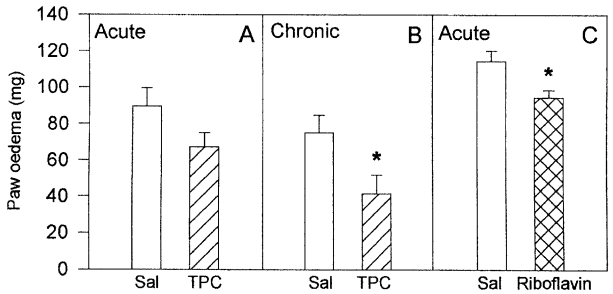


Fig. 7. Effect of B vitamins on formaldehyde-induced paw oedema. In panel (A), mice were treated with the association thiamine/pyridoxine/cyanocobalamin (TPC, 200 mg/kg, i.p.) 1 h before the subcutaneous injection of formaldehyde (0.92%, 20 μl). In panel (B), the animals were treated with the association thiamine/pyridoxine/cyanocobalamin (100 mg/kg, i.p.) for 7 days and formaldehyde was injected 1 h after the last thiamine/pyridoxine/cyanocobalamin dose. In panel (C), mice were treated with riboflavin (25 mg/kg, i.p.) 1 h before the subcutaneous injection of formaldehyde. Four hours after formaldehyde injection, the mice were killed and both hindpaws were cut at the knee joint and weighed. Oedema was defined as the weight difference between the injected and the non-injected contralateral hindpaw. Means ± S.E.M. for groups of 5–6 mice are shown. * $P < 0.05$.

### *3.5. Effect of naloxone on the antinociceptive effect induced by acute B vitamins treatment in the acetic acid-induced constriction model*

Fig. 6 shows the effect of i.p. injection of naloxone (10 mg/kg) on the antinociceptive effect induced by thiamine/pyridoxine/cyanocobalamin (100 mg/kg, i.p.; (A)) or riboflavin (25 mg/kg, i.p.; (B)) in the acetic acid-induced constriction model. The antinociceptive effect induced by thiamine/pyridoxine/cyanocobalamin or riboflavin was not changed when naloxone was administered 30 min before the injection of the B vitamin. However, the dose of naloxone used abolished the antinociceptive effect induced by morphine (2 mg/kg, i.p.) in the same model (Fig. 6C).

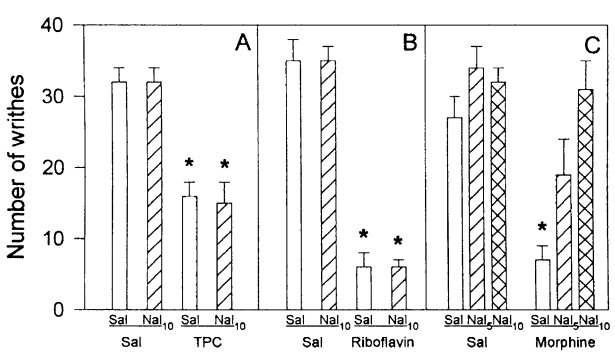


Fig. 6. Effect of naloxone on the antinociception induced by B vitamins or morphine in the acetic acid-induced constrictions model. Naloxone (Nal, 5 or 10 mg/kg) was injected intraperitoneally 30 min before treatment with the association thiamine/pyridoxine/cyanocobalamin (TPC, 200 mg/kg, i.p.; (A)), riboflavin (25 mg/kg, i.p.; (B)) or morphine (2 mg/kg, i.p.; (C)). One hour after treatment with thiamine/pyridoxine/cyanocobalamin, riboflavin or morphine, acetic acid (0.6%, 10 ml/kg) was injected intraperitoneally and writhes were counted for a period of 20 min. Means ± S.E.M. for groups of 6–11 mice are shown. * $P < 0.05$.

### *3.6. Effect of acute and chronic B vitamin treatment on the hindpaw oedema induced by subcutaneous injection of formaldehyde*

Fig. 7A shows that the i.p. injection (−1 h) of thiamine/pyridoxine/cyanocobalamin (100 mg/kg) induced a partial, but not statistically significant, reduction (25%) of the hindpaw oedema evaluated 4 h after the subcutaneous injection of formaldehyde (0.92%, 20 μl). A greater, and significant, inhibition (45%) of formaldehyde-induced hindpaw oedema was observed when the animals were treated with thiamine/pyridoxine/cyanocobalamin (100 mg/kg, i.p.) for 7 days, the last dose being injected 1 h before the injection of formaldehyde (Fig. 7B). I.p. injection (−1 h) of riboflavin at the dose of 50 mg/kg also partially (22%) inhibited the formaldehyde-induced hindpaw oedema (Fig. 7C).

## 4. Discussion

B vitamins, in particular thiamine, pyridoxine and riboflavin, induced a marked antinociceptive effect in the acetic acid- and formaldehyde-induced nociception models, and also partially reduced formaldehyde-induced oedema. The antinociceptive effect was observed after acute or chronic treatment, depending on the B vitamin or the nociceptive model used.

It is unlikely that the antinociceptive effect induced by the B vitamins results from a non-specific muscle-relaxant or sedative effect, since the performance of the animals in

the rota-rod test was not reduced by the treatment with the association thiamine/pyridoxine/cyanocobalamin or riboflavin. Although small changes in muscle tone or a minor central nervous system depressor effect may not be reliably detectable in the rota-rod test, observation of the animals did not indicate that a general behavioural inhibition occurs after treatment with B vitamins. In addition, it must be emphasised that a reduced number of adverse reactions has been reported after treatment of patients with B vitamins and central nervous system depressor effects and motor incoordination are not included among the reactions (Bernstein, 1990; Schoenen et al., 1998; Wyatt et al., 1999).

Initially, the number of acetic acid-induced constrictions was reduced by i.p. or per os administration of thiamine/pyridoxine/cyanocobalamin. Bartoszyk and Wild (1990) described similar results in the benzoquinone-induced constriction model in rats, but the doses of the B vitamins that inhibited the nociceptive response by 50% were 6.5-fold higher than those used in the present study to reduce the nociceptive response to acetic acid to the same level. This difference may be related to the different animal and nociceptive stimuli used in our study.

Isolated administration of thiamine or pyridoxine, but not of cyanocobalamin, also inhibited the nociceptive response induced by acetic acid. This indicates that thiamine and pyridoxine most likely account for the analgesic effect of the B vitamin associations. The effect induced by thiamine or pyridoxine was not as marked as that observed when the B vitamins were administered in association, indicating that the antinociception induced by the association results from a sum of individual effects. Interestingly, riboflavin per se induced an antinociceptive effect even when administered at doses much lower than those of the other B vitamins. To the best of our knowledge, this is the first demonstration of the antinociceptive effect induced by riboflavin in experimental animals.

These results, when analysed together, do not allow the proposal of a mechanism for the antinociceptive effect induced by the B vitamins. The acetic acid-induced nociceptive response may involve both direct stimulation of the nociceptive afferent fibers due to the pH reduction and the synthesis of inflammatory mediators (Ribeiro et al., 2000). It has been shown that nonsteroidal antiinflammatory drugs (Vaz et al., 1996), opioid analgesics (Vaz et al., 1996) and even tricyclic antidepressants (Gray et al., 1998) may inhibit the nociceptive response in the acetic acid model.

The B vitamins also inhibited the second phase of the nociceptive response induced by formaldehyde. Although acute treatment with thiamine/pyridoxine/cyanocobalamin partially inhibited the nociceptive response, a statistically significant reduction was observed only after 7 days of treatment with thiamine/pyridoxine/cyanocobalamin, thiamine or pyridoxine. It is not clear why the B vitamins induced an antinociceptive effect only after prolonged treatment in this model, in opposition to the results observed in the acetic acid model. However, the antinociceptive effect in the formaldehyde model which was observed only after prolonged treatment, may be related to some clinical findings indicating that the relief of pain associated with premenstrual tension, carpal tunnel syndrome and migraine is reached only after weeks of treatment with thiamine/pyridoxine/cyanocobalamin (Bernstein, 1990; Kasdan and Janes, 1987; Wyatt et al., 1999). However, once again showing a different characteristic, riboflavin induced an antinociceptive effect after a single dose, much lower than those of thiamine/pyridoxine/cyanocobalamin, thiamine or pyridoxine that induced a similar effect.

These results suggest that the effect of B vitamins may resemble that of antiinflammatory drugs, more than that of drugs that act predominantly in the CNS. The nociceptive response induced by formaldehyde has two phases that may involve different mechanisms (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987; Tjølsen et al., 1992; Vaz et al., 1996). The first phase results from direct chemical stimulation of the nociceptive afferent fibers, mainly C fibers (Heapy et al., 1987), while the second phase results from the action of inflammatory mediators released locally and also from the facilitation of synaptic transmission in the spinal cord (Tjølsen et al., 1992).

In support of a mechanism of action that resembles more that of antiinflammatory drugs, the B vitamins did not increase the latency to nociceptive behaviour in the hot plate model. Bartoszyk and Wild (1990) have found opposing results, but the vitamin doses used were three times higher than the highest dose used in the present study.

Recent studies have shown that the antinociceptive effect of some nonsteroidal antiinflammatory drugs (Pini et al., 1997; Tortorici et al., 1996; Björkman et al., 1990; Herrero and Max Headley, 1996) may be inhibited by naloxone, indicating the involvement of endogenous opioid mechanisms. We observed that the antinociceptive effect of the B vitamins in the acetic acid model was not changed when the animals were previously treated with naloxone, indicating that opioid mechanisms may not be involved.

A mechanism that may involve predominantly inhibition of the synthesis or action of inflammatory mediators is also supported by the results obtained with formaldehyde-induced hindpaw oedema. The treatment with B vitamins partially, but significantly, reduced the increase in hindpaw volume induced by formaldehyde. The hindpaw oedema induced by the local injection of carrageenan and endotoxin is associated with the production of different inflammatory mediators, including cytokines, eicosanoids, bradykinin and nitric oxide (Damas and Remacle-Volon, 1992; Medeiros et al., 1995; Vaz et al., 1996). It is possible that the hindpaw oedema induced by formaldehyde involves the production of similar inflammatory mediators. In support of this hypothesis, Saareks et al. (1998) have shown that pyridoxine inhibits the synthesis of prostaglandin $E_2$, thromboxane $B_2$ and leucotriene $E_4$ in humans.

It is likely that the mechanisms involved in the antinociceptive effect of B vitamins can vary among different members of the group. In support of this hypothesis, riboflavin induced an antinociceptive effect after acute treatment in the formaldehyde model, while thiamine, pyridoxine or the association thiamine/pyridoxine/cyanocobalamin only inhibited the second phase of the nociceptive response after treatment for 7 days. However, analysis of all the results together suggests that the mechanisms involved in the antinociceptive effect induced by B vitamins result from an inhibition of the synthesis and/or action of inflammatory mediators, since an antinociceptive effect was not observed in the hot-plate model. In this model, only the second phase of the formaldehyde-induced nociceptive response was inhibited, the antinociceptive effect was not reversed by naloxone, and there was a partial reduction of formaldehyde-induced hindpaw oedema.

In summary and conclusion, thiamine, pyridoxine, riboflavin or the association thiamine/pyridoxine/cyanocobalamin, induced a marked antinociceptive effect in two chemical models of nociception. The antinociceptive effect induced by riboflavin was reported for the first time. The antinociceptive effect induced by riboflavin was observed after treatment with reduced doses and even after acute treatment in a model in which the other B vitamins only induced an effect after treatment for 7 days. These results may prompt not only further research on the antinociceptive effect induced by this B vitamin, but perhaps also clinical studies, as there is only one report showing that riboflavin may be useful in migraine prophylaxis.

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