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# The Intermediation Role of Central Cyclooxygenase Products $TXA_2$, $PGF_{2\alpha}$, PGE, and PGD in Orexin-evoked Cardiovascular Effects

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# Abstract

Centrally injected some prostaglandins (PG) and orexin (OX) produce similar cardiovascular responses. We have recently reported that both central cyclooxygenase (COX) and central lipoxygenase (LOX) enzymes mediate the cardiovascular effects of OX. In the current study, we aimed to investigate the mediating effects of thromboxane (TX) $A_2$, PGD, PGE, and $PGF_{2a}$, as COX pathway subproducts known to be active in cardiovascular control, on cardiovascular responses elicited by OX.

Intracerebroventricular (i.c.v.) injection of OX increased cardiovascular levels in normotensive male Sprague Dawley rats. Moreover, central pretreatment with the $TXA_2$ synthesis inhibitor furegrelate, $PGF_{2\alpha}$ receptor antagonist, $PGF_{2\alpha}$-dimethylamine, PGE, and PGD receptor antagonist AH6809 partially attenuated the centrally administered OX -induced pressor and tachycardic cardiovascular responses in rats.

In conclusion, our results show that i.c.v. injection of OX increases blood pressure and heart rate. Moreover, $TXA_2$, $PGF_{2\alpha}$, PGE, and PGD mediate, at least in part, the centrally applied OX -evoked pressor and tachycardic responses. The results suggest that centrally injected OX -evoked pressor and tachycardia responses may also be mediated by arachidonic acid metabolites other than $TXA_2$, $PGF_{2\alpha}$, PGE, and PGD.


# Highlights

- Central injection of OX augments MAP and HR.
- The $TXA_2$ mediates the OX-produced cardiovascular responses.
- The central $PGF_{2a}$ intermediates the OX-produced cardiovascular responses.
- The central PGE and PGD are involved in the OX-produced cardiovascular responses.

# Introduction

Orexin (OX), also known as hypocretin, is a hypothalamic neuropeptide. It is produced from a common precursor molecule, pre-orexin (De Lecea et al. 1998), and binds to two G-coupled receptors, namely OX-1 and OX-2 (Sakurai et al. 1998). The OX neurons are located in the hypothalamus and are also found in other regions of the brain (Date et al. 1999; Nambu et al. 1999; Peyron et al. 1998). A considerable body of evidence points to the involvement of the OXergic system in modulating a variety of physiological processes, including food intake and energy expenditure, motivation, circadian rhythms of sleep and wakefulness, memory, cognitive functions, and the neuroendocrine system (Willie et al. 2001). In addition, the OXergic system plays an important role in regulating the cardiovascular system (Samson et al. 1999; Shirasaka et al. 1999). Data from several studies have shown that central injection of OX increases mean arterial blood pressure (MAP), heart rate (HR), renal sympathetic nerve activity, and plasma catecholamines in both awake and anesthetized rats (Altinbas et al. 2021; Samson et al. 1999; Shirasaka

et al. 1999). On the other hand, previous studies in this research area have reported that the OX-evoked cardiovascular effects are mediated by the vasopressinergic (Al-Barazanji et al. 2001), renin-angiotensin (Lin et al. 2002), cholinergic (Antunes et al. 2001), and histaminergic systems (Jochem 2009).

AA released from membrane phospholipids by activation of phospholipase $A_2$ ($PLA_2$) forms prostaglandins (PG) or leukotrienes via the COX or lipoxygenase LOX enzyme pathway (Smith et al. 2000; Wlodawer and Samuelsson 1973). In previous studies, researchers have documented that AA and its metabolites are involved in cardiovascular control (Tassoni et al. 2008). Centrally injected melittin, $PLA_2$ activator and AA increased arterial pressures in normotensive and hypotensive rats (Yalcin and Erturk 2007; Yalcin and Aydin 2009; Yalcin et al. 2009). It could be considered that centrally injected melittin- or AA -induced cardiovascular responses are the result of possibly central COX pathway products. This is because it is known that as AA -COX pathway products centrally $PGE_2$, $PGD_2$ (Ariumi et al. 2002; Hoffman et al. 1981; Siren, 1982a; Siren 1982b; Wasserman et al. 1977), $PGF_{2\alpha}$ (Hoffman et al. 1981; Siren 1982c) and also thromboxane (TX) $A_2$ (Yalcin and Savci 2004) play a role in cardiovascular modulation. Recently, it was reported that centrally injected OX increased the hypothalamic total extracellular concentration of PG, and central COX and LOX enzymes also mediated OX -induced cardiovascular responses (Altinbas et al. 2021). However, it is unknown which AA metabolites involved in cardiovascular modulation accompany OX -induced cardiovascular effects. Therefore, in the current study, we investigated the possible role of $TXA_2$, PGE, PGD, and $PGF_{2\alpha}$, which are synthesized in a substep of the COX -pathway, in the cardiovascular effects induced by centrally administered OX.

# Methods

## Animals

A total of 98 adult, male Sprague-Dawley rats (280–340 g) obtained from Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey, were used for these experiments. Four or five rats were housed per cage under controlled conditions of temperature (20–22 $^0$C), humidity (60–70 %), and lighting (12 h light/dark cycle) and provided with food and water ad libitum. The Animal Care and Use Committee of Bursa Uludag University approved all experimental procedures in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Each animal was studied separately, in a single experiment, and each experimental group consisted of seven rats.

## Surgical procedures

Under sevoflurane (2-4 % / 100 % $O_2$) anesthesia, the left femoral artery was cannulated with heparinized saline (100 U/ml) using a PE 50 tube filled with heparinized saline (100 U/ml) to measure mean arterial pressure (MAP) and heart rate (HR). The tip of the tube was covered and exteriorized at the neck of the rat. For i.c.v. drug application, a burr hole was drilled through the skull 1.5 mm lateral to the midline and

1.0 mm posterior to the bregma. A 22-gage stainless steel cannula was lowered 4.5 mm below the skull surface and secured to the skull with acrylic cement. After surgery, the rats were placed in individual cages and allowed to recover from the anesthesia for 4-5 h.

### Measurement of cardiovascular parameters

The arterial cannula was connected to a volumetric pressure transducer (BPT 300, BIOPAC Systems Inc., California, USA), which was connected to the MP36 system (BIOPAC Systems Inc.) to measure the cardiovascular parameters of the rats. The rats were allowed to stabilize for 30 minutes before the experiments, and baseline measurements MAP and HR were recorded within this period. Blood pressure was expressed as MAP (mmHg), and HR was expressed as beats per minute (bpm).

### Experimental protocols

In the present study, cardiovascular responses to i.c.v. injection of OX were first investigated as the main control for the study. After baseline measurement, the changes in cardiovascular parameters were recorded for the next 60 min in OX (1.5 nmol) or saline (5 µl) i.c.v. injected normotensive rats. The dose of OX was chosen from the effective dose used in our previous study (Altinbas et al. 2021).

To demonstrate the mediation of central $TXA_2$, $PGF_{2\alpha}$, PGE and PGD in cardiovascular effects evoked by OX in normotensive rats, pretreatment with the $TXA_2$ synthesis inhibitor furegrelate (250 and 375 µg; i.c.v.), the $PGF_{2\alpha}$ receptor antagonist $PGF_{2\alpha}$ dimethylamine (50 µg; i.c.v.), PGE and PGD receptor antagonist AH6809 (10 µg; i.c.v.), saline (5 µl; i.c.v.) or 30% DMSO (5 µl; i.c.v.) was performed 5 min before the injection of OX (1.5 nmol; i.c.v.) or saline (5 µl; i.c.v.), and cardiovascular parameters were recorded for the next 60 min in rats. The dose of furegrelate, $PGF_{2\alpha}$ dimethylamine, and AH6809 was chosen from our previous studies (Erkan et al. 2017; Yalcin et al., 2006).

### Drugs and i.c.v. injections

OX (Sigma-Aldrich Co., Deisenhofen, Germany), furegrelate and $PGF_{2\alpha}$ dimethylamine (Cayman Chemical Company, Ann Arbor, MI, USA) solutions were prepared in saline, AH6809 (Cayman Chemical Company, Ann Arbor, MI, USA) solution was prepared in 30 % DMSO on the day of experiment.

I.c.v. injections were performed using an injection cannula consisting of 28-gage stainless steel tubing connected to a 10-µl microsyringe with polyethylene tubing. The drugs were injected i.c.v. with an injection volume of 5 µl over a 60-s period. During injection, an air bubble moving in the polyethylene tubing was closely monitored to ensure that the drug was fully delivered.

### Statistical analysis

All values are expressed as mean ± standard error of the mean (S.E.M.) with $p < 0.05$ as significance level. Statistical analysis was done using two-way analysis of variance with repeated measures (RM - ANOVA; two-way) and *Bonferroni*'s post- ANOVA test.

# Results

### Effects of centrally injected OX on MAP and HR

Intracerebroventricularly (i.c.v.) injected OX (1.5 nmol) showed pressor and tachycardic effects in normotensive conscious animals ($p < 0.05$; Fig. 1). OX at a dose of 1.5 nmol caused an increase in MAP of approximately 15 mmHg. The increase in MAP after injection of OX began in the first few minutes, and the maximum increase in MAP occurred 5 min after administration of OX. The pressure response persisted up to 20 minutes after OX injection (Fig. 1a). Simultaneously, central injection of OX caused an increase in HR, which began in the first minute after injection and lasted up to 15 min, similar to MAP (Fig.1b). OX at a dose of 1.5 nmol caused an increase of approximately 45 beats/min per minute in HR of the rats (Fig.1b). Data are given in Online Resource 1.

### The mediating role of central $TXA_2$, $PGF_{2\alpha}$, PGE and PGD on the OX -evoked cardiovascular effects.

Central pretreatment with the $TXA_2$ synthesis inhibitor furegrelate (250 and 375 µg; i.c.v.), the $PGF_{2\alpha}$ receptor antagonist $PGF_{2\alpha}$ dimethylamine (50 µg; i.c.v.) and the PGE (EP1, EP2, EP3) and PGD (DP1) receptor antagonist AH6809 (10 µg; i.c.v.) did not alter basal cardiovascular parameters in rats. However, pretreatment with frugrelate (Fig. 2a,b), $PGF_{2\alpha}$-dimethylamine (Fig. 3a,b) and AH6809 (Fig. 4a,b) partially blocked the pressor and tachycardic effects produced by OX ($p < 0.05$). Data are given in Online Resource 1.

# Discussion

The results showed that central injection of OX caused the pressor cardiovascular effect by increasing the levels of MAP and HR in normotensive conscious rats. The intriguing finding of the current study was that the cardiovascular effects of OX were mediated in part by $TXA_2$, PGE, PGD and $PGF_{2\alpha}$, as AA-COX pathway.

The i.c.v. injection of OX increased MAP and HR, resulting in a cardiovascular effect similar to the effect we reported previously (Altinbas et al. 2021). First, Samson et al. and Shirasaka et al. 1999 reported that OX -induced cardiovascular effects. In these studies, centrally administered OX was found to increase MAP, HR, and renal sympathetic nerve activity in both awake and anesthetized rats (Samson et al. 1999; Shirasaka et al. 1999). The later findings proved beyond doubt that the central sympathoexcitatory effect plays a direct role in the cardiovascular effects of OX (Chen et al. 2000; Dun et al. 2000; Antunes et al. 2001). Intracisternal and intrathecal (T2-T3) administration of OX also elicited the same cardiovascular responses as i.c.v. injection. (Antunes 2001; Chen et al. 2000; Dun et al. 2000; Shahid et al. 2011). Intragastric or intraperitoneal injection of OX antagonists to rats exposed to novelty stress caused alterations in tachycardic and pressor responses in rats (Beig et al. 2015). In addition, OX microinjections administered within different regions of the central autonomic network triggered changes in the levels of MAP and HR (Antunes et al. 2001; Chen et al. 2000; Ciriello and de Oliveira 2003; Ciriello et al. 2003; De

Oliveira et al. 2003; Dun et al. 2000; Follwell and Ferguson 2002; Huang et al. 2010; Li et al. 2018; Shih and Chuang 2007; Shirasaka et al. 2001; Smith et al. 2007; Tupone et al. 2011; Van Den Top et al. 2003). In addition, it has been suggested that pre- OX knockout mice and rats with genetically ablated OX neurons have lower resting blood pressure compared to their wild-type controls (Kayaba et al. 2003; Schwimmer et al. 2010). These reports provide evidence that OX plays a role in cardiovascular control, as demonstrated in our study.

Our recent study suggested that there is an interaction between the central OX and AA cascade (Altinbas et al. 2021). In this study, we showed that i.c.v. injection of OX activates the COX and LOX pathways in cardiovascular responses. In the same article, we also performed a microdialysis study reporting that central administration of OX increased total extracellular AA metabolite levels in the posterior hypothalamus. However, this study did not explain which AA metabolites mediate the OX -induced cardiovascular effects. The most remarkable finding of this work is that $TXA_2$, PGE, PGD, and $PGF_{2\alpha}$ as AA-COX metabolites partially mediate the OX-induced cardiovascular responses. These results are consistent with those mentioned in previous studies on the cardiovascular effects of AA metabolites. Previously, researchers have shown that centrally administered $TXA_2$ affected MAP and HR (Yalcin and Savci 2004). Moreover, i.c.v. pretreatment with a $TXA_2$ synthase inhibitor (Yalcin et al. 2006) or a PGD, PGE, or $PGF_{2\alpha}$ receptor antagonist (Erkan et al. 2017) partially blocked the cardiovascular effects of central injection of AA. Recent research found that injection of PGE into the lateral ventricle or directly into the different brain areas altered cardiovascular scores (Hoffman et al. 1981; Osborne and Kurosawa 1994; Siren 1982a; Zhang et al. 2003). Similarly, central $PGF_{2\alpha}$ application caused pressor and positive chronotropic effects and tachycardia (Hoffman et al. 1981; Ono and Furukawa 1983; Rao et al. 1987; Siren 1982c). In addition, central application of $PGD_2$ to the i.c.v. in rats resulted in an increase in blood pressure and heart rate values (Förstermann et al. 1983; 1985; Siren 1982b). In another study, $PGE_2$ and $PGD_2$ levels were found to increase significantly in the medulla oblongata and hypothalamus during hemorrhagic shock (Cernak et al. 1984).

In conclusion, the results of our work suggest that i.c.v. injection of OX elicits pressor and tachycardic cardiovascular responses. Interestingly, the result of the study shows that some AA-COX pathway end products, including $TXA_2$, PGE, PGD and $PGF_{2\alpha}$, partially mediate the OX-induced cardiovascular responses. Perhaps higher doses of furegrelate, $PGF_{2\alpha}$ dimethylamine, and AH6809 could block OX-induced cardiovascular responses, but higher doses of the drugs caused adverse effects in the animals. However, furegrelate (Yalcin et al. 2006), $PGF_{2\alpha}$ dimethylamine, and AH6809 (Erkan et al. 2017), used at the same dose as the one administered in the present study, were reported to partially block the cardiovascular responses created by AA. In our study, the partial mediation effects of $TXA_2$, PGE, PGD, and $PGF_{2\alpha}$ as AA-metabolites on MAP and HR suggest that each metabolite might individually mediate the cardiovascular responses induced by OX. For, although not specifically tested in the current study, prostacyclin, LOX, and other COX pathway products may play a role in cardiovascular effects formed by central administration of OX as another AA end product. In addition, the possible effects of subproducts

of the central LOX pathway, which has an active role in cardiovascular control (Guvenc-Bayram et al. 2020), on OX-produced cardiovascular responses may be investigated in future studies. Consequently, the OXergic system and the AA cascade, including the AA-COX pathway products, were found to interact in the central nervous system with respect to cardiovascular control.

# Declarations

**Ethical Approval** The Animal Care and Use Committee of Bursa Uludag University approved all experimental procedures in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. (Date 02.12.2014 / No.2014-16/01).

**Consent to Participate** Only laboratory animals were used in this study.

**Consent to Publish** The authors state that they use laboratory animals in this study.

**Author Contributions** BA, MY and GG performed in the experiments. BA and MY analyzed the data and wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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**Competing Interests** The authors declare no competing interests.

**Data availability** All data generated in this study are included in this paper.

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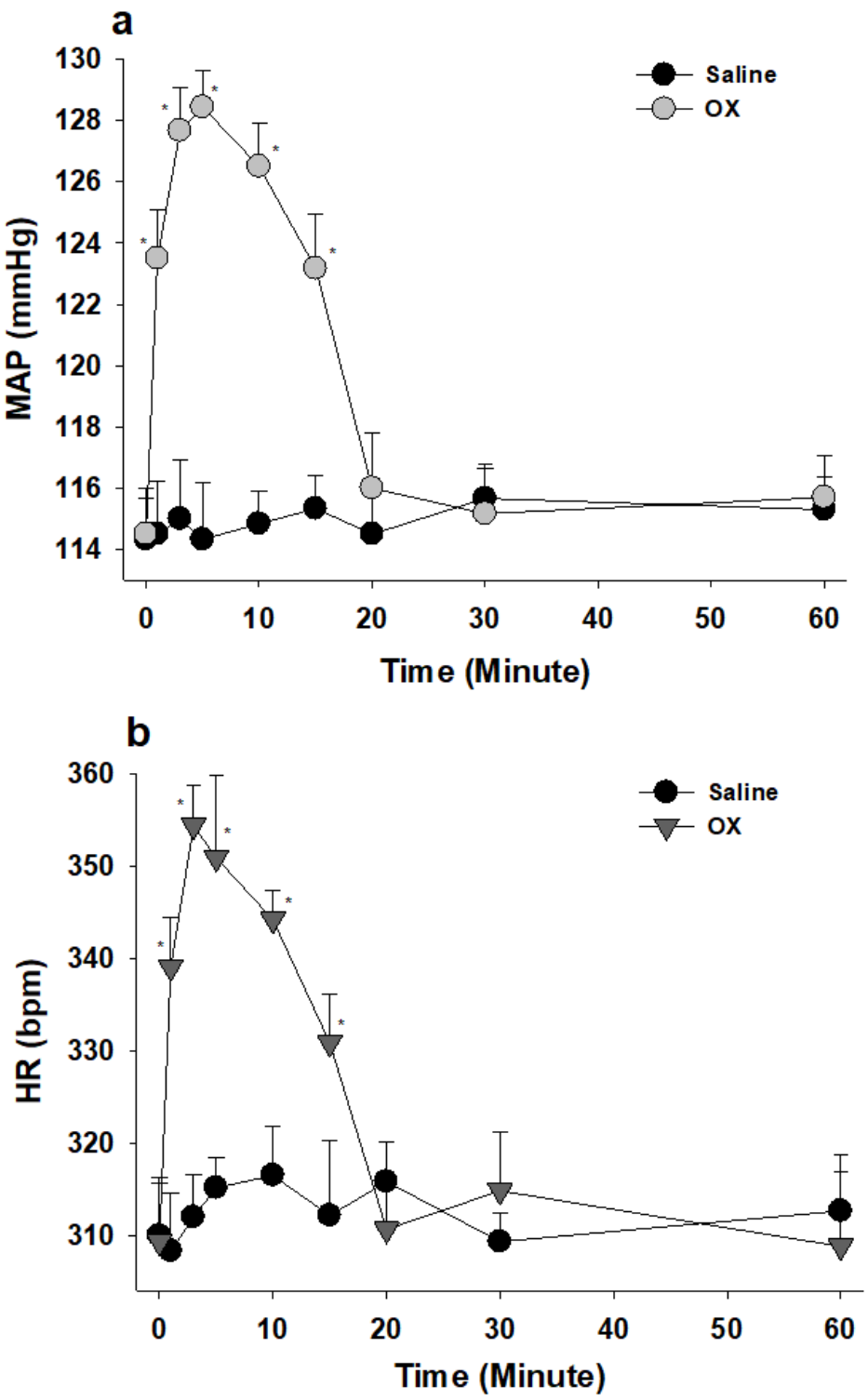
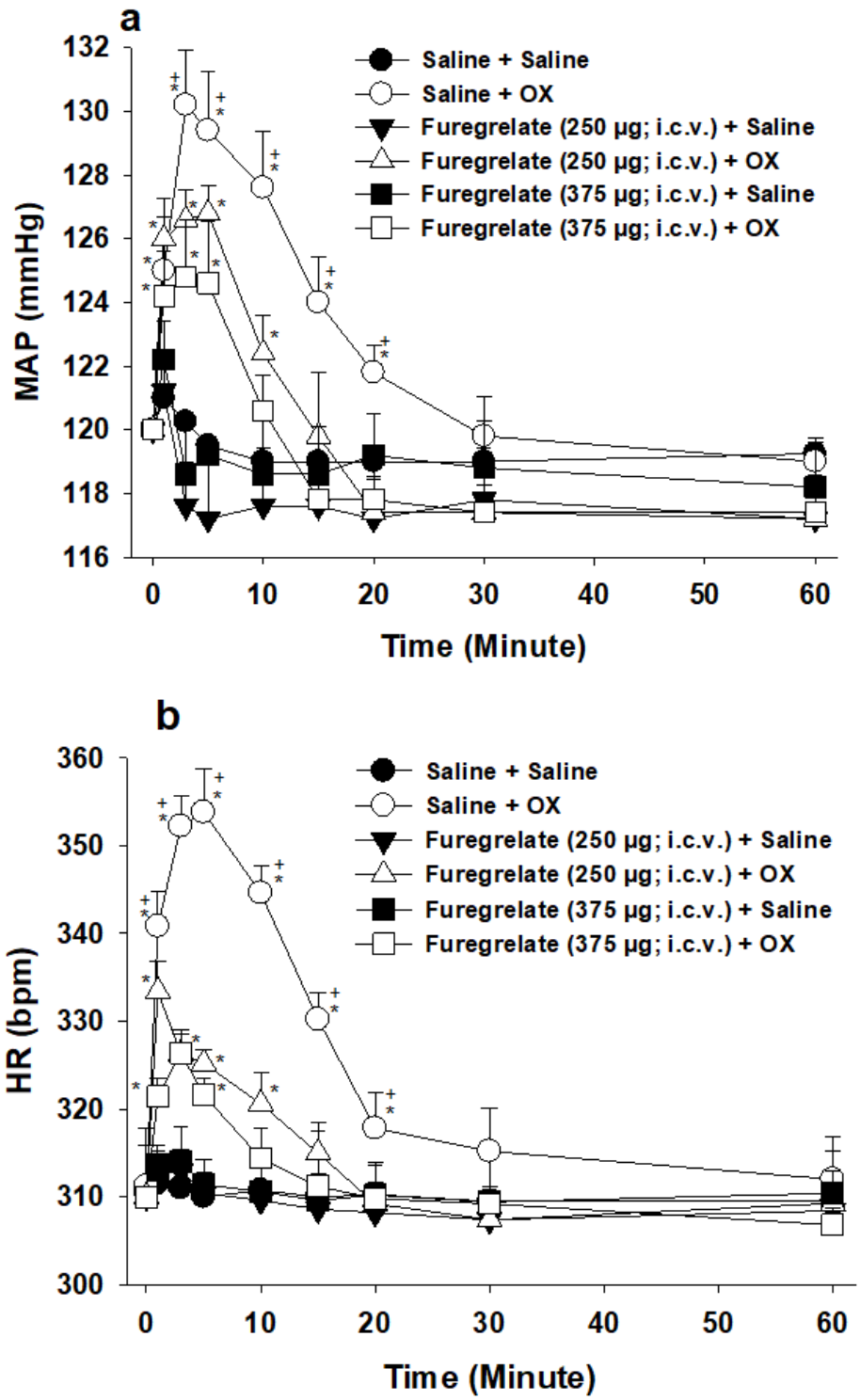
# Figures



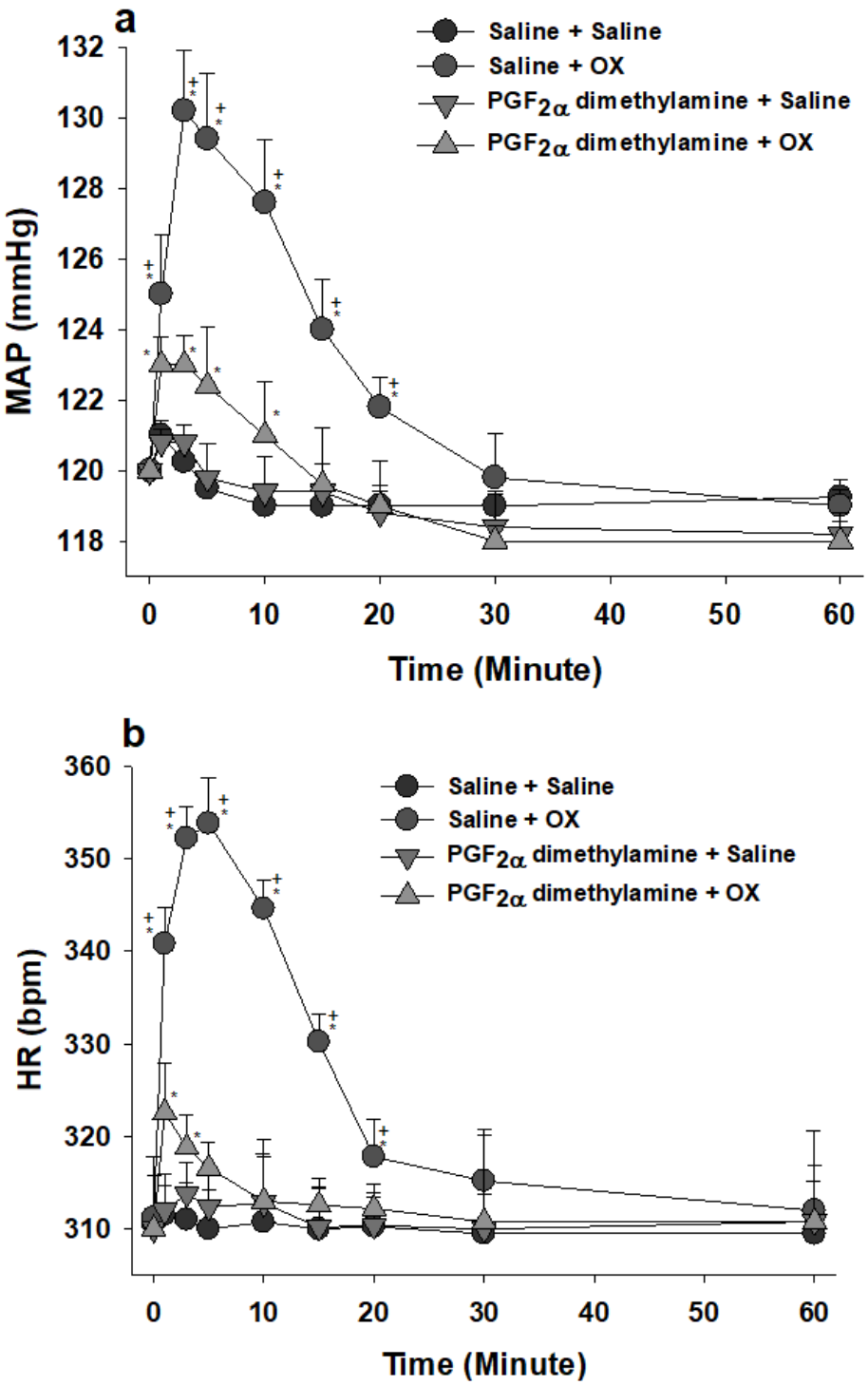

## Figure 1

MAP and HR effects of centrally administered OX OX (1.5 nmol; i.c.v.) or saline (5 µl; i.c.v.) was injected after baseline MAP (a) and HR (b) measurements had been obtained. After the injections, the MAP and HR of the animals were monitored for 60 min. *p < 0.05 significantly different from the saline group.




## Figure 2

Inhibition of OX-produced cardiovascular effect with frugrelate Furegrelate (250 and 375 µg; i.c.v.) or saline (5 µl; i.c.v.) was administered 5 min before OX (1.5 nmol; i.c.v.) or saline (5 µl; i.c.v.) injection, and then MAP (a) and HR (b) were monitored for the next 60 min. *p < 0.05 was considered significantly different from the value of the “Saline + Saline” and “Furegrelate + Saline” groups, and +p < 0.05 was considered significantly different from the value of the “Furegrelate + OX” group.




## Figure 3

Inhibition of OX-produced cardiovascular effect with PGF2α dimethylamine PGF2α dimethylamine (50 µg; i.c.v.) or saline (5 µl; i.c.v.) was administered 5 min before OX (1.5 nmol; i.c.v.) or saline (5 µl; i.c.v.) injection, and then MAP (a) and HR (b) were monitored for the next 60 min. $p < 0.05$ was considered significantly different from the value of the “Saline + Saline” and “PGF2α dimethylamine + Saline”

groups, and +p < 0.05 was considered significantly different from the value of the “PGF2α dimethylamine + OX” group.

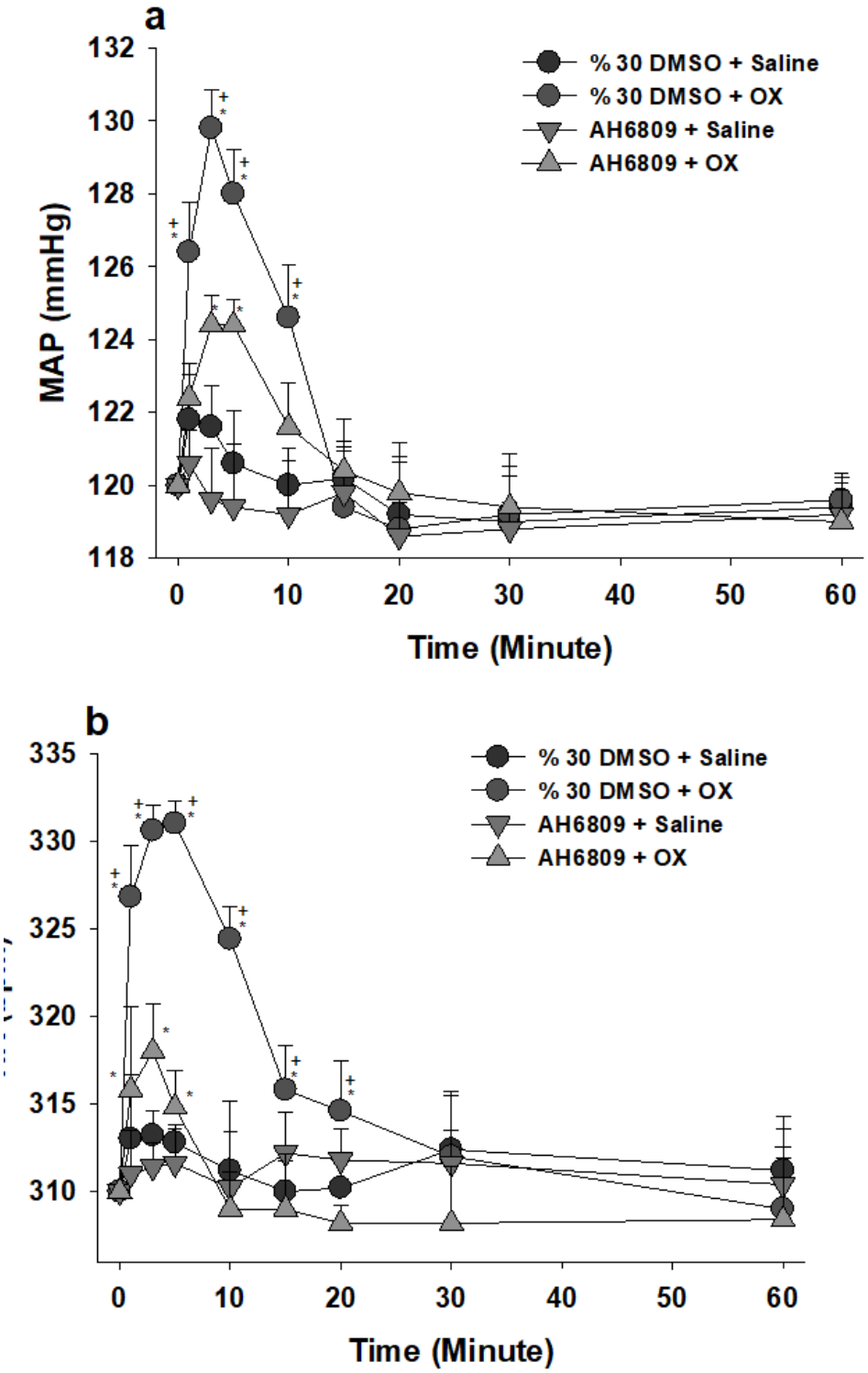



**Figure 4**

Inhibition of OX-produced cardiovascular effect with AH6809 AH6809 (10 µg; i.c.v.), or 30 % DMSO (5 µl; i.c.v.) was administered 5 min before OX (1.5 nmol; i.c.v.) or saline (5 µl; i.c.v.) injection, and then MAP (a) and HR (b) were monitored for the next 60 min. p < 0.05 was considered significantly different from the

value of the “30 % DMSO + Saline” and “AH6809 + Saline” groups, and +p < 0.05 was considered significantly different from the value of the “AH6809 + OX” group.