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Dual effect of cAMP on the writhing response in mice

Gerly A.C. Brito [a], Sandra N.R. Saraiva [a], João L.A.A. Falcão [b], Mariana L. Vale [b], Aldo A.M. Lima [b], Fernando Q. Cunha [c], Ronaldo A. Ribeiro [b,*]

[a] *Department of Morphology, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil*

[b] *Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará — UFC, Rua Cel. Nunes de Melo, 1127, 60430-270 Fortaleza, CE, Brazil*

[c] *Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil*

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Abstract

The intraperitoneal injection of agents that increase the intracellular level of cyclic AMP (cAMP), reduced significantly the number of writhes induced by acetic acid and zymosan in mice. However, dibutyryl cyclic AMP (Db-cAMP) induced a dual response: (a) low doses caused antinociception, and (b) a high dose potentiated the nociceptive effect of a low concentration of acetic acid. High doses of Db-cAMP also reversed the antinociceptive effect of dexamethasone and the depletion of resident peritoneal cells. We also demonstrated that a low dose of Db-cAMP, forskolin or dexamethasone inhibited the production of tumor necrosis factor-α and interleukin-1β by macrophages stimulated by zymosan. In conclusion, this study suggests that cAMP has a dual effect in the writhing model: an antinociceptive effect due to its modulatory action on resident peritoneal cells, thus, reducing the synthesis of mediators involved in the nociceptive response, and a nociceptive effect by directly sensitizing the nociceptive neuron.

Keywords: cAMP; Dibutyryl-cAMP; Cholera toxin; Forskolin; Aminophylline; Nociception

1. Introduction

Previous studies have demonstrated the importance of resident cells in inflammatory pain. Macrophages and mast cells signal the presence of foreign material and damage by releasing cytokines and classical inflammatory mediators (Ferreira, 1980; Cunha et al., 1991, 1992; Malaviya et al., 1996; Ribeiro et al., 2000). The cytokines released by the resident cells mediate mechanical inflammatory hyperalgesia in rats or the writhing response in mice, mainly via the production of cycloxygenase products and sympathomimetic mediators, the final hyperalgesic mediators (Ferreira et al., 1988; Cunha et al., 1991; Thomazzi et al., 1997; Ribeiro et al., 2000; Duarte et al., 1988).

It has been suggested that cyclic adenosine monophosphate (cAMP) participates as a second messenger in inflammatory hyperalgesia, triggered by the final hyperalgesic mediators in the nociceptive neuron terminal. This idea is in line with the general observation that receptors of hyperalgesic compounds, such as prostaglandin and sympathetic agents, are coupled to adenylate cyclase. Moreover, this idea is supported by the fact that stable cyclic AMP analogues, the adenylate cyclase activator, forskolin, or inhibitors of the phosphodiesterases enhance the mechanical and thermal hyperalgesic effects of prostaglandin E_2 (Taiwo and Levine, 1991; Taiwo et al., 1989; Cunha et al., 1999).

Conversely, it has also been demonstrated that an increase in the intracellular level of cAMP has an antiinflammatory effect mediated by a reduction in the release of cytokines from macrophages (Renz et al., 1988), or histamine and leukotrienes from mast cells (Marone et al., 1987).

The role of intracellular cAMP can be demonstrated by the use of: (1) direct adenylate cyclase-activating agents such as forskolin (Seamon and Daly, 1986) or indirect adenylate-cyclase activators like cholera toxin, which activates G_s (Pierce et al., 1971); (2) membrane-permeable analogues of cAMP (e.g., dibutyryl cyclic AMP; Db-cAMP); or (3) phosphodiesterase inhibitors [methylxanthines, like aminophylline; rolipram (Kuehl et al., 1987; Teixeira et al., 1997)]. Since the effects of cAMP include

* Corresponding author. Tel.: +55-85-288-8349; fax: +55-85-288-8333.

E-mail address: ribeiror@ufc.br (R.A. Ribeiro).

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upregulation of the neuron terminal and reduction of the release of inflammatory mediators by resident cells, we decided to use the mouse writhing model to study the participation of cAMP in inflammatory pain. This model may be used to clarify the role of this intracellular messenger on resident cells which are the main source of hyperalgesic cytokines (Ribeiro et al., 2000).

2. Materials and methods

2.1. Animals

Male Swiss mice, 25–30-g body weight, from the animal colony of the Federal University of Ceará, were used for the nociceptive tests. The animals received water and food ad libitum. The ethical guidelines as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals were followed throughout the experiments described.

2.2. Drugs and toxins

The following drugs were used: zymosan A (Sigma, USA), glacial acetic acid (Merck, Brazil), dexamethasone (Dohme, USA), N^6,2′-*O*-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid (dibutyryl cyclic AMP; Sigma), forskolin (Calbiochem), cholera toxin solution (List. Biol. Lab.), aminophylline (Sandoz). All substances used were diluted in 0.9% saline.

2.3. Nociceptive test

Nociceptive activity was tested in mice using the writhing model (Collier et al., 1968). The nociceptive stimuli were injected into the peritoneal cavities of mice, which were placed in a large glass cylinder, and the intensity of nociception was quantified by counting the total number of writhes occurring between 0 and 30 min after stimulus injection. The writhing response consists of contraction of the abdominal muscle together with stretching of the hind limbs. The nociceptive stimuli were zymosan (1 mg/mouse; 0.20 ml) and acetic acid (0.20 ml of solutions at concentrations of 0.3% or 0.6% v/v).

2.4. Depletion of peritoneal resident cells by peritoneal washing

The method used has been previously described (Ribeiro et al., 1991). Briefly, Swiss mice were anesthetized with ether, and three hypodermic needles were inserted into the abdominal cavity. Sterile saline 0.9% (10 ml) was injected through a subxyphoid needle. The abdominal cavity was then gently massaged for 1 min and the peritoneal liquid was collected via the two needles inserted into the inguinal region. This operation was repeated three times. More than 80% of the peritoneal macrophages were removed in the lavage fluid and almost all the volume of injected saline was recovered. Control (sham) mice underwent insertion of the three needles and were manipulated in the same way, but no fluid was injected or withdrawn. Thirty minutes later, the animals were used in the nociceptive test.

2.5. Effect of cholera toxin, forskolin or aminophylline and Db-cAMP intraperitoneal pretreatments on acetic acid- and zymosan-induced nociceptive activity

In order to define the role of cAMP in nociception, we tested the effect of substances that increase the cAMP intracellular level in the mouse writhing model. Thus, mice were pretreated i.p. with cholera toxin (160 ng/0.2 ml) 1 h before, or with forskolin (0.5 nmol), aminophylline (0.25 nmol), Db-cAMP (0.025 nmol) or saline (control), 10 min before the i.p. injection of acetic acid (0.20 ml of a 0.6% v/v solution) or zymosan (1 mg/mouse). Nociceptive activity was evaluated as described in Section 2.3.

2.6. Effect of pretreatment with Db-cAMP on acetic acid-induced nociceptive activity

Db-cAMP (2.5×10^{-4}, 0.025, 2.5, 250 nmol) or saline (control) was injected i.p., 10 min before administration of the 0.6% or 0.3% acetic acid solutions. Nociceptive activity was evaluated as described in Section 2.3.

2.7. Effect of Db-cAMP on dexamethasone-induced antinociceptive action

Since the injection of Db-cAMP caused a dual response, i.e. low doses caused antinociception and a high dose potentiated the nociception of lower concentrations of acetic acid, we decided to verify whether a higher dose of Db-cAMP was able to reverse the analgesic effect of dexamethasone, which is mediated by the inhibitory effect of glucocorticoids on the release of nociceptive mediators. Thus, dexamethasone (2 mg/kg; s.c.) or saline was injected 1 h before 0.6% acetic acid or zymosan. The groups treated with dexamethasone received i.p. injection of Db-cAMP (250 nmol) or saline 10 min before the nociceptive stimuli. Nociceptive activity was evaluated as described in Section 2.3.

2.8. Effect of depletion of peritoneal cavity resident cells on Db-cAMP activity on acetic acid-induced nociception

In order to investigate whether the increase in the intracellular level of cAMP in the resident peritoneal cells could account for the antinociceptive effect of the agents that increased the cAMP concentration, we tested the antinociceptive effect of Db-cAMP in peritoneal cavities depleted of resident cells. The depletion of the resident cells was carried out by lavage of the peritoneal cavity.

Peritoneal washing was performed as described in Section 2.4. Db-cAMP (250 or 2.5 nmol) or saline (0.25 ml) was injected i.p. in Swiss mice, 30 min after peritoneal washing. After 10 min, acetic acid (0.6%) was injected. Acetic acid was also injected i.p. in a control group not submitted to peritoneal washing (sham). Nociceptive activity was evaluated as described in Section 2.3.

2.9. Effect of intraperitoneal injection Db-cAMP in animals with washed cavities or unwashed cavities

Db-cAMP (250 nmol) was i.p. injected, as the stimulus, in control animals or in animals with a washed peritoneal cavity (performed as described in Section 2.4). Nociceptive activity was evaluated as described in Section 2.3.

2.10. Effect of Db-cAMP, forskolin and dexamethasone pretreatment on tumor necrosis factor-α and interleukin-1β production by peritoneal cells harvested from peritoneal cavities stimulated with zymosan

Mice were pretreated with saline, Db-cAMP (0.025 nmol), forskolin (0.5 nmol), 10 min before, or dexamethasone (2 mg/kg), 1 h before the stimulation with zymosan. The control group received just saline (0.2 ml). After 15 min, the peritoneal cavities were washed with Roswell Park Memorial Institute media (RPMI; 2 ml/cavity), and the exudates were centrifuged at $300 \times g$ for 10 min. The pelleted cells were resuspended in 500 μl of RPMI with 10% fetal calf serum, counted, and 5×10^5 cells were plated onto 48-well plastic tissue culture plates. The concentrations of tumor necrosis factor-α and interleukin-1β in the supernatants, after culture for 12 h, were determined by enzyme-linked immunosorbent assay (ELISA), as described previously (Cunha et al., 1993). Briefly, microtiter plates were coated overnight at 4°C with antibody against murine tumor necrosis factor-α or interleukin-1β (10 μg/ml). After non-specific binding sites were blocked, the samples and standard at various dilutions were added in duplicate and incubated at 4°C for 24 h. The plates were washed three times with buffer, and a second biotinylated polyclonal antibody against tumor necrosis factor-α or interleukin-1β, diluted 1/1000, was added (100 μl/well). After a further incubation at room temperature for 1 h, the plates were washed and 100 μl of avidin–horseradish peroxidase diluted 1:5000 was added. Then, 100 μl of color reagent *O*-phenylenediamine was added 15 min later, and the plates were incubated in the dark at 37°C for 15–20 min. The enzyme reaction was stopped with H_2SO_4 and absorbance at 490 nM was measured. The results are reported as means ± S.E.M. for three animals.

2.11. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test was used. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Effect of cholera toxin, forskolin, aminophylline and Db-cAMP on acetic acid- and zymosan-induced nociceptive activity in the writhing model

Cholera toxin (160 ng), forskolin (0.5 nmol), aminophylline (0.25 nmol) and Db-cAMP (0.025 nmol) significantly reduced ($P < 0.05$; acetic acid: saline = 47.70 ± 6.87, cholera toxin = 10.71 ± 4.47, forskolin = 11.76 ± 1.33, aminophylline = 13.41 ± 2.82, Db-cAMP = 21.28 ± 3.00, and zymosan: saline = 11.60 ± 1.30, cholera toxin = 5.71 ± 0.81, forskolin = 3.46 ± 1.66, aminophylline = 2.90 ± 1.63, Db-cAMP = 4.57 ± 1.68) the number of writhes induced by acetic acid (0.2 ml, 0.6% v/v) or

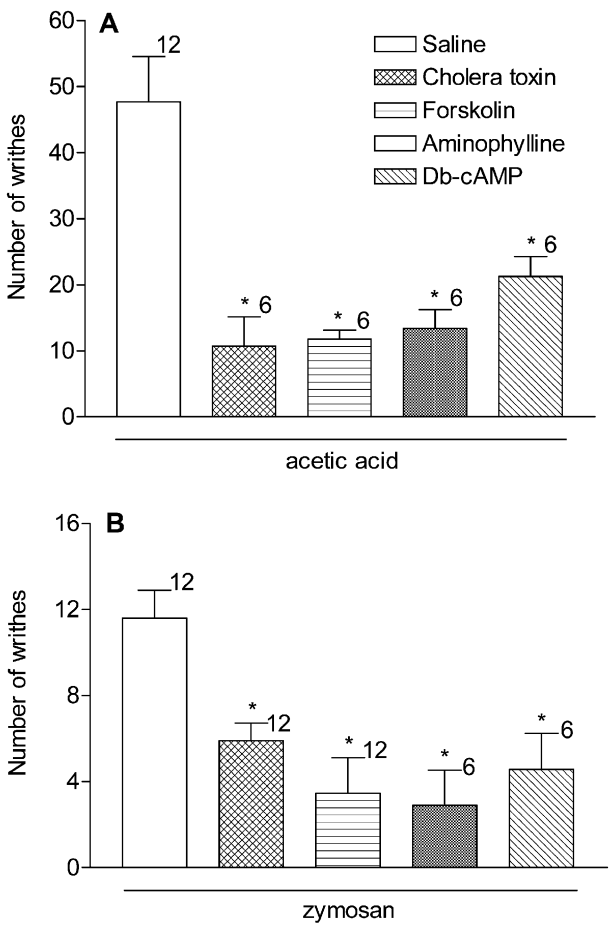

Fig. 1. Effect of cholera toxin, forskolin, aminophylline and Db-cAMP i.p. injections, on acetic acid- and zymosan-induced writhes in mice. Animals were injected i.p. with saline or cholera toxin (160 ng) 1 h before, or forskolin (0.5 nmol), aminophylline (0.25 nmol), Db-cAMP (0.025 nmol), 10 min before acetic acid (0.2 ml of solution, 0.6% v/v) or zymosan (1 mg/mouse) i.p. injection. The number of writhes was determined for 30 min after acetic acid (Panel A) or zymosan (Panel B) injection. Bars represent means ± S.E.M. for the number of mice indicated at the top of each column. * Indicates a statistically significant difference ($P < 0.05$) from the control group (saline) determined by ANOVA and Tukey's test.

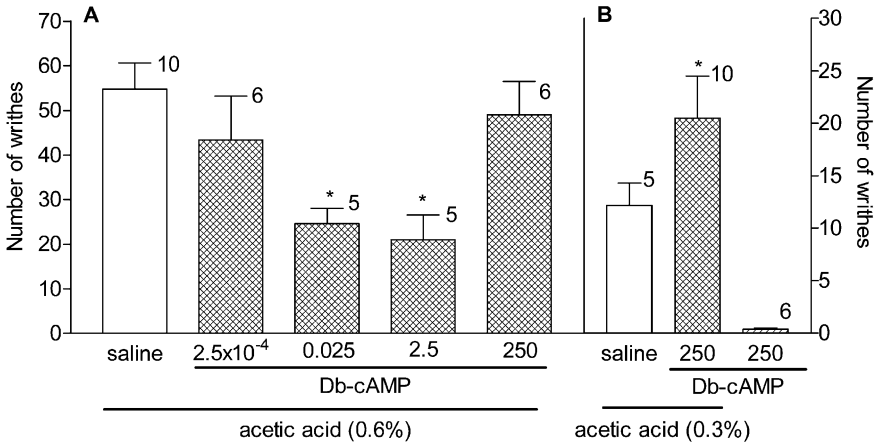

Fig. 2. Effect of Db-cAMP on nociception induced by acetic acid. Saline or Db-cAMP (2.5×10^{-4}, 0.025, 2.5, or 250 nmol) was injected 10 min before acetic acid (0.2 ml of solution, 0.6% v/v) injection (Panel A). Saline or Db-cAMP (250 nmol) was injected 10 min before acetic acid (0.2 ml of solution, 0.3% v/v), and Db-cAMP (250 nmol) was also injected alone (Panel B). The number of writhes was determined for 30 min after the stimuli injection and the results are presented as means ± S.E.M. for the number of mice indicated at the top of each column. * Indicates a statistically significant difference ($P < 0.05$) between the control (saline) and Db-cAMP groups determined by ANOVA and Tukey's test.

zymosan (1 mg/mouse), compared to the effect of saline (Fig. 1A,B).

3.2. Effect of different doses of Db-cAMP on acetic acid-induced nociceptive activity in the writhing model

The i.p. injection of Db-cAMP 10 min before acetic acid (0.2 ml, 0.6% v/v) induced a dual response: low doses (2.5×10^{-4}, 0.025 and 2.5 nmol) caused a dose-dependent antinociceptive effect, while a higher dose (250 nmol) was not able to induce antinociception (saline = 54.80 ± 5.82; Db-cAMP 2.5×10^{-4} nmol = 43.35 ± 9.85; 0.025 nmol = 24.60 ± 3.47; 2.5 nmol = 21.0 ± 5.55; 250 nmol = 49.00 ± 7.46 writhes) (Fig. 2A). In contrast to the antinociception caused by the lower doses, the i.p. injection of a high dose of Db-cAMP (250 nmol), 10 min before administration of the nociceptive stimulus, potentiated the nociceptive activity of a low dose of acetic acid (0.2 ml, 0.3% v/v; $P < 0.05$; Db-cAMP 250 nmol = 20.5 ± 4.01 vs. saline = 12.2 ± 2.08) when compared to the effect of saline pretreatment. Db-cAMP (250 nmol) alone was not able to induce writhes during a 30-min period following i.p. injection (Fig. 2B).

3.3. Effect of resident peritoneal cell depletion on acetic acid-induced nociceptive activity in the writhing model and reversal of this effect by Db-cAMP

Considering that lower doses of Db-cAMP exerted an antinociceptive effect, while the higher dose of Db-cAMP (250 nmol) potentiated the effect of a low dose of acetic acid (0.2 ml, 0.3% v/v), we decided to verify the possible importance of resident peritoneal cells on this dual activity of cAMP. Thus, resident peritoneal cells were depleted (80% reduction) by the peritoneal washing method, 30 min before acetic acid (0.6% v/v) i.p. injection. The washing procedure does not interfere with the nociceptive responsiveness induced by a stable prostacyclin analogue (Ribeiro et al., 2000). Acetic acid-induced writhes were significantly reduced by the washing procedure compared to the sham procedure ($P < 0.05$; washed = 10.25 ± 2.17, vs. sham = 49.66 ± 7.69). Intraperitoneal injection of Db-cAMP, 250 but not 2.5 nmol, 10 min before acetic acid

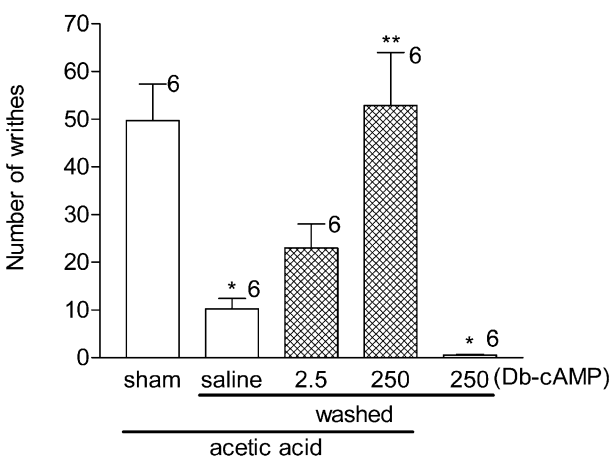

Fig. 3. Effect of Db-cAMP on acetic acid-induced writhes in Swiss mice after the depletion of peritoneal resident cells. Saline or Db-cAMP (2.5 or 250 nmol) was injected i.p., 30 min after the depletion of peritoneal resident cells by washing of the peritoneal cavity. Acetic acid (0.2 ml of solution, 0.6% v/v) was injected 10 min after saline or Db-cAMP (in mice submitted to peritoneal washing) injections, and Db-cAMP (250 nmol) was injected alone after peritoneal washing. The first bar represents the sham group that was not submitted to peritoneal washing and which received acetic acid. The number of writhes was determined for 30 min after acetic acid injection. Bars represent means ± S.E.M. of the number of mice indicated at the top of each column. * $P < 0.05$ represents a statistically significant difference (ANOVA; Tukey's test) between the test group and the sham group, and ** $P < 0.05$ represents statistically significant difference (ANOVA; Tukey's test) in relation to the washed group that received saline.

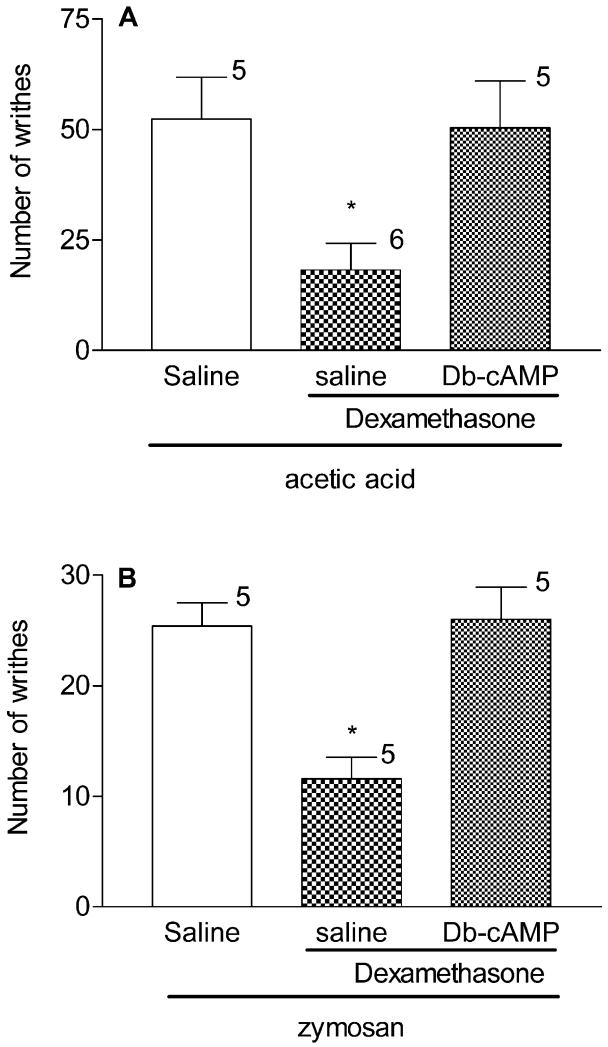

Fig. 4. Effect of Db-cAMP on dexamethasone antinociceptive action. Dexamethasone (2 mg/kg; s.c.) or saline was injected i.p., 1 h before acetic acid (0.2 ml of solution, 0.6% v/v) or zymosan (1 mg/mouse) i.p. administration. The groups treated with dexamethasone received i.p. injection of Db-cAMP (250 nmol) or saline 10 min before the nociceptive stimuli. The number of writhes was determined for 30 min after acetic acid (Panel A) or zymosan (Panel B) injection. Bars represent means ± S.E.M. for the number of mice indicated at the top of each column. * Indicates a statistically significant difference ($P < 0.05$) from the control group (saline) determined by ANOVA and Tukey's test.

0.6% v/v i.p. injection significantly reversed ($P < 0.05$; 250 nmol washed = 52.83 ± 11.13, vs. saline washed = 10.25 ± 2.17 writhes) the antinociceptive effect of the depletion of resident cells by the washing procedure. Db-cAMP (250 nmol) alone was not able to induce writhes during a 30-min period after i.p. injections in mice submitted to peritoneal washing (Fig. 3).

3.4. Effect of Db-cAMP on dexamethasone antinociceptive action

We verified that the inhibition of the release of the nociceptive inflammatory mediators by dexamethasone administration significantly reduced ($P < 0.05$; acetic acid: saline + dexamethasone = 18.16 ± 6.1 vs. saline = 52.4 ± 9.42, and zymosan: saline + dexamethasone = 11.6 ± 1.91 vs. saline = 25.4 ± 2.11 writhes) the acetic acid- or zymosan-induced nociceptive activity. The injection of a higher dose of Db-cAMP (250 nmol) reversed ($P < 0.05$; acetic acid: Db-cAMP + dexamethasone = 50.4 ± 10.6 vs. saline + dexamethasone = 18.16 ± 6.1 and zymosan: Db-cAMP + dexamethasone = 26.0 ± 2.93 vs. saline + dexamethasone = 11.6 ± 1.91 writhes) the dexamethasone-antinociceptive effect (Fig. 4).

3.5. Effect of Db-cAMP, forskolin and dexamethasone pretreatment on cytokine release

Db-cAMP, forskolin and dexamethasone administration before zymosan i.p. injection significantly reduced ($P <$

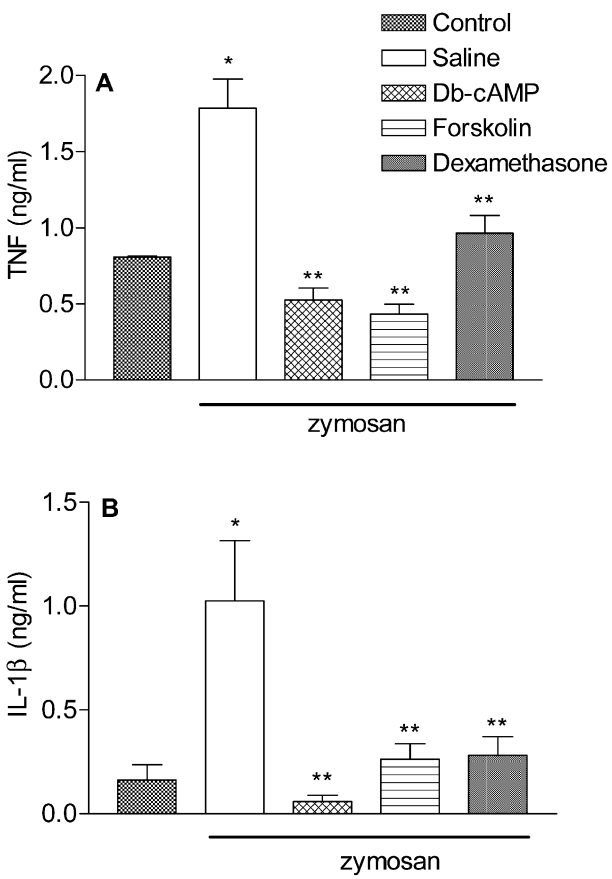

Fig. 5. Effect of Db-cAMP, forskolin and dexamethasone pretreatment on tumor necrosis factor-α and interleukin-1β production by peritoneal cells, harvested from peritoneal cavities stimulated with zymosan. Mice were pretreated with saline, Db-cAMP (0.025 nmol), forskolin (0.5 nmol), 10 min before, or dexamethasone (2 mg/kg) 1 h before stimulation with zymosan (1 mg/mouse). The control group received saline (0.2 ml). After 15 min, the peritoneal cavities were washed, and the concentrations of tumor necrosis factor-α and interleukin-1β in the supernatants after culture for 12 h were determined by ELISA. The bars represent means ± S.E.M. for three animals. * * Indicates a statistically significant difference ($P < 0.05$) in relation to saline group and * indicates a statistically significant difference in relation to control group determined by ANOVA and Tukey's test.

0.05; tumor necrosis factor-α: Db-cAMP = 0.52 ± 0.07, forskolin = 0.43 ± 0.06, dexamethasone = 0.96 ± 0.11 vs. saline = 1.78 ± 0.18, and interleukin-1β: Db-cAMP = 0.05 ± 0.03, forskolin = 0.26 ± 0.07, dexamethasone = 0.28 ± 0.08 vs. saline = 1.02 ± 0.28 ng/ml) tumor necrosis factor-α and interleukin-1β release induced by zymosan, compared to control (Fig. 5A,B).

4. Discussion

In this study, we demonstrated that substances which increase intracellular cAMP levels, such as forskolin (direct adenylate cyclase activator), cholera toxin (G_s protein activator), aminophylline (phosphodiesterase inhibitor), and low doses of Db-cAMP (membrane-permeable analogue of cAMP), reduce the number of writhes induced by acetic acid and zymosan in mice. These data suggest that an increase in the intracellular cAMP level may have an antinociceptive effect in the mouse writhing model. These findings are in line with those provided by the current literature, which report a down-regulatory role of cAMP on the release of inflammatory mediators.

It has been demonstrated that phosphodiesterase inhibitors display antiinflammatory activity in models of asthma, glomerulonephritis and rheumatoid arthritis (Moore and Willoughby, 1995; Teixeira et al., 1997; Torphy, 1998; Schudt et al., 1995; Tsuboi et al., 1996). Moreover, in vitro studies have demonstrated that agents which elevate the intracellular level of cAMP reduce the immunological release of leukotrienes from mast cells (Marone et al., 1987), leukotrienes B_4 from neutrophils (Ham et al., 1983) and inflammatory cytokines from macrophages and neutrophils (Renz et al., 1988; Teixeira et al., 1997). Thus, cAMP may participate in an endogenous mechanism that down-regulates the inflammatory response.

It has been proposed that, in rats, cytokines produced by resident cells have an important role in inflammatory pain. Tumor necrosis factor-α induces the release of interleukin-1β and interleukin-6, which stimulate the production of cyclo-oxygenase products, and interleukin-8, which induces the production of sympathomimetic amines (Dinarello et al., 1986; Cunha et al., 1991, 1992; Ferreira et al., 1988). These two biochemical pathways have also been previously demonstrated in the mouse writhing model (Duarte et al., 1988; Thomazzi et al., 1997). Furthermore, it has been shown that resident peritoneal macrophages and mast cells have an important role in nociception induced by acetic acid and zymosan in the writhing model, since they are the source of the cytokines that mediate this response (Ribeiro et al., 2000). Based on these data, one possible explanation for the antinociceptive effect of the agents used in this study, is that the increase in the intracellular level of cAMP in resident peritoneal cells results in a down-regulation of the release of cytokines that mediate nociception. In fact, we demonstrated in the present study that the intraperitoneal administration of Db-cAMP or forskolin, common with dexamethasone, inhibited the production of tumor necrosis factor-α and interleukin-1β stimulated by zymosan. These results confirmed previous data, which showed that substances which elevate the intracellular level of cAMP also down-regulate the production of tumor necrosis factor-α and other cytokines (Katakami et al., 1988; Renz et al., 1988). Confirming the importance of cytokines on the nociceptive effect of zymosan and acetic acid, our laboratory has demonstrated that pretreatment of the peritoneal cavity with antisera against tumor necrosis factor-α and interleukin-1β reduces the nociceptive responses induced by zymosan and acetic acid and that peritoneal cells harvested from peritoneal cavities injected with zymosan or acetic acid release both tumor necrosis factor-α and interleukin-1β (Ribeiro et al., 2000).

In contrast to the resident peritoneal cells, in which the increase of intracellular level of cAMP causes antinociception as discussed above, the increase of cAMP in the nociceptive neuron terminal seemed to cause nociception. It has been demonstrated that the receptors for the main hyperalgesic substances such as prostaglandins, bradykinin and sympathomimetics are associated with adenylate cyclase (Taiwo and Levine, 1991; Cunha et al., 1999). Moreover, cAMP analogues or substances that promote an increase in the intracellular levels of cAMP sensitize the rat peripherical nociceptor to thermal and mechanical stimuli (Mizumura et al., 1993; Ferreira and Nakamura, 1979; Cunha et al., 1999). In order to investigate whether a higher dose of Db-cAMP could also sensitize the nerve terminal in the writhing model, we tested different doses of this agent. We found that a high dose of this agent had no analgesic effect, in contrast to low doses of Db-cAMP, which reduced the number of writhes induced by acetic acid. Indeed, a higher dose of Db-cAMP amplified the nociceptive effect of low doses of acetic acid, and reversed the antinociceptive effect of dexamethasone, a well known inhibitor of prostaglandin and cytokine synthesis, agents which mediate the increase of cAMP in the sensitive neuron (Flower and Blackwell, 1979; Staruch and Wood, 1985; Beutler et al., 1986). Furthermore, high doses of Db-cAMP also reversed the decrease in writhing response induced by acetic acid in mice whose peritoneal cavities had been washed with saline.

It has previously been demonstrated that lavage of the peritoneal cavities of mice decreases the nociceptive response of animals injected with zymosan or acetic acid, since this procedure removes the resident peritoneal cells with a parallel reduction in the release of the mediators that sensitize the nociceptors (Thomazzi et al., 1997; Ribeiro et al., 2000). Thus, the explanation for the fact that low doses of cAMP caused antinociception while a high dose potentiated the nociception induced by acetic acid, is that cAMP in low doses acts on resident cells to inhibit the production of endogenous nociceptive mediators, while

higher doses directly sensitize the nociceptors to the algesic stimuli, which activate the sensitized receptors. In this context, we demonstrated that Db-cAMP injected alone in the peritoneal cavities of the mice was not able to induce a writhing response. These data confirm that cAMP acts by sensitizing the nociceptor, but it is not able to activate the nociceptor by itself. It is important to remember that there are two groups of inflammatory mediators: those that sensitize nociceptive receptors and those that activate sensitized receptors. Substances released by resident cells, such as cytokines, prostaglandins or sympathomimetics, are known to mediate sensitization of the pain receptor via cAMP. After sensitization of the pain receptor, previously ineffective stimuli become painful, as they are now able to activate the sensitized nociceptor (Poole et al., 1999). In the mouse writhing model, the activation of the pain receptor may be mediated by bradykinin, by the reduction of pH induced by acetic acid, or by mechanical stimulation, due to the increase in intestine peristalsis induced by the stimuli (Correa et al., 1996).

In conclusion, this study suggests that, in the mouse writhing model, cAMP has a dual effect: an antinociceptive effect, due to the modulatory action of this second messenger in resident peritoneal cells, reducing the synthesis of mediators involved in the nociceptive response, and a nociceptive effect, due to direct sensitization of the nociceptive receptor.

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