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Arachidonic Acid Inhibits Norepinephrine Release through Blocking of Voltage-sensitive Ca^{2+} Channels in PC12 Cells

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We studied the mechanism of arachidonic acid on the secretion of a neurotransmitter in rat pheochromocytoma PC12 cells. Arachidonic acid inhibited the 70 mM K^+-induced secretion of norepinephrine. Arachidonic acid also inhibited the 70 mM K^+-induced Ca^{2+} mobilization which is due to the opening of the voltage-sensitive Ca^{2+} channels (VSCC). Both the half maximal inhibitory concentration (IC_{50}) of the norepinephrine secretion and VSCC coincided at 30 μM. The major oxidized metabolites of arachidonic acid, prostaglandins did not mimic the inhibitory effect of arachidonic acid. Nordihydroguaiaretic acid (NDGA) and indomethacin which are inhibitors of lipoxygenase and cyclooxygenase, respectively, did not block the inhibitory effect of arachidonic acid. The results suggest that arachidonic acid serves as a signal itself, not in the form of metabolites. The pretreatment of various K^+ channel blockers such as 4-aminopyridine, tetraethylammonium, glipizide, or glibenclamide also did not show any effect on the inhibitory effect of arachidonic acid. Through these results we suggest that arachidonic acid regulates VSCC directly and affects the secretion of neurotransmitters.

Arachidonic acid is one of the polyunsaturated fatty acids in the plasma membranes. Arachidonic acid itself and its oxidized metabolites, serve as an important signal in various tissues. The sources of arachidonic acid include phospholipase A_2 (PLA_2)-mediated phospholipid hydrolysis, lysosomal hydrolysis of low-density lipoprotein (LDL) and modification of diacylglycerol which is produced by phospholipase C (PLC) or phospholipase D (PLD) (Burgoyne and Morgan, 1990).

The physiological actions of arachidonic acid have been studied in many tissues. The most interesting activity among the reported action of arachidonic acid is the regulation of ion channel activity. Arachidonic acid is known to have the following effect: inhibition of K^+ channel (Wang et al., 1992; Damron et al., 1993), inhibition of delayed-rectifier K^+ channel (Honore et al., 1994), inhibition of ATP-sensitive K^+ channels, activation of ATP-insensitive K^+ channels in rat atrial cells (Kim and Clapham, 1989; Kim and Duff, 1990), or activation of K^+ channel (Ordway et al., 1989). Arachidonic acid inhibits voltage-sensitive Ca^{2+} channels (VSCC) (Shimada and Somlyo, 1992; Keyser and Alger, 1990; Damron and Dorman, 1993) and Cl^- channels (Anderson and Welsh, 1990; Hwang et al., 1990). Fraser et al. reported that arachidonic acid inhibits Na^+ channels and GABA release (1993).

Though there has been many reports on arachidonic acid, the cellular action mechanism of arachidonic acid is still controversial (Wang et al., 1992). It has been reported that arachidonic acid modulates protein kinase A (PKA) , protein kinase C (PKC) (Bell and Burns, 1991; Danesch et al., 1994), NADPH oxidase (Sakata et al., 1987), and the GTPase-activating protein (Han et al., 1991). The change in the lipid environment of the ion channel, the inhibition of PKA-induced phosphorylation, and the obstruction of the channel inner mouth are also suggested as possible mechanisms.

The rat pheochromocytoma cell, PC12 has been a good model cell line for the study of the neuronal system. We observed that arachidonic acid inhibits the secretion of norepinephrine and Ca^{2+} increase induced by the opening of VSCC in PC12 cells. We focused on the arachidonic acid-induced inhibition of norepinephrine release, and tried to describe the effect and the mechanism of action.

Materials and Methods

Materials

Arachidonic acid, prostaglandin E_2 (PGE_2), PGD_2, PGF_2, and PGI_2, indomethacin, nordihydroguaiaretic acid (NDGA), nifedipine, 4-aminopyridine, tetraethyl-

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ammonium, cAMP, and sulfinpyrazone were purchased from Sigma. Glipizide, glibenclamide, and ω-conotoxin GVIA were obtained from Research Biochemical International. Fura-2/AM was obtained from Molecular Probes. [^{3}H]Norepinephrine and [^{3}H]adenosine were purchased from NEN. RPMI 1640 and penicillin/streptomycin were purchased from GIBCO. Bovine calf serum and horse serum were obtained from HyClone.

Cell Culture

Rat pheochromocytoma PC12 cells, were grown in RPMI 1640 supplemented with 10% (v/v) heat-inactivated bovine calf serum, 5% (v/v) heat-inactivated horse serum, and 1% (v/v) penicillin/ streptomycin in a humidified atmosphere of 5% CO_2 at 37℃. The culture medium was changed every two days and the cells were subcultured weekly.

Measurement of [^{3}H]norepinephrine secretion

Catecholamine secretion from PC12 cells was measured in 24 well plates following the method reported by Suh and Kim (1994). In brief, [^{3}H] norepinephrine (1 μCi/ml) was loaded by incubating cells with RPMI 1640 for 1 h at 37℃ in 5% CO_2/95% air. The cells were washed two times and then incubated in Locke's solution (154 mM NaCl, 5.6 mM KCl, 5.6 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES buffer adjusted to pH 7.4) for 15 min to stabilize the cells. Then the cells were incubated again with fresh Locke's solution for 15 min to measure basal secretion. The cells were subsequently stimulated with the drugs under test for 15 min. The medium was removed from each well and residual catecholamines were extracted from the cells by adding 10% trichloroacetic acid. The radioactivity was measured with a scintillation counter. The amount of [^{3}H] norepinephrine secreted was calculated as percentage of total [^{3}H] norepinephrine content.

[Ca^{2+}]$_i$ Measurement

[Ca^{2+}]$_i$ was determined using the fluorescent Ca^{2+} indicator fura-2 as reported previously (Choi and Kim, 1996). Briefly, the PC12 cell suspension was incubated in fresh serum-free RPMI 1640 medium with 3 μM fura-2/AM for 50 min at 37℃ under continuous stirring. The loaded cells were then washed twice with Locke's solution. Sulfinpyrazone (250 μM) was added to all solutions to prevent dye leakage. For the fluorimetric measurement of [Ca^{2+}]$_i$, 1×10^6 cells/ml were placed into a quartz cuvette in the thermostatically controlled cell holder at 37℃ and continuously stirred. Fluorescence ratios were monitored with dual excitation at 340 and 380 nm and emission at 500 nm. Calibration of the fluorescent signal in terms of [Ca^{2+}]$_i$ was performed as described by Grynkiewicz *et al.* (1985) using the following equation

$$[Ca^{2+}]_i = K_D[(R - R_{min})/(R_{max} - R)](S_{f2}/S_{b2})$$

where R is the ratio of fluorescence emitted by excitation at 340 and 380 nm. S_{f2} and S_{b2} are the proportionality coefficients at 380 nm excitation of Ca^{2+}-free fura-2 and Ca^{2+}-saturated fura-2, respectively. In order to obtain R_{min}, the fluorescence ratios of the cell suspension were measured successively at a final concentration of 4 mM EGTA, 30 mM Trizma base, and 0.1% Triton X-100. Then the cell suspension was treated with $CaCl_2$ for a final concentration of 4 mM Ca^{2+} and the fluorescence ratios were measured to obtain R_{max}.

Determination of intracellular cyclic AMP

Cyclic AMP generation in intact cells was determined by measuring the formation of [^{3}H] cAMP from [^{3}H] adenine nucleotide pools as described previously (Park and Kim, 1996). Briefly, cells were loaded with [^{3}H] adenine (2 μCi/ml) in complete medium for 24 h. After the loading, the cells were washed two times with Locke's buffer solution. 1×10^6 cells were aliquoted in Eppendorf tube for stimulation. The stimulation reaction was stopped by adding 1 ml ice-cold 5% (v/v) TCA containing 1 μM non-labeled cAMP. The tubes were left on ice for 30 min to extract the water soluble components including cAMP and ATP. Tubes were centrifuged at 15,000 × g for 15 min to precipitate the cell debris. [^{3}H] cAMP and [^{3}H] ATP were separated by using sequential chromatography on Dowex AG50W-X4 (200-400 mesh) cation exchanger and neutral alumina column. [^{3}H] ATP fraction was obtained by elution with 2 ml of distilled water from Dowex column and then the sequential elution with 3.5 ml distilled water was loaded to the alumina column. The alumina column was eluted by 4 ml imidazole buffer solution (100 mM, pH 7.2) into scintillation vials containing 15 ml scintillation fluid to count the [^{3}H] cAMP. Increase of intracellular cAMP concentration was presented as [^{3}H] cAMP/([^{3}H] ATP + [^{3}H] cAMP) × 10^3.

Analysis of data

All quantitative data are expressed as mean ± SEM. The results were analyzed for differences using the one-way ANOVA. We calculated IC_{50} by the AllFit for Windows (De Lean et al., 1978). Differences were considered to be significant only for $P < 0.05$.

Results

To test the physiological role of arachidonic acid on the exocytotic process, PC12 cells were treated with

arachidonic acid and norepinephrine secretion was measured. Arachidonic acid reduced the 70 mM K^+-induced norepinephrine secretion in a concentration-dependent manner (Fig. 1). The half maximal inhibitory concentration (IC_{50}) was 30 μM.

In addition, arachidonic acid also inhibited the activity of VSCC in PC12 cells. As shown in Fig. 2, arachidonic acid reduced the membrane depolarization-induced increase in cytosolic Ca^{2+} level which was triggered by the treatment of 70 mM K^+. The effect of arachidonic acid once again shows a concentration-dependent manner (Fig. 2B). Arachidonic acid shows slow and sustained increase in $[Ca^{2+}]_i$ at above 300 μM, which might be due to the nonspecific perturbation of the membrane structure (data not shown). The IC_{50} was 30 μM which is well coincided to the results of norepinephrine secretion.

L-type and N-type VSCC are mainly expressed in PC12 cells (Park and Kim, 1996). We tested the subtype of VSCC which is the inhibitory target of the arachidonic acid signaling with nifedipine and ω-conotoxin GVIA which are the specific antagonists for L-type and N-type VSCC, respectively. We, however, found that arachidonic acid inhibits both types of VSCC (data not shown).

Arachidonic acid can be a substrate for cyclooxygenase (converted to prostaglandins and thromboxanes) and lipoxygenase (converted to leukotrienes). We tested the possibility that the arachidonic acid effect is mediated by the downstream product prostaglandins. Pretreatment with 10 μM PGE_2 did not influence the 70 mM K^+-induced $[Ca^{2+}]_i$ increase (Fig. 3). We treated other types of prostaglandins, PGD_2, PGF_2, and PGI_2, and did not detect any inhibitory activity (data not shown).

We also tested whether arachidonic acid acts through the generation of another second messenger, the cAMP. However, arachidonic acid and PGE_2 did not produce cAMP in PC12 cells, whereas forskolin, an activator of adenylyl cyclase, successfully increased cytosolic cAMP level (Fig. 4). The data suggest that there is no active receptor for the prostaglandins.

To confirm the action of the metabolized form of arachidonic acid, we tested the effect of NDGA and indomethacin, which are the inhibitors of lipoxygenase and cyclooxygenase, respectively. Because auto-

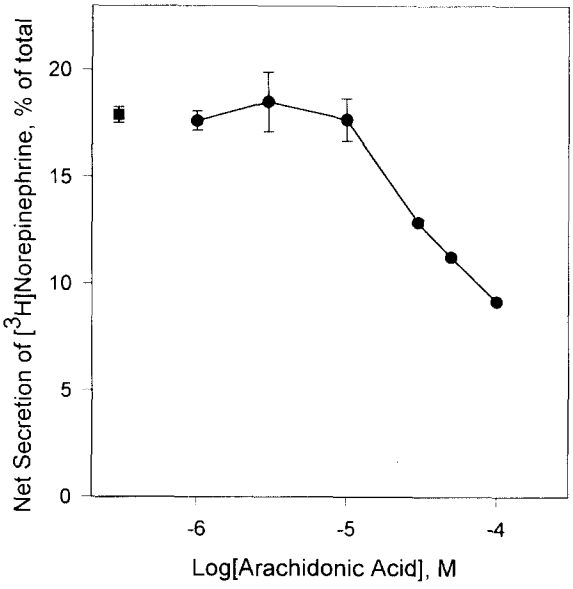

Fig. 1. Effect of arachidonic acid on 70 mM K^+-induced [^{3}H] norepinephrine secretion in PC12 cells. Cells were preincubated with indicated concentrations of arachidonic acid for 15 min to [^{3}H] norepinephrine-loaded cells and challenged with 70 mM K^+ (circle). The 70 mM K^+-induced [^{3}H] norepinephrine secretion in the absence of arachidonic acid also indicated (square). The amount of secreted [^{3}H] norepinephrine was measured as described in Materials and Methods and is presented as percentage of total radioactivity of cells. The experiments were performed three times independently and the results were reproducible. Each point was obtained from triplicate experiments and is shown as mean + SEM.

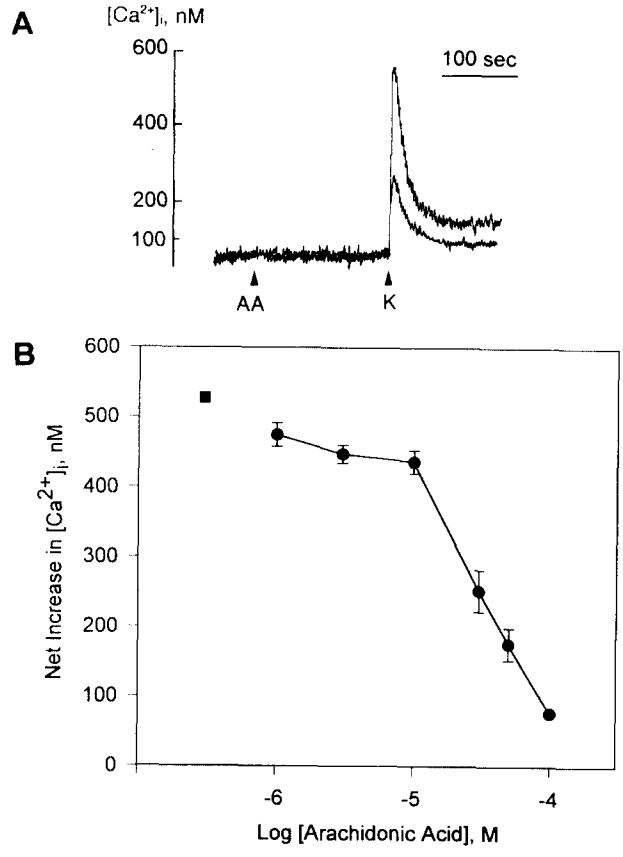

Fig. 2. Effect of arachidonic acid on 70 mM K^+-induced $[Ca^{2+}]_i$ rise in PC12 cells. A, Fura- 2/AM loaded PC12 cells were pretreated with (lower trace) or without (upper trace) 50 μM arachidonic acid, then challenged with 70 mM K^+. Typical Ca^{2+} transients are presented. B, Arachidonic acid inhibits 70 mM K^+-induced $[Ca^{2+}]_i$ rise in a concentration-dependent manner. PC12 cells were treated with various concentrations of arachidonic acid for 3 min and then stimulated with 70 mM K^+ (circle). The 70 mM K^+-induced $[Ca^{2+}]_i$ increase without arachidonic acid treatment was also indicated (square). The quantity of $[Ca^{2+}]_i$ was calibrated as described in Materials and Methods. The peak height of each stimulation was compared with the control $[Ca^{2+}]_i$ rise evoked by 70 mM K^+ in the absence of arachidonic acid. The experiments were performed four times independently and the results were reproducible. Each point is the mean + SEM.

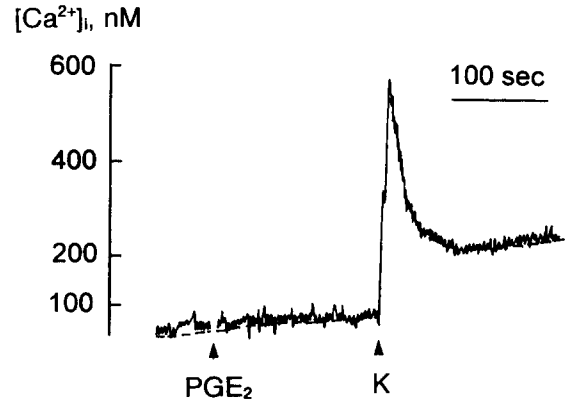

Fig. 3. Effect of PGE_2 on 70 mM K^+-induced $[Ca^{2+}]_i$ rise in PC12 cells. Fura-2/AM-loaded PC12 cells were pretreated with (dotted trace) or without (continuous trace) 10 μM PGE_2, then challenged with 70 mM K^+ (K). Typical Ca^{2+} transients from more than four separate experiments are presented.

fluorescence of these agents distort the fluorescence of fura-2, we could not confirm the effect with the $[Ca^{2+}]_i$ levels. So we examined the possibility with norepinephrine secretion (Fig. 5). Indomethacin did not affect the inhibitory effect of arachidonic acid. NDGA itself partially decreased the 70 mM K^+-induced secretion. However, the pretreatment with NDGA did not block the additional inhibitory effect of arachidonic acid. In addition, PGE_2 did not show the regulatory effect on the 70 mM K^+-induced norepinephrine secretion.

Many reports showed that arachidonic acid regulates several kinds of K^+ channels (Kim and

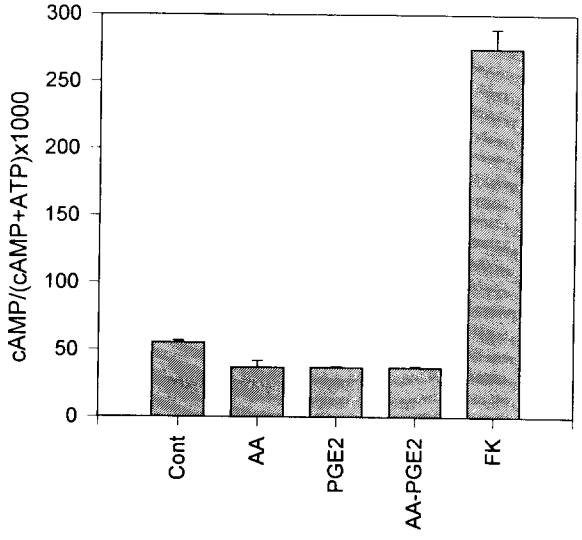

Fig. 4. PGE_2, arachidonic acid, and forskolin-induced cAMP production in PC12 cells. [^{3}H] Adenine-loaded PC12 cells were preincubated with 1 mM IBMX for 15 min, then treated with 10 μM PGE_2, 50 μM arachidonic acid, or 1 μM forskolin for 15 min in the presence of 1 mM IBMX. The experiments were performed three times independently and the results were reproducible. Each results are the mean + SEM from triplicate assays.

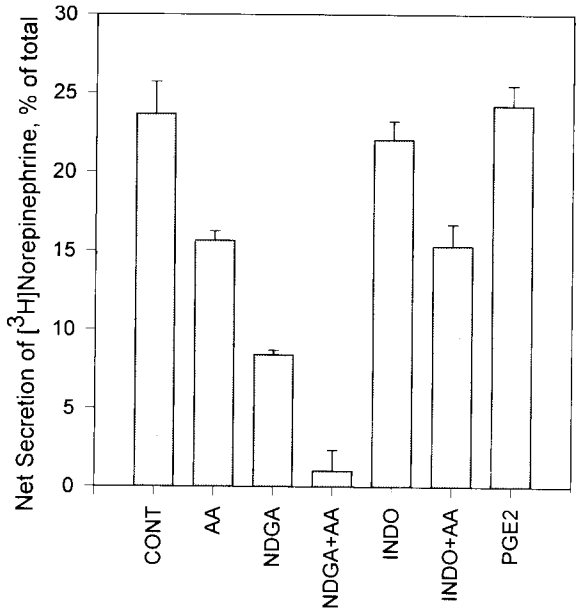

Fig. 5. Effects of NDGA and indomethacin on inhibitory effect of arachidonic acid. [^{3}H] norepinephrine-loaded cells were preincubated with 50 μM arachidonic acid (AA) with or without 50 μM NDGA or 50 μM indomethacin (INDO) for 15 min, then challenged with 70 mM K^+. 10 μM PGE_2 was also tested as arachidonic acid. The amount of secreted [^{3}H] norepinephrine is presented as the percentage of total radioactivity of cells. Each point was obtained from triplicate experiments and is shown as mean + SEM. The experiments were performed three times independently.

Clapham, 1989; Wang et al., 1992; Damron etal., 1993; Honore et al., 1994). We tested whether arachidonic acid activates K^+ channels, because the activation of K^+ channel could inhibit VSCC. Pretreatment with the inhibitors for K^+ channel, 4-aminopyridine (1 mM), tetraethylammonium (1 mM) did not block the arachidonic acid-induced VSCC inhibition (Fig. 6). Pretreatment with the inhibitors for K_{ATP} channel, glipizide (10 μM) and glibenclamide (1 μM), also did not block the arachidonic acid-induced inhibition of VSCC.

Discussion

Arachidonic acid is a major component of membrane lipids. Metabolites of arachidonic acid, have a variety of biological effects and can also act as second messengers. Low concentrations of arachidonic acid and other fatty acids are present in the extracellular space conditions, and consequently they could be important regulators of normal cellular functions.

Though Fraser et al. (1993) reported that arachidonic acid inhibits GABA release, the mechanism of arachidonic acid has not been elucidated clearly. We studied the arachidonic acid effect on the norepinephrine secretion in PC12 cells.

Until now, the signaling of arachidonic acid has been classified in two categories. One is the meta-

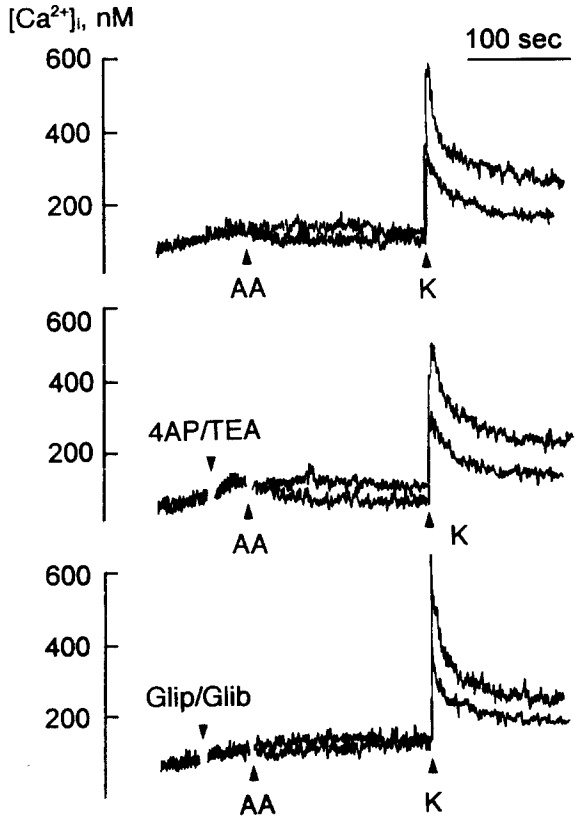

Fig. 6. Involvement of K^+ channels on the action of arachidonic acid. (Up) Fura-2/AM-loaded PC12 cells were pretreated with 50 μM arachidonic acid then challenged with 70 mM K^+ without inhibitor treatments. Cells were pretreated with (lower trace) or without (upper trace) 50 μM arachidonic acid and both traces are depicted. (Middle) 1 mM 4-aminopyridine and 1 mM tetraethylammonium were pretreated (4AP/TEA) with arachidonic acid (AA) and treated with 70 mM K^+ (K). (Down) 10 μM glipizide and 1 μM glibenclamide (Glip/Glib) were pretreated with arachidonic acid (AA) and treated with 70 mM K^+ (K). The expriments were performed in the same batch of the cells to compare the inhibition. Typical Ca^{2+} transients from more than four separate experiments are presented.

bolites of arachidonic acid, such as prostaglandin, thromboxane and leukotriene (Needleman et al., 1986). Arachidonic acid differs from other fatty acids in that it is a substrate for cyclooxygenase and lipoxygenase. Arachidonic acid is transformed to prostaglandin, leukotriene, and thromboxane with the help of these enzymes. In this case, the production of arachidonic acid is the rate-determining step of production of prostaglandins or leukotrienes. Since the products are the agonists for prostanoid and leukotriene receptors, they are involved in the activation of PLC or adenylyl cyclase, subsequent activation of PKC or PKA. A lot of the studies which report the action mechanism of arachidonic acid show prostanoid-, or leukotriene-dependent mechanisms.

However, we did not find any positive evidence for the involvement of metabolites of the arachidonic acid cascade in the inhibitory effect of norpenephrine secretion in PC12 cells. Our result using NDGA and indomethacin could be served as a negative evidence; there is no regulatory effect of indomethacin and NDGA on the action of arachidonic acid. Korn and Horn (1990) reported that NDGA itself inhibits VSCC by a mechanism distinct from other Ca^{2+} channel antagonists or arachidonic acid metabolism. Though NDGA inhibited the VSCC- induced secretion, it could not reverse the effect of arachidonic acid.

Furthermore, PGE_2 did not affect the increase in $[Ca^{2+}]_i$ through VSCC and showed no detectable increase in cytosolic cAMP. The results suggest that there is no active prostanoid receptor for the metabolites of arachidonic acid. Because it is reported that an increased cAMP level inhibits VSCC in PC12 cells (Park and Kim, 1996) and PGE_2 is a good stimulant for adenylyl cyclase in many types of cells (Needleman et al., 1986), the existence of adenylyl cyclase-linked prostanoid receptor might explain the mechanism of arachidonic acid signaling. However, our results indicate that the metabolite of arachidonic acid does not act as a negative regulator for VSCC.

It is possible that arachidonic acid may directly inhibit the channel activity (Ordway et al., 1991). In this case, arachidonic acid does not produce any detectable second messengers such as cAMP or Ca^{2+}. The mechanism of direct action of arachidonic acid is not fully understood. Arachidonic acid has been suggested as a modifier of cellular surface receptors or channels, and a regulator of membrane fluidity. In this case, the target of arachidonic acid could vary. Arachidonic acid could regulate VSCC directly, or it could regulate K^+ channels; the decrease of calcium influx could be due to the activation of K^+ channels in the central nervous systems (Trussell and Jackson, 1987).

We tested the possibility of K^+ channel involvement in the arachidonic acid-induced inhibition of Ca^{2+} channels in PC12 cells. In the presence of two blockers of K^+ channels (4-aminopyridine and tetraethylammonium), the inhibition of VSCC by arachidonic acid was not affected (Fig. 6), suggesting that the inhibition of Ca^{2+} channels may not be due to the activation of K^+ channels. In addition, it has been suggested that K_{ATP} channels cause a hyperpolarization which reduces the probability of opening VSCC, leading to the relaxation of smooth muscle (Quast, 1993). Our result, which tested the involvement of K_{ATP} channels using glipizide and glibenclamide as blockers of the K_{ATP} channels, shows that VSCC was not influenced by the presence of these blockers. The results suggest that K_{ATP} channels are not regulated by arachidonic acid in PC12 cells.

It is widely accepted that VSCC is an important component in the propagation of neuronal signals. The involvement of VSCC is clearly shown in the neurotransmitter release. The physiological relevance of our study exists in the elucidation of cellular

mechanisms of arachidonic acid-induced inhibition of neurotransmitter release through blocking of VSCC. It will be tested by the experiments using the patch clamp technique, which serves as a direct evidence supporting our hypothesis. Because many extracellular signaling molecules activate PLA_2 then produce arachidonic acid, it is suggested that the inhibitory activity of arachidonic acid on VSCC could be one of the important regulatory mechanisms of neurotransmission in the neuronal system.

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References

Anderson MP and Welsh KJ (1990) Fatty acid inhibits apical membrane chloride channels in airway epithelia. *Proc Natl Acad Sci USA* 87: 7334-7338.

Bell RM and Burns DJ (1991) Lipid activation of protein kinase C. *J Biol Chem* 266: 4661- 4664.

Burgoyne RD and Morgan A (1990) The control of free arachidonic acid levels. *Trend Biochem Sci* 15: 365-366.

Choi SY and Kim KT (1996) Characterization of Na^+ influx mediated by ATP^{4-}-activated P_2 purinoceptor in PC12 cells. *Br J Pharmacol* 118: 935-940.

Damron DS and Dorman RV (1993) Involvement of phospholipase A_2 and arachidonic acid in the depolarization-evoked accumulation of Ca^{2+} in hippocampal mossy fiber nerve endings. *Neurochem Res* 18: 1231-1237.

Damron DS, Van Wagoner DR, Moravec CS, and Bond M. (1993) Arachidonic acid and endothelin potentiate Ca^{2+} transient in rat cardiac myocytes via inhibition of distinct K^+ channels. *J Biol Chem* 268: 27224-27344.

Damron DS and Bond M (1993) Modulation of Ca^{2+} cycling in cardiac myocytes by arachidonic acid. *Circ Res* 72: 376-386.

Danesch U, Weber PC, and Sellmayer A (1994) Arachidonic acid increases c-fos and Egr-1 mRNA in 3T3 fibroblasts by formation of prostaglandin E_2 and activation of protein kinase C. *J Biol Chem* 269: 27258-27263.

De Lean A, Munson PJ and Rodbard D (1978) Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* 235: E97-E102.

Fraser DD, Hoehn K, Weiss S, and MacVicar BA (1993) Arachidonic acid inhibits sodium currents and synaptic transmittion in cultured striatal neurons. *Neuron* 11: 633-644.

Grynkiewicz G, Poenie M, and Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260: 3440-3450.

Han JW, McCormick F, and Macara IG (1991) Regulation of Ras-GAP and the neurofibromatosis-a gene product by eicosanoids. *Science* 252: 576-579.

Honore E, Barhanin J, Attali B, Lesage F, and Lazdunski M (1994) External blockade of the major cardiac delayed-rectifier K^+ channel (Kv1.5) by polyunsaturated fatty acids. *Proc Natl Acad Sci USA* 91: 1937-1944.

Huang JMC, Xian H, and Bacaner M (1992) Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci USA* 89: 6452-6456.

Hwang TC, Guggino SE, and Guggino WB (1990) Direct modulation of secretory chloride channels by arachidonic acid and other cis-unsaturated fatty acids. *Proc Natl Acad Sci USA* 87: 5706-5709.

Karnovsky MJ, Kleinfeld AM, Hoover RL, and Klausner RD (1982) The concept of lipid domains in membranes. *J Cell Biol* 94: 1-6.

Keyser DO and Alger BE (1990) Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals. *Neuron* 5: 545-553.

Khurana G and Bennett MR (1993) Nitric oxide and arachidonic acid modulation of calcium currents in post-ganglionic neurones of avian cultured ciliary ganglia. *Br J Pharmacol* 109: 480-485.

Kim D and Clapham DE (1989) Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* 244: 1174-1178.

Kim D and Duff RA (1990) Regulation of K^+ channels in cardiac myocytes by free fatty acids. *Circ Res* 67: 1040-1046.

Korn SJ and Horn R (1990) Nordihydroguaiaretic acid inhibits its voltage-activated Ca^{2+} currents independently of lipoxygenase inhibition. *Mol Pharmacol* 38: 524-530.

Klausner RD, Kleinfeld AM, Hoover RL, and Karnovsky MJ (1980) Lipid domains in membranes. *J Biol Chem* 255: 1286-1295.

Needleman P, Turk J, Jakschik BA, Morrison AR, and Lefkowith JB (1986) Arachidonic acid metabolism. *Annu Rev Biochem* 55: 69-102.

Ordway RW, Walsh JV, and Singer JJ (1989) Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* 244: 1176-1179.

Ordway RW, Singer JJ, and Walsh JV (1991) Direct regulation of ion channels by fatty acids. *Trends Neurosci* 14: 96-100.

Park TJ and Kim KT (1996) Cyclic AMP-independent inhibition of voltage-sensitive calcium channels by forskolin in PC12 cells. *J Neurochem* 66: 83-88.

Quast U (1993) Do the K^+ channel openers relax smooth muscle by opening K^+ channels? *Trends Pharmacol Sci* 14: 332-337.

Sakata A, Ida E, Tominaga M, and Onoeu K (1987) Arachidonic acid acts as an intracellular activator of NADPH-oxidase in Fc receptor-mediated superoxide generation in macrophages. *J Immunol* 138: 4353-4359.

Shimada T and Somlyo AP (1992) Modulation of voltage-dependent Ca^{2+} channel current by arachidonic acid and other long-chain fatty acids in rabbit intestinal smooth muscle. *J Gen Physiol* 100: 27-44.

Suh BC and Kim KT (1994) Inhibition by ethaverine of catecholamine secretion through blocking of L-type Ca^{2+} channel in PC12 cells. *Biochem Pharmacol* 47: 1262-1266.

Trussell LO and Jackson MB (1987) Dependence of an adenosine-activated potassium current on a GTP-binding protein in mammalian central neurons. *J Neurosci* 7: 3306-3316.

Wang W, Cassola A, and Giebisch G (1992) Arachidonic acid inhibits the secretory K^+ channel of cortical collecting duct of rat kidney. *Am J Physiol* 262: F554-F559.

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