PRE-EXISTING LIPOPOLYSACCHARIDE MAY INCREASE THE RISK OF HEATSTROKE IN RATS

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Background- We attempted to ascertain whether pre-existing inflammatory state [caused by exogenous administration of lipopolysaccharide (LPS)] exacerbated multiorgan dysfunction in experimental heatstroke.

Procedure- Immediately after the start of heat stress (42°C), anesthetized rats were divided into 2 major groups and given 0.9% NaCl solution (10 mL/kg of body weight, i.v.) or LPS (10 mg/kg of body weight, i.v.). Upon heat exposure, the occurrence of both hyperthermia (>42.0°C) and hypotension (mean arterial pressure <50 mmHg) was taken as the time point for heatstroke onset. Results- The LPS-treated, but not the saline-treated, animals underwent the heat stress for 52 minutes, displayed heatstroke syndromes. As compared to those of the saline controls, the LPS-treated rats had higher extent of activated inflammation (evidenced by increased plasma levels of interleukin-1β, tumor necrosis factor-α, and interleukin-6), hypercoagulable state (evidenced by increased levels of prothrombin time, activated partial thromboplastin time, and D-dimer, but decreased levels of both protein C and platelet counts), and multiorgan apoptosis and dysfunction (evidenced by increased plasma levels of creatinine, blood urea nitrogen, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase). Conclusions- Our results suggest that pre-existing inflammatory state may exacerbate the multiorgan injury during heat exposure. This tends to promote that pre-existing infection or sepsis may increase the risk of heatstroke.

The experimental protocol was approved by the Animal Ethic Committee of the Southern Medical University under guidelines of the National Medical and Health Research Council of China. Animal care and experiments were conducted according to the Guide for the Care and Use of Laboratory Animals published by the USA National Institutes of Health (NIH publication No. 85-23, revised 1996).
DOUBLE TRACING ANALYSIS OF PROSTAGLANDIN EP3 RECEPTOR-EXPRESSING PREOPTIC NEURONS THAT PROJECT TO THE DORSOMEDIAL HYPOTHALAMUS AND ROSTRAL RAPHE PALLIDUS

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The central mechanism of inducing fever is triggered by an action of prostaglandin (PG) E$_2$ on neurons in the preoptic area (POA) through the EP3 subtype of PGE receptor. EP3 receptor (EP3R)-expressing POA neurons directly project to the dorsomedial hypothalamus (DMH) and the rostral raphe pallidus nucleus (rRPa), both of which are key sites for the control of thermoregulatory effectors. Physiologically, febrile responses in brown adipose tissue (BAT) thermogenesis require DMH neuronal activation while those in cutaneous vasoconstriction do not and, when animals are cooled, BAT thermogenesis and skin vasoconstriction are activated at different threshold temperatures. Thus, we hypothesize that BAT thermogenesis and skin vasoconstriction during fever development are independently controlled by separate sets of neuronal pathways: PGE$_2$ pyrogenic signaling is transmitted from EP3R-expressing POA neurons via a direct projection to the DMH to activate BAT thermogenesis and via another direct projection to the rRPa to increase skin vasoconstriction. In this case, DMH-projecting and rRPa-projecting neurons would form segregated populations in the EP3R-expressing neuronal group in the POA.

In this study, we sought direct anatomical evidence to test this hypothesis with a double-tracing experiment in which two types of the retrograde tracer, cholera toxin b subunit (CTb), were injected into the DMH (Alexa488-conjugated CTb) and the rRPa (Alexa594-conjugated CTb) of rats and the resulting retrogradely labeled populations of EP3R-immunoreactive neurons in the POA were identified with confocal microscopy. We counted CTb-labeled cells in the EP3R-immunoreactive regions in the POA and found substantial numbers of EP3R-immunoreactive neurons in both the DMH-projecting and the rRPa-projecting populations. However, very few EP3R-immunoreactive POA neurons were labeled with both the CTb from the DMH and that from the rRPa, although a substantial number of neurons that were not immunoreactive for EP3R were double-labeled with both CTbs.

The paucity of the EP3R-expressing neurons that send collaterals to both the DMH and the rRPa suggests that pyrogenic signals are sent independently to these caudal brain regions from the POA and that such pyrogenic outputs from the POA might reflect different control mechanisms for BAT thermogenesis and for cutaneous vasoconstriction by separate sets of POA neurons.

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POSSIBLE INTERACTION BETWEEN PROSTAGLANDIN E2 AND GABA IN THE MECHANISMS OF FEVER IN THE REGION IMMEDIATELY ADJACENT TO THE OVLT

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Febrile responses to pyrogens are activated by the production of prostaglandin (PG)E2. Previous histological, microinjection, and electrophysiological studies have defined a region immediately adjacent to the organum vasculosum of the lamina terminalis (peri-OVLT) in the ventromedial preoptic area as the specific site at which PGE2 acts to produce fever. Unilateral microinjection of PGE2 into the peri-OVLT elicited thermogenic, tachycardic, cutaneous vasoconstrictive, and hyperthermic responses simultaneously in urethane-chloralose-anesthetized rats. The magnitude of these responses increased dose-dependently over the range of 57 fmol–2.8 pmol, except for the vasoconstrictive response. Microinjection of a GABA_A receptor antagonist, bicuculline methiodide or gabazine (5–20 pmol), into the PGE2-sensitive site in the peri-OVLT region also elicited responses similar to those induced by PGE2. Although administration of a GABA_A receptor agonist, muscimol (10 pmol), microinjected into the same site alone usually had no effect on the rate of whole-body O2 consumption, heart rate or colon and skin temperatures, all PGE2-induced responses were blocked 10 min after the muscimol pretreatment and recovered at 50–90 min. Pretreatment with the vehicle, saline, had no effect on the PGE2-induced responses. These results suggest that spontaneous release of GABA and tonic activation of GABA_A receptors in the peri-OVLT region prevent the elevation in the body core temperature under normal circumstances and that PGE2-induced febrile responses are mediated by inhibition of the GABAergic transmission in this area. However, it is also possible that PGE2 acted on postsynaptic PGE2 receptors that inhibit the signal transduction pathway of GABA_A receptors in the same postsynaptic cell. Moreover, I cannot strictly rule out the possibility that PGE2 and GABAergic agents affected two different populations of neurons that control febrile responses independently.
Evidence has accumulated to suggest that systemic administration of lipopolysaccharide (LPS), in addition to elevating tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) as well as fever, induces overproduction of glutamate, hydroxyl radicals and prostaglandin E₂ (PGE₂) in the rabbit's hypothalamus. Current study was attempted to assess whether Curcumin exerts its antipyresis by reducing circulating pro-inflammatory cytokines and hypothalamic glutamate, hydroxyl radicals and PGE₂ in rabbits. The microdialysis probes were stereotaxically and chronically implanted into the preoptic anterior hypothalamus of rabbit brain for determination of glutamate, hydroxyl radicals, and PGE₂ in situ. It was found that systemic administration of LPS (2 µg/kg) induced increased levels of both core temperature and hypothalamic levels of both glutamate and hydroxyl radicals accompanied by increased plasma levels of TNF-α, IL-1β, and IL-6. The rise in both the core temperature and hypothalamic glutamate and hydroxyl radicals could also be induced by direct injection of TNF-α, IL-1β, or IL-6 into the lateral ventricle of rabbit brain.

Pretreatment with Curcumin (5-40 mg/kg, i.p.) one hour before an i.v. dose of LPS significantly reduced the LPS-induced overproduction of circulating TNF-α, IL-1β, and IL-6, and brain glutamate, PGE₂, and hydroxyl radicals. Both the febrile response and overproduction of both glutamate and hydroxyl radicals in the hypothalamus caused by central administration of TNF-α, IL-1β, or IL-6 could be suppressed by curcumin. These results indicate that systemic injection of Curcumin may exert its antipyresis by inhibiting the glutamate-hydroxyl radicals-PGE₂ pathways in the hypothalamus and circulating TNF-α, IL-1β, and IL-6 accumulation during LPS-fever.
HYPERBARIC OXYGEN CAUSES REDUCTION OF ENDOTOXIN-RELATED SYSTEMIC INFLAMMATION AND FEVER IN RABBITS

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\textbf{Background}-To ascertain whether hyperbaric oxygen therapy inhibits the increase of glutamate, hydroxyl radicals, and prostaglandin E\textsubscript{2} in the hypothalamus and reduces fever during lipopolysaccharide-induced systemic inflammation in rabbits. \textbf{Procedure}-Adult male New Zealand white rabbits, weighing between 2.2 and 3.2 kg at the start of the study, were used. The pyrogen assay was carried out with unanesthetized animals restrained in rabbit stocks. The microdialysis probes were stereotaxically and chronically implanted into the preoptic anterior hypothalamus of rabbit brain (the essential thermoregulatory center) for assessment of glutamate, hydroxyl radicals, and prostaglandin E\textsubscript{2} in situ. For measurement of serum cytokines, 5 ml of blood was withdrawn from the marginal ear vein of each rabbit. The amounts of the cytokines tumor necrosis factor-\(\alpha\), interleukin-1\(\beta\), and interleukin-6 in the serum were determined by using double-antibody sandwich ELISA (R&D systems, Minneapolis, MN, USA) according to the manufacturer’s instruction. \textbf{Results}-Intravenous administration of lipopolysaccharide (2 \(\mu\)g/kg) caused increased levels of both core temperature and hypothalamic glutamate, hydroxyl radicals, and prostaglandin E\textsubscript{2} accompanied by increased plasma levels of tumor necrosis factor-\(\alpha\), interleukin-1\(\beta\), and interleukin-6. Treatment with hyperbaric oxygen (100\% at 253 kPa) once a day for consecutive 7 days prior to or 1 hour after injecting lipopolysaccharide significantly reduced the lipopolysaccharide-induced elevation of core temperature, circulating tumor necrosis factor-\(\alpha\), interleukin-1\(\beta\), and interleukin-6, and hypothalamic glutamate, hydroxyl radicals and prostaglandin E2. Direct injection of tumor necrosis factor-\(\alpha\) (20 ng), interleukin-1\(\beta\) (20 ng), or interleukin-6 (10 ng) into the lateral cerebral ventricle also caused a rise in both core temperature and hypothalamic glutamate and hydroxyl radicals, which could be attenuated by treatment with hyperbaric oxygen. \textbf{Conclusion}-Hyperbaric oxygen can be used as a prophylactic as well as a therapeutic agent for prevention or suppression of endotoxin-related systemic inflammation and fever in rabbits.
LPS-INDUCED CELLULAR ACTIVATION IN RAT AREA POSTREMA MICROGLIAL CELLS

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Background - During the process of bacterial infection, neurons and glial cells of the area postrema (AP) at the level of medulla oblongata are accessible to circulating, bacteria-related “pathogen-associated molecular patterns” (PAMPs) as well as circulating proinflammatory cytokines released from immune-competent cells like macrophages, due to the lack of a functional endothelial blood-brain barrier. In addition, cytokines released from AP-intrinsic cells may reach AP neurons and glial cells. Procedure - To study specific PAMP- and cytokine-mediated cellular activation, an AP primary microculture was established from topographically excised brain tissue of 5-6 days old rat pups. The Fura-2 ratio imaging technique was used to quantify PAMP- and cytokine-induced calcium signalling in AP microcultures superfused with oxygenated HEPES buffer containing PAMPs [lipopolysaccharide (LPS), muramyldipeptide (MDP), fibroblast-stimulating lipopeptide-1 (FSL-1)] or cytokines [interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)]. Subsequent immunolabeling with antisera directed against cell-specific marker proteins allowed identification of neurons, astrocytes and microglial cells. Cellular expression of TNF-α and inducible nitric oxide synthase (iNOS) due to LPS stimulation was verified by specific immunocytochemistry. Concentrations of proinflammatory cytokines (TNF-α, IL-6) released into the supernatant of LPS-stimulated AP microcultures were determined by use of specific bioassays. Results - Application of LPS induced fast, transient rises in intracellular calcium concentration ([Ca^{2+}]i) in 4 % of neurons, 2.5 % of astrocytes but 10 % of microglial cells investigated. Pre-incubation of AP microcultures with LPS for 18 h caused a complete abrogation of LPS-induced calcium signalling, indicative of endotoxin tolerance development. Only very few AP cells responded to stimulations with MDP or FSL-1. In the supernatants of LPS-treated AP-microcultures, significant amounts of bioactive TNF-α and IL-6 were detected, and immunocytochemical analysis revealed iNOS and TNF-α expression exclusively in microglial cells. Again, pre-circulation of AP microcultures with LPS for 18 h caused a marked suppression of LPS-induced cytokine release. When stimulated with TNF-α, rapid elevations of [Ca^{2+}]i could be detected in 8 % of all neurons and astrocytes, with limited responses of microglial cells, whereas IL-6 proved to be unable to activate AP-intrinsic cells. Conclusions - The demonstration of direct cellular responses of AP-intrinsic microglial cells to LPS-stimulation raises the intriguing possibility that the AP can act as a sensor for circulating PAMPs, mainly the TLR4-agonist LPS. In addition or consequence, TNF-α either as circulating or microglial proinflammatory cytokine activated AP-intrinsic astrocytes and neurons, which might transmit the immune signal to (extra-)hypothalamic structures involved in brain-controlled illness responses. Supported by Deutsche Forschungsgemeinschaft (DFG) (GE 649/6-1).
FEVER ASSOCIATED WITH INTRACEREBRAL HEMORRHAGE; INVESTIGATION OF ITS MOLECULAR MECHANISM IN A RAT MODEL

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Background: Fever is common in patients with intracerebral hemorrhage (ICH), although its molecular mechanism is unknown. We examined if the prostaglandin system is involved in fever associated with ICH as in the case of fever in infectious/inflammatory diseases. Procedure: Rats were implanted with temperature data logger in abdominal cavity at least one week before the experiment. To induce ICH, we injected collagenase into one side of the preoptic area under isoflurane anesthesia. In addition, some rats were intraperitoneally injected with cyclooxygenase (COX) inhibitors. Either 4 h or 28 h after collagenase injection, rats were killed by an overdose of pentobarbital, and cerebrospinal fluid and brain were sampled for biochemical and histological assays. Results: Abdominal temperature ($T_{ab}$) started to rise at around 3 h after injection of collagenase and the hyperthermia persisted over 24 h. Hemorrhage was evident when the brains were examined at 4 h and 28 h after collagenase injection. Injection of saline or heat-inactivated collagenase resulted in much smaller increase in $T_{ab}$. At both 4 h and 28 h after injection, prostaglandin E$_2$ (PGE$_2$) contents in cerebrospinal fluid and brain tissue were higher in collagenase-injected rats than those in saline-injected ones. Intraperitoneal injection of diclofenac, a non-specific cyclooxygenase (COX) inhibitor, suppressed collagenase-induced hyperthermia on both the first and second days. NS398, a COX-2 specific inhibitor, suppressed both $T_{ab}$ and PGE$_2$ increase when administered on the second day. However, NS398 exerted less suppressive effects when administered on the first day. Immunohistochemistry revealed that COX-2 was induced mainly in endothelial cells of the penumbra and subarachnoidal space. Conclusion: These results indicate that PGE$_2$ is involved in ICH-induced fever, and PGE$_2$ is synthesized mainly by the action of COX-1 on the first day and COX-2 on the second day.
SPATIOTEMPORAL ACTIVATION OF THE TRANSCRIPTION FACTOR NF-IL6 DURING THE TIME COURSE OF LPS-INDUCED FEVER IN THE RAT BRAIN

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Background- Intraperitoneal injection of lipopolysaccharide (LPS), a compound of gram negative bacterial cell walls, is known to induce brain-controlled sickness type responses such as fever. In the course of LPS-induced systemic inflammation early genomic activation of brain-cells occurs in a largely characterized distribution pattern, which can be monitored by immunohistochemical detection of the nuclear factor κB (NFκB) and the signal transducer and activator of transcription 3 (STAT3) and is linked to the initiation of the febrile response. Here we aimed to investigate if NF-IL6, a member of the C/CAAT enhancer binding protein family of transcription factors might be another possible participant in mediating LPS-induced activation of brain cells during fever. Procedure- Rats were injected systemically with LPS (100 µg/kg) or saline and brains were analyzed by immunohistochemistry 4, 6, 8 and 10 hours later. To determine the phenotype of reactive cells, NF-IL6-immunohistochemistry was combined with the detection of specific cell marker proteins. In addition, RT-PCR for NF-IL6-responsive inflammatory target genes was performed. Results- Moderate to strong LPS-induced nuclear NF-IL6-immunoreactivity (IR) occurred in a time dependent manner within circumventricular organs, namely the vascular organ of the lamina terminalis, the subfornical organ, the area postrema and the median eminence, brain structures characterized by a leaky blood-brain barrier. Furthermore, nuclear NF-IL6-IR was observed in the pituitary gland, the choroid plexus, the meninges as well as distinct large blood vessels throughout the entire brain. As for cellular phenotypes, we were able to demonstrate LPS-induced nuclear NF-IL6-IR in endothelial, microglial and ependymal cells, astrocytes, perivascular macrophages and neurons. Interestingly, the percentage of NF-IL6-reactive cells increased in parallel to the extend of late phases of the febrile response with a peak at 8h in most of the brain structures investigated. Conclusions- The present results suggest a role for NF-IL6 in the maintenance of LPS-induced fever rather than its initiation, which was hypothesized for STAT3 and NFκB and even does not rule out the possibility of NF-IL6 being part of the mechanisms implicated in defervescence. The definite physiological significance of NF-IL6 activation and its participation in the manifestation of brain-controlled sickness type responses remain to be elucidated.
HYPOTHALAMIC NATRIURETIC PEPTIDE INHIBITS FEVER INDUCED IN RATS BY SYSTEMIC ADMINISTRATION OF LPS

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Background - It has been reported that natriuretic peptides (NPs) - such as atrial NP (ANP), brain NP (BNP), and C-type NP (CNP) - and their receptors are present inside the blood-brain barrier, and that those contribute to blood-pressure and body-fluid regulation. Recently, we found that a NP-receptor antagonist, HS-142-1, when administered intracerebroventricularly, has an ability to inhibit the fever induced in rats by intravenous (i.v.) injection of bacterial endotoxin (lipopolysaccharide; LPS), suggesting that NPs are acting as antipyretic peptides within the brain. In this study, we investigated whether NPs within the hypothalamic preoptic area, a brain site involved in fever induction, act as an endogenous antipyretic in rats made febrile by systemic administration of LPS.

Procedure – The animals used in this study were male Wistar rats, weighing 270-350g. They were housed in individual plastic cages with wood-chip bedding in a room maintained at 26±1°C. Body temperature was measured using a biotelemetry system (Data Science, Inc., St Paul, MN). For intrapreoptic (i.p.o.) injection, a stainless-steel cannula was implanted in each rat under general anesthesia [sodium pentobarbitone (50 mg/kg, i.p.)] so that its tip lay in the right preoptic area using standard stereotaxic technique. For i.v. injections, a polyvinyl tube was inserted into the jugular vein so that its tip lay in the superior caval vein near the right atrium in animals anesthetized with sodium pentobarbitone (50 mg/kg, i.p.). Saline, the NP-receptor (A-type and B-type) antagonist HS-142-1, or ANP was given i.p.o. at 220 min after i.v. injection of LPS (the time-point of the start of the latest phase of the LPS fever), because we previously showed an inhibition of the same phase of the fever by intracerebroventricular injection of HS-142-1. In a separate experiment, we examined the effect of an i.v. injection of LPS on mRNA expressions for NPs within the hypothalamus of rats. Expressions of mRNA were analyzed by real-time RT-PCR.

Results – An i.v. injection of LPS induced a triphasic fever, the third phase of which was significantly enhanced by an i.p.o. injection of HS-142-1. In contrast, an i.p.o. treatment with ANP attenuated the third phase of the LPS-induced fever. When given i.v., LPS induced marked expressions of mRNAs coding for ANP and CNP within the hypothalamus.

Conclusions – These results suggest that the NPs produced within the hypothalamus may act on the preoptic NP receptors to inhibit the LPS-induced fever in rats.
STUDY OF PYROGENIC MEDIATORS INVOLVED IN LIVE E. COLI- INDUCED FEVER IN RATS

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Background – ET-1, BK, IL-1, TNF-α and IL-6 are important mediators of fever induced by lipopolysaccharide from Gram-negative bacteria (LPS). Nowadays we investigated the involvement of these mediators on live E. coli-induced fever in rats. Procedure – E. coli (2.5 x 10^8, i.p., 0.5 ml), ET-1 (100 fmol), BK (10 nmol), TNF-α (250 ng) or IL-1β (3.12 ng), or IL-6 (300 ng) were injected i.c.v., in 2µl, in male Wistar rats (200g). Pre-treatment were done i.c.v. (2µl) with: 3 pmol of ET_A (BQ-123) or ET_B (BQ-788) receptor antagonists; 20 nmol of B_1 (DALBK) or B_2 (icatibant) receptor antagonists; soluble tumour necrosis factor receptor I (sTNFRI, 500 ng), IL-1 receptor antagonist (IL-1ra, 200 µg) or monoclonal anti IL-6 antibody (5 µg) 15 min before pyrogenic stimuli. Body temperature (bT, °C) was measured by biotelemetry, every 30 min, during 6 or 24h. Peritoneal exudates, serum and CSF from rats receiving E. coli (2.5x10^8 CFU) was collected 0.5, 3, and 24 h after injection and cytokines concentration was measured by ELISA. Results – TNF-α, IL-1β and IL-6 were detected 3 h after the i.p. injection of 2.5 x 10^8 CFU of E. coli on serum of animals (145.6 ± 31.1; 950.5 ± 206.9; 942 ± 354; pg/ml, respectively). In the peritoneal exudates 0.5 and 3 h after 2.5 x 10^8 CFU of E. coli it was detected: TNFα (606 ± 190; 193..5 ± 24.9 pg/ml, respectively), IL-1β (3564 ± 748.5; 2899± 829 pg/ml, respectively) and IL-6 (2706.5 ± 1105; 5252.6 ± 1073.2 pg/ml, respectively). In these animals, cytokines were not found in the CSF. Pre-treatment with monoclonal anti IL-6 antibody reduced (3h, °C: from 38 ± 0.2 to 37.2 ± 0.1) while IL-1ra, sTNFRI, BQ-788, BQ-123, icatibant and DALBK did not alter this response. Conclusions – Fever induced by 2.5 x 10^8 UFC of E. coli seems to depend on central action of IL-6 but not on IL-1, TNF, ET or BK. Financial support: CAPES, CNPq, and FAPESP.
FEVER, SURVIVAL RATE AND INCREASE OF PGE2 IN THE CEREBROSPINAL FLUID (CSF) AFTER CECAL LIGATION AND PUNCTURE (CLP) IN RATS. EFFECT OF ACETAMINOPHEN.

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Background - Sepsis is the systemic response to severe infection in which fever is the most frequent manifestation. In this study we investigated the changes in body temperature, survival rate and the levels of PGE\textsubscript{2} in the CSF induced by bacterial infection. The effect of acetaminophen in this response was also investigated. Procedure - The animals was anesthetized with tribromoethanol and submitted to cecal ligation and puncture (CLP, 16-gauge needle) or sham surgery. Body temperature (bT) was measured by biotelemetry in male Wistar rats (200-250 g b.w.), every 30 min, during 48 h, after surgical procedure (sham or CLP). The survival was monitored by 7 days. PGE\textsubscript{2} was measured in the cerebrospinal fluid (CSF) by using ELISA kits. Results - The fever (in the early 8 h) and the decreased survival rate induced by CLP was puncture dependent. Besides to induce a long lasting fever four punctures increased PGE\textsubscript{2} in the CSF at 6, 12 and even when the fever was absent (24 and 48h). Acetaminophen 300 mg/kg (per os, 1h after surgery) attenuated the fever and reduced the PGE\textsubscript{2} (6h) level after CLP while 150 mg/kg did not alter the fever and CSF PGE\textsubscript{2}. In addition, at doses of 150 and 300mg/kg acetaminophen increased (from 50 to 80%) the survival rate of animals. Conclusions - Although CLP induces fever and increases the CSF PGE\textsubscript{2} level the fever course does not follow PGE\textsubscript{2} increase in the CSF. The mechanism by which acetaminophen exerts the pro-survival effect seems not to be related to PGs inhibition synthesis since, at 150 mg/kg acetaminophen did not altered the CSF PGE\textsubscript{2} but increased the survival rate.

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TNF-α AND IL-6, BUT NOT IL-1, ARE INVOLVED IN RANTES-INDUCED FEVER IN RATS

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Background - In rats, RANTES (Regulated on activation, normal T cells expressed and secreted) is involved in fever induced by lipopolysaccharide (LPS) via CCR1 and CCR5 receptors. Here the position of RANTES among the cytokines cascade working in the fever to LPS was investigated. Procedure - RANTES (25 pg/rat) was injected intrahypothalamically (i.h.) in male Wistar rats. Control animals received vehicle only. Body temperature was measured for up to 6 h by radio-telemetry system. IL-1 receptor antagonist (IL-1ra; 20 µg/rat, i.h.), soluble tumour necrosis factor receptor I (sTNFRI; 50 ng/rat, i.h.) and anti-rat IL-6 antibody (IL-6Ab; 0.5 µg/rat, i.h.) were administered before RANTES or IL-1β (300 pg/rat, i.h.), TNF-α (25 ng/rat, i.h.) and IL-6 (30 ng/rat, i.h.), respectively. After injection of RANTES, the levels of IL-1β, TNF-α and IL-6 were determined (2.5 h) in cerebrospinal fluid (CSF) using ELISA. Met-RANTES, antagonist of CCR1 and CCR5 receptors was given i.v. (100 µg/kg) before IL-1β, TNF-α or IL-6. After i.h. injection of RANTES nuclear factor-κB (NF-κB) activation (EMSA) and COX-2 mRNA (RT-PCR) expression were determined in the hypothalamus 0.5 and 2.5 h, respectively. Results - IL-1ra and sTNFRI reduced the fever induced by IL-1β and TNF-α, respectively, but not that induced by RANTES. IL-6Ab reduced the fever to IL-6 as well as the fever induced by RANTES. I.v. injected Met-RANTES reduced the fever induced by TNF-α but did not change that induced by IL-1β or IL-6. I.h. injected RANTES increased IL-6 CSF concentration while IL-1β and TNF-α were not detected in this fluid. Finally, RANTES increased NF-κB activation and COX-2 mRNA expression. Conclusions - These results suggest that in the fever cascade of mediators induced by LPS, the chemokine RANTES synthesis/release is preceded by TNF-α and that IL-6 and PGE2 (formed via COX-2) is situated downstream RANTES. The NF-κB activation after RANTES might represent the signalization via for induction of IL-6 and COX-2 synthesis. Support: FAPESP, CNPq.
THE RELEVANCE OF PGE\textsubscript{2} IN LPS-, ET-1- AND Tityus serrulatus VENOM (Tsv)-INDUCED FEVER

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\textbf{Background} – Indomethacin (INDO) does not alter the endothelin-1 (ET-1)-induced fever, but impede the increase of PGE\textsubscript{2} in the CSF. The fever induced by Tsv is also insensible to INDO while dipyrone (DIP) abolishes this response. This study aimed to re-evaluate the involvement of PGE\textsubscript{2} on LPS-, Tsv- and ET-1-induced fever. \textbf{Procedure} – Male Wistar rats received vehicle, DIP (120mg/kg) or INDO (2mg/kg) ip 30 min before LPS from \textit{E. coli} (5\textmu g/kg, iv), ET-1 (1 pmol, icv), Tsv (150\textmu g/kg, ip) or saline/artificial (a)CSF. The body temperature (°C) was measured every 30 minutes for up 2 or 3h by telemetry. The animals were anesthetized, CSF (cerebrospinal fluid) and hypothalamus were collected and the PGE\textsubscript{2} levels measured by ELISA. \textbf{Results} – LPS (3h: 1.3±0.1°C), ET-1 (3h: 0.8±0.1°C) and Tsv (2h: 2.1±0.1°C) induced a significant fever. DIP (76.9%) and INDO (46.2%) reduced the LPS-induces fever, but only DIP reduced the ET-1- (87.5%) and Tsv- (95.2%) induced fever. The CSF PGE\textsubscript{2} level remained undetectable in all control groups. The hypothalamic PGE\textsubscript{2} contents after icv aCSF was 619±111; after ip or iv saline were 390±41 and 486±44 pg/g of tissue, respectively. LPS and ET-1 increased the CSF (1511±286 and 416±74 pg/ml, respectively) and hypothalamic (2331±491 and 820±73 pg/g of tissue, respectively) PGE\textsubscript{2} concentration. INDO decreased the CSF and hypothalamic PGE\textsubscript{2} after LPS (78% and 66%, respectively) and ET-1 (no detectable and 87%, respectively). After LPS, DIP decreased the CSF PGE\textsubscript{2} concentration (84%), without change the hypothalamic PGE\textsubscript{2} contents (2105±281 pg/g of tissue). Dipyrone impeded the PGE\textsubscript{2} increase in the CSF without alters the enhancement in the hypothalamic content of PGE\textsubscript{2} (758±93 pg/g of tissue) after ET-1. The ip injection of Tsv does not alter the basal CSF and hypothalamic (388±30 pg/g of tissue) PGE\textsubscript{2} concentration. However, INDO and DIP reduced this basal hypothalamic concentration by 98% and 88%, respectively. \textbf{Conclusions} – These results strongly corroborate with ours previous findings that PGE\textsubscript{2} is not involved in the ET-1 and Tsv-induced fever and that the antipyretic effect of DIP does not rely on the blockage of PGE\textsubscript{2} synthesis. \textbf{Financial support:} CNPq, FAPESP.
FEVER, SICKNESS BEHAVIOR AND CIRCULATING CYTOKINES IN RESPONSE TO MACROPHAGE-ACTIVATING LIPOPEPTIDE-2 AND LIPOPOLYSACCHARIDE IN TOLL-LIKE RECEPTOR-2-DEFICIENT MICE AND CD36-DEFICIENT RATS

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Background: Macrophage-activating lipopeptide-2 (MALP-2) from Mycoplasma fermentans seems to induce an innate immune response via activation of Toll-like receptors (TLRs) 2 and 6. CD36 is regarded as a cellular sensor of diacylated lipopeptides such as MALP-2 and may be required for the array of MALP-2-induced effects in vivo. We therefore tested the responses of TLR2-knockout mice (TLR2-KO) and wildtype mice (C57-BL6), and of CD36 deficient spontaneously hypertensive rats (SHR) and their genetic controls (Wistar Kyoto rats, WKY) to systemic stimulations with the TLR2/6 agonist MALP-2 and the TLR4 agonist lipopolysaccharide (LPS).

Methods: TLR2-KO, C57-BL6, SHR and WKY were intra-abdominally implanted with radiotransmitters for recording of body temperature (fever) and motor activity. Food and water intake were also measured by a telemetric device to determine a possible development of anorexia and adipsia as characteristic components of brain-controlled sickness responses. Circulating levels of tumor necrosis factor (TNF) and interleukin-6 (IL-6) were measured by use of specific bioassays. Inflammatory activation of the brain was determined by the quantification of a nuclear translocation of the transcription factor STAT3 (signal transducer and activator of transcription 3) in brain areas, relevant for the manifestation of fever and sickness behavior.

Results: Fever and formation of TNF and IL-6 induced by intraperitoneal injections of MALP-2 were completely blunted in TLR2-KO mice, while LPS-induced responses were not impaired in these animals when compared to those of C57-BL6 wildtype mice. In SHR lacking CD36 an attenuation of fever and sickness behavior was observed in response to MALP-2, but even to a higher degree in response to LPS, when compared to WKY controls. Circulating cytokines and numbers of nuclear STAT3 signals in relevant areas of the brain were identical in SHR and WKY after stimulation with both pyrogens, indicating that the inflammatory activation of the brain in response to MALP-2 (and LPS) is not impaired by the lack of CD36.

Conclusions: These results demonstrate unequivocally that TLR2 is essential for the manifestation of MALP-2-induced (but not for LPS-induced) inflammatory responses. A moderate participation of CD36 in MALP-2-induced sickness- and cytokine-responses can not be ruled out, but is rather unlikely since LPS-induced inflammatory responses were also attenuated in SHR. The observed attenuations of MALP-2 and LPS-induced fevers in SHR may rather be caused by some of the endocrine abnormalities in these rats resulting in stronger endogenous antipyretic capacities.
THE ROLE OF PERIPHERAL AND CENTRAL CYCLOOXYGENASE PRODUCTS IN THE MANIFESTATION OF BRAIN-CONTROLLED SICKNESS RESPONSES DURING LOCALIZED INFLAMMATION INDUCED BY MACROPHAGE-STIMULATING LIPOPEPTIDE-2 (MALP-2)

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Background: Rats systemically treated with MALP-2 show brain-controlled acute-phase response symptoms such as fever or depressed motor activity. These responses are most likely mediated via activation of Toll-like receptors -2 and -6 and the subsequent induction of proinflammatory cytokines including tumor necrosis factor α and interleukin-6. Here we investigated whether an inflammatory activation of the brain can be demonstrated in response to systemic intraperitoneal or local injections of MALP-2 into a subcutaneous air pouch, and whether local (peripheral) or central cyclooxygenase (COX)-2-dependent formations of prostaglandin E2 (PGE2) are involved in MALP-2-induced illness responses.

Methods: In rats, body temperature, motor activity and food and water intake were measured by a telemetric device. Local (intra-pouch) and circulating levels of PGE2 were measured by use of an ELISA. Inflammatory activation of the brain in response to systemic or local stimulation with MALP-2 was determined by the immunohistochemical detection of a nuclear translocation of the transcription factors nuclear factor (NF)κB and signal transducer and activator of transcription (STAT)3 as well as the appearance of COX-2 in brain areas relevant for the induction of centrally controlled signs of illness.

Results: Local (intra-pouch) treatment with the preferential COX-2 inhibitor meloxicam attenuated, but not abolished fever, some of the other illness responses induced by local injections of MALP-2 into the pouch as well as plasma PGE2 levels and blunted intra pouch formation of PGE2. In the brain, systemic stimulation with MALP-2 induced nuclear STAT3- and NFκB-translocation in the brain vasculature and the sensory circumventricular organs, which was accompanied by a prominent increase in COX-2 immunoreactivity (IR) in endothelial cells. Local MALP2-treatment induced a moderate STAT3-activation and a small but significant increase in COX2-IR while hardly any NFκB-activation could be observed in the brains of these animals.

Conclusions: We were able to demonstrate that the activation of the brain STAT3(NFκB)-COX2 singling cascade seems to be involved in the manifestation of brain controlled illness symptoms induced by systemic and local inflammatory stimulation with MALP-2. In addition, the present data as well suggest a small but significant contribution of locally (peripheral) produced PGE2 to MALP-2-induced activation of brain controlled sickness responses like fever.
LPS FEVER AND ITS ENDOGENOUS PYROGEN AND FINAL MEDIATOR IN PIGEONS

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Background - It is well known that injections of bacterial endotoxin (LPS: lipopolysaccharide) induce fever in mammals. However, little is known about the febrile response of birds. Furthermore, conflicting results have been reported questioning the ability of birds to respond to LPS with fever. To investigate the ability to develop fever in birds, pigeons were injected I.V. with LPS. Interleukins and prostaglandins have been considered to be an endogenous pyrogen and a final mediator of fevers respectively in mammals. To investigate an involvement of interleukins in fever in birds, endogenous pyrogen (EP) was produced from blood of pigeon and rabbit, and pigeons were injected I.V. with each EP. To investigate an involvement of prostaglandins in fevers in birds, pigeons were injected I.V. with indomethacin (INDO) or aspirin, blockers of prostaglandin synthesis, at various times before or after LPS injections. Procedure – The animals were housed in individual cages in a climatic chamber at 26 ± 1 °C with light on at 09:00 and off at 21:00. Core temperatures (Tcore) were measured by a biotelemetry system (DATAQUEST® LabPRO™, Data Science Inc. USA). A battery-operated transmitter was implanted intraperitoneally. LPS at a dose of 10 µg/kg was injected at 13:00 through a chronically implanted catheter. Pigeon or rabbit EP at a dose of 1ml/kg was I.V. injected into either pigeons or rabbits at 13:00. INDO at a dose of 10 mg/kg was I.V. injected either 30 or 15 min before LPS or 2 or 4 h after. Aspirin at a dose of 100 mg/kg was I.V. injected 15 min before LPS. Results – I.V. injection of LPS at 13:00 evoked in pigeons a biphasic effect of Tcore, so that LPS induced with a latency of 30 min first decrease of Tcore, and 90 min after LPS, Tcore increased, obtaining maximum values from 18:00 to 20:00. Pigeon EP induced fever only in pigeons and rabbit EP induced fever only in rabbits. When INDO or aspirin was injected 30 or 15 min before LPS, it diminished the initial decrease of Tcore by more than 50 %, whereas the I.V. injection of these drugs 2 or 4 h after LPS did not affect the febrile rise of Tcore. Conclusions – These results suggest that pigeons were able to develop fever following I.V. injections of LPS, and that pigeon EP and rabbit EP are different from each other, and that prostaglandins are not involved in the febrile elevation of Tcore in pigeons, but appear to participate in the decrease of Tcore, which shortly follows the I.V. injection of LPS.
EFFECTS OF SIMULATED RECURRENT MYCOPLASMA INFECTION ON FEVER, GROWTH AND LEARNING AND MEMORY

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Introduction- Systemic infection is associated with fever and a suite of behavioural and cognitive responses collectively known as “sickness behaviour”. In developing countries, repeated infections with Mycoplasma or Gram-positive organisms (Staphylococcus, Streptococcus) are major causes of morbidity and mortality and may put infected individuals at particular risk of growth failure. We used the synthetic lipopeptide FSL-1 (fibroblast-stimulating lipopeptide-1), derived from Mycoplasma salivarium, to investigate the effects of simulated recurrent Mycoplasma infection on body temperature, cage activity, body mass, food intake, and learning and memory in growing rats. Methods- Male Sprague-Dawley rats were assigned randomly to receive three intraperitoneal (i.p.) injections of either FSL-1 (500µg.kg⁻¹ in PBS) or phosphate-buffered saline (PBS; 1ml.kg⁻¹), spaced 10d apart. Temperature and activity-sensitive radiotransmitters continuously measured core body temperatures as well as cage activity. Body mass and food intake were measured daily. Spatial learning and memory were tested in the Morris water maze. Results- FSL-1 treated rats had a significant increase in body temperature and decrease in night-time cage activity, food intake and body mass, compared with PBS-treated rats. The magnitudes of the fever, lethargy and anorexia induced by FSL-1 were not significantly different following the three successive injections. Conclusion- Repeated administration of FSL-1, at 10d intervals, induced the acute phase response including fever and brain-controlled sickness responses, without the development of pyrogenic tolerance. Repeated administration of FSL-1 did not leave residual impairment of learning and memory in growing rats.
DISTINGUISHING TONIC AND FEBRILE CONTROLS OF VASOCONESTRICTION BY PREOPTIC NEURONS

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Background - The preoptic area plays a pivotal role in mammalian temperature regulation. Heat conservation by cutaneous vasoconstriction is under tonic inhibitory control from neurons in the preoptic area. The preoptic area is also believed to comprise the key site where peripheral temperature, core temperature and febrile stimuli such as prostaglandin E₂ (PGE₂) interact to regulate body temperature. Experiments to localize and perhaps separate these functions within the preoptic area, however, have been limited by the spatial resolution of the methods employed. Methods - We used 15-30 nl injections of a short-acting inhibitory stimulus (GABA, 300 mM) to make a high resolution map of the preoptic regions providing tonic inhibition of sympathetic cutaneous vasoconstrictor (CVC) nerve activity, which was recorded in the tail of the anaesthetised rat (urethane, 1.5 g/kg, i.v.). Results – As expected, GABA injections into some but not all preoptic sites, activated tail CVC activity. Two distinct GABA-sensitive preoptic regions were identified: a rostromedial locus (RMPO) around the OVLT, and a second region (CLPO) that was centred approximately one millimetre caudolateral to RMPO. The two regions gave similar responses to GABA. Injecting PGE₂ (0.2 or 1 ng in 15 nl) into these two regions caused different effects. When injected into GABA-sensitive sites of the RMPO, PGE₂ caused a prompt rise in tail CVC activity and raised core temperature. When injected into GABA-sensitive sites in the central and caudal parts of the CLPO, PGE₂ was ineffective. Attenuated, delayed responses followed PGE₂ injections into intermediate sites. Conclusions - These results suggest that neurons in two distinct preoptic regions tonically inhibit rat tail vasoconstrictor activity, but only the RMPO neurons mediate tail vasoconstriction for the febrile response.
DISSECTING THE CONTRIBUTION OF PRENATAL FEVER AND HYPOFERREMIA
INDUCED BY TURPENTINE IN THE DEVELOPMENT OF SCHIZOPHRENIA-LIKE
BEHAVIOURS

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Background. Maternal infection or inflammation during the first 2 trimesters of human pregnancy is associated with greater incidence of schizophrenia (SCZ) in the adult offspring. Interleukin-6 (IL-6), a pro-inflammatory cytokine released during infection/inflammation, is thought to play a central role. IL-6 is fundamental in the induction of fever and of a decrease in the circulating levels of non-heme iron (hypoferremia) in the mother, which is the source of iron for the fetus. Both fever and hypoferremia may result in alterations in neurodevelopment, leading to increased risk of SCZ. We investigated the role these two factors on the effects of a prenatal aseptic inflammatory insult with turpentine oil (TURP) in an animal model. Procedure. To elicit an inflammatory response in pregnant rats at gestational day (GD) 15, we injected i.m. 100 µL of TURP or saline (SAL) as control. Body temperature (BT) was recorded 0, 8, 10 and 24 h after. One batch of dams was sacrificed 11 h after injection and blood was collected for circulating IL-6 and iron determination. Another batch of dams was allowed to give birth and the adult (60 days old) offspring were analyzed for pre-pulse inhibition (PPI) of acoustic startle and acute amphetamine (AMPH)-induced locomotion [2 mg/kg of body weight (BW)]. In a third batch of dams, SAL and TURP were co-administered with iron-dextran (1 daily i.p. injection of 20 mg/kg of BW from GD 15 to 18), in order to test the effect of hypoferremia. Behavioral responses of the offspring were correlated with maternal fever to investigate whether a link exists between these variables. Results. TURP treatment induced a significant febrile response in the GD 15 mothers, with a peak of 38.03 °C at 10 h, which receded within 24 h. This response was accompanied with a 6-fold rise in circulating IL-6 levels and a decrease in serum iron levels (from 246±30 in SAL to 82± 11 µg/dL in TURP). In the adult offspring, those animals whose mothers were treated with TURP presented a deficit in PPI but no changes in the response to a single injection of AMPH. Interestingly, maternal BT at the peak of fever significantly correlated with PPI (r\textsuperscript{2}=0.39, p<0.05) and locomotion after AMPH (r\textsuperscript{2}=0.7, p<0.01). Finally, PPI deficits induced by TURP were reversed by prenatal iron co-treatment. Conclusions. Our correlation analyses suggest that maternal fever may contribute to the development of behavioral alterations in the adult offspring, as well as reduced iron supply.
CALORIE RESTRICTION ATTENUATES SICKNESS BEHAVIOUR

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Background- Calorie restriction (CR) has been shown to have health promoting benefits, such as extending the mean and maximum life spans of numerous species, and inhibiting the development of certain cancers. CR alters the release of some cytokines and reduces mortality after exposure to a bacterial infection. Although cytokine responses after CR have been investigated sickness behaviour (fever, anorexia, cachexia) has not. The purpose of the present study was to examine the effect of CR on the development of sickness behaviour.

Procedure- Ten to sixteen week old adult male C57BL/6J mice were fed ad lib or exposed to either a 25% calorie restriction (CR25%) or 50% calorie restriction (CR50%) for 28 days. On the 29th day the mice were injected intraperitoneally with 50µg/kg of lipopolysaccharide. Changes in core body temperature, locomotor activity, body weight, and food and water intake were determined.

Results- CR50% mice demonstrated a full attenuation of all sickness behaviour measures in comparison to controls; however, the CR25% mice only showed a partial attenuation of sickness behaviour. The CR25% mice displayed a shorter-lived fever with the same peak in comparison to the controls (p < .001), whereas the CR50% mice did not develop fevers (p<.05 to p <.001 for the duration of the fever in controls). There were two distinct groups of CR25% mice, those with fevers (n=7), which still reached the same peak as controls but with a shorter duration, and those without (n=3). Neither CR25% nor CR50% mice exhibited anorexia (p < .001) and both had reduced cachexia (p < .001). The CR groups demonstrated a significant decline in core body temperature during the 4 week CR period (both p<.001); CR50% animals demonstrated the largest decline. There was a significant difference between the CR25% and CR50% mice (p<.001).

Conclusions- CR results in a suppression of sickness behaviour in a dose dependent manner. This may be due to CR causing a reduction in metabolism and/or influencing several central nervous, endocrine, and immune mechanisms. Possible mechanisms that could be involved in this attenuation of sickness behaviour include leptin, glucocorticoids, neuropeptide Y, and ghrelin due to their known involvement in the inflammatory response and altered levels after weight change.
EFFECT OF HYPOTHERMIA ON MOTOR FUNCTION OF ADULT RATS AFTER NEONATAL HYPOXIC ISCHEMIC BRAIN INSULT

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Background - Perinatal hypoxic-ischemic (HI) encephalopathy (HIE) is often related to pediatric mortality and long-term neurological impairment, and HI with hyperthermia insult worsens the neonatal prognosis more than with normothermia. Clinical trials have suggested that neonatal systemic or brain hypothermia soon after HI insult might improve the prognosis. The aim of this study was to test the effects of neonatal systemic hyperthermia soon after hyperthermic HI insult on adult motor ability using a rat HIE model and employing the Rotarod test.

Methods - Animals. Forty-five 7-day-old neonatal Wistar rats were anesthetized by isoflurane inhalation, and the left common carotid artery was surgically ligated. Sham-operated rats (n=11, SG) were also established by employing the same surgical procedure without arterial ligation. After the operation, the pups were returned to their dams for a 1-hr recovery. All the rats including SG were placed in a hyperthermic chamber for 15 min with humidified 8.0% oxygen. Then, all the rats except SG were placed in a chamber at 37°C (n=18, NG) and 34°C (n=16, HG) for 12 hrs. All the pups were subsequently returned to the dams and raised until 8 wks old.

Rotarod test. The Rotarod Treadmill was composed of a rotating rod with a diameter of 9 cm, controller, and a recording pedal switch. A rat was placed on the rotating rod, and the interval until it fell to the floor was measured. Measurements were performed for 3 consecutive days with a rotational frequency of 5, 5, and 7 rpm on the 1st, 2nd, and 3rd days, respectively.

Anatomy. Soon after the completion of the measurements, each rat’s brain was removed under deep anesthesia and the ratios (BWI) of the width of left versus right cerebral hemispheres were measured.

Results - The mean length of stay on the rod on the 2nd day for NG, HG, and SG was 42.3 ± 4.0, 57.6 ± 1.7, and 58.0 ± 13 sec (mean ± SE), with p<0.05 between NG versus HG or SG, although there was no significance in those on the 1st and 3rd days. BWI in NG was smaller than those in SG and HG (p<0.05).

Conclusion - Hyperthermic HIE might impair motor function, and hypothermia soon after HIE might inhibit this impairment.
ROLE OF PREOPTIC OPIOID RECEPTORS IN THE HYPOXIA-INDUCED ANAPYREXIA

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Background - Evidence indicates that endogenous opioids are involved in body temperature (Tb) regulation in mammals but no data exist about the involvement of the specific opioid receptors, mu, kappa and delta, in the anapyrexia induced by hypoxia. Thus, we investigated the participation of these opioid receptors in the anteroventral preoptic region (AVPO) in hypoxic anapyrexia. Procedure - Tb of conscious Wistar rats was monitored by SubCue dataloggers before and after intra-AVPO microinjection of the selective kappa-opioid receptor antagonist nor-binaltorphimine dihydrochloride (nor-BNI; 0.1 and 1.0µg/100nL/animal), the selective mu-opioid receptor antagonist CTAP (0.1 and 1.0µg/100nL/animal), the selective delta-opioid receptor antagonist Naltrindole (0.06 and 0.6µg/100nL/animal) or saline (vehicle, 100nL/animal), during normoxia and hypoxia (7% inspired O\textsubscript{2}). Results - Under normoxia, no treatment had any effect on Tb but exposure to hypoxia caused Tb to reduce in vehicle groups. Intra-AVPO microinjection of Nor-BNI and CTAP attenuated and intensified the hypoxia-induced anapyrexia, respectively, while naltrindole treatment had no effect on this response. Conclusions - Our results indicate that endogenous opioids modulate anapyrexia induced by hypoxia by acting on mu and kappa receptors in the AVPO inducing inhibitory and excitatory effects, respectively. Moreover, delta opioid receptors in the AVPO seem not to participate in this mechanism. Financial Support: FAPESP.
EFFECT OF BACLOFEN ON HYPOTHALAMIC NEURONS: ENHANCEMENT OR SUPPRESSION OF THERMOSENSITIVITY BY DRUG-DRUG INTERACTIONS

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Baclofen is a GABA-mimetic agent, which exerts its activity at GABA\textsubscript{B} receptors. Baclofen is at least as effective as diazepam in reducing spasticity and produces much less sedation. Previous studies on the effects of GABAergic agents on temperature sensitive neurons in rat hypothalamus have shown that the temperature sensitivity of the neurons is only changed by ligands of GABA\textsubscript{B}-receptors and this effect has been restricted to the warm-sensitive neurons.

Extracellular recordings were made from neurons in slices of the preoptic area/anterior hypothalamus (PO/AH) of rats, to investigate the effects of the GABA\textsubscript{B}-receptor agonist baclofen on neuronal response characteristics, and its interactions with GABA\textsubscript{A}-receptor antagonist bicuculline, as well as \(\mu\)-opioid receptor agonist PL-017 on the level of central temperature controller.

Baclofen decreased tonic activity (firing rate) in all types of neurons, but increased temperature sensitivity (temperature coefficient, TC) in warm-sensitive neurons. The increase in temperature sensitivity due to the GABA\textsubscript{B} agonist baclofen was significantly enhanced by co-perfusion of the GABA\textsubscript{A} antagonist bicuculline, indicating an interaction of GABA\textsubscript{A} and GABA\textsubscript{B} receptor-mediated mechanisms with regard to neuronal thermosensitivity. The tonic activity (in all type of PO/AH neurons), as well as the temperature sensitivity (in warm-sensitive neurons), was inhibited by selective \(\mu\)-opioid receptor agonist PL-017. Remarkably, the effect on temperature sensitivity was abolished and there was absence of synergism in regard to firing rate decrease when baclofen and PL-017 were applied simultaneously.

Our results are step of understanding the complicated mechanisms of action and interactions of the drugs on the level of central temperature controller – the neurons of the PO/AH.
MOLECULAR GENETICS OF DRUG-INDUCED THERMOGENIC RESPONSES: A CRITICAL ROLE FOR MITOCHONDRIAL UNCOUPLING PROTEIN 3

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Uncoupling proteins (UCPs) are highly-conserved members of the mitochondrial anion carrier superfamily that regulate proton leak across the inner mitochondrial membrane. The first uncoupling protein to be identified three decades ago, UCP1, is both necessary and sufficient for cold-induced thermogenesis and cold adaptation (survival) in mice. However, adult humans express negligible amounts of brown fat and UCP1, and no other analogous mitochondrial thermogenic pathway has been identified that mediates facultative thermogenesis of any type in man. Unlike UCP1, the homolog UCP3 is enriched in skeletal muscle, an established thermogenic organ in man. Using wild type and UCP3 knockout mice, we previously reported that C57Bl6/J UCP3-null mice almost completely lack the thermogenic and lethal responses to the widely-abused sympathomimetic amphetamine drugs 3,4-methylenedioxymethamphetamine and methamphetamine. Consistent with these observations, a recent publication revealed that hyperthermia induced by these agents is independent of brown fat and UCP1. Here, we demonstrate the generality of UCP3 in chemically-induced facultative thermogenic responses, and provide a more critical assessment of the genetics of facultative thermogenesis regulated by UCP3 in multiple strains of mice. First, using implantable microtelemetry devices to provide a minimally invasive method of temperature and activity assessments, we show that sympathomimetic agents generate comparable thermogenic responses in different background strains of mice, notably wild type B129 and C57Bl6/J mice. This is significant, because it rules out that the C57Bl6/J UCP3-null mice, which were originally created by gene-targeting in B129 stem cells, carried a B129 thermogenic defect or mutation that produced the thermogenic phenotype independently from UCP3. Second, we show that the thermogenic responses to other thermogenic inducers, such as lipopolysaccharide, a mediator of physiologic thermogenesis during bacterial infection, are also fully UCP3-dependent. These data strongly support a conserved role for UCP3 in certain types of thermogenic responses and rule out that any issues with the C57Bl6/J mouse account for the thermogenic defects in UCP3 knockout mice. Finally, UCP3 antagonists may be useful treatments for the mitigation of hyperthermia and excessive febrile syndromes.
THE RESPONSE OF BODY TEMPERATURE DAILY RHYTHMS TO PROPRANOLOL IN AGING DESERT ADAPTED GOLDEN SPINY MICE ACOMYS RUSSATUS

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Background – Studies carried out in our laboratory revealed that golden spiny mice Acomys russatus, a diurnal desert adapted species, responded to propranolol, a beta adrenergic blocker, by increasing melatonin secretion. Furthermore, aging A. russatus manifested arrhythmic body temperature (Tb) daily variation. As a well adapted desert rodent A. russatus has low resting metabolic rates (RMR) over 40% lower than the values expected for its body mass from alometric equations. These low RMR values contribute to an increase in its life span. The hypothesis for our study was: “If propranolol increases melatonin secretion in A. russatus than it should have the potential to restore Tb daily rhythms of aging mice”.

Methods – Two different age groups were studied aging mice (over two and a half years with no previous treatment) young mice (adults less than 10 months). Mice were acclimated to a photoperiod of 12L:12D at an ambient temperature of 28±1°C. Body (rectal) temperature (Tb) was compared between the two age groups. Mice of the aging group were injected with saline (control group) for two weeks and Tb and 6-sulphatoxymelatonin (6-SMT) daily rhythms were measured. At the second stage the aging mice were treated with propranolol (Sigma 4.5mg/Kg. Wb i.p) for two weeks and at the end of the treatment Tb and 6-SMT daily rhythms were measured.

Results – A clear difference in patterns of Tb daily rhythm is noted between the aging and young mice. Propranolol treatment significantly restored Tb daily rhythms of aging mice and furthermore it increased levels of melatonin during the dark phase compared with the control levels and showing a typical pattern of melatonin secretion as evaluated from 6-SMT with higher levels at dark phase and significantly lower values during photophase.

Conclusions – Our results show that propranolol has the potential to restore the Tb daily rhythms of aging A. russatus. Endogenous melatonin production during the dark phase plays a role in this restoration.
HEAT SHOCK PROTEIN 72 OVEREXPRESSION PREVENTS MICE FROM HEAT-INDUCED THERMOREGULATORY DEFICITS AND LETHALITY BY REDUCING HYPOTHALAMIC ISCHEMIA AND OXIDATIVE DAMAGE

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Background-This study was attempted to assess whether enhanced heat shock protein (HSP) 72 expression is able to attenuate thermoregulatory deficit as well as lethality in mice during heatstroke by reducing oxidative and ischemic damage in their hypothalamus. Procedure-Transgenic mice that were heterozygous for a porcine HSP72 gene ([+] HSP72), transgene negative littermate controls ([-]HSP72), and normal Institute of Cancer Research (ICR) strain mice were subjected to environmental heat stress. The heat stressed mice were returned to the normal room temperature (26°C) after the end of the heat exposure. Mice that survived on day 4 of heat treatment were considered survivors. The effects of heat stress (42.4°C for 1 h) on malondialdehyde (MDA), reduced-form glutathione (GSH), oxidized-form GSH (GSSG), glutathione peroxidase (GPx), glutathione reductase (GR), ATP, cellular ischemia (e.g., glutamate and lactate-to-pyruvate ratio) and damage (e.g., glycerol) markers, nitric oxide metabolites (NOx), dihydroxybenzoic acid (DHBA), and pro-inflammatory cytokines in the hypothalamus of these groups of animals were assessed in free moving state. Results-When the [-]HSP72 mice underwent heat, the fraction survival and core temperature at +4h of body heating were found to be 0 of 12 and 34.2°C±0.4, respectively. Overexpression of HSP72 significantly prevented the [+HSP72 mice from heat-induced death (fraction survival, 12/12) and hypothermia (core temperature, 37.4°C±0.3). [-]HSP72 had ATP depletion and increment of cellular ischemia and damage markers, free radicals (e.g., NOx and DHBA), oxidative stress (evidenced by increased levels of MDA and GSSG/GSH ratio and decreased levels of GR and GPx), and pro-inflammatory cytokines in their hypothalamus during heat stress. However, all the oxidative and ischemic damage, ATP depletion, and inflammatory response in the hypothalamus caused by heat were significantly reduced in [+HSP72. Conclusions-These results indicate that enhanced HSP72 expression in the hypothalamus prevents mice from heat-induced thermoregulatory deficit and lethality by reducing hypothalamic oxidative and ischemic damage and energy depletion. (The experimental protocol has been approved by the Animal Ethic Committee of Chi Mei Medical Center under guidelines of NIH publication No.85-23, revised 1996).
CASTRATION CAN ACT VIA TESTOSTERONE DEPLETION TO PROTECT MICE FROM HEAT-INDUCED HYPOTHALAMIC APOPTOSIS AND DEGENERATION

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Background - Heatstroke is characterized by hyperpyrexia, multiple organ failure, and predominant central nervous system dysfunction. Many evidences showed that testosterone exhibits the negative effects on many disease models such as sepsis, shock and severe injury. However, it remains unknown on the heatstroke animal model. Here we demonstrated castration can protect mice from heat-induced hypothalamic neuronal damage and lethality. Procedure - ICR male mice (6- to 8-wk-old) were random divided into four groups. The first group of mice was exposed to room temperature and used as normothermic controls. Another three groups: sham-operated mice, castrated mice with vehicle treatment, and castrated mice with testosterone replacement, were all subjected to whole body hyperthermia (WBH) at 41.2°C for 1 hour and then allowed to recover at room temperature (25°C). Mice that survived on day 4 of heat treatment were considered survivors. Plasma concentration of testosterone was measured by enzyme immunoassay. For heat-induced apoptotic study, mice were sacrificed at 2.5 hours post-WBH to excise the organs for TUNEL assays or H-E staining. Results – The fraction survival and core temperature of sham-operated mice at + 4 h post-WBH were found to be 5/15 and 34.4°C±0.3°C, respectively. Castration decreased the plasma levels of testosterone almost to zero, protected the mice from heat-induced death (fraction survival, 13/15) and reduced the hypothermia (core temperature, 37.3°C). The beneficial effects of castration in lethality and hypothermia can be significantly reduced by testosterone replacement. Heat-induced apoptosis (indicated by TUNEL) and neuronal damage (indicated by cell shrinkage and pyknosis of nucleus) in the hypothalamus were significantly prevented by castration and also reversed it by testosterone supplement. Conclusions - Castration can act via testosterone depletion to protect mice from heatstroke-induced hypothalamic apoptosis, degeneration and lethality. (The experimental protocol has been approved by the Animal Ethic Committee of Chi Mei Medical Center under guidelines of NIH publication No.85-23, revised 1996).
HYPER-HYDROXYETHYL STARCH RESCUES RATS FROM HEATSTROKE-INDUCED DEATH

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Background: We hypothesized that hyperhydroxyethyl starch (HyperHAES), which contains 6% HAES and 7.2% NaCl, would be superior to 6% HAES or 7.2% NaCl treatment alone during experimental heatstroke. Procedure: Anesthetized rats, immediately after the onset of heatstroke, were divided into five major groups and given the following: a) nothing; b) 0.9% NaCl solution (4 ml/kg of body weight, i.v.); c) 7.2% NaCl solution (4 ml/kg of body weight, i.v.); d) 6% HAES (4 ml/kg of body weight, i.v.); and e) HyperHAES (4 ml/kg of body weight, i.v.). Another group of rats, under urethane anesthesia, were exposed to room temperature (24°C) and used as normothermic controls. Urethane-anesthetized rats were exposed to ambient temperature of 43°C to induce heatstroke. Results: When the untreated or 0.9% NaCl solution-treated rats underwent heat exposure, their survival time values were found to be 17-23 mins. Resuscitation with 7.2% NaCl solution, 6% HAES, or HyperHAES, immediately at the onset of heatstroke, their survival time values respectively are 43, 32, or 219 mins. As compared with values for normothermic controls, the 0.9% NaCl solution-treated heatstroke rats had lower mean arterial pressure, cerebral perfusion pressure, cerebral blood flow, brain partial pressure of oxygen, and plasma levels of protein C. In contrast, the 0.9% NaCl solution-treated heatstroke rats had higher values of intracranial pressure, core and brain temperatures, brain levels of glutamate, glycerol, lactate-to-pyruvate ratio, neuronal damage scores, plasma levels of prothrombin time, partial thromboplastin time, D-dimer, and tumor necrosis factor-α, blood urea nitrogen, creatinine, aspartate and alanine aminotransferase, and alkaline phosphatase. The hypotension, intracranial hypertension, cerebral hypoperfusion, and hypoxia, increment of cerebral ischemia and damage markers, hypercoagulable state, and tumor necrosis TNF-α overproduction were all significantly attenuated by HyperHAES therapy. Conclusions: Our results suggest that HyperHAES seems superior to 7.2% NaCl solution or HAES treatment alone during heatstroke. HyperHAES improves survival during experimental heatstroke by attenuating multiorgan dysfunction.
ACTIVATED PROTEIN C CAN BE USED AS A PROPHYLACTIC AS WELL AS A THERAPEUTIC AGENT FOR HEATSTROKE FORMATION

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Background. The present study was attempted to assess the prophylactic as well as the therapeutic effects of human recombinant activated protein C (APC) (drotrecogin alfa, activated) in experimental heatstroke. Procedure. Anesthetized rats, one hour before the start or immediately after the termination of heat stress, were divided into 2 major groups and given vehicle solution (normal saline 2 ml per kilogram of body weight, intravenously) or APC (1-10 mg in 2 ml of normal saline per kilogram of body weight, intravenously). They were exposed to ambient temperature of 40°C for 100 mins to induce heatstroke. Results. When vehicle-pretreated anesthetized rats were exposed to environmental heat stress, their survival time values were found to be 57-71 mins. Pretreatment or posttreatment with APC significantly increased survival time (122-221 mins). All vehicle-pretreated heatstroke animals displayed systemic inflammation (evidenced by increased tumor necrosis factor-α, interleukin-1α, and interleukin-6) and activated coagulation (evidenced by increased levels of activated partial thromboplastin time, prothrombin time and D-dimer and decreased levels of both platelet count and protein C. Biochemical assay also revealed that both renal and hepatic dysfunction (e.g., increased plasma levels of blood urea nitrogen, creatinine, adenine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were noted during heatstroke. A significant decrease in both cerebral blood flow and partial pressure of oxygen in hypothalamus were also observed in vehicle-pretreated heatstroke animals. These heatstroke reactions were significantly reduced by pretreatment or posttreatment with APC. Conclusions. The results indicate that human recombinant APC can be used as a prophylactic as well as a therapeutic agent for experimental heatstroke by ameliorating systemic inflammation, hypercoagulate state, and multiple organ dysfunction. (The experimental protocol has been approved by the Animal Ethic Committee of Chi Mei Medical Center under guidelines of NIH publication No. 85-23, revised 1996).
SURVIVAL PROLONGATION AND NEUROPROTECTION IN EXPERIMENTAL HEATSTROKE BY COMBINATION DRUG TREATMENT

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**Background-** The clinical diagnosis of heatstroke (an extremely mortal disease) was suggested when hyperthermia was accompanied by circulatory shock (arterial hypotension), cerebral ischemia and damage. Several lines of evidence indicate that the increased plasma cytokines (e.g., interleukin-1 \(\beta\) (IL-1\(\beta\)) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), and elevated brain levels of monoamines, glutamate and free radicals productions may be implicated in pathogenesis during heatstroke. Treatment with a combined therapeutic approach has been repeatedly advocated in the cerebral ischemia experiments. The aim of this study was to investigate whether the combined agent (dexamethasone and hydroxyethyl starch) has beneficial efficacy to improve heatstroke-induced neuronal damage in experimental heatstroke by attenuating the concentration of monoamines, and hydroxyl radical productions in rat brain and plasma levels of cytokines and lipid peroxidation associated with heatstroke.

**Procedure-** Urethane-anesthetized rats underwent instrumentation for the measurement of colonic temperature, mean arterial pressure, local striatal cerebral blood flow, heart rate, and neuronal damage score. Rats were exposed to an ambient temperature (43\(^\circ\)C) to induce heatstroke. The normothermic control rats were placed in chamber with room temperature, 24\(^\circ\)C. Concentrations of the ischemic and damage markers, dopamine (DA), serotonin (5-HT), and hydroxyl radical productions in corpus striatum, and the levels of IL-1 \(\beta\), TNF-\(\alpha\) and malondialdehyde (MDA) in plasma were observed during heatstroke in rats.

**Results-** In our results, it was significantly decreased in values of cerebral ischemic and cellular injury markers after immediate treatment with the combined agent in rats. The combined agent also diminished the heatstroke-induced high plasma levels of cytokines and MDA, and high cerebral striatal levels of dopamine, serotonin and hydroxyl radicals in rats, and led to ameliorate the condition of heatstroke-induced central neuronal damage.

**Conclusions-** Immediate treatment with this combined agent confers significant protection against heatstroke-induced arterial hypotension, systemic inflammation, cerebral ischemia, cerebral monoamines and free radicals productions overload, and improves the survival time in heatstroke rats.
A HYPERBARIC OXYGEN THERAPY APPROACH IN HEAT STROKE WITH MULTIPLE ORGAN DYSFUNCTION

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\textbf{Background-} Heatstroke is a life-threatening illness characterized by body hyperthermia and multiple organ dysfunction or failure. Hyperbaric oxygen therapy is a noninvasive medical strategy in which a person breathes 100\% oxygen at a pressure greater than normal. We attempted to assess whether a hyperbaric oxygen therapy approach is beneficial in heatstroke with multiple organ dysfunction. \textbf{Procedure-} Anesthetized rats, immediately after the onset of heatstroke, were randomized into the following groups and given: a) hyperbaric oxygen (100\% O\textsubscript{2} at 253kpa for 1 h); or b) normal air. They were exposure to 43\textdegree{}C temperature to induce heatstroke. A heatstroke patient was refractory to conventional temperature control measures such as NSAIDS or external cooling devices (cooling blankets) even applied for several hours. Then, the patient was subjected to hyperbaric oxygen therapy. \textbf{Results-} When the untreated rats underwent heat stress, their survival time values were found to be 20-24 min. Resuscitation with hyperbaric oxygen increased the survival time to new values of 152-176 min. All untreated heatstroke rats displayed cerebroventricular dysfunction (evidenced by hypotension, intracranial hypertension, and cerebral hypoperfusion, hypoxia, and ischemia), hypercoagulable state, and tissue ischemia/injury. The cerebrovascular dysfunction, hypercoagulable state, tissue ischemia/injury, and brain oxidative stress that occurred during heatstroke were all suppressed by hyperbaric oxygen therapy. A 49-year-old male laborer, suffering from heatstroke syndromes (e.g., hyperpyrexia, seizure and coma, and hypotension), was admitted to an emergency unit of our medical center. The patient displayed multiple organ dysfunction with rhabdomyolysis, hepatic, renal, respiratory, and cerebral dysfunction, and disseminated intravascular coagulation (DIC). Both hyperpyrexia and multiple organ dysfunction was resistant to conventional treatment measures. Hyperbaric oxygen was adopted to rescue the patient from heatstroke-induced death. Before treatment, analyses of serum revealed hypercoagulable state or DIC as well as signs of rhabdomyolysis, and renal and hepatic failure. In addition, pulmonary edema, coma, hypotension, and hyperpyrexia occurred. Hyperbaric oxygen therapy was used successfully to combat these syndromes and to rescue the patient from heatstroke death. \textbf{Conclusions-} The current results show that hyperbaric oxygen therapy is beneficial in treating heatstroke with multiple organ dysfunction. (The experimental protocol has been approved by the Animal Ethic Committee of Chi Mei Medical Center under guidelines of NIH publication No.85-23, revised 1996).
PREMARIN CAN ACT VIA ESTROGEN RECEPTORS TO RESCUE MICE FROM HEATSTROKE-INDUCED LETHALITY

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**Background**-It has been shown that premarin, a water-soluble estrogen sulfate, may improve survival during heatstroke by ameliorating inflammatory responses and cardiovascular dysfunction in an anesthetized rat model. However, it is not known whether Premarin can act via estrogen receptors to rescue mice from heatstroke-induced lethality.

**Procedure**-Unanesthetized, unrestrained mice were exposed to ambient temperature of 42.4°C to induce heatstroke. Another group of mice was exposed to room temperature (24°C) and used as normothermic controls. They were given isotonic sodium chloride solution, Premarin (0.1-1.0 mg/kg of body weight, i.p.), or Premarin (1 mg/kg of body weight, i.p.) plus the nonselective estrogen receptor antagonist ICI 182, 780 (0.25 mg/kg of body weight, i.p.) 1 h after the termination of heat stress. Their physiologic and biochemical parameters were continuously monitored. Mice that survived on day 4 of heat treatment were considered survivors.

**Results**-When the vehicle-treated mice underwent heat, the fraction survival and core temperature at +4h of body heating were found to be 0 of 12 and 34.4°C±0.3°C, respectively. Administration of Premarin (1 mg/kg) 1 h after the cessation of heat stress rescued the mice from heat-induced death (fraction survival, 12/12) and reduced the hypothermia (core temperature, 37.3°C). The beneficial effects of Premarin in ameliorating lethality and hypothermia can be abolished by simultaneous administration of ICI 182, 780. Both interleukin-10 (an anti-inflammatory cytokine) and estradiol in the serum were increased significantly in heat-stressed mice administered Premarin compared with vehicle-treated heat-stressed group. Heat-induced apoptosis in the spleen, liver, and kidney were significantly reduced by Premarin. The increased levels of cellular ischemia and damage markers and inducible nitric oxide synthase in the hypothalamus during heatstroke were decreased significantly by Premarin therapy. The levels of proinflammatory cytokines and renal and hepatic dysfunction markers in plasma that are up-regulated in heat stressed mice were significantly lower in Premarin-administered mice.

**Conclusions**-The data indicate that Premarin may act via estrogen receptors to rescue mice from heatstroke-induced lethality. (The experimental protocol has been approved by the Animal Ethic Committee of Chi Mei Medical Center under guidelines of NIH publication No.85-23, revised 1996).
TEMPORAL SEQUENCING OF BRAIN ACTIVATIONS IN MENOPAUSAL HOT FLASHES

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Background - Thermoreception and thermoregulation are essential evolutionary endowments for homeotherms. In general, interoceptive systems in the brain, of which thermoreception is a specific instance, are thought to involve spinothalamocortical pathways. These may be initiated in primitive brain structures in the medulla, such as the dorsal raphé (DR). The DR projects to the insula, which is involved in interoception, the perception of internal bodily events. In humans, in vivo functional imaging has identified activity in the DR, the insula, and the dorsolateral prefrontal cortex (PFC) to exogenously-applied thermal stimulation. Hot flashes (HFs) are the most common symptom of menopause, affecting millions of women. They consist of sensations of intense heat, as well as sweating and peripheral vasodilation. Here, we sought to delineate the temporal sequence of the brain activations underlying HF events using fMRI.

Procedure - Twenty postmenopausal women with frequent HFs were heated ventrally and dorsally at 42°C for 2 h while sternal skin conductance (SSC) was recorded to detect HFs. These occurred in all women. fMRI/BOLD time series data were analyzed for a 30 image period around HF onset in 3 equi-temporal windows (Baseline, Pre HF, Post HF). The DR, the insula, and the PFC were bilaterally defined in stereotactic space. The BOLD/time series data were analyzed with Statistical Parametric Mapping (SPM). Results - Activation of the DR occurred 8 sec before the SSC response, suggesting that neuronal activity in this area precedes HF onset by up to 14 sec. This was followed by significant (T=2.4, P<.02) activation of the insula, which, in turn, was followed by significant (T=3.5, P<.002) activation of the PFC.

Conclusions - These data demonstrate, for the first time in humans, an orderly progression of neuronal activation beginning in the brainstem (DR), and moving to progressively higher brain areas in the cerebral cortex (insula, followed by PFC). A better understanding of the mechanisms underlying thermoregulation in general, and HFs in particular, could lead to better treatments for the latter. Supported by NIH Merit Award R37AG05233 to RRF.
MODELLING THERMOREGULATION IN PATIENTS UNDERGOING SURGERY

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\textbf{Background} - Nearly all patients administered anesthesia become hypothermic. Under normal physiological conditions, the core-to-peripheral temperature gradient is maintained by tonic vasoconstriction. By the induction of anesthesia, the threshold for vasoconstriction is shifted to lower temperatures. Hence, heat redistribution takes place from the warm core to the colder periphery, leading to hypothermia. For preventing peri-operative hypothermia, more knowledge is needed about the physical and physiological aspects of the impaired thermoregulatory system during anesthesia. \textbf{Procedure} - A computer model, ThermoSEM, has been developed that describes heat transfer during surgery. The model consists of three parts: 1) a passive part, which gives a simplified description of the human geometry and the passive heat transfer processes, 2) an active part that takes into account the thermoregulatory system and the effects caused by administration of anesthesia and 3) submodels, through which it is possible to adjust the boundary conditions. The active model was derived combining a pharmacological model and patient data taken during aortic valve surgery. The pharmacological model was used to calculate the anesthetic agent concentration in the blood. Anesthetic drugs lower the threshold for vasoconstriction in linear proportion to increased plasma concentration. A linear relation was derived between the anesthesia concentration calculated with help of the pharmacological model and the vasoconstriction threshold found in the aortic valve patients. \textbf{Results} - The model was validated against experimental data of healthy subjects, cardiac patients and orthopedic patients and showed good agreement. If the boundary conditions and initial conditions are accurately known, the model predicts core temperatures with a bias ranging from 0.15°C for healthy subjects to 0.5°C for patients during heart surgery (with a measured temperature drop of 7°C). Skin temperature bias is smaller than 1°C. \textbf{Conclusions} – The thermophysiological model ThermoSEM is able to predict temperature responses of healthy persons and patients during various standard surgery conditions.
CAN THE BODY TEMPERATURES CHANGES DETECT FLUID ABSORPTION DURING TRANSURETHRAL RESECTION OF PROSTATE?

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Background- One of the major complications of transurethral resection of prostate (TURP) is TURP syndrome which is caused by intraoperative absorption of irrigation solution through open prostatic venous sinuses. Hyponatremia is the deteriorating factor of the symptoms but at the same time is the useful detector of fluid absorption when “monopolar” TURP is performed with nonconductive irrigants (such as D-sorbitol). Recently developed “bipolar” transurethral resection in saline (TURis) system decreased the incidence of intraoperative hyponatremia because it allows use of normal saline solution as irrigant. Substituted detector of fluid absorption in this system for serum sodium concentration is needed for the management of those aged patients with poor cardiopulmonary function. We hypothesized that irrigant solution (kept at 40°C before use) absorption influences body temperatures during TURP and changes in temperatures can detect the fluid absorption.

Subjects and methods- Nine ASA physical status I I patients (age: 74±6 yr, mean ±SD) undergoing TURP with TURis system under spinal anesthesia were enrolled in this study. Body temperatures (tympanic membrane, finger tip, planter) as well as blood pressure, heart rate, central venous pressure (CVP), serum electrolytes concentration such as chloride (s-Cl), potassium and base excess were measured every 20 minutes during surgery. Data were statistically analyzed using repeated measures of ANOVA followed by Bonferroni’s test (significant when p<0.05). Time course changes of data and inter-factor correlations were assessed.

Results- Spinal anesthesia significantly increased planter skin temperature and decreased tympanic membrane temperature in early phase of the anesthesia. But there were no significant changes in temperatures after that during surgery. Significant increase in s-Cl (99±2 vs 104±5 mEq/L, before vs 60 minutes after the start of surgery) showed significant correlation with CVP increase (R=0.79), suggesting intraoperative fluid absorption. Intraoperative changes in temperatures showed no correlations with s-Cl increase nor with CVP increase.

Conclusion- Body temperatures changes could not detect fluid absorption during TURP with TURis system.
DO SESSIONS OF CRYOSTIMULATION HAVE INFLUENCE ON WHITE BLOOD CELLS COUNT, LEVEL OF IL6 AND THE TOTAL OXIDATIVE AND ANTIOXIDATIVE STATUS IN HEALTHY MEN?

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\textbf{Background} - The influence of extremely low temperatures on the human body and physiological reactions are not fully recognized. It has been postulated that cryostimulation could modify immunological reactions, leukocytes mobilization and levels of cytokines. \textbf{Procedure} - The aim of this research was to estimate the influence of a ten sessions 3-minute-long exposures to cryogenic temperature (-130°C) on the white blood cells count, level of IL6 and the total oxidative and antioxidative status in 15 young, clinically healthy men. Blood samples were obtained in the morning before cryostimulation, again 30 min after treatment and the next day in the morning, both during the 1\textsuperscript{st} and 10\textsuperscript{th} session. The white blood cells (WBC) count, level of IL6 and total lipid peroxides as the total oxidative status (TOS) and the total antioxidative status (TAS), were measured.

\textbf{Results} - After completing a total of ten whole-body therapy sessions a significant increase in WBC count, especially lymphocytes and monocytes was noted. There was an increase in level of IL6 after 1\textsuperscript{st} and the last cryostimulation the most pronounced after 10\textsuperscript{th} session. On the contrary the TAS level decreased significant after the treatment. \textbf{Conclusions} – Repeated expositions to extremely low temperatures use in cryostimulation have mobilization effect on immunological system.