Prevention of catecholamine release from adrenal chromaffin cells by phospholipase A2-and lipoxygenase-inhibitors

Nobuyuki SASAKAWA, Satoshi YAMAMOTO, Konosuke KUMAKURA and Ryuichi KATO
Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

Accepted May 20, 1983

In various secretory cells, metabolites of membrane phospholipids play significant roles in the secretory process of lysosomal enzymes, hormones and other biologically active substances. Our recent findings suggest that an activation of phospholipase A2 and a lipoxygenase product(s) play an important role in glucose-induced insulin secretion in pancreatic islets (1). Adrenal medulla is another useful model for studying the cellular mechanism of the secretory function. Catecholamines are released from adrenal chromaffin cells by a Ca2+-dependent exocytotic process, and the voltage-dependent Ca2+-channel plays a significant role in the entry of Ca2+, akin to the glucose-induced insulin release from the pancreatic islet. Therefore, in order to elucidate whether phospholipase A2 stimulation and resultant arachidonate metabolites play a role in the mechanisms of catecholamine release from adrenal chromaffin cells, we studied the effects of phospholipase A2-, cyclooxygenase-, and lipoxygenase-inhibitors on catecholamine release in the primary culture of bovine adrenal chromaffin cells.

Adrenal chromaffin cells were isolated from fresh bovine adrenal glands as described by Kumakura et al. (2). The purified chromaffin cells were cultured by modification of the method described by Kilpatrick et al. (3). The cells were plated on a 16 mm diameter well of a 4-well plastic multidish at a concentration of 3 x 10^5 cells per well in Dulbecco's modified Eagle's Medium, supplemented with 10% fetal calf serum, and cultured at 37°C in an atmosphere of 95% air / 5% CO2. The culture medium contained the following antibiotics: penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (40 μg/ml) and mycostatin (25 units/ml). The medium also contained fluorodeoxyuridine (10 μM), cytosinearabinoside (10 μM) and uridine (5 μM) to prevent the proliferation of non-neuronal cells, and it was replaced every 3 to 4 days. Cell viability estimated by the 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.

Cultured cells were first washed with 95% O2 and 5% CO2 saturated Krebs-Ringer bicarbonate glucose buffer (pH 7.2–7.4) of the following composition (mM): NaCl, 119; KCl, 4.7; CaCl2, 2.2; MgCl2, 1.2; KH2PO4, 1.2; NaHCO3, 25 and glucose, 11 containing 0.5% bovine serum albumin, and then they were incubated with one milliliter of the same buffer. To determine the effects of various inhibitors, the cells were preincubated at 37°C for 10 min with different concentrations of mepacrine or p-bromophenacyl...
bromide or for 15 min with nordihydroguaiaretic acid, BW755C, indomethacin and aspirin. After preincubation, carbamylcholine (final concentration: 0.1 mM) or potassium chloride (final concentration: 56 mM) was added to the medium, and the incubation was continued for 10 min to measure catecholamine release. In the cases of p-bromophenacyl bromide and mepacrine, the preincubation medium was changed to a new solution which contained carbamylcholine (0.1 mM) or potassium chloride (56 mM) but no inhibitors. Cellular and released catecholamines (norepinephrine and epinephrine) in the medium were extracted with 0.4N perchloric acid and analyzed with a high performance liquid chromatograph (Waters Assoc., Milford, MA) equipped with an electrochemical detector (Bioanalytical Systems Inc., W. Lafayette, IN) (Felice et al.) (4). Released catecholamines were expressed as the percent of the total cellular catecholamine content. The spontaneous release (about 4% of cellular content) was subtracted from the secretion data.

When the cultured adrenal chromaffin cells were incubated with 0.1 mM carbamylcholine for 10 min, the amount of released catecholamine was 23.4±1.2% of the total cellular catecholamine content. When the cells were pretreated with mepacrine or p-bromophenacyl bromide, the carbamylcholine-induced release was inhibited in a concentration-related fashion (Fig. 1a). Catecholamine release was inhibited to 15% or 20% of the control release either by 0.1 mM mepacrine or p-bromophenacyl bromide. As shown in Fig. 1b, nordihydroguaiaretic acid reduced carbamylcholine-induced catecholamine release in a concentration-dependent manner. BW755C also inhibited the carbamylcholine-induced catecholamine release. Approximately, 80% and 70% inhibitions were observed with 0.1 mM nordihydroguaiaretic acid and 0.1 mM BW755C, respectively.

Indomethacin (1–100 μM) failed to inhibit the carbamylcholine-induced catecholamine release. Aspirin (0.01–1 mM) also showed no inhibitory effect. At a concentration of 0.1 mM, aspirin rather enhanced the carbamylcholine-induced catecholamine release.
When the cells were stimulated with 56 mM potassium chloride for 10 min, the amount of released catecholamine was 12.2±0.6% of the total cellular content. At 0.1 mM mepacrine, p-bromophenacyl bromide, nordihydroguaiaretic acid and BW755C also inhibited the high potassium-induced catecholamine release by 30%, 65%, 60% and 30%, respectively (Table 1). All of the aforementioned inhibitors showed no effects on the viability of cells and the spontaneous release of catecholamines, even at the maximum concentrations which we used (data not shown).

Mepacrine, a phospholipase A<sub>2</sub> inhibitor, prevented the carbamylcholine-induced catecholamine release from cultured adrenal chromaffin cells, consistent with a recent report by Izumi et al. (5). Mepacrine also inhibited the high potassium-induced catecholamine release. High potassium-induced catecholamine release was inhibited only to 70% of the control release by 0.1 mM mepacrine; however, the same concentration of mepacrine inhibited the carbamylcholine-induced catecholamine release by 85%. This may be due to the blockade of the acetylcholine receptor coupled ionic channel by the above drug (6). Moreover, mepacrine may interact with the voltage dependent Ca<sup>2+</sup> channel and also may act as a calmodulin inhibitor. Thus, we examined the effect of p-bromophenacyl bromide which is a direct inhibitor of phospholipase A<sub>2</sub> (7) on catecholamine release. Our experimental results showed that p-bromophenacyl bromide inhibited the carbamylcholine-induced catecholamine release. Moreover, p-bromophenacyl bromide also inhibited the high potassium-induced catecholamine release. These results suggest that an activation of phospholipase A<sub>2</sub> is involved in the cellular mechanism of catecholamine release from the adrenal chromaffin cells.

In general, activation of membrane phospholipase A<sub>2</sub> results in the release of arachidonic acid from membrane phospholipids, and the released arachidonic acid is metabolized through the cyclooxygenase or lipoxygenase pathway. It has been reported that prostaglandin E<sub>2</sub>, a cyclooxygenase product, inhibits acetylcholine-induced catecholamine release from adrenal medulla slices (8). In the present study, 0.1 mM aspirin, a cyclooxygenase inhibitor, slightly enhanced the carbamylcholine-induced catecholamine release. However, another cyclooxygenase inhibitor, indomethacin (1–100 μM), showed no enhancing effect. Moreover, neither indomethacin nor aspirin showed any inhibitory effects on carbamylcholine-induced catecholamine release. Thus, it is unlikely that cyclooxygenase products (such as prostaglandins) play a major role in the regulation of catecholamine release from adrenal

**Table 1. Effects of mepacrine, p-bromophenacyl bromide, BW755C and nordihydroguaiaretic acid on the high potassium-induced catecholamine release from cultured adrenal chromaffin cells**

| Inhibitor                  | % Release   |
|----------------------------|-------------|
| None                       | 12.2±0.60   |
| Mepacrine                  | 8.6±0.60*   |
| p-Bromophenacyl bromide    | 4.2±0.48*   |
| Nordihydroguaiaretic acid  | 4.6±0.50*   |
| BW755C                     | 8.4±0.45*   |

Catecholamine release (10-min period) is expressed as % release which represents the percentage of total cellular catecholamine content. The spontaneous release (about 4% of cellular content) was subtracted from the secretion data. Each value represents the mean±S.E. (n=6). *: P<0.05 compared with the value of catecholamine release induced by potassium chloride alone.
Nordihydroguaiaretic acid and BW755C, which are known to inhibit lipoxygenase or both cyclooxygenase (9) and lipoxygenase (10), inhibited the catecholamine release in a concentration-dependent manner. These lipoxygenase inhibitors also inhibited the high potassium-induced catecholamine release. Therefore, our present results suggest that a lipoxygenase product(s) rather than a cyclooxygenase product(s) plays an important role in the mechanism of catecholamine release from adrenal chromaffin cells.

In conclusion, the present findings suggest that the activation of phospholipase A2 and the resultant arachidonate metabolite(s) formed by the lipoxygenase pathway play a significant role in the cellular mechanism of catecholamine release from bovine adrenal chromaffin cells. However, it does not exclude that these inhibitors have multiple actions on the secretory process. Therefore, further experiments are necessary in order to obtain more direct evidence.

References
1) Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. and Kato, R.: Prevention of glucose-induced insulin secretion by lipoxygenase inhibitor. Eur. J. Pharmacol. 78, 225–227 (1982)
2) Kumakura, K., Guidotti, A. and Costa, E.: Primary cultures of chromaffin cells: Molecular mechanisms for the induction of tyrosine hydroxylase mediated by 8-Br-cyclic AMP. Mol. Pharmacol. 16, 865–876 (1979)
3) Kilpatrick, D.L., Ledbetter, F.H., Carson, K.A., Kirschner, A.G., Slepetis, R. and Kirschner, N.: Stability of bovine adrenal medulla cells in culture. J. Neurochem. 36, 679–692 (1980)
4) Felice, L.J., Felice, J.D. and Kissinger, P.T.: Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. J. Neurochem. 31, 1461–1465 (1978)
5) Izumi, F., Yanagihara, N., Wada, A. and Sakurai, S.: Intracellular regulation of exocytosis. In Synthesis, Storage and Secretion of Adrenal Catecholamines, Edited by Izumi, F., Oka, M. and Kumakura, K., p. 159–168. Pergamon Press, Oxford (1982)
6) Tsai, M.C., Oliveira, A.C., Albuquerque, E.X., Eldefrawi, M.E. and Eldefrawi, A.T.: Mode of action of quinacrine on the acetylcholine receptor ionic channel complex. Mol. Pharmacol. 16, 382–392 (1979)
7) Vallee, E., Gougat, J., Navarro, J. and Delahayes, J.F.: Anti-inflammatory and platelet anti-aggregant activity of phospholipase A2 inhibitors. J. Pharmacol. 31, 588–592 (1979)
8) Gutman, Y. and Boonyaviroj, P.: Mechanism of PGE inhibition of catecholamine release from adrenal medulla. Eur. J. Pharmacol. 55, 129–136 (1979)
9) Higgs, G.A., Flower, R.J. and Vane, J.R.: A new approach to anti-inflammatory drugs. Biochem. Pharmacol. 28, 1958–1961 (1979)
10) Hamberg, M.: On the formation of thromboxane B2 and 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (12h-20:4) in tissues from the guine pig. Biochim. Biophys. Acta. 431, 651–654 (1976)