Centrally administered neuromedin U elevates plasma adrenaline by brain prostanoid TP receptor-mediated mechanisms in rats

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Abstract

Neuromedin U is a hypothalamic peptide involved in energy homeostasis and stress responses. The peptide, when administered intracerebroventricularly (i.c.v.), decreases food intake and body weight while increasing body temperature and heat production. We examined the effect of i.c.v. administered neuromedin U on plasma catecholamines with regard to the brain prostanoid using anesthetized rats. Neuromedin U (0.1, 0.5 and 1 nmol/animal, i.c.v.) effectively elevated plasma adrenaline (a maximal response was obtained at 0.5 nmol/animal), but had little effect on plasma noradrenaline. However, intravenously administered neuromedin U (0.5 nmol/animal) had no effect on plasma catecholamines. Neuromedin U (0.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline was effectively reduced by intracerebroventricular pretreatments with indomethacin (an inhibitor of cyclooxygenase) (0.6 and 1.2 μmol/animal), furegrelate (an inhibitor of thromboxane A_2 synthase) (0.9 and 1.8 μmol/animal) and (+)-S-145 (a blocker of prostanoid TP receptors) (250 and 625 nmol/animal), respectively. The neuromedin U-induced adrenaline response was also abolished by acute bilateral adrenalectomy. These results suggest that centrally administered neuromedin U evokes the secretion of adrenaline from the adrenal medulla by brain prostanoid TP receptor-mediated mechanisms in rats.

Keywords: Brain; Neuromedin U; Cyclooxygenase; Thromboxane A_2; Adrenal medulla

1. Introduction

Neuromedin U is a peptide initially isolated from porcine spinal cord and named due to its strong contractile properties in the uterus (Minamino et al., 1985). The peptide was later identified in the brain, spinal cord and intestine of other species (O'Harte et al., 1991; Kage et al., 1991; Austin et al., 1994). Rat and mouse neuromedin U consists of 23 amino acids and their C-terminal regions, which are essential for the peptide's activity, are highly conserved compared to other species (Conlon et al., 1988; Minamino et al., 1988). Neuromedin U-like immunoreactivity is found in the spinal cord, several brain regions including the hypothalamus, pituitary and thyroid glands in addition to the gastrointestinal and urogenital tracts (Brighton et al., 2004). While several peripheral activities such as contraction of smooth muscle and blood vessels and hypertensive effects have been described for neuromedin U (Minamino et al., 1985; Sumi et al., 1987; Gardiner et al., 1990), its role in the central nervous system remains poorly understood.

Neuromedin U-like immunoreactivity and mRNA in the central nervous system are abundant in nuclei of the brain stem and hypothalamus. Within the hypothalamus, neuromedin U-like immunoreactive cell bodies and mRNA expression are restricted to the arcuate nucleus and median eminence (Ballesta et al., 1988; Howard et al., 2000). The paraventricular, ventromedial and dorsomedial nuclei receive dense innervation of neuromedin U immunoreactive fibers (Ballesta et al., 1988). Neuromedin U receptor subtype 2 mRNA is also demonstrated in the hypothalamus including paraventricular nucleus (Guan et al., 2001). Centrally administered neuromedin U has been reported to decrease food intake and body weight in free-feeding rats (Howard et al., 2000;

Nakazato et al., 2000; Hanada et al., 2001; Ivanov et al., 2002). On the other hand, the peptide activates stress responses such as locomotor activity (Nakazato et al., 2000), non-exercise thermogenesis (Novak et al., 2006), the hypothalamo-pituitary-adrenal axis (Wren et al., 2002) and sympathetic nervous system (Chu et al., 2002; Sato et al., 2007). Hypothalamic paraventricular nucleus has been shown to be a control center for the sympathetic nervous system (Swanson and Sawchenko, 1980) in addition to the hypothalamo-pituitary-adrenal axis (Herman and Cullinan, 1997). Therefore, the brain neuromedin U seems to associate with the regulation of both energy intake and expenditure. Although the energy expenditure seems to be mainly carried out by activation of the sympathetic nervous system, the central excitatory mechanisms are largely undefined.

Recently, we reported that centrally administered stress-related neuropeptides, vasopressin and bombesin, elicit adrenal secretion of both adrenaline and noradrenaline via the brain thromboxane A_2–mediated mechanisms, while centrally administered corticotropin-releasing factor (CRF) elicits adrenal adrenaline secretion and sympathetic noradrenaline release via the brain thromboxane A_2– and prostaglandin E_2-mediated mechanisms, respectively, in rats (Yokotani et al., 1995, 2005; Okada et al., 2003). In the present study, therefore, we examined the mechanisms involved in the centrally administered neuromedin U-induced elevation of plasma catecholamines with regard to the brain prostanoids using urethane-anesthetized rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 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*. Under urethane anesthesia (1.0 g/kg/animal, 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. In some experiments, acute bilateral adrenalectomy [plus hydrocortisone (5 mg/kg/animal, i.m.)] or sham-operation (plus 200 µl saline/animal, i.m.) was done just before these cannulations into the femoral artery and vein by an abdominal midline incision (Yokotani et al., 2005; Shimizu et al., 2006). After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for intracerebroventricular administration of test substances using 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 (1986). Three hours were allowed to elapse before the application of neuromedin U or blocking reagents.

Neuromedin U dissolved in sterile saline was slowly injected into the right lateral ventricle in a volume of 10 µl/animal using a 25-µl Hamilton syringe. Each animal received only one dose of neuromedin U (or vehicle). Water-soluble indomethacin-Na,

furegrelate and (+)-S-145 dissolved in sterile saline were intracerebroventricularly (i.c.v.) administered in a volume of 5 µl/animal using a 10-µl Hamilton syringe. In the case of using blocking reagents, neuromedin U was i.c.v. administered 30 min after the application of indomethacin-Na and 60 min after the application of furegrelate or (+)-S-145, due to their slightly elevating effects on the basal plasma levels of catecholamines. Each animal also received only one dose of blocking reagents (or vehicle).

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

2.2. Measurement of plasma catecholamines

Blood samples (250 µl) were collected through an arterial catheter and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically with high performance liquid chromatography (HPLC) (Shimizu et al., 2004). Briefly, after centrifugation, the plasma (100 µl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of twice deionized water, 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA and 1 ng of 3,4-dihydroxybenzylamine as an internal standard. The tube was shaken for 10 min and the alumina was washed three times with 4 ml of ice-cold twice deionized water. Then, catecholamines adsorbed onto the alumina were eluted with 300 µl 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, Eicompack CA-50DS, 2.1 x 150 mm (Eicom); mobile phase, 0.1 M NaH_2PO_4-Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l disodium EDTA, 0.75 g/l 1-octanesulfonate sodium and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 µl. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. By this assay, coefficients of variation for intra- and inter-assay were 3.0% and 3.7%, respectively, and 0.5 pg of noradrenaline and adrenaline was accurately determined.

2.3. Treatment of data and statistics

All values are expressed as the means±S.E.M. of the net changes 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. 1-4). When only two means were compared, an unpaired Student's *t*-test or Welch's *t*-test was used (Fig. 5). *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: neuromedin U (Peptide Institute, Osaka, Japan); water-soluble indomethacin sodium trihydrate (a kind gift from Merck, Rahway, NJ, U.S.A); furegrelate sodium (Biomol Research Lab., Plymouth Meeting, PA, U.S.A.); (+)-S-145 [(+)-(1*R*,2*S*,3*S*,4*S*)-(5*Z*)-7-(3-[4-^{3}H]-phenylsulphonyl-aminobicyclo[2.2.1]hept-2-yl)hept-5-enoic acid] (a kind gift from Shionogi Pharmaceutical Co. Ltd., Osaka, Japan); hydrocortisone (Sigma Aldrich Fine Chemicals, St. Louis, MO, U.S.A.). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effect of neuromedin U on plasma catecholamines

I.c.v. administered vehicle (10 µl saline/animal) and blood sampling for 5 times for 60 min had no effect on the basal plasma levels of either adrenaline or noradrenaline (Fig. 1).

Neuromedin U [0.1, 0.5 and 1 nmol (0.26, 1.3 and 2.6 µg)/animal, i.c.v.] effectively elevated plasma level of adrenaline, but a maximal response was obtained at 0.5 nmol/animal. The responses in each dose of neuromedin U reached a maximum 5 min after administration of this peptide and then declined toward the basal level (Fig. 1). On the other hand, neuromedin U (0.1, 0.5 and 1 nmol/animal, i.c.v.) slightly, but not significantly, elevated the plasma level of noradrenaline (Fig. 1).

Intravenous administration of neuromedin U (0.5 nmol/animal) had no effect on plasma levels of both catecholamines (data not shown).

3.2. Effect of indomethacin (an inhibitor of cyclooxygenase) on the centrally administered neuromedin U-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 µl saline/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of adrenaline and noradrenaline. Pretreatment with indomethacin [1.2 µmol (500 µg)/animal, i.c.v.] had no effect on the basal plasma levels of catecholamines (Fig. 2).

Indomethacin [0.6 and 1.2 µmol (250 and 500 µg)/animal, i.c.v.] significantly reduced the neuromedin U (0.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline, while the reagent had no effect on the neuromedin U-induced noradrenaline response (Fig. 2).

3.3. Effect of furegrelate (an inhibitor of thromboxane A_2 synthase) on the centrally administered neuromedin U-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 µl saline/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of adrenaline and noradrenaline. Pretreatment with furegrelate [1.8 µmol (500 µg)/animal, i.c.v.] had no effect on the basal plasma levels of both catecholamines (Fig. 3).

Furegrelate [0.9 and 1.8 µmol (250 and 500 µg)/animal, i.c.v.] reduced the neuromedin U (0.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline, while the reagent had no effect on the neuromedin U-induced noradrenaline response (Fig. 3).

3.4. Effect of (+)-S-145 (a blocker of prostanoid TP receptors) on the centrally administered neuromedin U-induced elevation of plasma catecholamines

Pretreatment with (+)-S-145 [625 nmol (250 µg)/animal, i.c.v.] had no effect on the basal plasma levels of adrenaline and noradrenaline (Fig. 4).

(+)-S-145 [250 and 625 nmol (100 and 250 µg)/animal, i.c.v.] dose-dependently reduced the neuromedin U (0.5 nmol/animal, i.c.v.)-induced elevation of plasma

adrenaline, while the reagent had no effect on the neuromedin U-induced noradrenaline response (Fig. 4).

3.5. Effect of acute bilateral adrenalectomy on the centrally administered neuromedin U-induced elevation of plasma catecholamines

The basal plasma levels of noradrenaline were not influenced by bilateral adrenalectomy, while the basal adrenaline level was effectively, but not significantly, reduced by this procedure (Fig. 5).

Bilateral adrenalectomy abolished the neuromedin U (0.5 nmol/animal, i.c.v.)-induced elevation of plasma adrenaline, while the procedure largely had no effect on the neuromedin U-induced noradrenaline response (Fig. 5).

4. Discussion

Plasma adrenaline is mainly secreted from adrenal medulla (adrenaline-containing cells), while plasma noradrenaline reflects not only the release from sympathetic nerves but also the secretion from the adrenal medulla (noradrenaline-containing cells) (Verhofstad et al., 1985; Edwards et al., 1996; Suzuki and Kachi, 1996; Vollmer et al., 2000; Yamaguchi-Shima et al., 2007). In the present study, i.c.v. administered neuromedin U elevated plasma adrenaline but had little effect on plasma noradrenaline in rats. Since intravenously administered neuromedin U had no effect on the plasma catecholamines, the peptide seems to act on the central nervous system, thereby activating the central adrenomedullary outflow in rats.

We previously reported that central pretreatment with indomethacin or ketoprofen (inhibitors of cyclooxygenase) attenuates the i.c.v. administered CRF-, vasopressin-, bombesin- and histamine-induced elevation of plasma catecholamines in rats (Okuma et al., 1996; Yokotani et al., 2001; Okada et al., 2003; Shimizu et al., 2006). Since prostanoids (prostaglandins and thromboxane A_2) generated by several enzymes including cyclooxygenase have been demonstrated to act as a neuromediator and/or neuromodulator in the brain's actions including cardiovascular function (Wood et al., 1993; Zhang et al., 2003) and regulation of hormone secretion (Bernardini et al., 1989; Reimsnider and Wood, 2006), the brain prostanoids seem to be involved in these substances-induced activation of the sympatho-adrenomedullary outflow. In the present study, the adrenaline response induced by i.c.v. administered neuromedin U was attenuated by central pretreatment with indomethacin, suggesting the involvement

of brain prostanoids in the neuromedin U-induced activation of adrenomedullary outflow in rats.

Previously, we reported that microinjection of thromboxane A_2 mimetic into the hypothalamic paraventricular nucleus predominantly elevates plasma adrenaline by activation of the brain prostanoid TP receptors in rats (Murakami et al., 2002). Furthermore, brain thromboxane A_2 is involved in the centrally administered CRF-, vasopressin-, bombesin- and histamine-induced secretion of adrenaline from the adrenal medulla in rats (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006). Recently, Yalcin and Savci (2004) reported that activation of the brain prostanoid TP receptors elevates plasma adrenaline in addition to its pressor effect. In the present study, central pretreatments with furegrelate [an inhibitor of thromboxane A_2 synthase (Gorman et al., 1983)] and (+)-S-145 [a blocker of prostanoid TP receptors (Hanasaki and Arita, 1988; Mihara et al., 1989)] effectively attenuated the elevation of plasma adrenaline induced by i.c.v. administered neuromedin U, respectively. These lines of evidence suggest that centrally administered neuromedin U activates the adrenomedullary outflow by brain prostanoid TP receptor-mediated mechanisms in rats.

Although plasma adrenaline originates exclusively from the adrenal medulla, the contribution of the extramedullary chromaffin tissues cannot be excluded. Previously, we reported that bilateral adrenalectomy abolished the elevation of plasma adrenaline induced by i.c.v. administered CRF-, bombesin-, vasopressin- and histamine in rats (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006). These results indicate that these substances evoke the secretion of adrenaline from the adrenal medulla. In the present study, bilateral adrenalectomy abolished the neuromedin U-induced elevation

of plasma adrenaline, suggesting that centrally administered neuromedin U activates the secretion of adrenaline from the adrenal medulla in rats.

The hypothalamus, especially the paraventricular nucleus, has been considered to be the control center of the sympatho-adrenomedullar outflow (Swanson and Sawchenko, 1980; Jansen et al., 1995; Kenney et al., 2003). Neuromedin U directly activates the hypothalamic paraventricular nucleus in vitro and in vivo (Niimi et al., 2001; Ozaki et al., 2002; Qiu et al., 2003). Furthermore, this nucleus receives dense innervation of neuromedin U-immunoreactive fibers (Ballesta et al., 1988; Brighton et al., 2004). Neuromedin U receptor subtype 2, predominantly expressed in the central nervous system, is also abundantly found in the paraventricular nucleus (Howard et al., 2000; Graham et al., 2003; Brighton et al., 2004). These lines of evidence further suggest a role of brain neuromedin U in the central activation of adrenomedullary outflow in rats.

Recently, i.c.v. administered neuromedin U (0.05 and 0.5 nmol) has been shown to increase the mean blood pressure, heart rate and plasma noradrenaline in conscious rats (Chu et al., 2002). The reason for the difference in the neuromedin U-induced elevation of plasma catecholamines seems to be due to the experimental conditions, because many reflex responses are involved in conscious animals. In addition, microinjection of neuromedin U (5, 10 and 50 pmol) into the nucleus tractus solitarius decreased the mean blood pressure and heart rate in urethane-anesthetized rat (Tsubota et al., 2003). The nucleus tractus solitarius is the site of termination of the cardiovascular afferent fibers of baroreceptors and distribution of neuromedin U-containing neurons has been demonstrated (Ballesta et al., 1988). Up-regulation of c-Fos expression by i.c.v. administered neuromedin U has been reported in the nucleus

tractus solitarius in addition to the hypothalamic paraventricular nucleus (Ivanov et al., 2002). In the present study, the effect of a larger dose of neuromedin U (1 nmol) on the plasma adrenaline was smaller than that of neuromedin U (0.5 nmol). The results suggest a possibility that a large dose of neuromedin U activates nucleus tractus solitarius, thereby inhibiting the activity of the paraventricular nucleus and/or sympathetic preganglionic neurons in the spinal cord, since the nucleus tractus solitarius projects to the hypothalamic paraventricular nucleus and spinal cord, thereby modulating their activities (Loewy and Burton, 1978; Ter Horst et al., 1989).

In summary, we demonstrated here that centrally administered neuromedin U evokes the secretion of adrenaline from the adrenal medulla by brain prostanoid TP receptor-mediated mechanisms in rats.

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Legends to figures

Fig. 1. Effect of neuromedin U on plasma catecholamines. ΔAdrenaline and ΔNoradrenaline: increments of adrenaline and noradrenaline above the basal. Arrow indicates the administration of vehicle (saline 10 µl/animal, i.c.v.) or neuromedin U (0.1, 0.5 and 1 nmol/animal, i.c.v.). Each point represents the mean±S.E.M. *Significantly different from the vehicle-treated group with the Bonferroni method [adrenaline; at 5 min, $F(3,24)=7.76$, $P<0.017$: noradrenaline; at 30 min, $F(3,23)=6.59$, $P<0.017$]. The actual values for adrenaline and noradrenaline at 0 min were 414±51 and 315±23 pg/ml (n=28), respectively.

Fig. 2. Effect of indomethacin (an inhibitor of cyclooxygenase) on the centrally administered neuromedin U-induced elevation of plasma catecholamines. Indomethacin (0.6 and 1.2 µmol/animal, i.c.v.) or vehicle-1 (5 µl saline/animal, i.c.v.) was administered 30 min before the administration of neuromedin U (0.5 nmol/animal, i.c.v.) or vehicle-2 (10 µl saline/animal, i.c.v.). Arrows indicate the intracerebroventricular administrations of indomethacin/vehicle-1 and neuromedin U/vehicle-2. *Significantly different from the vehicle-1- and neuromedin U-treated group with the Bonferroni method [adrenaline; at 5 min, $F(2,14)=5.22$, $P<0.025$]. Other conditions were the same as those of Fig. 1. The actual values for adrenaline and noradrenaline at 0 min were 316±53 and 413±49 pg/ml in the vehicle-1-pretreated group (n=12); 411±68 and 246±38 pg/ml in the indomethacin (0.6 µmol/animal)-pretreated group (n=5); 390±157 and 261±88 pg/ml in the indomethacin (1.2 µmol/animal)-pretreated group (n=10), respectively.

Fig. 3. Effect of furegrelate (an inhibitor of thromboxane A_2 synthase) on the centrally administered neuromedin U-induced elevation of plasma catecholamines. Furegrelate (0.9 and 1.8 μmol/animal, i.c.v.) or vehicle-1 (5 μl saline/animal, i.c.v.) was administered 60 min before the administration of neuromedin U (0.5 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). *Significantly different from the vehicle-1- and neuromedin U-treated group with the Bonferroni method [adrenaline: at 5 min, $F(2,15)=7.40$, $P<0.025$; at 10 min, $F(2,14)=4.44$, $P<0.025$]. Other conditions were the same as those of Figs. 1 and 2. The actual values for adrenaline and noradrenaline at 0 min were 351±59 and 416±45 pg/ml in the vehicle-1-pretreated group (n=12); 408±77 and 322±85 pg/ml in the furegrelate (0.9 μmol/animal)-pretreated group (n=6); 377±113 and 467±45 pg/ml in the furegrelate (1.8 μmol/animal)-pretreated group (n=12), respectively.

Fig. 4. Effect of (+)-S-145 (a blocker of prostanoid TP receptors) on the centrally administered neuromedin U-induced elevation of plasma catecholamines. (+)-S-145 (250 and 625 nmol/animal, i.c.v.) or vehicle-1 (5 μl saline/animal, i.c.v.) was administered 60 min before the administration of neuromedin U (0.5 nmol/animal, i.c.v.) or vehicle-2 (10 μl saline/animal, i.c.v.). *Significantly different from the vehicle-1- and neuromedin U-treated group with the Bonferroni method [adrenaline; at 5 min, $F(2,17)=15.83$, $P<0.025$]. Vehicle-1-treated groups were cited from Fig. 3. Other conditions were the same as those in Figs. 1-3. The actual values for adrenaline and noradrenaline at 0 min were 112±24 and 197±38 pg/ml in the (+)-S-145 (250

nmol/animal)-pretreated group (n=7); 98±22 and 234±52 pg/ml in the (+)-S-145 (625 nmol/animal)-pretreated group (n=11), respectively.

Fig. 5. Effect of acute bilateral adrenalectomy on the centrally administered neuromedin U-induced elevation of plasma catecholamines. Acute bilateral adrenalectomy [plus hydrocortisone (5 mg/kg/animal, i.m.)] or sham-operation (plus 200 µl saline/animal, i.m.) was done 3 hr before the application of neuromedin U (0.5 nmol/animal, i.c.v.). Arrow indicates the administration of neuromedin U. *Significantly different from the sham-operated group with an unpaired Student's *t*-test or Welch's *t*-test [adrenaline; at 5 min, $F(4,5)=16.09$, $P<0.05$, at 10 min, $F(4,5)=9.22$, $P<0.05$, at 30 min, $F(4,5)=7.63$, $P<0.05$: noradrenaline; at 30 min, $F(4,5)=1.09$, $P<0.05$]. Other conditions were the same as those in Figs. 1-4. The actual values for adrenaline and noradrenaline at 0 min were 164±55 and 193±37 pg/ml in sham-operated group (n=5) and 86±78 and 145±38 pg/ml in bilateral adrenalectomized group (n=6), respectively.

Figure-1

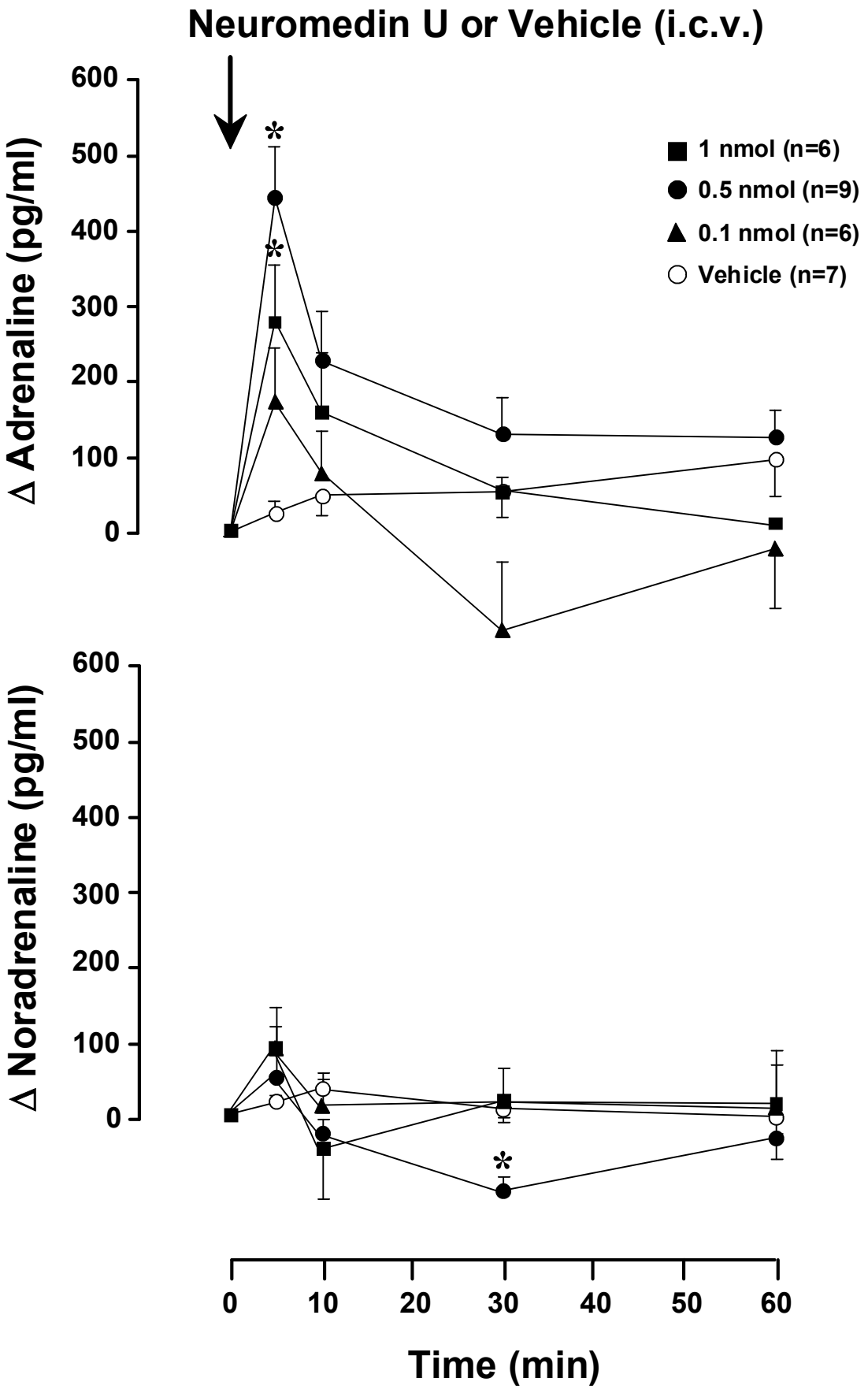

Neuromedin U or Vehicle (i.c.v.)
1 nmol (n=6)
0.5 nmol (n=9)
0.1 nmol (n=6)
Vehicle (n=7)
Δ Adrenaline (pg/ml)
Δ Noradrenaline (pg/ml)
600
500
400
300
200
100
0
*
*
*
0
10
20
30
40
50
60
Time (min)

Figure-2

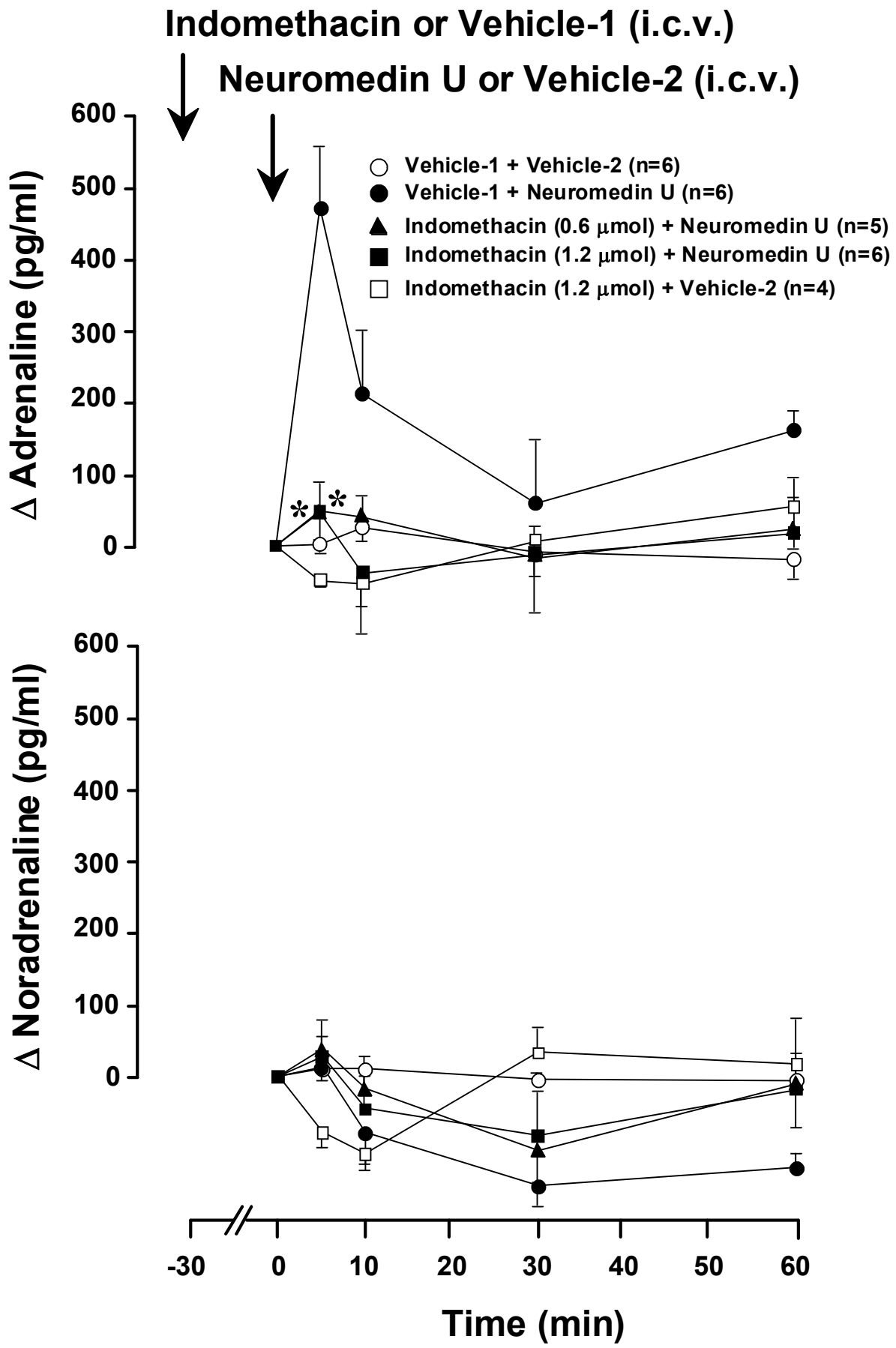

Indomethacin or Vehicle-1 (i.c.v.)
Neuromedin U or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=6)
Vehicle-1 + Neuromedin U (n=6)
Indomethacin (0.6 μmol) + Neuromedin U (n=5)
Indomethacin (1.2 μmol) + Neuromedin U (n=6)
Indomethacin (1.2 μmol) + Vehicle-2 (n=4)
Δ Adrenaline (pg/ml)
Δ Noradrenaline (pg/ml)
600
500
400
300
200
100
0
*
*
-30
0
10
20
30
40
50
60
Time (min)

Figure-3

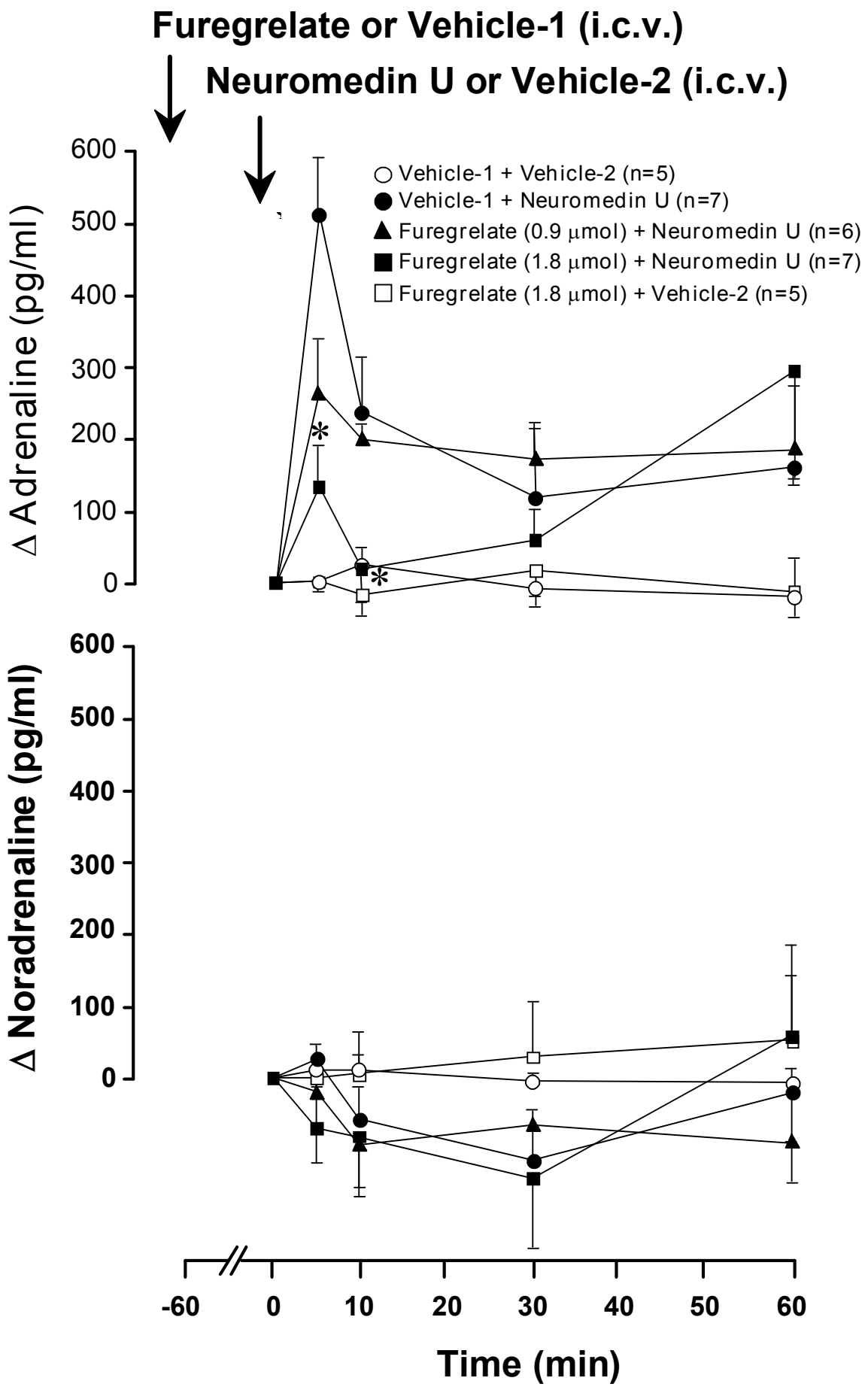

Furegrelate or Vehicle-1 (i.c.v.)
Neuromedin U or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=5)
Vehicle-1 + Neuromedin U (n=7)
Furegrelate (0.9 μmol) + Neuromedin U (n=6)
Furegrelate (1.8 μmol) + Neuromedin U (n=7)
Furegrelate (1.8 μmol) + Vehicle-2 (n=5)
Δ Adrenaline (pg/ml)
Δ Noradrenaline (pg/ml)
600
500
400
300
200
100
0
-60
0
10
20
30
40
50
60
Time (min)

Figure-4

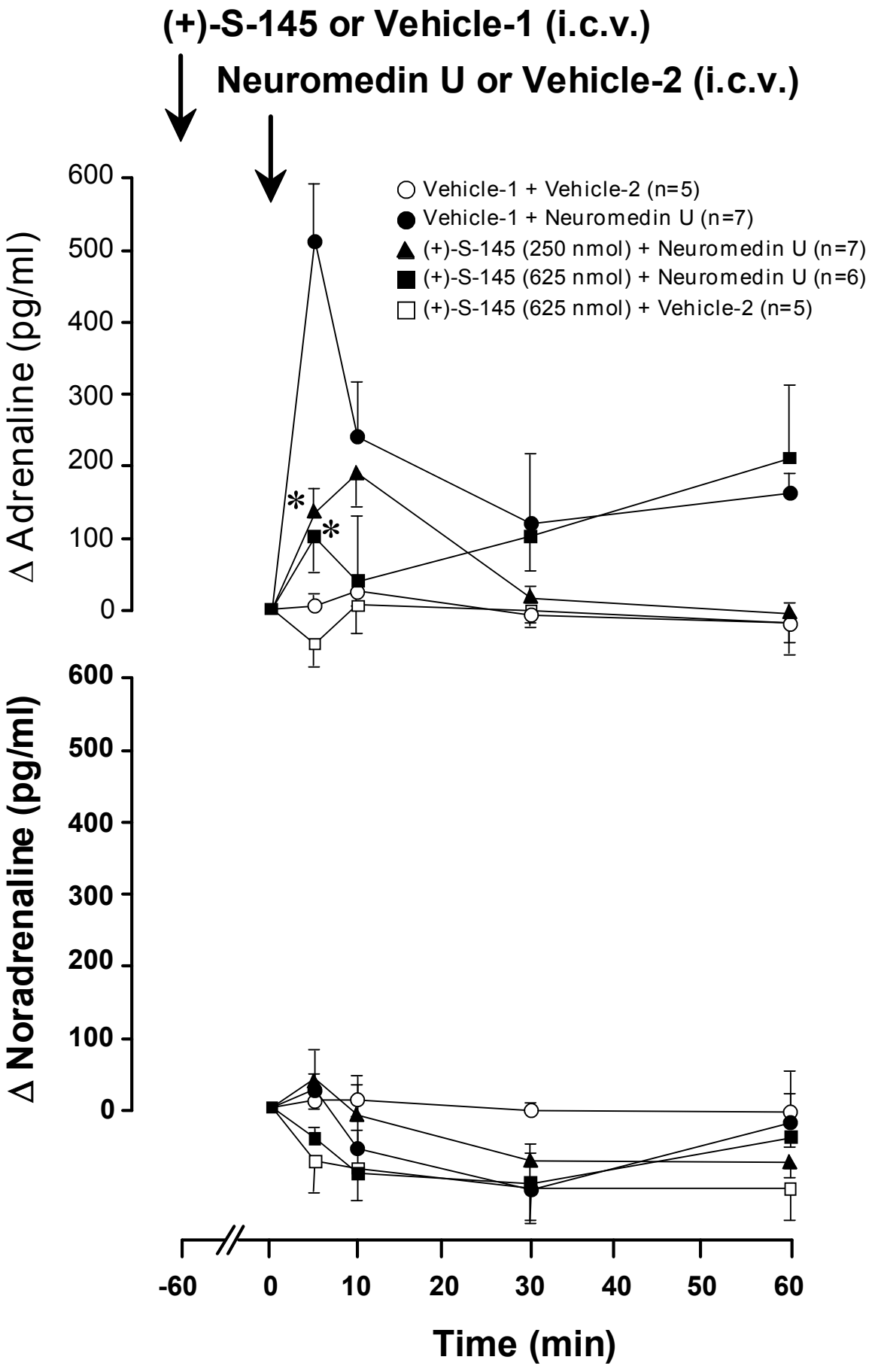
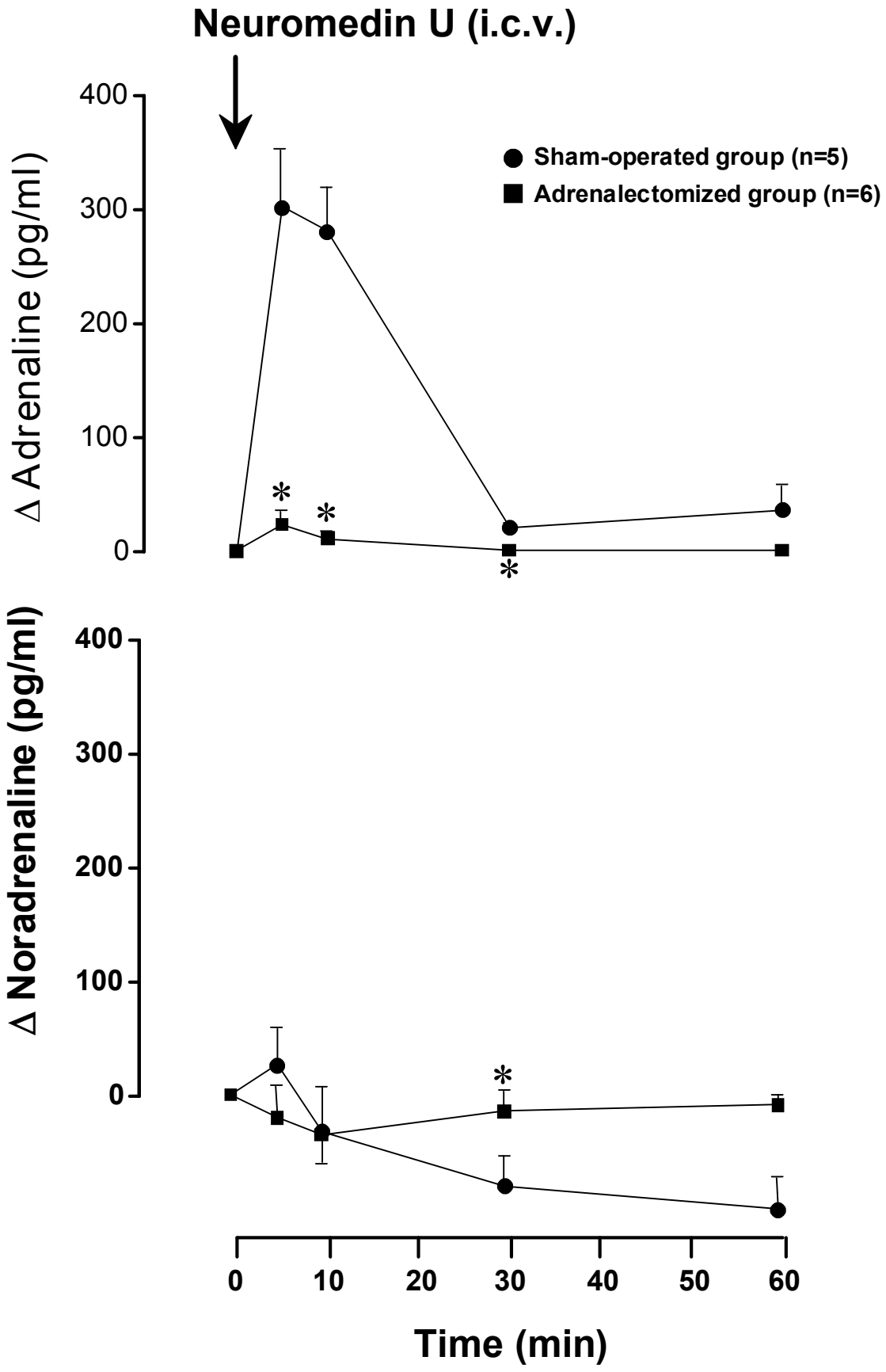

(+)-S-145 or Vehicle-1 (i.c.v.)
Neuromedin U or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=5)
Vehicle-1 + Neuromedin U (n=7)
(+)-S-145 (250 nmol) + Neuromedin U (n=7)
(+)-S-145 (625 nmol) + Neuromedin U (n=6)
(+)-S-145 (625 nmol) + Vehicle-2 (n=5)
Δ Adrenaline (pg/ml)
Δ Noradrenaline (pg/ml)
600
500
400
300
200
100
0
*
*
-60
0
10
20
30
40
50
60
Time (min)

Figure-5


Neuromedin U (i.c.v.)
Sham-operated group (n=5)
Adrenalectomized group (n=6)
Δ Adrenaline (pg/ml)
400
300
200
100
0
*
*
*
Δ Noradrenaline (pg/ml)
400
300
200
100
0
*
0
10
20
30
40
50
60
Time (min)