Roles of brain phosphatidylinositol-specific phospholipase
C and diacylglycerol lipase in centrally administered
histamine-induced adrenomedullary outflow in rats

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Abstract

Recently, we reported that intracerebroventricularly (i.c.v.) administered histamine evokes the secretion of noradrenaline and adrenaline from adrenal medulla by brain cyclooxygenase-1- and thromboxane A₂-mediated mechanisms in rats. These results suggest the involvement of brain arachidonic acid cascade in the histamine-induced activation of the central adrenomedullary outflow. Arachidonic acid is released mainly by phospholipase A₂ (PLA₂)-dependent pathway or phospholipase C (PLC)/diacylglycerol lipase-dependent pathway. In the present study, histamine (27 nmol/animal, i.c.v.)-induced elevation of plasma noradrenaline and adrenaline was dose-dependently reduced by U-73122 (PLC inhibitor) (10 and 100 nmol/animal, i.c.v.), ET-18-OCH₃ (phosphatidylinositol-specific PLC inhibitor) (10 and 30 nmol/animal, i.c.v.) and RHC-80267 (diacylglycerol lipase inhibitor) (1.3 and 2.6 μmol/animal, i.c.v.). However, mepacrine (PLA₂ inhibitor) (1.1 and 2.2 μmol/animal, i.c.v.) and D609 (phosphatidylcholine-specific PLC inhibitor) (30, 100 and 300 nmol/animal, i.c.v.) had no effect. These results suggest the involvement of brain phosphatidylinositol-specific PLC and diacylglycerol lipase in the centrally administered histamine-induced activation of the adrenomedullary outflow in rats.

Keywords: Adrenal medulla; Brain; Histamine;
Phosphatidylinositol-specific phospholipase C;
Diacylglycerol lipase

1. Introduction

Oxygenated metabolites of arachidonic acid such as prostaglandins and thromboxane A₂ have been demonstrated to act as neuromediator and/or neuromodulator in the brain actions including cardiovascular function (Chiu and Richardson, 1983; Wood et al., 1993; Zhang et al., 2003) and regulation of hormone secretion (Brooks et al., 1986; Bernardini et al., 1989; Reimsnider and Wood, 2006). Previously, we reported the involvement of brain arachidonic acid cascade in the centrally administered corticotropin-releasing factor-, arginine-vasopressin- and bombesin-induced elevation of plasma noradrenaline and adrenaline, since these responses were attenuated by central pretreatment with indomethacin, an inhibitor of cyclooxygenase, in rats (Okuma et al., 1997; Okada et al., 2003b). In addition, centrally administered arachidonic acid elevated plasma levels of both catecholamines and these elevations were attenuated by central pretreatment with indomethacin in rats (Yokotani et al., 2000). These results suggest the involvement of active metabolites of brain arachidonic acid in activation of the central sympatho-adrenomedullary outflow in rats.

Arachidonic acid has been shown to be released mainly by two different pathways: (1) phospholipase A₂ (PLA₂) hydrolyzes the *sn*-2 ester bond of membrane phospholipids, thereby releasing arachidonic acid (Irvine, 1982; Balsinde

et al., 2002); (2) phospholipase C (PLC) cleaves the phosphodiester bond, resulting in the formation of diacylglycerol, which can be hydrolyzed by diacylglycerol lipase to yield arachidonic acid (Bell et al., 1979; Irvine, 1982; Moscat et al., 1986; Hou et al., 1996; Tang et al., 2006). Previously, we reported that the brain PLA₂ is involved in the centrally administered melittin (PLA₂ activator)-induced elevation of plasma noradrenaline and adrenaline (Yokotani et al., 2000), and that the brain PLC-diacylglycerol lipase pathway is involved in the centrally administered corticotropin-releasing factor-, arginine-vasopressin- and bombesin-induced elevation of plasma catecholamines in rats (Okada et al., 2003b; Shimizu et al., 2004, 2005).

Recently, we reported that central pretreatment with ketoprofen, an inhibitor of cyclooxygenase-1, attenuated the centrally administered histamine-induced elevation of plasma noradrenaline and adrenaline in rats (Shimizu et al., 2006). In the present experiment, therefore, we aimed to clarify which phospholipase is involved in the histamine-induced elevation of plasma catecholamines using 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), 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 papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for intracerebroventricular administration of test substances using stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of 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 histamine or the application of blocking reagents.

Histamine 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 histamine (or vehicle). Mepacrine and tricyclodecane-9-yl xanthate potassium salt (D609) dissolved in sterile saline were intracerebroventricularly (i.c.v.) administered in a volume of 5 μ l/animal using a 10- μ l Hamilton syringe. 1-[6-[[(17β) -3-Methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U-73122) and 1,6-bis(cyclohexyloximinocarbonylamino)hexane (RHC-80267) dissolved in 2.5 μ l of 100% *N,N*-dimethylformamide (DMF)/animal were i.c.v. administered using a 10- μ l Hamilton syringe. 1-*O*-Octadecylinin-2-*O*-methyl-*sn*-glycero-3-phosphorylcholine (ET-18-OCH₃) dissolved in 100% DMF was i.c.v. administered using a 10- μ l Hamilton syringe in a volume of 5 μ l/animal due to its low solubility in DMF. In the case of using blocking reagents, histamine was i.c.v. administered 30 min after application of U-73122, RHC-80267, ET-18-OCH₃ and D609, and 180 min after application of mepacrine due to their slightly elevating effects on the basal plasma levels of catecholamines. Each animal also received only one dose of blocking reagents (or vehicles).

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

2.2. Measurement of plasma catecholamines

Blood samples (250 μ l) were collected through an arterial catheter and were preserved on ice during experiments. Plasma were 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 μ l) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double 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 double deionized water. Then, catecholamines adsorbed onto the alumina were eluted with 300 μ 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 a Ag/AgCl reference electrode; column, Eicompac 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 EDTA dihydrate, 0.75 g/l sodium 1-

octanesulfonate and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 μ l. 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 were accurately determined.

2.3. Treatment of data and statistics

All values are expressed as the means $\pm$ S.E.M. The data were analyzed by repeated-measure analysis of variance, followed by post-hoc analysis with the Bonferroni method. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: mepacrine (quinacrine) dihydrochloride (Research Biochemicals, Natick, MA, USA); U-73122, RHC-80267 and D609 (Biomol Research Lab., Plymouth Meeting, PA, USA); ET-18-OCH₃ (Sigma Aldorich Fine Chemicals, St. Louis, MO, U.S.A.). Histamine dihydrochloride and all other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effect of mepacrine (an inhibitor of PLA₂) on the centrally administered histamine-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 noradrenaline and adrenaline (Fig. 1). Pretreatment with mepacrine [2.2 µmol (1000 µg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 1).

Since we previously reported that histamine [5.4, 27 and 54 nmol (1, 5 and 10 µg)/animal, i.c.v.] dose-dependently elevated plasma levels of both catecholamines (Shimizu et al., 2006), we used the dose of 27 nmol (5 µg)/animal in the present experiment. Administration of histamine [27 nmol (5 µg)/animal, i.c.v.] rapidly increased plasma levels of noradrenaline and adrenaline. These responses reached a maximum 10 min after administration of histamine and then declined toward their basal levels (Fig. 1). The histamine-induced elevation of both catecholamines was not influenced by mepacrine [1.1 and 2.2 µmol (500 and 1000 µg)/animal, i.c.v.] (Fig. 1).

3.2. Effect of U-73122 (an inhibitor of PLC) on the centrally administered histamine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (2.5 µl DMF/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 2). Pretreatment with U-73122 [100 nmol (46 µg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 2).

The histamine [27 nmol (5 µg)/animal, i.c.v.]-induced elevation of both catecholamines was attenuated by U-73122 in a dose-dependent manner [10 and 100 nmol (4.6 and 46 µg)/animal, i.c.v.] (Fig. 2).

3.3. Effects of ET-18-OCH₃ (an inhibitor of phosphatidylinositol-specific PLC) and D609 (an inhibitor of phosphatidylcholine-specific PLC) on the centrally administered histamine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (5 µl DMF or saline/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 3A and B). Pretreatment with ET-18-OCH₃ [30

nmol (15.7 µg)/animal, i.c.v.] and D609 [300 nmol (80 µg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 3A and B).

ET-18-OCH₃ [10 and 30 nmol (5.2 and 15.7 µg)/animal, i.c.v.] dose-dependently reduced the histamine [27 nmol (5 µg)/animal, i.c.v.]-induced elevation of plasma levels of noradrenaline and adrenaline (Fig. 3A). On the other hand, the histamine-induced response was not influenced by D609 [30, 100 and 300 nmol (8, 27 and 80 µg)/animal, i.c.v.] (Fig. 3B).

3.4. Effect of RHC-80267 (an inhibitor of diacylglycerol lipase) on the centrally administered histamine-induced elevation of plasma catecholamines

Treatments with vehicle-1 (2.5 µl DMF/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 4). Pretreatment with RHC-80267 [2.6 µmol (1000 µg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 4).

The histamine [27 nmol (5 µg)/animal, i.c.v.]-induced elevation of both catecholamines was attenuated by RHC-

80267 in a dose-dependent manner [1.3 and 2.6 μmol (500 and 1000 μg)/animal, i.c.v.] (Fig. 4).

4. Discussion

PLA₂ plays critical roles in the synthesis of arachidonic acid. To date, twelve groups of mammalian PLA₂ have been identified (Six and Dennis, 2000; Balsinde et al., 2002). They are classified into two major categories, secreted PLA₂ and cytosolic PLA₂. Mepacrine (quinacrine) is widely used as a nonselective inhibitor of PLA₂, thereby inhibiting both secreted PLA₂ and cytosolic PLA₂ (Fioretti et al., 2004; Hirose et al., 2007). Mepacrine has been shown to inhibit the release of arachidonic acid induced by *N*-methyl-D-aspartate at concentrations that inhibit PLA₂ activity, but not affect PLC activity, in primary culture of cerebellar granule cells (Lazarewicz et al., 1990). Mepacrine also blocks melittin (a PLA₂ activator)-stimulated prostaglandin E₂ release from renal cortex slices (Churchill et al., 1990). Previously we reported that central pretreatment with mepacrine (1.1 µmol/animal) abolished the centrally administered melittin-induced elevation of plasma noradrenaline and adrenaline in rats (Yokotani et al., 2000). In the present experiment, however, central pretreatment with a large dose of mepacrine (2.2 µmol/animal) was ineffective on the centrally administered histamine-induced elevation of plasma catecholamines, although the responses evoked by histamine was much smaller than those evoked by melittin. The result suggests the

involvement of the other phospholipase than PLA₂ in the histamine-induced elevation of plasma catecholamines in rats.

Arachidonic acid may also be released through PLC- and diacylglycerol lipase-mediated pathway. This pathway was reported to be a source of arachidonic acid in human platelets (Bell et al., 1979), murine macrophages (Moscat et al., 1986), rat pancreatic acini (Hou et al. 1996) and rabbit aortae (Tang et al., 2006). U-73122 has been shown to be a selective inhibitor of PLC in human platelets and polymorphonuclear neutrophils (Bleasdale et al., 1990; Smith et al., 1990) and to antagonize the hyperalgesia evoked by histamine H₁ receptor agonist [2-(3-trifluoromethylphenyl)histamine]-induced activation of brain PLC in mice (Galeotti et al., 2004). In the present experiment, central pretreatment with U-73122 effectively reduced the centrally administered histamine-induced elevation of plasma catecholamines. The result suggests the involvement of the brain PLC in the histamine-induced elevation of plasma catecholamines in rats.

There are two types of PLCs: phosphatidylinositol-specific PLC and phosphatidylcholine-specific PLC. Phosphatidylinositol-specific PLC hydrolyzes phosphatidylinositol phosphate(s) and phosphatidylcholine-specific PLC hydrolyzes phosphatidylcholine, thereby both generating diacylglycerol. The source of early production of diacylglycerol has been shown to be exclusively mediated

by phosphatidylinositol-specific PLC, while phosphatidylcholine is the source of the sustained production of diacylglycerol by phosphatidylcholine-specific PLC (Diaz-Meco et al., 1989; Exton, 1994; Sbrissa et al., 1998; Rebecchi and Pentyala, 2000). Histamine has been reported to contract cat duodenal smooth muscle cells by phosphatidylinositol-specific PLC-mediated mechanisms (Jeong et al., 2003), and also to evoke Ca^{2+} current in guinea-pig ileal smooth muscle cells by phosphatidylcholine-specific PLC-mediated mechanisms (Unno et al., 1998). Then, we examined which PLC is involved in the centrally administered histamine-induced elevation of plasma catecholamines using ET-18-OCH₃ and D609. ET-18-OCH₃ has been shown to selectively inhibit phosphatidylinositol-specific PLC with an IC₅₀ value of 9.6 μM in fibroblasts and adenocarcinoma cells (Powis et al., 1992). D609 selectively inhibits phosphatidylcholine-specific PLC with an IC₅₀ value of 94 μM in human monocytic cells (Schutze et al., 1992). In the present experiment, central pretreatment with ET-18-OCH₃ (30 nmol/animal) effectively reduced the centrally administered histamine-induced elevation of plasma catecholamines, while central pretreatment with D609 was even ineffective on the histamine-induced responses at doses up to 300 nmol/animal. These results suggest the involvement of the brain phosphatidylinositol-specific PLC

in the histamine-induced elevation of plasma catecholamines in rats.

Diacylglycerol can be hydrolyzed by diacylglycerol lipase to yield arachidonic acid. Diacylglycerol, produced by phosphatidylinositol-specific PLC from phosphatidylinositol phosphate(s), may function as an important cellular source of arachidonic acid. Tang et al. reported that the methacholine treatment of cultured rabbit aortic endothelial cells labeled with [^{14}C] arachidonic acid results in the release of free [^{14}C] arachidonic acid and in the decrease in radioactivity in phosphatidylinositol and diacylglycerol fractions of these cells (Tang et al., 2006). Furthermore, these responses induced by methacholine treatment were inhibited by U-73122 and RHC-80267, a selective inhibitor of diacylglycerol lipase (Levasseur et al., 1984; Natarajan et al., 1988). In the present experiment, central pretreatment with RHC-80267 effectively reduced the centrally administered histamine-induced elevation of plasma catecholamines, suggesting the involvement of brain diacylglycerol lipase in the histamine-induced elevation of plasma catecholamines in rats.

Phospholipase D (PLD) has also been reported to be involved in the production of arachidonic acid. PLD-derived phosphatidate is subsequently transformed by phosphatidate phosphohydrolase into diacylglycerol, which can be further converted to arachidonic acid (Hodgkin et al., 1998;

Carrasco and Merida, 2007). In fact, PLD-phosphatidate phosphohydrolase pathway was reported to be a source of diacylglycerol and arachidonic acid in rat osteoblast-like cells (Kaneki et al., 1998) and cultured vascular smooth muscle cells (Freeman, 2000). However, the major substrate of PLD is phosphatidylcholine, which contains relatively little arachidonic acid in position 2 (Exton, 1994; Hodgkin et al., 1998; Carrasco and Merida, 2007). On the other hand, 30-80% of total phosphatidylinositol is typically the *sn*-1-stearoyl-2-arachidonyl species (Hodgkin et al., 1998; Carrasco and Merida, 2007). Other phospholipids such as phosphatidylserine and phosphatidylethanolamine can function as a source of arachidonic acid, however these phospholipids are preferentially hydrolyzed by PLA₂ rather than PLC (Atsumi et al., 1997; Six and Dennis, 2000; Balsinde et al., 2002). These observations suggest that the brain phosphatidylinositol-specific PLC rather than PLA₂, phosphatidylcholine-specific PLC and PLD may metabolize mainly phosphatidylinositol phosphate(s) to diacylglycerol which functions as a source of arachidonic acid in response to the centrally administered histamine in rats.

Previously, we reported that the centrally administered histamine-induced elevation of plasma catecholamines was attenuated by central pretreatment with ketoprofen, an inhibitor of cyclooxygenase-1, and furegrelate, an inhibitor of thromboxane A₂ synthase, and also was abolished by bilateral adrenalectomy (Shimizu et al., 2006). These

results suggest that the brain arachidonic acid generated by histamine may be metabolized to thromboxane A₂ by brain cyclooxygenase-1 and thromboxane A₂ synthase, thereby evoking the secretion of noradrenaline and adrenaline from adrenal noradrenaline- and adrenaline-containing cells respectively in rats. The brain thromboxane A₂-mediated secretion of noradrenaline and adrenaline from adrenal medulla was also demonstrated by centrally administered arginine-vasopressin and bombesin in rats (Okada et al., 2003a; Yokotani et al., 2005).

In summary, we demonstrated here that the centrally administered histamine activates the central adrenomedullary outflow by brain phosphatidylinositol-specific PLC- and diacylglycerol lipase-dependent mechanisms, thereby increasing plasma levels of noradrenaline and adrenaline in rats.

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Legends to figures

Fig. 1. Effect of mepacrine (an inhibitor of PLA₂) on the centrally administered histamine-induced elevation of plasma catecholamines. Δ Noradrenaline and Δ Adrenaline: increments of noradrenaline and adrenaline above the basal. Mepacrine [1.1 and 2.2 μ mol (500 and 1000 μ g)/animal] or vehicle-1 (5 μ l saline/animal) was intracerebroventricularly (i.c.v.) administered 180 min before the administration of histamine [27 nmol (5 μ g)/animal, i.c.v.] or vehicle-2 (10 μ l saline/animal, i.c.v.). Arrows indicate the intracerebroventricular administrations of mepacrine/vehicle-1 and histamine/vehicle-2. Each point represents the mean $\pm$ S.E.M. The actual values for noradrenaline and adrenaline at 0 min were 431 $\pm$ 56 and 395 $\pm$ 77 pg/ml in the group pretreated with vehicle-1 (n=10); 432 $\pm$ 98 and 497 $\pm$ 197 pg/ml in the group pretreated with mepacrine (1.1 μ mol/animal) (n=5); 366 $\pm$ 60 and 474 $\pm$ 107 pg/ml in the group pretreated with mepacrine (2.2 μ mol/animal) (n=14), respectively.

Fig. 2. Effect of U-73122 (an inhibitor of PLC) on the centrally administered histamine-induced elevation of plasma catecholamines. U-73122 [10 and 100 nmol (4.6 and 46 μ g)/animal] or vehicle-1 (2.5 μ l DMF/animal) was i.c.v.

administered 30 min before the administration of histamine [27 nmol (5 µg)/animal, i.c.v.] or vehicle-2 (10 µl saline/animal, i.c.v.). * $p < 0.05$, significantly different from vehicle-1- and histamine-treated group. Other conditions were the same as those of Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 305±33 and 223±36 pg/ml in the group pretreated with vehicle-1 (n=11); 354±85 and 391±164 pg/ml in the group pretreated with U-73122 (10 nmol/animal) (n=6); 323±59 and 328±91 pg/ml in the group pretreated with U-73122 (100 nmol/animal) (n=11), respectively.

Fig. 3. Effects of ET-18-OCH₃ (an inhibitor of phosphatidylinositol-specific PLC) and D609 (an inhibitor of phosphatidylcholine-specific PLC) on the centrally administered histamine-induced elevation of plasma catecholamines. (A) ET-18-OCH₃ [10 and 30 nmol (5.2 and 15.7 µg)/animal] or vehicle-1 (5 µl DMF/animal) was i.c.v. administered 30 min before the administration of histamine [27 nmol (5 µg)/animal, i.c.v.] or vehicle-2 (10 µl saline/animal, i.c.v.). * $p < 0.05$, significantly different from vehicle-1- and histamine-treated group. Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 335±34 and 211±38 pg/ml in the group pretreated with DMF (5 µl/animal) (n=13); 308±62 and 238±38 pg/ml in the

group pretreated with ET-18-OCH₃ (10 nmol/animal) (n=6); 357±75 and 261±54 pg/ml in the group pretreated with ET-18-OCH₃ (30 nmol/animal) (n=10), respectively. (B) D609 [30, 100 and 300 nmol (8, 27 and 80 µg)/animal] or vehicle-1 (5 µl saline/animal) was i.c.v. administered 30 min before the administration of histamine [27 nmol (5 µg)/animal, i.c.v.] or vehicle-2 (10 µl saline/animal, i.c.v.). Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 370±60 and 350±87 pg/ml in the group pretreated with saline (5 µl/animal) (n=12); 397±144 and 319±173 pg/ml in the group pretreated with D609 (30 nmol/animal) (n=6); 338±60 and 360±104 pg/ml in the group pretreated with D609 (100 nmol/animal) (n=6); 380±66 and 377±64 pg/ml in the group pretreated with D609 (300 nmol/animal) (n=10), respectively.

Fig. 4. Effect of RHC-80267 (an inhibitor of diacylglycerol lipase) on the centrally administered histamine-induced elevation of plasma catecholamines. RHC-80267 [1.3 and 2.6 µmol (500 and 1000 µg)/animal] or vehicle-1 (2.5 µl DMF/animal) was i.c.v. administered 30 min before the administration of histamine [27 nmol (5 µg)/animal, i.c.v.] or vehicle-2 (10 µl saline/animal, i.c.v.). **p*<0.05, significantly different from vehicle-1- and histamine-

treated group. Vehicle-1-treated groups were cited from Fig. 2. Other conditions were the same as those of Figs. 1-3. The actual values for noradrenaline and adrenaline at 0 min were 213 ± 24 and 347 ± 80 pg/ml in the group pretreated with RHC-80267 ($1.3 \mu\text{mol/animal}$) ($n=6$); 214 ± 39 and 268 ± 102 pg/ml in the group pretreated with RHC-80267 ($2.6 \mu\text{mol/animal}$) ($n=13$), respectively.

Figure-1

Figure-2

Figure-3

Figure-4