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Antipyretic and antioxidant activities of 5-trifluoromethyl-4,5-dihydro-1H-pyrazoles in rats

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Abstract

The objective of this study was to determine the effect of eight 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles (TFDPs) on rat body temperature and baker's yeast-induced fever. TFDPs or vehicle (5% Tween 80 in 0.9% NaCl, 5 mL/kg) were injected subcutaneously and rectal temperature was measured as a function of time in 28-day-old male Wistar rats (N = 5-12 per group). Antipyretic activity was determined in feverish animals injected with baker's yeast (*Saccharomyces cerevisiae* suspension, 0.135 mg/kg, 10 mL/kg, *ip*). 3-Ethyl- and 3-propyl-TFDP (140 and 200 µmol/kg, respectively, 4 h after yeast injection) attenuated baker's yeast-induced fever by 61 and 82%, respectively. These two effective antipyretics were selected for subsequent analysis of putative mechanisms of action. We then determined the effects on cyclooxygenase-1 and -2 (COX-1 and COX-2) activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) oxidation *in vitro*, on tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels and on leukocyte counts in the washes of peritoneal cavities of rats injected with baker's yeast. While 3-ethyl- and 3-propyl-TFDP did not reduce baker's yeast-induced increases of IL-1β or TNF-α levels, 3-ethyl-TFDP caused a 42% reduction in peritoneal leukocyte count. 3-Ethyl- and 3-propyl-TFDP did not alter COX-1 or COX-2 activities *in vitro*, but presented antioxidant activity in the DPPH assay with an IC_{50} of 39 mM (25-62) and 163 mM (136-196), respectively. The data indicate that mechanisms of action of these two novel antipyretic pyrazole derivatives do not involve the classic inhibition of the COX pathway or pyrogenic cytokine release.

Key words: Pyrazole derivatives; Fever; Cytokine levels; Cyclooxygenase

Introduction

Fever is a regulated increase of body core temperature characterized by an increase thermoregulatory set point, which results from the interaction of the central nervous and immune systems (1). While fever is a hallmark of injury, infection and inflammation, it has also been considered to be the most important component of a complex host response to invading agents, called acute-phase response (2). Although there is evidence supporting the idea that fever enhances host defenses, some studies have suggested that raising core temperature to the febrile range may be harmful. Therefore, in clinical situations in which fever-associated risks outweigh benefits, antipyretic treatment is indicated (3).

Pyrazoles constitute an important group of organic compounds that have been extensively studied due to their numer-

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ous biological activities. Accordingly, dipyrone (also known as metamizole) is a potent antipyretic and analgesic pyrazole derivative, with little anti-inflammatory activity (4), that is used in several countries. Nevertheless, several adverse effects, including agranulocytosis, have been associated with its use (5). Therefore, the development of novel compounds having antipyretic and anti-inflammatory activities with improved safety profiles remains a clinical need.

A number of studies have reported the synthesis of novel pyrazole derivatives with analgesic (6-10) and antipyretic (11,12) activities. We have explored the hypothesis that benzene, which is present in salicylamide, could be mimicked with an appropriate 3- or 4-substituted 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles (TFDP) scaffold. In addition, the presence of a trifluoromethyl group within cyclic compounds, especially at a strategic position, has become an important aspect to be studied in pharmaceutical research due to the unique physical and biological properties of fluorine. For instance, the substitution of a methyl group with a trifluoromethyl group has resulted in added lipophilicity, facilitating absorption of molecules within biological systems and thereby improving the overall pharmacokinetic properties of drug candidates (13).

The compounds 3Et-TFDP and 4Me-TFDP belong to a series of recently synthesized pyrazole derivatives that have been screened for antinociceptive and antiedematogenic activity in mice. These compounds cause antinociception in the formalin test and decrease carrageenin-induced edema (14). Moreover, 3Et-TFDP and 4Me-TFDP produce antinociception in the complete Freund's adjuvant animal model of arthritis (15). Importantly, in these studies, the antinociceptive effect occurred in the absence of significant adverse effects, indicating that these compounds may be models for the development of new drugs with analgesic and anti-inflammatory properties. The potential antipyretic activities of 3Et-TFDP and 4Me-TFDP, however, have not been evaluated.

In the present study, we investigated whether a series of eight TFDPs, including 3Et-TFDP and 4Me-TFDP, attenuate baker's yeast-induced fever. Furthermore, in order to explore the possible mechanisms underlying the antipyretic action of these pyrazole derivatives, we determined whether these compounds alter the increase of baker's yeast-induced cytokines (tumor necrosis factor-α, TNF-α, and interleukin-1β, IL-1β) in the peritoneal fluid, cyclooxygenase-1 and -2 (COX-1 and COX-2) activities or 1,1-diphenyl-2-picrylhydrazyl (DPPH) oxidation *in vitro*.

Material and Methods

Chemicals

TFDPs (Figure 1) were synthesized by the Núcleo de Química de Heterociclos, as reported elsewhere (14), and were suspended in a 5% Tween 80 solution in 0.9% NaCl.

Commercially available dried baker yeast (*Saccharomyces cerevisae*; Saf do Brasil Produtos Alimentícios Ltda., Brazil) was suspended in pyrogen-free 0.9% NaCl in a water bath at 37°C for 5 min. Dipyrone (Hoechst, Brazil) was diluted in 0.9% NaCl. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems Inc. (USA) and the COX (ovine) Inhibitor Screening Assay Kit was obtained from Cayman Chemical Co. (USA). Other reagents were supplied by Sigma-Aldrich Chemical Co. (USA).

Animals

Male Wistar rats (28-30 days of age, 70-90 g), bred in our animal house were used. The animals were housed in groups of 8 to a cage at a controlled temperature (23 ± 1°C) with a 12-h light/dark cycle (lights on at 7:00 am) and with standard lab chow and tap water available *ad libitum*.

The animals were transferred to the experimental room 1 day before the experiments for acclimation to the environment. All temperature measurements were made between 8:00 am and 5:00 pm and room temperature was maintained at 23 ± 1°C. Each animal was used only once, and no more than 1 animal per litter was assigned to each group. Animals that presented initial (basal) body temperature below 36.4° or above 37.3°C were excluded from the experiments. The experiments were approved by the Committee on the Use and Care of Laboratory Animals of Universidade Federal de Santa Maria (protocol #23081.013228/2008-78).

Rectal temperature measurement

Rectal temperature (T_R) was measured using a lubricated thermocouple (external diameter: 2 mm) inserted 2.0 cm into the rectum of the animal for 1 min. The probe was linked to a digital device, which displayed the temperature at the tip of the probe with ±0.1°C precision and the values were recorded manually. In order to minimize the effects of stress associated with handling and injections on rectal temperature, all rats were habituated to the measuring procedure for two consecutive days. In these sessions, the animals were subjected to the same temperature measuring procedure described above.

The effect of TFDPs on the basal T_R of rats was evaluated. A dose-response curve was constructed for each com-

pound and the maximal dose that caused no effect on T_R was determined for testing in a subsequent set of experiments to determine whether each compound could attenuate baker yeast-induced fever. After the basal (the first temperature measure of the day) T_R measurement, the animals were injected subcutaneously (*sc*) with vehicle (5% Tween 80 in 0.9% NaCl, 5 mL/kg) or pyrazole derivatives: 3H-TFDP, 3Me-TFDP, 3Et-TFDP, 3Pr-TFDP, 3iPr-TFDP, 3Bu-TFDP, 3Ph-TFDP, or 4Me-TFDP at 60, 200, or 600 µmol/kg. T_R was recorded every hour up to 5 h, and are reported as the difference from the basal value.

Fever induction

The animals had their basal T_R measured and those with T_R between 36 and 36.8°C were injected with a pyrogenic dose of baker's yeast (0.135 g/kg, 10 mL/kg*, ip*), as reported elsewhere (11). T_R was recorded every hour, up to 4 h, when vehicle (5% Tween 80 in 0.9% NaCl, 5 mL/kg), 3H-TFDP, 3Me-TFDP, 3Et-TFDP, 3Pr-TFDP, 3iPr-TFDP, 3Bu-TFDP, 3Ph-TFDP, 4Me-TFDP (60, 200, or 600 µmol/kg) or dipyrone (140 µmol/kg, in a subset of animals) was administered. T_R was then monitored over the following 5 h. When the highest dose that had no effect on T_R did not attenuate yeast-induced fever, the next dose tested was two-thirds higher. To determine the most promising compounds for subsequent testing, we calculated the antipyretic activity index of the TFDPs in baker's yeast-induced fever, according to the following equation:

$$\text{Antipyretic activity index} = 1 - \frac{\Delta \text{Texp} - \Delta \text{Tcontrol}}{\Delta \text{Tyeast} - \Delta \text{Tcontrol}} \times 100\%$$

where ΔTexp = mean T_R variation (5- and 6-h values) of animals treated with baker's yeast/TFDP; ΔTcontrol = mean T_R variation (5- and 6 h-values) of animals treated with saline/5% Tween 80 in 0.9% NaCl; ΔTyeast = mean T_R variation (5- and 6 h-values) of animals treated with baker's yeast/5% Tween 80 in 0.9% NaCl.

Cell count in peritoneal wash

A pyrogenic dose of baker's yeast (0.135 g/kg, *ip*) or vehicle (0.9% NaCl) was injected intraperitoneally (*ip*) and 3 h later the animals were injected with a TFDP, which had an antipyretic effect (140-200 µmol/kg, *sc*) or vehicle (5% Tween 80 in 0.9% NaCl, 5 mL/kg, *sc*). One hour later, the animals were sacrificed by decapitation under thiopental anesthesia. The abdominal skin below the sternum was nicked, and a peritoneal wash was performed by *ip* injection of 10 mL 20 mM Tris-HCl buffer containing 2 mM EGTA, 2 mM EDTA and 0.2 mM PMSF. The abdominal cavity was gently massaged for 1 min and 8 mL peritoneal wash was collected by aspiration with a 20-mL syringe. If blood was detected by visual inspection, the sample was discarded. A 300-µL aliquot was used for cell count in a Neubauer chamber (1:3 dilution in Türk solution) by light microscopy, and a leukocyte count was performed by a subject who was not aware of the animal's previous treatments. The samples were centrifuged at 1500 *g* for 10 min, and 6 mL of the supernatant was lyophilized and stored at -80°C for later cytokine analysis.

TNF-α and IL-1β assays

Lyophilized peritoneal fluid samples were suspended in 1000 µL 1% bovine albumin solution and the levels of TNF-α and IL-1β were determined using commercially available monoclonal immunoassay kits for each cytokine (R&D Systems Inc., USA). The value ranges detected by this assay were 100-6400 pg/mL (IL-1β) and 5-2000 pg/mL (TNF-α). The intra-assay coefficients of variation for IL-1β and TNF-α assays were 1.73 ± 0.39 and 4.8 ± 0.92%, respectively. All samples were analyzed at the same time in a single ELISA plate (one plate for each cytokine). Both cytokine concentrations were estimated by colorimetric measurement at 450 nm using an ELISA plate reader and interpolation from a standard curve. Data are reported as pg/mL.

Cyclooxygenase-1 and cyclooxygenase-2 activities *in vitro*

The COX screening assay kit (Kit No. 560101, Cayman) was used according to manufacturer instructions to determine whether 3Et-TFDP and 3Pr-TFDP altered COX-1 and COX-2 activities. COX-1 and COX-2 initial activity tubes were prepared by placing 950 µL reaction buffer, 10 µL heme, 10 µL COX-1 and COX-2 enzymes in the respective tubes. Similarly, COX-1 and COX-2 inhibitor tubes were prepared by adding 20 µL inhibitor (compound under test and celecoxib) to each tube in addition to the above ingredients. The background tubes corresponding to inactivated COX-1 and COX-2 enzymes were obtained by placing the tubes containing enzymes in boiling water for 3 min. Reactions were initiated by adding 10 µL arachidonic acid to each tube and were quenched with 50 µL 1 M HCl. Prostaglandin H_2 (PGH_2) thus formed was reduced to $PGF_2\alpha$ by the addition of 100 µL $SnCl_2$. The prostaglandin produced in each well was quantified

using broadly specific prostaglandin antiserum that binds to major prostaglandins and reading the 96-well plate at 405 nm. The wells of the 96-well plate showing low absorption at 405 nm indicate low levels of prostaglandins in these wells and hence, lesser enzyme activity. Therefore, the COX inhibitory activities of the compounds could be quantified from the absorption of 405 nm of different wells of the 96-well plate. The results are reported as percent inhibition of COX-1 and COX-2 enzymes.

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging activity assay

The DPPH• method is based on the scavenging of the stable DPPH• radical by the antioxidant. The TFDP scavenging activity against DPPH• was measured by the method of Brand-Williams et al. (16). Briefly, DPPH• (Sigma-Aldrich Chemical Co., USA) was dissolved in 100% ethanol at a final concentration of 0.3 mM, and 250 µL was added to a medium containing different concentrations of the ethanolic TFDP solution. Appropriate controls were used for the amounts of ethanol in the samples. The reaction mixture was shaken vigorously and incubated for 30 min at room temperature. The inhibition of absorbance decay, measured at 518 nm, indicated the scavenging activity of the pyrazoles against DPPH•. Ascorbic acid was used as a positive control. Data are reported as percent inhibition of DPPH• absorbance decay. Mean values were obtained from duplicates.

Statistical analysis

Basal rectal temperatures and changes in rectal temperatures are reported as means ± SEM. Data were analyzed by two- or three-way analysis of variance (ANOVA), with time treated as a within-subject factor depending on the experimental design. *Post hoc* analysis was conducted using the F-test for simple effects. A value of $P < 0.05$ was considered to be statistically significant.

Results

TFDPs reduce basal rectal temperatures

A dose-response curve (60, 200 or 600 µmol/kg, *sc*) was constructed for each compound, and the maximal dose that caused no effect on T_R was determined. The pyrazole derivatives tested reduced the basal T_R at high doses (200-600 µmol/kg), as indicated by the statistically significant ($P < 0.05$) dose x time interactions shown in Figure 2.

Some TFDP reduce baker's yeast-induced fever

Figure 3A–H shows the effects of TFDPs used at the maximal dose that caused no effect on T_R, on baker's yeast-induced fever. The compounds 3Et-TFDP, 3Pr-TFDP, 3Ph-TFDP, and 4Me-TFDP exhibited significant antipyretic activity, $P < 0.05$.

Figure 4 shows the antipyretic activity of the pyrazole derivatives 3Et-TFDP and 3Pr-TFDP compared to the antipyretic effect of dipyrone (140 µmol/kg). Statistical analysis (ANOVA with repeated measures) of T_R change with time revealed a significant "treatment with time" interaction for compounds 3Et-TFDP and 3Pr-TFDP ($P < 0.001$). *Post hoc* analysis (F-test for simple effect) revealed that 3Et-TFDP and dipyrone attenuated baker's yeast-induced fever ($P < 0.05$; Figure 4A). Similarly, 3Pr-TFDP and dipyrone (Figure 4B) decreased T_R with time when compared to the control group ($P < 0.05$).

To identify the most promising compounds for subsequent testing, the antipyretic activity indexes of the TFDP compounds were determined. Compounds 3Et-TFDP and 3Pr-TFDP, at the doses of 140 and 200 µmol/kg, respectively, presented the highest antipyretic activity indexes (61% for compound 3Et-TFDP and 82% for 3Pr-TFDP). These compounds were selected for subsequent testing.

Effect of 3Et-TFDP and 3Pr-TFDP on the number of total cells present in rat cavity washes

Since peritoneal leukocyte migration is important for fever development (17), we investigated whether compounds 3Et-TFDP and 3Pr-TFDP altered number of leukocytes in the peritoneal cavity. As shown in Figure 5, injection of baker's yeast into the peritoneal cavity significantly increased leukocyte migration and treatment with compound 3Et-TFDP at the dose of 140 µmol/kg, administered 4 h after yeast injection, significantly reduced leukocyte influx ($P < 0.05$; Figure 5A). However, statistical analysis revealed no significant effect of treatment with compound 3Pr-TFDP (200 µmol/kg) on the baker's yeast-induced increase in the total number of leukocytes in the peritoneal cavity washes ($P > 0.05$; Figure 5B).

3Et-TFDP and 3Pr-TFDP do not modify increased TNF-α and IL-1β levels in peritoneal fluid during baker's yeast-induced fever

There is evidence that pro-inflammatory cytokines such as TNF-α and IL-1β play a role in systemic inflammation and sickness behavior, including fever (18). Since compounds 3Et-TFDP and 3Pr-TFDP attenuated baker's yeast-induced

fever, we determined whether they altered TNF-α and IL-1β levels in the peritoneal fluid. Statistical analysis revealed that *ip* injection of baker's yeast and vehicle significantly increased TNF-α and IL-1β levels in the peritoneal cavity ($P < 0.05$). However, treatment with compounds 3Et-TFDP and 3Pr-TFDP, administered 4 h after yeast injection, did not alter TNF-α levels (Figure 6A,B). Similarly, these compounds had no effect on IL-1β levels in the peritoneal fluid (Figure 6C,D).

Effects of 3Et-TFDP and 3Pr-TFDP on COX activity

Since COX inhibitors abolish lipopolysaccharide-induced fever (19), we investigated whether the compounds 3Et-TFDP and 3Pr-TFDP altered COX activity. *In vitro* assays demonstrated that 3Et-TFDP and 3Pr-TFDP had no effect on COX-1 and COX-2 activities. On the other hand, celecoxib (100 µM and 1 nM), which was included as an internal standard in the assay, did not inhibit COX-1, but inhibited COX-2 activity by 52 and 94%, respectively.

Effects of 3Et-TFDP and 3Pr-TFDP on DPPH scavenging activity

Compounds 3Et-TFDP and 3Pr-TFDP had DPPH scavenging activity. The IC_{50} values of ascorbic acid, 3Et-TFDP and 3Pr-TFDP were 0.024 (0.015-0.038), 39 (25-62), and 163 mM (136-196), respectively.

Discussion

In the current study, we showed that a series of TFDPs decreased T_R with time and attenuated baker's yeast-induced fever in young rats. Specifically, we showed that compounds 3Et-TFDP, 3Pr-TFDP, 3Ph-TFDP, and 4Me-TFDP exhibited significant antipyretic activity. Among these, the pyrazole derivatives 3Et-TFDP and 3Pr-TFDP presented the most effective antipyretic activity against baker's yeast-induced fever and were selected for additional testing. It has been shown that administration of 3Et-TFDP produces rapid and long-lasting antinociception, whose maximum effect also occurs 1 h after its administration (15). It should be noted, however, that antinociceptive doses were five times larger than those used to cause antipyresis in the present study. Baker's yeast-induced fever coincided with an increase in total leukocytes in the peritoneal wash, which was attenuated by compound 3Et-TFDP. None of the compounds inhibited baker's yeast-induced increases of TNF-α and IL-1β in the peritoneal wash, or inhibited COX activity *in vitro*. Interestingly, 3Et-TFDP and 3Pr-TFDP derivatives showed significant DPPH radical scavenging activity.

The antipyretic screening was performed using the baker's yeast-induced fever protocol in rats, which is particularly suitable to evaluate new antipyretics, since it is sensitive to classic and novel antipyretic drugs (11). The pyrazole derivatives 3H-TFDP, 3Et-TFDP, 3Pr-TFDP, 3Bu-TFDP, 3Ph-TFDP, and 4Me-TFDP significantly decreased the T_R of pyretic animals within 2 h after administration. It is clear from the results that substitutions in position 3 of the pyrazole moiety altered antipyretic activity. In this respect, it is interesting to note that compounds that have been described as good analgesics in previous studies (14,15) also presented significant antipyretic activity in the current study. A preliminary evaluation of the structure-activity relationship has indicated that increasing the length of the carbon chain at position 3 of the pyrazole ring up to three carbons increased antipyretic activity (H<ethyl<propyl<butyl), with the exception of 3Me-TFDP, that presented no antipyretic activity. Increasing the carbon side chain increases lipophilicity and may alter binding affinity to selected targets, causing potential pharmacokinetic and pharmacodynamic changes. Considering that substitution of the linear chain for a ramified group (*i*-propyl) also increased lipophilicity but decreased antipyretic activity, it is possible that linear chain elongation may alter the pharmacodynamic properties, although other actions cannot be ruled out at present.

The currently described antipyretic activity of TFDP is, to some extent, in agreement with previous studies that have shown a significant antipyretic effect for 5-trichloromethyl pyrazole derivatives (11). However, comparing the corresponding 5-trifluoromethyl to the 5-trichloromethyl analogues, and to 3-methyl and 3-phenyl substitutions, reveals that while the 5-trifluoromethyl compound with a 3-methyl substitution loses activity, the 5-trifluoromethyl compound with a 3-phenyl substitution maintains antipyretic activity. This suggests that substituting the 5-trichloromethyl for a 5-trifluoromethyl group does not alter antipyretic activity, unless the substituent in position 3 of the pyrazole ring is a methyl group. Interestingly, if the methyl group is transferred to position 4 of the ring (compound 4Me-TFDP), the 5-trifluoromethyl derivative maintains its antipyretic activity.

Leukocyte recruitment is considered to be a central feature of the inflammatory response (20). We showed that the pyrazole derivative 3Et-TFDP, administered 4 h after yeast injection, significantly reduced baker's yeast-induced leukocyte influx to the peritoneal cavity, suggesting that the antipyretic effect of this compound may involve inhibition of leukocyte migration.

The injection of baker's yeast increased TNF-α and IL-1β levels in the peritoneal wash. These data agree with reports showing that levels of TNF-α increase in the early phases of the fever induced by *S. cerevisiae* mannans, the pyrogenic component of baker's yeast (21). Nevertheless, treatment with the pyrazole derivatives 3Et-TFDP and 3Pr-TFDP did

not decrease TNF-α and IL-1β levels in peritoneal fluid. These results suggest that mechanisms other than decreased cytokine levels underlie the currently described antipyretic effect of the tested compounds.

There are several lines of clinical and experimental evidence indicating that cyclooxygenases play an important role in fever development (22). However, specific assays of ovine COX-1 and COX-2 activities revealed that 3Et-TFDP and 3Pr-TFDP do not alter COX-1 or COX-2 activities. Therefore, a target other than COX may be involved in the currently described antipyretic effect of these compounds.

DPPH radical scavenging activity is a standard assay for screening the radical scavenging activity of specific compounds (16). The pyrazole derivatives 3Et-TFDP and 3Pr-TFDP neutralized the DPPH radical, indicating a slight antioxidant activity, particularly if compared with ascorbic acid. These results are in agreement with the previously reported antioxidant activity of selected pyrazole derivatives (23), and are particularly interesting, since accumulating evidence suggests a redox modulation of the inflammatory response and fever. The fact that a number of antioxidants of different chemical natures decrease LPS- (24-27) and baker's yeast- (28) induced fever and that ascorbate potentiates the antipyretic action of acetaminophen suggests that the antipyretic activity of these compounds may be related to their antioxidant activity. Therefore, one might propose that the antipyretic action of these pyrazole derivatives is related to their antioxidant activity. However, it must also be considered that the antioxidant activity of these compounds is weak, compared to ascorbic acid, and that mechanisms of action other than antioxidant activity may occur. In line with this view, it is possible that 3Et-TFDP and 3Pr-TFDP may share the mechanisms of dipyrone, a potent antipyretic and analgesic pyrazolonic derivative, which displays remarkably low anti-inflammatory and weak reversible COX-blocking activity (29,30). In this regard, some of its biological actions have been shown to depend on conversion to at least two active metabolites, 4-methylaminoantipyrine and 4-aminoantipyrine (30,31). Accordingly, given that 3Et-TFDP and dipyrone showed similar potency and efficacy in transiently attenuating yeast-induced fever, it is reasonable to propose that the antipyretic effects of 3Et-TFDP and other analogs may be due to direct antipyretic actions as such, or may result from effects of their metabolites. Furthermore, like dipyrone, 3Et-TFDP and 3Pr-TFDP may act by activation of the nitric oxide-cyclic GMP pathway at the periphery (32) or displacement of glutamate binding (33). These possibilities remain to be studied, and further investigations are needed to determine the mechanisms by which 3Et-TFDP and 3Pr-TFDP cause antipyresis.

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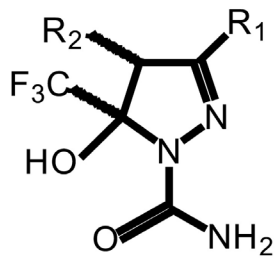

	3H-TFDP	3Me-TFDP	3Et-TFDP	3Pr-TFDP	3iPr-TFDP	3Bu-TFDP	3Ph-TFDP	4Me-TFDP
R_1	H	Me	Et	Pr	*i*-Pr	Bu	Ph	H
R_2	H	H	H	H	H	H	H	Me

Figure 1. Chemical structure of 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles (TFDPs).

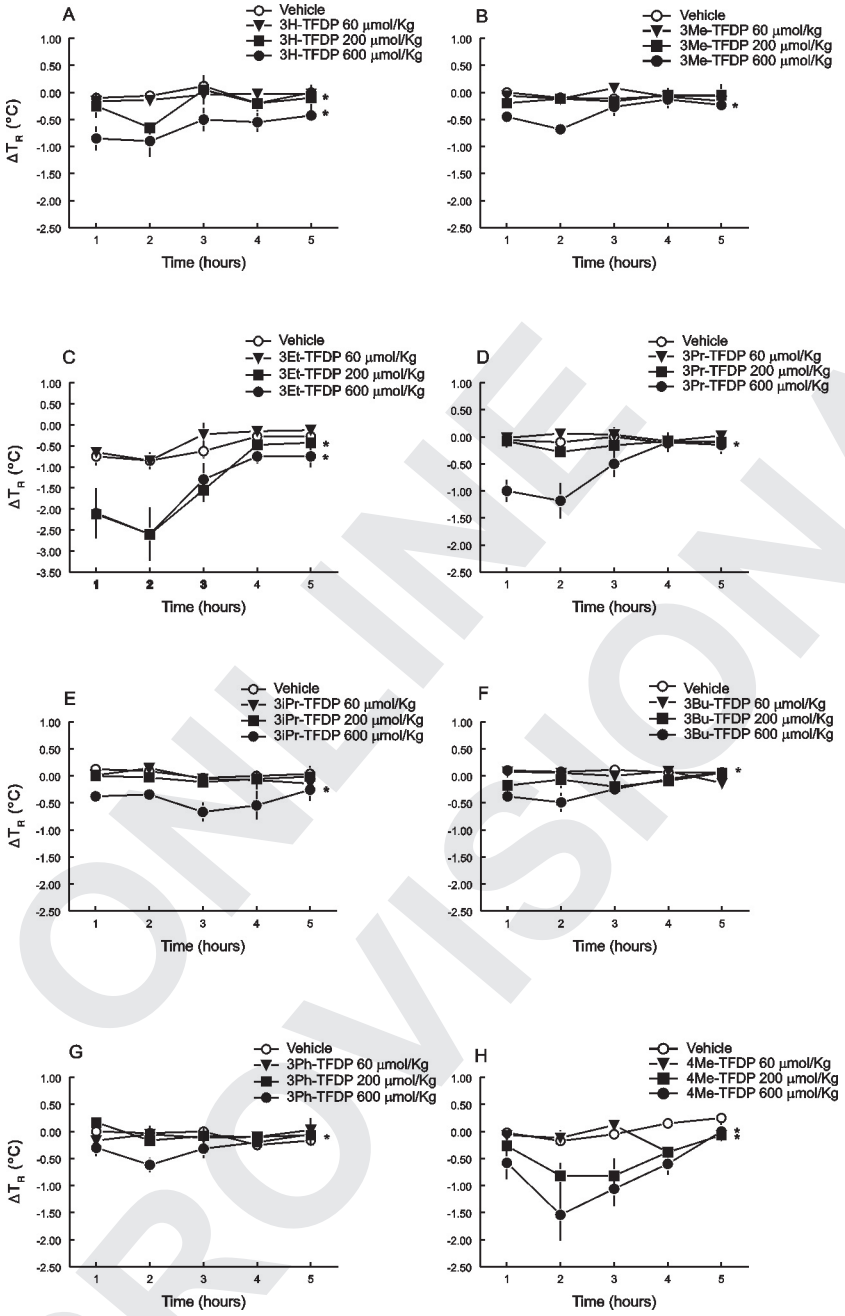

Figure 2. Effect of vehicle (5% Tween 80 in 0.9% NaCl) or TFDPs (60, 200 or 600 µmol/kg, *sc*) on rectal temperature (T_R) change. Data are reported as means ± SEM change from baseline T_R (N = 5-9 per group). TFDP = 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles. The significant dose by time interactions are: 3H-TFDP [$F(12,56) = 2.24$; $P < 0.05$], 3Me-TFDP [$F(12,76) = 2.78$; $P < 0.05$], 3Et-TFDP [$F(12,48) = 4.26$; $P < 0.05$], 3Pr-TFDP [$F(12,68) = 7.85$; $P < 0.05$], 3iPr-TFDP [$F(3,29) = 4.66$; $P < 0.05$], 3Bu-TFDP [$F(12,68) = 3.92$; $P < 0.05$], 3Ph-TFDP [$F(12,80) = 2.37$; $P < 0.05$], 4Me-TFDP [$F(12,68) = 3.18$; $P < 0.05$]. *$P < 0.05$ compared to vehicle (F-test for a simple effect). In this set of experiments the mean basal temperature was 36.9 ± 0.3°C.

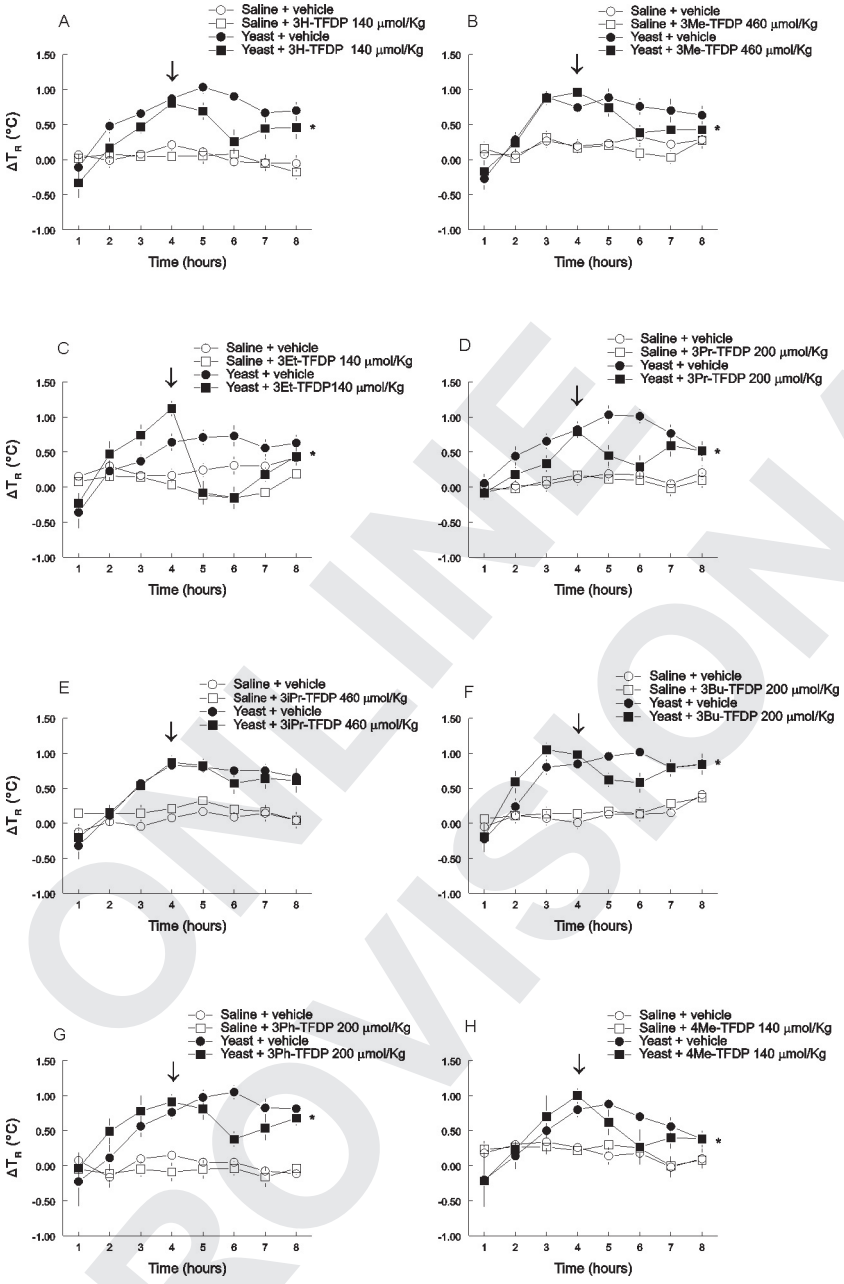

Figure 3. Effect of vehicle (5% Tween 80 in 0.9% NaCl) or TFDP (at the highest dose administered *sc* that had no effect on rectal temperature (T_R), 4 h after yeast injection) on baker's yeast-induced fever (0.135 g/kg). Baker's yeast was injected *ip* at zero time. The arrow indicates the time of injection of the drugs tested. Data are reported as means ± SEM change from baseline rectal temperature (N = 6-12 per group). TFDP = 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole. *P < 0.05 compared to vehicle (F-test for the simple effect). The significant dose by time interactions are: 3Et-TFDP [F(1,36) = 6.97; P < 0.05], 3Pr-TFDP [F(1,43) = 9.66; P < 0.05], 3Ph-TFDP [F(1,29) = 4.99; P < 0.05], and 4Me-TFDP [F(1,18) = 4.58; P < 0.05]. In this set of experiments the mean basal temperature was 36.8 ± 0.16°C.

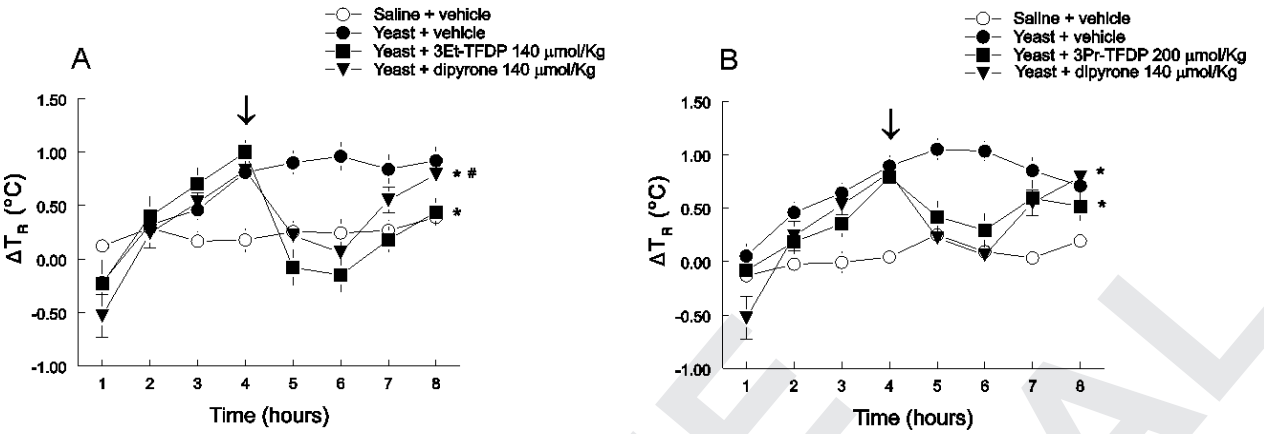

Figure 4. Effect of subcutaneous administration of vehicle (5% Tween 80 in 0.9% NaCl), 3Et-TFDP (140 µmol/kg; A), 3Pr-TFDP (200 µmol/kg; B) or dipyrone (140 µmol/kg) on baker's yeast-induced fever (0.135 g/kg, *ip*). Baker's yeast was injected *ip* at zero time. The arrow indicates the time of injection of the drugs tested. Data are reported as means ± SEM change from baseline rectal temperature (N = 6-12 per group). TFDP = 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles; T_R = rectal temperature. *P < 0.001 compared to vehicle. #P < 0.05 compared to dipyrone (F-test for the simple effect). In this set of experiments the mean basal temperature was 36.8 ± 0.14°C.

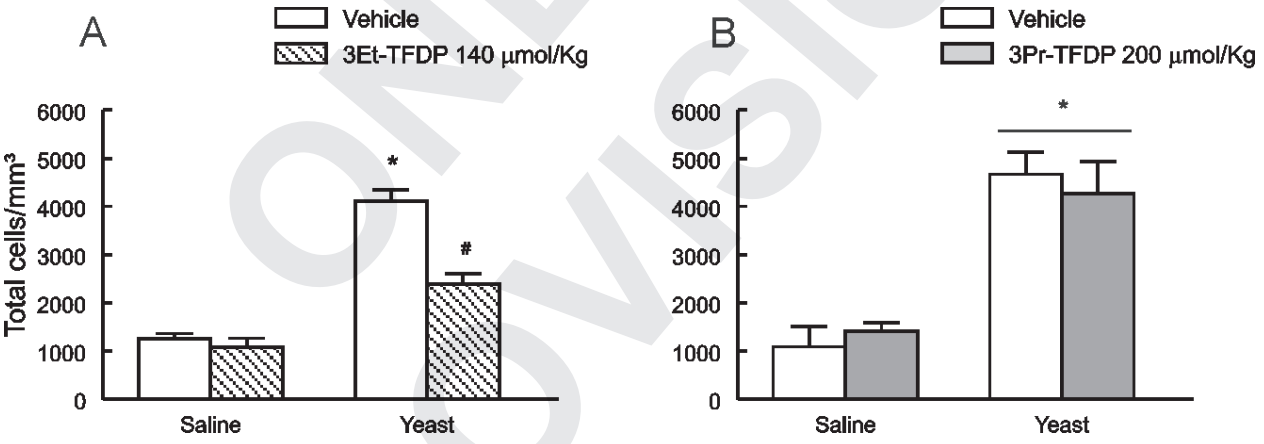

Figure 5. Effect of subcutaneous administration of vehicle (5% Tween 80 in 0.9% NaCl), 3Et-TFDP (140 µmol/kg; A) or 3Pr-TFDP (200 µmol/kg; B) on the number of total cells present in rat cavity washes in the presence or absence of baker's yeast. Values represent the mean number of cells/mm^3 (N = 5-7 per group). TFDP = 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles. *P < 0.05 compared to saline; #P < 0.05 compared to yeast-vehicle.

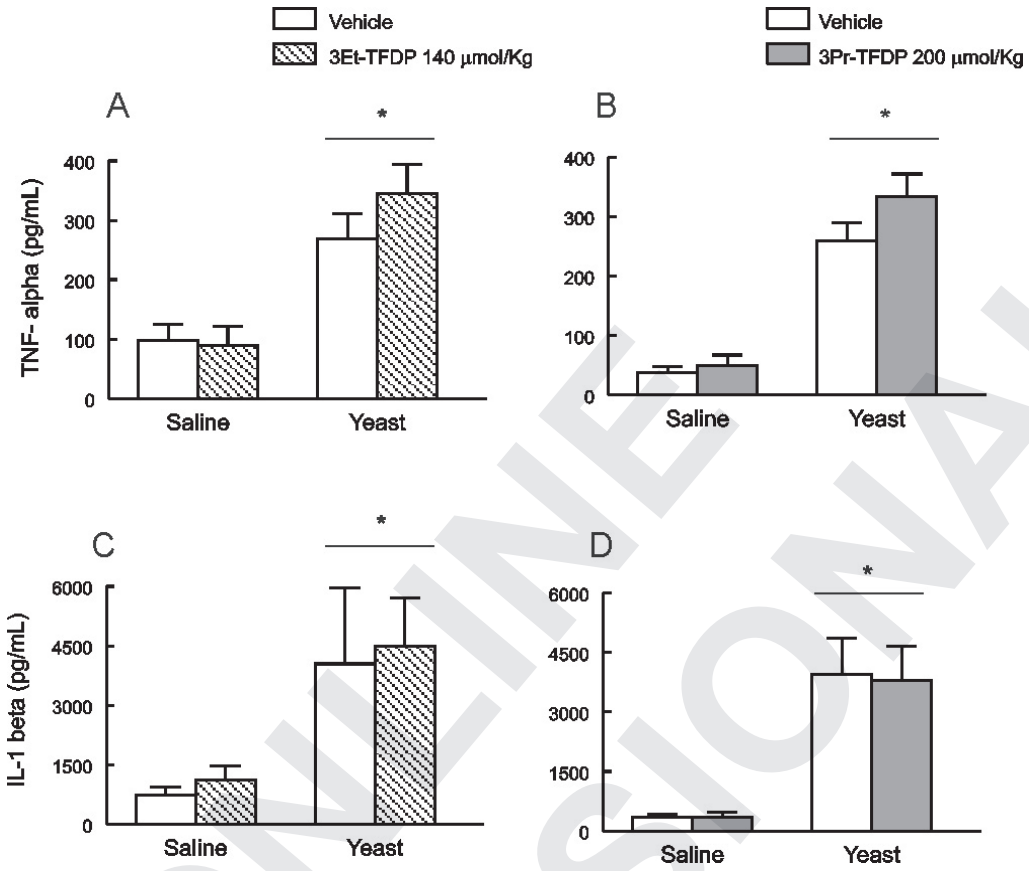

Figure 6. Effect of subcutaneous administration of vehicle (5% Tween 80 in 0.9% NaCl), 3Et-TFDP (140 µmol/kg, 4 h after yeast injection) or 3Pr-TFDP (200 µmol/kg, 4 h after yeast injection) on TNF-α (A, B) and IL-1β (C, D) levels in peritoneal fluid in the presence or absence of baker's yeast (N = 5-7 per group). TFDP = 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazoles. *P < 0.05 compared to saline (two-way ANOVA).