Brain cyclooxygenase and prostanoid TP receptors are involved in centrally administered epibatidine-induced secretion of noradrenaline and adrenaline from the adrenal medulla in rats

Takahiro Shimizu*, Kunihiko Yokotani

Department of Pharmacology, School of Medicine, Kochi University, Nankoku, Kochi 783-8505, Japan

* Corresponding author. Tel./fax: +81-88-880-2328

E-mail address: shimizu@kochi-u.ac.jp (T. Shimizu)

Abstract

Plasma adrenaline mainly originates from adrenaline-containing cells in the adrenal medulla, whereas plasma noradrenaline reflects not only the release from sympathetic nerves but also the secretion from noradrenaline-containing cells in the adrenal medulla. The present study was undertaken to examine the mechanisms involved in centrally administered epibatidine (a potent agonist of nicotinic acethylcholine receptors)-induced elevation of plasma catecholamines with regard to the brain prostanoid. Intracerebroventricularly (i.c.v.) administered epibatidine (1, 5 and 10 nmol/animal) effectively elevated plasma noradrenaline and adrenaline. The epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines was attenuated by hexamethonium (an antagonist of nicotinic acethylcholine receptors) (0.9 and 1.8 μmol/animal, i.c.v.), indomethacin (an inhibitor of cyclooxygenase) (0.6 and 1.2 μmol/animal, i.c.v.) and (+)-S-145 (an antagonist of prostanoid TP receptors) (0.6 and 1.3 μmol/animal, i.c.v.), and abolished by acute bilateral adrenalectomy. On the other hand, intravenously administered epibatidine (5 nmol/animal) was largely ineffective on the plasma levels of catecholamines, and intravenous pretreatment with hexamethonium (1.8 μmol/animal) had no effect on the epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines. These results suggest that centrally administered epibatidine activates the brain nicotinic acethylcholine receptors, thereby evoking the secretion of noradrenaline and adrenaline from the adrenal medulla by brain cyclooxygenase- and prostanoid TP receptor-mediated mechanisms in rats.

Keywords: Epibatidine; Brain nicotinic acethylcholine receptor; Cyclooxygenase; Prostanoid TP receptor; Adrenal medulla

1. Introduction

Smoking is a leading cause of cardiovascular diseases including high blood pressure (Lakier, 1992) and nicotine is one of the components of cigarette smoke. The effects of peripherally administered nicotine on the sympatho-adrenomedullary system have been clearly shown to evoke the release of noradrenaline and adrenaline from sympatho-adrenomedullary system by activation of peripheral nicotinic acethylcholine receptors (Watts, 1960; Wang et al., 2000; Yokotani et al., 2001, 2002). Centrally administered nicotine also evokes pressor response and elevation of plasma catecholamine by activation of the brain nicotinic acethylcholine receptors in rats (Kiritsy-Roy et al., 1990; Buccafusco and Yang 1993; Tseng et al., 1993, 1994). However, the effect of microinjected nicotine into the brain seems to vary according to the injected nuclei. Nicotine administered into the rostral ventrolateral medulla increases blood pressure and renal sympathetic nerve activity (Tseng et al., 1993, 1994), whereas nicotine administration into the nucleus tractus solitarius induces hypotension, probably by an enhancement of inhibitory baroreflex (Tseng et al., 1993, 1994; Ashworth-Preece et al., 1998). Chronic treatment of nicotine is able to intensify and accelerate the development of hypertension in spontaneously hypertensive rats (Bui et al., 1994; Ferrari and Fior-Chadi, 2007), in which the central dysregulation of sympatho-adrenomedullary outflow has been suggested to be involved (Barron and Van Loon, 1989; Wyss and Carlson, 2001; Guyenet, 2006). However, the precise mechanisms of this alkaloid-induced central modulation of the sympatho-adrenomedullary outflow are largely undefined.

We recently reported that centrally administered stress-related neurotransmitters such as vasopressin, bombesin and histamine elicit adrenal secretion of both noradrenaline and adrenaline from noradrenaline- and adrenaline-containing cells in the adrenal medulla via the brain thromboxane A_2-mediated mechanisms, whereas centrally administered corticotropin-releasing factor (CRF) and glucagon-like peptide 1 (GLP-1) elicits adrenaline secretion from adrenal adrenaline-containing cells and noradrenaline release from sympathetic nerves via the brain thromboxane A_2- and prostaglandin E_2-mediated mechanisms, respectively, in rats (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006; Arai et al., 2008). In the present study, therefore, we examined the mechanisms involved in the centrally administered epibatidine (a potent agonist of nicotinic acethylcholine receptors)-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 (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 ml/h), epibatidine or hexamethonium, and the femoral artery was cannulated for collecting blood samples. In some experiments, acute bilateral adrenalectomy [plus hydrocortisone (5 mg/kg, 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; Sasaki et al., 2008). 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 (Paxinos and Watson, 1986). Three hours were allowed to elapse before the application of epibatidine or blocking reagents.

Epibatidine dissolved in 100% *N*,*N*-dimethylformamide (DMF) was slowly injected into the right lateral ventricle in a volume of 2.5 μl/animal using a 10-μl Hamilton syringe. Each animal received only one dose of epibatidine or vehicle.

Hexamethonium, water-soluble indomethacin-Na 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. When blocking reagents were used, epibatidine was i.c.v. administered 30 min after the application of hexamethonium or indomethacin-Na and 60 min after the application of (+)-S-145, due to their slightly elevating effects on the basal plasma levels of catecholamines. When epibatidine was injected intravenously (i.v.), the epibatidine solution (500 μl) dissolved in 0.5% DMF in saline was slowly injected via a cannula inserted into the femoral vein. Intravenous administration of hexamethonium was also carried out via the cannula in a volume of 500 μl saline/animal. 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 (1,500 *g* for 10 min, at 4°C), 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. The extraction efficiency of catecholamines was about 83.8±1.6% (n=4). 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 sodium 1-octanesulfonate and 15% methanol at a flow rate 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. 1A, 2A, 3 and 4). When only two means

were compared, an unpaired Student's *t*-test or Welch's *t*-test was used (Figs. 1B, 2B and 5). *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: (±)-epibatidine dihydrochloride hydrate (epibatidine), hexamethonium chloride and hydrocortisone (Sigma Aldrich Fine Chemicals, St. Louis, MO, U.S.A.); water-soluble indomethacin sodium trihydrate (a kind gift from Merck Rahway, NJ, U.S.A.); (+)-S-145 [(+)-(1*R*,2*S*,3*S*,4*S*)-(5*Z*)-7-(3-phenylsulphonyl-aminobicyclo[2.2.1]hept-2-yl)hept-5-enoic acid] (a kind gift from Shionogi Pharmaceutical Co. Ltd., Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effect of centrally administered epibatidine on plasma levels of catecholamines

I.c.v. administered vehicle (2.5 μl DMF/animal) had no effect on the basal plasma levels of either noradrenaline or adrenaline, respectively (Fig. 1A). Epibatidine [1 nmol (0.3 μg)/animal, i.c.v.] had little effect on the plasma levels of noradrenaline and adrenaline, but this chloroalkaloid [5 and 10 nmol (1.4 and 2.8 μg)/animal, i.c.v.] significantly elevated plasma levels of noradrenaline and adrenaline; maximal noradrenaline responses were obtained by a dose of 10 nmol/animal (i.c.v.) and maximal adrenaline responses were obtained by a dose of 5 nmol/animal (i.c.v.), respectively (Fig. 1A). These responses reached a maximum 5 min after the administration of epibatidine and then gradually declined toward their basal levels.

Intravenous administration of vehicle (500 μl of 0.5% DMF in saline) had no effect on the basal plasma levels of either noradrenaline or adrenaline (Fig. 1B). Epibatidine (5 nmol/animal, i.v.) largely unaffected the plasma levels of both catecholamines (Fig. 1B).

3.2. Effect of hexamethonium (an antagonist of nicotinic acethylcholine receptors) on the centrally administered epibatidine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 μl saline/animal, i.c.v.)/hexamethonium [1.8 μmol (500 μg)/animal, i.c.v.] and vehicle-2 (2.5 μl DMF/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline, respectively (Fig. 2A).

Epibatidine (5 nmol/animal, i.c.v.)-induced elevation of noradrenaline was significantly reduced by hexamethonium (1.8 μmol/animal, i.c.v.), while the reagent-induced elevation of plasma adrenaline was dose-dependently reduced by hexamethonium [0.9 and 1.8 μmol (250 and 500 μg)/animal, i.c.v.] (Fig. 2A).

Treatments with vehicle-1 (500 μl saline/animal, i.v.)/hexamethonium (1.8 μmol/animal, i.v.) and vehicle-2 (2.5 μl DMF/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline, respectively (Fig. 2B). Pretreatment with hexamethonium (1.8 μmol/animal, i.v.) had little effect on the epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines (Fig. 2B).

3.3. Effect of indomethacin (an inhibitor of cyclooxygenase) on the centrally administered epibatidine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 μl saline/animal, i.c.v.)/indomethacin [1.2 μmol (500 μg)/animal, i.c.v.] and vehicle-2 (2.5 μl DMF/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline, respectively (Fig. 3).

Epibatidine (5 nmol/animal, i.c.v.)-induced elevation of noradrenaline and adrenaline was dose-dependently reduced by indomethacin [0.6 and 1.2 μmol (250 and 500 μg)/animal, i.c.v.] (Fig. 3).

3.4. Effect of (+)-S-145 (an antagonist of prostanoid TP receptors) on the centrally administered epibatidine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 µl saline/animal, i.c.v.)/(+)-S-145 [1.3 µmol (500 µg)/animal, i.c.v.] and vehicle-2 (2.5 µl DMF/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline, respectively (Fig. 4).

Epibatidine (5 nmol/animal, i.c.v.)-induced elevation of both catecholamines was significantly reduced by (+)-S-145 [0.6 and 1.3 µmol (250 and 500 µg)/animal, i.c.v.] (Fig. 4).

3.5. Effect of bilateral adrenalectomy on the centrally administered epibatidine-induced elevation of plasma catecholamines

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

In sham-operated rats, epibatidine (5 nmol/animal, i.c.v.) effectively elevated plasma levels of noradrenaline and adrenaline (Fig. 5). The reagent-induced elevation of both catecholamines was abolished by bilateral adrenalectomy (Fig. 5).

4. Discussion

In the preliminary experiment, centrally administered nicotine (250 and 500 nmol/animal, i.c.v.) dose-dependently evoked a rapid increase of plasma levels of adrenaline and noradrenaline within 3 min after the administration of this alkaloid, and quickly returned to the preadministered basal levels, as shown by Kiritsy-Roy et al. (1990). Due to the rapid response of nicotine, we used epibatidine in the present experiments to clarify the mechanisms involved in the brain nicotinic acetylcholine receptor-induced elevation of plasma catecholamines in rats. Epibatidine obtained from the skin of the Ecuadoran frog *Epipedobates tricolar* (Daly et al., 1978) is a highly potent nicotinic acethylcholine receptor agonist having broad spectrum activity on the nicotinic acethylcholine receptors (Qian et al., 1993; Badio and Daly, 1994; Lembeck, 1999). In the present experiment, centrally administered epibatidine (5 and 10 nmol/animal) dose-dependently elevated plasma levels of noradrenaline and adrenaline in rats. A possibility has arisen that the centrally administered chloroalkaloid leaks out into the systemic circulation, thereby peripherally activating nicotinic receptors located on the sympathetic nerves and adrenal medulla. However, peripherally administered epibatidine (5 nmol/animal) had little effect on the plasma catecholamines. Furthermore, centrally administered epibatidine (5 nmol/animal)-induced responses were effectively attenuated by central pretreatment with hexamethonium (an antagonist of nicotinic acethylcholine receptors), but not influenced by peripheral pretreatment with the same dose of this reagent. These results suggest that centrally administered epibatidine acts on the nicotinic acethylcholine

receptors in the brain, thereby elevating plasma levels of noradrenaline and adrenaline in rats.

Prostanoids (prostaglandins and thromboxane A_2) generated by several enzymes, including cyclooxygenase, have been demonstrated to act as a neurotransmitter and/or neuromodulator in the brain's actions such as cardiovascular function (Wood et al., 1993; Zhang et al., 2003) and regulation of hormone secretion (Bernardini et al., 1989; Reimsnider and Wood, 2006). Previously, we reported that central pretreatment with indomethacin or ketoprofen (inhibitors of cyclooxygenase) attenuated the centrally administered CRF-, vasopressin-, histamine-, GLP-1- and bombesin-induced elevation of plasma noradrenaline and adrenaline (Okada et al., 2003; Shimizu et al., 2006; Arai et al., 2008; Lu et al., 2008), suggesting the involvement of the brain prostanoids in these substances-induced elevation of plasma catecholamines in rats. In the present study, central pretreatment with indomethacin effectively attenuated the elevation of plasma noradrenaline and adrenaline induced by centrally administered epibatidine. The result also suggests the involvement of brain prostanoids in the epibatidine-induced elevation of plasma catecholamines in rats.

Plasma adrenaline is mainly secreted from adrenaline-containing cells in the adrenal medulla, whereas plasma noradrenaline reflects not only the release from sympathetic nerves but also the secretion from noradrenaline-containing cells in the adrenal medulla (Edwards et al., 1996; Suzuki and Kachi, 1996; Vollmer et al., 2000; Yamaguchi-Shima, et al., 2007). We previously reported that centrally administered prostaglandin E_2 (but not prostaglandin D_2, I_2 and F_{2alpha}) elevates plasma noradrenaline from sympathetic nerves by activation of the brain prostanoid EP_3 receptors in rats (Yokotani et al., 1995, 2005; Murakami et al. 2002) and also 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, the brain prostanoid TP receptors are involved in the centrally administered vasopressin-, bombesin- and histamine-induced secretion of both noradrenaline and adrenaline from noradrenaline- and adrenaline-containing cells in the adrenal medulla and in the centrally administered CRF- and GLP-1-induced secretion of adrenaline from adrenaline-containing cells in the adrenal medulla in rats (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006; Arai et al., 2008). The activation of brain TP receptors has been shown to elevate blood pressure and plasma catecholamines in hemorrhaged hypotensive rats (Yalcin and Savci, 2004). In the present study, central pretreatment with (+)-S-145 [an antagonist of prostanoid TP receptors (Hanasaki and Arita 1988; Mihara et al. 1989)] effectively attenuated the elevation of plasma noradrenaline and adrenaline induced by centrally administered epibatidine. The result suggests a possibility that centrally administered epibatidine elicits the secretion of both noradrenaline and adrenaline from the adrenal medulla by brain prostanoid TP receptor-mediated mechanisms in rats.

To further explore the source of noradrenaline and adrenaline elicited by centrally administered epibatidine, we examined the effect of acute bilateral adrenalectomy on the epibatidine-induced elevation of plasma catecholamines. Previously, we reported that bilateral adrenalectomy abolished the elevation of plasma noradrenaline and adrenaline induced by centrally administered vasopressin, bombesin and histamine (Okada et al., 2003; Yokotani et al., 2005; Shimizu et al., 2006). In the present study, bilateral adrenalectomy abolished the centrally administered epibatidine-

induced elevation of both plasma noradrenaline and adrenaline. The result suggests that centrally administered epibatidine elicits the secretion of both noradrenaline and adrenaline from the adrenal medulla in rats.

In the central nervous system, nicotinic acethylcholine receptors are preferentially located at presynaptic terminals, thereby regulating the release of many neurotransmitters (Wonnacott, 1997; Vizi and Lendvai, 1999; Gotti and Clementi, 2004; Sher et al., 2004) such as glutamate in the rat prefrontal cortex, striatal synaptosomes and nucleus tractus solitarius (Marchi et al., 2002; Zhao et al., 2007; Dickinson et al., 2008). Glutamate excites the hypothalamic paraventricular nucleus (Boudaba et al., 1997; Li et al., 2004), which has been considered to be the control center of the sympatho-adrenomedullary outflow (Swanson and Sawchenko, 1980; Jansen et al., 1995; Kenny et al., 2003). Previously, we reported that *N*-methyl-D-aspartate applied into this nucleus using dialysis probe elicited concomitant overflow of hypothalamic thromboxane B_2 (a stable metabolite of thromboxane A_2) with the elevation of plasma noradrenaline and adrenaline in rats (Okada et al., 2000). These lines of evidence suggest a possibility that activation of the hypothalamic nicotinic acethylcholine receptors evokes the secretion of noradrenaline and adrenaline from adrenal medulla by glutamate- and thromboxane A_2-mediated mechanisms in rats. Further experiments are required to explore the mechanisms for central nicotinic acethylcholine receptors to activate the central adrenomedullary outflow in rats.

In summary, we demonstrated here that centrally administered epibatidine activates central nicotinic acethylcholine receptors, thereby evoking the secretion of noradrenaline and adrenaline from the adrenal medulla by brain cyclooxygenase- and prostanoid TP receptor-mediated mechanisms in rats.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 20590702) from Japan Society for the Promotion of Science, a Grant from The Smoking Research Foundation in Japan, and The Kochi University President's Discretionary Grant.

References

Anton, A.H., Sayre, D.F., 1962. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138, 360-375.

Arai, J., Okada, S., Yamaguchi-Shima, N., Shimizu, T., Sasaki, T., Yorimitsu, M., Wakiguchi, H., Yokotani, K., 2008. Role of brain prostanoids in glucagon-like peptide-1-induced central activation of sympatho-adrenomedullary outflow in rats. Clin. Exp. Pharmacol. Physiol. 35, 965-970.

Ashworth-Preece, M., Jarrott, B., Lawrence, A.J., 1998. Nicotinic acetylcholine receptors in the rat and primate nucleus tractus solitarius and on rat and human inferior vagal (nodose) ganglia: evidence from in vivo microdialysis and [^{125}I] alpha-bungarotoxin autoradiography. Neuroscience 83, 1113-1122.

Badio, B., Daly J.W.,1994. Epibatidine, a potent analgetic and nicotinic agonist. Mol. Pharmacol. 45, 563-569.

Barron, B.A., Van Loon, G.R., 1989. Role of sympathoadrenomedullary system in cardiovascular response to stress in rats. J. Anton. Nerv. Syst. 28, 179-187.

Bernardini, R., Chiarenza, A., Calogera, A.E., Gold, P.W., Chrousos, G.P., 1989. Arachidonic acid metabolites modulate rat hypothalamic corticotropin-releasing hormone secretion in vitro. Neuroendocrinology 50, 708-715.

Boudaba, C., Schrader, L.A., Tasker, J.G., 1997. Physiological evidence for local excitatory synaptic circuits in the rat hypothalamus. J. Neurophysiol. 77, 3396-3400.

Buccafusco, J.J., Yang, X., 1993. Mechanism of the hypertensive response to central injection of nicotine in conscious rats. Brain Res. Bull. 32, 681-684.

Bui, L.M., Keen, C.L., Dubick, M.A., 1994. Influence of 12-week nicotine treatment and dietary copper on blood pressure and indices of the antioxidant system in male spontaneous hypertensive rats. Biol. Trace Elem. Res. 46, 67-87.

Daly, J.W., Brown, G.B., Mensah-Dwumah, M., Myers, C.W., 1978. Classification of skin alkaloids from neotropical poison-dart frogs (Dendrobatidae). Toxicon. 16, 163-188.

Dickinson, J.A., Kew, J.N., Wonnacott, S., 2008. Presynaptic alpha 7- and beta 2-containing nicotinic acetylcholine receptors modulate excitatory amino acid release from rat prefrontal cortex nerve terminals via distinct cellular mechanisms. Mol. Pharmacol. 74, 348-359.

Edwards, S.L., Anderson, C.R., Southwell, B.R., McAllen, R.M., 1996. Distinct preganglionic neurons innervate noradrenaline and adrenaline cells in the cat adrenal medulla. Neuroscience 70, 825-832.

Ferrari, M.F., Fior-Chadi, D.R., 2007. Chronic nicotine administration. Analysis of the development of hypertension and glutamatergic neurotransmission. Brain Res. Bull. 72, 215-224.

Gotti, C., Clementi, F., 2004. Neuronal nicotinic receptors: from structure to pathology. Prog. Neurobiol. 74, 363-396.

Guyenet, P.G., 2006. The sympathetic control of blood pressure. Nat. Rev. Neurosci. 7, 335-346.

Hanasaki, K., Arita, H., 1988. Characterization of a new compound, S-145, as a specific TXA_2 receptor antagonist in platelets. Thromb. Res. 50, 365-376.

Jansen, A.S., Nguyen, X.V., Karpitskiy, V., Mettenleiter, T.C., Loewy, A.D., 1995. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 270, 644-646.

Kenny, M.J., Weiss, M.L., Haywood, J.R., 2003. The paraventricular nucleus: an important component of the central neurocircuitry regulating sympathetic nerve outflow. Acta. Physiol. Scand. 177, 7-15.

Kiritsy-Roy, J.A., Mousa, S.A., Appel, N.M., Van Loon, G.R., 1990. Tolerance to nicotine-induced sympathoadrenal stimulation and cross-tolerance to stress: differential central and peripheral mechanisms in rats. Neuropharmacology 29, 579-589.

Lakier, J.B., 1992. Smoking and cardiovascular disease. Am. J. Med. 93, 8S-12S.

Lembeck, F., 1999. Epibatidine: high potency and broad spectrum activity on neuronal and neuromuscular nicotinic acetylcholine receptors. Naunyn. Schmiedebergs Arch. Pharmacol. 359, 378-385.

Li, D.P., Chen, S.R., Pan, H.L., 2004. VR1 receptor activation induces glutamate release and postsynaptic firing in the paraventricular nucleus. J. Neurophysiol. 92, 1807-1816.

Lu, L., Shimizu, T., Nakamura, K., Yokotani, K., 2008. Brain neuronal/inducible nitric oxide synthases and cyclooxygenase-1 are involved in the bombesin-induced activation of central adrenomedullary outflow in rats. Eur. J. Pharmacol. 590, 177-184.

Marchi, M., Risso, F., Viola, C., Cavazzani, P., Raiteri, M., 2002. Direct evidence that release-stimulating alpha7 nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. J. Neurochem. 80, 1071-1078.

Mihara, S., Hara, S., Ueda, M., Ide, M., Fujimoto, M., 1989. Antagonistic actions of S-145 on vascular and platelet thromboxane A_2 receptors. Eur. J. Pharmacol. 171, 179-187.

Murakami, Y., Okada, S., Nishihara, M., Yokotani, K., 2002. Roles of brain prostaglandin E_2 and thromboxane A_2 in the activation of the central sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 452, 289-294.

Okada, S., Murakami, Y., Nishihara, M., Yokotani, K., Osumi, Y., 2000. Perfusion of the hypothalamic paraventricular nucleus with N-methyl-D-aspartate produces thromboxane A_2 and centrally activates adrenomedullary outflow in rats. Neuroscience 96, 585-590.

Okada, S., Murakami, Y., Yokotani, K., 2003. Role of brain thromboxane A_2 in the release of noradrenaline and adrenaline from adrenal medulla in rats. Eur. J. Pharmacol. 467, 125-131.

Paxinos, G., Watson, C., 1986. In: Paxinos, G., Watson, C. (Eds.), The Rat Brain in Stereotaxic Coordinates. Academic Press, Boston.

Qian, C., Li, T., Shen, T.Y., Libertine-Garahan, L., Eckman, J., Biftu, T., Ip, S., 1993. Epibatidine is a nicotinic analgesic. Eur. J. Pharmacol. 250, R13-R14.

Reimsnider, S., Wood, C.E., 2006. Differential modulation of ovine fetal ACTH secretion by PGHS-1 and PGHS-2. Neuroendocrinology 83, 4-11.

Sasaki, T., Shimizu, T., Wakiguchi, H., Yokotani, K., 2008. Centrally administered neuromedin U elevates plasma adrenaline by brain prostanoid TP receptor-mediated mechanisms in rats. Eur. J. Pharmacol. 592, 81-86.

Sher, E., Chen, Y., Sharples, T.J., Broad, L.M., Benedetti, G., Zwart, R., McPhie, G.I., Pearson, K.H., Baldwinson, T., De Filippi, G., 2004. Physiological roles of neuronal nicotinic receptor subtypes: new insights on the nicotinic modulation of neurotransmitter release, synaptic transmission and plasticity. Curr. Top. Med. Chem. 4, 283-297.

Shimizu, T., Okada, S., Yamaguchi-Shima, N., Yokotani, K., 2004. Brain phospholipase C-diacylglycerol lipase pathway is involved in vasopressin-induced release of noradrenaline and adrenaline from adrenal medulla in rats. Eur. J. Pharmacol. 499, 99-105.

Shimizu, T., Okada, S., Yamaguchi, N., Sasaki, T., Lu, L., Yokotani, K., 2006. Centrally administered histamine evokes the adrenal secretion of noradrenaline and adrenaline by brain cyclooxygenase-1- and thromboxane A_2-mediated mechanisms in rats. Eur. J. Pharmacol. 541, 152-157.

Suzuki, T., Kachi, T., 1996. Similarities and differences in supporting and chromaffin cells in the mammalian adrenal medullae: an immunohistochemical study. Anat. Rec. 244, 358-365.

Swanson, L.W., Sawchenko, P.E., 1980. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology 31, 410-417.

Tseng, C.J., Appalsamy, M., Robertson, D., Mosqueda-Garcia, R., 1993. Effects of nicotine on brain stem mechanisms of cardiovascular control. J. Pharmacol. Exp. Ther. 265, 1511-1518.

Tseng, C.J., Ger, L.P., Lin, H.C., Tung, C.S., 1994. The pressor effect of nicotine in the rostral ventrolateral medulla of rats. Clin. J. Physiol. 37, 83-87.

Vizi, E.S., Lendvai, B., 1999. Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. Brain Res. Brain Res. Rev. 30, 219-235.

Vollmer, R.R., Balcita-Pedicino, J.J., Debnam, A.J., Edwards, D.J., 2000. Adrenal medullary catecholamine secretion patterns in rats evoked by reflex and direct neural stimulation. Clin. Exp. Hypertens. 22, 705-715.

Wang, M., Okada, S., Murakami, Y., Yokotani, K., 2000. Nicotine-induced noradrenaline release from the isolated rat stomach by activation of L- and N-type calcium channels. Jpn. J. Pharmacol. 83, 102-106.

Watts, D.T., 1960. The effect of nicotine and smoking on the secretion of epinephrine. Ann. N. Y. Acad. Sci. 90, 74-80.

Wonnacott, S., 1997. Presynaptic nicotinic ACh receptors. Trends. Neurosci. 20, 92-98.

Wood, C.E., Cudd, T.A., Kane, C., Enkelke, K., 1993. Fetal ACTH and blood pressure responses to thromboxane mimetic U-46619. Am. J. Physiol. 265, R858-R862.

Wyss, J.M., Carlson, S.H., 2001. The role of the nervous system in hypertension. Curr. Hypertens. Rep. 3, 255-262.

Yalcin, M., Savci, V., 2004. Restoration of blood pressure by central injected U-46619, a thromboxane A_2 analog, in hemorrhaged hypotensive rats: investigation of different brain areas. Pharmacology 7, 177-187.

Yamaguchi-Shima, N., Okada, S., Shimizu, T., Usui, D., Nakamura, K., Lu, L., Yokotani, K., 2007. Adrenal adrenaline- and noradrenaline-containing cells and celiac sympathetic ganglia are differentially controlled by centrally administered

corticotropin-releasing factor and arginine-vasopressin in rats. Eur. J. Pharmacol. 564, 94-102.

Yokotani, K., Nishihara, M., Murakami, Y., Hasegawa, T., Okuma, Y., Osumi, Y., 1995. Elevation of plasma noradrenaline levels in urethane-anaesthetized rats by activation of central prostanoid EP_3 receptors. Br. J. Pharmacol. 115, 672-676.

Yokotani, K., Okada, S., Murakami, Y., Nakamura, K., 2001. Nicotinic receptors involved in gastric noradrenaline release evoked by electrical stimulation of the splanchnic nerve in rats. Eur. J. Pharmacol. 423, 149-155.

Yokotani, K., Okada, S., Nakamura, K., 2002. Characterization of functional nicotinic acetylcholine receptors involved in catecholamine release from the isolated rat adrenal gland. Eur. J. Pharmacol. 446, 83-87.

Yokotani, K., Okada, S., Nakamura, K., Yamaguchi-Shima, N., Shimizu, T., Arai, J., Wakiguchi, H., Yokotani, K., 2005. Brain prostanoid TP receptor-mediated adrenal noradrenaline secretion and EP_3 receptor-mediated sympathetic noradrenaline release in rats. Eur. J. Pharmacol. 512, 29-35.

Zhang, Z.H., Wei, S.G., Francis, J., Felder, R.B., 2003. Cardiovascular and renal sympathetic activation by blood-borne TNF-alpha in rat: the role of central prostaglandins. Am. J. Physiol. Regul. Integr. Comp. Physiol. 284, R916-R927.

Zhao, R., Chen, H., Sharp, B.M., 2007. Nicotine-induced norepinephrine release in hypothalamic paraventricular nucleus and amygdala is mediated by N-methyl-D-aspartate receptors and nitric oxide in the nucleus tractus solitarius. J. Pharmacol. Exp. Ther. 320, 837-844.

Legends to figures

Fig. 1. Effect of centrally and peripherally administered epibatidine on the plasma levels of catecholamines. ΔNoradrenaline and ΔAdrenaline: increments of noradrenaline and adrenaline above the basal level. Each point represents the mean±S.E.M. (A) Arrow indicates the administration of vehicle (DMF 2.5 μl/animal, i.c.v.) or epibatidine (1, 5 and 10 nmol/animal, i.c.v.). *Significantly different from the vehicle-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(3,14)=20.78$, $P<0.017$; at 10 min, $F(3,14)=13.07$, $P<0.017$: adrenaline; at 5 min, $F(3,14)=6.30$, $P<0.017$; at 10 min, $F(3,14)=7.75$, $P<0.017$; at 30 min, $F(3,14)=4.53$, $P<0.017$; at 60 min, $F(3,13)=5.85$, $P<0.017$]. The actual values for noradrenaline and adrenaline at 0 min were 186±22 and 211±35 pg/ml (n=18), respectively. (B) Arrow indicates the administration of vehicle (0.5% DMF/saline 500 μl/animal, i.v.) or epibatidine (5 nmol/animal, i.v.). *Significantly different from the vehicle-treated group with Welch's *t*-test [adrenaline; at 30 min, $F(3,5)=32.3$, $P<0.05$]. The actual values for noradrenaline and adrenaline at 0 min were 146±9 and 176±48 pg/ml (n=10), respectively.

Fig. 2. Effect of hexamethonium (an antagonist of nicotinic acethylcholine receptors) on the centrally administered epibatidine-induced elevation of plasma catecholamines. Arrows indicate the administrations of hexamethonium/vehicle-1 and epibatidine/vehicle-2. Other conditions were the same as those of Fig. 1. (A) Hexamethonium (0.9 and 1.8 μmol/animal) or vehicle-1 (5 μl saline/animal) was i.c.v. administered 30 min before the administration of epibatidine (5 nmol/animal, i.c.v.) or

vehicle-2 (2.5 μl DMF/animal, i.c.v.). *Significantly different from vehicle-1- and epibatidine-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(2,13)=8.07$, $P<0.025$; at 10 min, $F(2,13)=6.05$, $P<0.025$: adrenaline; at 5 min, $F(2,13)=33.87$, $P<0.025$; at 10 min, $F(2,13)=49.78$, $P<0.025$; at 30 min, $F(2,13)=11.34$, $P<0.025$; at 60 min, $F(2,13)=10.70$, $P<0.025$]. The actual values for noradrenaline and adrenaline at 0 min were 299±62 and 304±107 pg/ml in the vehicle-1-pretreated group (n=9); 419±88 and 606±134 pg/ml in the hexamethonium (0.9 μmol/animal)-pretreated group (n=6); 223±22 and 316±64 pg/ml in the hexamethonium (1.8 μmol/animal)-pretreated group (n=9), respectively. (B) Hexamethonium (1.8 μmol/animal) or vehicle-1 (500 μl saline/animal) was i.v. administered 30 min before the administration of epibatidine (5 nmol/animal, i.c.v.) or vehicle-2 (2.5 μl DMF/animal, i.c.v.). The actual values for noradrenaline and adrenaline at 0 min were 376±83 and 290±62 pg/ml in the vehicle-1-pretreated group (n=10); 164±15 and 328±40 pg/ml in the hexamethonium (1.8 μmol/animal)-pretreated group (n=10), respectively.

Fig. 3. Effect of indomethacin (an inhibitor of cyclooxygenase) on the centrally administered epibatidine-induced elevation of plasma catecholamines. Arrows indicate the administration of indomethacin/vehicle-1 and epibatidine/vehicle-2. Indomethacin (0.6 and 1.2 μmol/animal) or vehicle-1 (5 μl saline/animal) was i.c.v. administered 30 min before the administration of epibatidine (5 nmol/animal, i.c.v.) or vehicle-2 (2.5 μl DMF/animal, i.c.v.). Other conditions were the same as those of Figs. 1 and 2. *Significantly different from vehicle-1- and epibatidine-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(2,11)=7.76$, $P<0.025$; at 10 min,

$F(2,11)=5.17$, $P<0.025$: adrenaline; at 5 min, $F(2,11)=10.17$, $P<0.025$; at 10 min, $F(2,11)=3.94$, $P<0.025$]. Vehicle-1-treated groups were the same as those of Fig. 2A. The actual values for noradrenaline and adrenaline at 0 min were 193±69 and 418±63 pg/ml in the indomethacin (0.6 μmol/animal)-pretreated group (n=4); 208±32 and 299±59 pg/ml in the indomethacin (1.2 μmol/animal)-pretreated group (n=9), respectively.

Fig. 4. Effect of (+)-S-145 (an antagonist of prostanoid TP receptors) on the centrally administered epibatidine-induced elevation of plasma catecholamines. Arrows indicate the administration of (+)-S-145/vehicle-1 and epibatidine/vehicle-2. (+)-S-145 (0.6 and 1.3 μmol/animal) or vehicle-1 (5 μl saline/animal) was i.c.v. administered 60 min before the administration of epibatidine (5 nmol/animal, i.c.v.) or vehicle-2 (2.5 μl DMF/animal, i.c.v.). Other conditions were the same as those in Figs. 1-3. *Significantly different from the vehicle-1- and epibatidine-treated group with the Bonferroni method [noradrenaline; at 5 min, $F(2,12)=9.95$, $P<0.025$; at 10 min, $F(2,12)=5.28$, $P<0.025$: adrenaline; at 5 min, $F(2,12)=10.54$, $P<0.025$; at 10 min, $F(2,12)=4.69$, $P<0.025$]. The actual values for noradrenaline and adrenaline at 0 min were 276±56 and 179±49 pg/ml in the vehicle-1-pretreated group (n=10); 246±45 and 145±27 pg/ml in the (+)-S-145 (0.6 μmol/animal)-pretreated group (n=5); 303±39 and 161±24 pg/ml in the (+)-S-145 (1.3 μmol/animal)-pretreated group (n=9), respectively.

Fig. 5. Effect of acute bilateral adrenalectomy on the centrally administered epibatidine-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 h before the application of epibatidine (5 nmol/animal, i.c.v.). Arrow indicates the administration of epibatidine. Other conditions were the same as those in Figs. 1-4. *Significantly different from the sham-operated group with an unpaired Student's *t*-test or Welch's *t*-test [noradrenaline; at 5 min, $F(4,4)=6.51$, $P<0.05$; at 10 min, $F(4,4)=5.92$, $P<0.05$; at 30 min, $F(4,4)=1.36$, $P<0.05$; at 60 min, $F(4,4)=2.13$, $P<0.05$: adrenaline; at 5 min, $F(4,4)=50.97$, $P<0.05$; at 10 min, $F(4,4)=2.88$, $P<0.05$]. The actual values for noradrenaline and adrenaline at 0 min were 172±21 and 129±36 pg/ml in sham-operated group (n=5) and 191±50 and 51±28 pg/ml in bilateral adrenalectomized group (n=5), respectively.

Figure-1

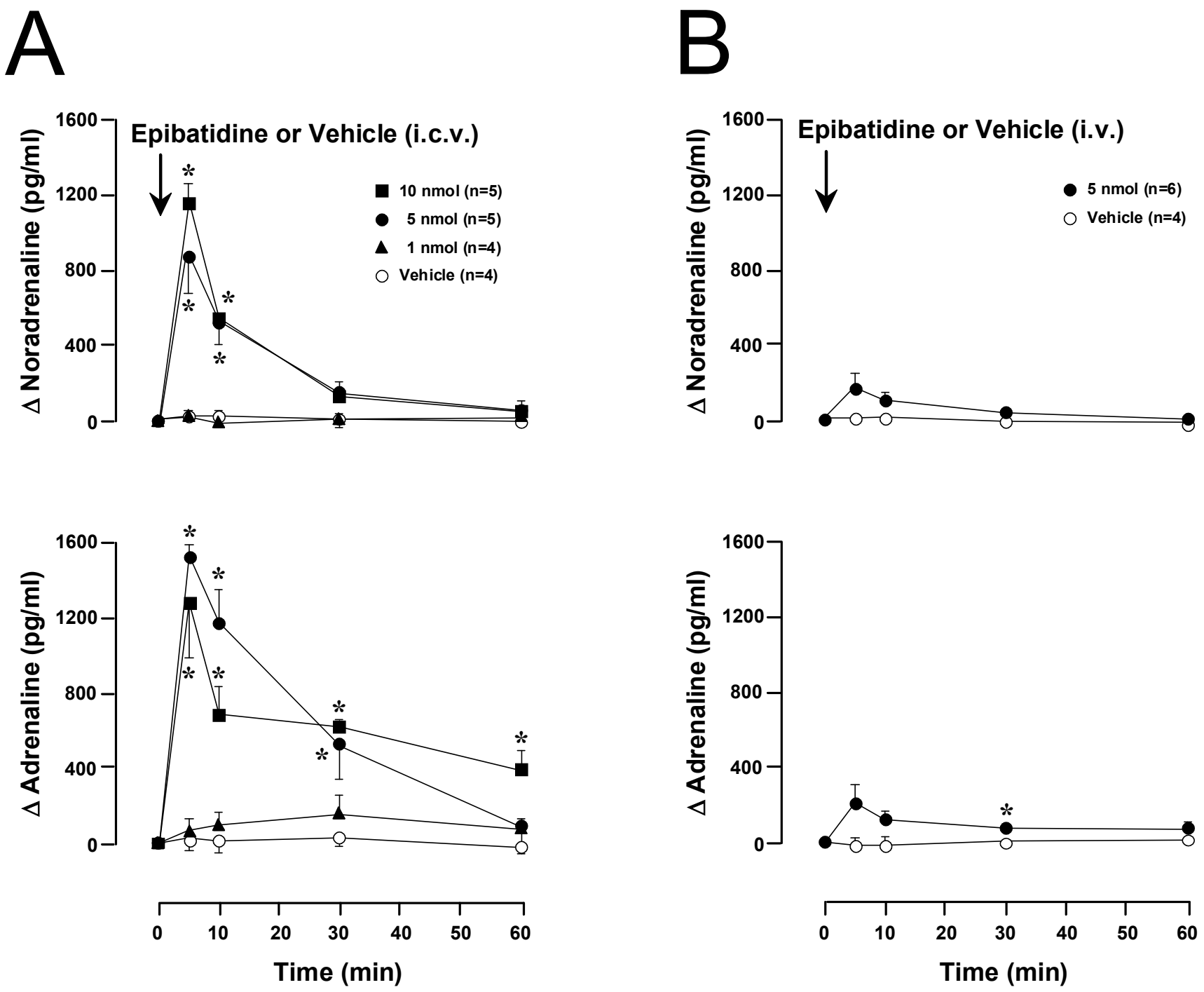

A
B
Epibatidine or Vehicle (i.c.v.)
Epibatidine or Vehicle (i.v.)
10 nmol (n=5)
5 nmol (n=5)
1 nmol (n=4)
Vehicle (n=4)
5 nmol (n=6)
Vehicle (n=4)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
1600
1200
800
400
0
0
10
20
30
40
50
60
Time (min)

Figure-2

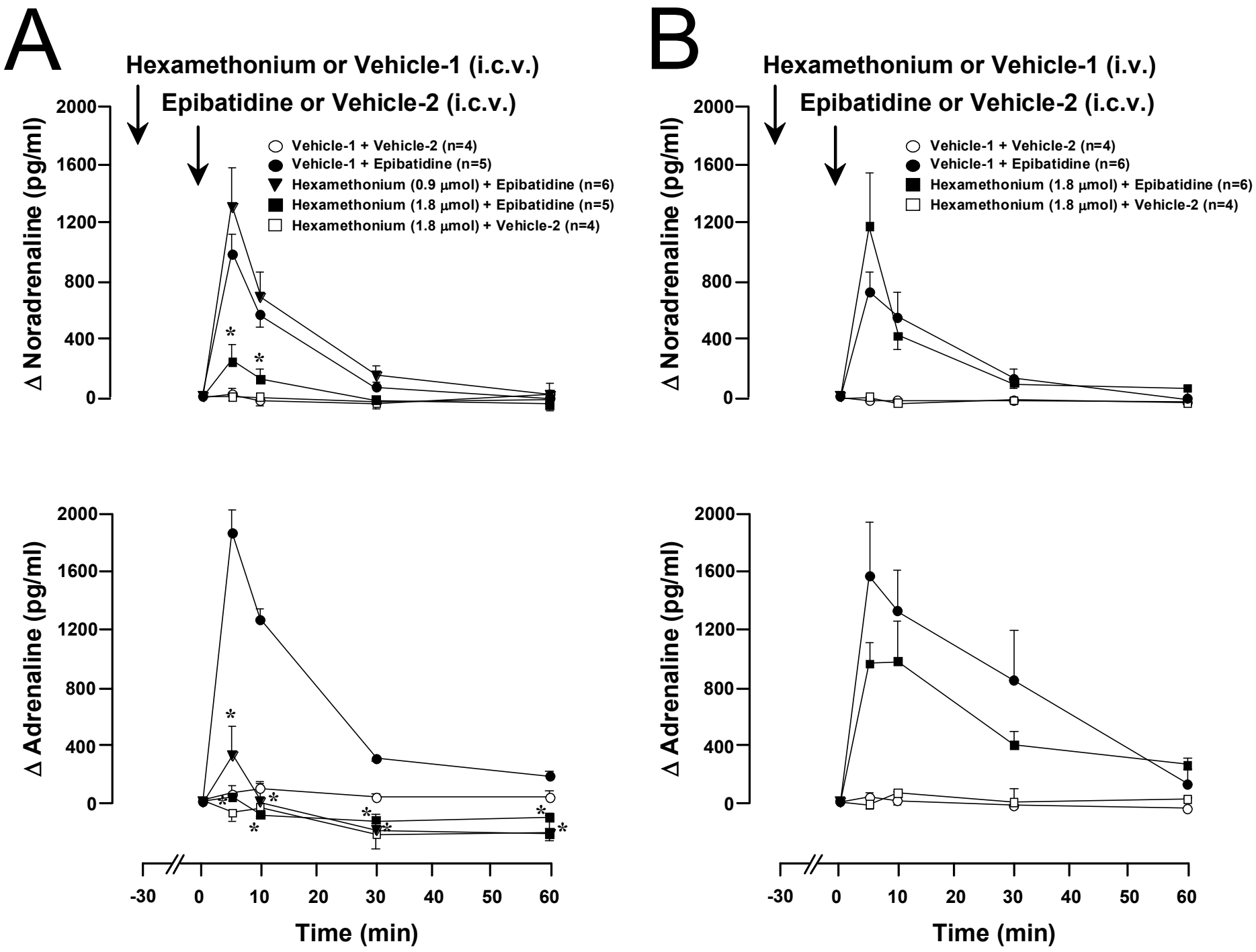

A
Hexamethonium or Vehicle-1 (i.c.v.)
Epibatidine or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
Vehicle-1 + Epibatidine (n=5)
Hexamethonium (0.9 μmol) + Epibatidine (n=6)
Hexamethonium (1.8 μmol) + Epibatidine (n=5)
Hexamethonium (1.8 μmol) + Vehicle-2 (n=4)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
2000
1600
1200
800
400
0
-30
0
10
20
30
40
50
60
Time (min)
B
Hexamethonium or Vehicle-1 (i.v.)
Epibatidine or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
Vehicle-1 + Epibatidine (n=6)
Hexamethonium (1.8 μmol) + Epibatidine (n=6)
Hexamethonium (1.8 μmol) + Vehicle-2 (n=4)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
Time (min)

Figure-3

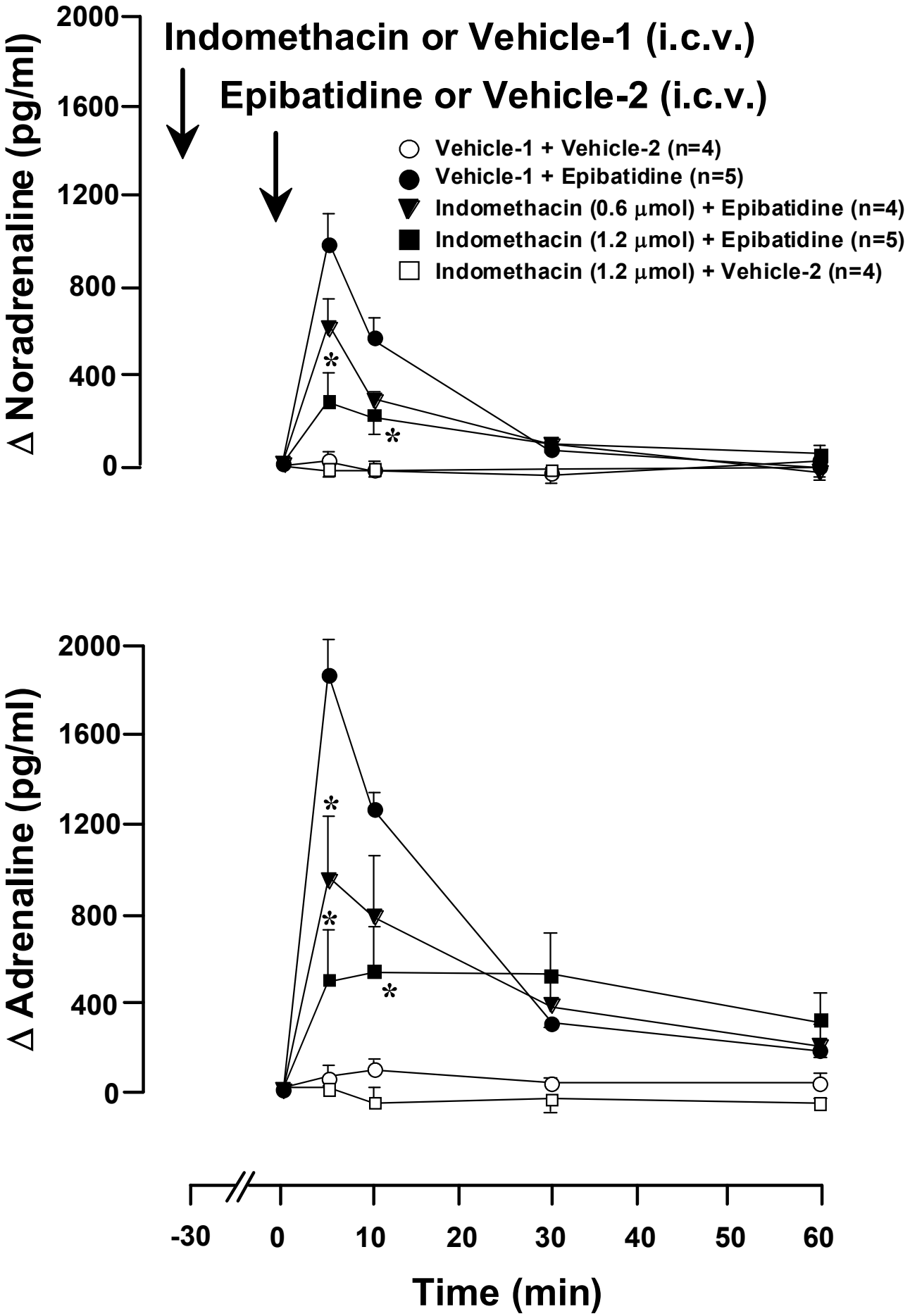

Indomethacin or Vehicle-1 (i.c.v.)
Epibatidine or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=4)
Vehicle-1 + Epibatidine (n=5)
Indomethacin (0.6 μmol) + Epibatidine (n=4)
Indomethacin (1.2 μmol) + Epibatidine (n=5)
Indomethacin (1.2 μmol) + Vehicle-2 (n=4)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
2000
1600
1200
800
400
0
*
-30
0
10
20
30
40
50
60
Time (min)

Figure-4

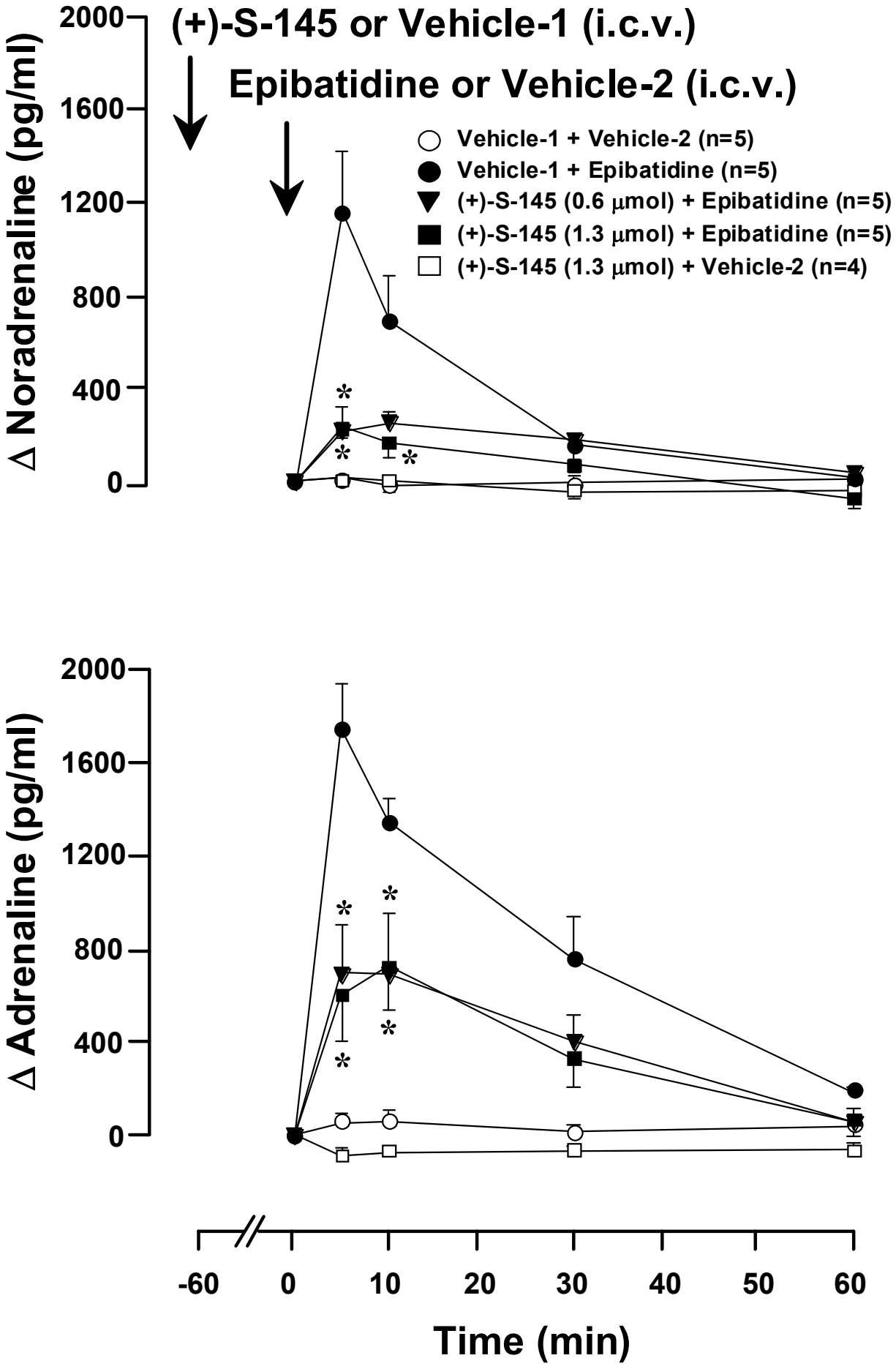
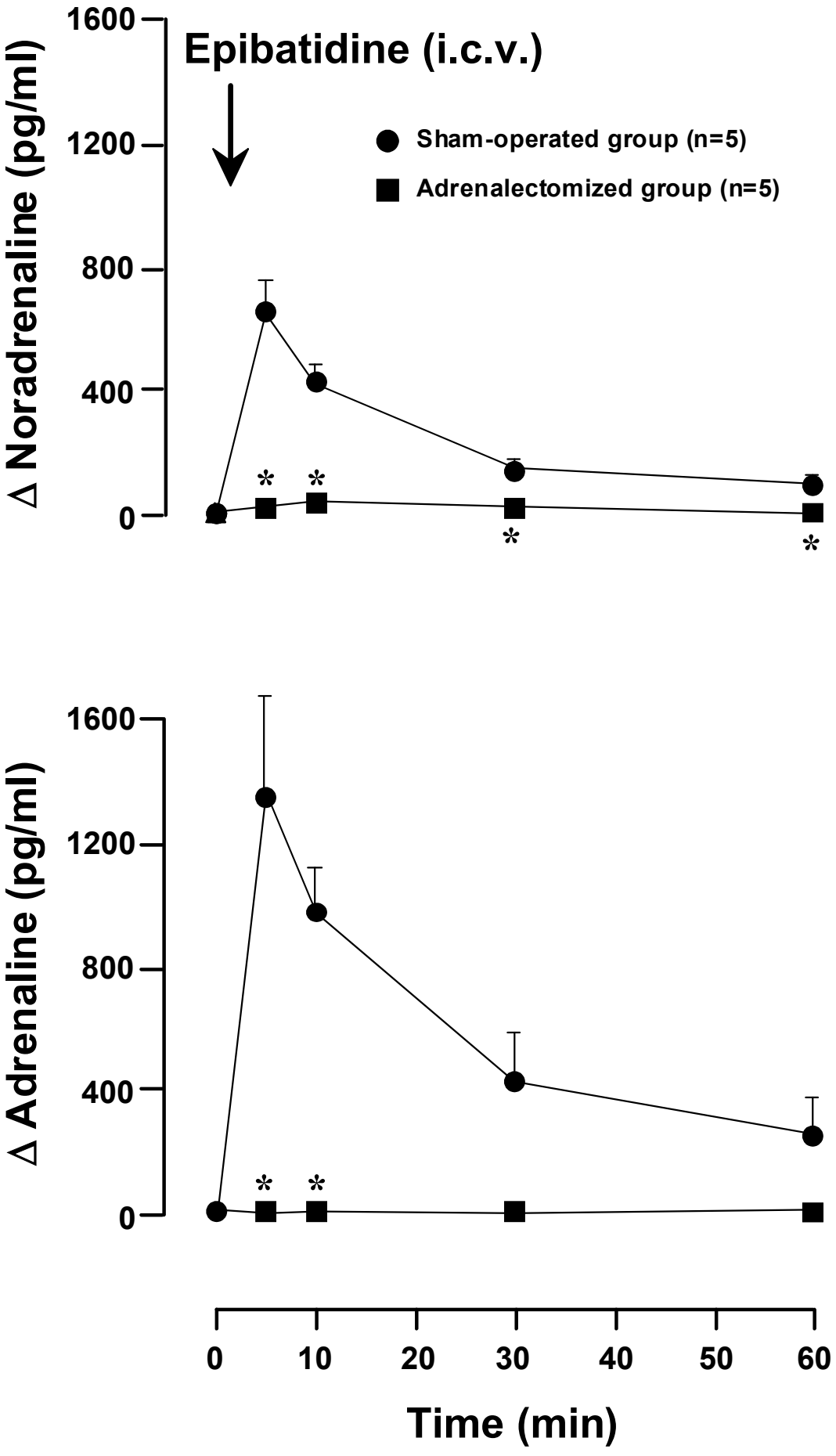

(+)-S-145 or Vehicle-1 (i.c.v.)
Epibatidine or Vehicle-2 (i.c.v.)
Vehicle-1 + Vehicle-2 (n=5)
Vehicle-1 + Epibatidine (n=5)
(+)-S-145 (0.6 μmol) + Epibatidine (n=5)
(+)-S-145 (1.3 μmol) + Epibatidine (n=5)
(+)-S-145 (1.3 μmol) + Vehicle-2 (n=4)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
2000
1600
1200
800
400
0
-60
0
10
20
30
40
50
60
Time (min)

Figure-5


Epibatidine (i.c.v.)
Sham-operated group (n=5)
Adrenalectomized group (n=5)
Δ Noradrenaline (pg/ml)
Δ Adrenaline (pg/ml)
1600
1200
800
400
0
*
0
10
20
30
40
50
60
Time (min)