# Involvement of a capsaicin-sensitive TRPV1-independent mechanism in lipopolysaccharide-induced fever in chickens

Motamed Elsayed Mahmoud[1, 3)], Yasutake Shimizu[1)]*, Takahiko Shiina[1)], Hideki Nikami[2)], Reem Mahmoud Dosoky [3)], Moustafa Mohamed Ahmed [3)] and Tadashi Takewaki[1)]

[1)] Department of Basic Veterinary Science, Laboratory of Physiology, The United Graduate School of Veterinary Sciences, and [2)] Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, and [3)] Department of Animal Hygiene, Animal Behavior, Faculty of Veterinary Medicine, Assiut University, Egypt

*CORRESPONDENCE to: Shimizu, Y., Department of Basic Veterinary Science, Laboratory of Physiology, the United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Tel & Fax: 058-293-2940, e-mail: yshimizu@gifu-u.ac.jp

**Abstract**

It has been demonstrated that capsaicin blocks lipopolysaccharide (LPS)-induced fever in mammals. In this study, we investigated TRPV1 (transient receptor potential ion channel of vanilloid subtype-1)-independent action of capsaicin on LPS-induced fever in chickens. The chicken is a valuable model for this purpose because chicken TRPV1 has been shown to be insensitive to capsaicin and thus the effects of capsaicin can be attributed to TRPV1-independent mechanisms. Administration of capsaicin (10 mg/kg, iv) to conscious unrestrained chicks at 5 days of age caused a transient decrease in body temperature. This effect of capsaicin was not observed in chicks that had been pretreated twice with capsaicin, indicating that the capsaicin-sensitive pathway can be desensitized. LPS (2 mg/kg, ip) induced fever that lasted for about 2.5 hours, but fever was not induced in chicks that had been pretreated with capsaicin for 2 days. The preventive effect of capsaicin on LPS-induced fever was not blocked by capsazepine, an antagonist for TRPV1, but the antagonist per se blocked the febrile response to LPS. These findings suggest that a capsaicin-sensitive TRPV1-independent mechanism may be involved in LPS-induced fever.

***Keywords***: *capsaicin, capsazepine, chicken, fever, LPS, TRPV1*

## 1. Introduction

Administration of lipopolysaccharides (LPS), cell wall products of gram negative bacteria, induces fever in mammals. The key events associated with LPS-induced fever are processing of LPS by macrophages and endothelial cells in the liver, lung and brain, releasing of endogenous pyrogenic cytokines such as interleukin (IL)-1, IL-6 and TNF- , and subsequent triggering of central thermosensory neurons by ultimate fever mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO) (Ivanov *et al.*, 2002; Ivanov and Romanovsky, 2004; Gray *et al.*, 2005; Steiner *et al.*, 2006a, b; Romanovsky, 2007). Both heat-producing and heat-conserving mechanisms are thereby activated, resulting in an elevation of body temperature. LPS also causes fever in birds, including domestic chickens, despite the fact that birds have higher body temperature than that in mammals, and LPS can stimulate chicken peritoneal macrophages to release pyrogenic cytokines as in mammalian macrophages (Cheema *et al*., 2003; El-Mahmoudy *et al.*, 2002). Since inhibitors of PGE2 and NO block fever in both mammals and birds, the febrile response to LPS would be mediated by COX2/PGE2 and iNOS/NO pathways (Johnson *et al.*, 1993a, b; Baert *et al.*, 2005; Gray *et al.*, 2005). Fundamental mechanisms responsible for LPS-induced fever in chickens may therefore be identical to those in mammals.

Recently, it has been demonstrated that pretreatment with capsaicin (8-methyl-N-vanillyl-6-noenamide, CAP) blocks the early phase of LPS-induced fever in rats (Dogan *et al.*, 2004). It is generally accepted that CAP acts on a ligand-gated nonselective cation channel termed TRPV1 (transient receptor potential ion channel of vanilloid subtype-1) (Székely *et al.*, 2000; Dogan *et al.*, 2004; Petervari *et al.*, 2005), which is mainly expressed by sensory neurons. In contrast to the fact that CAP defunctionalizes afferent neurons of the vagus nerve, these neurons do not express

TRPV1 (Holzer, 1998). In addition, the inhibitory effect of CAP pretreatment on LPS-induced fever does not seem to be related to TRPV1, because a selective TRPV1 agonist (resiniferatoxin) fails to mimick the CAP effect and a competitive TRPV1 antagonist, capsazepine, does not block the CAP effect (Dogan *et al.*, 2004). These findings indicate that there is a non-neural TRPV1-independent mechanism for the action of CAP. In accordance with this, it has been shown in LPS-stimulated murine macrophages that CAP inhibits the activation of NF- B and production of PGE2 and NO via a TRPV1-independent pathway (Oh *et al.*, 2001; Kim *et al.*, 2003; Park *et al.*, 2004).

Chicken TRPV1 has been identified and characterized. Interestingly, chicken TRPV1 is activated by heat or protons as is its counterpart in mammals but is insensitive to capsaicin (Jordt and Julius, 2002). In agreement with this, birds are generally indifferent to the burning pain sensation induced by capsaicin and prefer to eat pungent rather than non-pungent hot peppers (Clapham, 1997; Romisch, 2002). However, chickens are not completely insensitive to CAP. For instance, a single CAP injection in a chicken causes hypothermia, and repeated injections induce impaired thermoregulation at high ambient temperature and loss of ability to discriminate between warm and cool drinking water (Mason and Maruniak, 1983; Sann *et al.*, 1987). Thus, it is reasonable to assume that these responses of chickens to CAP may be related to the putative TRPV1-independent mechanism. In other words, the chicken would serve as a valuable model to analyze the TRPV1-independent pathway.

In view of the advantage of the chicken model to explore the TRPV1-independent mechanism for the CAP effect, we consider it of interest to examine how CAP affects LPS-induced fever in chickens. Our results provide a firm conclusion

that the inhibitory effect of CAP on LPS-induced fever can be attributed to a TRPV1-independent mechanism.

## 2. Materials and Methods

### *2.1. Animals*

Newly hatched male chicks (White Leghorn line), specific pathogen free, were brought from Goto Chick Company (Gifu, Japan) at one day of age with a body weight range of 40 ± 7 g. Each chick had free access to feed and water. The chicks were maintained on a 12:12 light-dark cycle and kept in standard thermostatically controlled cages to match chicks' requirements (initial setting of 35°C with temperature reduction of 1°C every day). The study was approved by the Animal Care and Use Committee of Gifu University.

### *2.2. Measurement of colonic temperature*

Colonic temperature was measured in conscious unrestrained chicks in their home cages under neutral ambient temperature of 33 °C. A lubricated thermistor probe (model XN-64, Technol Seven, Yokohama, Japan) was inserted gently 5 cm beyond the vent and the colonic temperature was monitored using a peripheral processor connected to a computerized medical system (Chuo Electronic Co., Hong Kong). Baseline temperature recordings were made for one hour, and chicks exhibiting no stress fever (Jones *et al.*, 1983) were used for the experiment. Each chick was used only once. To avoid circadian variations in colonic temperature recordings, measurements were started at 8 a.m.

### *2.3. Injections of capsaicin and capsazepine*

Both CAP and capsazepine were purchased from Sigma (St Louis, MO, U.S.A.). CAP and capsazepine were dissolved in ethanol (99.8%) and then mixed

with an equal volume of Tween 80 solution and finally diluted in 0.9% saline (final ratio: 10% ethanol, 10% Tween 80 and 80% saline). CAP (10 mg/kg) and/or capsazepine (40 mg/kg) were injected into the brachial vein at 3, 4 and 5 days of age. A vehicle solution was injected into control chicks.

*2.4. Injection of LPS*

A purified lyophilized phenol extract of *Escherichia coli* endotoxin (0111:B4; Sigma, St Louis, MO) was dissolved in sterile saline, aliquoted, and frozen at – 20°C. Five-days-old chicks were injected with LPS (2 mg/kg, ip). An injection of an equivalent volume of saline was used as a control. The dose of LPS was determined on the basis of the results of an earlier study (Johnson *et al.*, 1993a). All injections were conducted between 9 a.m. and 10 a.m.

*2.5. Preparation of primary culture of chicken peritoneal macrophages*

Abdominal exudate cells were collected as previously described (Sabet *et al.,* 1977) followed with modifications (Hussain and Qureshi, 1997). Briefly, a Sephadex suspension (Sephadex G-50 super fine, Sigma) was prepared in distilled water, mixed, and washed several times in sterile saline solution (0.8%) and finally resuspended in saline (0.85%) at a concentration of 3% (bead dry weight/volume of saline). Chicks (5 days of age, 60-80 g in body weight) were injected intraperitoneally with Sephadex solution at 1ml/50g body weight in the left anterior abdominal quadrant. After 72 hours, chicks were exsanguinated via the carotid artery, abdominally de-feathered and de-skinned, and the abdominal cavity was flushed with 70% ethanol. Then the peritoneal cavity was rinsed and aspirated with 5 ml of 1× HBSS and 0.5 U/ml of heparin. The procedure was repeated and approximately 10 ml of peritoneal wash was recovered per chick. The cells were harvested by centrifugation at 1000 g for 10 min. Then pooled cells from 10 to 20 chicks were resuspended in RPMI 1640

containing 10% heat-inactivated fetal bovine serum, 1% chicken serum, 200 μg/ml gentamycin and 100 U/ml penicillin. Cell counts and viability assessment were made using trypan blue exclusion stain. The cells were suspended in the culture medium and seeded in 15-ml tissue culture flasks to obtain purified macrophages. After 2 h of incubation at 41 °C and 5% $CO_2$, non-adherent cells were washed away and were re-incubated overnight after replacing the medium with fresh medium. Cells harvested by scraping from culture flasks were resuspended in serum-free medium and inoculated at 1.0 $X10^6$ cells /ml in a 12-well culture plate.

Cells were incubated with CAP (10 μM) and/or capsazepine (40 μM) in the presence or absence of LPS (10 μg/ml) at 41 °C. After 24 hours of incubation, culture supernatants were collected for nitrite assay.

*2.6. Measurement of nitrite*

Since the level of nitrite reflects NO synthesis, the level of nitrite, a stable product of NO, was measured in 96-well microtiter plates by Griess reaction as described by Green *et al*. (1982). In brief, samples (100 μl/well) were incubated with an equal volume of Griess solution (1% sulfanilamide in 5% phosphoric acid + 1% α-naphthylamine in distilled water, Sigma, St. Louis, MO, USA) in darkness at room temperature for 10 min. Optical densities at a wavelength of 540 nm were evaluated by an ELISA reader (MPRA4, Tokyo, Japan). A standard curve was made by various concentrations of sodium nitrite to calculate concentrations of nitrite in the culture supernatant.

*2.7. Statistical analysis*

Data are presented as means ± S.D. Statistical analysis was performed by using the Statistical Analysis System program (SAS Institute, Cary, NC, USA).

Tukey's Studentized Range (HSD) was selected for comparisons among treatments. A difference was considered to be significant if $P < 0.05$.

## 3. Results

### *3.1. Effects of single and repeated injections of CAP on colonic temperature of chicks*

Intravenous administration of CAP (10 mg/kg) in 5-day-old chicks for the first time induced a significant decrease in colonic temperature (down to 40.4 ± 0.5 °C from the control value of 41.0± 0.3 °C ($F$ (3, 349) = 12.4, $P < 0.05$) (Fig.1). The decrease in colonic temperature lasted for 1.5 hours, and colonic temperature recovered to the basal temperature within 6 hours after CAP injection. In contrast, no such reduction in colonic temperature was observed in chicks that had been pretreated successively on days 3 and 4 (Fig.1). Vehicle injection did not cause any significant changes in colonic temperature in any group (Fig.1).

### *3.2. Effect of LPS injection on colonic temperature of chicks*

An intraperitoneal injection of LPS (2 mg/kg) induced a rise in colonic temperature of about 1 °C in 5-day-old chicks kept at a thermoneutral ambient temperature of 33°C (Fig. 2). The rise in colonic temperature began 2 hours after LPS injection and lasted for 2.5 hours. Peak colonic temperature, 42.1 ± 0.3 °C ($F$ (1, 437) = 43.4, $P < 0.04$), was observed at 3 hours after the injection. Then colonic temperature gradually decreased and reached the control level by 6 hours. In saline-treated chicks, there was no significant change in colonic temperature.

### *3.3. Effect of CAP pretreatment on LPS-induced fever in chicks*

Next, we injected LPS into 5-day-old chicks that had been pretreated successively with CAP on days 3 and 4. As shown in Fig. 3A, the LPS-induced rise in colonic temperature was abolished by CAP pretreatment ($F$ (1, 261) = 6.34, $P <$

0.02). Saline injection resulted in no significant changes in colonic temperature (Fig.3A).

*3.4. Effect of simultaneous pretreatments with CAP and capsazepine on LPS-induced fever in chicks*

To see if the preventive effect of pretreatments with CAP on LPS-induced fever would be mediated by TRPV1, an antagonist for the receptor, capsazepine, was injected simultaneously with CAP during pretreatment sessions. The effect of CAP was still evident and febrile response to LPS was absent even after application of the antagonist with CAP ($F$ (1, 195) = 11.4, $P < 0.03$) (Fig. 3B). In another group of chicks, capsazepine was injected in the absence of CAP as a control. Unexpectedly, capsazepine itself showed a preventive effect similar to that of CAP on LPS-induced rise in colonic temperature ($F$ (1, 227) = 4.17, $P < 0.03$) (Fig. 3C).

*3.5. Nitrite production*

We investigated the effects of capsaicin and/or capsazepine on inducible nitric oxide production by LPS-stimulated macrophages. Isolated chicken peritoneal macrophages were incubated with LPS (10 μg/ml) in the presence or absence of CAP and/or capsazepine. Non-treated macrophages, without LPS stimulation, produced minimal levels of nitrite (1.9 ± 1.3 μM /1X $10^6$ cells). However, 24 hours after LPS stimulation, nitrite production was markedly increased (18.1± 3.4 μM) as shown in Fig. 5. Nitrite production was reduced by CAP (10 μM) to 4.6 ± 2.4 μM, but this effect was not abated by co-administration of its competitive antagonist capsazepine. Nevertheless, capsazepine (40 μM) alone attenuated nitrite production in LPS-stimulated macrophages (F (3, 24) = 1.6, P < 0.02) (Fig. 5).

## 4. Discussion

Recently, it has been reported that a CAP-sensitive TRPV1-independent pathway may be related to febrile response in rats. This view is largely dependent on pharmacological evidence that pretreatment with CAP resulted in a loss of the first phase of LPS-induced fever, but the effect of CAP was neither mimicked by the ultrapotent TRPV1 agonist resiniferatoxin nor blocked by the TRPV1 antagonist capsazepine (Dogan *et al*., 2004). We considered that it would be worthwhile to validate the TRPV1-independent mechanism for the CAP effect by using an alternative model. Since chicken TRPV1 has been shown to be CAP-insensitive, effects of CAP itself and/or responses altered by its pretreatment might be exclusively brought about via the TRPV1-independent pathway in chickens. Therefore, in the present study, we used chickens and obtained the following findings. Firstly, CAP transiently decreased the body temperature of young (5 days post-hatching) chickens; this effect was abolished by injection of CAP repeatedly for 2 days. Secondly, LPS induced monophasic fever in young chickens, but fever was not observed after pretreatment with CAP for 2 days. Finally, the preventive effect of CAP on LPS-induced fever was not blocked by capsazepine. Also, in isolated macrophages from chickens, both CAP and capsazepinbe had the same inhibitory effect on LPS-induced NO production. These findings suggest that a CAP-sensitive TRPV1-independent mechanism that may involve NO production by macrophages mediated induction of fever by LPS.

The present results showed that a single intravenous injection of CAP (10 mg/kg) induces a transient decrease in body temperature in 5-day-old chicks but that this hypothermia totally disappeared when chicks were pretreated with the same dose of CAP on days 3 and 4 successively (Fig.1). These results are consistent with an earlier

observation in mature chickens (Sann *et al.*, 1987). CAP-induced hypothermia and its disappearance by repeated administrations of the drug have also been observed in mammals (Jancso-Gabor *et al.*, 1970; Szikszay *et al.*, 1982; Kobayashi *et al.*, 1998). It has been suggested that CAP induces hypothermia through autonomic responses such as vasodilatation and salivation (Jancso-Gabor *et al.*, 1970) or panting (Sann *et al.*, 1987). Although the CAP effects on body temperature are apparently similar, the initiating mechanisms may be completely different in mammals and birds. In mammals, the hypothermic effect of CAP is mediated entirely by TRPV1 (Caterina *et al.*, 2000). The desensitization phenomenon of TRPV1 by CAP has been well established, providing a reasonable explanation for the fact that the effect of CAP disappeared after repeated administration in mammals. In contrast, chicken TRPV1 is insensitive to capsaicin (Jordt and Julius, 2002). Thus, the hypothermia induced by CAP in chickens may not be related to TRPV1. The mechanism by which CAP induces hypothermia in birds has not been elucidated. It should be noted, however, that the putative TRPV1-independent mechanism can be completely desensitized by repeated administration of CAP in chicks (Fig.1). We considered this desensitized condition caused by CAP to be suitable for analyzing the role of the CAP-sensitive TRPV1 independent pathway in LPS-induced fever.

A number of thermoregulatory disturbances are produced after LPS injection in both mammals and birds (Jones *et al.*, 1983; Johnson *et al.*, 1993a; Rudaya *et al.*, 2005). The fundamental mechanisms responsible for LPS-induced fever in mammals are apparently similar in many aspects to those in birds (Johnson *et al.*, 1993a; Gray *et al.*, 2005; Steiner *et al.*, 2006a, b). It has been demonstrated that a CAP-sensitive mechanism is involved in LPS-induced fever in mammals. CAP-sensitive mechanisms are composed of both TRPV1-dependent and -independent pathways.

Evidence for contribution of the TRPV1-independent pathway to LPS-induced fever in mammals has been provided by experiments using rats whose TRPV1-positive neurons are desensitized by intraperitoneal injection of CAP (Dogan *et al*., 2004) or using TRPV1-null mutant mice (Iida *et al.*, 2005). However, these methods for eliminating TRPV1-mediated actions of CAP would be complicated to be evaluated, because effects of other stimuli for TRPV1, such as heat or acid stimuli, are also eliminated. In contrast, chickens are a natural model for inactive TRPV1 regarding the CAP receptor with intact responsiveness to other stimuli. Our results (Figs. 2 and 3A) showing that CAP pretreatment in chicks abolished the rise in body temperature after LPS injection clearly demonstrate that the CAP-sensitive TRPV1-independent mechanism plays a pivotal role in LPS-induced fever.

TRPV1-independent effects of CAP in mammals have been demonstrated by in vitro experiments. In LPS-stimulated macrophages, CAP inhibits the activation of NF- B, release of the pro-inflammatory cytokine TNF-α (Park *et al.*, 2004), expression of iNOS, activation of COX2 and subsequent production of NO and PGE2 (Kim *et al.*, 2003). These are substantially TRPV1-independent, because mammalian macrophages do not express TRPV1 (Kim *et al.*, 2003; Schaumacher *et al.*, 2000). In agreement with this, the TRPV1 antagonist capsazepine failed to block the ability of CAP to inhibit NO and PGE2 synthesis in macrophages (Kim *et al.*, 2003). On other hand, capsazepine itself mimicked CAP inhibition of NF- B, TNF-α, iNOS and NO production (Oh *et al.,* 2001; Kim *et al.,* 2003). Recently, it has been shown that the inhibitory effect of CAP on TNF-α production in LPS-stimulated macrophages is related to the peroxisome proliferator-activated receptors (PPAR) ligand-like action of the drug (Park *et al.*, 2004). Although the nature of the CAP-sensitive TRPV1-

independent mechanism in chickens is unclear at present, it seems worthwhile for future investigation to focus on PPAR as a target for CAP.

The lack of an antagonizing effect of capsazepine has been considered to be supportive evidence for TRPV1-independent actions of CAP. Consistent with this, co-administration of capsazepine with CAP did not inhibit the effects of CAP on LPS-induced fever (Fig. 3B) and on NO production by LPS-stimulated macrophages (Fig. 4). However, an unexpected effect of capsazepine was also observed; that is, capsazepine itself, similar to CAP, exerted an inhibitory effect on LPS-induced fever (Fig. 3C). Taken together, these findings suggest that both drugs act as agonists to the TRPV1-independent pathway. These non-competing, but rather identical, effects of a TRPV1 agonist and antagonist are unlikely to be specific for chicks, since capsazepine inhibits LPS-induced activation of NF- B, release of TNF-α, NO production and PGE-2 synthesis as does CAP in mammalian macrophages (Oh *et al.*, 2001; Kim *et al*., 2003; Mori *et al.*, 2006).

In summary, the contribution of CAP-sensitive TRPV1-independent mechanisms to LPS-induced fever has been assessed by using a chicken model. The results of the present study demonstrate that these mechanisms play a key role in LPS-induced fever in newly hatched chicks. Considering that fundamental mechanisms for thermoregulation and pathological events related to fever are similar in mammals and birds, the conclusion of this study would be applicable to mammals.

**Acknowledgments**

The authors are grateful to Goto Chick Company, Gifu, Japan for kindly providing one-day-old male Leghorn chicks. This work was supported by a Grant-in-

Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

**References**

Baert, K., Duchateau, L., De Boever, S., Cherlet, M., De Backer, P., 2005. Antipyretic effect of oral sodium salicylate after an intravenous of E.coli LPS injection in broiler chicken. Br. Poult. Sci. 46, 137-43.

Caterina, M.J., Lefller, A., Malberg, A.B., Martin, W.J., Trafton, J., Petersenzeit, K.R., Koltzenberg, M., Basbaum, A.I., Julius, D., 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288, 306-313.

Cheema, M.A., Qureshi, M.A., Havenstein, G.B., 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82, 1519-1529.

Clapham, DE., 1997. Some like it hot: spicing up ion channels. Nature 389, 783-784.

Dogan, M.D., Patel, S., Rudaya, A.Y., Steiner, A.A., Székely, M., Romanovsy, A.A., 2004. Lipopolysaccharide fever is initiated via capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. Br. J. Pharmacol. 143, 1023-1032.

El-Mahmoudy, A., Matsuyama, H., Borgan, M.A., Shimizu, Y., El-Sayed, M.G., Minamoto, N., Takewaki, T., 2002. Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. Int. Immunopharmacol. 2, 1603-1611.

Gray, D.A., Maloney, S.K., Kamerman, P.R., 2005. Lipopolysaccharide-induced fever in Pekin ducks is mediated by prostaglandins and nitric oxide and modulated by adrenocortical hormones. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R1258-R1264.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 126, 131-138.

Holzer, P., 1998. Neural injury, repair, and adaptation in the GI tract. II. The elusive action of capsaicin on the vagus nerve. Am. J. Physiol. Gastrointest. Liver Physiol. 275, G8-G13.

Hussain, I., Quershi, M.A., 1997. Nitric oxide synthase activity and mRNA expression in chicken macrophages. Pout. Sci. 76, 1524-1530.

Iida, T., Shimizu, I., Nealen, M.L., Campbell, A., Caterina, M., 2005. Attenuated fever response in mice lacking TRPV1. Neurosci. Lett. 378, 28-33.

Ivanov, A.I., Romanovsky, A.A., 2004. Prostaglandin E2 as a mediator of fever: synthesis and catabolism. Front. Biosci. 9, 1977-1993.

Ivanov, A.I., Pero, R.S., Sheck, A.C., Romanovsky, A.A., 2002. Prostaglandin E2-synthesizing enzymes in fever: differential transcriptional regulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283, R1104-R1117.

Jancsó-Gábor, A., Szolcsányi, j., Janscó, N., 1970. Stimulation and desensitization of the hypothalamic heat sensitive structures by capsaicin in rats. J. Physiol. 208, 449-459.

Johnson, R.W., Curtis, S.E., Dantzer, R., Bahr, J.M., Kelly, K.W., 1993a. Sickness behavior in birds caused by peripheral or central injection of endotoxin. Physiol. Behav. 53, 343-348.

Johnson, R.W., Curtis, S.E., Dantzer, R., Kelly, K.W., 1993b. Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol. Behav. 53, 127-131.

Jones, C.A., Edens, F.W., Denbow, D.M., 1981. Rectal temperature and blood chemical responses of young chickens given E. coli endotoxin. Poult. Sci. 60, 2189-2194.

Jones, C.A., Edens, F.W., Denbow, D.M., 1983. Influence of age on the temperature response of chickens to Escherichia coli and Salmonella typhimurium endotoxins. Poult. Sci. 62, 1553-1558.

Jordt, S.E., Julius, D., 2002. Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108, 421-430.

Kim, C.-S., Kawada, T., Kim, B.-S., Han, I.-S., Choe, S.-Y., Kurata, T., Yu, R., 2003. Capsaicin exhibits anti-inflammatory property by inhibiting IkB-degradation in LPS stimulated peritoneal macrophages. Cell. Signal. 15, 299-306.

Kobayashi, A., Osaka, T., Namba, Y., Inoue, S., Lee, T.H., Kimura, S., 1998. Capsaicin activates heat loss and heat production simultaneously and independently in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 275, R92-R98.

Mason, J.R., Mruniak, J.A., 1983. Behavioral and physiological effects of capsaicin in red-winged blackbirds. Pharmacol. Biochem. Behav. 19, 857-862.

Mori, A., Lehmann, S., O'kelly, J., Kumagai, T., Desmond, J.C., Pervan, M., McBride, W.H., Kizaki, M., Koeffler, H.P., 2006. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Res. 66, 3222-3229.

Oh, G.-S., Pae, H.-O., Seo, W.-G., Kim, N.-Y., Pyun, K.H., Kim, I.-K., Shin, M.-K., Chung, H.-T., 2001. Capsazepine, a vanilloid receptor antagonist, inhibits the expression of inducible nitric oxide synthase gene in lipopolysaccharide-

stinulated RAW 264.7 macrophages through the inactivation of nuclear transcription factor-kappa B. Int. Immunopharmacol. 1, 777-784.

Park, J.-Y., Kawada, T., Han, I.-S., Kim, B.-S., Goto, T., Takahashi, N., Fushiki, T., Kurata, T., Yu, R., 2004. Capsaicin inhibits the production of tumor necrosis factor α by LPS-stimulated murine macrophages, RAW 264.7: a PPARγ ligand-like action as a novel mechanism. FEBS Lett. 572, 266-270.

Petervari, E., Garami, A., Pakai, E., Székely, M., 2005. Effect of perineural capsaicin treatment of the abdominal vagus on endotoxin fever and on a non-febrile thermoregulatory event. J. Endotoxin Res. 11, 260-266.

Romanovsky, A.A., 2007. Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R37-R46.

Romisch, K., 2002. Some like it hot. Trends Biochem. Sci. 27, 174.

Rudaya, A.Y., Steiner, A.A., Robbins, J.R., Dragic, A.S., Romanovsky, A.A., 2005. Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R1244- R1252.

Sabet, T., Wen-Chenq, H., Stanisz, M., El-Domeiri, A. and Van Alten, P., 1977. A simple method for obtaining peritoneal macrophages from chickens. J. Immunol. Methods 14, 103-110.

Sann, H., Harti, G., Pierau, F.K., Simon, E., 1987. Effect of capsaicin upon afferent and efferent mechanisms of nociception and temperature regulation in birds. Can. J. Physiol. Pharmacol. 65, 1347-1354.

Schaumacher, M.A., Moff, I., Sudanaqunta, S.P., Levine, J.D., 2000. Molecular cloning of an N-terminal splice variant of the capsaicin receptor. Loss of N-

terminal domain suggests functional divergence among capsaicin receptor subtypes. J. Biol. Chem. 275, 2756-2762.

Steiner, A.A., Chakravarty, S., Rudaya, A.Y., Herkenham, M., Romanovsky, A.A., 2006 a. Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoeitic cells. Blood 107, 4000-4002.

Steiner, A.A., Ivanov, A.I., Serrats, J., Hosokawa, H., Phayre, A.N., Robbins J.R., Roberts J.L., Kobayashi, S., Matsumura, K., Sawchenko, P.E., Romanovsky, A.A., 2006 b. Cellular and molecular basis of the initiation of fever. PLoS Biol. 4, 1517-1524.

Szikszay, M., Obál, F.Jr., Obál, F., 1982. Dose-response relationships in the thermoregulatory effects of capsaicin. Naunyn-Schmiedebergs Arch. Pharmacol. 320, 97-100.

Székely, M., Balasko, M., Kulchitsky, V.A., Simons, C.T., Ivanov, A.I., Romanovsky, A.A., 2000. Multiple neural mechanisms of fever. Auton. Neurosci. 85, 78-82.

**Figure legends**

**Fig. 1: Effects of capsaicin on colonic temperature in chicks.**

Colonic temperature was measured in conscious unrestrained 5-day-old chicks in their home cages under neutral ambient temperature of 33 °C. Capsaicin (CAP; 10 mg/kg) was given intravenously to chicks with (●, n = 6) or without (○, n = 10) pretreatment of CAP at 3 and 4 days of age (upper panel). A vehicle was intravenously injected as a control into 6 chicks pretreated with CAP (●) and into 10 chicks not pretreated with CAP (○) (lower panel). Values are means ± SD for pretreated chicks. * $P < 0.05$ vs non-pretreated chicks.

**Fig. 2: Effects of LPS injection on colonic temperature in chicks.**

Chicks at 5 days of age were injected intraperitoneally with 2 mg/kg LPS (●) or a vehicle (0.9% saline, ○) at time zero, and colonic temperature was measured. Values are means ± SD for 8 LPS-injected or 12 vehicle-injected chicks. * $P < 0.05$ vs vehicle-injected chicks.

**Fig. 3: Effects of CAP and/or capsazepine (CPZ) pretreatment on LPS-induced fever.**

Chicks at 3 and 4 days of age were pretreated with CAP (10 mg/kg) in the absence [A] or presence [B] of capsazepine (40 mg/kg) or with capsazepine (40 mg/kg) alone [C], and then LPS (2 mg/kg) was injected intraperitoneally at 5 days of age (upper panels). Saline was intraperitoneally injected as a control into chicks in respective conditions (lower panels). Values are means ± SD for 5-7 chicks. * $P <$ 0.05 vs vehicle-pretreated chicks.

**Fig. 4: Effects of CAP and/or capsazepine (CPZ) on nitrite production by LPS-stimulated peritoneal macrophages.**

Sephadex-elicited chicken peritoneal macrophages ($1.0 \times 10^6$ cells /ml) in a 12-well culture plate were incubated with medium alone or with reagents for 24 hours, and the culture plate was incubated with medium alone or with reagents for 24 hours and then the supernatant was analyzed for nitrite levels. Each value is mean ± SD from three separate experiments. Each experiment consisted of 2 to 3 replicate cultures. * $P < 0.05$ vs non-treated cells.

Fig. 1

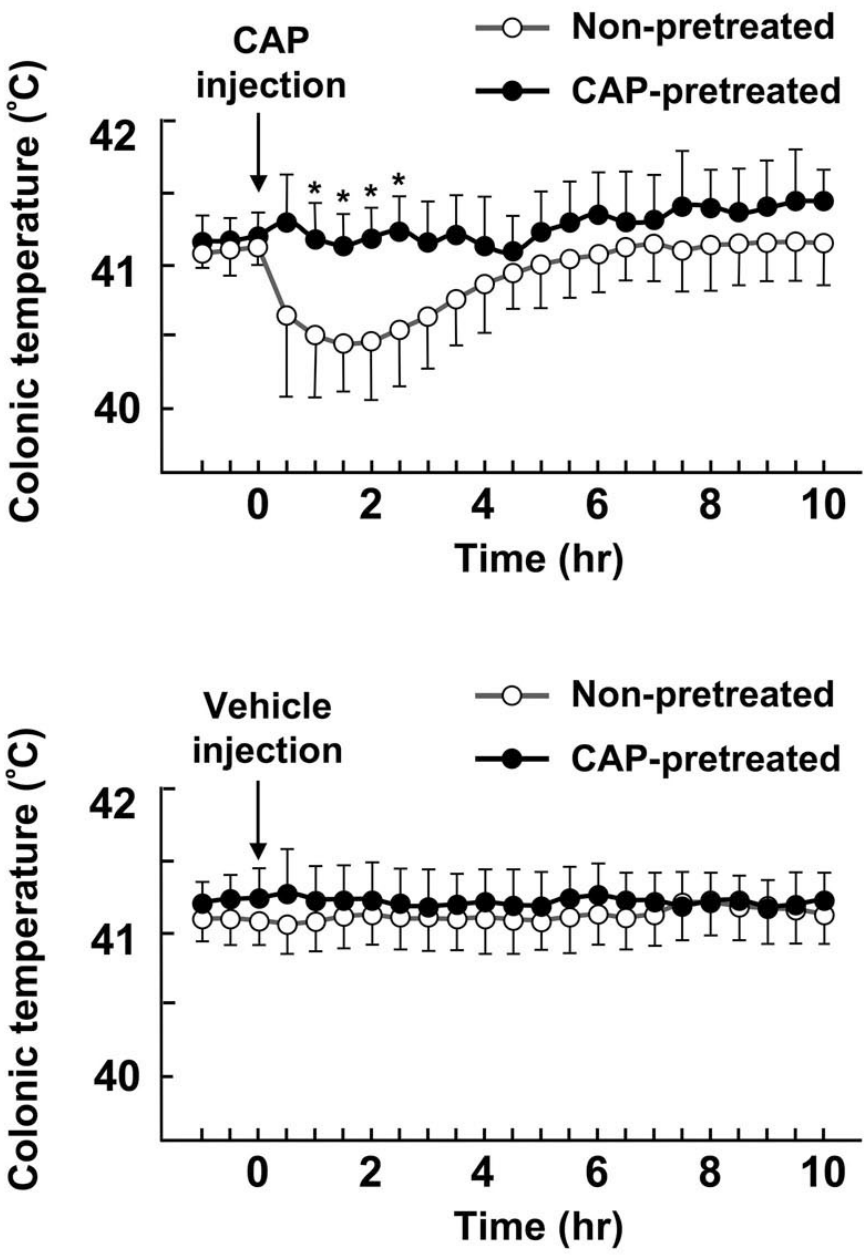

CAP
injection
Non-pretreated
CAP-pretreated
Colonic temperature (°C)
42
41
40
0
2
4
6
8
10
Time (hr)
Vehicle
injection
Non-pretreated
CAP-pretreated
Colonic temperature (°C)
42
41
40
0
2
4
6
8
10
Time (hr)

Fig. 2

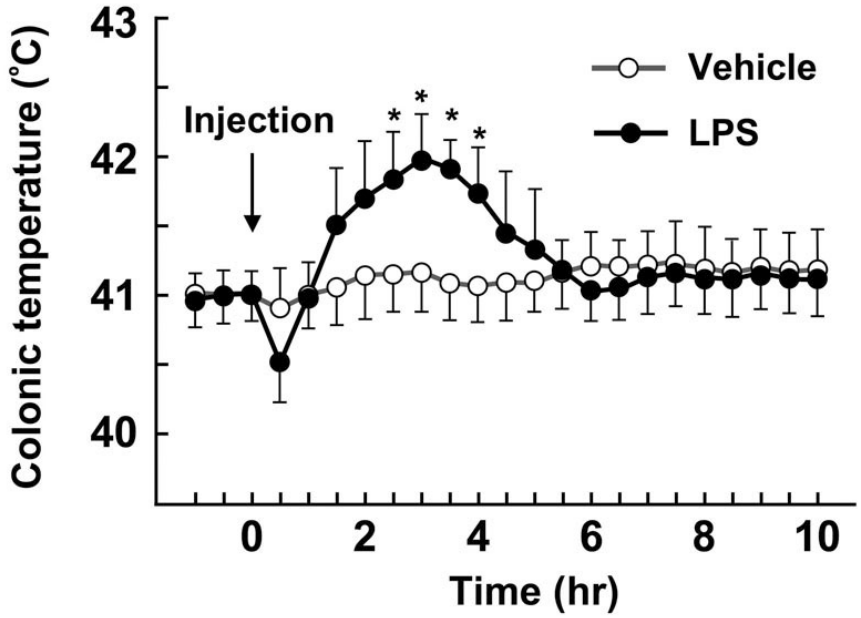

Colonic temperature (°C)
43
42
41
40
Injection
Vehicle
LPS
*
*
*
*
0
2
4
6
8
10
Time (hr)

Fig.3

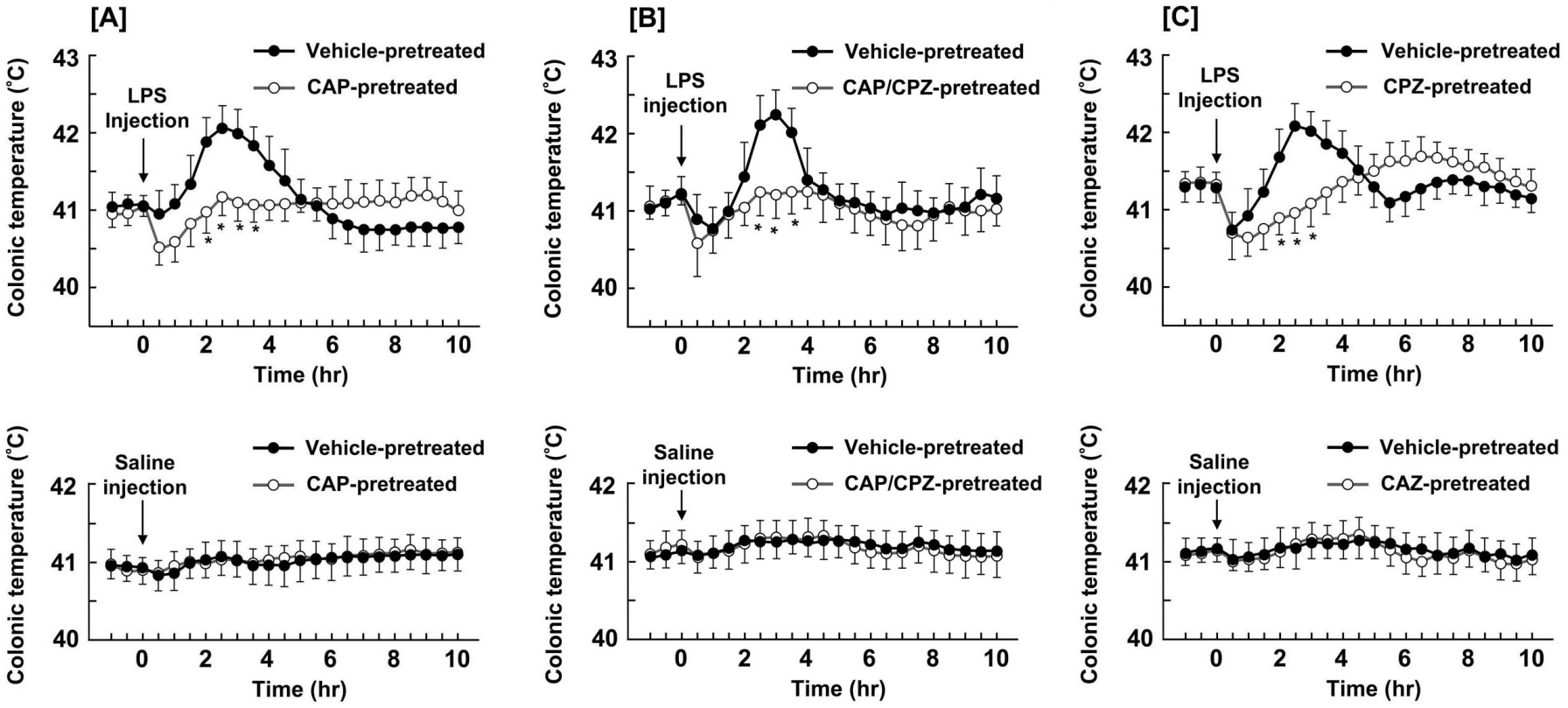
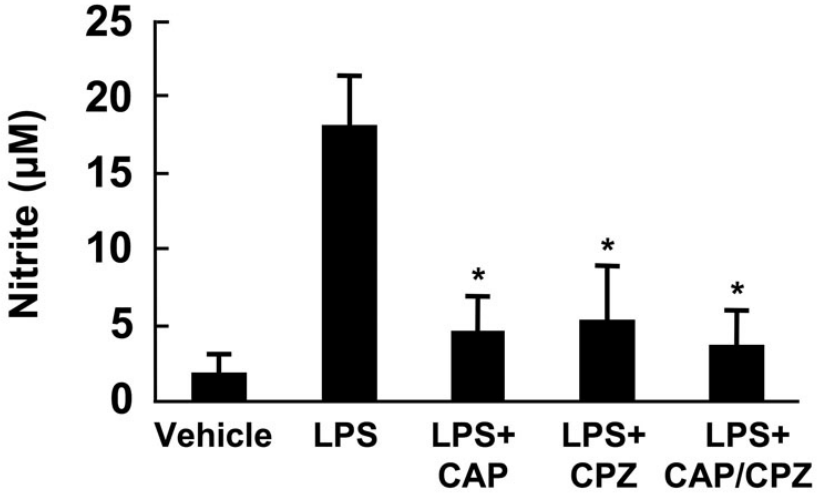

[A]
[B]
[C]
Vehicle-pretreated
CAP-pretreated
CAP/CPZ-pretreated
CPZ-pretreated
CAZ-pretreated
LPS Injection
LPS injection
Saline injection
Colonic temperature (°C)
Time (hr)
43
42
41
40
0
2
4
6
8
10
*

Fig. 4


Nitrite (μM)
25
20
15
10
5
0
*
*
*
Vehicle
LPS
LPS+ CAP
LPS+ CPZ
LPS+ CAP/CPZ