



**ROLE OF THE BRAIN PROSTANOIDS IN GLUCAGON-LIKE PEPTIDE-1-INDUCED CENTRAL ACTIVATION OF SYMPATHO-ADRENOMEDULLARY OUTFLOW IN RATS**

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6 **ROLE OF THE BRAIN PROSTANOIDS IN GLUCAGON-LIKE**  
7 **PEPTIDE-1-INDUCED CENTRAL ACTIVATION OF**  
8 **SYMPATHO-ADRENOMEDULLARY OUTFLOW IN RATS**  
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26 Short title: GLP-1 and sympatho-adrenomedullary outflow  
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## SUMMARY

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1. The aim of the present study was to characterize the source of plasma catecholamines induced by centrally administered glucagon-like peptide-1 (GLP-1), with regard to the brain prostanoids, in urethane-anesthetized rats.

2. GLP-1 and other compounds were administered intracerebroventricularly (i.c.v.), and blood samples were collected via a cannula inserted into the femoral artery. Catecholamines were extracted from plasma with activated alumina and were assayed electrochemically with high-performance liquid chromatography.

3. GLP-1 (0.3, 1.0 and 3.0 nmol/animal) dose-dependently elevated plasma levels of noradrenaline and adrenaline, and GLP-1 (1.0 nmol)-induced response was reduced by exendin (5-39) (a selective GLP-1 receptor antagonist) in a dose-dependent manner (5 and 10 nmol/animal). The GLP-1-induced elevation of both catecholamines was abolished by indomethacin (an inhibitor of cyclooxygenase ) (1.2  $\mu$ mol/animal), while baicalein (a lipoxygenases inhibitor) (1.2  $\mu$ mol/animal) had no effect.

4. Furegrelate (an inhibitor of thromboxane A<sub>2</sub> synthase) (1.8  $\mu$ mol/animal) and (+) S-145 (a thromboxane A<sub>2</sub> receptor antagonist) (625 nmol/animal) attenuated the GLP-1-induced elevation of plasma adrenaline, but these reagents had no effect on the elevation of plasma noradrenaline. The GLP-1-induced elevation of plasma adrenaline was abolished by acute bilateral adrenalectomy, but the procedure had no effect on the elevation of plasma noradrenaline.

5. These results suggest that centrally administered GLP-1 induces the secretion of adrenaline from the adrenal medulla by brain thromboxane A<sub>2</sub>-mediated mechanisms, while the peptide evokes the release of noradrenaline from sympathetic nerves by brain prostanoid other than thromboxane A<sub>2</sub>-mediated mechanisms in rats.

Key words: cyclooxygenase, glucagon-like peptide-1, plasma catecholamine, sympatho-adrenomedullary system, thromboxane A<sub>2</sub>.

## INTRODUCTION

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Glucagon-like peptide-1 (GLP-1) was reported originally to be a peptide hormone released from the enteroendocrine L cells in the intestine to stimulate insulin secretion from the pancreas.<sup>1-3</sup> GLP-1 is also a brain neuropeptide synthesized in the caudal regions of the nucleus of the solitary tract.<sup>4,5</sup> Although the sites of GLP-1 synthesis in the brain are quite limited, GLP-1-immunoreactive nerve fibers and GLP-1 receptors are widespread throughout the brain.<sup>6</sup> It has been shown that intravenous and intracerebroventricular administration of GLP-1 receptor agonists increases blood pressure and heart rate and induces c-Fos-like immunoreactivity in autonomic regulatory neurons in the brain.<sup>7-9</sup> These observations suggest a role for GLP-1 in the regulation of several autonomic responses in the brain.<sup>10</sup> However, the central mechanisms underlying these actions of GLP-1 remain largely undefined.

Previously, we reported that intracerebroventricular pretreatment with indomethacin, an inhibitor of cyclooxygenase, effectively reduced the increase of plasma noradrenaline and adrenaline induced by intracerebroventricularly (i.c.v.)-administered corticotrophin-releasing factor (CRF),<sup>11</sup> arginine-vasopressin,<sup>12</sup> and bombesin.<sup>13,14</sup> These results suggest the involvement of brain prostanoids in the central activation of the sympatho-adrenomedullary outflow in rats. In the present study, therefore, we investigated the mechanisms involved in i.c.v.-administered GLP-1-induced elevation of plasma catecholamines with regard to the brain prostanoids in urethane-anesthetized rats.

## METHODS

### Experimental procedures

Male Wistar rats weighing approximately 350 g were maintained in an air-conditioned room at 22-24°C under a constant day-night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. With the rats under urethane anesthesia (1.0 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 mL/h), and the femoral artery was cannulated for collecting blood samples, as described previously.<sup>15</sup> For some animals, acute bilateral adrenalectomy (plus hydrocortisone 5 mg/kg, i.m.) or a sham operation (plus 200 µL saline/animal, i.m.)<sup>16</sup> was carried out just before the experiments through bilateral dorsal incisions using an aseptic surgical technique.

Next, the animal was placed in a stereotaxic apparatus, as described previously.<sup>14</sup> A hole was drilled in the skull for intracerebroventricular administration of test substances through a stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP, -0.8; L, 1.5; V, 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas of Paxinos and Watson.<sup>17</sup> Three hours were allowed to elapse before the application of GLP-1 or other reagents.

GLP-1 and other reagents (except for baicalein) were dissolved in sterile saline and injected slowly into the right cerebral ventricle in a volume of 5 µL with a 10-µL Hamilton syringe. In the case of baicalein, the reagent was dissolved in *N, N*-dimethyl formamide (DMF) and injected slowly into the right cerebral ventricle in a volume of 2.5 µL with a 10-µL Hamilton syringe. Exendin (5-39) (a GLP-1 receptor antagonist) and (+) S-145 (a thromboxane A<sub>2</sub> receptor antagonist) were administered i.c.v. 15 min before GLP-1 administration. Water-soluble indomethacin-Na (a cyclooxygenase inhibitor), baicalein (a lipoxygenase inhibitor) and furegrelate (a thromboxane A<sub>2</sub> synthase inhibitor) were administered i.c.v. 30 min before GLP-1 administration.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Kochi University.

### Measurement of plasma catecholamines

Blood samples (250  $\mu$ L) were collected through an arterial catheter and preserved on ice during the experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre,<sup>18</sup> with a slight modification, and were assayed electrochemically with high-performance liquid chromatography (HPLC).<sup>15</sup> Briefly, after centrifugation, plasma (100  $\mu$ L) was transferred to a sample tube containing 30 mg of activated alumina, 1 ng of 3,4-dihydroxybenzylamine as an internal standard, and 3 mL of 0.5 M Tris buffer (pH 8.6) containing 0.1 M disodium EDTA. The tube was shaken for 10 min, and the alumina was washed three times with 4 mL of ice-cold, double-deionized water. Then, the catecholamines adsorbed onto the alumina were eluted with 300  $\mu$ L of 4% acetic acid containing 0.1 mM disodium EDTA.

A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France), and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with HPLC. Analytical conditions were as follows: detector +450 mV potential against an Ag/AgCl reference electrode; column, Eicompact CA-50DS, 2.1 x 150 mm (Eicom); mobile phase, 0.1 M  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  buffer (pH 6.0) containing 50 mg/L disodium EDTA, 750 mg/L 1-octane sulfate sodium (Nacalai Tesque, Kyoto, Japan) and 15% methanol, at a flow rate of 0.18 mL/min. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. This assay could determine 0.5 pg of noradrenaline and adrenaline accurately.

### Treatment of data and statistics

Results are expressed as the mean $\pm$ S.E.M. of the net change above the respective basal values. The data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post-hoc analysis with the Bonferroni method for comparing a control to all other means (Figs. 1, 2 and 4B). When only two means were compared, the data were

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6 analyzed with an unpaired Student's t-test (Figs. 3, 4A and 5). *P* values less than 0.05  
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8 were taken to indicate statistical significance.  
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## 10 11 12 13 **Compounds** 14

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16 GLP-1 (7-36 amide) and exendin (5-39) were purchased from Peptide Institute (Osaka,  
17 Japan). Baicalein and furegrelate sodium were obtained from Biomol Research Labs  
18 (Plymouth Meeting, PA, USA). Water-soluble indomethacin sodium trihydrate and (+)  
19 S-145 were kind gifts from Merck (Rahway, NJ, USA) and Shionogi Pharmaceutical Co.  
20 Ltd. (Osaka, Japan), respectively. All other reagents were purchased from Nacalai  
21 Tesque (Kyoto, Japan).  
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## RESULTS

### Effect of GLP-1 on the plasma levels of catecholamines

Administration of vehicle (5  $\mu$ L of saline/animal) and blood sampling five times over a 60-min period had no effect on the basal plasma levels of either noradrenaline or adrenaline (Fig. 1).

GLP-1 dose-dependently elevated plasma levels of noradrenaline and adrenaline (Fig. 1). The noradrenaline response to GLP-1 (0.3 and 1.0 nmol/animal) reached a maximum at 20-30 min after administration of the peptide, and then gradually declined to near basal levels, while the response to GLP-1 (3.0 nmol/animal) reached a maximum at 20 min after administration of the peptide, and elevated levels were maintained throughout the experiments. The plasma adrenaline response to GLP-1 (0.3, 1.0 and 3.0 nmol/animal) reached a maximum at 20 min after administration of the peptide, and then gradually declined to near basal levels (Fig. 1).

### Effect of exendin (5-39) on the GLP-1-induced elevation of plasma catecholamines

Pretreatment with exendin (5-39) (a GLP-1 receptor antagonist) (10 nmol/animal) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 2).

Pretreatment with vehicle (5  $\mu$ L of saline/animal) slightly, but not significantly, potentiated the effect of GLP-1 (1.0 nmol/animal) on the plasma level of noradrenaline (Fig. 2). The GLP-1-induced elevation of plasma catecholamines was attenuated by pretreatment with exendin (5-39) in a dose-dependent manner (5 and 10 nmol/animal) (Fig. 2).

### Effects of indomethacin and baicalein on the GLP-1-induced elevation of plasma catecholamines



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6 Pretreatment with indomethacin (a cyclooxygenase inhibitor) (1.2  $\mu\text{mol}$  [500  $\mu\text{g}$ ]/animal)  
7 or baicalein (a lipoxygenase inhibitor) (1.2  $\mu\text{mol}$  [400  $\mu\text{g}$ ]/animal) had no effect on the  
8 basal plasma levels of noradrenaline and adrenaline, respectively (Fig. 3, A and B).  
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11 Pretreatment with indomethacin (1.2  $\mu\text{mol}$ /animal) effectively reduced the elevation  
12 of both catecholamines induced by GLP-1 (1.0 nmol/animal) (Fig. 3A). In contrast,  
13 pretreatment with baicalein (1.2  $\mu\text{mol}$ /animal) slightly, but not significantly, elevated the  
14 GLP-1-induced responses (Fig. 3B).  
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### 21 **Effects of furegrelate and (+) S-145 on the GLP-1-induced elevation of plasma** 22 **catecholamines** 23 24

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26 Pretreatment with furegrelate (a thromboxane  $A_2$  synthase inhibitor) (1.8  $\mu\text{mol}$  [500  
27  $\mu\text{g}$ ]/animal) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig.  
28 4A). Pretreatment with furegrelate significantly reduced the elevation of adrenaline  
29 induced by GLP-1 (1.0 nmol/animal), but the reagent had no effect on the elevation of  
30 noradrenaline induced by this peptide (Fig. 4A).  
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35 According to our previous report,<sup>14</sup> we used (+) S-145 (a thromboxane  $A_2$  receptor  
36 antagonist) at doses of 250 and 625 nmol/animal in the present experiment (Fig. 4B).  
37 Pretreatment with this reagent (625 nmol/animal) had no effect on the basal plasma levels  
38 of noradrenaline and adrenaline. Pretreatment with this reagent effectively attenuated the  
39 GLP-1 (1.0 nmol/animal)-induced elevation of adrenaline in a dose-dependent manner  
40 (250 and 625 nmol/animal), while it slightly, but not significantly, reduced the elevation  
41 of noradrenaline induced by this peptide (Fig. 4B).  
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### 50 **Effect of bilateral adrenalectomy on the GLP-1-induced elevation of plasma** 51 **catecholamines** 52 53

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55 The basal plasma levels of noradrenaline and adrenaline were reduced slightly by sham  
56 operation. The basal plasma level of noradrenaline was reduced slightly, but not  
57 significantly, by bilateral adrenalectomy, while the basal plasma level of adrenaline was  
58 reduced significantly by the procedure (Fig. 5).  
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6 The GLP-1 (1.0 nmol/animal)-induced elevation of plasma adrenaline was  
7 abolished by bilateral adrenalectomy, while the procedure had no effect on the  
8 peptide-induced elevation of plasma noradrenaline (Fig. 5).  
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## DISCUSSION

In the present experiment, centrally administered GLP-1 effectively elevated plasma levels of catecholamines (adrenaline > noradrenaline), and these elevations were suppressed dose-dependently by exendin (5-39), a GLP-1 receptor antagonist.<sup>19</sup> Several lines of evidence indicate that centrally administered GLP-1 induces a dose-dependent increase in blood pressure and heart rate.<sup>9,20,21</sup> Furthermore, it has been shown that an activation of the GLP-1 receptors in the rat brain induces c-Fos expression in neurons involved in autonomic control sites, including the hypothalamic paraventricular nucleus and medullary neurons providing output to sympathetic preganglionic neurons.<sup>9</sup> Taken together, it would be reasonable to assume that centrally administered GLP-1 elevates plasma catecholamines via an activation of brain GLP-1 receptors in rats.

Previously, we reported that the brain cyclooxygenase is involved in centrally administered CRF-, arginine-vasopressin-, and bombesin-induced elevations of plasma noradrenaline and adrenaline using indomethacin in rats.<sup>11,12,13</sup> The elevation of plasma noradrenaline induced by centrally administered interleukin-1 $\beta$  was also attenuated by central pretreatment with indomethacin.<sup>22</sup> Since indomethacin is a potent inhibitor of the prostaglandin-forming cyclooxygenase,<sup>23</sup> active products of cyclooxygenase seem to be involved in the elevations of plasma catecholamines induced by these peptides in rats. However, it remains possible that active products of the brain lipoxygenases are involved in the elevations of plasma catecholamines induced by these peptides.

In the next experiment, therefore, we examined the effects of centrally administered indomethacin and baicalein, a lipoxygenase inhibitor,<sup>24</sup> on the GLP-1-induced elevation of plasma catecholamines. These reagents were directly administered into the lateral ventricle of the rat brain. The elevation of plasma catecholamines induced by GLP-1 was abolished by central pretreatment with indomethacin (1.2  $\mu$ mol/animal). In contrast, the same dose of baicalein had no attenuating effect on the GLP-1-induced elevation of plasma catecholamines. Collectively, it would be reasonable to assume that the active products of cyclooxygenase seem to be involved in the GLP-1-induced elevation of plasma catecholamines in rats.

We have already reported that furegrelate, a selective thromboxane A<sub>2</sub> synthase inhibitor,<sup>25</sup> abolished the elevation of plasma adrenaline induced by centrally administered CRF, arginine-vasopressin, or bombesin.<sup>11,12,14</sup> Furthermore, injection of a

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6 thromboxane A<sub>2</sub> mimetic into the hypothalamic paraventricular nucleus predominantly  
7 elevates plasma adrenaline.<sup>26</sup> These results suggest the involvement of brain  
8 thromboxane A<sub>2</sub> in the elevation of plasma adrenaline induced by these peptides. Then,  
9 we examined the effect of furegrelate on the GLP-1-induced elevation of plasma  
10 catecholamines. The GLP-1-induced elevation of plasma adrenaline was reduced by  
11 centrally administered furegrelate, while the reagent was without effect on the  
12 GLP-1-induced elevation of plasma noradrenaline. Furthermore, (+) S-145, a  
13 thromboxane A<sub>2</sub> receptor antagonist,<sup>14</sup> also attenuated the GLP-1-induced elevation of  
14 plasma adrenaline, but not that of noradrenaline. Based on these results, it would be  
15 reasonable to assume that the brain thromboxane A<sub>2</sub> is involved in the GLP-1-induced  
16 elevation of plasma adrenaline.  
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25 In general, plasma noradrenaline reflects the activity of the sympathetic nervous  
26 system, while plasma adrenaline reflects the activity of the adrenomedullary system.  
27 Recently, we demonstrated that centrally administered arginine-vasopressin, bombesin  
28 and histamine elicit adrenal secretion of both noradrenaline and adrenaline by brain  
29 thromboxane A<sub>2</sub>-mediated mechanisms, while centrally administered CRF elicits  
30 sympathetic noradrenaline release and adrenal adrenaline secretion by brain  
31 prostaglandin E<sub>2</sub>- and thromboxane A<sub>2</sub>-mediated mechanisms, respectively, in rats.<sup>14-16</sup>  
32 Furthermore, it has been reported that noradrenaline-containing cells and  
33 adrenaline-containing cells in the adrenal medulla are innervated separately by  
34 preganglionic neurons in the spinal cord.<sup>27</sup> More recently, using c-Fos expression, we  
35 demonstrated that adrenaline- and noradrenaline-containing cells in the adrenal medulla  
36 and celiac sympathetic ganglia are differentially controlled by brain thromboxane A<sub>2</sub> and  
37 prostanoids other than thromboxane A<sub>2</sub> (probably prostaglandin E<sub>2</sub>), respectively, in  
38 rats.<sup>28</sup> In the last experiment, we performed acute bilateral adrenalectomy to determine  
39 the origin of plasma catecholamines induced by centrally administered GLP-1. Bilateral  
40 adrenalectomy abolished the centrally administered GLP-1-induced elevation of plasma  
41 adrenaline, while the procedure had no effect on the elevation of plasma noradrenaline.  
42 These results suggest that centrally administered GLP-1 evokes noradrenaline release  
43 from sympathetic nerves and adrenaline secretion from the adrenal gland in rats.  
44 GLP-1-containing nerve terminals densely innervate neurons in the hypothalamic  
45 paraventricular nucleus,<sup>29</sup> which has been recognized as a regulatory center of the  
46 sympatho-adrenomedullary system.<sup>30</sup> These observations further support the present  
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6 results that central GLP-1-containing neurons regulate central  
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8 sympatho-adrenomedullary outflow in rats.

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10 In summary, we demonstrated here that centrally administered GLP-1 evokes  
11 adrenal adrenaline secretion by brain thromboxane A<sub>2</sub>-mediated mechanisms and  
12 sympathetic noradrenaline release by mechanisms involving prostaglandins other than  
13 thromboxane A<sub>2</sub> (probably prostaglandin E<sub>2</sub>) in rats.  
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## FIGURE LEGENDS

**Fig. 1** Effect of GLP-1 on plasma catecholamine levels. Arrow indicates the administration of GLP-1 (0.3, 1.0 and 3.0 nmol/animal, i.c.v.) or vehicle (5  $\mu$ L saline/animal, i.c.v.).  $\circ$ , vehicle ( $n = 4$ );  $\blacktriangle$ , 0.3 nmol ( $n = 9$ );  $\bullet$ , 1.0 nmol ( $n = 10$ );  $\blacksquare$ , 3.0 nmol ( $n = 5$ ). \*Significantly different ( $P < 0.05$ ) from vehicle (5  $\mu$ L saline)-treated control with Bonferoni method. Each point represents the mean $\pm$ S.E.M. The actual values for noradrenaline and adrenaline at 0 min were 400 $\pm$ 46 and 322 $\pm$ 45 pg/mL ( $n = 28$ ), respectively.

**Fig. 2** Effect of exendin (5-39) (a GLP-1 receptor antagonist) on the GLP-1-induced elevation of plasma catecholamines. Exendin (5-39) (5 and 10 nmol/animal, i.c.v.) or vehicle-1 (5  $\mu$ L saline/animal, i.c.v.) was administered 15 min before GLP-1 (1.0 nmol/animal, i.c.v.) or vehicle-2 (5  $\mu$ L saline/animal, i.c.v.).  $\circ$ , vehicle-1 plus vehicle-2 ( $n = 4$ );  $\square$ , exendin (5-39) (10 nmol/animal) plus vehicle-2 ( $n = 4$ );  $\bullet$ , vehicle-1 plus GLP-1 ( $n = 16$ );  $\blacktriangle$ , exendin (5-39) (5 nmol/animal) plus GLP-1 ( $n = 5$ );  $\blacksquare$ , exendin (5-39) (10 nmol/animal) plus GLP-1 ( $n = 6$ ). \*Significantly different ( $P < 0.05$ ) from vehicle-1 (5  $\mu$ L saline)- and GLP-1-treated group with Bonferoni method. Other conditions were the same as those in Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 376 $\pm$ 56 and 263 $\pm$ 56 pg/mL in the vehicle-1 (5  $\mu$ L saline)-pretreated group ( $n = 20$ ), 580 $\pm$ 68 and 376 $\pm$ 83 pg/mL in the exendin (5-39) (5 nmol)-pretreated group ( $n = 5$ ), 204 $\pm$ 23 and 180 $\pm$ 54 pg/mL in the exendin (5-39) (10 nmol)-pretreated group ( $n = 10$ ), respectively.

**Fig. 3** Effects of indomethacin (a cyclooxygenase inhibitor) and baicalein (a lipoxygenase inhibitor) on the GLP-1-induced elevation of plasma catecholamines. (A) Indomethacin (1.2  $\mu$ mol/animal, i.c.v.) or vehicle-1 (5  $\mu$ L saline/animal, i.c.v.) was administered 30 min before GLP-1 (1.0 nmol/animal, i.c.v.) or vehicle-2 (5  $\mu$ L saline/animal, i.c.v.).  $\circ$ , vehicle-1 plus vehicle-2 ( $n = 4$ );  $\triangle$ , indomethacin plus vehicle-2 ( $n = 4$ );  $\bullet$ , vehicle-1 plus GLP-1 ( $n = 12$ );  $\blacktriangle$ , indomethacin plus GLP-1 ( $n = 7$ ). (B) Baicalein (1.2  $\mu$ mol/animal, i.c.v.) or vehicle-1 (2.5  $\mu$ L DMF/animal, i.c.v.) was administered 30 min before GLP-1 (1.0 nmol/animal, i.c.v.) or vehicle-2 (5  $\mu$ L saline/animal, i.c.v.).  $\circ$ , vehicle-1 plus vehicle-2 ( $n = 3$ ),  $\square$ , baicalein plus vehicle-2 ( $n$

= 5); ●, vehicle-1 plus GLP-1 ( $n = 6$ ); ■, baicalein plus GLP-1 ( $n = 5$ ). \*Significantly different ( $P < 0.05$ ) from vehicle-1 (5  $\mu\text{L}$  saline/2.5  $\mu\text{L}$  DMF)- and GLP-1-treated group with Student's  $t$ -test in (A) and (B), respectively. Other conditions were the same as those in Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were  $430 \pm 64$  and  $224 \pm 21$  pg/mL in the vehicle-1 (5  $\mu\text{L}$  saline)-pretreated group ( $n = 16$ ),  $457 \pm 82$  and  $500 \pm 100$  pg/mL in the indomethacin-pretreated group ( $n = 11$ ) in (A);  $354 \pm 47$  and  $221 \pm 65$  pg/mL in the vehicle-1 (2.5  $\mu\text{L}$  DMF)-pretreated group ( $n = 9$ ),  $314 \pm 42$  and  $341 \pm 77$  pg/mL in the baicalein-pretreated group ( $n = 10$ ) in (B), respectively.

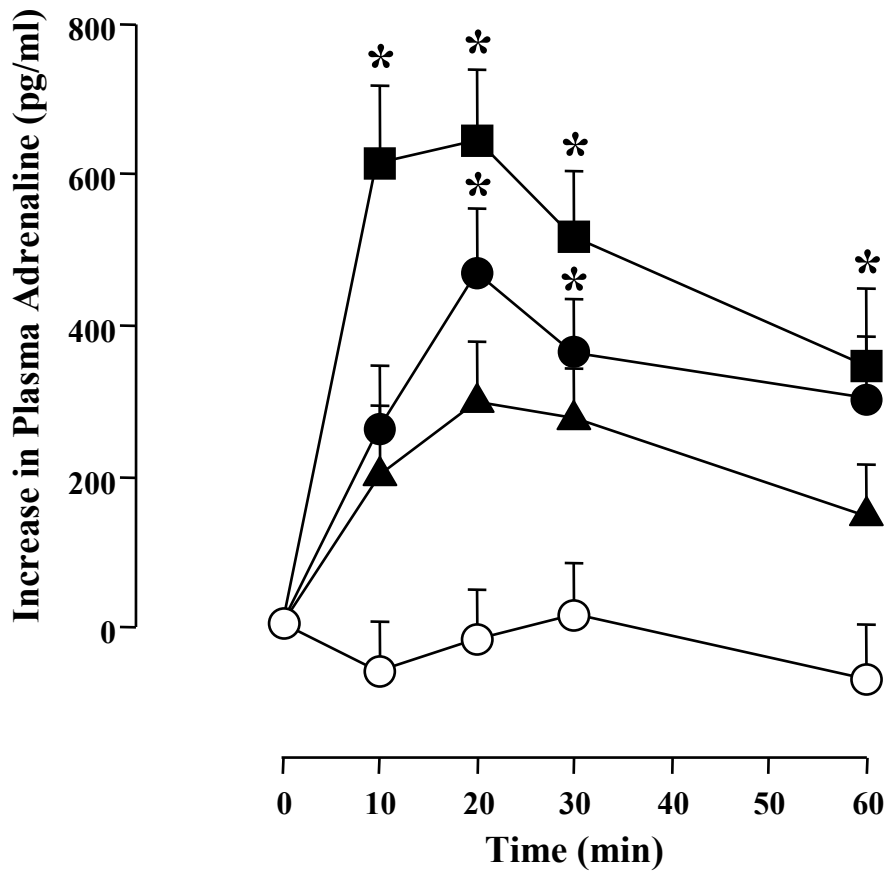
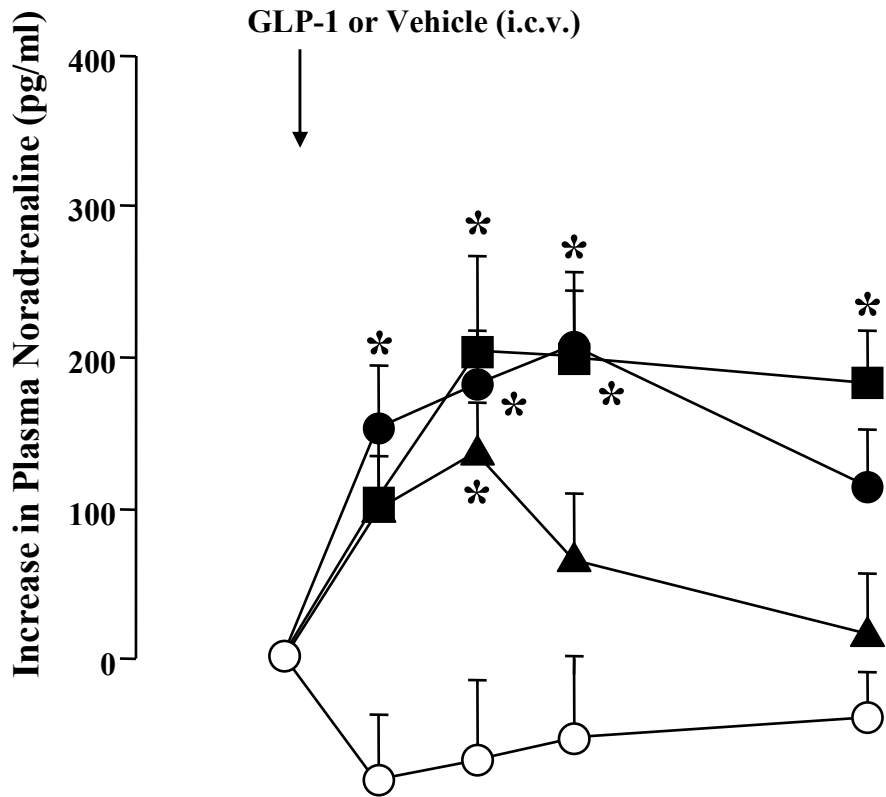
**Fig. 4** Effects of furegrelate (a thromboxane  $A_2$  synthase inhibitor) and (+) S-145 (a thromboxane  $A_2$  receptor antagonist) on the GLP-1-induced elevation of plasma catecholamines. (A) Furegrelate (1.8  $\mu\text{mol}/\text{animal}$ , i.c.v.) or vehicle-1 (5  $\mu\text{L}$  saline/animal, i.c.v.) was administered 30 min before GLP-1 (1.0 nmol/animal, i.c.v.) or vehicle-2 (5  $\mu\text{L}$  saline/animal, i.c.v.). ○, vehicle-1 plus vehicle-2 ( $n = 4$ ) (cited from Fig. 3A); ▽, furegrelate plus vehicle-2 ( $n = 6$ ); ●, vehicle-1 plus GLP-1 ( $n = 12$ ) (cited from Fig. 3A); ▼, furegrelate plus GLP-1 ( $n = 6$ ). (B) (+) S-145 (250 and 625 nmol/animal, i.c.v.) or vehicle-1 (5  $\mu\text{L}$  saline/animal, i.c.v.) was administered 15 min before GLP-1 (1.0 nmol/animal, i.c.v.) or vehicle-2 (5  $\mu\text{L}$  saline/animal, i.c.v.). ○, vehicle-1 plus vehicle-2 ( $n = 4$ ) (cited from Fig. 2); □, (+) S-145 (625 nmol/animal, i.c.v.) plus vehicle-2 ( $n = 3$ ); ●, vehicle-1 plus GLP-1 ( $n = 16$ ) (cited from Fig. 2); ▲, (+) S-145 (250 nmol) plus GLP-1 ( $n = 4$ ); ■, (+) S-145 (625 nmol) plus GLP-1 ( $n = 6$ ).

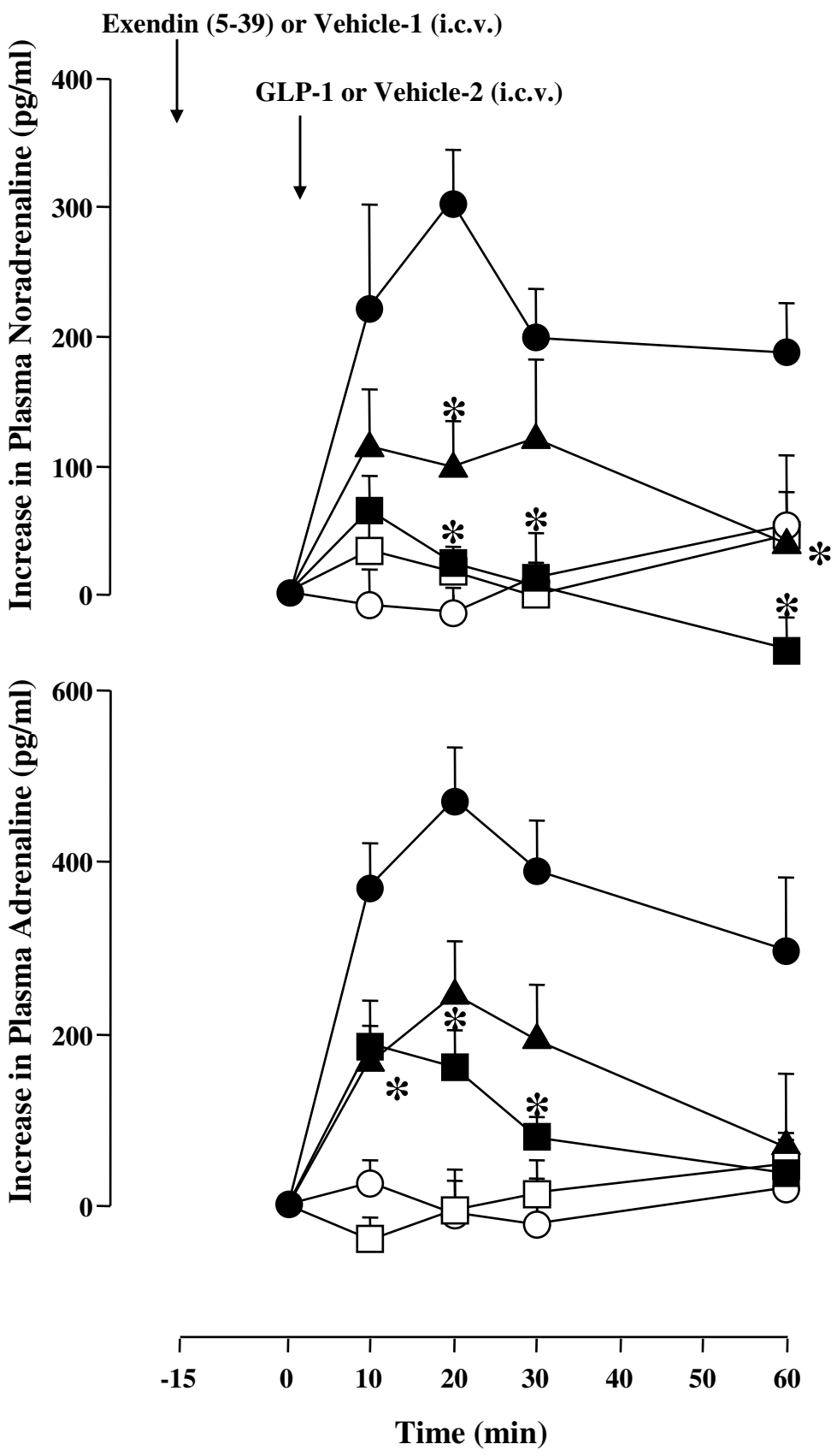
\*Significantly different ( $P < 0.05$ ) from vehicle-1 (5  $\mu\text{L}$  saline)- and GLP-1-treated group with Student's  $t$ -test in (A) and Bonferoni method in (B), respectively. Other conditions were the same as those in Figs. 1-3. The actual values for noradrenaline and adrenaline at 0 min were  $430 \pm 64$  and  $224 \pm 21$  pg/mL in the vehicle-1 (5  $\mu\text{L}$  saline)-pretreated group ( $n = 16$ ),  $425 \pm 43$  and  $319 \pm 40$  pg/mL in the furegrelate-pretreated group ( $n = 12$ ) in (A);  $376 \pm 56$  and  $263 \pm 56$  pg/mL in the vehicle-1 (5  $\mu\text{L}$  saline)-pretreated group ( $n = 20$ ),  $143 \pm 11$  and  $155 \pm 58$  pg/mL in the (+) S-145 (250 nmol)-pretreated group ( $n = 4$ ),  $251 \pm 55$  and  $126 \pm 30$  pg/mL in the (+) S-145 (625 nmol)-pretreated group ( $n = 9$ ) in (B), respectively.

**Fig. 5** Effect of acute bilateral adrenalectomy on the GLP-1-induced elevation of catecholamines. Hydrocortisone (5 mg/kg) or saline (200  $\mu\text{L}/\text{animal}$ ) was administered

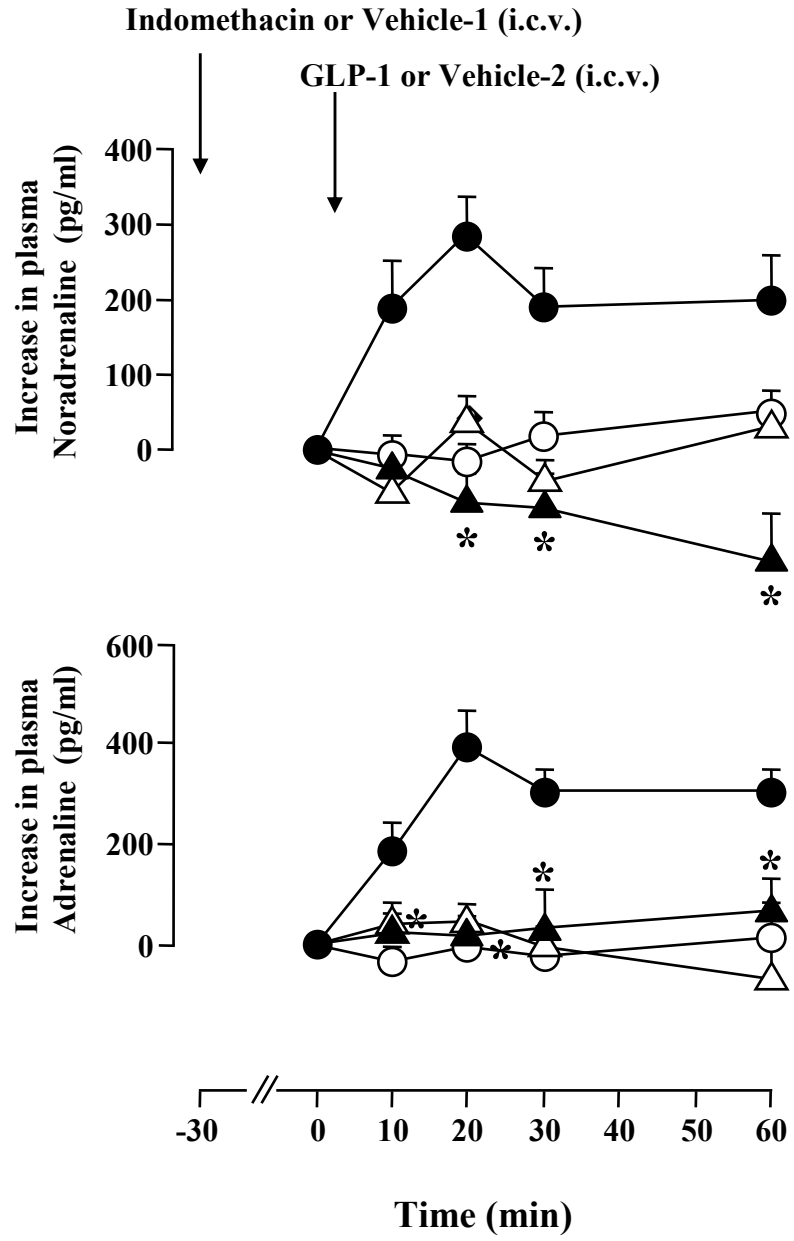
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6 intramuscularly in the adrenalectomized group (■) or sham-operated group (●),  
7 respectively. Arrow indicates the administration of GLP-1 (1.0 nmol/animal, i.c.v.).  
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9 \*Significantly different ( $P < 0.05$ ) from sham-operated group with Student's t-test. Other  
10 conditions were the same as those in Figs. 1-4. The actual values for noradrenaline and  
11 adrenaline at 0 min were  $281 \pm 81$  and  $143 \pm 36$  pg/mL in the sham-operated group ( $n = 5$ )  
12 and  $99 \pm 17$  and  $52 \pm 6$  pg/mL in the bilateral adrenalectomized group ( $n = 5$ ), respectively.  
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For Peer Review

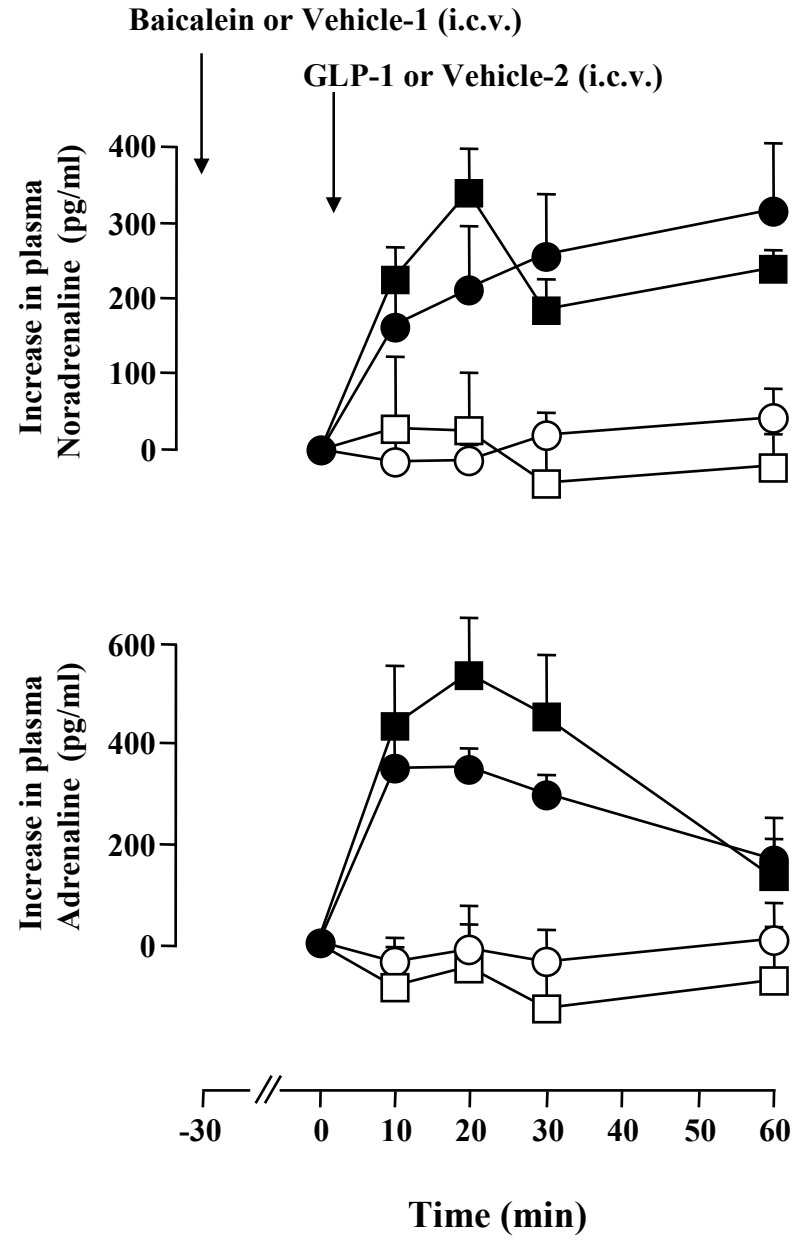




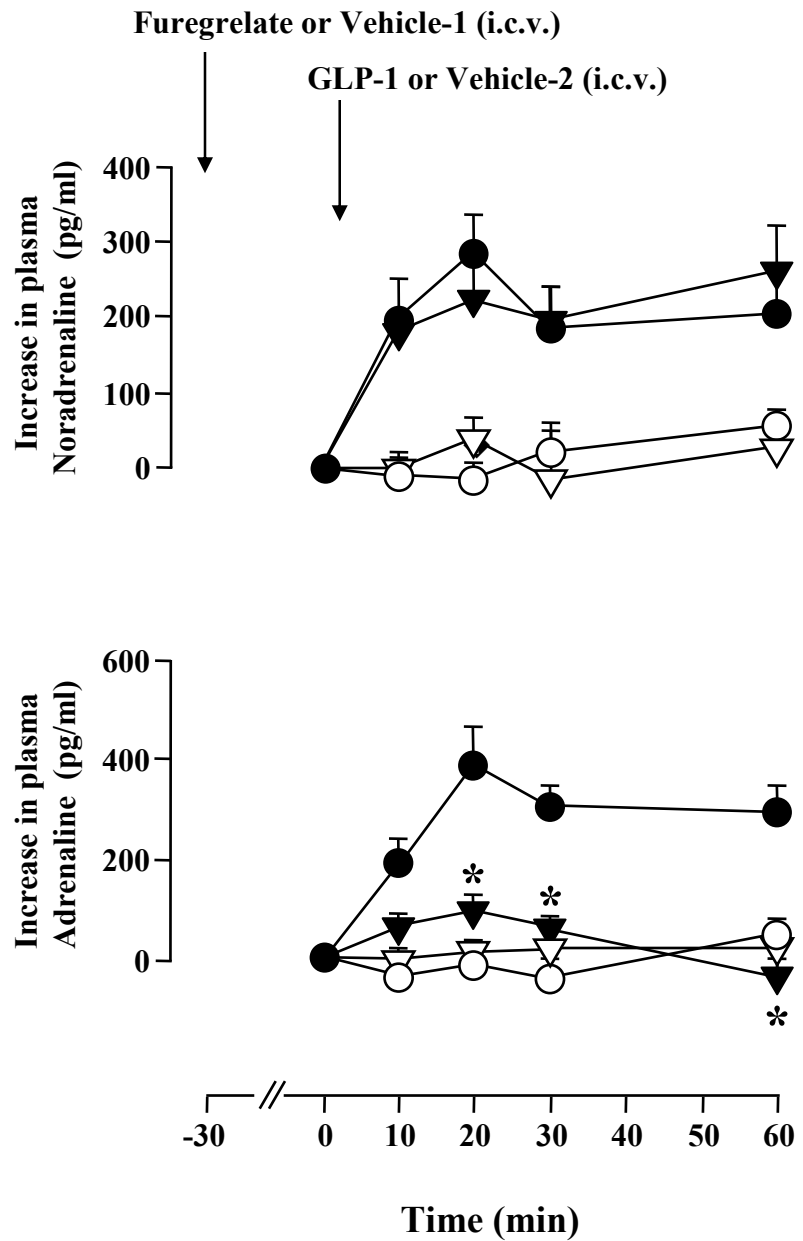
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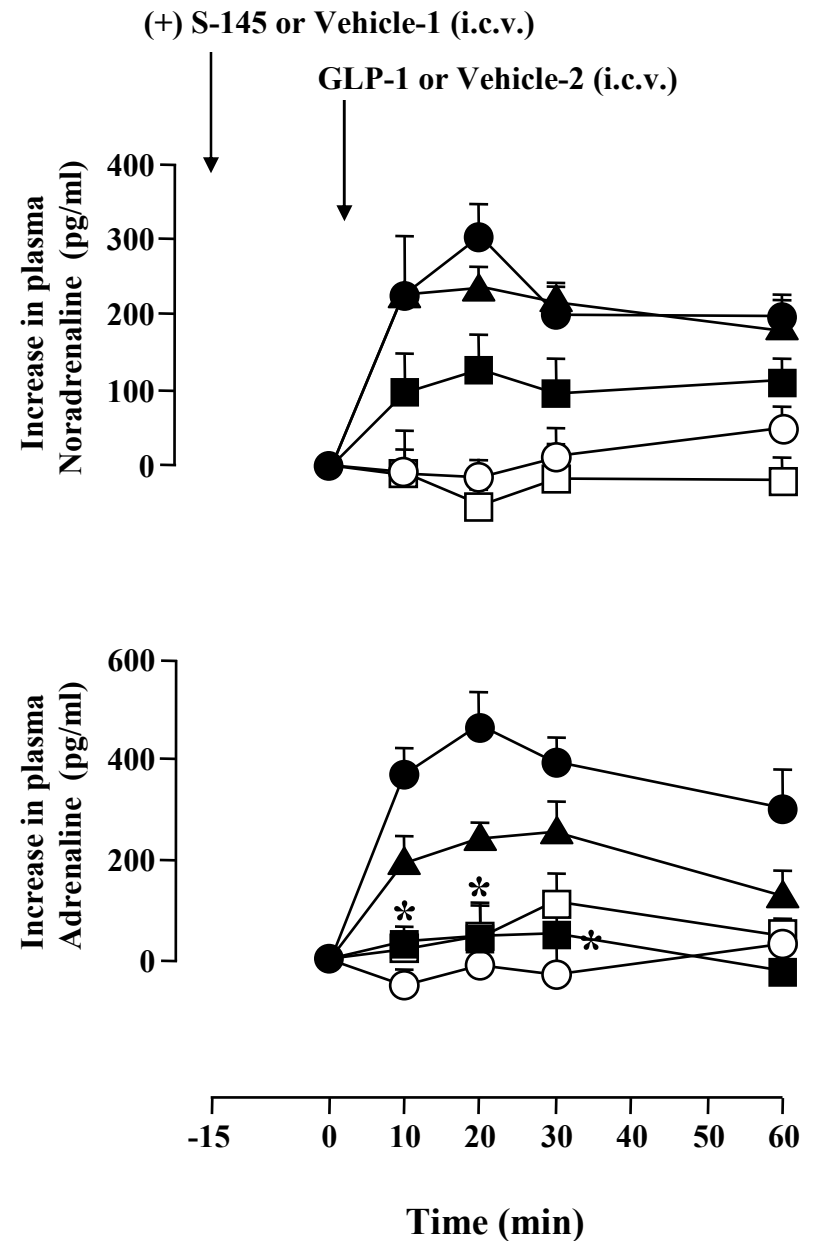
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