Modulation of Acetylcholine Release from Guinea-Pig Ileum Myenteric Plexus by Arachidonic Acid Cascade Inhibitors

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Abstract—Effects of arachidonic acid and mepacrine on ACh release from guinea-pig ileum myenteric plexus were investigated. Mepacrine (1-8 μM) inhibited the ACh release in a concentration-dependent manner. Arachidonic acid counteracted the inhibitory effect of mepacrine, but PGE2 did not. The inhibition induced by a combination of mepacrine and indomethacin on nicotine-induced ACh release was prevented by arachidonic acid, while that on spontaneous ACh release was prevented by arachidonic acid and PGE2 added simultaneously. The roles of arachidonic acid and PGs in the ACh release will be discussed.

Previously, in our laboratory, it has been shown that the release of ACh induced by nicotine was inhibited by the cyclooxygenase inhibitor indomethacin (IND) and reversed by adding prostaglandin E2 (PGE2) (1). This suggests the contribution of endogenous PGs in maintaining the excitability of the cholinergic neurons in the myenteric plexus. In some endocrine tissues, exogenously applied arachidonic acid was shown to increase the release of lactogen and prolactin, and the increase was not prevented by IND (2, 3). This indicated that arachidonic acid, a precursor of PGs, itself may also contribute to the release of these hormones. There exist further evidences that arachidonic acid increases the intracellular Ca2+ concentration by mobilizing Ca2+ from intra or extracellular store sites (2, 4), but the role of arachidonic acid in the neurotransmission is still unknown.

The purpose of the present study is to elucidate the role of arachidonic acid in the ACh release from guinea-pig ileum myenteric plexus using an inhibitor of phospholipase A2, mepacrine (5, 6).

Male guinea-pigs, weighing 300 to 700 g, were killed by a blow on the head and bled. The longitudinal muscle with adherent myenteric plexus was prepared from the ileum, excluding the last 10 cm of ileal portion (7). These strips were mounted in an organ bath containing 3 ml Tyrode’s solution of the following composition (mM): NaCl, 136.9; KCl, 2.7; CaCl2, 1.8; MgCl2, 1.05; NaHCO3, 0.4; glucose, 5.6. Physostigmine salicylate (5 μM) and choline chloride (1 μM) were added. The bath fluid was kept at 37°C and aerated with 95% O2–5% CO2 gas. Experiments on ACh release were carried out as previously described (8). In each experiment, stimulations by nicotine or high K+ solution were performed twice with an interval of 19 min. The first stimulation (S1) was carried out in the absence and the second stimulation (S2) in the presence of drugs. When the effects of drugs on the ACh release were studied, the drug was added to the medium 17 min before S2. The high K+ (50 mM) Tyrode’s solution was kept isosmotic by appropriate changes in the Na+ concentration. The samples for the estimation of ACh release were assayed on guinea-pig ileum longitudinal strips as previously described (1). To remove the added nicotine, arachidonic acid and PGs, the collected samples were gently shaken with one tenth volume of Amberlite XAD-2 for 15 min and then filtered (9). The ACh release due to each stimulation was calculated by subtracting the release collected immediately preceding the resting period from the total release during the stimulation period. All
results were shown as \( S_2/S_1 \times 100(\%) \). For the spontaneous ACh release, the spontaneous release (\( R_2 \)) immediately before \( S_2 \) was expressed as a percent of that (\( R_1 \)) immediately before \( S_1 \). The results were analyzed using Student’s \( t \)-test.

In the control experiments, the absolute amount of released ACh varied with different preparations, but no significant difference in ACh release was observed between the first (\( S_1 \)) and second (\( S_2 \)) stimulation. Therefore, the ratio of an amount of nicotine-induced ACh release to that of spontaneous ACh release was relatively constant throughout all the experiments.

Mepacrine at concentrations between 1 and 8 \( \mu \)M inhibited the ACh release induced by nicotine (6.16 \( \mu \)M) in a concentration dependent manner (Fig. 1). Mepacrine also significantly inhibited the spontaneous ACh release, but the degree of the inhibition was less than that in the nicotine-induced ACh release (Table 1). High concentration of mepacrine (10 \( \mu \)M) produced more potent inhibition on both the spontaneous and the nicotine-induced ACh release (Fig. 1), but this inhibitory effect was partly prevented by increasing the concentration of choline in the medium from 1 to 10 \( \mu \)M, while the effects of mepacrine of lower concentration were not affected. It seems likely that mepacrine also inhibits the high affinity uptake of choline besides inhibiting phospholipase A2 at higher concentration, as suggested by Jope and

![Fig. 1. Effect of different concentration of mepacrine on the nicotine-induced ACh release from guinea-pig ileum myenteric plexus in the presence of choline at a concentration of 1 (○) or 10 (●) \( \mu \)M. Each point represents the mean for 3 or 4 experiments. Vertical lines show standard errors of the means. ACh release was expressed as the ratio \( S_2/S_1 \). S_1 was carried out in absence and \( S_2 \) in the presence of mepacrine.](image)

**Table 1.** Comparison of the effects of arachidonic acid and PGE2 on the inhibitory effects of mepacrine and indomethacin on the ACh release from guinea-pig ileum myenteric plexus

| Treatment | Control | Mepacrine (5 \( \mu \)M) | Indomethacin (2.8 \( \mu \)M) | Mepacrine +Indomethacin |
|-----------|---------|-------------------------|-----------------------------|------------------------|
| Nicotine-induced ACh release | S2/S1 (\%) | 98.2±6.8 (8) | 34.2±5.7**(4) | 34.4±12.2**(4) | 13.3±7.5**(6) |
| none | | | | | |
| Arachidonic acid (10 \( \mu \)M) | | 73.6±1.5a (3) | 68.8±4.4b (4) | 62.2±2.8c (4) |
| PGE2 (14.3 nM) | | 30.8±4.2 (4) | | |
| Arachidonic acid +PGE2 | | | | |
| Spontaneous ACh release | R2/R1 (\%) | 119.5±12.1 (8) | 69.6±2.9* (4) | 33.2±6.2**(4) | 38.9±4.7**(6) |
| none | | | | | |
| Arachidonic acid (10 \( \mu \)M) | | 123.3±18.8a (3) | 45.5±6.5 (4) | |
| PGE2 (14.3 nM) | | 79.9±2.8 (4) | 58.9±4.9b (4) | 40.9±7.1 (6) |
| Arachidonic acid +PGE2 | | | 60.6±6.9c (4) | |

\( S_2/S_1 \) represents the ratio of output between the second (\( S_2 \)) and first (\( S_1 \)) stimulation periods and \( R_2/R_1 \) between the second (\( R_2 \)) and first (\( R_1 \)) resting periods immediately before the respective stimulation periods. \( S_1 \) and \( R_1 \) were carried out in the absence of and \( S_2 \) and \( R_2 \) in the presence of drugs. Values are the means±S.E.M. The number of observations are shown in parentheses. \*P<0.05, **P<0.01, as compared with the corresponding control. \aP<0.05, as compared with mepacrine alone. \bP<0.05, as compared with indomethacin alone. \cP<0.05, as compared with mepacrine and indomethacin.
In the following experiments, mepacrine was used at a concentration of 5 \mu M since it produced near maximal inhibition of the ACh release without affecting choline uptake into the nerve endings. The inhibition of the nicotine-induced ACh release by 5 \mu M mepacrine was comparable to that by 2.8 \mu M IND, which was shown in previous experiments (1) (Table 1). Arachidonic acid (10 \mu M) significantly prevented the inhibitory effect of mepacrine on the nicotine-induced ACh release, but PGE$_2$ (14.3 nM) did not (Table 1). Similar results were obtained in the spontaneous ACh release. On the other hand, the ACh release induced by high K$^+$ concentration (50 mM) was not affected significantly by mepacrine. The values of the relative ACh release were 105.8±6.1 (mean±S.E., n=3) and 89.6±10.4 (n=3) for the control and mepacrine, respectively.

In many tissues, arachidonic acid rapidly metabolized to PGs. Then, the possibility whether the above mentioned effects of arachidonic acid are due to PGs was examined. A combination of mepacrine and IND further decreased the nicotine-induced ACh release to an extent which exceeded that of IND alone. On the spontaneous ACh release, mepacrine was less effective than IND, and the combined effect of mepacrine and IND was similar to that of IND alone (Table 1). Arachidonic acid significantly prevented the inhibitory effect of mepacrine in the presence of IND on the nicotine-induced ACh release, but an addition of PGE$_2$ showed no further prevention. The reason why PGE$_2$ was ineffective in that case is not clear at present. In contrast, the inhibitory effect of mepacrine in the presence of IND on the spontaneous ACh release was slightly but significantly prevented only by a combination of arachidonic acid and PGE$_2$ (Table 1).

Morphological study showed that the activation of phospholipase A$_2$ resulted in an aggregation and lysis of synaptic vesicles (11) and their fusion to the plasma membrane in bovine brain synaptic vesicles (12). Exogenously applied phospholipase A$_2$ also caused the enhanced release of $[^3]$H]nor-epinephrine (13) and $[^3]$H]ACh (14) from the isolated synaptosomes of rat or guinea-pig cerebral cortex, respectively. These results suggest that an activation of phospholipase A$_2$ has some important role in the release of neurotransmitters. In the present study, mepacrine inhibited both the spontaneous and the nicotine-induced ACh release from guinea-pig ileum myenteric plexus, and this inhibition was prevented by arachidonic acid, but not by PGE$_2$. Therefore, we concluded that arachidonic acid acted by restoring the lowered arachidonic acid levels in the tissue by mepacrine. The fact that the restoring effect of arachidonic acid on the inhibition of the spontaneous and the nicotine-induced ACh release by mepacrine was abolished or diminished by IND suggests the contribution of both arachidonic acid and PGs in the ACh release from the myenteric plexus. It is well known that arachidonic acid is metabolized to lipoxygenase products other than cyclooxygenase products, PGs (15). However, lipoxygenase products can not be considered to participate in the effect of arachidonic acid on the ACh release since Nakahata et al. (16) reported results suggesting that these products rather inhibited the ACh release from guinea-pig small intestine.

Baba et al. (17) showed that mepacrine decreased high K$^+$ or veratridine-induced $[^3]$H]ACh release from rat brain synaptosomes by inhibiting the depolarization-induced calcium uptake. In the present experiments, however, mepacrine did not affect high K$^+$-induced release of ACh. Although the reason for the different results is not clear at present, the difference of tissue or species of animals should be taken into consideration. Previously, we have demonstrated that contributions of PGs to evoked ACh release differ with different tissues (18).

In summary, the present experiments suggest that both arachidonic acid and PGs have some significant role in maintaining the excitability of the cholinergic neurons in the myenteric plexus and that these substances contribute to different extents to the spontaneous and the nicotine-induced ACh release.
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