ELSEVIER

European Journal of Pharmacology 398 (2000) 341–347

ejp

www.elsevier.nl/locate/ejphar

Brain phospholipase A_2-arachidonic acid cascade is involved in the activation of central sympatho-adrenomedullary outflow in rats

Kunihiko Yokotani *, Muchung Wang, Yoshinori Murakami, Shoshiro Okada, Masakazu Hirata

Department of Pharmacology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

Received 29 November 1999; received in revised form 27 March 2000; accepted 31 March 2000

Abstract

The present experiments were designed to explore the role of the brain phospholipase A_2-arachidonic acid cascade in the activation of central sympatho-adrenomedullary outflow in rats, using melittin (an activator of phospholipase A_2) and arachidonic acid. Intracerebroventricularly administered melittin (2.5, 10, and 25 μg/animal) or arachidonic acid (75, 150, 300 μg/animal) effectively and dose dependently elevated plasma levels of adrenaline and noradrenaline. The elevation of both catecholamines induced by melittin (10 μg/animal) was abolished by centrally administered mepacrine (an inhibitor of phospholipase A_2), but not by neomycin (an inhibitor of phospholipase C). However, mepacrine had no effect on the increase induced by arachidonic acid (150 μg/animal). Indomethacin (an inhibitor of cyclooxygenase) abolished all responses induced by melittin and arachidonic acid. Furegrelate (an inhibitor of thromboxane A_2 synthase) abolished the elevation of adrenaline induced by melittin and arachidonic acid, but had no effect on the elevation of noradrenaline induced by these compounds. These results suggest that activation of the brain phospholipase A_2-arachidonic acid cascade facilitates the central sympatho-adrenomedullary outflow in rats. Brain thromboxane A_2 is involved in the activation of central adrenomedullary outflow and an active metabolite of arachidonic acid other than thromboxane A_2 may be involved in activation of the central sympathetic outflow.

Keywords: Brain phospholipase A_2; Catecholamines, plasma; Sympatho-adrenomedullary outflow, central; Melittin; Mepacrine

1. Introduction

Phospholipase A_2 hydrolyzes the *sn*-2 ester bond of membrane phospholipids with the release of arachidonic acid (Flower and Blackwell, 1976; Irvine, 1982; Axelrod, 1990). In addition, phospholipase C cleaves the phosphodiester bond, resulting in the formation of a 1,2-diglyceride; arachidonic acid is then released from the diglyceride by the sequential actions of diglyceride lipase (Bell et al., 1979). A portion of the released arachidonic acid is metabolized rapidly to oxygenated products by several distinct enzyme systems, including cyclooxygenase, and the products of the arachidonic acid cascade may act as intracellular or intramembrane signaling molecules.

Previously we reported that intracerebroventricularly administered prostaglandin E_2 activates the central sympathetic outflow by activation of brain prostanoid EP_3 receptors in rats (Yokotani et al., 1988, 1995a, 1996). Furthermore, intracerebroventricularly administered interleukin-1β activates the central sympathetic outflow in rats and this response is attenuated by centrally administered indomethacin, an inhibitor of cyclooxygenase (Yokotani et al., 1995b; Murakami et al., 1996). Interleukin-1 has been shown to interact with phospholipase A_2 thereby increasing prostaglandin synthesis in rabbit chondrocytes (Chang et al.,1986; Kerr et al., 1989). This evidence suggests that the brain phospholipase A_2-arachidonic acid cascade plays a role in the activation of central sympathetic outflow.

The present study was, therefore, designed to further explore the relationship between brain phospholipase A_2-arachidonic acid cascade and central sympatho-adrenomedullary outflow in rats, using melittin. Melittin is a polypeptide component of bee venom which activates phospholipase A_2, thereby increasing arachidonic acid release and prostaglandin synthesis (Hassid and Levine, 1977; Argiolas and Pisano, 1983; Churchill et al., 1990).

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

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00276-4

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 350 g were maintained in a 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 paper (Yokotani et al., 1995a).

Three hours after the animal was 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). Melittin and arachidonic acid were dissolved in sterile saline and slowly injected into the lateral ventricle in a volume of 10 μl using a 50-μl Hamilton syringe. Mepacrine, neomycin and indomethacin–Na were also dissolved in sterile saline and administered into the right lateral ventricle 60 min before the application of melittin or arachidonic acid.

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 (Yokotani et al., 1995a). 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 an 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 5 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-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 rate of 0.22 ml/min. The amount of

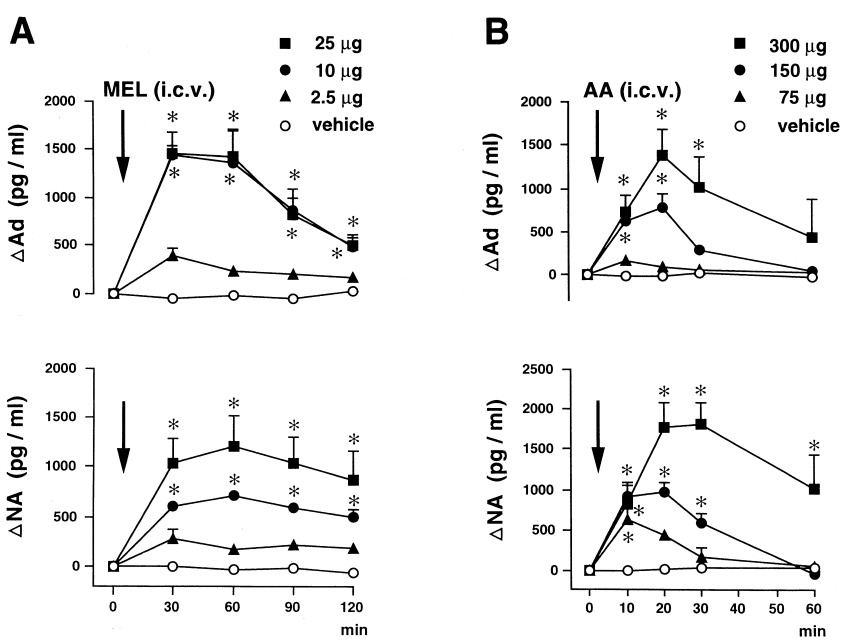

Fig. 1. Effects of melittin and arachidonic acid on plasma levels of adrenaline and noradrenaline. Arrow indicates intracerebroventricular (i.c.v.) administration of melittin (MEL) (2.5, 10 and 25 μg/animal) in (A) and arachidonic acid (AA) (75, 150 and 300 μg/animal) in (B). ΔAd and ΔNA, increase of adrenaline (Ad) and noradrenaline (NA) above basal. A: vehicle (saline 10 μl/animal)($n = 5$), 2.5 μg MEL ($n = 4$), 10 μg MEL ($n = 5$) (cited from Fig. 2A); 25 μg MEL ($n = 5$). B: vehicle ($n = 5$), 75 μg AA ($n = 4$), 150 μg AA ($n = 5$) (cited from Fig. 2B), 300 μg AA ($n = 5$). Each point represents the mean ± S.E.M. * Significantly different ($p < 0.05$) from vehicle-treated control. The actual values for Ad and NA at 0 min were 159.8 ± 28.8 and 306.2 ± 26.9 pg/ml ($n = 19$) in (A), 182.7 ± 17.8 and 314.1 ± 19.7 pg/ml ($n = 19$) in (B), respectively.

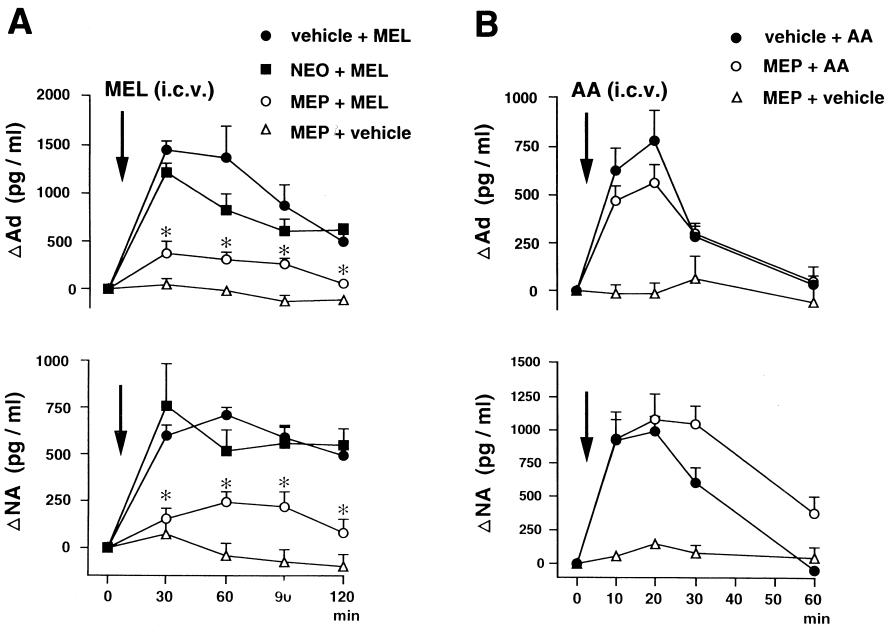

Fig. 2. Effects of mepacrine and neomycin on the melittin- or arachidonic acid-induced elevation of plasma adrenaline (Ad) and noradrenaline (NA). Mepacrine (MEP) (500 μg/animal, i.c.v.) or neomycin (NEO) (500 μg/animal, i.c.v.) was administered 60 min before the administration of melittin (MEL) (10 μg/animal, i.c.v.) or arachidonic acid (AA) (150 μg/animal, i.c.v.). (A) MEP + vehicle ($n = 5$), vehicle + MEL ($n = 5$), MEP + MEL ($n = 5$), NEO + MEL ($n = 4$). (B) MEP + vehicle ($n = 4$), vehicle + AA ($n = 5$), MEP + AA ($n = 5$). * Significantly different ($p < 0.05$) from animals treated with vehicle + MEL in (A) or with vehicle + AA in (B). Other conditions were the same as those in Fig. 1. The actual values for Ad and NA at 0 min were 261.2 ± 54.9 and 488.9 ± 48.1 pg/ml ($n = 10$) in the MEP-pretreated group, 485.5 ± 197.2 and 510.3 ± 46.8 pg/ml ($n = 4$) in the NEO-pretreated group in (A), 200.4 ± 33.3 and 348.4 ± 42.0 pg/ml ($n = 9$) in the MEP-pretreated group in (B), respectively.

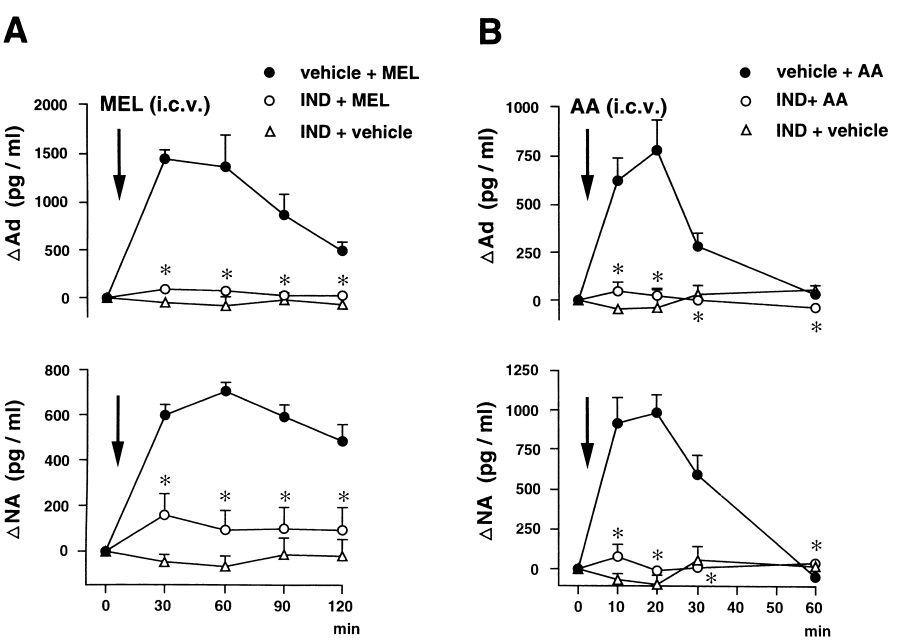

Fig. 3. Effect of indomethacin on the melittin- or arachidonic acid-induced elevation of plasma adrenaline (Ad) and noradrenaline (NA). Indomethacin (IND) (500 μg/animal, i.c.v.) was administered 60 min before the administration of melittin (MEL)(10 μg/animal, i.c.v.) or arachidonic acid (AA) (150 μg/animal, i.c.v.). (A) IND + vehicle ($n = 4$), vehicle + MEL ($n = 5$) (cited from Fig. 2A), IND + MEL ($n = 5$). (B) IND + vehicle ($n = 4$), vehicle + AA ($n = 5$) (cited from Fig. 2B), IND + AA ($n = 5$). * Significantly different ($p < 0.05$) from animals treated with vehicle + MEL in (A) or with vehicle + AA in (B). Other conditions were the same as those in Figs. 1 and 2. The actual values for Ad and NA at 0 min in IND-pretreated group were 198.0 ± 35.5 and 367.2 ± 35.3 pg/ml ($n = 10$) in (A), 185.5 ± 25.8 and 318.1 ± 47.4 pg/ml ($n = 9$) in (B), respectively.

catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine, an internal standard. This assay method could accurately determine 0.5 pg of adrenaline and noradrenaline.

2.3. Treatment of data and statistics

All values are expressed as the means ± S.E.M. The data were analyzed by repeated-measure ANOVA using Statview 4.0 (Abacus Concept, CA, USA), followed by post hoc analysis with the Bonferroni method for comparing a control to all other means (Fig. 1). When only two means were compared, an unpaired Student's *t*-test was used (Figs. 2–4). *P* values less than 0.05 were taken to be significant.

2.4. Compounds

The following drugs were used: arachidonic acid sodium and neomycin sulfate (Sigma, St. Louis, MO, USA); furegrelate sodium (Biomol Research Lab., Plymouth Meeting, PA, USA); water-soluble indomethacin sodium trihydrate (Merck, Rahway, NJ, USA); synthetic melittin and mepacrine (quinacrine dihydrochloride) (Research Biochemicals, Natik, MA, USA). All other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of melittin and arachidonic acid on plasma catecholamines

Intracerebroventricularly (i.c.v.) administered vehicle (10 μl saline/animal) and blood sampling five times over a 120-min period in (A) or a 60-min period in (B) did not affect the basal plasma levels of either adrenaline or noradrenaline (Fig. 1A and B).

Administration of melittin (2.5, 10, and 25 μg/animal, i.c.v.) dose dependently elevated plasma levels of adrenaline and noradrenaline (Fig. 1A). The plasma levels of adrenaline and noradrenaline reached a maximum 30–60 min after the administration of melittin and then gradually declined toward the basal level.

Administration of arachidonic acid (75, 150, 300 μg/animal, i.c.v.) rapidly and dose dependently elevated the plasma levels of adrenaline and noradrenaline (Fig. 1B). The levels of both catecholamines reached a maximum 20–30 min after the administration of arachidonic acid and then declined toward the basal level.

Intravenous administration of melittin (10 μg/animal) or arachidonic acid (150 μg/animal) had no effect on plasma catecholamine levels (data not shown).

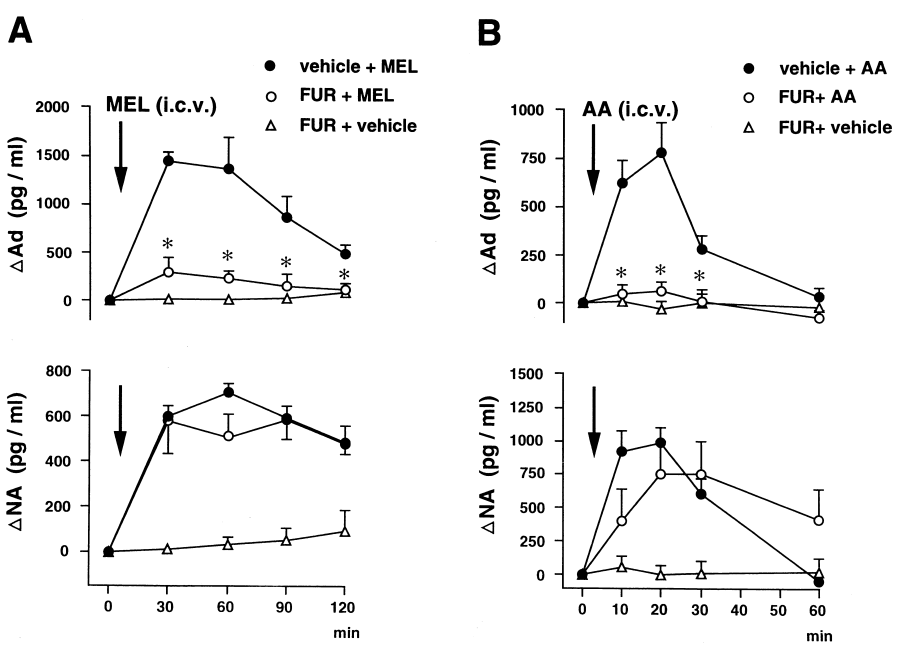

Fig. 4. Effect of furegrelate on the melittin- or arachidonic acid-induced elevation of plasma adrenaline (Ad) and noradrenaline (NA). Furegrelate (FUR) (500 μg/animal, i.c.v.) was administered 60 min before the administration of melittin (MEL) (10 μg/animal, i.c.v.) or arachidonic acid (AA) (150 μg/animal, i.c.v.). (A) FUR + vehicle ($n = 4$), vehicle + MEL ($n = 5$) (cited from Fig. 2A), FUL + MEL ($n = 4$). (B) FUR + vehicle ($n = 4$), vehicle + AA ($n = 5$) (cited from Fig. 2B), FUR + AA ($n = 5$). * Significantly different ($p < 0.05$) from animals treated with vehicle + MEL in (A) or with vehicle + AA in (B). Other conditions were the same as those in Figs. 1–3. The actual values for Ad and NA at 0 min in FUR-pretreated group were 108.6 ± 13.2 and 448.5 ± 39.7 pg/ml ($n = 8$) in (A), 169.0 ± 20.6 and 470.2 ± 67.2 pg/ml ($n = 9$) in (B), respectively.

3.2. Effects of mepacrine and neomycin on the melittin- or arachidonic acid-induced elevation of plasma catecholamines

Administration of mepacrine (500 μg/animal, i.c.v.) did not affect the basal plasma levels of either catecholamine, but neomycin (500 μg/animal, i.c.v.) slightly elevated the basal level of adrenaline (Fig. 2A and B).

Mepacrine effectively reduced the melittin (10 μg/animal, i.c.v.)-induced elevation of plasma adrenaline and noradrenaline, but neomycin had no effect on the melittin-induced elevation of both catecholamines (Fig. 2A). The levels of adrenaline and noradrenaline at 30 min were 375.9 ± 123.6 and 152.4 ± 54.3 pg/ml in the mepacrine-plus melittin-treated group ($n = 5$) and 1210.9 ± 105.3 and 760.9 ± 246.8 pg/ml in the neomycin-plus melittin-treated group ($n = 4$). The former values were significantly different from those for the vehicle-plus melittin-treated group (1444.7 ± 90.4 and 599.3 ± 53.0 pg/ml, $n = 5$) (Fig. 2A).

On the other hand, mepacrine (500 μg/animal, i.c.v.) had no effect on the increase in plasma catecholamines induced by arachidonic acid (150 μg/animal, i.c.v.) (Fig. 2B).

3.3. Effect of indomethacin on the melittin- or arachidonic acid-induced elevation of plasma catecholamines

Administration of indomethacin (500 μg/animal, i.c.v.) had no effect on the basal plasma levels of catecholamines (Fig. 3A and B).

Indomethacin completely abolished the elevation of both catecholamines induced by melittin (10 μg/animal, i.c.v.) (Fig. 3A). The levels of adrenaline and noradrenaline at 30 min were 91.2 ± 28.4 and 161.3 ± 92.4 pg/ml in the indomethacin-plus melittin-treated group ($n = 5$). These values were significantly different from those for the vehicle- plus melittin-treated group (1444.7 ± 90.4 and 599.3 ± 53.0 pg/ml, $n = 5$).

Indomethacin also completely abolished the elevation of both catecholamines induced by arachidonic acid (150 μg/animal, i.c.v.) (Fig. 3B). The levels of adrenaline and noradrenaline at 20 min were 25.5 ± 35.6 and -2.8 ± 35.3 pg/ml in the indomethacin-plus arachidonic acid-treated group ($n = 5$). These values were significantly different from those for the vehicle-plus arachidonic acid-treated group (780.4 ± 155.2 and 985.3 ± 111.6 pg/ml, $n = 5$) (Fig. 3B).

3.4. Effect of furegrelate on the melittin- or arachidonic acid-induced elevation of plasma catecholamines

Administration of furegrelate (500 μg/animal, i.c.v.) had no effect on the basal plasma levels of catecholamines (Fig. 4A and B).

Furegrelate completely abolished the elevation of adrenaline, but not that of noradrenaline, induced by melittin (10 μg/animal, i.c.v.) (Fig. 4A). The level of adrenaline at 30 min was 298.7 ± 152.8 pg/ml in the furegrelate-plus melittin-treated group ($n = 4$). This value was significantly different from that for the vehicle-plus melittin-treated group (1444.7 ± 90.4 pg/ml, $n = 5$).

Furegrelate also completely abolished the elevation of adrenaline, but not that of noradrenaline, induced by arachidonic acid (150 μg/animal, i.c.v.) (Fig. 4B). The levels of adrenaline at 20 min were 65.6 ± 47.8 pg/ml in the furegrelate- plus arachidonic acid-treated group ($n = 5$). This values was significantly different from that for the vehicle- plus melittin-treated group (780.4 ± 155.2 pg/ml, $n = 5$).

4. Discussion

Melittin activates phospholipase A_2 by its interaction with the membrane bound phospholipid side chains (Nishiya, 1991), which results in accelerated lipid hydrolysis of the phospholipase A_2-phospholipid complex and thereby increases arachidonic acid release. However, melittin has also been shown to stimulate the breakdown of phosphoinositides by activation of phospholipase C (Fletcher et al., 1991; Choi et al., 1992). In the present study, centrally administered melittin and arachidonic acid effectively elevated plasma levels of catecholamines. The melittin-induced response was not influenced by neomycin, an inhibitor of phospholipase C, but was abolished by mepacrine, which inhibits phospholipase A_2 activity, thereby inhibiting the production of arachidonic acid (Hirata et al., 1979; Vallee et al., 1979; Vigo et al., 1980; Hofmann et al., 1982). In addition, the arachidonic acid-induced elevation of both catecholamines was not influenced by mepacrine. These results suggest that the activation of brain phospholipase A_2 facilitates the central sympathoadrenomedullary outflow by the release of arachidonic acid. The ability of mepacrine to block the melittin-induced effect is consistent with a previous finding that mepacrine blocks melittin-stimulated prostaglandin E_2 release in renal cortex slices (Churchill et al., 1990).

Arachidonic acid itself has been implicated in physiological processes such as modulation of ion channels and regulation of the activity of many enzymes such as protein kinase A, protein kinase C and NADPH oxidase (Katsuki and Okuda, 1995). The reactions of the prostaglandin biosynthesis from arachidonic acid are catalyzed by cyclooxygenase, which is inhibited by indomethacin. In the present study, the centrally administered arachidonic acid-induced elevation of plasma catecholamines was attenuated by indomethacin. The elevation of plasma catecholamines induced by melittin was also attenuated by this reagent. From these results, it seems likely that cyclooxygenase-generated active metabolites of arachidonic acid

may be involved in the melittin-and arachidonic acid-induced facilitation of central sympatho-adrenomedullary outflow.

The elevation of plasma adrenaline levels caused by centrally administered melittin or arachidonic acid was abolished by indomethacin and also by furegrelate, an inhibitor of thromboxane A_2 synthase. Recently, we reported that central thromboxane A_2 may be involved in facilitation of the central adrenomedullary outflow, because the centrally administered nitric oxide donor (3-morpholino-sydnonimine)-induced elevation of plasma adrenaline was abolished by centrally administered thromboxane A_2 synthase inhibitors or thromboxane A_2 receptor antagonists in rats (Murakami et al., 1998). The present results suggest that central thromboxane A_2 is involved in the melittin-and arachidonic acid-induced facilitation of central adrenomedullary outflow.

The elevation of plasma noradrenaline levels caused by centrally administered melittin or arachidonic acid was also attenuated by indomethacin, but was not influenced by furegrelate. Therefore, it seems likely that the activation of central sympathetic outflow evoked by these reagents is mediated by an active metabolite of arachidonic acid other than thromboxane A_2. Feuerstein et al. (1982) have already shown that prostaglandin E_2 injected into the lateral cerebral ventricle of the rat increases plasma levels of catecholamines, especially noradrenaline. We also reported that centrally administered prostaglandin E_2 facilitates central sympathetic outflow by activation of brain prostanoid EP_3 receptors in rats (Yokotani et al., 1988, 1995a, 1996). From this evidence, it seems likely that the activation of central sympathetic outflow caused by melittin or arachidonic acid is mediated by central prostaglandin E_2-mediated mechanisms.

The central nervous system appears to contain multiple phospholipases A_2 (Farooqui et al., 1997b). Six phospholipase A_2 isoforms have been cloned in the rat: low molecular weight secreted forms of phospholipase A_2 (types IB, IIA, IIC and V); Ca^{2+}-dependent high-molecular weight phospholipase A_2 (type IV); Ca^{2+}-independent high molecular weight phospholipase A_2 (type VI). Types IIC, V and VI are expressed in the rat hypothalamus (Molloy et al., 1998), which is considered to be the center for central sympatho-adrenomedulary outflow (Swanson and Sawchenko, 1983). Although the precise role of phospholipase A_2 in neural function is not fully understood, there is a growing body of evidence suggesting the involvement of these enzymes in neurotransmitter release (Moskowitz et al., 1982), in addition to the following, e.g., long-term potentiation (Williams et al., 1989), membrane repair (Nakamura, 1993) and neurodegeneration during ischemia (Edgar et al., 1982). Furthermore, several studies have recently implicated phospholipase A_2 in a variety of psychiatric diseases such as Alzheimer's disease (Stephenson et al., 1996) and schizophrenia (Gattaz et al., 1995; Farooqui et al., 1997a). The multiplicity of phospholipases A_2 may provide for diversity in function and for specificity in the regulation of enzyme activity in response to a wide range of extracellular signals. Further studies are required to resolve the question of which type of brain phospholipase A_2 may be involved in the activation of the central sympatho-adrenomedullary outflow.

In summary, we demonstrated here that the activation of the brain phospholipase A_2-arachidonic acid cascade facilitates the central sympatho-adrenomedullary outflow in rats. Brain thromboxane A_2 is involved in the activation of central adrenomedullary outflow. An active metabolite of arachidonic acid other than thromboxane A_2 (probably prostaglandin E_2) may be involved in the activation of central sympathetic outflow.

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.

Argiolas, A., Pisano, J.J., 1983. Facilitation of phospholipase A_2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. J. Biol. Chem. 258, 13697–13702.

Axelrod, J., 1990. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. Biochem. Soc. Trans. 18, 503–507.

Bell, R.L., Kennerly, D.A., Stanford, N., Majerus, P.W., 1979. Diglyceride lipase: a pathway for arachidonate release from human platelets. Proc. Natl. Acad. Sci. U. S. A. 76, 3238–3241.

Chang, J., Gilman, S.C., Lewis, A.J., 1986. Interleukin 1 activates phospholipase A_2 in rabbit chondrocytes: a possible signal for IL 1 action. J. Immunol. 136, 1283–1287.

Choi, O.H., Padgett, W.L., Daly, J.W., 1992. Effects of the amphiphilic peptides melittin and mastoparan on calcium influx, phosphoinositide breakdown and arachidonic acid release in rat pheochromocytoma PC12 cells. J. Pharmacol. Exp. Ther. 260, 369–375.

Churchill, P.C., Rossi, N.F., Churchill, M.C., Ellis, V.R., 1990. Effect of melittin on renin and prostaglandin E_2 release from rat renal cortical slices. J. Physiol. 428, 233–241.

Edgar, A.D., Strosznajder, J., Horrocks, L.A., 1982. Activation of ethanolamine phospholipase A_2 in Brain during ischemia. J. Neurochem. 39, 1111–1116.

Farooqui, A.A., Rapoport, S.I., Horrocks, L.A., 1997a. Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. Neurochem. Res. 22, 523–527.

Farooqui, A.A., Yang, H.C., Rosenberger, T.A., Horrocks, L.A., 1997b. Phospholipase A_2 and its role in brain tissue. J. Neurochem. 69, 889–901.

Feuerstein, G., Adelberg, S.A., Kopin, I.J., Jacobowitz, D.M., 1982. Hypothalamic sites for cardiovascular and sympathetic modulation by prostaglandin E_2. Brain Res. 231, 335–342.

Fletcher, J.E., Jiang, M.S., Gong, Q.H., Smith, L.A., 1991. Snake venom cardiotoxins and bee venom melittin activate phospholipase C activity in primary cultures of skeletal muscle. Biochem. Cell Biol. 69, 274–281.

Flower, R.J., Blackwell, G.J., 1976. The importance of phospholipase-A_2 in prostaglandin biosynthesis. Biochem. Pharmacol. 25, 285–291.

Gattaz, W.F., Schmitt, A., Maras, A., 1995. Increased platelet phospholipase A_2 activity in schizophrenia. Schizophr. Res. 16, 1–6.

Hassid, A., Levine, L., 1977. Stimulation of phospholipase activity and prostaglandin biosynthesis by melittin in cell culture and in vivo. Res. Commun. Chem. Pathol. Pharmacol. 18, 507–517.

Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Schiffmann, E.,

Axelrod, J., 1979. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. Proc. Natl. Acad. Sci. U. S. A. 76, 2640–2643.

Hofmann, S.L., Prescott, S.M., Majerus, P.W., 1982. The effects of mepacrine and *p*-bromophenacyl bromide on arachidonic acid release in human platelets. Arch. Biochem. Biophys. 215, 237–244.

Irvine, R.F., 1982. How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. 204, 3–16.

Katsuki, H., Okuda, S., 1995. Arachidonic acid as a neurotoxic and neurotrophic substance. Prog. Neurobiol. 46, 607–636.

Kerr, J.S., Stevens, T.M., Davis, G.L., McLaughlin, J.A., Harris, R.R., 1989. Effects of recombinant interleukin-1 beta on phospholipase A_2 activity, phospholipase A_2 mRNA levels, and eicosanoid formation in rabbit chondrocytes. Biochem. Biophys. Res. Commun. 165, 1079–1084.

Molloy, G.Y., Rattray, M., Williams, R.J., 1998. Genes encoding multiple forms of phospholipase A_2 are expressed in rat brain. Neurosci. Lett. 258, 139–142.

Moskowitz, N., Schook, W., Puszkin, S., 1982. Interaction of brain synaptic vesicles induced by endogenous Ca^{2+}-dependent phospholipase A_2. Science 216, 305–307.

Murakami, Y., Yokotani, K., Okuma, Y., Osumi, Y., 1996. Nitric oxide mediates central activation of sympathetic outflow induced by interleukin-1 beta in rats. Eur. J. Pharmacol. 317, 61–66.

Murakami, Y., Yokotani, K., Okuma, Y., Osumi, Y., 1998. Thromboxane A_2 is involved in the nitric oxide-induced central activation of adrenomedullary outflow in rats. Neuroscience 87, 197–205.

Nakamura, S., 1993. Involvement of phospholipase A_2 in axonal regeneration of brain noradrenergic neurones. NeuroReport 4, 371–374.

Nishiya, T., 1991. Interaction of melittin and phospholipase A_2 with azobenzene-containing phospholipid. J. Biochem. 109, 383–388.

Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinates. In: Paxinos, G., Watson, C. (Eds.), Academic Press, Boston.

Stephenson, D.T., Lemere, C.A., Selkoe, D.J., Clemens, J.A., 1996. Cytosolic phospholipase A_2 ($cPLA_2$) immunoreactivity is elevated in Alzheimer's disease brain. Neurobiol. Dis. 3, 51–63.

Swanson, L.W., Sawchenko, P.E., 1983. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci. 6, 269–324.

Vallee, E., Gougat, J., Navarro, J., Delahayes, J.F., 1979. Anti-inflammatory and platelet anti-aggregant activity of phospholipase-A_2 inhibitors. J. Pharm. Pharmacol. 31, 588–592.

Vigo, C., Lewis, G.P., Piper, P.J., 1980. Mechanisms of inhibition of phospholipase A_2. Biochem. Pharmacol. 29, 623–627.

Williams, J.H., Errington, M.L., Lynch, M.A., Bliss, T.V., 1989. Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. Nature 341, 739–742.

Yokotani, K., Yokotani, K., Okuma, Y., Osumi, Y., 1988. Sympathoadrenomedullary system mediation of the prostaglandin E_2-induced central inhibition of gastric acid output in rats. J. Pharmacol. Exp. Ther. 244, 335–340.

Yokotani, K., Nishihara, M., Murakami, Y., Hasegawa, T., Okuma, Y., Osumi, Y., 1995a. 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., Okuma, Y., Osumi, Y., 1995b. Recombinant interleukin-1β inhibits gastric acid secretion by activation of central sympatho-adrenomedullary outflow in rats. Eur. J. Pharmacol. 279, 233–239.

Yokotani, K., Okuma, Y., Osumi, Y., 1996. Inhibition of vagally mediated gastric acid secretion by activation of central prostanoid EP_3 receptors in urethane-anaesthetized rats. Br. J. Pharmacol. 117, 653–656.