The effects of selective and non-selective cyclooxygenase inhibitors on endothelin-1-induced fever in rats

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SUMMARY

It was previously shown that sustained fever can be induced in rats by central injection of endothelin-1 (ET-1). This peptide appears to participate into the mechanism(s) of lipopolysaccharide (LPS)-induced fever, which is reduced by pre-treatments with ETB receptor antagonists. In this study we compared the effects of a non-selective cyclooxygenase (COX) inhibitor, indomethacin, with those of two selective COX-2 inhibitors, celecoxib and lumiracoxib, on ET-1-induced fever in rats. Fever induced in conscious animals by ET-1 (1 pmol, i.c.v.) or LPS (5 µg kg⁻¹, i.v.) was prevented by pre-treatments with celecoxib (5 and 10 mg kg⁻¹) or lumiracoxib (5 mg kg⁻¹), given by oral gavage 1 h before stimuli. Lower doses of celecoxib had partial (2.5 mg kg⁻¹) or no effect (1 mg kg⁻¹). Indomethacin (2 mg kg⁻¹, i.p.) partially inhibited fever induced by LPS but had no effect on ET-1-induced fever. The levels of prostaglandin (PG)E₂ and PGF₂α in the cerebrospinal fluid (CSF) of pentobarbital-anesthetized rats were significantly increased 3 h after the injection of LPS or ET-1. The latter increase was abolished by celecoxib at all tested doses and by indomethacin. In conclusion, selective COX-2 inhibitors were able to prevent ET-1-induced fever, indicating a role for COX-2 in this phenomenon. However, the fact that reduced CSF PGs levels obtained with indomethacin and a low dose of celecoxib are not accompanied by changes in fever induced by ET-1, along with the lack of inhibitory effects of indomethacin on ET-1 fever, suggest that the latter might also involve COX-2-independent mechanisms.

Keywords: COX-2 inhibitors, indomethacin, prostaglandins, cerebrospinal fluid, LPS
Introduction

It is now well established the prostaglandins (PGs) represent the final mediators of fever induced by exogenous and endogenous pyrogens, through an action on PGs receptor-expressing neurons in the preoptic area (POA) of the anterior hypothalamus (33). In fact, PGE$_2$ induces fever when injected centrally (13, 33, 38) and its levels in the cerebrospinal fluid (CSF) and in the POA rise in parallel with the generation of fever caused by several stimuli (9, 12, 16, 19, 23, 27, 35, 42). Moreover, the inhibition of PGs synthesis by cyclooxygenase (COX) inhibitors attenuates fever in humans and experimental animals, whereas these drugs do not affect fever induced by the administration of PGs (4, 7, 12, 13, 37, 39). PGF$_{2\alpha}$ also induces fever when injected centrally (13, 34) and its levels are increased in rat CSF in response to lipopolysaccharide (LPS) (10).

We have previously observed that endothelin-1 (ET-1), a member of the ET family of peptides (29), acts as a mediator of LPS-induced fever (15). In fact, i.c.v. pre-treatment with the selective ET$_B$ receptor antagonist BQ-788 blunted fever induced by i.v. LPS or i.c.v. ET-1 (15). The rise in core temperature induced by i.c.v. ET-1 was accompanied by such thermoeffector response as cutaneous vasoconstriction, measured as a decrease in tail skin temperature (ASC Fabricio, unpublished observation). The simultaneous occurrence of increased core temperature and cutaneous vasoconstriction defines the effect of ET-1 as a real fever, since the elevation in core temperature accompanied by tail skin vasodilation is rather defined as hyperthermia (2).

Fever induced by i.c.v. injection of 100 fmol ET-1 in the rat is not modified by the pre-treatment with a non-selective COX inhibitor, indomethacin, whereas ET$_B$ receptor
blockade does not affect fever induced by centrally administered interleukin (IL)-1β or tumour necrosis factor-α (15), whose pyrexic effects are effectively suppressed by indomethacin (13). Taken together, these findings led us to propose that ET-1, similarly to corticotrophin-releasing hormone, macrophage inflammatory protein-1, pre-formed pyrogenic factor and IL-8 (13, 31, 46, 47), may act as a mediator of a putative COX-independent pathway of fever triggered by LPS in rats (15).

Another line of evidence shows that COX-2 isoform plays a crucial role in fever. The deletion of genes encoding COX-2 (26) or PGE receptor subtype EP3 (44) prevents febrile response to LPS. Selective COX-2 inhibitors suppressed both LPS-induced fever (7, 45, 48) and the rise in PGE2 levels in the CSF (45). Furthermore, the induction of COX-2 was closely correlated with fever in terms of both timing and magnitude (7). Although ETs were shown to increase COX-2 expression and PGs production in a variety of cells including rat astrocytes (24), to our knowledge no study has been carried out to investigate the effects of selective COX-2 inhibitors, also referred to as coxib, on ET-1-induced fever. Here we investigated the effect of two coxib agents, celecoxib and lumiracoxib, on fever induced by centrally injected ET-1, and compared these effects with those of indomethacin. The relationship between fever and the levels of pyrogenic prostaglandins, PGE2 and PGF2α, in the CSF were also investigated following the above treatments. LPS was taken as a positive control in this experimental paradigm.
Methods

Animals

Experiments were conducted using male Wistar rats weighing 180-200 g, housed individually at 24 ± 1°C under a 12:12 h light-dark cycle (lights on at 06:00 h) with free access to food chow and tap water until the day of the experiment, when only water was made available. All experiments were previously approved by the institution’s ethical committee for research on laboratory animals and were performed in accordance with Brazilian legislation and the Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research, USA (20).

Intracerebral cannula implantation

Under anaesthesia with sodium pentobarbital (40 mg kg\(^{-1}\), i.p.), a permanent 22-gauge stainless steel guide cannula (0.7 mm OD, 10 mm long) was stereotaxically implanted into the right lateral ventricle at coordinates: 1.6 mm lateral to the midline, 1.5 mm posterior to bregma and 2.5 mm under the brain surface of the brain and incisor bar was lowered 2.5 mm below the horizontal zero (36). Cannulae were fixed to the skull with jeweller's screws embedded in dental acrylic cement. Animals were then treated with oxytetracycline hydrochloride (400 mg kg\(^{-1}\); i.m.) and allowed to recover for 1 week before the experiments. After each experiment, the animal was anaesthetised (as before) and the location of the cannula track verified histologically. Animals showing cannula misplacement or blockage upon injection, or abnormal weight gain patterns during the post-implantation period were excluded from the study.
Temperature measurements

Rectal temperature was measured in conscious and unrestrained rats for 1 min every 30 min for up to 6 h, in most cases by gently inserting a small thermistor probe (model 402 coupled to a model 46 telethermometer, Yellow Springs Instruments, Ohio, U.S.A.) 4 cm into the rectum, without removing them from their home cages. Experimental measurements were conducted at the thermoneutral zone for rats (17) in a temperature-controlled room (28 ± 1°C), following adaptation of the animals to this environment for at least 1 h. After this period, baseline temperature was determined 4 times at 30 min intervals before any injections, and only animals displaying mean basal rectal temperatures between 36.8 and 37.4°C were selected for the study. To minimise core temperature changes due to handling, animals were conditioned to this environment and procedure twice on the preceding day. The experiments involving indomethacin were conducted essentially as described above, except that core body temperature was measured by using battery-operated biotelemetry transmitters (Data Science, St. Paul, MN, USA) implanted in the peritoneal cavity at the same time as i.c.v. cannula implantation. These experiments revealed that ET-induced fever recorded using the radio-telemetry system (Fig.1B) was indistinguishable from that assessed by the rectal probe method (Fig.2B).

Cerebrospinal fluid sampling and determination of PG levels in the CSF

A single sample of CSF was collected from each animal according to the method described by Consiglio and Lucion (11) for estimation of immunoreactive PG levels.
Briefly, each rat was anaesthetised with pentobarbital (40 mg kg$^{-1}$) and fixed to the stereotaxic apparatus, with its body flexed downward. Thereafter, the skin covering the base of the skull and back of the neck was trichotomised and wet with a cotton swab soaked with ethanol to reveal a small depression between the occipital protuberance and the atlas. A scalp cannula connected to a syringe was then inserted through the depression into the cisterna magna to collect 60-100 µl samples, which were centrifuged at 1,300 g for 15 min, frozen immediately and kept at -20ºC until assayed for PG immunoreactivity. Samples contaminated with blood were discarded. After dilution (1:5) of the CSF samples, PGE$_2$ or PGF$_{2\alpha}$ was measured using ELISA kits from Cayman Chemical Co. (Ann Arbor, MI, USA) following the procedures detailed in the instructions. Detection limits of PGE$_2$ and PGF$_{2\alpha}$ ELISA-assays were 15 and 8 pg/ml, respectively. The intra- and inter-assay coefficients of variation were < 10% for both assays. Cross-reactivity data were as follows: 43% with PGE$_3$, 18.7% with PGE$_1$, 1% with 6-keto-PGF$_{1\alpha}$, 0.25% with 8-iso-PGF$_{2\alpha}$, less than 0.01% with all other prostanoids tested (including PGF$_{2\alpha}$) in the PGE$_2$ assay; 51% with PGD$_2$, 1% with 6,15-diketo-13,14-dihydro PGF$_{1\alpha}$, 0.4% with PGE$_2$, 0.2% with 11-dehydro-TXB$_2$, less that 0.01% with all other prostanoids tested in the PGF$_{2\alpha}$ assay.

**Experimental protocols**

In a first set of experiments, we tested the effect of a non-selective inhibitor of COX, indomethacin (2 mg kg$^{-1}$, i.p.) or its vehicle (tris[hydroxymethyl] aminomethane.HCl, pH 8.2), 30 min prior to injection, on changes of temperature induced by *E. coli* LPS (5 µg kg$^{-1}$, i.v.), ET-1 (1 pmol, i.c.v.) or their vehicles, sterile saline (1 ml
kg\(^{-1}\), i.v.) and artificial cerebrospinal fluid (aCSF; composition mmol l\(^{-1}\): NaCl 138.6, KCl 3.35, CaCl\(_2\) 1.26, NaHCO\(_3\) 11.9, i.c.v.), respectively. Other animals received celecoxib (1; 2.5; 5 and 10 mg kg\(^{-1}\)), lumiracoxib (1 and 5 mg kg\(^{-1}\)) or vehicle (sterile water, 1 ml/animal), by oral gavage 1 h prior to stimuli.

In another series of experiments, we analysed the changes in the PGE\(_2\) and PGF\(_{2\alpha}\) levels in the CSF of rats 3 hours after the i.v. injection of 5 µg kg\(^{-1}\) LPS or the central injection of ET-1 (1 pmol, i.c.v.). We have chosen a 3-h interval between pyrogen administration and collection of CSF for PG determination as this allows sufficient time for transcription (25) and translation of the COX-2 protein (3), as well as the maximal raise of both PGs in CSF after LPS (19). Finally, we investigated the effect of indomethacin (2 mg kg\(^{-1}\)), celecoxib (1, 2.5 and 5 mg kg\(^{-1}\)) or their vehicles on the increase in CSF PGs levels induced by ET-1.

For i.c.v. injections of ET-1, a 31-gauge needle, connected by polyethylene tubing to a 25 µl Hamilton gas-tight syringe (Hamilton, Birmingham, UK), was lowered into the guide cannula so that it protruded 2.5 mm beyond its tip into the ventricle and a volume of 3 µl was slowly infused over 1 min to avoid abrupt increases in CSF volume. LPS (5 µg kg\(^{-1}\), 200 µl/rat) or the corresponding vehicle (sterile saline) were administered to the rats by intravenous injection via a lateral tail vein. Pyrogenic stimuli were always injected between 10:00 and 11:00 h to minimise possible diurnal variability.
Drugs

The following drugs were employed: ET-1, from Research Biochemicals International (Natick, MA, U.S.A.); LPS (E.coli 0111:B4) and sodium pentobarbital, from Sigma Chem. Co., St. Louis, U.S.A.; oxytetracycline hydrochloride (Terramicina®), from Pfizer Laboratories, São Paulo, Brazil; indomethacin, a gift from Merck, Sharp & Dohme, São Paulo, Brazil; celecoxib (Celebra®) from Pharmacia, São Paulo, Brazil; lumiracoxib, kindly provided by Novartis Pharma SpA (Origgio, Varese, Italy).

Statistical analysis

All variations in rectal temperature were expressed as changes from the mean basal value (i.e. ΔT, in °C). Values are presented as the mean ± standard error of the mean (SEM), and statistical comparisons were performed by one-way ANOVA followed by Tukey's test or by Student’s t-test for comparison between group means, when appropriate, using a SPSS® statistical software (SPSS Inc., Chicago, IL, U.S.A.). Differences were considered significant when P<0.05.
Results

Experiments on fever

Intravenous injections of 5 µg kg⁻¹ LPS produced a fever with a rapid rise, peaking at 2-3 h after administration; the rectal temperature remained elevated throughout a 6-h observation period (Fig. 1A, 2A and 3A). The central injection of ET-1 (1 pmol, i.c.v.) caused a slowly-developing and long-lasting fever (Fig. 1B, 2B and 3B). The pre-treatment of rats with indomethacin (2 mg kg⁻¹, i.p.) significantly reduced, but by no means abolished, the pyrogenic response to i.v. injection of LPS (Fig.1A). In sharp contrast, indomethacin did not affect fever induced by ET-1 (Fig. 1B).

On the other hand, both celecoxib (5 and 10 mg kg⁻¹) and lumiracoxib (5 mg kg⁻¹) prevented fever induced either by LPS or ET-1, whereas they did not modify the rectal temperature of control rats (Fig. 2 and 3). The effect of celecoxib or lumiracoxib on LPS or ET-1-induced fever was also expressed as the percentage of inhibition of the total response (Fig. 4). In LPS-induced fever (Fig. 4A) the estimated ID₅₀ was 3.4 mg kg⁻¹ (8.89 µmol kg⁻¹) for celecoxib and 1.26 mg kg⁻¹ (4.29 µmol kg⁻¹) for lumiracoxib. In ET-1-induced fever, the estimated ID₅₀ was 4.3 mg kg⁻¹ (11.3 µmol kg⁻¹) for celecoxib and 1.6 mg kg⁻¹ (5.45 µmol kg⁻¹) for lumiracoxib (Fig. 4B). Thus, on a molar basis, lumiracoxib was twice more potent than celecoxib as an antipyretic on both LPS and ET-1-induced fever.
Experiments on CSF PGs levels

Cisternal CSF of the animals was collected 3 h after injection, when both LPS and ET-1 have induced significant increases in core temperature (Fig. 5A).

Baseline CSF levels of PGE_2 and PGF_{2α} were 138.7 ± 55.3 and 70.5 ± 17.5 pg ml^{-1} respectively in animals receiving i.v. saline, and 212.4 ± 87.25 and 89.4 ± 25.05 pg ml^{-1} respectively in animals receiving i.c.v. aCSF (Fig. 5B and C). LPS induced a four-fold increase in PGE_2 levels and a three-fold increase in PGF_{2α} levels in the cisternal CSF compared to control animals. ET-1 induced a similar increase in PGF_{2α} (about three-fold) but was more effective than LPS in increasing CSF PGE_2 levels (about six-fold) compared to animals receiving aCSF (Fig. 5B and C). There appears to be no correlation between temperature and PGE_2 levels in CSF: LPS-induced fever is almost twice the magnitude of ET-induced fever, while PGE_2 levels in LPS-injected animals are lower than those of animals receiving ET-1.

Indomethacin, given at a dose (2 mg kg^{-1}, i.p.) that failed to modify fever induced by ET-1, abolished the increase in CSF PGs levels induced by this peptide (Fig.6A and B). Celecoxib was also able to prevent ET-1-induced increases in PGE_2 and PGF_{2α} levels in the CSF even at doses (1 and 2.5 mg kg^{-1}) which were ineffective in preventing fever induced by ET-1 (Fig. 6C and D).
**Discussion**

The present study confirms previous observations by our group that exogenous ET-1 induces fever in rats (15) and reveals that this effect is accompanied by an increase in PG levels in the CSF. Furthermore, it shows that selective COX-2 inhibitors reduce fever caused by i.c.v. ET-1 in the rat; this effect is accompanied by a reduction in ET-1-stimulated PG levels in the CSF. These findings suggest that ET-1 induces fever via an increase in COX-2-generated PGs.

The COX-2 isoform has been localised at the level of different cell types in the rat brain; firstly, COX-2 is inducible in endothelial cells of brain vasculature, where its expression can be stimulated by LPS and pyrogenic cytokines (3-7, 30), as well as in glial cells, where COX-2 is also sensitive to induction by endotoxin, pro-inflammatory cytokines and other factors, notably including ET-3 (14, 24, 32). Secondly, COX-2 gene expression is constitutive in neurons of various brain areas, including the hypothalamus (1). Since selective COX-2 inhibitors in this study were always given prior to the administration of pyrogenic stimuli, this experimental design does not allow to distinguish whether they act on constitutive COX-2 in neurons, or on the inducible isoform in endothelial and glial cells, or on both targets.

Findings presented here provide pharmacological evidence that COX-2 and its PGs products are involved in the mechanism ET-1 induce fever. Indeed, the antipyretic actions of lumiracoxib and celecoxib in our model correlate well with their selectivity in inhibiting COX-2 activity. Lumiracoxib is a highly selective COX-2 inhibitor that shows potent anti-inflammatory activity (8) and differs in a number of important structural aspects from currently available COX-2 selective inhibitors, including celecoxib (28). In our
experimental paradigm lumiracoxib proved to be a more potent antipyretic than celecoxib on fever induced by LPS or ET-1, which reflects different selectivity of the two drugs in inhibiting COX-2 in vitro: IC$_{50}$ ratio COX-1/COX-2 = 400 for lumiracoxib compared to an IC$_{50}$ ratio COX-1/COX-2 = 30 for celecoxib (8). Thus, our results confirm and extend previous observations supporting a role for COX-2 in the induction of fever (3-7, 26, 30, 45, 48).

Here we show that the ET-1-induced fever is accompanied by an increase of PG levels in the CSF in the rat. It is well known that a number of physiological and pathophysiological actions of ETs in the body are mediated through the activation of COX pathway (40, 41). That occurs in most, albeit not in all cases, following the activation of ET$_B$ receptor subtype (18, 24, 43), and we have previously shown that such receptor is specifically involved in fever (15). ET-1 and ET-3, which shares with ET-1 the ability to activate ET$_B$ receptors (29), increase COX-2 expression via ET$_B$ receptors in macrophages and astrocytes, respectively, to which a specific increase in PGE$_2$ production ensues (24, 43); PGE$_2$ production in ET-1-stimulated macrophages is almost completely inhibited by NS 398, a COX-2 selective inhibitor (43).

Taken collectively, our data and the evidence discussed above would fit into a simple theoretical model according to which ET-1 induces fever in the rat through an increase in brain PGs generated by the COX-2 isoform. However, we also found (confirming our previous observations) that the non-selective COX inhibitor indomethacin, given at doses that blunt PGs levels in the CSF, does not prevent fever induced by ET-1. This finding is difficult to explain, as one expects that indomethacin, insofar as it also blocks COX-2, should mimic all of the effects of selective COX-2 inhibitors in this paradigm.
Discrepancies between the effects of indomethacin and coxib drugs on ET-1-induced fever might reflect distinct effects of these drugs on PG-synthesizing enzymes, as well as PG-transporters, and PG-catabolism in fever-related sites, as recently reported (21, 22). One other discrepancy in our study concerns with the different potency of celecoxib in reducing ET-1-induced fever and ET-1-stimulated PGs production, respectively. Indeed, celecoxib inhibited ET-1-induced changes in CSF PGs from 1 mg kg\(^{-1}\) onward, which is apparently at odds with the inhibitory effect on fever, starting from 5 mg kg\(^{-1}\). Thus, the above discrepancies leave the space open to alternative action mechanisms of ET-1-induced fever, possibly involving COX-2-independent pathways.
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References


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Legends

Figure 1 Effect of indomethacin on fever induced by LPS or ET-1 in rats. Indomethacin (INDO, 2 mg kg\(^{-1}\), i.p.) or tris[hydroxymethyl]aminomethane.HCl (TRIS, pH 8.2) were injected 30 min before LPS (5 \(\mu\)g kg\(^{-1}\), i.v.; panel A) or ET-1 (1 pmol, i.c.v.; panel B). Control animals received sterile saline (SAL, 1 ml kg\(^{-1}\), i.v.) or artificial CSF (aCSF, i.c.v.). Values represent the means ± SEM of the changes in rectal temperature (\(\Delta T\)) of 4 to 7 animals. *\(P < 0.05\) when compared to corresponding value of LPS-treated group.

Figure 2 Effect of celecoxib on fever induced by LPS or ET-1 in rats. Animals were pre-treated with celecoxib (1, 2.5 or 5 mg kg\(^{-1}\)) or sterile water (H\(_2\)O, 1 ml/animal) by oral gavage 1 h before injection of LPS (5 \(\mu\)g kg\(^{-1}\), i.v.; panel A) or ET-1 (1 pmol, i.c.v.; panel B). Control animals received sterile saline (SAL, 1 ml kg\(^{-1}\), i.v.) or artificial CSF (aCSF, i.c.v.). 10 mg kg\(^{-1}\) celecoxib completely prevented LPS and ET-1-induced fever (results not shown). Values represent the means ± SEM of the changes in rectal temperature (\(\Delta T\)) of 5 to 10 animals. *\(P < 0.05\) when compared to corresponding value of LPS-treated group in ‘A’ or ET-1-treated group in ‘B’.

Figure 3 Effect of lumiracoxib on fever induced by LPS or ET-1 in rats. Animals were pre-treated with lumiracoxib (1 or 5 mg kg\(^{-1}\)) or sterile water (H\(_2\)O, 1 ml/animal) by oral gavage 1 h before the injection of LPS (5 \(\mu\)g kg\(^{-1}\), i.v.; panel A) or ET-1 (1 pmol, i.c.v.; panel B). Control animals received sterile saline (SAL, 1 ml kg\(^{-1}\), i.v.) or artificial CSF (aCSF, i.c.v.). Values represent the means ± SEM of the changes in rectal temperature (\(\Delta T\)) of 5 to
6 animals. *$P < 0.05$ when compared to corresponding value of LPS-treated group in ‘A’ or ET-1-treated group in ‘B’.

**Figure 4** Concentration (mg kg$^{-1}$) of celecoxib and lumiracoxib plotted on a log scale against the percentage inhibition of LPS or ET-1-induced fever. The hatched lines are those calculated for best fit. Values represent the means ± SEM of inhibition induced by celecoxib or lumiracoxib on fever induced by LPS (panel A) or ET-1 (panel B) as shown in figures 3 and 4.

**Figure 5** Increase in core temperature and in CSF PGs levels induced by i.v. LPS or i.c.v. ET-1 in rats. Panel A: Core temperatures of animals just before anaesthesia for CSF collection. The levels of PGE$_2$ (panel B) or PGF$_{2\alpha}$ (panel C) were determined 3 h after the injection of LPS (5 µg kg$^{-1}$, i.v.) or ET-1 (1 pmol, i.c.v.). Control animals received sterile saline (SAL, i.v.) or artificial CSF (aCSF, i.c.v., 3 µl). Values represent the means ± SEM of 4 to 8 animals per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs respective control group.

**Figure 6** Effect of COX inhibitors on changes in CSF PGs levels induced by ET-1 in rats. Panel A and B: Indomethacin (INDO, 2 mg kg$^{-1}$, i.p.) or tris[hydroxymethyl]aminomethane.HCl (TRIS, pH 8.2) were injected 30 min before endothelin-1 (ET-1, 1 pmol, i.c.v.) or artificial CSF (aCSF, 3 µl). Panel C and D: Animals were pre-treated with celecoxib (CEL; 1, 2.5 or 5 mg kg$^{-1}$) or vehicle (H$_2$O) by oral gavage 1 h before the injection of ET-1 or aCSF. Levels of PGE$_2$ (panel A and C) and PGF$_{2\alpha}$ (panel B and D)
were determined 3 h after the injection of ET-1. Values represent the means ± SEM of the changes in CSF PGs levels of 4 to 5 animals. *P<0.05, **P<0.01 vs vehicle / ET-1 group.
Figure 1
Figure 2

A

B
Figure 4

A

% inhibition of LPS-induced fever

Log Dose (mg/kg)

B

% inhibition of ET-1-induced fever

Log Dose (mg/kg)
Figure 5

A

$\Delta T (^\circ \text{C})$

1.75

1.25

0.75

0.25

-0.25

B

$\text{PGE}_2 (\text{pg/ml})$

2000

1500

1000

500

0

C

$\text{PGF}_{2\alpha} (\text{pg/ml})$

400

300

200

100

0

SAL  LPS

l.v.

aCSF  ET-1

l.c.v.
Figure 6

A

PGE$_2$ (pg/ml)

TRIS/aCSF  TRIS/ET-1  INDO/ET-1

B

PGF$_{2\alpha}$ (pg/ml)

TRIS/aCSF  TRIS/ET-1  INDO/ET-1

C

PGE$_2$ (pg/ml)

C  0  1  2.5  5 mg kg$^{-1}$ CEL

ET-1

D

PGF$_{2\alpha}$ (pg/ml)

H$_2$O/aCSF  H$_2$O/ET-1  CEL 5 mg kg$^{-1}$/ET-1