Relationships between Adenylate Cyclase and Na\textsuperscript{+},K\textsuperscript{+}-ATPase in Rat Pancreatic Islets*

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We tested the hypothesis that the adenylate cyclase system and Na\textsuperscript{+},K\textsuperscript{+}-ATPase are reciprocally related in rat pancreatic islets. We studied the effect of theophylline, caffeine, and dibutyryl cyclic AMP on Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in a membrane preparation from collagenase-isolated rat islets.

Theophylline, caffeine, or dibutyryl cyclic AMP, in concentrations of 1 mM, all inhibited Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity (44, 62, and 43%, respectively). Kinetic analysis indicated that theophylline and dibutyryl cAMP inhibit Na\textsuperscript{+},K\textsuperscript{+}-ATPase by different mechanisms; theophylline decreased $V_{\text{max}}$ and decreased apparent $K_{m}$ (ATP), whereas dibutyryl cAMP decreased $V_{\text{max}}$ and increased apparent $K_{m}$ (ATP).

Similar inhibition of Na\textsuperscript{+},K\textsuperscript{+}-ATPase by theophylline or dibutyryl cAMP was noted in a particulate fraction from rat kidney and in a purified porcine brain Na\textsuperscript{+},K\textsuperscript{+}-ATPase preparation.

The adenylate cyclase system and Na\textsuperscript{+},K\textsuperscript{+}-ATPase may act reciprocally in pancreatic islets and in other tissues. In the β cell this relationship may be essential in coordinating consumption of ATP in the stimulated, as opposed to the resting, state.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats (300 g) were allowed food ad libitum.

Materials

Collagenase (from Clostridium histolyticum) was obtained from Boehringer Mannheim. Other reagents were obtained from Sigma. Water for the enzyme assay solutions was passed through an Aquafine UV water sterilizer and a Corning MP-12A Megapure distilling apparatus. The resulting water had a specific resistance of 18 megohm/cm at 25 °C.

Islet Isolation

Anesthesia was induced by intraperitoneal sodium pentobarbital, 60 mg/kg. Islets were isolated by a method modified from those of Lacy and Kostianovsky (12) and Gotoh et al. (13). The gastrointestinal tract was resected en bloc, and the pancreas was distended via the biliary duct with 15 ml of Hanks’ balanced salt solution, containing collagenase (2 mg/ml), and digested at 37 °C for 28 min without agitation. The digestate was washed four times with Hanks’ balanced salt solution. Islets were visualized using a dissecting microscope and picked with a Pasteur pipette.

The preparation of islets for the enzyme assay was as previously described (6) and is summarized below.

Islet Storage

Islets were harvested into a solution containing 0.25 M sucrose and 1 mM Tris-EDTA and frozen at -70 °C until homogenized for the enzyme assay. Islets were collected (250-350/rat) over a few days and pooled into each collecting tube until 1000 islets/tube were accumulated.

Membrane Preparation

Islets—One thousand islets were used for each experiment. Islets were thawed and homogenized in a 2-ml Ten Broeck tissue grinder with six or seven vigorous passes of the plunger. The homogenates were centrifuged at 35,000 × g for 35 min at 5 °C. The pellet was resuspended in 2.6 ml of cold 1 mM Tris-EDTA (pH 7.4) and divided

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1 The abbreviation used is: Bt-cAMP, dibutyryl cyclic AMP.

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Enzyme Analysis

Na\textsuperscript{+}, K\textsuperscript{+}-ATPase—Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity was defined as the difference in inorganic phosphate (Pi) liberated from ATP in the presence and absence of Na\textsuperscript{+} and K\textsuperscript{+}. Previous studies in our laboratory have demonstrated no significant difference between basal enzyme activity defined this way and ouabain-inhibitable activity (6). Since effects of any chemical upon Na\textsuperscript{+}, K\textsuperscript{+}-ATPase could be due to interactions with ouabain, rather than to effects of such chemicals, we have used solutions that do not contain the cardiac glycoside.

The incubation solution consisted of 0.5 mM EDTA, 21 mM glycyglycine, 21 mM histidine, 3.6 mM MgCl\textsubscript{2} with or without 100 mM NaCl, and 20 mM KCl. An islet membrane aliquot (0.1 ml) was added to 0.5 ml of the incubation solution. Ten microliters of test drug in 3 mM Tris buffer, or buffer alone, was then added. Samples were mixed and incubated for 10 min. Next, 50 ml of Tris-ATP (final concentration 3 mM) was added to all tubes, and the samples were incubated in a 37 °C shaker water bath for 10 min. The reaction was stopped by immersing the tubes in an ice bath and adding 50 ml of ice-cold 50% trichloroacetic acid. Samples were centrifuged at 1100 X g for 10 min, and supernatants were assayed spectrophotometrically for inorganic phosphate by the method of Fiske and Subbarow (14). Corrections were made for spontaneous hydrolysis of ATP with tubes containing ATP but no tissue. Mg\textsuperscript{2+}-ATPase activity was defined as the amount of P\textsubscript{i} liberated from ATP in the presence of Mg\textsuperscript{2+} (without Na\textsuperscript{+} and K\textsuperscript{+}). Enzyme activity was expressed as micromoles of P\textsubscript{i}/mg of protein/h.

Purified porcine cerebral cortical Na\textsuperscript{+}, K\textsuperscript{+}-ATPase (catalog no. A7510) was obtained from Sigma. This enzyme was diluted with Tris buffer or buffer alone, was then added. Samples were mixed and incubated for 10 min. Next, 50 ml of Tris-ATP (final concentration 3 mM) was added to all tubes, and the samples were incubated in a 37 °C shaker water bath for 10 min. The reaction was stopped by immersing the tubes in an ice bath and adding 50 ml of ice-cold 50% trichloroacetic acid. Samples were centrifuged at 1100 X g for 10 min, and supernatants were assayed spectrophotometrically for inorganic phosphate by the method of Fiske and Subbarow (14). Corrections were made for spontaneous hydrolysis of ATP with tubes containing ATP but no tissue. Mg\textsuperscript{2+}-ATPase activity was defined as the amount of P\textsubscript{i} liberated from ATP in the presence of Mg\textsuperscript{2+} (without Na\textsuperscript{+} and K\textsuperscript{+}). Enzyme activity was expressed as micromoles of P\textsubscript{i}/mg of protein/h.

Enzyme Kinetic Studies—Kinetic experiments were performed by measuring Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity at increasing concentrations of ATP in the presence or absence of the test substance.

Protein Assay—Protein was determined by the method of Lowry et al. (15).

Statistical Analysis—Values are expressed as the mean ± S.E. Comparisons between test substances and appropriate controls were made using Student’s unpaired t test.

RESULTS

The dose response for inhibitory effects of theophylline upon islet Na\textsuperscript{+}, K\textsuperscript{+} ATPase is shown in Fig. 1A. Control activity of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase was 8.39 ± 0.87 µmol of P\textsubscript{i}/mg of protein/h. Over a dose range of 0.5–5 mM, theophylline evoked a dose-dependent inhibition of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity. Empirically, the approximate half-maximal inhibitory dose of theophylline was found to be 1 mM. Fig. 1B shows that control Mg\textsuperscript{2+}-ATPase activity was 30.77 ± 3.57 µmol of P\textsubscript{i}/mg of protein/h, and this activity was not affected by theophylline.

The effects of other substances that alter the activity of the adenylate cyclase system are shown in Fig. 2A. Caffeine, as well as BtζAMP, inhibited Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity, 1 mM caffeine effecting 62% inhibition and 1 mM BtζAMP causing 43% reduction in specific activity, about equipotent with 1 mM theophylline (44%). Neither of these substances changed Mg\textsuperscript{2+}-ATPase activity (control 31.56, caffeine 34.16, BtζAMP 29.49 µmol of P\textsubscript{i}/mg of protein/h (Fig. 2B).

To study the mechanism by which theophylline and BtζAMP inhibit Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, enzyme kinetic studies were performed in the presence and absence of 1 mM of these substances. When presented as an Eadie-Hofstee plot (Fig. 3), theophylline decreased both V\textsubscript{max} and apparent K\textsubscript{m} (ATP). Similar to the effect of theophylline, BtζAMP decreased V\textsubscript{max}, however, in contrast to theophylline, BtζAMP increased apparent K\textsubscript{m} (ATP).

Table I summarizes these kinetic findings with statistical analysis. Apparent K\textsubscript{m} was determined for individual experiments. The mean value for theophylline and BtζAMP was compared with that of control incubations using the unpaired t test. The changes in V\textsubscript{max} and apparent K\textsubscript{m} described above, were statistically significant.

Table II summarizes effects of theophylline and BtζAMP upon renal and brain Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, and Mg\textsuperscript{2+}-ATPase activities. These compounds had effects upon Na\textsuperscript{+}, K\textsuperscript{+}-ATPase which simulated those observed in the islet preparation. Theophylline and BtζAMP tended to enhance renal Mg\textsuperscript{2+}-ATPase, but this was not significant. This tendency was not seen in either islets or brain.
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FIG. 2. A, inhibition by caffeine (1 mM) and Bt2cAMP (1 mM) of rat pancreatic islet Na⁺,K⁺-ATPase. Results shown as mean ± S.E. *, p < 0.001 versus control. B, lack of effects of caffeine (1 mM) and Bt2cAMP (1 mM) on rat pancreatic islet Mg²⁺-ATPase. Results shown as mean ± S.E.

DISCUSSION

Both theophylline and Bt2cAMP inhibited islet Na⁺,K⁺-ATPase activity; however, neither acted by classical competitive or noncompetitive mechanisms. Both substances reduced the observed maximum velocity of the reaction. However, theophylline increased and Bt2cAMP decreased the measured affinity for ATP. Neither substance affected Mg²⁺-ATPase activity.

In the presence of glucose, theophylline, and Bt2cAMP both depolarize pancreatic B-cells and induce hormone release (9). Depolarization, accompanied by insulin secretion, is also produced by ouabain, the classic inhibitor of Na⁺,K⁺-ATPase, and the electrical and secretory patterns can be quite similar to those with theophylline, depending on the concentration of ouabain and background glucose (16); thus, there may be an interplay between these substances upon the secretory mechanism, which these present studies begin to investigate.

Glucose itself increases islet cAMP, probably via metabolic pathways, rather than directly (17). Our previous data (6) indicate that glucose can inhibit islet Na⁺,K⁺-ATPase. These present experiments point out that there can be indirect inhibition of Na⁺,K⁺-ATPase via cAMP production.

Current ideas about glucose-induced insulin release suggest that a major depolarizing and secretogenic step is ATP blockage of K⁺ channels (18, 19). Using ⁸⁷Rb⁺ as a tracer which mimics K⁺, at low (3 mM) background glucose levels, both theophylline and Bt2cAMP reduce islet K⁺ efflux (9). Reduced K⁺ efflux is not mandatory for insulin release, since ouabain potently increases islet K⁺ efflux. All three substances (theophylline, Bt2cAMP, and ouabain) enhance biphasic insulin release when optimal background glucose is present (16). Secretory properties of cholinergic stimuli are characterized by ouabain-like induction of K⁺(Rb⁺) efflux. This has been attributed to effects on K⁺(Rb⁺) permeability, but Na⁺,K⁺-ATPase activity was not measured (20). We have described the inhibition of islet Na⁺,K⁺-ATPase by arginine and ouabain (6). We propose that the secretogenic properties of

TABLE I

| Na⁺,K⁺-ATPase inhibition by theophylline and Bt2cAMP | Data are derived from Fig. 3. Vₘₐₓ (μmol Pi/mg protein/h) describes the ordinate intercept of the v versus v/s plot. |
|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| **Vₘₐₓ (μmol Pi/mg protein/h)** | **Apparent Kₐ (ATP)** |
| Control | 12.04 ± 0.21 | 589.4 ± 49.0 |
| Theophylline (1 mM) | 6.15 ± 0.17∗ | 394.7 ± 24.9∗ |
| Bt2cAMP (1 mM) | 6.77 ± 0.46∗ | 588.3 ± 65.0∗ |

* p < 0.01 versus control.

TABLE II

| Na⁺/K⁺/Mg²⁺ activity | Kidney | Brain |
|-----------------------|--------|-------|
| Control | 39.8 ± 2.85/25.5 ± 2.46 | 22.9 ± 1.80/1.64 ± 0.26 |
| Theophylline | | |
| 1 mM | 25.6 ± 0.42/30.9 ± 1.00 | 16.2 ± 1.46/1.28 ± 0.50 |
| 2 mM | 21.0 ± 1.07/31.9 ± 1.28 | 12.3 ± 2.14/1.14 ± 0.23 |
| Bt2cAMP | | |
| 1 mM | 23.5 ± 0.66/29.3 ± 1.20 | 14.8 ± 0.49/1.32 ± 0.20 |
| 2 mM | 18.2 ± 2.05/34.0 ± 3.19 | 8.9 ± 1.59/1.9 ± 0.58 |

* Statistically significant from control, p at least < 0.05.
cholinerger agonists and arginine may be due to ouabain-like effects on the islet B-cell plasma membrane.

Glucose and leucine inhibit K⁺ efflux, and this is attributed to closure of ATP-sensitive K⁺ channels (18-21). Similarly, tolbutamide, the sulfonyl urea secretory stimulant, can close K⁺ channels, while effects of an inhibitor, diazoxide, have been ascribed to opening of these channels (22).

In contrast, the amino acid secretagogues arginine and lysine increase K⁺ efflux (23), but the authors theorize that the depolarizing and secretogenic effects of catolic amino acids are due to accumulation of these substances in B-cells.

Based on our observations, we suggest that Na⁺,K⁺-ATPase and adenylate cyclase act reciprocally during the process of secretion, since theophylline and β-2CAMP inhibit Na⁺,K⁺-ATPase. Reciprocity is further suggested by experiments in pancreatic islets showing that ouabain will enhance glucose-induced cAMP when a CAMP phosphodiesterase inhibitor is present (24). Reciprocity of these two enzyme systems has been reported in non-islet tissues; cAMP has been shown to inhibit Na⁺,K⁺-ATPase in rat brain (25). This is consistent with our present findings in porcine cerebral cortex and in rat renal particulate fractions. Thus, it seems reasonable to consider modulation of Na⁺,K⁺-ATPase by the adenylate cyclase system to be widespread in a variety of tissues.

Because adenylate cyclase activation is associated with insulin release, this enzyme system should utilize ATP preferentially during secretion. On the other hand, since, in a variety of tissues, Na⁺,K⁺-ATPase is associated with cellular polarization, this enzyme would utilize ATP to maintain the nonsecretory state.

Suppression of ATPases would be one mechanism to provide ATP at the plasma membrane for closure of ATP-sensitive K⁺ channels, adenylate cyclase activity, phosphatidylinositol turnover, and a variety of other cell functions. Some secretagogues might stimulate secretion in a ouabain-like fashion. These would enhance K⁺ efflux. Others, like glucose, while suppressing Na⁺,K⁺-ATPase (6) but also providing ATP during oxidation might evoke more intricate interactions with other islet structures, ion channels, and metabolic pathways.

REFERENCES

1. Winegrad, A I (1987) Diabetes 36, 396-406
2. Jørgensen, P L (1982) Biochem. Biophys. Acta 694, 27-68
3. Smith, T W (1988) N Engl J Med 318, 358-365
4. Yun, E S (1978) Neuroscience 3, 367-384
5. Lacy, P E (1970) Diabetes 19, 895-905
6. Levin, S R, Kasson, B G, and Drews, J F (1979) J Clin Invest 62, 692-701
7. Fagin, J A, Ikura, K, and Levin, S R (1987) Diabetes 36, 1448-1452
8. Ikura, K, and Levin, S R (1983) J. Membr. Biol. Transp 19, 993-997
9.Henquen, J C (1985) Acta Physiol. Soc. Scandinavica 16, 37-48
10. Charles, M A, Lawecks, R, Schmid, F G, Forsham, P H, and Grodsky, G M (1973) Science 179, 569-571
11. Valverde, I (1979) Science 206, 225-227
12. Lacy, P E, and Kostanovsky, M (1967) Diabetes 16, 35-39
13. Gotoh, M, Makihata, T, Sato, T, Porter, J, Bonner-Weir, S, O'Hara, C, and Monaco, A P (1987) Transplantation 43, 725-730
14. Fuske, C H, and Subbarow, Y (1925) J Biol Chem 66, 375-400
15. Lowry, O H, Rosebrough, N J, Farr, A L, and Randall, R J (1951) J. Biol Chem 193, 265-275
16. Henquen, J C, and Meissner, H P (1962) J. Biol Chem 193, 265-275
17. Larkin, R G, and Dunlop, M E (1984) in Receptors, Membranes and Transport Mechanisms in Medicine (Doyne, A E, and Mendelsohn, F A O, eds) pp 137-147, Elsevier Science Publishers B V, Amsterdam
18. Cook, D L, and Hales, C N (1984) Nature 311, 271-273
19. Ashcroft, F M, Harrison, D E, and Ashcroft, S J H (1984) Nature 312, 446-448
20. Henquen, J C, Garcia, M C, Bozem, M, Hermans, M P, and Nenquen, M (1988) Endocrinology 122, 2134-2142
21. Ribelet, B, and Can, S (1987) Proc Natl Acad Sci U S A 85, 1721-1725
22. Trube, G, Roseman, P, and Ohno-Shosaku, T (1986) Pflugers Arch 407, 493-499
23. Charles, S, Tamagawa, T, and Henquen, J C (1982) Biochim Biophys Acta 705, 301-308
24. Gagerman, E, Hellman, B, and Takeda, I-B (1979) Endocrinology 104, 1000-1002
25. Sen, A, K, Murphy, R, Stancer, H, Awad, G, Godse, B, and Grof, P (1976) in Membranes and Diseases (Rola, J, Hoffman, J, and Leaf, A, eds) pp 109-122, Raven Press, New York
26. Cleland, W W (1970) in The Enzymes (Boyer, P D, ed) 3rd Ed., Vol II, pp 89-81, Academic Press, New York.
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