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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 with the use of mice lacking 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 (WT) mice *in vitro* through interaction with TP and FP, respectively. The cytokine-induced increase in beating rate was markedly inhibited in atria from mice lacking either TP or FP. The tachycardia induced in WT mice by injection of lipopolysaccharide (LPS) was greatly attenuated in $TP^{-/-}$ or $FP^{-/-}$ mice and was completely absent in mice lacking both TP and FP. Propranolol failed to block the LPS-induced increase in heart rate in WT 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.

The right atrium was isolated from WT mice or mice deficient in each type of prostanoid receptor, and was examined for 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 WT atrium in a concentration-dependent manner from the basal rate of 260 beats min^{-1} to a plateau level of > 400 beats min^{-1}, which effects appeared 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 10^{-9} M and reached a plateau at 3×10^{-8} M. This positive chronotropic effect of $PGF_{2\alpha}$ was not observed in the atrium of mice lacking FP ($FP^{-/-}$ mice), indicating that it was mediated by FP (**Fig. 1a**). In contrast, PGD_2, PGE_2, and PGI_2 each exhibited a positive chronotropic effect in both WT atria and atria derived from mice lacking their respective receptors (**Fig. 1b–e**). The chronotropic actions of these prostanoids were not apparent with atria from $FP^{-/-}$ mice, however, 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 WT atrium; the first phase of this effect was apparent at 3×10^{-12} M and reached a plateau of 370 beats min^{-1} at 10^{-10} M, and the second phase was apparent at 3×10^{-8} M and reached a level of 400 beats min^{-1} at 10^{-6} M (**Fig. 1f**). The first phase of the response was not observed in atria from $TP^{-/-}$ mice, which revealed more clearly the second phase of the response at submicromolar I-BOP concentrations. In contrast, atria from $FP^{-/-}$ mice 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 WT atrium induced by stimulation of both FP and TP was similar to that induced by stimulation of FP alone. These observations suggest that TP likely induces tachycardia 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 heart to induce an increase in heart 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 WT 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 negative chronotropic effects elicited by epinephrine and acetylcholine, respectively, did not differ substantially among the atria from WT 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 affect significantly $PGF_{2}\alpha$-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 exhibit automaticity after they are freed from control by the nodal pacemaker. The auricle does not contain any pacemaker tissue. The isolated nodal area manifested a beating rate (281 ± 7 beats min^{-1}) 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) exhibited potent positive chronotropic effects in the nodal area, as did epinephrine (**Fig. 2b**). The transitional area acquired automaticity on isolation but showed a beating rate (251 ± 16 beats min^{-1}) 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 (**Fig. 2c**), suggesting that the expression of FP and TP may differ between the pacemakers of 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 increased diastolic depolarization rate, shortened action potential duration, and thus increased beating rate in a concentration-dependent manner in an electrophysiological experiment using guinea-pig nodal cells (our unpublished

observation), suggesting direct actions of these prostanoids on the pacemaker cells. It remains to be determined, however, 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 WT, $FP^{-/-}$, and $TP^{-/-}$ mice under basal conditions and found no significant difference (data not shown), suggesting that the basal production of $PGF_{2\alpha}$, TXA_2 or both, was 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 atrium preparation to stimulate the production of endogenous prostanoids. These agents are typical inflammatory cytokines that are released in large amounts into the circulation under conditions of systemic inflammation, and they stimulate prostanoid synthesis in various organs[4]. Indeed, the cytokine mixture significantly increased syntheses of both TXA_2 and $PGF_{2\alpha}$ in the WT atrium (**Fig. 3a**) and induced a biphasic increase in the beating rate of the WT atrium, with the first and second peaks apparent 20 and 80 min after cytokine addition (**Fig. 3b**). In addition, IL-1β alone could induce a similar degree of increase in beating rate to that induced by the cytokine mixture (data not shown). The first peak was not observed with the atrium of $TP^{-/-}$ mice, whereas the second-phase increase was not seen in the $FP^{-/-}$ atrium (**Fig. 3c**), indicating that the first and second phases were mediated by TXA_2 and $PGF_{2\alpha}$, respectively. Both phases were abolished by pre-treatment of the WT atrium with indomethacin (**Fig. 3c**). 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 play 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.

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 cell wall constituent of gram-negative bacteria, are a well-established model of acute inflammation[4], and we therefore administered this polymer to mice in order to stimulate the production of inflammatory cytokines. Administration of LPS to WT 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 $TP^{-/-}$ mice, the early phase, although not completely absent, was greatly diminished, whereas the late phase was similar to that apparent in WT mice (**Fig. 4b**), suggesting that the TXA_2-TP system mediates predominantly the early phase of LPS-induced tachycardia. In $FP^{-/-}$ mice, LPS induced only the early phase of the increase in heart rate (**Fig. 4b**), 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 ($FP^{-/-}$ $TP^{-/-}$ mice) and examined the effect of LPS on their heart rate. The heart rate of LPS-treated $FP^{-/-}$ $TP^{-/-}$ mice (**Fig. 4b**) did not differ substantially from that of vehicle-treated WT mice (**Fig. 4a**), indicating that TXA_2 and $PGF_2\alpha$ indeed mediate all components of LPS-induced tachycardia *in vivo*. Moreover, pre-treatment of WT mice with indomethacin, which inhibits both cyclooxygenase (COX) -1 and COX-2, rate-limiting enzymes of prostanoid synthesis, also prevented the effect of LPS on heart rate (**Fig. 4c**), confirming the role of prostanoids in LPS-induced tachycardia. Furthermore, we 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, pre-treatment of WT mice with propranolol reduced the basal heart rate by ~100 beats min^{-1} but failed to block the effect of LPS (**Fig. 4d**). In addition, propranolol also reduced the basal

heart rate without changing the profiles of heart rate in both $FP^{-/-}$ and $TP^{-/-}$ 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 is due to the direct positive chronotropic effects 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 conditions of systemic inflammation[5]. This demand is met in part by an adaptive hyper-dynamic 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 hyper-dynamic 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 hyper-dynamic state; these drugs reduce tachycardia as well as fever. The effect of NSAIDs on heart rate has been thought to be due to a reduction in sympathetic tone caused by their antipyretic action. However, 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 mice showed a defective febrile response to LPS[9], was nevertheless similar to that in WT 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 that if the present mechanism works also in humans. However, there have been little report suggesting a role of prostanoids in the regulation of heart rate, indicating that few investigators suspected 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 NSAID on inflammatory tachycardia. Michie *et al.* 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, Bernard *et al.* examined the effect of ibuprofen on heart rate in 455 septic patients, and found its potent and prompt suppressive action on tachycardia[11]. These reports suggest that the prostanoids play a role in inflammatory tachycardia also in humans. However, the roles of prostanoids in inflammatory tachycardia in humans remain to be examined further.

The contribution of prostanoids to the establishment of the hyper-dynamic state has been largely unknown. We recently showed both that PGE_2 functions as a critical mediator of the febrile response by acting in the preoptic area *via* EP_3 (ref. 9), and that it also participates in the activation of the hypothalamic-pituitary-adrenal axis during the acute phase response by acting at 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 demonstrate that prostanoids play 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 $EP_4^{-/-}$ mice, and WT control mice share a genetic background similar to that of C57BL/6. $EP_4^{-/-}$ mice have the mixed genetic background of 129/svOla and C57BL/6; F2-WT mice, with a genetic background similar to that

of $EP4^{-/-}$ mice, were used as a control for experiments with these latter animals (**Fig. 1d**). $FP^{-/-}$ $TP^{-/-}$ mice were generated by crossing $FP^{-/-}$ and $TP^{-/-}$ mice. All experiments, which were approved by the Asahikawa Medical College Committee on Animal Research, were performed with 8- to 12-week-old female mice.

Isolation of atria and measurement of beating rate

The heart was excised from mice anesthetized by intraperitoneal injection of ketamine (100 mg per kilogram of body mass) and xylazine (5 mg kg^{-1}), and the right atrium was separated in Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose). The atrium was then mounted in an organ bath filled with 10 ml of Krebs-Henseleit solution that was aerated with 95% O_2 and 5% CO_2 at 36 ± 0.5°C. The beating rate of the atrium was measured 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^{-1}), TNF-α (20 ng ml^{-1}), and IFN-γ (10 ng ml^{-1}) 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 WT mice into three parts in Krebs-Henseleit solution. The action potentials from each portion of the tissue were monitored with a needle electrode connected to an electrocardiograph. The tissue was stimulated with $PGF_2\alpha$ (10 nM) or I-BOP (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.

In vivo measurements of heart rate and blood pressure

Heart rate and blood pressure of awake mice were measured by the tail-cuff method with a Softron BP–98A instrument (Tokyo, Japan) as described[19]. In the present 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^{-1}. When examining the effects of an adrenergic antagonist or COX inhibitors, propranolol (1 mg kg^{-1}) and indomethacin (10 mg kg^{-1}) were injected intraperitoneally 30 min before LPS injection, and SC560 (10 mg kg^{-1}) or SC58125 (10 mg kg^{-1}) 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's *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).

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Figure Legends

Figure 1 Effects of exogenous prostanoids on the beating rate of the isolated right atrium. (**a**) Effects of $PGF_2\alpha$ on WT (closed circles) and $FP^{-/-}$ (open circles) atria. (**b**) Effects of PGD_2 on WT (closed circles), $FP^{-/-}$ (open circles), and $DP^{-/-}$ (open squares) atria. (**c**) Effects of PGE_2 on WT (closed circles), $FP^{-/-}$ (open circles), $EP_1^{-/-}$ (closed squares), $EP_2^{-/-}$ (open squares), and $EP_3^{-/-}$ (open triangles) atria. (**d**) Effects of PGE_2 on F2-WT (closed circles) and $EP_4^{-/-}$ (open circles) atria. (**e**) Effects of PGI_2 on WT (closed circles), $FP^{-/-}$ (open circles), and $IP^{-/-}$ (open squares) atria. (**f**) Effects of I-BOP on WT (closed circles), $FP^{-/-}$ (open circles), and $TP^{-/-}$ (closed squares) atria. All data are means ± s.e.m. of values from five or six atria.

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 WT 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**) Effects of $PGF_2\alpha$ (10 nM), I-BOP (1 nM), and epinephrine (Epi, 1 μM) on the beating rate originating in the nodal area. Data are means ± s.e.m. of values from seven atria. $*P < 0.05$ versus respective control. (**c**) Effects of $PGF_2\alpha$ (10 nM), I-BOP (1 nM), and epinephrine (1 μM) on the beating rate originating in the transitional area. Data are means ± s.e.m. of values from six atria. $*P < 0.05$ versus respective control. In (**b**) and (**c**), the control beating rate shown by the closed column was higher than that shown by the open column because the blockers of the autonomic nervous system were not included when examining the effects of epinephrine.

Figure 3 Role of endogenous prostanoids in the effect of inflammatory cytokines on the beating rate of the right atrium. (**a**) Isolated atria from WT mice were stimulated with a mixture

of IL-1β (20 ng ml^{-1}), TNF-α (20 ng ml^{-1}), and IFN-γ (10 ng ml^{-1}). The concentrations of TXB_2 (open column) and $PGF_{2\alpha}$ (closed column) in the bathing medium were measured at 20 and 80 min after the addition of the cytokine mixture. Data are means ± s.e.m. of values from six atria. Basal concentrations of these two prostanoids were below the detection limits. (**b**) Isolated atria from WT mice were incubated in the presence (open circles) or absence (closed circles) of the cytokine mixture, and the beating rate was monitored for 3 h. Data are means ± s.e.m. of values from six to nine atria. (**c**) Atria isolated from $FP^{-/-}$ (open circles), $TP^{-/-}$ (closed circles) mice, or indomethacin-treated WT atria (closed triangles), were incubated with the cytokine mixture and analyzed as in **b**. Data are means ± s.e.m. of values from seven or eight atria.

Figure 4 Role of endogenous prostanoids in the induction of tachycardia by LPS *in vivo*. (**a**) WT mice were injected intraperitoneally with either LPS (10 mg kg^{-1}, open circles) or vehicle (closed circles), and the heart rate was monitored for 100 min. Data are means ± s.e.m. of values from eight to 10 mice. (**b**) Responses to LPS in $FP^{-/-}$ (open circles), $TP^{-/-}$ (closed circles) or $FP^{-/-}$ $TP^{-/-}$ (closed triangles) mice. Data are means ± s.e.m. of values from five to seven mice. Dashed line indicates the time course of heart rate in WT mice. (**c**) Effects of SC560 (10 mg kg^{-1}, open circles), SC58125 (10 mg kg^{-1}, closed circles) or indomethacin (10 mg kg^{-1}, closed triangles) on LPS-induced tachycardia in WT mice. Dashed line indicates the time course of heart rate in LPS-stimulated WT mice. Data are means ± s.e.m. of values from five mice. (**d**) Effect of propranolol on LPS-induced tachycardia in WT mice. Propranolol (1 mg kg^{-1}, closed circles) or vehicle (open circles) was injected intraperitoneally 30 min before LPS. Data are means ± s.e.m. of values from five to 10 mice.

Figure 1

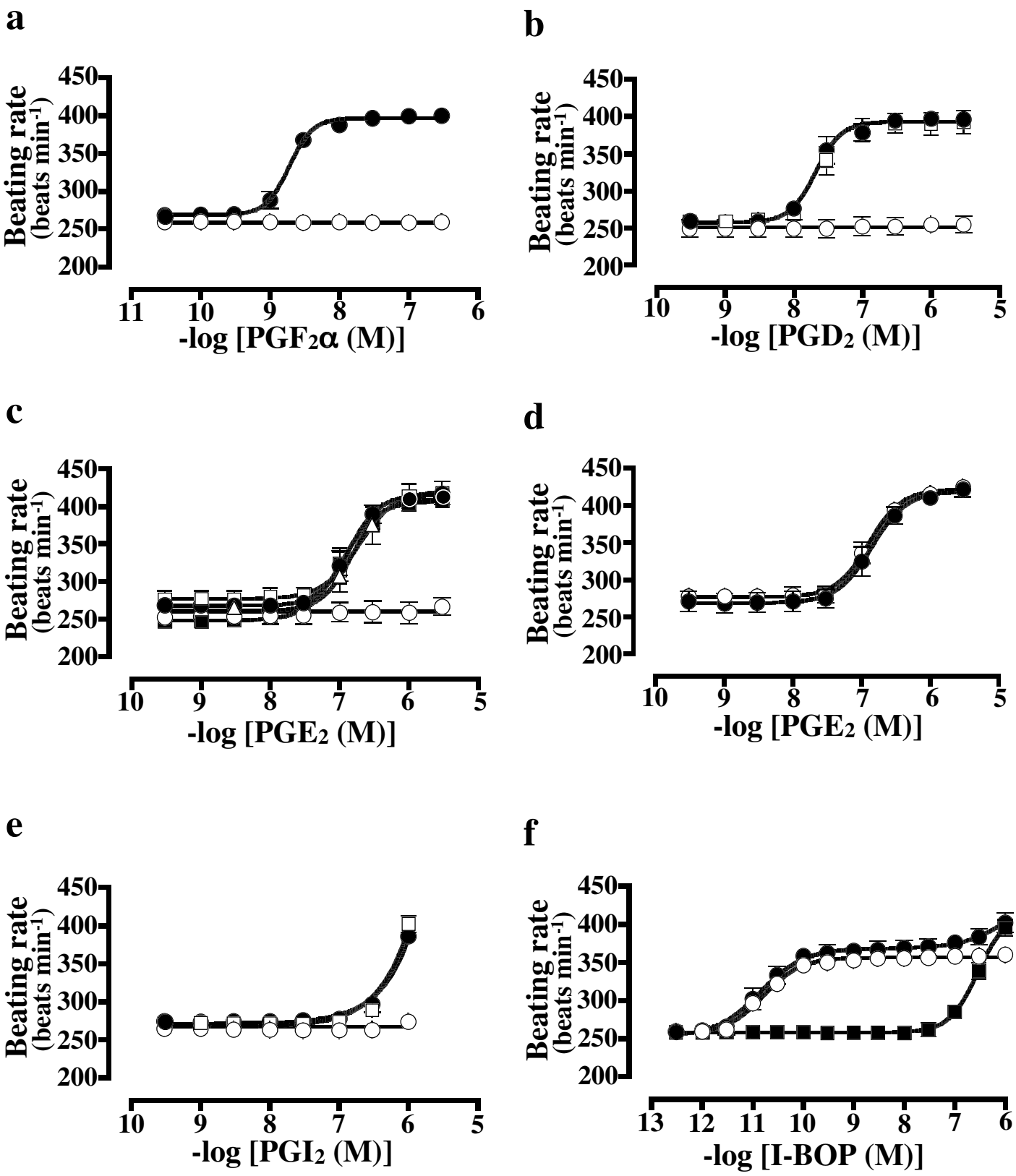

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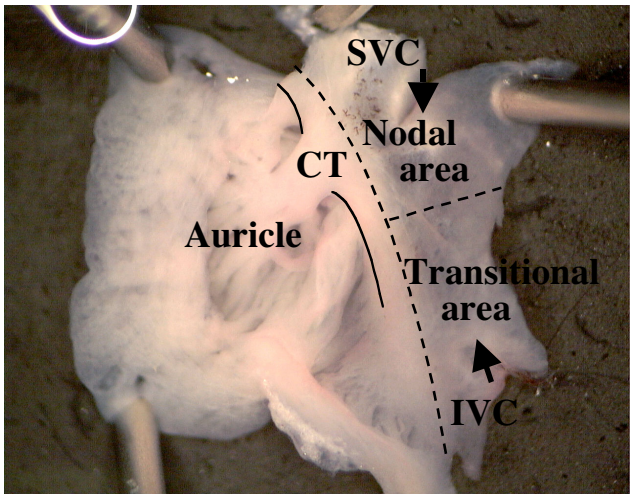
Figure 2

a



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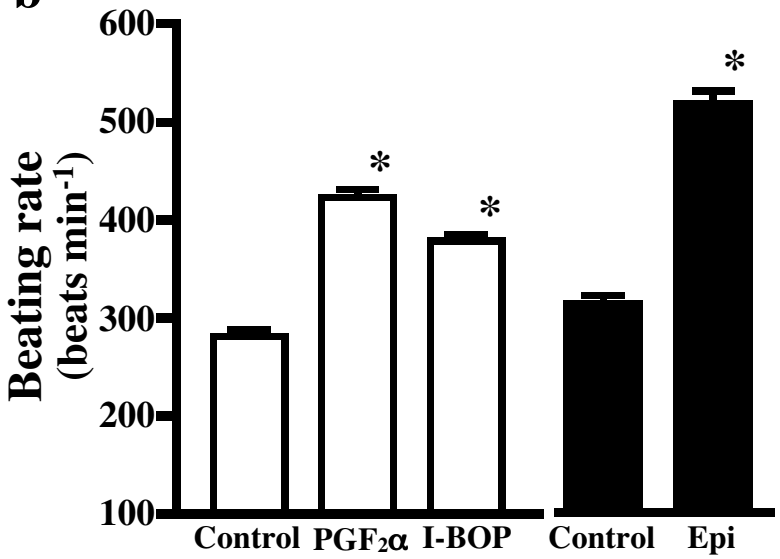

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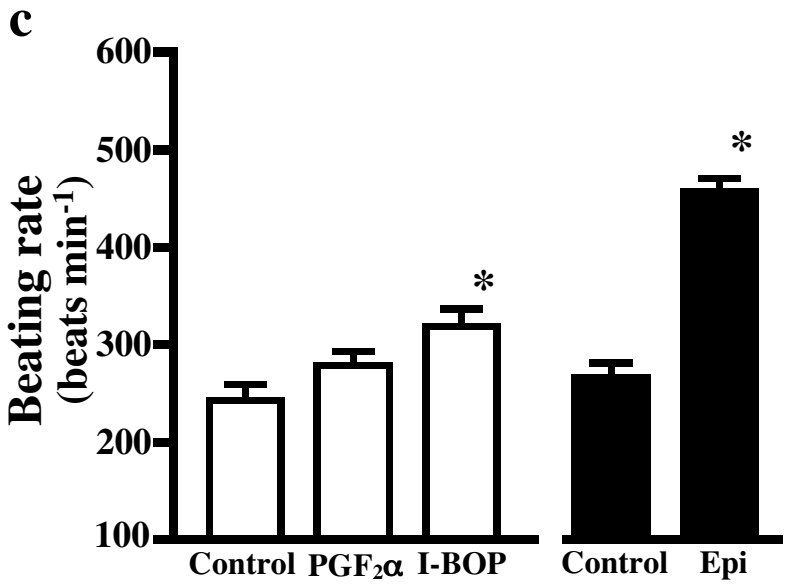

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Figure 3

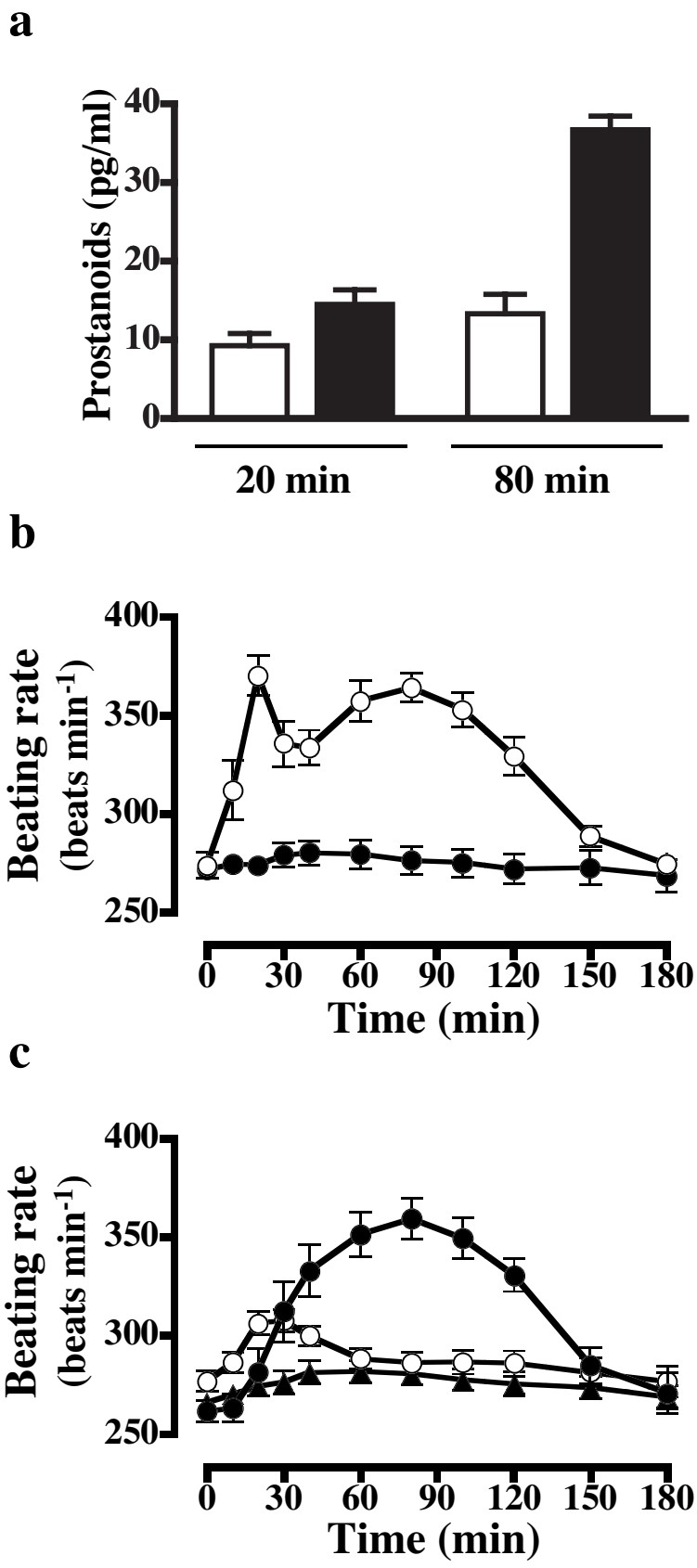

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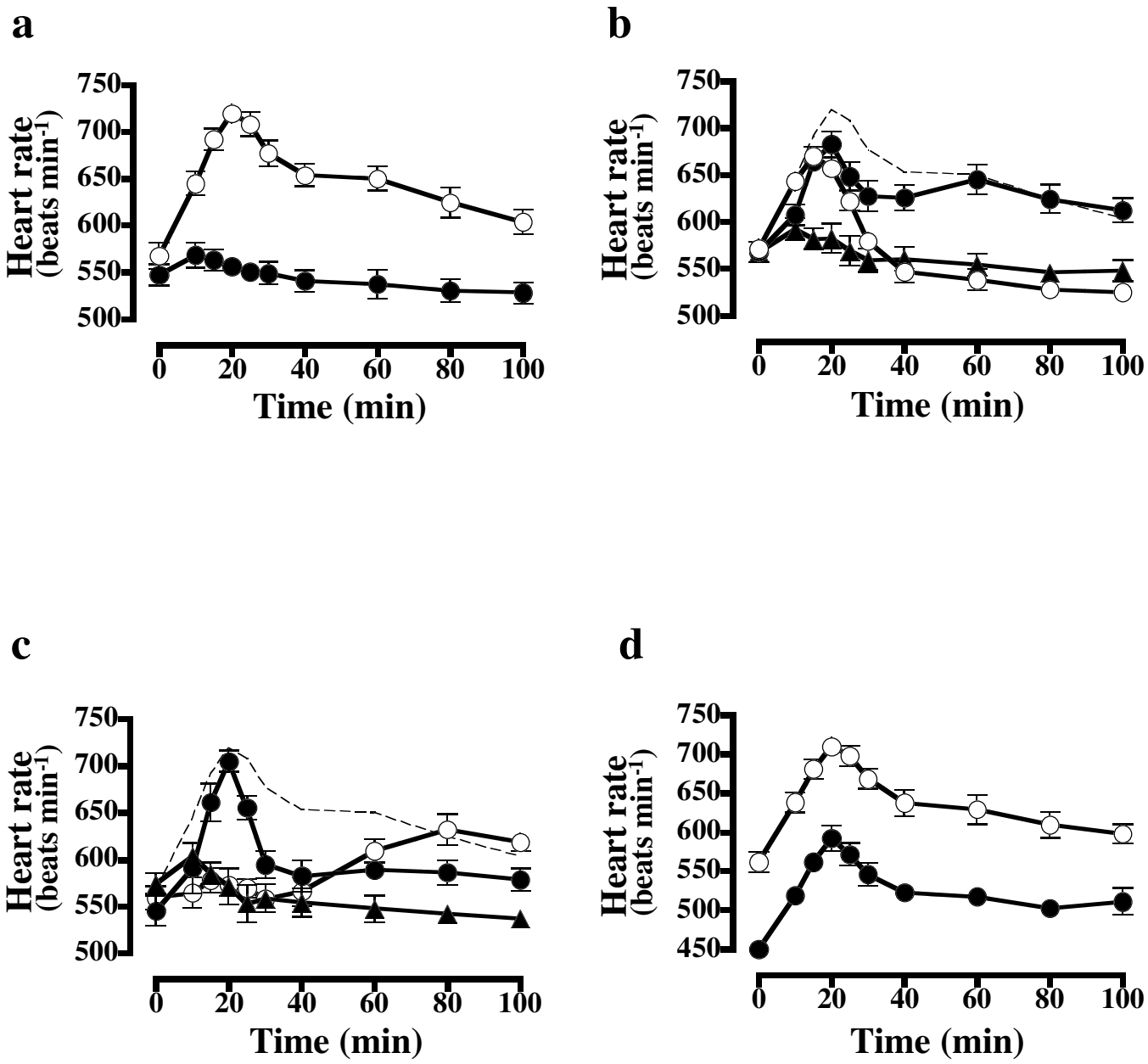
Figure 4



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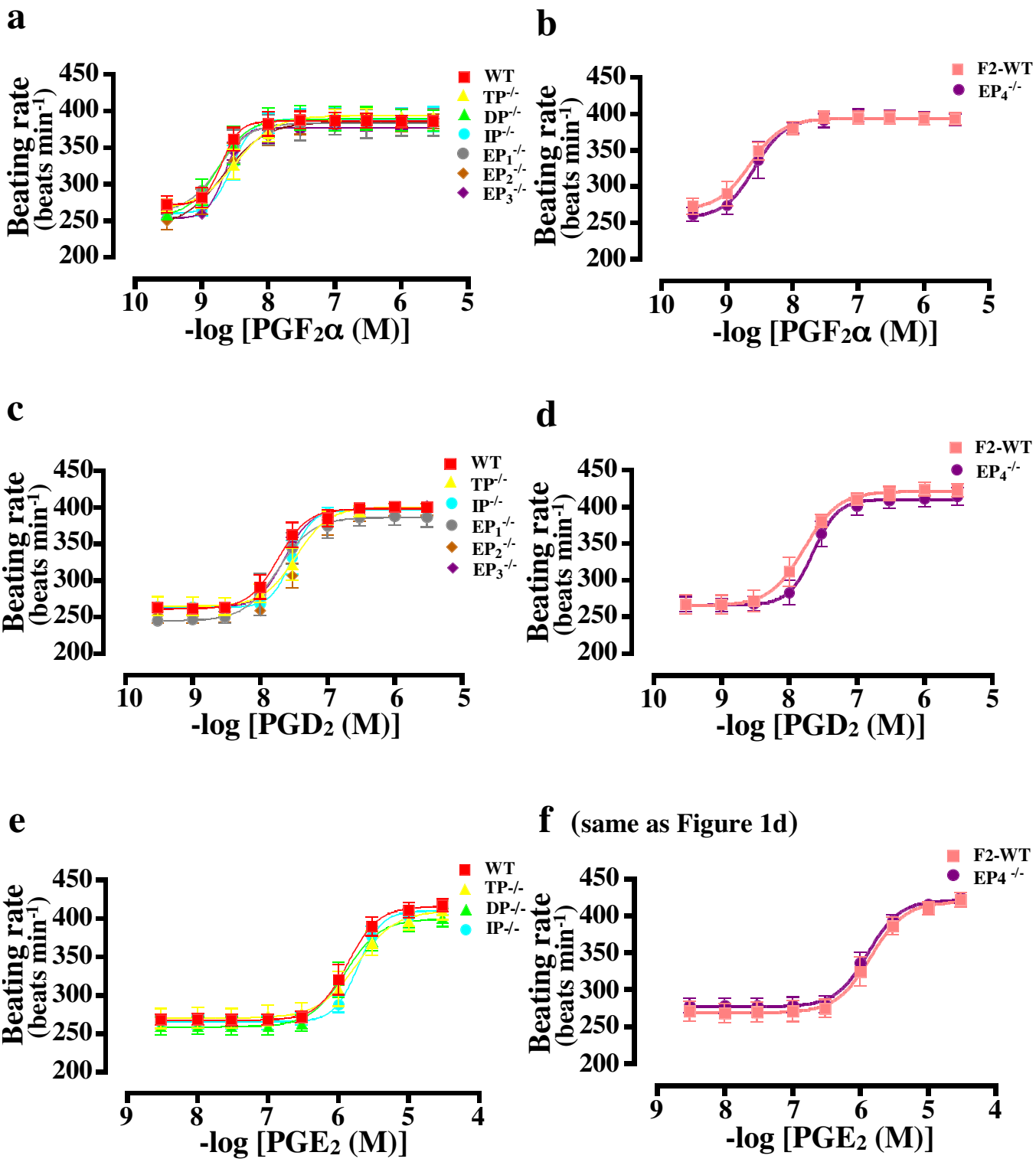
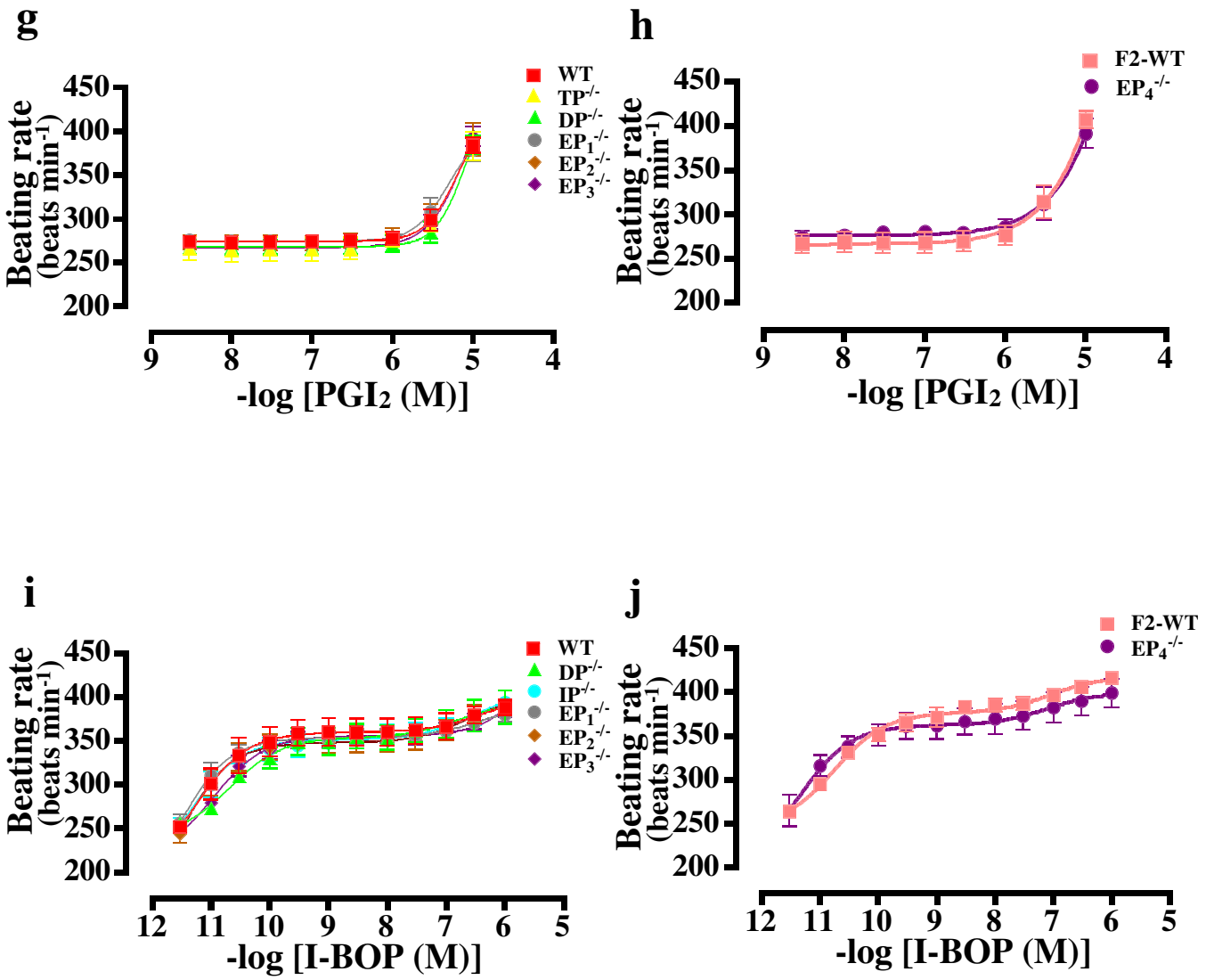
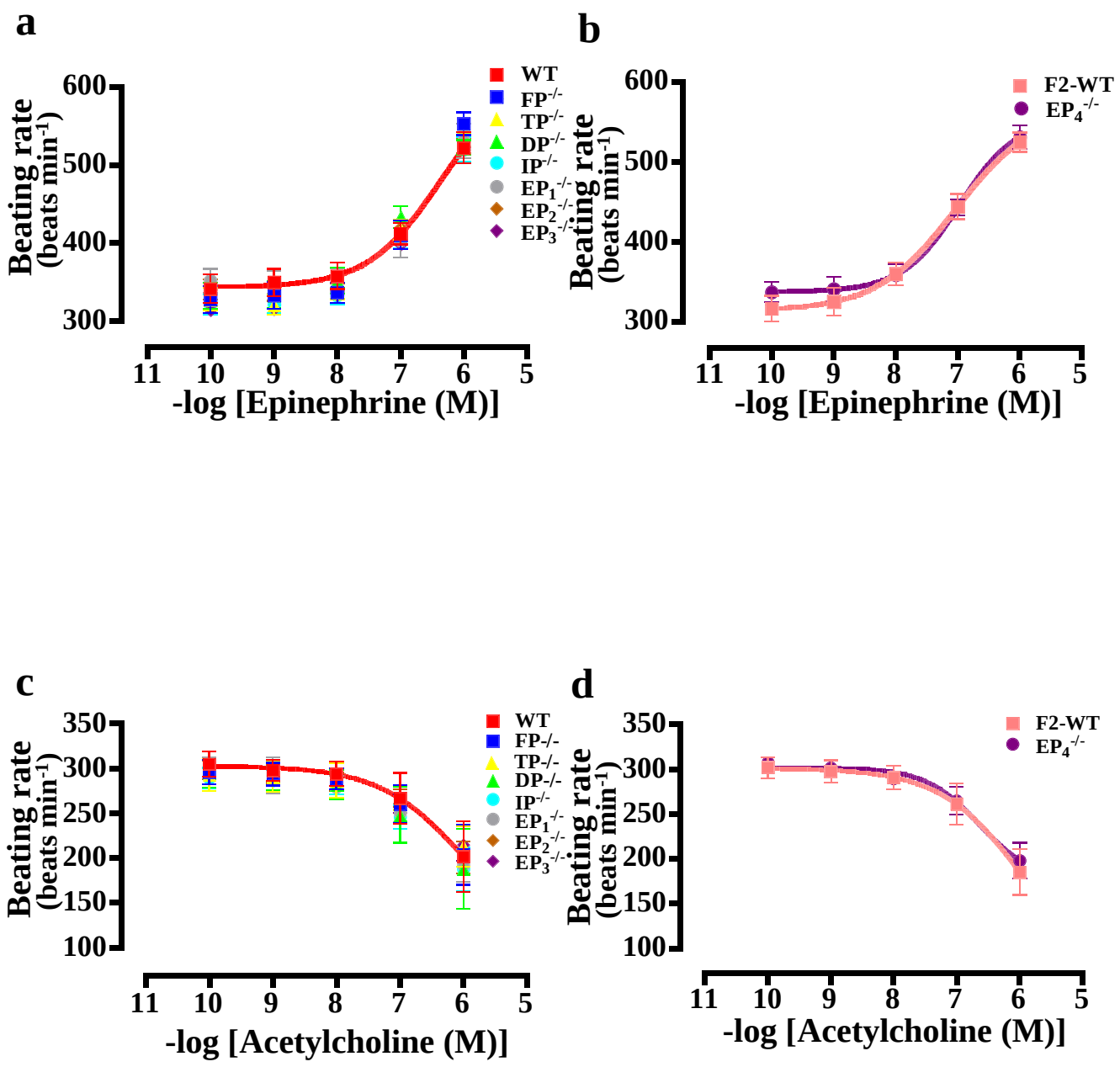
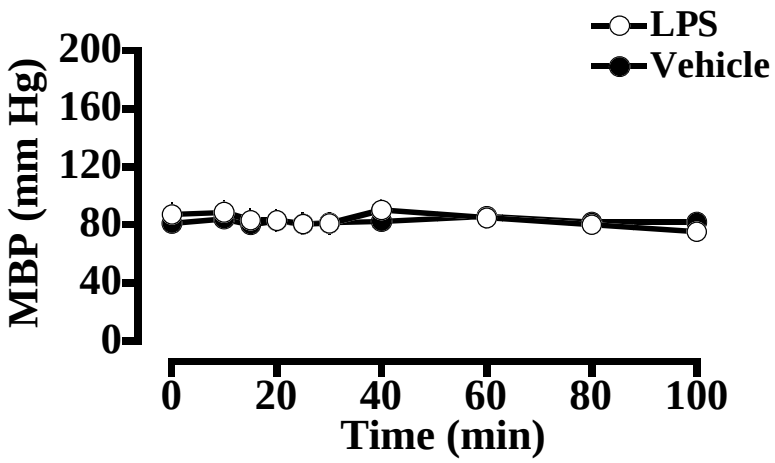

a
Beating rate (beats min-1)
450
400
350
300
250
200
10 9 8 7 6 5
-log [PGF2α (M)]
WT
TP-/-
DP-/-
IP-/-
EP1-/-
EP2-/-
EP3-/-
b
Beating rate (beats min-1)
-log [PGF2α (M)]
F2-WT
EP4-/-
c
Beating rate (beats min-1)
-log [PGD2 (M)]
WT
TP-/-
IP-/-
EP1-/-
EP2-/-
EP3-/-
d
Beating rate (beats min-1)
-log [PGD2 (M)]
F2-WT
EP4-/-
e
Beating rate (beats min-1)
9 8 7 6 5 4
-log [PGE2 (M)]
WT
TP-/-
DP-/-
IP-/-
f (same as Figure 1d)
Beating rate (beats min-1)
-log [PGE2 (M)]
F2-WT
EP4 -/-


g
Beating rate (beats min-1)
450
400
350
300
250
200
9
8
7
6
5
4
-log [PGI2 (M)]
WT
TP-/-
DP-/-
EP1-/-
EP2-/-
EP3-/-
h
Beating rate (beats min-1)
F2-WT
EP4-/-
-log [PGI2 (M)]
i
Beating rate (beats min-1)
12
11
10
9
8
7
6
5
-log [I-BOP (M)]
WT
DP-/-
IP-/-
EP1-/-
EP2-/-
EP3-/-
j
Beating rate (beats min-1)
F2-WT
EP4-/-
-log [I-BOP (M)]

Supplementary Figure 1. Effects of exogenous prostanoids on the beating rate of the isolated right atrium. a and **b**, Effects of $PGF_2\alpha$. **c** and **d**, Effects of PGD_2. **e** and **f**, Effects of PGE_2. **g** and **h**, Effects of PGI_2. **i** and **j**, Effects of I-BOP. The excised atrium was mounted in an organ bath filled with Krebs-Henseleit solution containing propranolol (1 μM), phenoxybenzamine (1 μM), atropine (1 μM) and indomethacin (5 μM). The beating rate of the atrium was measured by monitoring tension with a force-displacement transducer. Each prostanoid was added cumulatively to the bath. All animals, with the exception of $EP_4^{-/-}$ mice, and WT control mice share a genetic background similar to that of C57BL/6. In contrast, $EP_4^{-/-}$ mice have the mixed genetic background of 129/svOla and C57BL/6; F2-WT mice, with a genetic background similar to that of $EP_4^{-/-}$ mice, were used as a control for experiments with these latter animals (**b**, **d**, **f**, **h** and **j**). Data represent the effects of the prostanoids and I-BOP, which were not included in Fig. 1. Data in WT atria and **f**, however, are presented again for convenience. All data are means ± s.e.m. of values from five or six atria.


a
b
c
d
Beating rate (beats min-1)
600
500
400
300
350
250
200
150
100
11 10 9 8 7 6 5
-log [Epinephrine (M)]
-log [Acetylcholine (M)]
WT
FP-/-
TP-/-
DP-/-
IP-/-
EP1-/-
EP2-/-
EP3-/-
F2-WT
EP4-/-

Supplementary Figure 2. Effects of epinephrine and acetylcholine on the beating rate of the isolated right atrium. a and **b**, Effects of epinephrine. **c** and **d**, Effects of acetylcholine. The excised atrium was mounted in an organ bath filled with Krebs-Henseleit solution. The beating rate of the atrium was measured by monitoring tension with a force-displacement transducer. Epinephrine or acetylcholine was added cumulatively to the bath. All animals, with the exception of $EP_4^{-/-}$ mice, and WT control mice share a genetic background similar to that of C57BL/6. In contrast, $EP_4^{-/-}$ mice have the mixed genetic background of 129/svOla and C57BL/6; F2-WT mice, with a genetic background similar to that of $EP_4^{-/-}$ mice, were used as a control for experiments with these latter animals (**b** and **d**). All data are means ± s.e.m. of values from five or six atria.


LPS
Vehicle
MBP (mm Hg)
200
160
120
80
40
0
0
20
40
60
80
100
Time (min)

Supplementary Figure 3. Effects of LPS on blood pressure. Blood pressure of awake mice were measured by the tail-cuff method after LPS injection. MBP, mean blood pressure.