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Thromboxane A_2 and prostaglandin $F_{2\alpha}$ mediate inflammatory tachycardia

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Systemic inflammation induces various adaptive responses including tachycardia. Although inflammation-associated tachycardia has been thought to result from increased sympathetic discharge caused by inflammatory signals of the immune system[1], definitive proof has been lacking. Prostanoids, including prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane (TX) A_2, exert their actions through specific receptors: DP, EP (EP_1, EP_2, EP_3, EP_4), FP, IP and TP, respectively[2]. Here we have examined the roles of prostanoids in inflammatory tachycardia using mice that lack each of these receptors individually. The TXA_2 analog I-BOP and $PGF_{2\alpha}$ each increased the beating rate of the isolated atrium of wild-type mice *in vitro* through interaction with TP and FP receptors, respectively. The cytokine-induced increase in beating rate was markedly inhibited in atria from mice lacking either TP or FP receptors. The tachycardia induced in wild-type mice by injection of lipopolysaccharide (LPS) was greatly attenuated in TP-deficient or FP-deficient mice and was completely absent in mice lacking both TP and FP. The β-blocker propranolol did not block the LPS-induced increase in heart rate in wild-type animals. Our results show that inflammatory tachycardia is caused by a direct action on the heart of TXA_2 and $PGF_{2\alpha}$ formed under systemic inflammatory conditions.

We isolated the right atrium from wild-type mice or mice deficient in each type of prostanoid receptor, and examined chronotropic effects of a series of prostanoids. Adrenergic and muscarinic antagonists as well as indomethacin were added to the incubation medium to inhibit effects of the autonomic nervous system or of endogenous prostanoids, respectively. All the prostanoids tested increased the beating rate of the wild-type atrium in a concentration-dependent manner from the basal rate of 260 beats/min to a plateau level of >400 beats/min; these effects occurred within 3 min of the addition and continued for more than 1 h, although the potencies were different among these compounds (**Fig. 1a–e** and **Supplementary Fig. 1** online). For example, the response to $PGF_{2\alpha}$ was first detected at 1 nM and reached a plateau at 30 nM. This positive chronotropic effect of $PGF_{2\alpha}$ was not observed in the atrium of mice lacking FP (*Ptgfr*$^{-/-}$ mice), indicating that it was mediated by FP (**Fig. 1a**). In contrast, PGD_2, PGE_2 and PGI_2 each showed a positive chronotropic effect in both wild-type atria and atria derived from mice lacking their respective receptors; these effects, however, were not apparent with FP-deficient atria, suggesting that they were mediated not by their cognate receptors but by FP (**Fig. 1b–e**).

The stable TP agonist I-BOP induced a biphasic increase in the beating rate of the wild-type atrium; the first phase of this effect was apparent at 3 pM and reached a plateau of 370 beats/min at 100 pM, and the second phase was apparent at 30 nM and reached a level of 400 beats/min at 1 μM (**Fig. 1f**). The first phase of the response was not observed in atria of mice lacking TP (*Tbxa2r*$^{-/-}$ mice), which showed more clearly the second phase of the response at submicromolar I-BOP concentrations. In contrast, FP-deficient atria showed only the first phase of the response to I-BOP. These results thus indicated that TP and FP mediate the first and second phases of the biphasic chronotropic effect of I-BOP, respectively. A small but reproducible difference was apparent between the beating rates attained by stimulation of FP or TP alone, whereas the maximal beating rate of the wild-type atrium induced by stimulation of both FP and TP was similar to that induced by stimulation of FP alone. These observations suggest that TP probably increases the beating rate by the same signaling mechanism as does FP, but with a lower efficacy. Both $PGF_{2\alpha}$ and TXA_2 thus each act directly on the atrium to induce an increase in beating rate through interaction with their cognate receptors. Furthermore, PGD_2, PGE_2 and PGI_2 each exert positive chronotropic actions by cross-reacting with FP at relatively high concentrations, which might not be achieved under physiological conditions.

We next examined whether such direct stimulation of the heart by prostanoids contributes to the chronotropic actions of the autonomic nervous system. Atria from wild-type mice or from mice deficient in FP or TP were incubated with either epinephrine or acetylcholine in the absence of indomethacin and other blockers. The positive or nega-

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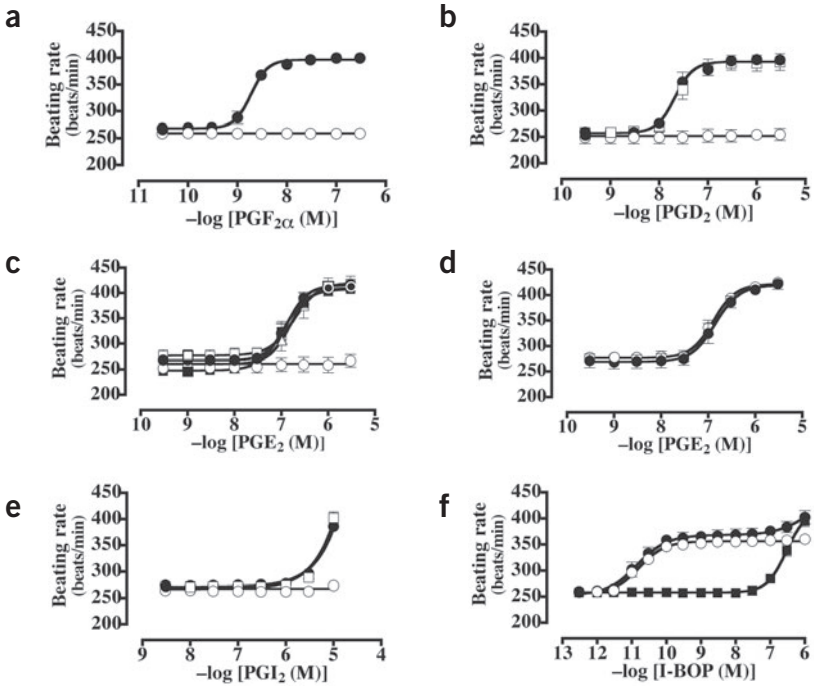

Figure 1 Effects of exogenous prostanoids on the beating rate of the isolated right atrium. (**a**) Effects of $PGF_{2\alpha}$ on wild-type (closed circles) and FP-deficient (open circles) atria. (**b**) Effects of PGD_2 on wild-type (closed circles), FP-deficient (open circles), and DP-deficient (from *Ptgdr*$^{-/-}$ mice, open squares) atria. (**c**) Effects of PGE_2 on wild-type (closed circles), FP-deficient (open circles), EP_1-deficient (from *Ptger1*$^{-/-}$ mice, closed squares), EP_2-deficient (from *Ptger2*$^{-/-}$ mice, open squares), and EP_3-deficient (from *Ptger3*$^{-/-}$ mice, open triangles) atria. (**d**) Effects of PGE_2 on F_2 wild-type (closed circles) and EP_4-deficient (from *Ptger4*$^{-/-}$ mice, open circles) atria. (**e**) Effects of PGI_2 on wild-type (closed circles), FP-deficient (open circles), and IP-deficient (from *Ptgir*$^{-/-}$ mice, open squares) atria. (**f**) Effects of I-BOP on wild-type (closed circles), FP-deficient (open circles), and TP-deficient (closed squares) atria. All data are means ± s.e.m. ($n = 5$ or 6).

tive chronotropic effects elicited by epinephrine and acetylcholine, respectively, did not differ substantially among the atria from wild-type mice and from the various types of receptor-deficient animals (**Supplementary Fig. 2** online), suggesting that these actions of epinephrine and acetylcholine are independent of prostanoids. Conversely, both propranolol and atropine did not significantly affect the $PGF_{2\alpha}$-induced increase in beating rate (data not shown), suggesting that FP signaling was independent of adrenergic or muscarinic signaling.

To determine whether prostanoids act on pacemaker tissue, we divided the right atrium into three portions: the nodal area, transitional area and auricle (**Fig. 2a**). The nodal area contains the sinoatrial node, and the transitional area contains transitional cells[3], which show automaticity after they are freed from control by the nodal pacemaker. The auricle does not contain any pacemaker tissue. The isolated nodal area showed a beating rate (281 ± 7 beats/min) similar to that of the whole atrium, confirming that it contained the sinoatrial node (**Fig. 2b**). Both $PGF_{2\alpha}$ (10 nM) and I-BOP (1 nM) showed potent positive chronotropic effects in the nodal area, as did epinephrine. The transitional area acquired automaticity on isolation but showed a beating rate (251 ± 16 beats/min) lower than that of the nodal area (**Fig. 2c**). I-BOP significantly increased the beating rate of this tissue, as did epinephrine, but $PGF_{2\alpha}$ had no such effect, indicating that the expression of FP and TP might differ between the nodal and transitional areas. The isolated auricle did not show automaticity, and no rhythm was induced by either $PGF_{2\alpha}$ or I-BOP (data not shown). These results thus indicate that $PGF_{2\alpha}$ and I-BOP each exert their chronotropic effects by acting on the confined regions of the atrium that contain the pacemaker cells. In addition, we found that both I-BOP and $PGF_{2\alpha}$ significantly ($P < 0.05$) increased diastolic depolarization rate, shortened action potential duration, and thus increased beating rate in a concentration-dependent manner in an electrophysiological experiment using isolated guinea-pig nodal cells (K.O., K.Y., T.I. & F.U., unpublished observation), suggesting direct actions of these prostanoids on the pacemaker cells. It remains to be determined whether FP and TP are expressed in the pacemaker cells or which type(s) of ion channel mediates the actions of these prostanoids.

We next determined whether endogenously produced prostanoids also elicit positive chronotropic effects in the isolated right atrium. We first compared beating rates among atria from wild-type, FP-deficient and TP-deficient mice under basal conditions in the absence of indomethacin and found no significant difference (data not shown), suggesting that the basal production of both $PGF_{2\alpha}$ and TXA_2 were not sufficient to affect this parameter. We therefore administered a mixture of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ to the atrial preparation to stimulate the production of endogenous prostanoids. These agents are typical inflammatory cytokines that are released in large amounts into the circulation under systemic inflammatory conditions and stimulate prostanoid

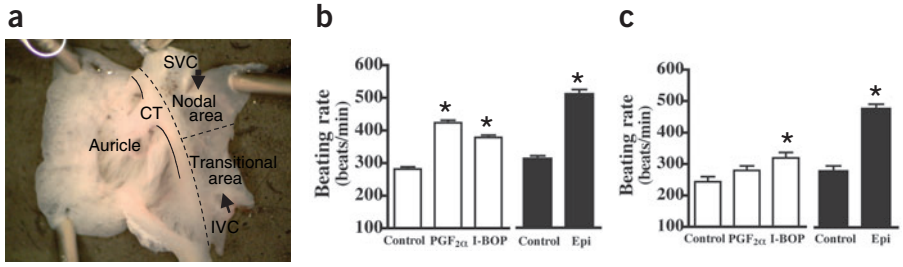

Figure 2 Distinct sites of action of $PGF_{2\alpha}$ and I-BOP within the right atrium. (**a**) Endocardial view of the right atrium. The right atrium from a wild-type mouse was divided into three parts as indicated by the dotted lines. The solid line indicates the crista terminalis (CT), and the arrows indicate the inlets of the superior vena cava (SVC) and inferior vena cava (IVC). (**b**,**c**) Effects of $PGF_{2\alpha}$, I-BOP and epinephrine (Epi) on the beating rate originating in the nodal area (**b**) and in the transitional area (**c**). Data are means ± s.e.m. ($n = 6$ or 7). *$P < 0.05$ versus respective control. In **b**, the control beating rate shown by the closed column was significantly ($P < 0.05$) higher than that shown by the open column because β-blocker was not included when examining the effect of epinephrine.

synthesis in various organs[4]. Indeed, the cytokine mixture significantly ($P < 0.05$) increased syntheses of both TXA_2 and $PGF_{2\alpha}$ in the wild-type atrium (**Fig. 3a**) and induced a biphasic increase in the beating rate, with the first and second peaks apparent 20 and 80 min after cytokine addition (**Fig. 3b**). IL-1β alone could also induce a similar degree of increase in the beating rate to that induced by the cytokine mixture (data not shown). The first peak was not observed with the TP-deficient atrium, whereas the second-phase increase was not seen in the FP-deficient atrium (**Fig. 3c**), indicating that the first and second phases were mediated by TXA_2 and $PGF_{2\alpha}$, respectively. In addition, both phases were abolished by pretreatment of the wild-type atrium with indomethacin, which inhibits both cyclooxygenase (COX)-1 and COX-2, rate-limiting enzymes of prostanoid synthesis. These results indicate that inflammatory cytokines stimulate the production of TXA_2 and $PGF_{2\alpha}$ in the right atrium, and that these prostanoids mediate distinct phases of the positive chronotropic response to the cytokines. It is noteworthy, however, that platelets might have a role as a source of TXA_2 under a systemic inflammatory condition, in which platelets would be activated and release TXA_2 in large amounts.

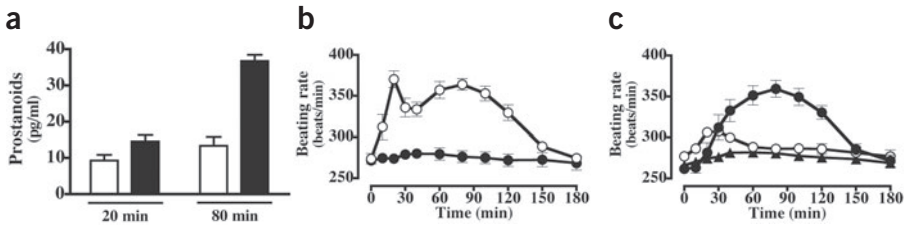

Figure 3 Role of endogenous prostanoids in cytokine-stimulated increase in the beating rate of the right atrium. (**a**) Isolated atria from wild-type mice were stimulated with the cytokine mixture. The concentrations of TXB_2 (open column) and $PGF_{2\alpha}$ (closed column) in the bathing medium were measured. Data are means ± s.e.m. ($n = 6$). Basal concentrations of these two prostanoids were below the detection limits. (**b**) Isolated atria from wild-type mice were incubated in the presence (open circles) or absence (closed circles) of the cytokine mixture, and the beating rate was monitored. Data are means ± s.e.m. ($n = 6$–9). (**c**) Atria isolated from FP-deficient (open circles), TP-deficient (closed circles), or indomethacin-treated wild-type atria (closed triangles), were incubated with the cytokine mixture. Data are means ± s.e.m. ($n = 7$ or 8).

Finally, we investigated whether the $PGF_{2\alpha}$-FP system and the TXA_2-TP system mediate tachycardia *in vivo* under systemic inflammatory conditions. Mice injected with LPS, a major constituent of the cell wall of Gram-negative bacteria, are a well established model of acute inflammation[4], and we therefore administered this polymer to mice to stimulate the production of inflammatory cytokines. Administration of LPS to wild-type mice induced a biphasic increase in heart rate characterized by a transient peak (early phase) at 20 min followed by a sustained increase (late phase) that persisted for at least 100 min after LPS injection (**Fig. 4a**). In TP-deficient mice, the early phase, although not completely absent, was greatly diminished, whereas the late phase was similar to that apparent in wild-type mice (**Fig. 4b**), suggesting that the TXA_2-TP system mediates predominantly the early phase of LPS-induced tachycardia. In FP-deficient mice, LPS induced only the early phase of the increase in heart rate, indicating that the late phase of LPS-induced tachycardia is mediated by the $PGF_{2\alpha}$-FP system.

To verify further the contributions of TXA_2 and $PGF_{2\alpha}$, we generated mice deficient in both FP and TP (*Ptgfr*$^{-/-}$ *Tbxa2r*$^{-/-}$ mice) and examined the effect of LPS on their heart rate. The heart rate of LPS-treated *Ptgfr*$^{-/-}$ *Tbxa2r*$^{-/-}$ mice did not differ substantially from that of vehicle-treated wild-type mice (**Fig. 4b**), indicating that TXA_2 and $PGF_{2\alpha}$ indeed mediate all components of LPS-induced tachycardia *in vivo*. Moreover, pretreatment of wild-type mice with indomethacin also prevented the effect of LPS on heart rate (**Fig. 4c**), confirming the role of prostanoids in LPS-induced tachycardia. We further examined the relative contribution of COX-1 and COX-2 to LPS-induced tachycardia using their selective inhibitors, SC560 and SC58125, respectively. SC560 suppressed the early phase, and SC58125 suppressed the late phase of LPS-induced tachycardia, indicating that COX-1 and COX-2 were involved preferentially in the early and late phases, respectively (**Fig. 4c**). In contrast, pretreatment of wild-type mice with propranolol reduced the basal heart rate by ~100 beats/min but did not block the effect of LPS (**Fig. 4d**). In addition, propranolol reduced also the basal heart rate without changing the profiles of heart rate in both FP-deficient mice and TP-deficient mice (data not shown). Furthermore, LPS had no significant effect on blood pressure during the experimental period (**Supplementary Fig. 3** online), excluding the possibility that LPS-induced tachycardia was the result of a hemodynamic change. These results indicate that the LPS-induced increase in heart rate is not mediated by enhancement of the activity of the sympathetic nervous system but rather results from the direct positive chronotropic actions of TXA_2 and $PGF_{2\alpha}$ on the heart.

The demand for oxygen and nutrients to combat invading microorganisms increases greatly in peripheral tissues under systemic inflammatory conditions[5]. This demand is met in part by an adap-

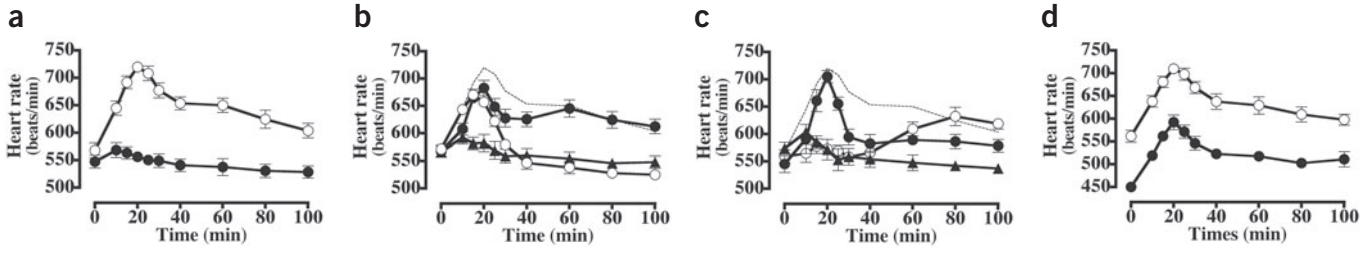

Figure 4 Role of endogenous prostanoids in LPS-induced tachycardia *in vivo*. (**a**) Wild-type mice were injected with either LPS (open circles) or vehicle (closed circles). Data are means ± s.e.m. ($n = 8$–10). (**b**) Responses to LPS in FP-deficient (open circles), TP-deficient (closed circles) or FP- and TP-deficient (closed triangles) mice. Data are means ± s.e.m. ($n = 5$–7). (**c**) Effects of SC560 (open circles), SC58125 (closed circles) or indomethacin (closed triangles) on LPS-induced tachycardia in wild-type mice. Data are means ± s.e.m. ($n = 5$). In **b** and **c**, dashed lines indicate the time course of heart rate in LPS-stimulated wild-type mice. (**d**) Effect of propranolol on LPS-induced tachycardia in wild-type mice. Propranolol (closed circles) or vehicle (open circles) was injected 30 min before LPS. Data are means ± s.e.m. ($n = 5$–10).

tive hyperdynamic state characterized by tachycardia, tachypnea and fever, as is apparent during the early phase of septic shock[6]. The immune system and central nervous system are thought to mediate the development of this hyperdynamic state in a coordinated manner through the actions of various cytokines and autonomic nerves[1,7,8]. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, are frequently used to alleviate the symptoms that accompany such a hyperdynamic state; these drugs reduce tachycardia as well as fever. The effect of NSAIDs on heart rate has been thought to result from a reduction in sympathetic tone caused by their antipyretic action. We have now shown that the suppressive effect of the β-blocker propranolol on heart rate in LPS-treated mice was achieved by slowing the basal heart rate rather than by preventing the induction of tachycardia. In addition, a pattern of tachycardia in response to LPS in mice lacking EP_3, which showed a defective febrile response to LPS[9], was nevertheless similar to that in wild-type mice (data not shown), suggesting that febrile response and inflammatory tachycardia are independent events mediated by the different prostanoids. Our results thus indicate that NSAIDs act directly at a site in the atrium to suppress tachycardia under systemic inflammatory conditions.

It is an important issue whether the present mechanism works also in humans. There have been few reports suggesting a role of prostanoids in the regulation of heart rate, indicating that few investigators suspect a direct relationship between prostanoids and heart rate under systemic inflammatory conditions. There have been, however, several pioneering works reporting a potent suppressive effect of NSAIDs on inflammatory tachycardia. One study examined the effect of ibuprofen, a popular NSAID, on heart rate in healthy volunteers after administration of LPS, and found its potent suppressive effect on tachycardia[10]. In addition, another study examined the effect of ibuprofen on heart rate in 455 individuals with septicemia, and found its potent and prompt suppressive action on tachycardia[11]. These reports suggest that the prostanoids have a role in inflammatory tachycardia in humans, but their role remains to be characterized further.

The contribution of prostanoids to the establishment of the hyperdynamic state has been largely unknown. We recently showed that PGE_2 functions as a crucial mediator of the febrile response by acting in the preoptic area through EP_3 (ref. 9), and that it also participates in the activation of the hypothalamic-pituitary-adrenal axis during the acute-phase response through EP_1 and EP_3 (ref. 12). These previous findings, together with our present observations that $PGF_{2\alpha}$ and TXA_2 mediate inflammatory tachycardia, thus show that prostanoids have central roles in the acute-phase response as part of the defense of the body against microbial invasion.

METHODS

Mice. Generation and maintenance of mice lacking each of the prostanoid receptors have been described[9,13–18]. These animals, with the exception of *Ptger4*$^{-/-}$ mice (mice lacking EP_4), and wild-type control mice share a genetic background similar to that of C57BL/6. *Ptger4*$^{-/-}$ mice have the mixed genetic background of 129/svOla and C57BL/6; F_2 wild-type mice, with a genetic background similar to that of *Ptger4*$^{-/-}$ mice, were used as a control for experiments with these latter animals (**Fig. 1d**). *Ptgfr*$^{-/-}$ *Tbxa2r*$^{-/-}$ mice were generated by crossing *Ptgfr*$^{-/-}$ and *Tbxa2r*$^{-/-}$ mice. All experiments, which were approved by the Asahikawa Medical College Committee on Animal Research, were performed with 8–12-week-old female mice.

Isolation of atria and measurement of beating rate. We excised the heart from mice anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg), and separated the right atrium in Krebs-Henseleit solution. The atrium was then mounted in an organ bath filled with 10 ml of the solution aerated with 95% O_2 and 5% CO_2 at 36 ± 0.5 °C. We measured the beating rate of the atrium by monitoring tension with a force-displacement transducer connected to a polygraph recorder. After a stabilization period of 1 h under 0.5 g of tension, each prostanoid, epinephrine or acetylcholine was added cumulatively to the bath. When examining the effects of prostanoids, we minimized neuronal effects by adding propranolol (1 μM), phenoxybenzamine (1 μM) and atropine (1 μM) to the bathing solution and we also inhibited the endogenous production of prostanoids by adding indomethacin (5 μM). When examining the effect of inflammatory cytokines, we stimulated the atrium with a mixture of IL-1β (20 ng/ml), TNF-α (20 ng/ml), and IFN-γ (10 ng/ml) in the presence of propranolol, phenoxybenzamine and atropine (each at 1 μM). We also measured the concentrations of TXB_2, a stable metabolite of TXA_2, and $PGF_{2\alpha}$ in the bathing medium at 20 and 80 min after the cytokine addition using EIA kits (Cayman Chemical).

Electrocardiograph recordings. To examine the site of action of prostanoids, we divided the right atrium of wild-type mice into three portions in Krebs-Henseleit solution. The action potentials from each portion were monitored with a needle electrode connected to an electrocardiograph. The tissue was stimulated with $PGF_{2\alpha}$ (10 nM) or I-BOP ([1s (1α, 2β (5Z), 3α (1E, 3S*), 4α)]-7-[3-(3-hydroxy-4-4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1] heptan-2-yl]-5-heptanoic acid[18]; 1 nM) in the presence of propranolol, phenoxybenzamine, atropine and indomethacin. The effect of epinephrine (1 μM) was examined in the absence of these compounds.

Heart rate and blood pressure measurements. We measured heart rate and blood pressure of conscious mice by the tail-cuff method with a BP–98A instrument (Softron) as described[19]. In this study, we referred heart rate to represent cycle length of heart contraction. After an acclimatization period of 20 min, the basal heart rate and blood pressure were measured and then LPS (026:B6, Sigma) was injected intraperitoneally at a dose of 10 mg/kg. When examining the effects of an adrenergic antagonist or COX inhibitors, propranolol (1 mg/kg) and indomethacin (10 mg/kg) were injected intraperitoneally 30 min before LPS injection, and SC560 (10 mg/kg, Cayman Chemical) or SC58125 (10 mg/kg) were injected intraperitoneally 120 min before LPS injection. Although indomethacin inhibits both COX-1 and COX-2, SC560 and SC58125 are selective inhibitors for COX-1 and COX-2, respectively[20,21].

Statistical analysis. Data are presented as means ± s.e.m. and the significance of differences was evaluated by Student *t*-test. A value of $P < 0.05$ was considered statistically significant. Analysis and graphing of the data were performed with Prism III software (GraphPad Software).

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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