Muscarinic Autoinhibition and Modulatory Role of Protein Kinase C in Acetylcholine Release from the Myenteric Plexus of Guinea Pig Ileum

Noriko Saitoh[1], Ritsuko Fujimoto[1], Toshiaki Ishii[1,2], Hideaki Nishio[1], Tadayoshi Takeuchi[1,2] and Fumiaki Hata[1,2,*]

[1]*Department of Veterinary Pharmacology, College of Agriculture and* [2]*Department of Molecular Physiology and Biochemistry, Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai 593, Japan*

Received December 17, 1996 Accepted March 12, 1997

ABSTRACT—The modulatory role of protein kinase C on phospholipase A_2, activation of which had been suggested to result in acetylcholine release from cholinergic neurons, was studied in longitudinal muscle preparations with the myenteric plexus of guinea pig ileum. The relationship of muscarinic autoinhibition to the modulation was also examined. Phorbol-12,13-dibutyrate (PDBu), an activator of protein kinase C, dose-dependently increased spontaneous and electrical field stimulation-induced acetylcholine releases from the preparation. The inhibitors of protein kinase C, staurosporine and calphostin C, inhibited the stimulatory effects of PDBu, but neither inhibitor affected spontaneous or electrical field stimulation-induced acetylcholine release in the absence of PDBu. On the other hand, atropine significantly increased electrical field stimulation-induced release by blocking a muscarinic autoinhibitory mechanism. Under the autoinhibition blocked condition, U73122, an inhibitor of phospholipase C, and staurosporine significantly inhibited the effect of atropine on electrical field stimulation-induced release. An inhibitor of phospholipase A_2, mepacrine, inhibited PDBu-induced acetylcholine release and also inhibited the effect of atropine on electrical field stimulation-induced release. An activator of phospholipase A_2, melittin, and a product of the phospholipase, arachidonic acid, increased the spontaneous and electrical field stimulation-induced releases. These results suggest that the phospholipase C - protein kinase C system modulates acetylcholine release from cholinergic neurons by activating phospholipase A_2 in the myenteric plexus of guinea pig ileum, and the activation of muscarinic autoreceptor may negatively modulate acetylcholine release at a point upstream of the system.

Keywords: Acetylcholine release, Protein kinase C, Autoinhibition, Ileal myenteric plexus (guinea pig), Phospholipase A_2

Several reports suggest a role for phospholipase A_2 in the secretory process of endocrine and exocrine cells. For example, the activators of phospholipase A_2, melittin and mastoparan increased the release of prolactin from bovine pituitary cells (1), release of insulin from rat pancreatic acinar cells (2) and release of luteinizing hormone from rat anterior pituitary cells (3). There is also a report indicating stimulatory effects of melittin on high K^+-induced acetylcholine release from PC 12 cells (4). Mepacrine, an inhibitor of phospholipase A_2, is reported to inhibit catecholamine secretion from adrenal chromaffin cells (5). In longitudinal muscle-myenteric plexus preparations of guinea pig ileum, we found that mepacrine significantly inhibited acetylcholine release induced by electrical field stimulation from nerve terminals of cholinergic neurons (6, 7) and that 5-lipoxygenase metabolites of arachidonic acid had a positive modulatory effect on the acetylcholine release (8). From these findings, it is likely that activation of phospholipase A_2 and subsequent arachidonic acid metabolism are important in the acetylcholine release process in the preparation.

4β-Phorbol-12,13-dibutyrate (PDBu) or 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), an activator of protein kinase C, increased acetylcholine release induced by electrical stimulation from rabbit hippocampus in vitro (9) and the release induced by electrical stimulation or a high K^+ concentration from the slices of the caudate nucleus of guinea pigs (10). A synaptosome preparation is known to contain high levels of protein kinase C (11) and its substrate proteins (12). In addition, there are some reports about the effect of TPA on the peripheral nervous system: TPA increased acetylcholine release stimulated by

* To whom correspondence should be addressed[(1)].

electrical stimulation from neuro-muscular junctions in rats (13), mice (14) and frogs (15), and it also increased norepinephrine release from sinus nodes of guinea pigs (16).

On the other hand, protein kinase C was reported to modulate phospholipase A_2 activity in mouse peritoneal macrophages (17), human platelets (18) and human monocytic cells (19). It has also been reported that phospholipase A_2 was activated by TPA by phosphorylation via protein kinase C in macrophages (20), and protein kinase C increased the sensitivity of phospholipase A_2 to Ca^{2+} in platelets (21) and Chinese hamster ovary cells transfected with and expressing the m_5 muscarinic receptor (22). These findings suggested that it would be interesting to study whether protein kinase C modulates phospholipase A_2 activity involved in the regulation of neurotransmitter release. We, therefore, studied the relationship between protein kinase C and phospholipase A_2 in acetylcholine release in longitudinal muscle preparations with the myenteric plexus of guinea pig ileum. In our studies, we have always used physostigmine to prevent the hydrolysis of released acetylcholine. Acetylcholine accumulated by acetylcholinesterase inhibition led to inhibition of acetylcholine release via presynaptic muscarinic receptor stimulation in guinea pig ileum myenteric plexus (23–25). We also studied the relationship between the modulation of phospholipase A_2 by protein kinase C and the autoinhibition mechanism.

MATERIALS AND METHODS

Male guinea pigs, weighing 300–700 g, were lightly anesthetized with diethylether and then killed by bleeding. The longitudinal muscle of the ileum with the myenteric plexus attached was prepared as described previously (6). The preparations were mounted in an organ bath containing 3 ml of Tyrode solution of the following composition: 136.9 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4 and 5.6 mM glucose. Physostigmine salicylate (5 μM) and choline chloride (1 μM) were added to the bathing medium, which was kept at 37℃ and bubbled with 95% O_2 and 5% CO_2.

Release of acetylcholine induced by electrical field stimulation

As shown in the protocol (Fig. 1), the strips were equilibrated for 15 min by perfusion with Tyrode solution at a rate of 1–2 ml/min. Then perfusion was stopped and the bathing medium was replaced by 3 ml of fresh Tyrode solution. After a 1-min spontaneous acetylcholine release period, the fluid was replaced again by 3 ml of the fresh solution. The collected fluid was used for determining spontaneous acetylcholine release (R_1). Then the strips were stimulated by electrical field stimulation as described elsewhere (8). The parameters for electrical field stimulation were as follows: supramaximal voltage (50 V), pulse duration 0.5 msec, 200 pulses at 10 Hz in most studies. In another series of experiments to examine the frequency dependency of stimuli, stimuli at 1–50 Hz were delivered. These procedures were repeated to collect samples of R_2 and S_2. The first stimulation was carried out in the absence (S_1) of test drug(s), and the second was in the presence (S_2) of test drug(s). For measuring electrical field stimulation-induced release of acetylcholine, the bathing fluid was collected 40 sec after stimulation. At the end of experiment, the strips were blotted and weighed so release could be expressed as acetylcholine released per g tissue. The amount of acetylcholine released in response to each stimulation (S_1', S_2') was calculated by subtracting the release during the 1-min resting period (spontaneous release, R_1, R_2) immediately preceding stimulation from the total release (S_1, S_2) during stimulation. All results are shown as relative acetylcholine release defined as $R_2/R_1 \times 100$ and $S_2'/S_1' \times 100$.

Assay of released acetylcholine

The samples collected were assayed for acetylcholine

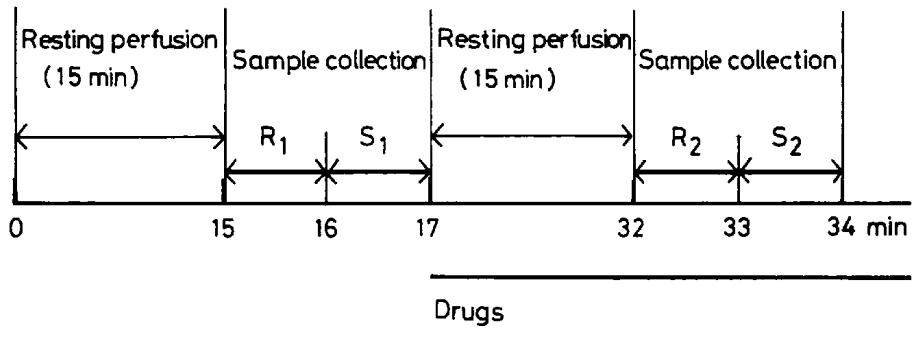

Fig. 1. Protocol for experiments on acetylcholine release.

using isolated strips of longitudinal muscle obtained from the most distal region of the guinea pig ileum (26). The intestinal preparations were exposed to various concentrations of authentic acetylcholine (2.7–5.4 nM) for 15 sec. PDBu, melittin, arachidonic acid and inhibitors of protein kinase C or phospholipase A_2 did not have any appreciable effect on the bioassay at the concentrations contained in the samples. The active substance in the bath fluid was considered to be acetylcholine because the contraction of the muscle strip induced by bath fluid was prevented by atropine and was lost after treatment of the bath fluid with alkali and boiling for a few minutes (7).

In another series of experiments, acetylcholine released in the medium that contained atropine was assayed by HPLC. For analysis by HPLC, acetylcholine in aliquots of the medium was precipitated with KI_3 in the presence of tetraethylammonium as a coprecipitant and 50 pmol ethylhomocholine as an internal standard. The quaternary ammonium compounds precipitated were dissolved with about 1 ml of acetonitrile and the solution was passed through a Bio Rad AG 1×8 anion exchange resin column to trap I_3^- in the solution. The effluents were evaporated to dryness under reduced pressure, and the dried samples were stored in the refrigerator until assay. At use, the dried samples were dissolved with 50 μl water and passed through a filter (0.45-μm pore size), and 50 μl of the filtrates was injected into the HPLC assay system for acetylcholine determination. Acetylcholine was determined by Yanaco reversed-phase HPLC (Yanaco, Kyoto), developed first by Potter et al. (27), using a postcolumn enzyme (acetylcholinesterase plus choline oxidase) reactor (Eicom AC-Enzympak; Eicom, Kyoto) instead of the enzyme-flow system of the original method.

Statistical analyses

All data in the text are expressed as the mean±S.E.M. The statistical significance of the difference between two mean values was assessed by the unpaired Student's *t*-test. For comparison of one control with several experimental groups, the significance of difference was assessed by one-way analysis of variance followed by Dunnett's test.

Drugs

PDBu, staurosporine, melittin, mepacrine and arachidonic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA); 2-(12-hydroxydodeca-5,10-dinyl)-3,5,6-trimethyl-1,4-benzoquionone (AA861) and 1-[6-[{17β-3-methoxyestra-1,3,5(10)-trien-17-yl}amino]hexyl]-1*H*-pyrrole-2,5-dione (U73122) from Wako Pure Chemical (Osaka); and calphostin C from Kyowa Medex (Tokyo). All other chemicals were of analytical grade. Stock solutions of PDBu (1 mM) and calphostin C (1 mM) were prepared in dimethyl sulfoxide (DMSO). Stock solutions of arachidonic acid (10 mM), U73122 (100 μM), AA861 (1 mM) and staurosporine (100 μM) were prepared in ethanol. Before use, those solutions were diluted appropriately with Tyrode solution. DMSO and ethanol at the concentrations used did not affect the release of acetylcholine and the bioassay.

RESULTS

In control experiments, the spontaneous release (R_1) immediately before the first stimulation was apparently constant (646±109 pmol/g tissue per min, n=11). The value for the relative amounts of acetylcholine release immediately before the second stimulation (R_2) was 109.0±6.9% of the first one (R_1). Electrical field stimulation (200 pulses at 10 Hz) of the longitudinal muscle-myenteric plexus preparation resulted in significant release of acetylcholine: 930±66 pmol/g tissue (value above the spontaneous release, S_1'; n=9) collected during the period of electrical field stimulation and the subsequent 40 sec. Under control conditions, release on the second stimulation (S_2') was 110.9±6.0% of that on the first (S_1).

Effects of phorbol ester on spontaneous and electrical field stimulation-induced release of acetylcholine from longitudinal muscle-myenteric plexus preparations of guinea pig ileum

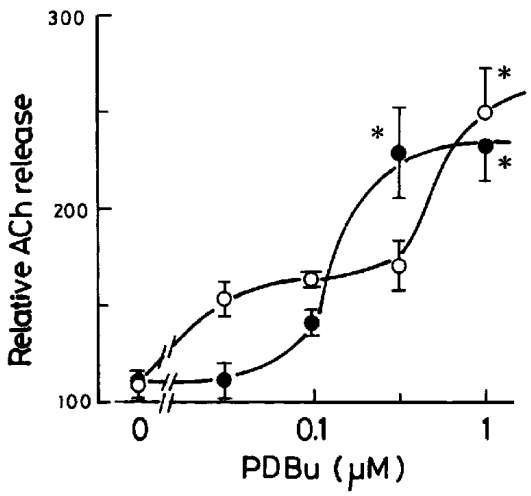

Fig. 2. Effects of phorbol-12,13-dibutyrate (PDBu) on spontaneous (○) and electrical field stimulation-induced (●) acetylcholine (ACh) release from longitudinal muscle preparations with the myenteric plexus of guinea pig ileum. Relative acetylcholine release was determined without or with the indicated concentrations of PDBu and expressed as $R_2/R_1\times100$ and $S_2'/S_1'\times100$. Points and bars represent means and standard errors for 3 to 11 experiments. Significances of differences from the value in the absence of PDBu: *$P<0.05$ by Dunnett's test. For further details, see Materials and Methods.

PDBu, an activator of protein kinase C, increased the spontaneous and electrical field stimulation-induced acetylcholine releases dose-dependently (Fig. 2). The preparations were stimulated with electrical pulses at 10 Hz in most experiments of the present study, which were submaximal for induction of acetylcholine release. The maximal acetylcholine release (197±41% of the submaximal release at 10 Hz, n=6) was induced by electrical pulses at supramaximal intensity (50 Hz). PDBu further increased the maximal acetylcholine release to 385±61% (significantly different from the value without PDBu, $P<0.05$, n=5). The stimulatory effect of PDBu on acetylcholine release was counteracted by staurosporine, an inhibitor of protein kinase C: staurosporine at 100 nM almost completely counteracted the effect of PDBu (Fig. 3). Another inhibitor of protein kinase C, calphostin C at 1 μM, counteracted the effect of PDBu moderately. The effects of higher concentrations of the drug could not be examined due to its low solubility. These two inhibitors did not have any significant effect on the releases in the absence of PDBu (Fig. 3).

Effects of inhibitors of phospholipase A_2 and 5-lipoxygenase on the stimulatory effects of PDBu

Mepacrine was previously shown to inhibit electrical field stimulation-induced acetylcholine release. We, therefore, examined the effect of the inhibitor on the PDBu-stimulated acetylcholine releases. Mepacrine at 5 μM significantly inhibited the stimulatory effects of PDBu on the spontaneous and electrically stimulated acetylcholine releases (Fig. 4). In this study also, the inhibitor inhibited the spontaneous and electrically stimulated release in control experiments as we showed previously, that is, in the absence of PDBu (Fig. 4).

Since 5-lipoxygenase metabolites of arachidonic acid were previously shown to modulate the acetylcholine release, we next examined the effect of an inhibitor of 5-lipoxygenase on PDBu-stimulated acetylcholine releases. AA861, a selective inhibitor of 5-lipoxygenase, at a concentration of 30 μM completely counteracted the stimulatory effects of PDBu on the spontaneous and electrical field stimulation-induced releases (Fig. 4). It also inhibited the two releases in the absence of PDBu (Fig. 4).

Effects of atropine on spontaneous and electrical field stimulation-induced release of acetylcholine

Atropine at concentrations ranging from 0.01 to 10 μM did not have any significant effect on the spontaneous acetylcholine release. However, the muscarinic receptor antagonist enhanced electrical field stimulation-induced acetylcholine release concentration-dependently: at 0.1 μM, moderately and at 1 μM, maximally to 280% of the control (Fig. 5), indicating very strong autoinhibition by released acetylcholine that physostigmine protected from

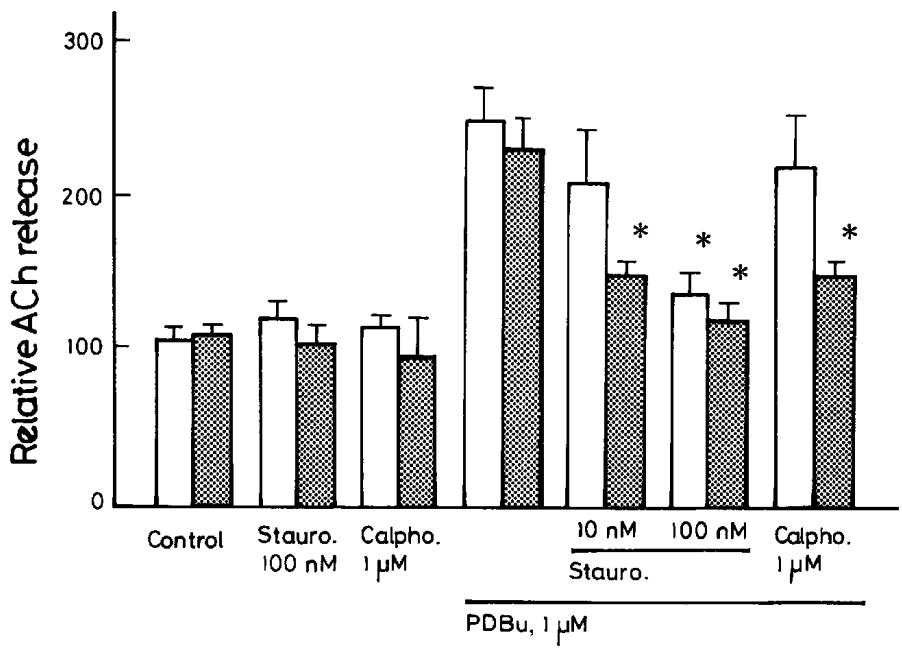

Fig. 3. Effects of staurosporine and calphostin C (1 μM) on spontaneous (open columns) and electrical field stimulation-induced (shaded columns) acetylcholine release in the absence or presence of PDBu (1 μM). Columns and bars represent means and standard errors for 3 to 11 experiments. Significances of differences from the corresponding value in the presence of PDBu alone: *$P<0.05$ by Dunnett's test.

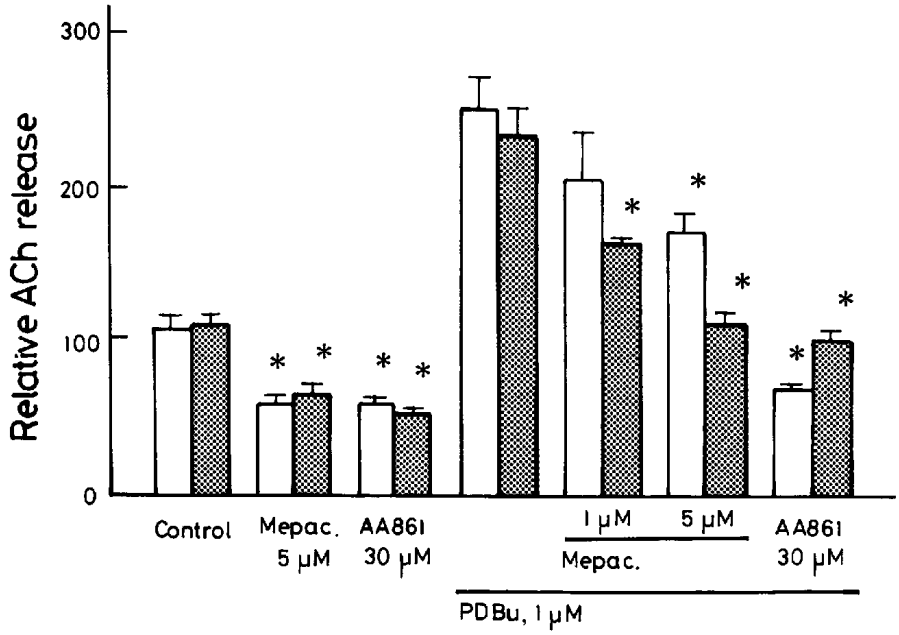

Fig. 4. Effects of mepacrine and AA861 (30 μM) on spontaneous (open columns) and electrical field stimulation-induced (shaded columns) acetylcholine release in the absence or presence of PDBu (1 μM). Columns and bars represent means and standard errors for 3 to 11 experiments. Significant differences from the corresponding control value or from the corresponding value in the presence of PDBu alone (*$P<0.05$ by Dunnett's test) are indicated.

hydrolysis under the present experimental conditions. Muscarine (up to 1 μM) in the presence of physostigmine did not have any effect on the spontaneous and electrical field stimulation-induced releases (data not shown).

Effects of inhibitors of protein kinase C and phospholipase C on enhanced acetylcholine release induced by atropine

Next, we examined whether the phospholipase C-protein kinase C system is associated with the mechanism of muscarinic autoinhibition in the acetylcholine release. Staurosporine at 100 nM significantly inhibited the stimulatory effect of atropine on electrical field stimulation-induced acetylcholine release (under the autoinhibition-blocked condition). U73122, an inhibitor of phospholipase C, also significantly inhibited the stimulatory effect of atropine on electrical field stimulation-induced acetylcholine release (Fig. 6). In contrast, in the absence of atropine (under the autoinhibition-working condition), staurosporine (Fig. 3) and U73122 (Fig. 6B) had no effect on the electrically-induced release.

Mepacrine and AA861 significantly inhibited the acetylchlone release in the presence of atropine (Fig. 6).

Effects of melittin and arachidonic acid on acetylcholine release from the myenteric plexus of guinea pig ileum

We examined whether activation of phospholipase A_2 results in increased acetylchloine release from longitudinal muscle-myenteric plexus preparations. Melittin, an activator of phospholipase A_2, increased the spontaneous and electrically-induced acetylcholine releases (Fig. 7).

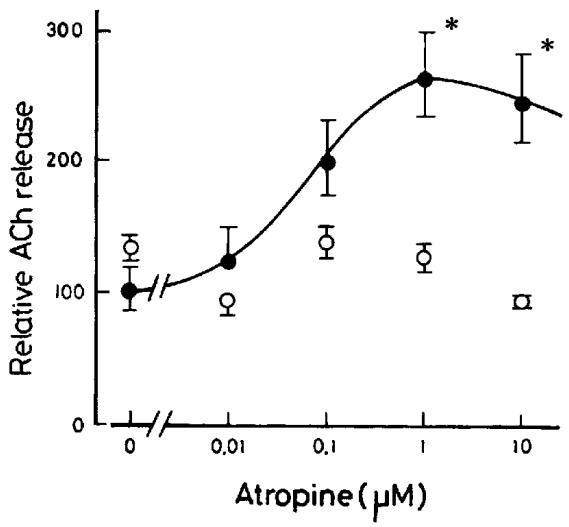

Fig. 5. Effects of atropine on spontaneous (○) and electrical field stimulation-induced (●) acetylcholine release. Relative acetylcholine release was determined without or with the indicated concentrations of atropine. Points and bars represent means and standard errors for 3 to 17 experiments. Significances of differences from the value in the absence of atropine: *$P<0.05$ by Dunnett's test.

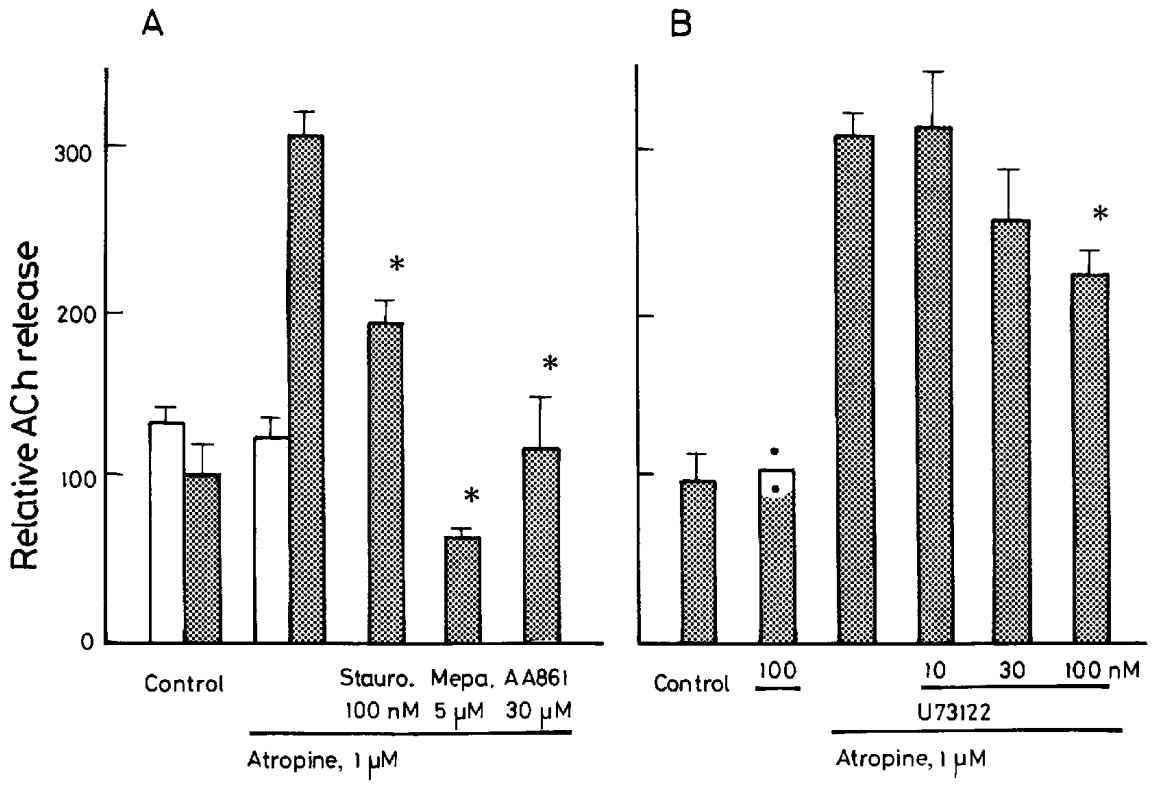

Fig. 6. Effects of staurosporine (100 nM), mepacrine (5 μM), AA861 (30 μM) (A) and U73122 (B) on electrical field stimulation-induced acetylcholine release in the presence of atropine. Electrical field stimulation-induced release (shaded columns) was determined in the absence or presence of atropine (1 μM) without or with indicated concentrations of the inhibitors. Open columns indicate spontaneous release. Columns and bars represent means and standard errors for 3 to 5 experiments. Value for U73122 alone is the mean of 2 experiments. Significances of differences from the value in the presence of atropine alone: $^{*}P<0.05$ by Dunnett's test.

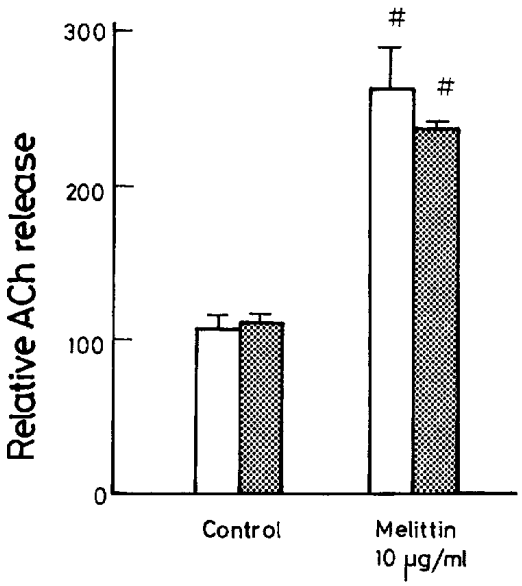

Fig. 7. Effects of melittin on spontaneous (open columns) and electrical field stimulation-induced (shaded columns) acetylcholine release. Columns and bars represent the mean and standard errors for 3 to 11 experiments. Significant differences from the corresponding control value at $^{\#}P<0.01$ by Student's *t*-test are shown.

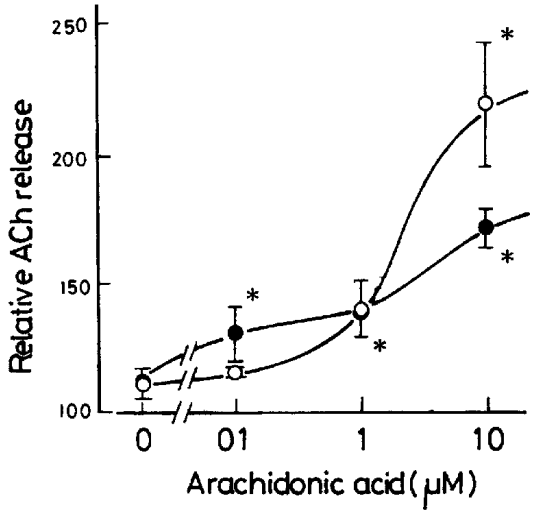

Fig. 8. Effects of arachidonic acid on spontaneous (○) and electrical field stimulation-induced (●) acetylcholine release. Relative acetylcholine releases were determined without or with the indicated concentrations of arachidonic acid. Points and bars represent means and standard errors for 3 to 11 experiments. Significance of difference from the value in the absence of arachidonic acid: $^{*}P<0.05$ by Dunnett's test.

Exogenous arachidonic acid, a possible product formed by activated phospholipase A_2, also increased the two releases concentration-dependently (Fig. 8). Staurosporine (up to 100 nM) did not have any significant effect on the releases enhanced by melittin (electrically-induced release by 10 μg/ml melittin with 100 nM staurosporine was 100.9%, n=2, of that by melittine alone) or arachidonic acid (electrically-induced release by 1 μM arachidonic acid with 100 nM staurosporine was 108.8%, n=3, of that by arachidonic acid alone).

DISCUSSION

We previously reported that mepacrine inhibited electrical field stimulation-induced acetylcholine release from the myenteric plexus of guinea pig ileum (6, 7) and that metabolites of arachidonic acid, such as leukotriene D_4 and 5-HETE, reversed the inhibitory effect (8). From these results, we suggested an important role of 5-lipoxygenase metabolites in the release of acetylcholine. TPA was reported to increase electrical field stimulation- or Ca^{2+} ionophore-induced acetylcholine release from the myenteric plexus of guinea pig ileum (10). In the present study, PDBu also increased spontaneous and electrical field stimulation-induced acetylcholine release from the same tissue preparations as those in these previous studies. Since protein kinase C inhibitors reversed the stimulatory effects of PDBu on acetylcholine release, it seems that PDBu increased acetylcholine release by activating endogenous protein kinase C. Interestingly, the stimulatory effects of PDBu were also inhibited by mepacrine, a phospholipase A_2 inhibitor, indicating that activation of phospholipase A_2 occurs at a point downstream of activation of protein kinase C. Arachidonic acid and melittin, a product and an activator of phospholipase A_2, respectively, increased the release of acetylcholine. Staurosporine did not affect the acetylcholine releases enhanced by arachidonic acid and melittin. From these findings, it seems likely that protein kinase C activates phospholipase A_2, which in turn results in production of 5-lipoxygenase metabolites and subsequent positive modulation of acetylcholine release. The inhibitory effects of AA861 on spontaneous and PDBu-induced acetylcholine release also support the above idea (Fig. 4).

Atropine, by which muscarinic autoreceptors were blocked, very significantly increased electrical field stimulation-induced acetylcholine release, although it did not affect the spontaneous release. Muscarine did not affect both the releases. These results suggest that muscarinic autoreceptors were fully activated by released acetylcholine during electrical field stimulation due to the presence of physostigmine in the medium. In the absence of atropine, namely, under the autoinhibition-working condition, staurosporine did not affect the control releases in the absence of PDBu (either spontaneous or electrical field stimulation-induced, Fig. 3). In contrast, in the presence of atropine, under the autoinhibition-blocked condition, it significantly inhibited electrical field stimulation-induced release (Fig. 6A). These results suggest that in the absence of atropine, protein kinase C is maximally inhibited by the autoinhibition mechanism, and therefore, the inhibitor of protein kinase C could not further inhibit the kinase, while PDBu could directly activate the kinase. U73122 at 100 nM, one of the most novel inhibitors of phospholipase C also inhibited the effect of atropine on electrical field stimulation-induced release. Thus, the phospholipase C - protein kinase C system may usually function, but not under the autoreceptor-fully activated conditions. It was suggested that m_1, m_3 and m_5 subtype of muscarinic receptors positively couple to the phospholipase C - protein kinase C system. In the longitudinal muscle with myenteric plexus preparation of guinea pig ileum, the subtype of the muscarinic autoreceptor could not be defined by the study with some available muscarinic antagonists such as pirenzepine, AF-DX 116 and 4-DAMP (N. Saitoh, T. Takeuchi and F. Hata, unpublished data). Therefore, it can not be discussed at present whether the relation of the muscarinic autoreceptor to the phospholipase C - protein kinase C system is direct or indirect. Spontaneous release was not increased by atropine and was not inhibited by staurosporine in the presence of atropine. So, it seems that autoinhibition and modulation by protein kinase C are not associated with spontaneous release.

In the present study, U73122 did not necessarily show a strong effect (Fig. 6B). Unexpectedly, higher concentrations (>1 μM) of U73122 were not only less effective than a concentration of 100 nM on electrically-induced release but somewhat stimulative on the spontaneous release (data not shown). These findings are compatible with an agonistic effect of higher concentrations of the drug in prolactin secretion from GH_3 rat pituitary cells (28), although an exact mechanism is still unknown. The inhibitory effect of staurosporine on the acetylcholine release enhanced by atropine was also incomplete, even at the concentration that completely inhibited the PDBu-induced enhanced release. Therefore, mechanisms of autoinhibition other than inhibition of the phospholipase C - protein kinase C system can not be excluded at present.

Spontaneous and electrical field stimulation-induced acetylcholine releases enhanced by PDBu were inhibited by mepacrine and AA861 (Fig. 4). The stimulatory effect of atropine on electrical field stimulation-induced acetylcholine release was also inhibited by both the antagonists (Fig. 6A). These results also suggest that phospholipase C - protein kinase C system is present at a point

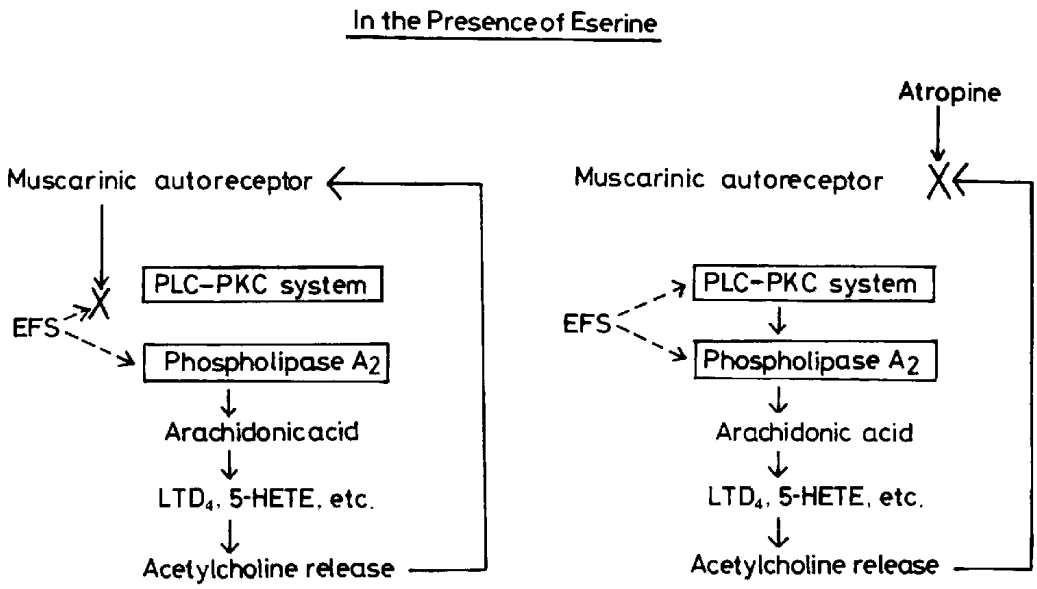

Fig. 9. Pharmacologically inferred relationship of muscarinic autoinhibition with the modulation of the phospholipase C (PLC) - protein kinase C (PKC) system in acetylcholine release from longitudinal muscle preparations with the myenteric plexus of guinea pig ileum. EFS: electrical field stimulation, LTD_4: leukotriene D_4, 5-HETE: 5-hydroxyeicosatetraenoic acid.

upstream of the phospholipase A_2 - 5-lipoxygenase system.

Protein kinase C has been suggested to activate phospholipase A_2 by increasing its sensitivity to calcium ions (21, 22). Although, in the absence of calcium ions, PDBu did not induce the release of acetylcholine (data not shown), in the presence of calcium ions, it increased the maximum response induced by electrical stimulation at 50 Hz in the present study. These results suggest a direct, calcium-dependent stimulatory effect of protein kinase C on phospholipase A_2 in the release process of acetylcholine.

Mepacrine (and also AA861) significantly inhibited the control release in the absence of PDBu (either spontaneous or electrical field stimulation-induced, Fig. 4), whereas staurosporine did not affect (Fig. 3), indicating that protein kinase C-independent activation of phospholipase A_2 occurs. Protein kinase C-independent activation of phospholipase A_2 may be induced by calcium ions that enter into nerve terminals during excitation of neurons or by phosphorylation of phospholipase A_2 by some other protein kinase(s) rather than protein kinase C: epidermal growth factor activates phospholipase A_2 through tyrosine kinase in cultured mesangial cells (29, 30), and protein kinase A activates but calcium calmodulin kinase II inhibits phospholipase A_2 in rat brain synaptosomes (31).

The present findings together with our previous findings (8) are summarized in Fig. 9. Activation of phospholipase A_2 is most important in a modulatory system of the acetylcholine release, and in turn produces 5-lipoxygenase metabolites of arachidonic acid and links to the process of acetylcholine release from cholinergic nerve terminals in the myenteric plexus. The phospholipase C-protein kinase C system positively modulates the activity of phospholipase A_2 when nerve terminals are excited. When excess acetylcholine was released, the autoinhibitory mechanism negatively modulates the acetylcholine release at a point upstream of the phospholipase C - protein kinase C system.

Acknowledgments

This was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sports and Culture of Japan.

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