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EFFECTS OF INHIBITORS OF ARACHIDONIC ACID METABOLISM ON CALCIUM UPTAKE AND CATECHOLAMINE RELEASE IN CULTURED ADRENAL CHROMAFFIN CELLS

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Abstract—The possibility that arachidonic acid metabolism is involved in the secretory process in cultured adrenal chromaffin cells was investigated by studying the effects of lipoxygenase inhibitors and cyclooxygenase inhibitors on $^{45}Ca^{2+}$ uptake and catecholamine release. Lipoxygenase inhibitors, which have different chemical structures, such as nordihydroguaiaretic acid (NDGA), 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline (BW755C) and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) all prevented the catecholamine release evoked by carbamylcholine and high K^+. In contrast, cyclooxygenase inhibitors, such as aspirin and indomethacin failed to inhibit the carbamylcholine-evoked catecholamine release. Lipoxygenase inhibitors also inhibited $^{45}Ca^{2+}$ uptake into the cells stimulated by carbamylcholine and high K^+. Lipoxygenase inhibitors inhibited $^{45}Ca^{2+}$ uptake and catecholamine release with similar potency. Slightly higher concentrations of lipoxygenase inhibitors were required to inhibit high K^+-evoked effects compared to those evoked by carbamylcholine. The inhibitory effects of these inhibitors on carbamylcholine-evoked catecholamine release was different in its nature from the inhibitory effect of verapamil, a blocker of the Ca^{2+} channel, and was not due to a competitive antagonism at cholinergic receptor site. Moreover, these lipoxygenase inhibitors did not inhibit the binding of [^{3}H]nitrendipine to chromaffin cell homogenate. The data suggest that lipoxygenase inhibitors prevent the catecholamine release from cultured adrenal chromaffin cells by blocking Ca^{2+} uptake. It might be possible that lipoxygenase product(s) is involved in the Ca^{2+} translocation system in these cells.

Secretion of catecholamine from adrenal medullary chromaffin cells is Ca^{2+} dependent and is initiated by an increase in the cellular Ca^{2+} uptake [1, 2]. Recently, it has been reported that phospholipase A_2 (phosphatide 2-acylhydrolase, EC 3.1.1.4) inhibitors prevent the catecholamine release and cellular Ca^{2+} uptake in these cells [3–5]. If nicotinic agonists or some secretagogues really stimulate phospholipase A_2, lysophosphatides and arachidonic acid would be formed from membrane phospholipids. In general, released arachidonic acid is enzymatically oxidized through two major pathways. The cyclooxygenase pathway leads to production of prostaglandin and thromboxanes. The lipoxygenase pathway oxidizes arachidonic acid to hydroxy fatty acids or to leukotrienes. Inhibitors of arachidonic acid metabolism prevent the secretion of hormones or lysosomal enzyme release, suggesting that arachidonic acid metabolism may play a significant role in the secretory process of certain cell systems [6–13]. Recently, we reported that lipoxygenase products may play important roles in the secretory mechanism of insulin from isolated pancreatic islets [9–11, 13]. Therefore, in this report, we have investigated the effects of inhibitors of arachidonic acid metabolism on Ca^{2+} uptake and catecholamine release in cultured adrenal chromaffin cells.

MATERIALS AND METHODS

Materials. The following materials were obtained from the companies indicated: $^{45}CaCl_2$ (32 Ci/g) and [^{3}H]nitrendipine (72.5 Ci/mmole), New England Nuclear, Boston, Massachusetts; carbamylcholine chloride, NDGA,† indomethacin, fluorodeoxyuridine, cytosine arabinoside and uridine, Sigma Chemical Company, St. Louis, Missouri; AA861, Takeda Chemical Industries Ltd., Osaka, Japan; BW755C, The Wellcome Research Laboratories, Beckenham, Kent, U.K.; nitrendipine and nifedipine, Yamanouchi Pharm. Co., Ltd., Tokyo, Japan; Dulbecco's Modified Eagle's Medium, mycostatin and fetal calf serum, Gibco, Grant Island, New York; penicillin G, Banyu Pharmaceutical Co., Ltd., Tokyo, Japan; streptomycin sulfate, Meijiseika, Kaisha, Ltd., Tokyo, Japan; gentamicin sulfate, Schering Co., U.S.A., Kenilworth, New Jersey.

Primary culture of adrenal chromaffin cells. Fresh bovine adrenal glands were obtained from a local slaughterhouse, and chromaffin cells were isolated

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† Abbreviations: NDGA, nordihydroguaiaretic acid; AA861, 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl) 1-4,-benzoquinone; BW755C, 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline; Tris, tris(hydroxymethyl)aminomethane; 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; leukotriene B_4, 5,12,-dihydroxy-6,8,10,14-eicosatetraenoic acid; leukotriene C_4, 5-hydroxy-6-gulutathionyl-7,9,11,14-eicosatetraenoic acid; leukotriene D_4, 5-hydroxy-6-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid.

by treatment with 0.025% collagenase as described by Kumakura *et al.* [14]. The purified chromaffin cells were cultured by the method described by Kilpatrick *et al.* [15] with slight modification. The cells were plated on a 16 mm diameter well of a 4-well plastic multidish and 270 ml plastic culture flask at a concentration of 3–6 × 10^5 cells per ml in Dulbecco's modified Eagle's Medium, supplemented with 10% fetal calf serum, and cultured at 37° in an atmosphere of 95% air/5% CO_2. The culture medium contained the following antibiotics; penicillin G (100 units/ml), streptomycin sulfate (100 μg/ml), gentamicin sulfate (40 μg/ml) and mycostatin (25 units/ml). The medium also contained fluorodeoxyuridine (10 μM), cytosine arabinoside (10 μM) and uridine (5 μM) to prevent the proliferation of non-neuronal cells, and was replaced every 3–4 days. Cell viability estimated by a trypan blue exclusion test was above 95% after 10 days of culture. The cells were used for experiments between 4 and 10 days of culture.

Catecholamine release. Cultured cells were first washed with 95% O_2 and 5% CO_2 saturated Krebs–Ringer bicarbonate glucose buffer (pH 7.2–7.4) of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.2, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11 containing 0.5% bovine serum albumin, and were incubated with 1 ml of the same buffer. To determine the effects of inhibitors and some drugs the cells were preincubated at 37° for 10 min with different concentrations of each drug. After preincubation, carbamylcholine (final concentration: 0.3 mM) or KCl (final concentration: 56 mM) was added to the medium and the incubation was continued for another 3 min to measure catecholamine release. Cellular catecholamines (norepinephrine plus epinephrine) and released catecholamines (norepinephrine plus epinephrine) in the medium were extracted with 0.4 N perchloric acid and analyzed by a high performance liquid chromatography (Waters Assoc., Milford, Massachusetts) equipped with an electrochemical detector (Bioanalytical Systems Inc., W. Lafayette, Indiana) [16].

$^{45}Ca^{2+}$ uptake. After preincubation of the cells in 0.45 ml Krebs–Ringer bicarbonate glucose buffer in the presence and absence of inhibitors at 37° for 10 min, 0.05 ml buffer containing $^{45}CaCl_2$ (1 μCi/well) and each secretagogue was added to each well. After 1 min, the radioactive medium was removed to stop the uptake, and the cells were immediately washed four times with the ice-cold buffer. The cells were solubilized in 1% Triton X-100 solution in water for 30 min and the radioactivity was determined.

[^{3}H]Nitrendipine binding. The cultured chromaffin cells were collected by centrifugation at 1000 r.p.m. for 5 min. They were washed twice with a phosphate buffered saline containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.3 mM KH_2PO_4. Washed cells were suspended in 50 mM Tris–HCl buffer (pH 7.5) and were homogenized twice with a sonicator for 20 sec in ice. The homogenate was centrifuged at 600 *g* for 5 min and the resultant supernatant (0.27 mg protein/ml) was incubated with 2 nM [^{3}H]nitrendipine in total vol. 1 ml at 37° for 45 min. Incubation was terminated by the addition of 5 ml ice cold buffer followed by rapid filtration onto Whatman GF/B glass fiber filters and three additional 5 ml washes. Radioactivity trapped on the filters was counted in 10 ml of ACS aqueous scintillation mixture (Amersham) after 24 hr at room temperature. Unlabeled nitrendipine (20 μM) was used to define nonspecific binding. All procedures were done in the dark because of the extreme light-sensitivity of the dihydropyridines.

RESULTS

Figure 1A shows the effects of various concentrations of inhibitors on catecholamine (norepinephrine plus epinephrine) release from cultured adrenal chromaffin cells. When the cells were incubated with carbamylcholine (0.3 mM) for 3 min, the amount of released catecholamines was 19.5 ± 0.7% of the total cellular catecholamine content. When the cells were pretreated with lipoxygenase inhibitors, i.e. NDGA, AA861 and BW755C, for 10 min, the carbamylcholine-evoked catecholamine release was inhibited in a concentration-dependent manner. The inhibitory potency of lipoxygenase inhibitors on carbamylcholine-evoked catecholamine release is in the following order: AA861 > NDGA > BW755C and the apparent IC_{50} values for the inhibition were 10 μM, 20 μM and 100 μM respectively. In contrast, cyclooxygenase inhibitors, aspirin and indomethacin, failed to inhibit the carbamylcholine-evoked catecholamine release. These inhibitors showed no effect on the spontaneous catecholamine release. Figure 1B shows the effects of lipoxygenase and cyclooxygenase inhibitors on $^{45}Ca^{2+}$ uptake of cultured adrenal chromaffin cells. Lipoxygenase inhibitors, NDGA, BW755C and AA861, all inhibited the carbamylcholine-evoked $^{45}Ca^{2+}$ uptake in a concentration-dependent manner. In contrast, cyclooxygenase inhibitors, aspirin and indomethacin, did not prevent the carbamylcholine-evoked $^{45}Ca^{2+}$ uptake. These inhibitors showed no effect on basal $^{45}Ca^{2+}$ uptake of chromaffin cells. The potency of these inhibitors on $^{45}Ca^{2+}$ uptake and catecholamine release were similar.

Figure 2 shows the actions of lipoxygenase inhibitors and verapamil on carbamylcholine-evoked catecholamine release in the presence of various concentrations of extracellular Ca^{2+}. The inhibitory effect of verapamil (3 μM), which is a well-known blocker of Ca^{2+} channel [17], was overcome by an increase in extracellular Ca^{2+} concentration, while the inhibitory effects of lipoxygenase inhibitors, i.e. NDGA (10 μM), BW755C (50 μM), AA861 (10 μM), were not overcome by Ca^{2+}. These results suggest that the inhibitory effect of verapamil is competitively antagonized by Ca^{2+} but those of lipoxygenase inhibitors are not antagonized by Ca^{2+}.

In order to investigate the type of inhibition caused by lipoxygenase inhibitors, effects of carbamylcholine concentrations on the inhibitory effects of lipoxygenase inhibitors were examined. As shown in Fig. 3, the inhibitory effects of lipoxygenase inhibitors were not overcome by an increase in carbamylcholine concentration, suggesting that the inhibitory effects of these lipoxygenase inhibitors were not due to competitive antagonism at the cholinergic receptor site.

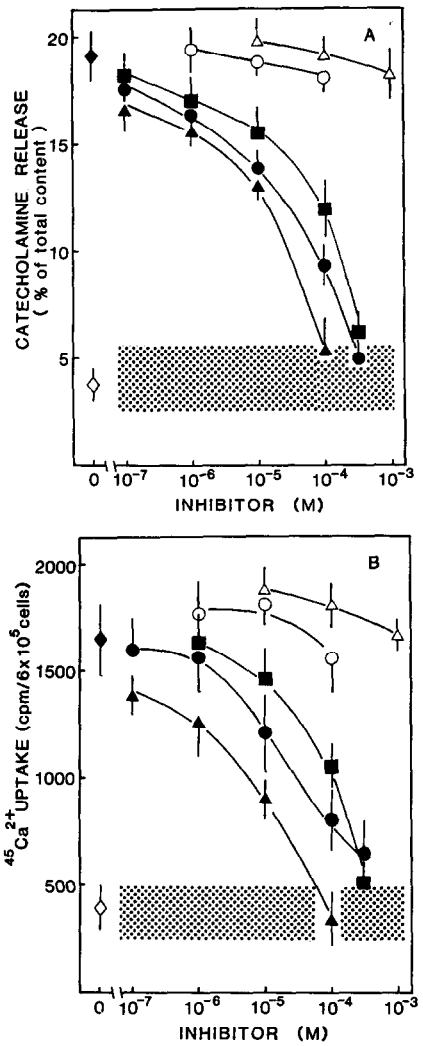

Fig. 1. Effects of lipoxygenase and cyclooxygenase inhibitors on carbamylcholine-evoked catecholamine release (A) and $^{45}Ca^{2+}$ uptake (B) in cultured adrenal chromaffin cells. (A) Cells were preincubated with various concentrations of inhibitors for 10 min, after preincubation cells were stimulated by carbamylcholine (0.3 mM) for 3 min. Released catecholamines in the medium were expressed as the percent of the total cellular content. (B) Cells were preincubated with various concentrations of inhibitors for 10 min, after preincubation cells were stimulated by carbamylcholine (0.3 mM) for 1 min. $^{45}Ca^{2+}$ in the cells was extracted with 1% Triton X-100 solution and counted by liquid scintillation counter. Each of these data represents the mean ± S.E. of triplicate determinations. The lower hatched areas indicate the basal catecholamine release and $^{45}Ca^{2+}$ uptake in the presence of various concentrations of inhibitors. Typical data obtained from two or three different cell preparations are presented. Symbols: ◇, basal value in the absence of inhibitors; ◆, control value (in the presence of 0.3 mM carbamylcholine) in the absence of inhibitors; △, + aspirin; ○, + indomethacin; ■, + BW755C; ●, + NDGA; ▲, + AA861.

Since these inhibitors blocked the carbamylcholine-stimulated events, the effects of these inhibitors on high K^+-evoked $^{45}Ca^{2+}$ uptake and catecholamine release were also examined.

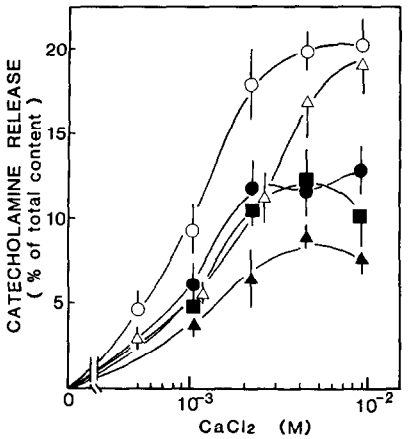

Fig. 2. Effects of various concentrations of Ca^{2+} on the inhibition of carbamylcholine-evoked catecholamine release by lipoxygenase inhibitors and verapamil. In this experiment, Locke's solution containing 154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 5.6 mM glucose, 5.0 mM Hepes (pH 7.5) was used. Cells were preincubated with verapamil (3 μM), BW755C (50 μM), NDGA (10 μM), AA861 (10 μM) for 10 min, after preincubation cells were stimulated by carbamylcholine (0.3 mM) for 3 min under various concentrations of Ca^{2+}. The spontaneous release (about 4% of cellular content) was subtracted from secretion data. The values represent the mean ± S.E. of triplicate determinations. Typical data obtained from two or three different cell preparations are presented. Symbols: ○, no inhibitor; △, + verapamil; ■, + BW755C; ●, + NDGA; ▲, + AA861.

Lipoxygenase inhibitors prevented the high K^+-evoked $^{45}Ca^{2+}$ uptake and catecholamine release with a similar potency in a concentration-dependent manner (Fig. 4A, B).

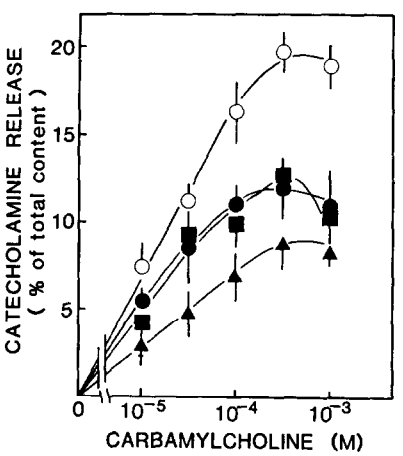

Fig. 3. Effects of change in carbamylcholine concentrations on the inhibitory effects of lipoxygenase inhibitors on catecholamine release. Cells were preincubated with BW755C (50 μM), NDGA (10 μM), AA861 (10 μM) for 10 min; after preincubation the cells were stimulated by various concentrations of carbamylcholine for 3 min. The spontaneous release (about 4% of cellular content) was subtracted from secretion data. The data indicate the mean ± S.E. of triplicate determinations. Typical data obtained from two or three different cell preparations are presented. Symbols: ○, no inhibitor; ■, + BW755C; ●, + NDGA; ▲, + AA861.

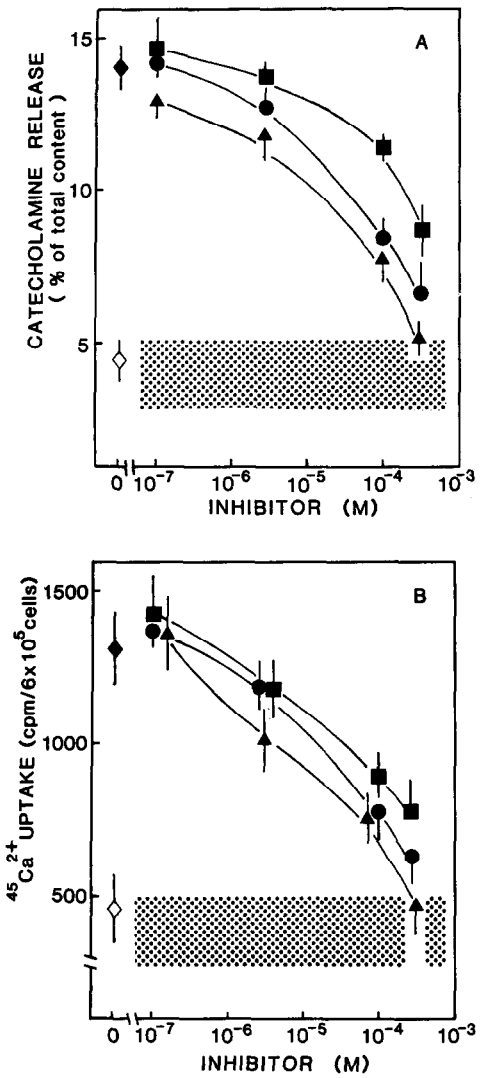

Fig. 4. Inhibitory effects of lipoxygenase inhibitors on the high K^+-evoked catecholamine release (A) and $^{45}Ca^{2+}$ uptake (B) in cultured adrenal chromaffin cells. (A) Cells were preincubated with various concentrations of inhibitors for 10 min, after preincubation cells were stimulated by high K^+ (56 mM) for 3 min. Released catecholamines in the medium were expressed as the percent of the total cellular content. (B) Cells were preincubated with various concentrations of inhibitors for 10 min, after preincubation cells were stimulated by high K^+ (56 mM) for 1 min. $^{45}Ca^{2+}$ in the cells was extracted with 1% Triton X-100 solution and counted by liquid scintillation counter. Each point indicates the mean ± S.E. of triplicate determinations. The lower hatched areas represent the basal catecholamine release and $^{45}Ca^{2+}$ uptake in the presence of various concentrations of inhibitors. Typical data obtained from two or three different cell preparations are presented. Symbols: ◇, basal value in the absence of inhibitors; ◆, control value (in the presence of 56 mM KCl) in the absence of inhibitors; ■, + BW755C; ●, + NDGA; ▲, + AA861.

Since lipoxygenase inhibitors prevented not only carbamylcholine-evoked $^{45}Ca^{2+}$ uptake but also high K^+-evoked $^{45}Ca^{2+}$ uptake, we cannot exclude the possibility that these lipoxygenase inhibitors inhibit $^{45}Ca^{2+}$ uptake by directly interacting on voltage-sensitive Ca^{2+} channels. Therefore, the effects of these inhibitors on the binding of the Ca^{2+}-channel antagonist, [^{3}H]nitrendipine, to the homogenate of the cultured chromaffin cells were examined. As shown in Fig. 5, increasing concentrations of unlabeled nitrendipine and nifedipine inhibited [^{3}H]nitrendipine binding to chromaffin cell homogenates at concentrations in the range of 0.1–10 μM. Nitrendipine inhibited high K^+-evoked $^{45}Ca^{2+}$ uptake with similar potency to that for inhibition of [^{3}H]nitrendipine binding (Fig. 5). Although lipoxygenase inhibitors prevented the high K^+-evoked $^{45}Ca^{2+}$ uptake, [^{3}H]nitrendipine binding was not affected by these drugs, suggesting that the inhibitory effects of lipoxygenase inhibitors on $^{45}Ca^{2+}$ uptake was not due to its direct interaction with the [^{3}H] nitrendipine binding site.

Although pertinent data are not presented, the effects of exogenously added lipoxygenase products or related fatty acids on catecholamine release were examined. Arachidonic acid significantly induced catecholamine release at 50 μM or above, but the obvious reduction in cell viability was observed at 50 μM; therefore this effect may be due mainly to its cell lytic effect. One of 5-lipoxygenase products, 5-HETE (10 μM) significantly induced catecholamine release (data not shown). However, the releasing effect was not concentration-dependent. Therefore, at present, it is not known whether this catecholamine releasing effect of 5-HETE is physio-

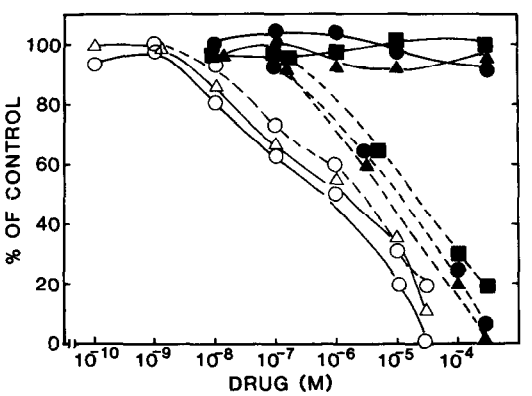

Fig. 5. Effects of dihydropyridines and lipoxygenase inhibitors on [^{3}H]nitrendipine binding and high K^+-evoked $^{45}Ca^{2+}$ uptake in cultured adrenal chromaffin cells. [^{3}H]Nitrendipine (2 nM) was incubated with the chromaffin cell homogenate (0.27 mg protein/ml) at 37° for 45 min as described under Materials and Methods. [^{3}H] Nitrendipine specifically bound was defined as the difference between total and nonspecific binding. For the $^{45}Ca^{2+}$ uptake experiment, the cells were preincubated with inhibitors for 10 min, after preincubation cells were stimulated by high K^+ (56 mM) for 1 min and cellular radioactivity was determined. Values indicated are expressed as percent of control specific binding and high K^+-evoked $^{45}Ca^{2+}$ uptake incubated in the absence of inhibitors. The data for binding (———) represent the means of duplicate determinations. The data for $^{45}Ca^{2+}$ uptake (– – – –) indicate the means of triplicate determinations. Typical data obtained from two or three different cell preparations are presented. Symbols: ●, NDGA; ■, BW755C; ▲, AA861; ○, nitrendipine; △, nifedipine.

logically significant or not. Moreover, another one of the 5-lipoxygenase products, leukotriene B_4, which behaves as a Ca^{2+} ionophore in liposomes [18] and is a potent inducer of stimulus–secretion coupling in neutrophils [19, 20], failed to induce $^{45}Ca^{2+}$ uptake and catecholamine release at 10 nM and 10 μM. Leukotriene C_4 (10 nM, 10 μM) and leukotriene D_4 (10 nM, 10 μM) also failed to evoke catecholamine release.

In all experiments, the cell viability was checked by a trypan blue exclusion test. The cell viability was always above 95%. The inhibitors at concentrations which we used did not reduce the cell viability.

DISCUSSION

The adrenal medullary chromaffin cell was one of the first secretory systems in which the requirement of Ca^{2+} to activate exocytosis was recognized [1, 2]. In certain secretory cells, it has been shown that activation of cellular phospholipase A_2 plays a significant role in the mechanism of Ca^{2+}-dependent cellular secretion [21]. Recently, several investigators reported that phospholipase A_2 inhibitors such as mepacrine and *p*-bromophenacyl bromide prevent the catecholamine release and cellular Ca^{2+} uptake in these cells [3–5]. In the mechanisms of stimulus–secretion coupling in intact adrenal chromaffin cells, much less is known as to whether the role of arachidonic acid metabolism is physiologically important or not. Therefore, in this paper we examined the effects of lipoxygenase and cyclooxygenase inhibitors on cellular Ca^{2+} uptake and catecholamine release using cultured adrenal chromaffin cells. In our present experiments, NDGA [22], BW755C [23] and AA861 [24] which have different chemical structures and inhibit lipoxygenase or both cyclooxygenase and lipoxygenase, prevented the carbamylcholine- and high K^+-evoked catecholamine secretion concomitant with the inhibition of cellular Ca^{2+} uptake. These results indicate that the inhibitory effects of these drugs on catecholamine release is mainly due to their inhibitory actions on the process of Ca^{2+} uptake.

In order to investigate the effects of lipoxygenase inhibitors on the secretory process distal to Ca^{2+} entry, we examined the effects of these inhibitors on A23187-evoked catecholamine release. The combination of A23187 (10 μM) and lipoxygenase inhibitors, however, acted to reduce cell viability; therefore we could not investigate the effects of lipoxygenase inhibitors on the secretory process at a step distal to Ca^{2+} entry.

In adrenal chromaffin cells, nicotinic agonists interact with the acetylcholine receptor and initiate the Ca^{2+} uptake both through the acetylcholine receptor coupled Ca^{2+}-channel and through the voltage-sensitive Ca^{2+}-channel [25]. High K^+ directly depolarizes cell membrane, resulting in the opening of the voltage-sensitive Ca^{2+}-channel [25]. Our results presented here show that lipoxygenase inhibitors prevent not only carbamylcholine-evoked $^{45}Ca^{2+}$ uptake but also high K^+-evoked $^{45}Ca^{2+}$ uptake, and the lipoxygenase inhibitors mainly inhibit a voltage-sensitive Ca^{2+} entry in either a direct or indirect way rather than inhibiting Ca^{2+} entry by interacting with the acetylcholine receptor or a step specific for the acetylcholine receptor coupled process. Since slightly higher concentrations of the lipoxygenase inhibitors were required to inhibit high K^+-evoked effects compared to those evoked by carbamylcholine, we cannot completely exclude the possibility that these lipoxygenase inhibitors also have some inhibitory effects on the step specific for the acetylcholine receptor coupled process.

Dihydropyridine derivatives, such as nitrendipine and nifedipine, appear to reduce an inward flux of Ca^{2+} by blocking voltage-sensitive Ca^{2+}-channels in some tissues [26, 27]. Recently, a number of investigators have reported the presence of a binding site for dihydropyridine derivatives, such as [^{3}H]-nitrendipine, in the membrane of various tissues [28–30]. Failure of lipoxygenase inhibitors to inhibit the [^{3}H]nitrendipine binding indicates that the inhibitory effects of these drugs on cellular Ca^{2+} uptake cannot be attributable to the direct interaction of these inhibitors on [^{3}H]nitrendipine binding sites. Although the apparent potency of the inhibition of [^{3}H]nitrendipine binding by dihydropyridine derivatives (IC_{50} = 1 μM) and the potency of nitrendipine to block the Ca^{2+} uptake (IC_{50} = 3 μM) in chromaffin cells were approx. 100–1000-fold less than those observed in the other tissues [28–31], the IC_{50} value of dihydropyridine derivatives for the inhibition of [^{3}H]nitrendipine binding was somewhat similar to the IC_{50} value of this drug for inhibition of $^{45}Ca^{2+}$ uptake. Therefore the [^{3}H]nitrendipine binding we observed seems to be pharmacologically significant.

Exogenously added lipoxygenase products or related fatty acids, which we tested, did not show obvious catecholamine releasing effects. However, there remains the question of whether exogenously added lipoxygenase products can exert a similar effect as endogenous lipoxygenase products in these chromaffin cells. Furthermore, the products which we tested are only a part of the various lipoxygenase products. In this laboratory, studies are under way in order to find endogenous lipoxygenase products which have obvious catecholamine releasing effects.

In conclusion, our present results suggest that lipoxygenase inhibitors, such as NDGA, BW755C and AA861, prevent the catecholamine release by inhibiting cellular Ca^{2+} uptake. The mechanism of inhibition of $^{45}Ca^{2+}$ uptake and catecholamine release by these three compounds seems to be different from competition at acetylcholine receptor site or those of the well-known Ca^{2+} channels inhibitors. Since the pharmacologically common effect of these three compounds with different chemical structures is the inhibition of lipoxygenase, if lipoxygenase activity plays a key role in the secretory process of catecholamine from adrenal chromaffin cells, it may be involved in the Ca^{2+} translocation systems in these cells. Actually, in granulocytes, some lipoxygenase products play a significant role in the membrane Ca^{2+} flux [32–34]. Another possibility is that these compounds have inhibitory effects on Ca^{2+} uptake by means of some unknown mechanisms. Because, at present, we cannot exclude the possibility that these compounds inhibit some membrane-associated processes.

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