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European Journal of Pharmacology 399 (2000) 243–249

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Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis

Janetti N. Francischi [a], Celina M. Yokoro [a], S. Poole [b], Wagner L. Tafuri [c], Fernando Q. Cunha [d], Mauro M. Teixeira [e,*]

[a] *Departament of Farmacologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[b] *National Institute for Biological Standards and Control, Potters Bar, Herts EN6 3QG, UK*
[c] *Departament of Patologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[d] *Departament of Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil*
[e] *Departament of Bioquímica e Imunologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627-Pampulha, 31270-901, Belo Horizonte, Brazil*

Received 25 November 1999; received in revised form 18 April 2000; accepted 26 April 2000

Abstract

There has been much interest in strategies which modulate tumour necrosis factor-α (TNF-α) levels and/or function in rheumatoid arthritis. The elevation of intracellular levels of cyclic AMP in leukocytes by phosphodiesterase 4 inhibitors is accompanied by significant inhibition of the production of TNF-α. Nevertheless, these drugs may enhance the hyperalgesia induced by a range of inflammatory mediators, including TNF-α. In the present study, we examined the effects of the phosphodiesterase 4 inhibitor rolipram on the local inflammatory infiltrate and hyperalgesia in a rat model of adjuvant-induced arthritis. Rolipram (3 mg/kg) was administered by oral gavage from day 10 to 14 after disease induction. Pretreatment with rolipram abrogated oedema formation and significantly inhibited hyperalgesia. Histopathological analysis revealed a marked inhibition of cellular influx as well as bone and cartilage destruction. Serum and local TNF-α levels were suppressed in treated animals whereas there were little changes in interleukin-1β levels. Although cyclic AMP elevating agents may affect nociceptor threshold to increase the hyperalgesic responses acutely, they also possess significant anti-inflammatory activity, which may hinder local mediator release and/or action. The anti-inflammatory effects of rolipram predominate during this chronic arthritis model in the rat.

Keywords: Arthritis; Phosphodiesterase 4; Hyperalgesia; Oedema; TNF-α (tumor necrosis factor-α); Rolipram

1. Introduction

Rheumatoid arthritis is a common chronic inflammatory disorder involving the synovial membranes of multiple joints in humans (Sewell and Trentham, 1993). Although there are reasonably good drugs used in the symptomatic relief of arthritis (e.g. non-steroidal anti-inflammatory drugs), there are few safe drugs, which modify fundamental pathologic processes responsible for the chronic inflammation (Cash and Klippel, 1994). Recently, there has been much interest in strategies, which inhibit tumour necrosis factor-α (TNF-α) levels and/or function as TNF-α appears to play a major pathophysiological role in rheumatoid arthritis (Feldman et al., 1998). Such interest stems from the clinically beneficial effects of anti-TNF-α treatment of rheumatic patients (Feldman et al., 1998, Moreland et al., 1997).

The elevation of intracellular levels of cyclic AMP in leukocytes is accompanied by significant inhibition of the production of TNF-α (Teixeira et al., 1997; Procopio et al., 1999). In this regard, strategies which elevate cyclic AMP may be beneficial in the treatment of rheumatoid arthritis. The levels of cyclic AMP inside cells are controlled by the degree of cyclic AMP production via adenylate cyclase-coupled receptors (e.g. β-adrenoceptors) and the metabolism of cyclic AMP by phosphodiesterases (Teixeira et al., 1997). The main phosphodiesterase activity present in leukocytes is the phosphodiesterases type 4 (Teixeira et al., 1997; Torphy, 1998). Blockade of these enzymes is associated with the inhibition of several leuko-

* Corresponding author. Tel.: +55-31-499-2723; fax: +55-31-499-2695.
E-mail address: mmtex@icb.ufmg.br (M.M. Teixeira).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00330-7

cyte functions, including inhibition of TNF-α production and the release of other inflammatory mediators and reactive oxygen species (Teixeira et al., 1997; Torphy, 1998; Au et al., 1998).

A few animal studies have evaluated the effect of phosphodiesterase 4 inhibitors in rodent models of rheumatoid arthritis (Sekut et al., 1995; Nyman et al., 1997; Ross et al., 1997). These studies demonstrate that treatment with the prototype phosphodiesterase 4 inhibitor rolipram is effective both prior to (Sekut et al., 1995) and when given after (Nyman et al., 1997; Ross et al., 1997) the arthritis-inducing stimulus. However, none of these studies evaluated any possible effect of rolipram on a major characteristic of rheumatoid arthritis, inflammatory hyperalgesia. In this respect, we have recently demonstrated that pretreatment of animals with locally injected rolipram significantly enhanced the hyperalgesia induced by several mediators, including that induced by TNF-α (Cunha et al., 1999). Thus, if phosphodiesterase 4 inhibitors are to be used in the treatment of rheumatoid arthritis, it is essential that their effects on hyperalgesia in animal models of arthritis are evaluated.

In the present study, we have used a model of adjuvant-induced arthritis in rats to evaluate the effect of the phosphodiesterase 4 inhibitor rolipram on the local inflammatory infiltrate and hyperalgesia. The local (in the paw) and circulating levels of TNF-α in control and treated rats were measured to assess the effectiveness of the dose of rolipram used. For comparison, we also assessed the local and systemic levels of interleukin-1α, a cytokine known to play an important role in arthritis (Breedveld, 1999), but usually less affected by phosphodiesterase 4 inhibitor pretreatment (reviewed by Torphy, 1998). Of note, rolipram was given orally after the induction of arthritis.

2. Material and methods

2.1. Animals

Female Holtzman rats (140–170 g) were used throughout this study. Animals were kept in cages (maximum of six animals per cage) at a temperature of 26 ± 3°C, and on a 12-h light–dark cycle. Water and food were given ad libitum. All experimental procedures described below have been approved by the local animal ethics committee.

2.2. Induction of arthritis by adjuvant

Rats were injected subcutaneously with a single dose of 0.2 ml mineral oil–water emulsion (10:1, v/v) containing 400 μg of dried *Mycobacterium butyricum* into the dorsal root of the tail under ether anaesthesia. The time of adjuvant injection is referred to as day 0.

2.3. Treatment of animals with rolipram

Rolipram (3 mg/kg) or vehicle were administered via oral gavage and animals ($n = 15$) were treated for 5 days. In the first day, two oral administrations were given and this was followed by single daily administrations. Treatment was initiated on day 10, when the first signs of joint inflammation and pain are usually noted (Tatsuo et al., 1994; Francischi et al., 1997). On day 14, a group of animals ($n = 10$) was sacrificed for histopathological and serological analysis. The remaining animals ($n = 5$) were followed for a further period of 7 days. Control animals received vehicle (alcohol 0.5%). The dose of rolipram used here has been previously shown to inhibit effectively the development of chronic inflammatory diseases in rats (Sekut et al., 1995).

2.4. Measurement of hindpaw hyperalgesia and oedema

The method for measuring hyperalgesia has been previously described elsewhere (Capetola et al., 1980; Tatsuo et al., 1994; Francischi et al., 1997). Briefly, the tendency of normal (naive), control and arthritic rats to vocalise following flexion of the tarsotibial joints of both hindpaws was tested daily for 22 days starting from day 0. The results are reported as the mean number (± S.E.M.) of vocalisations obtained following five flexions of hindlimb tarsotibial joints. Hindpaw volume (as an indicator of oedema) was

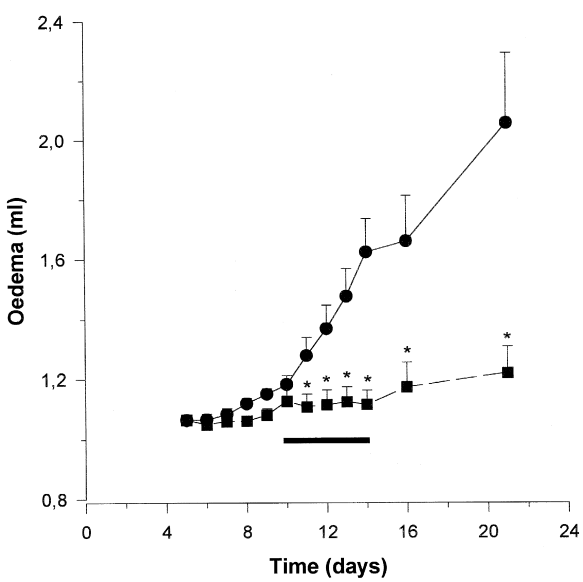

Fig. 1. Effect of the treatment with rolipram on hindpaw oedema in adjuvant-induced arthritis in rats. Adjuvant arthritis was induced and animals treated with rolipram (squares, 3 mg/kg, via oral gavage) or vehicle (circles, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the line). Oedema was measured using a hydroplethysmometer. Results are shown as the increase in paw volume (ml) and are the mean ± S.E.M. for 10 animals (days 0 to 14) or 5 animals (days 15 to 21) in each group. * $P < 0.05$.

measured daily using an Ugo Basile hydroplethysmometer (model 7150) after the test for hyperalgesia. The volume (ml) of one hindpaw was essentially the same as that of the contralateral paw (data not shown) and is reported as the mean ± S.E.M. All measurements were obtained at the same time of the day.

2.5. Histopathological processing and analysis

Fragments of tarso-metatarsal joints were collected 14 and 21 days after the induction of arthritis and fixed in 10% buffered formalin. The fragments were then treated with a 10% acidic nitric solution for decalcification, dehydrated, cleared, embedded in paraffin, cut (3–4 μm thick) and stained with Haematoxylin and Eosin. Tissue sections were analysed by one of the authors (WT) who was blind to the experimental groups. The following parameters were assessed and graded from absent to intense (− to + + +): oedema, synovial inflammation, juxta-articular erosion, accumulation of neutrophils, granulamotous tissue, tendon and skeletal muscle inflammation. The joints of at least three animals were observed in each experimental group.

2.6. Measurement of systemic levels of TNF-α and interleukin-1β

Serum was prepared by allowing blood to clot and retract at 37°C for 15 min and at 4°C for 30 min, respectively. The serum collected was then centrifuged twice at 10,000 × *g* for 10 min and stored at −20°C until TNF-α and interleukin-1β measurements. For the measurement of tissue cytokine levels, the subcutaneous tissue of the right hindpaw and that surrounding the tarsotibial joints was removed and placed on phosphate buffered saline containing 0.05% Tween 20, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzamethonium chloride, 10 mM EDTA and 20 KI aproptinin A. The tissue was homogenized, centrifuged at 3000 × *g* for 10 min and stored at −70°C until further analysis. TNF-α and interleukin-1β levels were evaluated using a standard sandwich ELISA technique as previously described (Rees et al., 1999).

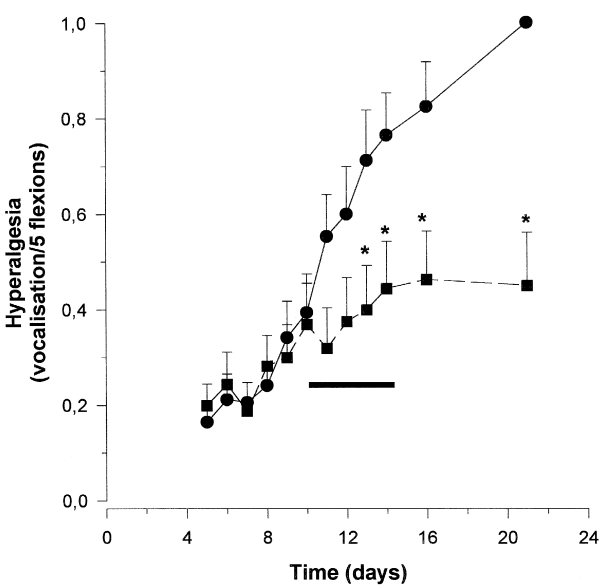

Fig. 2. Effect of the treatment with rolipram on hindpaw hyperalgesia in adjuvant-induced arthritis. Adjuvant arthritis was induced and animals treated with rolipram (squares, 3 mg/kg, via oral gavage) or vehicle (circles, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the line). Hyperalgesia was assessed by the ability of animals to vocalise following flexion of the tarsotibial joints of both hindpaws. Results are the mean number ± S.E.M. of vocalisations obtained for five flexions per paw in 10 animals (days 0 to 14) or 5 animals (days 15 to 21) in each group. $^{*}P < 0.05$.

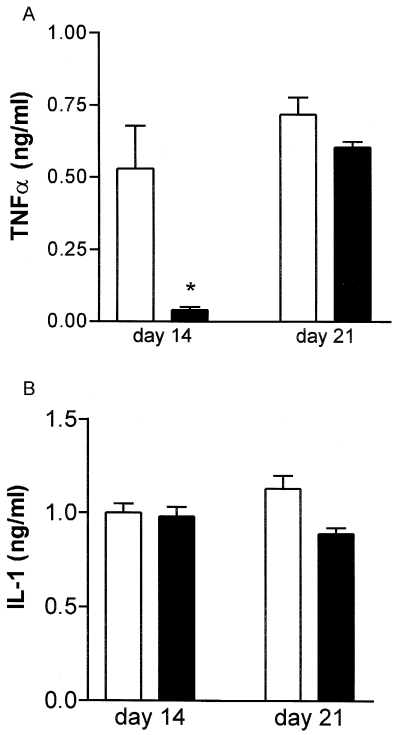

Fig. 3. Effects of the treatment with rolipram on the systemic levels of (A) TNF-α and (B) interleukin-1β in adjuvant-induced arthritis. Adjuvant arthritis was induced and animals treated with rolipram (circles, 3 mg/kg, via oral gavage) or vehicle (squares, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the arrows). Twenty four hours after the last administration of rolipram, animals were killed, blood taken, serum prepared and TNF-α levels assessed by ELISA. Results are shown as the mean ± S.E.M. for five animals in each group. $^{*}P < 0.01$.

2.7. Materials

Phenylmethylsulphonyl fluoride, benzamethonium chloride, EDTA, Tween 20 and aproptinin A were from Sigma (St. Louis, USA). Rolipram was a kind gift of Dr John Fozard, Novartis, Switzerland. Recombinant rat TNF-α and interleukin-1β, the coating and biotinylated sheep anti-rat TNF-α and anti-interleukin-1β antibodies were prepared at the National Institute for Biological Standards

and Control, United Kingdom. *M. butyricum* powder was purchased from Difco (lot 43887JC, Detroit, MO).

2.8. Statistical analysis

Data are presented as the mean ± S.E.M. of the shown number of experiments. Results were analysed using analysis of variance and the Student–Newman–Keuls post-hoc test. P values smaller than 0.05 were considered significant.

3. Results

3.1. Effects of the treatment with rolipram on hindpaw oedema and hyperalgesia

Animals injected with adjuvant usually start demonstrating measurable oedema and hyperalgesia around the 10th day after disease induction (Francischi et al., 1997). In the present experiment, significant hindpaw oedema (Fig. 1) and hyperalgesia (Fig. 2) were observed around day 11. Treatment with rolipram starting on day 10 until day 14 abrogated paw oedema (Fig. 1). Interestingly, hindpaw oedema in arthritic animals were still significantly inhibited 7 days (day 21) after the drug had been stopped, although an upward shift was already noticeable (Fig. 1). The inhibitory effects of rolipram treatment on hindpaw hyperalgesia is shown in Fig. 2. Significant inhibition of hyperalgesia was noted from day 14 and persisted until day 21 after treatment (Fig. 2).

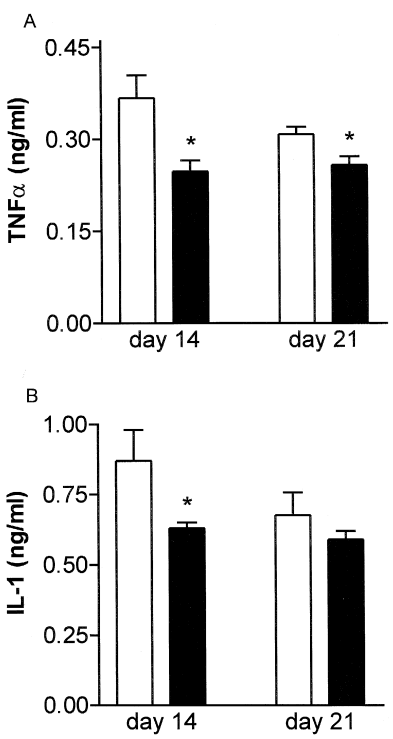

Fig. 4. Effects of the treatment with rolipram on the local levels of (A) TNF-α and (B) interleukin-1β in adjuvant-induced arthritis. Adjuvant arthritis was induced and animals treated with rolipram (circles, 3 mg/kg, via oral gavage) or vehicle (squares, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the arrows). Twenty four hours after the last administration of rolipram, animals were killed, the subcutaneous tissue of the right hindpaw and surrounding the tarsotibial joints were removed, homogeneized and IL-1β and TNF-α levels assessed by ELISA. Results are shown as the mean ± S.E.M. for five animals in each group. * $P < 0.05$.

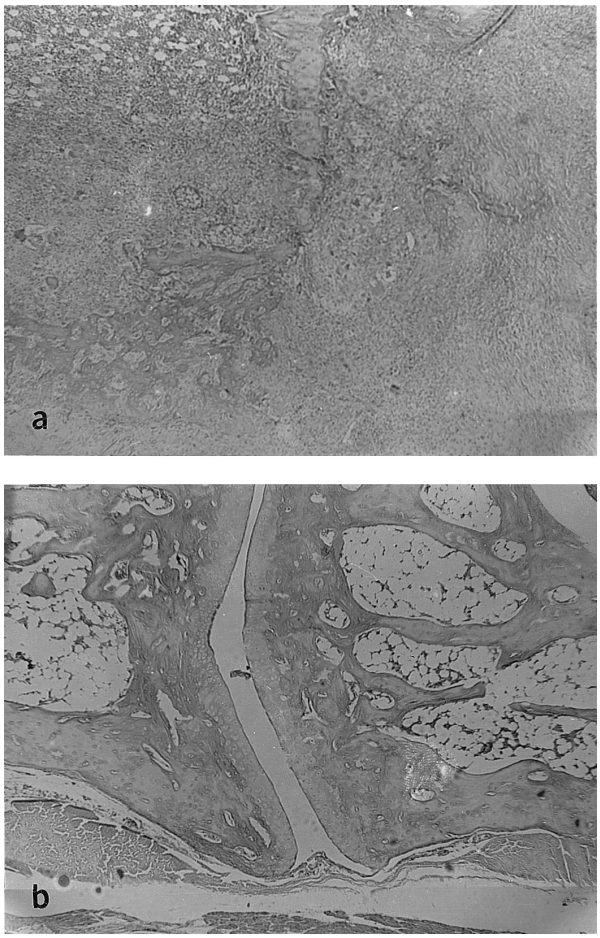

Fig. 5. Saggital sections of the tarso-metartasal joints 21 days after the induction of arthritis by adjuvant in rats. (a) Untreated animal showing an intense and diffuse inflammatory reaction with cartilage and bone erosion. (b) Rolipram-treated animal showing little inflammation and no destructive lesions. Haematoxylin and eosin 40× magnification.

3.2. Effects of the treatment with rolipram on systemic and local TNF-α and interleukin-1β levels

The levels of TNF-α in naive animals were below the detection limit of our assay (< 30 pg/ml). In the animals made arthritic by adjuvant, there was a detectable amount of TNF-α levels in serum and in the paws 14 and 21 days after disease induction (Figs. 3 and 4). Treatment of these animals with rolipram from days 10 to 14 significantly inhibited the increase in serum TNF-α levels at day 14, but had no significant effect on TNF-α levels at day 21

Table 1
Effects of the treatment with rolipram on joint inflammation in adjuvant-induced arthritis

	Untreated animals	Treated animals
Oedema	+ + +	+
Synovial inflammation	+ + +	+
Justa-articular erosion (cartilage and bone)	+ + +	−
Accumulation of neutrophils	+ +	−
Granulomatous tissue	+	−
Tendon and skeletal muscle inflammation	+ + +	−

Adjuvant arthritis was induced and animals treated with rolipram (3 mg/kg) or vehicle from days 10 to 14 after disease induction. Results shown are from animals killed 7 days (day 21) after the last dose of rolipram or vehicle was given and are the average of 4 animals in each group. The following legend applies: − absent, + mild, + + moderate and + + + intense.

(Fig. 3A). Local TNF-α levels were also significantly elevated in arthritic animals at days 14 and 21 after disease induction (Fig. 4A). TNF-α levels at days 14 and 21 were significantly inhibited by treatment with rolipram by 33% and 17%, respectively (Fig. 4A).

In contrast to its marked effects on systemic TNF-α levels, treatment with rolipram had little effect on interleukin-1β levels in serum (Fig. 3B). Nevertheless, interleukin-1β levels in the paws of treated animals 14 days after disease induction were approximately 30% lower than that in vehicle-treated animals (Fig. 4B).

3.3. Effects of the treatment with rolipram on the local inflammatory response

Fragments of hindpaws with the tarso-metatarsal joints of arthritic animals showed pronounced inflammation characterised by an intense and diffuse infiltration of mononuclear cells into the sub-synovial tissue with cartilage and bone destruction (Fig. 5A). Accumulation of neutrophils along the surface of the synovium and an intense oedema were also observed. In contrast, mononuclear cell infiltration was discrete in the treated animals. Moreover, they had a lower degree of sub-synovial inflammation and little cartilage or bone erosion was observed (Fig. 5B). A similar histopathological picture was observed on days 14 or 21 after disease induction (data not shown). Table 1 summarises the findings obtained in control and rolipram-treated groups on day 21 after disease induction.

4. Discussion

Adjuvant-induced arthritis in rats is a well-established experimental model for the study of the pathophysiology of various types of human arthritis, in special for the study of rheumatoid arthritis (Owen, 1980; Pearson, 1956; Pearson and Wood, 1963). In addition, it is a good chronic inflammatory model for development of potential analgesic and/or anti-inflammatory drugs useful for arthritis treatment (Colpaert et al, 1982).

In the present study, we showed that daily oral administration of the prototype phosphodiesterase 4 inhibitor rolipram effectively inhibited the increase in volume of the hindpaws of arthritic rats. The inhibition of hindpaw volume was associated with inhibition of both cell infiltration and local oedema formation as assessed by histology. Moreover, there was significantly lesser tissue destruction in treated animals as compared to vehicle control. These results are in remarkably good agreement with previous studies demonstrating an inhibitory effect of rolipram in other models of arthritis in mice (Ross et al., 1997) and rats (Sekut et al., 1995; Nyman et al., 1997). In addition, our results clearly demonstrate that the anti-inflammatory effects induced by rolipram were sustained for at least 7 days after the treatment had ceased. Similarly, Nyman et al. (1997) showed that rolipram stopped disease progression for several days in a collagen-induced arthritis model in rats. Overall, these results firmly demonstrate that inhibition of phosphodiesterase 4 may be of clinical benefit in the treatment of arthritis in humans.

We have recently demonstrated that when rolipram or other cyclic AMP elevating drugs were administered locally in an acute model of hyperalgesia, there was a marked increase in hyperalgesic responses following administration of a range of different stimuli, including TNF-α (Cunha et al., 1999). Such effects of rolipram and other cyclic AMP elevating agents appeared to be related to their ability to modify nociceptor threshold in sensory nerve endings (Cunha et al., 1999). Thus, it was important to evaluate any potential increase in the hyperalgesia in our model of adjuvant arthritis. To this end, rolipram was administered on day 10, a time when hyperalgesia is already present but not maximal. In contrast to its potentiating local effect on acute inflammatory pain, rolipram had no enhancing effect in this chronic model of hyperalgesia and inflammation. In fact, this is the first study to demonstrate a significant inhibitory effect of rolipram on the hyperalgesic responses in arthritic animals. The discrepancy between the two opposing effects of rolipram are the object of active research in our laboratories. One possibility to explain such discrepancy is that in the chronic model of arthritis, rolipram may be actively inhibiting leukocyte activation and mediator release (see for example, Au et al., 1998; reviewed in Torphy, 1998). By inhibiting the release of pro-inflammatory mediators, rolipram could potentially modulate the onset and maintenance of the hyperalgesic response. Another interesting possibility that we are now examining in more detail is the possibility that rolipram and other phosphodiesterase 4 inhibitor may have differential effects when applied locally or systemically. These differences may be related to the presence of more or lesser anti-inflammatory activity, which may depend on

the route of administration of the drug. In fact, systemic pretreatment with rolipram significantly inhibited the hyperalgesic responses and leukocyte infiltration induced by the intraplantar injection of carrageenan whereas local injection of the drug increased hyperalgesia and failed to affect leukocyte infiltration (Cunha et al., 1999 and data not shown).

There is much evidence demonstrating a direct effect of rolipram and other phosphodiesterase 4 inhibitors on the macrophage to inhibit TNF-α production (reviewed by Torphy, 1998). In addition, rolipram has been shown to block the secretion of TNF-α from macrophages by inhibiting T cell activation and expression of surface molecules (Kasyapa et al., 1999). The inhibitory effects of rolipram on TNF-α production has been shown to play a major role in the ability of the drug to inhibit collagen-induced arthritis in mice (Ross et al., 1997). Although the mechanisms underlying the anti-inflammatory of rolipram in our model were not determined, systemic and local TNF-α levels were substantially reduced in rolipram-treated animals arguing for a possible role of inhibition of this cytokine in the anti-inflammatory effects of rolipram. Interestingly, although systemic TNF-α levels were back to control levels after the drug was discontinued, levels of TNF-α in the paw were still significantly inhibited. Nevertheless, the level of inhibition at day 21 was lower than at day 14. This local increase in TNF-α levels may account, at least in part, for the tendency of oedema to increase towards the end of the observation period.

In addition to inhibiting TNF-α, phosphodiesterase inhibitors have been shown to modulate the production of other cytokines, including interleukin-1β (Torphy, 1998). In our experiments, treatment with rolipram significantly inhibited the increase in local, but not systemic, levels of interleukin-1β. In addition, the effects of rolipram on interleukin-1β production were marginal and not sustained. This is in agreement with previous studies demonstrating that interleukin-1β is inhibited in some but not all in vitro experiments of macrophage activation (reviewed by Torphy, 1998). The reasons for these discrepancies are not known but could be related to a greater effect of cAMP elevating agents on TNF-α mRNA stability (Verghese et al., 1995). Overall, these data argue for a possible role of inhibition of interleukin-1β for the anti-inflammatory and analgesic effects of rolipram. Thus, although rolipram may affect nociceptor threshold to increase hyperalgesic responses, it also possesses significant anti-inflammatory activity, which may hinder local mediator release and/or action. It appears that the anti-inflammatory effects of rolipram predominate during this chronic arthritis model in the rat.

In summary, we present data showing that oedema and hyperalgesia presented by arthritic rats were actively abrogated by oral administration of rolipram, a phosphodiesterase 4 inhibitor. We suggest that the reduction of hyperalgesia was secondary to the reduction of the local (at the joints) inflammatory response. Thus, phosphodiesterase 4 inhibitors may be useful in the treatment of rheumatoid arthritis as these drugs appear to modify the fundamental pathological process in the joint of affected animals. Any acute effect of these drugs on the hyperalgesic response are counterbalanced by their strong anti-inflammatory effects.

Acknowledgements

We are grateful to Webster Pimenta for his expert technical help. Work in our laboratories is supported by FAPEMIG, CNPq and FAPESP.

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