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Roles of brain prostaglandin E_2 and thromboxane A_2 in the activation of the central sympatho-adrenomedullary outflow in rats

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Abstract

We examined the effects of centrally administered active metabolites of the arachidonic acid cascade on activation of the central sympatho-adrenomedullary outflow using urethane-anaesthetized rats. Intracerebroventricularly (i.c.v.) administered prostaglandin E_2 (0.3 nmol/animal) significantly elevated plasma levels of noradrenaline while levels of adrenaline were not affected. Prostaglandin D_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 at the same dose (0.3 nmol/animal, i.c.v.) had no effect on plasma levels of either catecholamine. Thromboxane A_2 mimetic, 7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo [2.2.1]hept-2-yl], [1*S*-[1α,2α(*Z*),3β(1*E*,3*S*),4α]]-5-heptenoic acid (I-BOP) (5 and 10 pmol/animal) microinjected into the paraventricular nucleus of the hypothalamus significantly elevated plasma levels of adrenaline, but had little effect on plasma levels of noradrenaline. The I-BOP-induced (10 pmol/animal) elevation of plasma adrenaline levels was abolished by (+)-(1*R*,2*R*,3*S*,4*S*)-(5*Z*)-7-(3-[4-^{3}H]-phenylsulphonyl-aminobicyclo[2.2.1]hept-2-yl)hept-5-enoic acid sodium salt [(+)-S-145] (a blocker of thromboxane A_2 receptors) [625 nmol (250 μg)/animal, i.c.v.]. These results suggest that brain prostaglandin E_2 and thromboxane A_2 are respectively involved in the activation of the central sympathetic and adrenomedullary outflow in rats.

Keywords: Prostaglandin E_2; Thromboxane A_2; Brain; Noradrenaline; Adrenaline; Plasma; Sympathetic outflow; Adrenomedullary outflow

1. Introduction

Oxygenated metabolites of arachidonic acid such as prostaglandins and thromboxane A_2 are synthesized within the central nervous system of mammals (Wolfe, 1982). There is increasing evidence that prostaglandins are implicated in the central regulation of a variety of functions, including body temperature (Coceani et al., 1988; Milton, 1989), cardiovascular function (Hoffman and Schmid, 1979; Chiu and Richardson, 1983), hormone secretion (Behrman, 1979; Heaulme and Dray, 1984; Brooks et al., 1986) and behavioral activities (Johnson et al., 1993). Thromboxane A_2 has also been demonstrated to act as neuromediator and/or neuromodulator in the brain actions including elevation of arterial blood pressure and release of adrenocorticotropic hormone (Wood et al., 1993), release of corticotropin-releasing factor (CRF) (Bernardini et al., 1989) and inhibition of noradrenaline release from the hippocampus (Nishihara et al., 2000).

Previously, we reported that the elevation of plasma noradrenaline induced by intracerebroventricularly (i.c.v.) administered interleukin-1β was abolished by i.c.v. administered indomethacin, an inhibitor of cyclooxygenase (Murakami et al., 1996). Recently, we also reported that elevation of both plasma noradrenaline and adrenaline induced by i.c.v. administered CRF- and arachidonic acid was also abolished by i.c.v. administered indomethacin (Yokotani et al., 2000, 2001). These results suggest the involvement of active metabolites of the arachidonic acid cascade in the activation of the central sympatho-adrenomedullary outflow in rats. In the present study, therefore, we examined the effects of centrally administered prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 and thromboxane A_2 on plasma levels of catecholamines in urethane-anaesthetized rats.

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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 anaesthesia (1.2 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. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous paper (Yokotani et al., 1995).

Three hours after the animal had been placed in the stereotaxic apparatus, a stainless steel cannula (0.35 mm outer diameter) was inserted into the right lateral ventricle according to the rat brain atlas of Paxinos and Watson (1986). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP, −0.8; L, 1.5; H, 4.0 (AP, anterior from the bregma; L, lateral from the midline; H, below the surface of the brain). Prostaglandins (prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2) dissolved in sterile saline containing 0.5% ethanol were slowly injected into the right lateral ventricle in a volume of 10 μl, using a 50-μl Hamilton syringe. To avoid the possibility of whole brain ischemia after i.c.v. administration of 7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo [2.2.1]hept-2-yl], [1*S*-[1α,2α(*Z*),3β(1*E*,3*S*),4α]]-5-heptenoic acid (I-BOP) (a thromboxane A_2 mimetic), this reagent was microinjected into the paraventricular nucleus of the hypothalamus in a volume of 0.5 μl using a pulled glass micropipette (75 μm outer diameter of tip) and 1.0-μl Hamilton syringe. The stereotaxic coordinates of the tip of the glass micropipette were as follows (in mm): AP, 1.8; L, 0.3; H, 7.8. [(1*R*,2*R*,3*S*,4*S*)-(5*Z*)-7-(3-[4-^{3}H]-Phenylsulphonyl-aminobicyclo [2.2.1] hept-2-yl) hept-5-enoic acid sodium salt] (S-145, a blocker of thromboxane A_2 receptors) dissolved in saline was administered into the right lateral ventricle in a volume of 10 μl 60 min before administration of I-BOP.

At the end of the experiments, the brain was removed and fixed in 10% formalin to verify the correct placement of the glass pipette and the cannula. Sections sliced at 20 μm were prepared for microscopic study of the location of the injector tip. A typical placement of the pulled glass micropipette in the paraventricular nucleus of the hypothalamus is shown in Fig. 2.

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

2.2. Measurement of plasma catecholamines

Blood samples (250 μl) were collected through an arterial catheter. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically by high-performance liquid chromatography (Okada et al., 2000). Briefly after centrifugation, the plasma (100 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double deionized water, 1 ng of 3,4-dihydroxybenzylamine as internal standard and 1 ml of 1.5 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, 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-

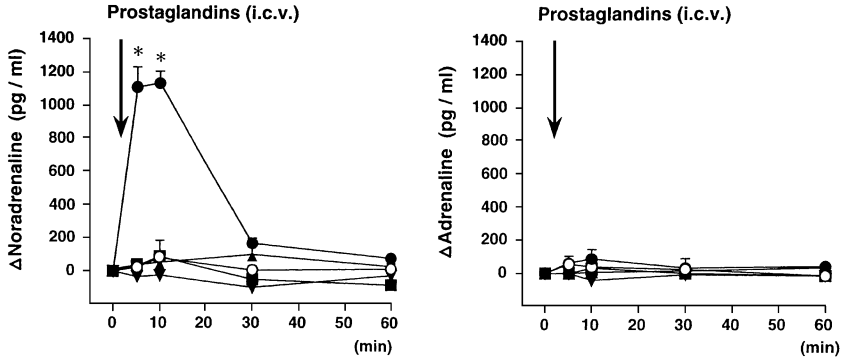

Fig. 1. Effects of intracerebroventricular (i.c.v.) administration of prostaglandins on the plasma levels of adrenaline and noradrenaline. Arrow indicates the administration of prostaglandins (0.3 nmol/animal, i.c.v.). Δ Adrenaline and Δ noradrenaline: net increases in plasma levels of adrenaline or noradrenaline from their respective basal levels. ○, Vehicle (saline 10 μl/animal); ●, prostaglandin E_2; ▲, prostaglandin D_2; ■, prostaglandin I_2; ▼, prostaglandin $F_{2\alpha}$. Each point represents the mean ± S.E.M. * Significantly different ($P<0.05$) from vehicle-treated control. The actual values for the basal plasma levels of noradrenaline and adrenaline were 290 ± 67 and 222 ± 46 pg/ml for vehicle-treated control group ($n=7$), 268 ± 14 and 181 ± 54 pg/ml for prostaglandin E_2-treated group ($n=5$), 343 ± 81 and 184 ± 82 pg/ml for prostaglandin D_2-treated group ($n=4$), 359 ± 88 and 183 ± 41 pg/ml for prostaglandin I_2-treated group ($n=4$), 337 ± 35 and 198 ± 79 pg/ml for prostaglandin $F_{2\alpha}$-treated group ($n=4$), respectively.

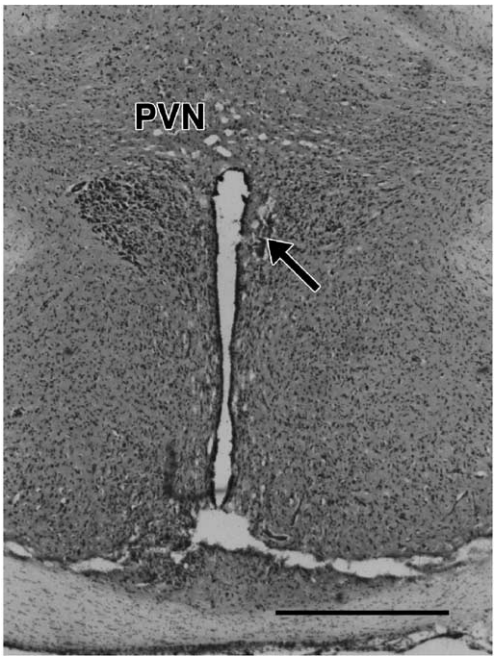

Fig. 2. Photomicrograph of a typical placement of the pulled glass micropipette in the paraventricular nucleus (PVN) of the hypothalamus. Arrow indicates the tip of the glass micropipette. Scale bar: 1000 μm.

le-Bel, France) and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with high-performance liquid chromatography. Analytical conditions were as follows: detector, +450 mV potential against a Ag/AgCl reference electrode; column, Eicompack CA-50DS, 2.1 × 150 mm (Eicom); mobile phase, 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l EDTA dihydrate, 750 mg/l 1-octane sulfate sodium (Nacalai Tesque, Kyoto, Japan) and 15% methanol at a flow of 0.22 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 adrenaline and noradrenaline accurately.

2.3. Treatment of data and statistics

Results were expressed as the means ± S.E.M. of the net changes above the respective basal values because of individual variations. The data were analyzed by repeated measures analysis of variance (ANOVA) followed by post hoc analysis with the Bonferroni method for comparing a control to all other means (Figs. 1 and 3). When only two means were compared, an unpaired Student's *t*-test was used (Fig. 5). *P* values less than 0.05 were taken to indicate significance.

2.4. Compounds

The following drugs were used: prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 (Sigma-Aldrich, St. Louis, MO, USA), I-BOP (Cayman Chemical, Ann Arbor, MI, USA) and (+)-S-145 (a kind gift from Shionogi Pharmaceutical, Osaka, Japan). All other reagents were of the highest grade available (Nacalai Tesque).

3. Results

3.1. Effects of i.c.v. administered prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 on plasma levels of catecholamines

After administration of vehicle (10 μl saline containing 0.5% ethanol, i.c.v.), blood sampling at 0, 5, 10, 30 and 60 min had no effect on the plasma levels of either noradrenaline or adrenaline (Fig. 1).

Administration of prostaglandin E_2 (0.3 nmol/animal, i.c.v.) caused a rapidly developing elevation of plasma levels of noradrenaline, while adrenaline levels were not affected (Fig. 1). The plasma noradrenaline levels reached their maximum (1111 ± 116 pg/ml) 10 min after the administration of prostaglandin E_2, and then rapidly declined

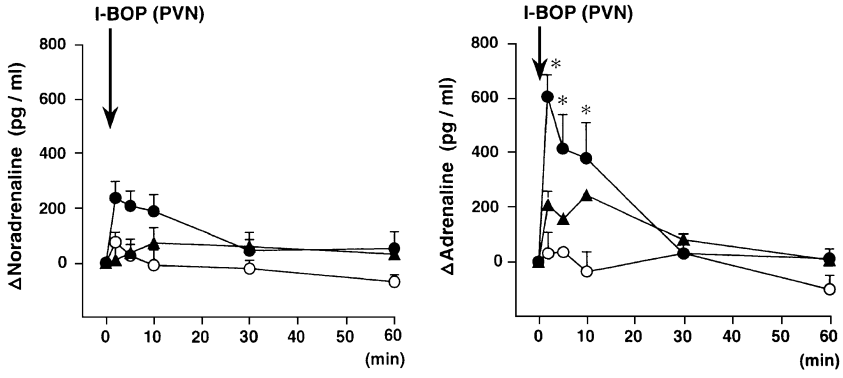

Fig. 3. Effect of I-BOP injected into the paraventricular nucleus of the hypothalamus on plasma levels of noradrenaline and adrenaline. ○, Vehicle (0.5 μl saline containing 0.5% ethanol/animal); ▲, I-BOP (5 pmol/animal); ●, I-BOP (10 pmol/animal). Other conditions were the same as those in Fig. 1. The actual values for the basal plasma levels of noradrenaline and adrenaline were 300 ± 49 and 224 ± 41 pg/ml for vehicle-treated group ($n = 5$), 312 ± 45 and 237 ± 23 pg/ml for I-BOP-treated (5 pmol/animal) group ($n = 4$), 312 ± 65 and 255 ± 64 pg/ml for I-BOP-treated (10 pmol/animal) group ($n = 6$), respectively. * Significantly different ($P < 0.05$) from the vehicle-treated control.

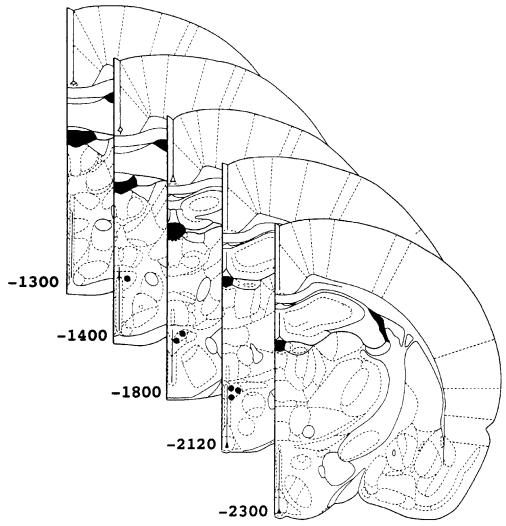

Fig. 4. Diagram of coronal sections of the rat brain showing each injection site in the I-BOP-treated (10 pmol/animal) group. ●, The tip of glass micropipette. Numbers in each section indicate the distance (μm) of the plate from the bregma. Plates were adapted from the CD-ROM rat brain atlas of Paxinos and Watson (1997). The site in plate −1300 lies between −800 and −1350, −1400 lies between −1350 and −1600, −1800 lies between −1600 and −1960, −2120 lies between −1960 and −2250, and −2300 lies between −2250 and −2700, respectively.

toward the basal levels. On the other hand, the same dose of prostaglandin D_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 had no effect on the plasma levels of catecholamines (Fig. 1).

Intravenous administration of prostaglandin E_2 (0.3 nmol/animal) had no effect on the plasma levels of catecholamines (data not shown).

3.2. Effect of I-BOP injected into the hypothalamus on plasma levels of catecholamines

I-BOP was injected into the paraventricular nucleus of the hypothalamus (Fig. 2). After microinjection of vehicle (saline containing 0.5% ethanol) into the paraventricular nucleus, blood sampling at 0, 2, 5, 10, 30 and 60 min had no effect on the plasma levels of either noradrenaline or adrenaline (Fig. 3). Microinjection of I-BOP (5 and 10 pmol/animal) into the paraventricular nucleus induced a rapid and dose-dependent elevation of plasma levels of adrenaline, but those of noradrenaline were slightly, but not significantly, elevated (Fig. 3). The plasma adrenaline levels reached their maximum (203 ± 55 pg/ml for 5 pmol/animal and 601 ± 83 pg/ml for 10 pmol/animal) 2 min after the administration of I-BOP, and then rapidly declined toward the basal levels. Injection sites of I-BOP (10 pmol/animal) are shown in Fig. 4. Intravenous administration of I-BOP (10 pmol/animal) had no effect on the plasma levels of catecholamines (data not shown).

3.3. Effects of (+)-S-145, a blocker of thromboxane A_2 receptors, on the I-BOP-induced elevation of plasma adrenaline levels

I.c.v. administered (+)-S-145 [625 nmol (250 μg)/animal] or its vehicle (10 μl of saline) had no effect on the basal plasma levels of catecholamines. Intraventricular pretreatment with (+)-S-145 abolished the elevation of plasma adrenaline levels induced by I-BOP (10 pmol/animal), but had no effect on the slight elevation of plasma noradrenaline levels induced by I-BOP (Fig. 5).

4. Discussion

We compared the effects of prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 on plasma levels of catecholamines. I.c.v. administered prostaglandin E_2 significantly elevated the plasma levels of noradrenaline, while those of adrenaline were not influenced. The other prostaglandins (prostaglandin D_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2) had little effect on the

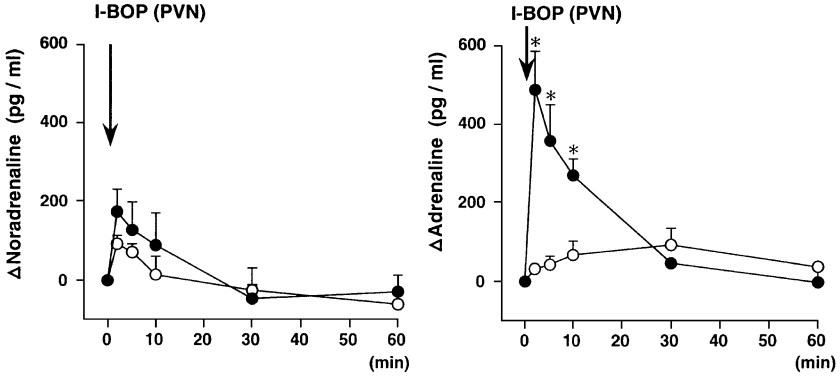

Fig. 5. Effect of (+)-S-145, a blocker of thromboxane A_2 receptors, on the I-BOP-induced elevation of plasma levels of noradrenaline and adrenaline. (+)-S-145 [625 nmol (250 μg)/animal, i.c.v.] or vehicle (10 μl saline, i.c.v.) was administered 60 min before microinjection of I-BOP (10 pmol/animal). ○, Vehicle plus I-BOP; ●, Vehicle plus I-BOP; ○, (+)-S-145 plus I-BOP. The actual values for the basal plasma levels of noradrenaline and adrenaline were 296 ± 74 and 229 ± 74 pg/ml in the vehicle- plus I-BOP-treated group ($n = 6$), 305 ± 60 and 223 ± 72 pg/ml in the (+)-S-145- plus I-BOP-treated group ($n = 5$), respectively. * Significantly different ($P < 0.05$) from the (+)-S-145- plus I-BOP-treated group.

plasma levels of either catecholamine. These results are consistent with previous reports in that prostaglandin E_2 injected into the rat lateral cerebral ventricle increased the plasma levels of catecholamines, especially noradrenaline (Okuno et al., 1982; Feuerstein et al., 1982). We have also reported that i.c.v. administered prostaglandin E_2 activates the central sympathetic outflow, thereby inhibiting the vagally mediated gastric acid secretion in rats (Yokotani et al., 1988).

Receptors coupled to prostaglandin E_2 have been pharmacologically divided into at least three subtypes (EP_1, EP_2 and EP_3) (Coleman et al., 1994). In the central nervous system, EP_3 prostanoid receptor mRNA is highly expressed in brain regions such as hippocampus, preoptic area and hypothalamus (Sugimoto et al., 1994). Previously, we reported that i.c.v. administered sulprostone (an agonist of EP_3/EP_1 prostanoid receptors) and misoprostol (an agonist of EP_3/EP_2 prostanoid receptors) effectively elevated only the plasma noradrenaline levels, but butaprost (an agonist of EP_2 prostanoid receptors) was ineffective (Yokotani et al., 1995, 1996). These results suggest the involvement of brain EP_3 receptors in the prostaglandin E_2-induced activation of the central sympathetic outflow. Other prostaglandins (prostaglandin D_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2) seem not to be involved in the regulation of the central sympatho-adrenomedullary outflow. A question has therefore arisen as to which of the active metabolites of arachidonic acid cascade is involved in the activation of the central adrenomedullary outflow.

Recently, we reported that the elevation of plasma adrenaline levels induced by i.c.v. administered 3-morpholinosydnonimine (a nitric oxide donor) was abolished by i.c.v. administered furegrelate and (+)-S-145 in rats (Murakami et al., 1998). The elevations of plasma adrenaline levels induced by i.c.v. administered CRF and arachidonic acid were also abolished by furegrelate (Yokotani et al., 2000, 2001). Since furegrelate is a selective inhibitor of thromboxane synthase (Gorman et al., 1983) and (+)-S-145 is a specific blocker of thromboxane A_2 receptors (Hanasaki and Arita, 1988), it is likely that brain thromboxane A_2 is involved in the activation of the central adrenomedullary outflow.

It is interesting to note that perfusion of the hypothalamic paraventricular nucleus with *N*-methyl-D-aspartate using microdialysis evoked thromboxane A_2 release into the perfusate of the microdialysis probe and a concomitant elevation of plasma adrenaline levels, and these responses were abolished by i.c.v. administered furegrelate (Okada et al., 2000). The hypothalamus, especially the paraventricular nucleus, has been considered to be the control center of the sympatho-adrenomedullary outflow (Swanson and Sawchenko, 1983). Results of a retrograde tracer study suggest a possible connection between the sympatho-adrenomedullary system and the paraventricular nucleus through the splanchnic nerve and spinal cord (Jansen et al., 1995). Thromboxane A_2 synthase is abundant in various regions of the hypothalamus, including the paraventricular nucleus (Wood et al., 1997). Thromboxane A_2 receptor mRNA has also been reported to be present in the brain (Namba et al., 1992; Gao et al., 1997). Therefore, we examined the effect of I-BOP, a selective agonist of thromboxane A_2 receptors, microinjected into the paraventricular nucleus of the hypothalamus, on plasma levels of catecholamines. I-BOP significantly elevated the plasma levels of adrenaline with a slight elevation of plasma noradrenaline levels. The I-BOP-induced elevation of plasma adrenaline levels was abolished by i.c.v. administered (+)-S-145, a selective blocker of thromboxane A_2 receptors, while a slight elevation of plasma noradrenaline levels was not influenced by (+)-S-145. These results suggest the involvement of thromboxane A_2 in the activation of the central adrenomedullary outflow in rats.

In conclusion, it seems likely that brain prostaglandin E_2 and thromboxane A_2 are respectively involved in the activation of the central sympathetic and adrenomedullary outflow in rats.

Acknowledgements

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