Characterization and pharmacological evaluation of febrile response on zymosan-induced arthritis in rats

Alexandre Kanashiro,1 Andréa C. Pessini,1 Renes R. Machado,1 David do C. Malvar,1 Fernando A. Aguiar,1 Denis Melo Soares,1 Mariana L. do Vale,2 and Glória E. Petto de Souza1

1Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil; and 2Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil

Submitted 23 June 2008; accepted in final form 18 February 2009

Zymosan-induced arthritis in rats

Kanashiro A, Pessini AC, Machado RR, Malvar DC, Aguiar FA, Soares DM, Vale ML, Souza GEP. Characterization and pharmacological evaluation of febrile response on zymosan-induced arthritis in rats. Am J Physiol Regul Integr Comp Physiol 296: R1631–R1640, 2009. First published February 25, 2009; doi:10.1152/ajpregu.90527.2008.—The present study investigated the febrile response in zymosan-induced arthritis, as well as the increase in PGE₂ concentration in the cerebrospinal fluid (CSF), along with the effects of antipyretic drugs on these responses in rats. Zymosan intra-articularly injected at the dose of 0.5 mg did not affect the body core temperature (Tc) compared with saline (control), whereas at doses of 1 and 2 mg, zymosan promoted a flattened increase in Tc and declined thereafter. The dose of 4 mg of zymosan was selected for further experiments because it elicited a marked and long-lasting Tc elevation starting at 3 1/2 h, peaking at 5 1/2 h, and remaining until 10 h. This temperature increase was preceded by a decrease in the tail skin temperature, as well as hyperalgesia and edema in the knee joint. No febrile response was observed in the following days. In addition, zymosan-induced fever was not modified by the sciatic nerve excision. Zymosan increased PGE₂ concentration in the CSF but not in the plasma. Oral pretreatment with ibuprofen (5–20 mg/kg), celecoxib (1–10 mg/kg), dipyrone (60–240 mg/kg), and paracetamol (100–200 mg/kg) or subcutaneous injection of dexamethasone (0.25–1.0 mg/kg) dose-dependently reduced or prevented the fever during the zymosan-induced arthritis. Celecoxib (5 mg/kg), paracetamol (150 mg/kg), and dipyrone (120 mg/kg) decreased CSF PGE₂ concentration and fever during zymosan-induced arthritis, suggesting the involvement of PGE₂ in this response.

Fever; anti-inflammatory drugs; paracetamol; dipyrone; celecoxib; prostaglandin E₂

Fever, a complex neuroimmune response, is an event of the acute phase response and is traditionally defined as a controlled elevation in body temperature in response to inflammation or invasion by infectious agents (51). Exogenous pyrogens stimulate host defense via interaction with associated membrane or intracellular receptors (27, 56, 57; for a review, see Ref. 1) and promote the release of several endogenous pyrogens, such as cytokines and chemokines. These mediators, in turn, are responsible for the synthesis/release of cyclooxygenase (COX)-2-dependent PGE₂, and other central mediators, which, acting on specific receptors, modulate the activity of thermoregulatory neurons in the preoptic area of the anterior hypothalamus via EP₃ receptors (17, 36, 51). This promotes an elevation of corporal temperature via a coordinated series of physiological and behavioral responses, such as peripheral vasoconstriction (7, 8, 48). Moreover, other pathways involving the component C₅a of the complement system, the blanch hepatic of the afferent vagus nerve, and the release of norepinephrine of the neural α-1 and glial α-2-adrenoceptors appear to be involved in the development of the febrile response in mammals (5).

Rheumatoid arthritis (RA), a chronic erosive inflammatory disease of the synovium whose pathogenesis is uncertain, is an autoimmune condition that affects ~0.5–1% of the population worldwide (18). Both development and progression of RA are determined by multiple variables, including immunological, genetic, and environmental factors (10). In addition, clinical studies have demonstrated that a great variety of extra-articular manifestations can appear during the rheumatoid arthritis onset and development, one of them being febrile response (26).

Zymosan-induced arthritis in rodents shows morphological similarities to human disease, such as immune cell infiltration, pannus development, and destruction of cartilage and bone, and has been widely used to study the pathogenesis of the disease and to identify potential new therapeutic drugs for clinical use (28, 52).

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen (a COX nonselective inhibitor) and celecoxib (a COX-2 selective inhibitor), and steroidal anti-inflammatory drugs, such as dexamethasone (arachidonic acid and proinflammatory proteins synthesis inhibitor), represent clinically important classes of agents commonly used in the treatment of conditions frequently associated with rheumatic diseases, including fever, inflammation, and joint pain (38). In addition, recent studies have demonstrated that paracetamol and dipyrone, which show weak anti-inflammatory but potent antipyretic and analgesic effects, inhibit preferentially COX-2 and both COX-1/-2, respectively (29, 30, 35, 37).

The protective functions of fever during infections are associated with its ability to decrease bacterial growth and stimulate the host defense mechanisms (61). Therefore, the febrile response may be dangerous for patients suffering from noninfectious clinical complications (41, 61). This means that antipyretic therapy may be necessary in patients when the body temperature observed during the febrile response exceeds its physiological benefits. However, the choice of an antipyretic drug must be made not only considering the fever inhibition but also the health conditions of the patient, which means that the drug cannot interfere with latent or controlled disease, mainly cardiovascular diseases.

In view of these considerations, the present study was performed aiming at 1) assessing whether the increase in body

http://www.ajpregu.org 0363-6119/09 $8.00 Copyright © 2009 the American Physiological Society R1631

temperature in zymosan-induced arthritis constitutes solely a hyperthermia or an integrated thermoregulatory response, i.e., fever, 2) investigating the febrile response time-course curve together with the typical symptoms of arthritis, such as hyperalgesia and edema development, 3) studying the role of neural pathways in this response, 4) investigating the effects of classic antipyretic (dipyridone, paracetamol) and anti-inflammatory drugs (ibuprofen, celecoxib, dexamethasone) both on fever and 5) on the level of prostaglandin PGE\(_2\) in the cerebrospinal fluid (CSF) after intra-articular zymosan injection.

**MATERIALS AND METHODS**

**Animals.** Experiments were conducted using male 180 – 220 g Wistar rats, individually housed at 24 ± 1°C under 12:12-h light-dark cycle (lights on at 0600) and which had free access to food and tap water until the day of the experiment itself, at which point, only water was made available to them. Each animal was used only once. Care and use of the animals were in accordance with the protocols previously approved by the Animal Care and Use Ethics Committee of the University of São Paulo, Ribeirão Preto Campus (Protocol nr. 06.1.1353.53.2) and Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research (1996).

**Zymosan-induced arthritis.** The rats were subjected to the intra-articular injection of different doses of zymosan (0.5, 1, 2, and 4 mg), suspended in sterile saline (50 μl), into their right knee joints. Control animals received only saline (55).

**Temperature measurements.** The body core temperature (T\(_c\)) was measured in conscious and unrestrained rats every 30 min for up to 8 h, by gently inserting, 4 cm into the rectum, a Vaseline-coated thermistor probe (model 402 coupled to a model 46 telethermometer; Yellow Springs Instruments, Yellow Springs, OH), without removing the animals from their cages. The food was removed 10 h before the beginning of the experiment. Experimental measurements were conducted in the thermoneutral zone of rats (23) in a temperature-controlled room (controlled at 27 ± 1°C). After this period, baseline temperatures were determined 3 or 4 times at 30-min intervals prior to any injection until 1000; only animals displaying mean basal rectal temperatures between 36.8 and 37.4°C were selected for the study.

To minimize core temperature changes due to handling, animals were twice habituated to this environment and procedure on the preceding day.

In the experiment in which the body temperature was measured for up to 72 h, we used a battery-operated radiotelemetry transmitter (Data Science, St. Paul, MN), implanted in the peritoneal cavity of each animal under deep anesthesia induced by intraperitoneal injection of xylazine (20 mg/kg) and ketamine (58 mg/kg). During the whole experiment, the animals were housed under standard conditions of temperature, relative humidity, and 12:12-h light-dark cycles, with free access to both food and water. After implantation of the radiotelemetry transmitter, the animals were treated with oxytetracycline hydrochloride (400 mg/kg im) and allowed to recover for 1 wk before the experiment. The signals from the transmitters were delivered through a computer-linked receiver to the Dataquest data acquisition system (Dataquest A.R.T. System). Preliminary experiments revealed that LPS-induced fever recorded using the radiotelemetry system was indistinguishable from that assessed by the rectal probe method (17).

The skin temperature (Tsk) was measured by attaching a thermistor probe (model 402 coupled to a model 46 telethermometer, Yellow Springs Instruments) to the lateral surface of the tail on the first distal third, without removing the rats from their home cages, for 1-min at 30-min intervals up to 6 h. The thermistor was fixed and isolated from the changes of ambient temperature by a sticker tape attached with an isolation tape. To avoid tail irritation, at the location of the thermistor insertion, a piece of micropore was added to the tape. On the day of the experiment, the basal skin temperature of each animal was determined by four measurements at 30-min intervals before any injection. Only animals displaying a basal skin temperature between 32.0 and 33.0°C were selected for the study (48). The heat loss index (HLI), used to evaluate thermoeffector responses of tail skin vasculature, was calculated according to the formula: HLI = (Tsk – Ta) / (Tc – Ta)\(^{-1}\), where Tsk is tail skin temperature, Ta is ambient temperature, and Tc is core body temperature. The values of HLI will vary from 0 to 1.0, representing states of maximum vasoconstriction to maximum vasodilatation, respectively (48).

**Rat knee joint incapacitation test.** The rat knee joint incapacitation test is described in detail elsewhere (55). In this test, a computer-assisted device measures the length of time that a specific hind paw fails to touch the surface of a rotating cylinder in a 1-min period (paw elevation time). Normally, animal paw elevation time is ~10–15 s. In previous experiments, incapacitation was studied in animals injected with zymosan (1 mg/animal) (47, 58) into the knee joints, and in the present study, we have modified this dose to 4 mg/cavity. The period for which the hind paw failed to touch the rotating cylinder was interpreted as being proportional to the pain felt by the animal. Paw elevation time was measured before zymosan administration (control time) and thereafter every hour for 8 h.

**Measurement of knee joint swelling.** Knee joint swelling was evaluated by measurement of the transverse diameters of each knee joint by a digital caliper (Digimatic Caliper, Mitsutoyo Corporation, Japan) and measured before zymosan administration (control time) and subsequently every hour for 8 h. Knee joint swelling is expressed in millimeters.

**Assessment of vascular permeability.** The method of Evans blue extravasation, as described by Lam and Ferrell (33), was used to assess plasma protein extravasation in the rat knee joint. It relies on the fact that Evans blue has high binding affinity to plasma proteins. Normally, the large plasma proteins and the bound Evans blue dye cannot pass through the endothelial gaps, and they are, therefore, restricted to the vascular compartment. However, when the endothelial gaps are enlarged, the plasma protein-Evans blue dye complex can escape to the interstitial tissues. Thus, measurement of the amount of Evans blue dye in the synovial capsule can provide an index of the relative vascular permeability.

Evans blue (25 mg/kg) was administered by endovenous injection 1 h before death. After 8 h of the saline (50 μl) or zymosan (4 mg/cavity) injection, the animals were killed, and the anterior and posterior synovial capsules and fat pad were dissected from each knee joint. The tissues obtained from each knee joint were then weighed, and the amount of Evans blue in the samples was estimated using a dye extraction technique. This meant that the tissue extracted was mixed with formamide (2 ml) in glass tubes and incubated at 40°C overnight. Each tube was centrifuged for 10 min at 2000 rpm, and 200 μl of the supernatant was separated for measurement of absorbance at 620 nm using a microplate reader (BioTek Instruments, Winooski, VT). The amount of dye recovered was calculated by comparing the absorbance of the fluid with that of a standard curve prepared with known concentrations of Evans blue solution.

**Excision of the sciatic nerve.** Under deep anesthesia induced by intraperitoneal injection of xylazine (20 mg/kg) and ketamine (58 mg/kg), the right sciatic nerve was exposed and then an ~0.5 cm-long nerve segment distal was excised. Sham animals, whose sciatic nerves were exposed but left intact, served as controls. After surgery, the animals were treated with oxytetracycline hydrochloride (400 mg/kg im). One week after the operation, rats received a single intra-articular injection of 4 mg of zymosan in the right knee, and then the rectal temperature was measured as described above.

**CSF sampling and determination of PG levels in the cisternal CSF and plasma.** A single sample of CSF was collected from each animal according to the method described by Consiglio and Lucion (15). Briefly, just before the CSF collection, each rat was anesthetized as described before and fixed to the stereotaxic apparatus, with its body flexed downward. The top of the head was trichotomized (to facilitate
the visualization of the area) and moistened with a cotton swab soaked in ethanol to reveal a small depression between the occipital protuberance and the atlas. A scalp (25-gauge) connected to a 1-ml syringe was then inserted vertically and centrally through this depression into the cisterna magna, and a gentle aspiration caused the CSF to flow through it, resulting in 50- to 100-μl samples. Frequently, it is not necessary to aspirate with the syringe, since the way the head is positioned exerts enough pressure to let the fluid flow spontaneously. Gentle movements of the needle are necessary during collection to prevent bleeding. Then the CSF samples were placed in Eppendorf tubes containing 2 μl of indomethacin (2.5 μg/μl) to stop PG production. Samples were maintained in the dark under ice until centrifugation at 1,300 g for 15 min at 4°C, and then they were immediately frozen to −20°C until analysis. When contaminated with blood, the samples were discarded. Immediately after CSF collection, the blood samples were collected by cardiac puncture directly into a tube containing heparin. The blood samples were centrifuged immediately at 2,000 g for 15 min at 4°C, and the resulting plasma was then stored at −20°C.

PGE2 was measured using the PGE2 Parameter Assay Kit (R&D Systems, Minneapolis, MN), which has a detection limit of 7.8 pg/ml. Cross-reactivity data were as follows: 17.5% with PGE1, 11.9% with PGE2, 7% with PGF1α, 6% with PGF2α, 2.5% with 6-Keto-PGF1α, and less than 0.1% with all other prostanoids tested. Intra- and inter-assay coefficients of variation were <11%. All samples were assayed according to the manufacturer’s instructions.

Experimental design. In the first set of experiments, we established the dose of zymosan able to induce an increase in the body temperature of rats. To do so, after being gently immobilized, rats received into their right knee joints injections of either zymosan at doses of 0.5, 1.0, 2.0, and 4.0 mg or sterile saline in a volume of 50 μl. Zymosan was suspended in sterile saline and the intra-articular injections were made aseptically. Zymosan was always injected between 10:00 and 11:00 AM to avoid circadian rhythm variations. After selecting the dose that promotes the highest increase in body temperature, 4 mg, we investigated whether the increase in body temperature constitutes a thermoregulatory response, namely, fever, i.e., an increase of the body temperature and decrease of the tail skin temperature.

Other experiments were performed to validate this arthritis model in the fever study. First, to examine the extent of febrile response induced by 4 mg of intra-articular injected zymosan, the Tc of animals was monitored night and day for up to 72 h by using a battery-operated radiotelemetry transmitter. Second, to investigate other symptoms that follow the arthritis development, both hyperalgesia (evaluated by rat knee joint incapacitation test) and edema/joint swelling (evaluated by measurement of knee joint swelling and vascular permeability) were measured. Third, we evaluated the possible involvement of the sciatic nerve as a neural pathway in the febrile response induced by intra-articular injection of zymosan. For that, the sciatic nerve was surgically removed (see Excision of sciatic nerve).

In another set of experiments, the effects of selective and nonselective COX inhibitors, steroidal anti-inflammatory drugs and the antipyretic drugs, dipyrone and paracetamol, were investigated. Thus, the animals were preadministered by mouth ibuprofen (5, 10, and 20 mg/kg), celecoxib (1, 5, and 10 mg/kg), dipyrone (60, 120, and 240 mg/kg), or paracetamol (100, 150, and 200 mg/kg) in a volume of 1 ml, 30 min prior to the intra-articular injection of zymosan. Oral administration was made by gavage, through a polyethylene cannula (PE-50, 4 cm length) connected to a needle (22 gauge) placed in a 1-ml plastic syringe. To ensure that the appropriate dose was administered or that the drug was correctly administered, the volume and the reactions of animals after drug administration were cautiously observed. Animals that presented cough or respiratory anguish (when the drug solution reaches the respiratory tract) were euthanized, and animals that regurgitated were excluded from the study. Dexamethasone (0.25, 0.5, and 1 mg/kg) in a volume of 0.2 ml was injected subcutaneously 1 h prior to the intra-articular injection of the stimulus. Control animals were pretreated equally with the same volume of the appropriate vehicles (pyrogen-free saline: celecoxib, ibuprofen, dipyrone, and dexamethasone; paracetamol: saline/ethanol/Tween 80, 9:1:0.05) and received only saline in their knee joint. The doses of all drugs employed in this study were based on previous studies in our laboratory (17, 36, 39, 54).

In the final set of the experiments, we analyzed first the presence of PGE2 in both cisternal CSF and plasma samples at two different time points (3 1/2 and 5 h). Finally, the effect of celecoxib (5 mg/kg po), paracetamol (150 mg/kg po), and dipyrone (120 mg/kg po) on the increase of CSF PGE2 levels induced by intra-articular zymosan administration was investigated as well. In accordance with the fever development and the alteration caused by these drugs, we chose the 5-h time point to measure the CSF PGE2 levels.

Drugs. The following reagents were used: Zymosan A and ibuprofen were purchased from Sigma-Aldrich (St. Louis, MO). Celecoxib (Celebro) came from Pharmacia (São Paulo, Brazil), dipyrone (so- metamizol, Aventis Pharma Deutschland, Berlin, Germany) and dexamethasone (Decadral) were obtained from Aché (São Paulo, Brazil). Paracetamol came from Sigma-Aldrich, ketamine (Ketamina Agener) was obtained from União Química Farmacêutica Nacional S.A. (São Paulo, Brazil), xylazine (Dopaser) was obtained from Calier Laboratories S.A., (Barcelona, Spain), oxytetracycline hydrochloride (Terramicina) was obtained from Pfizer (São Paulo, Brazil), and Evans Blue was obtained from Merck (Frankfurtur, Germany).

Statistical analyses. For data analysis, the baseline temperature prior to any injection was determined for each animal, and all variations in body, rectal, or tail skin temperature were expressed as changes from the mean basal value (i.e., as ΔT, in°C). All results are presented as means ± SE, and mean baseline temperatures were not statistically different among the groups included in any particular set of experiments. The levels of PGs, hyperalgesia, knee joint swelling, and vascular permeability were analyzed by one-way ANOVA followed by Tukey’s test. ΔT and HLI responses were compared across treatments and time points, which were analyzed by two-way ANOVA for repeated measures followed by the Bonferroni test. All data were analyzed using Prism computer software (GraphPad Prism, San Diego, CA). Differences were considered significant when P < 0.05.

RESULTS

Febrile response during zymosan-induced arthritis in rats. As shown in Fig. 1A, the intra-articular injection (into the right knee joint) of the minor dose of zymosan (0.5 mg) did not significantly affect the rectal temperature compared with saline-stimulated animals. At doses of 1, 2 and 4 mg, zymosan elicited a significant rectal temperature elevation over baseline values up to 8 h after administration. At a dose of 2 mg, zymosan promoted a significant increase in rectal temperature, which peaked at 5 1/2 h and declined thereafter, while at 1 mg, zymosan promoted a flattened increase in rectal temperature that was not statistically different from that promoted by 2 mg. However, the intra-articular injection of 4 mg zymosan elicited a markedly long-lasting rectal temperature elevation that started at 3 1/2 h, peaked at the 5 1/2 h, and remained until the end of experimental period, 8 h. For this reason, this dose was selected for further experiments.

In another set of experiments, animals were given an intra-articular injection of zymosan (4 mg) or of saline, and rectal and skin (HLI) temperatures were monitored during 6 h. The increase in rectal temperature induced by 4 mg/animal of zymosan was preceded by a significant decrease in tail skin

AJP-Regul Integr Comp Physiol • VOL 296 • MAY 2009 • www.ajpregu.org
temperature from 2 to 4 h, indicating vasoconstriction of the local vascular bed. On the other hand, saline injected in the animal knee joints did not alter rectal or tail skin temperature (Fig. 1, B and C).

The body temperature was also monitored for up to 72 h, with the aim of investigating whether the intra-articular injection of 4 mg of zymosan would be able to induce fever for more than 1 day or to alter the night circadian rhythm (increased body temperature during the hours of darkness) (Fig. 2, A–C). In this set of experiments, we could observe that the fever remained for up to 10 h after intra-articular injection of zymosan. However, in our experimental conditions, during the darkness period, the body temperature increased in the zymosan-injected animals as with the saline (control) -injected animals (Fig. 2, A–C). Moreover, no febrile response was observed in the light period of the second and third days (Fig. 2, B and C).

Additionally, to validate the zymosan administration as an arthritis inductor, two typical symptoms were also investigated by using joint incapacitation test (hyperalgesia), and knee joint swelling and vascular permeability tests (edema). Herein, we carried out the time course curve of hyperalgesia and knee joint swelling in the same experimental conditions used in the temperature measurement, and it was observed that the intra-articular injection of zymosan elicited a marked elevation of pain (evaluated by paw elevation) and knee joint swelling (by measurement of the transverse diameters of each knee joint), which started at 2 h, peaked at 3 h, and remained until the end of experimental period, 8 h (Fig. 3, A and B, respectively). Moreover, at the end of experiment (8 h), we also observed an increase in the knee vascular permeability (Fig. 3C).

In the present study, it was observed that the time course curve of febrile response was not altered when comparing sham animals to those that had their sciatic nerve removed (Fig. 4). The excision of sciatic nerve or sham surgery does not alter the basal temperature of rats or the febrile response induced by intra-articularly injected zymosan.

**Effect of anti-inflammatory (ibuprofen, celecoxib, and dexamethasone) and antipyretic (paracetamol, dipyrone) drugs on febrile response during zymosan-induced arthritis.** In the present study, under our experimental conditions, we observed that the febrile response during zymosan-induced arthritis was dose dependently inhibited by pretreating the rats with drugs that interfere with arachidonate metabolism, such as ibuprofen (a nonselective COX inhibitor; 5, 10, and 20 mg/kg), celecoxib (a selective COX-2 inhibitor; 1, 5, and 10 mg/kg), and dexamethasone (a suppressor of phospholipase A2 activity and cytokines synthesis; 0.25, 0.5, and 1 mg/kg) (Fig. 5, A–C). With the exception of a 1.0 mg/kg dose of celecoxib, all the different drugs and their respective doses reduced or inhibited the zymosan-induced fever. Thus, ibuprofen at doses of 10 and 20 mg/kg reduced the fever from 4 to 6 1/2 h; celecoxib at 5 mg/kg markedly reduced the fever from 4 h to 7 h, while at 10 mg/kg celecoxib fully abolished from 3 1/2 h to 8 h the fever after intra-articular administration of zymosan. Dexamethasone at 0.25 mg/kg reduced the fever from 4 h to 7 h, while at 0.5 and 1 mg/kg, it fully abolished the fever with intra-articular injection of zymosan.

In addition, it was observed that the febrile response during zymosan-induced arthritis was also dose-dependently reduced by pretreating the animals with dipyrone (60, 120, and 240 mg/kg) and paracetamol (100, 150, and 200 mg/kg), two
known classic antipyretic drugs (Fig. 6, A and B, respectively). At the highest dose, 240 mg/kg, dipyrone blocked the febrile response, while at 120 mg/kg, it extensively reduced the fever from 4 h to 7 h and, at 60 mg/kg, it promoted a feeble effect on the fever. It is worth noting that the effects of dipyrone were more durable and effective than that of paracetamol whose effect, at the highest dose, 200 mg/kg, was significant only from 3 to 5 h. Control animals treated with the vehicles (for paracetamol) or saline alone showed no significant variations in rectal temperature over baseline values for up to 8 h after zymosan administration. The pretreatment with all investigated drugs at their respective dose did not modify the basal temperatures of rats (data not shown).

Investigation of CSF and plasma PGE₂ levels and effect of celecoxib, dipyrone and paracetamol on CSF PGE₂ levels in the zymosan-induced arthritis. To investigate the participation of PGE₂ in the fever response in arthritic animals, both cisternal CSF and blood samples were collected 3 1/2 h and 5 h after intra-articular injection of zymosan (4 mg) or sterile saline into the right knee. Herein, it was observed that zymosan administration significantly increased the CSF PGE₂ levels at 5 h but not at 3 1/2 h (Fig. 7A) when compared with the control group. In addition, we observed no increase in the plasma PGE₂ levels (Fig. 7B) at both time points studied.

Finally, the cisternal CSF of the animals was collected 5 h after intra-articular injection of zymosan (4 mg) or sterile saline into the right knee in rats orally pretreated with celecoxib, dipyrone, and paracetamol. This time point was selected with the intention of correlating the antipyretic effect of the assayed drugs with the PGE₂ level in the CSF, due to the fact that, as shown above, celecoxib, dipyrone and paracetamol showed different profiles of fever reduction (Fig. 5B, 6, A and B, respectively), which indicates that celecoxib at 5 mg/kg and dipyrone at 120 mg/kg still kept their robust antipyretic effect, while the antipyretic effect of paracetamol at 150 mg/kg was more different from the control. Zymosan administration induced a significant increase in PGE₂ level in the CSF at 5 h in arthritic rats compared with control animals (Fig. 8A). Celecoxib (5 mg/kg po) brought the CSF PGE₂ level near to control values while dipyrone reduced 62.0% and paracetamol 37.8% the PGE₂ concentration in the CSF at the time point studied (5 h), showing a marked relationship between rectal temperature elevation (Fig. 8B) and the amount of this prostanoid in the CSF (Fig. 8A).

DISCUSSION

The current study showed that intra-articular injection of zymosan induced a characteristic joint inflammation recognized by pain and edema, which was accompanied by a long-lasting increase in body temperature that was characterized as an integrated febrile response since the increase in rectal temperature was preceded by a decrease in the tail skin temperature, an important fever-associated physiological response (25). This febrile response, observed only in the first day, was independent of the neural pathways (evaluated by sciatic nerve excision), associated by an increase of central PGE₂ concentration and sensitive for different antipyretic drugs.

Zymosan, an insoluble polysaccharide from the cell wall of yeast (Saccharomyces cerevisiae), has the property to activate the innate immune system cells and, subsequently, promote local inflammatory response and sickness behavior. In the knee joint, zymosan is able to simulate an inflammatory response like that observed in the arthritis development, in which both resident synovial and infiltrated immune cells produce several mediators of inflammation, including nitrogen and oxygen-derived free radicals, cytokines, leukotrienes, and prostaglandins, which have been associated with the degradation of articular cartilage (4, 46, 47). Frasnelli et al. (19) demonstrated that Toll-like receptor (TLR) 2 is the major pathway of proinflammatory signaling in zymosan-induced arthritis in mice. Furthermore, this stimulus has been used in the investigation of other aspects of arthritis-associated inflammatory response, including loss of mobility, articular hyperalgesia, edema, and fever (20, 21, 46, 47, 52).

In previous study, Gegout et al. (20) observed that intra-articular injection of zymosan increased the body temperature. The present study showed that this effect was, in fact, characterized as an integrated febrile response since the increase in rectal temperature was preceded by a decrease in the tail skin temperature. The reduction in tail skin temperature was reflected by the reduction of heat loss index (HLI; 48), indicating vasoconstriction of the local (tail) vascular bed with the intention of retaining heat, in response to the increased hypothalamic set point (44). The rat’s tail serves as a variable heat exchanger (25, 44), since its blood flow is regulated by the activity of sympathetic vasconstrictor nerves specifically controlled by neurons of the raphé magnus/pallidum nucleus (6),...
which takes place in the pyrogenic efferent pathway (16, 42, 43). Thus, it seems that mediators released from the inflamed joint reach the bloodstream and directly or indirectly (for instance, inducing the synthesis of PGs) stimulate the thermo-regulatory center (51), evoking a concerted thermoregulatory response, in which the reduced efficacy of the vasculature of the tail skin to dissipate body heat corroborates with fever development (9).

The absence of fever in the following days after arthritis installation may be explained by the fact that proinflammatory cytokines are produced mainly by synovial cells in joint tissues only at the beginning of inflammatory response to zymosan (46) and by the complex generation of endogenous antipyretic compounds in the brain (e.g., α-melanocyte stimulating hor-

Recently, evidence has suggested that peripheral afferent nerves, especially those contained in the vagus nerve, play an important signaling role between the inflammatory site and the brain. In this regard, subdiaphragmatic vagotomy blocks fever induced by intraperitoneal injection of either LPS or IL-1β (59, 60). It has been shown that sensory innervations by the sciatic nerve can influence pain and edema in inflammatory responses (13, 22). Thus, rats whose sciatic nerve has been excised before zymosan injection may be used to investigate whether the sensory nerves are involved in febrile response in zymosan-induced arthritis. Therefore, in the current study, we observed that the resection of sciatic nerve did not show significant temperature changes in the time course curve after zymosan administration, suggesting that neural pathways are not involved in the febrile response in zymosan-induced arthritis in rats. An additional possibility is that the intensity of the stimulus is high, so that the mediators locally generated can spill over into the systemic circulation, inducing fever by humoral pathway. It seems true for IL-6 (but not of IL-1 or TNF-α) in vagotomized rats and guinea pigs receiving a high dose of LPS (into the air pouch or subcutaneously implanted plastic chamber) (12, 40; for a review, see Ref. 51).

Gegout et al. (21) observed that an intra-articular injection of zymosan increased the body temperature that was reduced by indomethacin, a nonselective COX inhibitor. Supporting these findings (21) and their implication of COX-2 in the production

\[
\text{Fig. 3. Evaluation of hyperalgesia (by using joint incapacitation test) (A) and edema (by knee joint swelling and vascular permeability tests) (B and C, respectively) in zymosan-induced arthritis in rats. Zymosan (4 mg) or sterile saline was injected intra-articularly in a volume of 50 µl. *P < 0.05 compared with the saline group.}
\]

\[
\text{Fig. 4. Evaluation of resection of the sciatic nerve on febrile response in zymosan-induced arthritis in rats. Zymosan (4 mg) or sterile saline was injected intra-articularly in a volume of 50 µl. Values represent the means \pm SE of variation in rectal temperature (ΔT, °C) of each group. Rectal temperature was measured by telethermometry. Basal temperatures (mean \pm SE, °C) were the following: ○, 37.02 ± 0.12; □, 37.02 ± 0.02; ■, 37.00 ± 0.12; ●, 37.07 ± 0.07. *P < 0.05 compared with the saline group (sham operated). +P < 0.05 compared with the saline group (sciatic nerve removed).}
\]
of pyrogenic prostaglandins (34), we observed that the selective COX-2 inhibitor celecoxib, as well as ibuprofen, a non-selective COX-inhibitor, dose-dependently reduced the febrile response during zymosan-induced arthritis. Furthermore, celecoxib also inhibited the increase of the CSF PGE2 concentration in the CSF after zymosan intra-articular administration. These effects have been observed by our group after peripheral or central administration of exogenous (LPS) or endogenous pyrogenic substances (endothelin-1 and chemokines) (17, 36, 39). It is important to mention that zymosan-induced arthritis does not increase the plasma concentration of PGE2, suggesting that central, but not peripheral, generated PGE2 is involved in the febrile response during this inflammatory condition.

Also, in accordance with Gegout’s findings (21), dexamethasone reduced the fever induced by intra-articular injection of zymosan. This effect could be related with an indirect effect via anexin-1 on phospholipase A2 or to a direct effect on

Fig. 5. Effect of ibuprofen (A), celecoxib (B), or dexamethasone (C) on febrile response in zymosan-induced arthritis in rats. Ibuprofen (5, 10, and 20 mg/kg po), celecoxib (1, 5, and 10 mg/kg po), dexamethasone (0.25, 0.5, and 1 mg/kg sc), or vehicle was administered 30 min (ibuprofen, celecoxib) or 1 h (dexamethasone) prior to zymosan (4 mg) or sterile saline injected intra-articularly. Rectal temperature was evaluated by telethermometry. Values represent the means ± SE of the variation in rectal temperature (ΔT, °C) of each group. Basal temperatures (mean ± SE, °C) were the following: ■, 36.93 ± 0.10; ○, 36.96 ± 0.04; □, 36.97 ± 0.03; △, 36.9 ± 0.20; ■, 37.05 ± 0.10 (A); ■, 36.99 ± 0.07; □, 37.00 ± 0.03; ○, 37.02 ± 0.04; △, 37.02 ± 0.04; ■, 37.02 ± 0.08 (B); ■, 36.98 ± 0.02; □, 37.02 ± 0.05; ○, 36.96 ± 0.05; △, 37.02 ± 0.08; ■, 37.18 ± 0.12 (C). *P < 0.05 compared with the saline group. +P < 0.05 compared with the zymosan group.

Fig. 6. Effect of dipyrone (A) and paracetamol (B) on febrile response during zymosan-induced arthritis in rats. Dipyrone (60, 120, and 240 mg/kg po), paracetamol (100, 150, and 200 mg/kg po), or vehicle was administered 30 min prior to zymosan (4 mg) or sterile saline injected intra-articularly. Rectal temperature was evaluated by telethermometry. Values represent the means ± SE of the variation in rectal temperature (ΔT, °C) of each group. Basal temperatures (means ± SE, °C) were the following: ■, 36.93 ± 0.10; ○, 36.96 ± 0.07; □, 37.02 ± 0.07; △, 36.87 ± 0.07; ■, 37.00 ± 0.14 (A); ■, 36.97 ± 0.05; □, 36.87 ± 0.03; ○, 36.85 ± 0.02; △, 37.0 ± 0.05; ■, 36.83 ± 0.03 (B). *P < 0.05 compared with the saline group. +P < 0.05 compared with the zymosan group.
immune and synovial cells by inhibiting the synthesis/release of inflammatory/pyrogenic mediators and COX-2 expression, a mechanism related to the inhibition of NF-κB transmigration to the nucleus, one of the most important anti-inflammatory and immunosuppressive effects of glucocorticoids (2; for a review, see Ref. 50).

Finally, the present study demonstrated that dipyrone and paracetamol, two classic antipyretic and analgesic, but not anti-inflammatory drugs, showed different profiles in the antipyretic and in their abilities to reduce the PGE2 concentration in the CSF of zymosan-stimulated rats. At the dose selected, 120 mg/kg, dipyrone was more effective in reducing the fever and maintained this effect for hours while, paracetamol showed a short-time effect and, at the time studied (5 h after zymosan administration), we observed a weak antipyretic effect, which is in line with the minor reduction in the PGE2 concentration in the CSF. The reason to investigate the effect of dipyrone and paracetamol on fever induced by zymosan-induced arthritis comes from the fact that these drugs are clinically used in humans to treat pain and fever coming from an inflammatory chronic process, including rheumatoid arthritis (32). An important characteristic of paracetamol that deserves to be mentioned here is that this agent does not affect the renal, cardiovascular, and gastrointestinal systems, which allow the clinical use of this agent as an analgesic in arthritic patients (3).

Perspectives and Significance

Taken together, the present results showed that only the initial phase (first 10 hours) of the zymosan-induced arthritis is accompanied by a febrile response, which is sensitive to

![Fig. 7. Determination of PGE2 levels in cerebrospinal fluid (CSF) (A) and plasma (B) and rectal temperatures (C) in zymosan-induced arthritis in rats. Zymosan (4 mg; ZYM) or sterile saline (SAL) was injected intra-articularly in a volume of 50 μl. The CSF was harvested from cisterna magna of the rats, 3 1/2 and 5 h after intra-articular zymosan injection. PGE2 levels were determined by ELISA. Rectal temperature was evaluated by telethermometry, 3 1/2 and 5 h after intra-articular zymosan injection. Values represent means ± SE of the concentration (pg/ml) of PGE2 in the CSF and plasma and the variation in rectal temperature (ΔT, °C) of 5–8 animals. Basal temperatures (means ± SE, °C) were the following: SAL (3 1/2 h), 37.03 ± 0.06; ZYM (3 1/2 h), 37.05 ± 0.04; SAL (5-h), 37.08 ± 0.08; ZYM (5-h), 36.99 ± 0.06. *P < 0.05 compared with the corresponding saline group.](http://ajpregu.physiology.org/)

![Fig. 8. Effect of celecoxib, paracetamol, and dipyrone on changes in CSF PGE2 levels (A) and rectal temperatures (B) in zymosan-induced arthritis in rats. Celecoxib (CELE; 5 mg/kg po), paracetamol (PARA; 150 mg/kg po), dipyrone (DIP; 120 mg/kg po), or saline (SAL; control) was administered 30 min prior to zymosan (4 mg ia) or sterile saline (control) injection, as previously described. The CSF was harvested from cisterna magna of the rats, 5 h after intra-articular zymosan injection. PGE2 levels were determined by ELISA. Rectal temperature was evaluated by telethermometry, 5 h after intra-articular zymosan injection. Values represent the means ± SE of the concentration (pg/ml) of PGE2 in the CSF and the variation in rectal temperature (ΔT, °C) of 5–8 animals. Basal temperatures (means ± SE, °C) were the following: SAL, 36.99 ± 0.08; ZYM, 37.12 ± 0.07; ZYM plus CELE, 36.97 ± 0.05; ZYM plus DIP, 37.00 ± 0.06; ZYM plus PARA, 37.03 ± 0.06. *P < 0.05 compared with the SAL/zymosan group.](http://ajpregu.physiology.org/)
anti-inflammatory and antipyretic drugs able to inhibit the PGE2 synthesis. Because fever is known to increase the activity of immune cells by increasing phagocytosis and antibody formation (31, 45, 61), which can worsen the inflammatory status of immunologically induced arthritis, the control of fever by antipyretic/anti-inflammatory drugs certainly contributes to the reduction of knee joint lesion and pain. Moreover, the clinical use of paracetamol to treat pain and fever of patients with concurrent blood coagulation failure could be useful to prevent NSAIDs side effects such as platelet activity inhibition and bleeding. Further studies are now necessary to verify the effects of the drugs studied here on cytokines, chemokines, and C5a in the synovial fluid and blood, as well as cytokines, chemokines, and PGE2 in the hypothalamic tissue, aiming to better understand the real mechanism by which these drugs alter the course of inflammatory process and fever.

GRANTS
This study was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grants 05/59211-4 and 06/04917-9) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant 305802/2004-6), Brazil.

REFERENCES


46. Pettipher ER, Salter ED. Resident joint tissues, rather than infiltrating neutrophils and monocytes, are the predominant sources of TNF-α in zymosan-induced arthritis. *Cytokine* 8: 130–133, 1996.


