CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY IN NORMAL MOUSE PANCREATIC ISLETS

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1. Introduction

The potentiating effects of methylxanthines on insulin release in response to glucose and other agents have been attributed to an increase in the β-cell concentration of cyclic 3′,5′-AMP following inhibition of cyclic 3′,5′-AMP-phosphodiesterase (EC 3.1.4.c) [1, 2]; conversely the inhibition of glucagon-stimulated insulin release by imidazole has been ascribed to a stimulation of the β-cell phosphodiesterase [1]. However, very little is known about cyclic 3′,5′-nucleotide phosphodiesterase in this tissue; although extracts of normal mouse islets have been shown to catalyse the breakdown of radioactive cyclic 3′,5′-AMP [3] there are no data on the kinetics of the enzyme(s) responsible. Some kinetic information has been presented for the phosphodiesterase activity in a hamster islet carcinoma [4] but it is not known whether such results are typical of the normal β-cell. In the present study we describe a new sensitive assay for phosphodiesterase activity and report evidence that suggests that normal mouse islets of Langerhans contain at least two forms of cyclic nucleotide phosphodiesterase, with widely different K_m's for cyclic 3′,5′-AMP. Data are also given on the sensitivity of the low K_m phosphodiesterase towards some agents that might affect insulin release through modulation of phosphodiesterase activity.

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2. Methods

Islets were isolated by a collagenase method [5] from the pancreata of 3–4 week-old male white mice. Islet extracts were prepared by sonication (5–10 sec at position 2 on a Soniprobe, Dawe Instruments) of 150–200 islets in 200 μl of cold 0.1 M triethanolamine, 10 mM $MgSO_4$, 0.5 mM EDTA, pH 7.7. The sonicate was dialysed for 1–3 hr at 4° against 500 ml of the same buffer and then frozen. The frozen extracts were stored at −20° until used (within 4 days).

For assay of cyclic nucleotide phosphodiesterase activity, the islet extracts were diluted with buffer to a concentration equivalent to about 0.4 islets/μl. 10 μl of the diluted extract were incubated at 37° with 50 μl buffer containing $MgSO_4$ and EDTA as above and cyclic 3′,5′-AMP as required. Other additions are as described in the tables and figures. After 60 min the reaction was stopped by heating the reaction tubes at 80° for 4 min. The 5′-AMP formed was then converted to ATP by adding 20 μl of buffer containing 3 mg/ml phosphoenolpyruvate, 15 units/ml pyruvate kinase and 18 units/ml myokinase. The ATP formed was finally assayed photokinetically by a luciferin–luciferase method [6]. Tissue and medium blanks were carried through the whole procedure. The concentration of ATP in standards as well as that of cyclic 3′,5′-AMP in the reaction medium was spectrophotometrically determined (A_{260}) in each experiment.

Table 1
Recovery of 5′-AMP as ATP.

5′-AMP added (pmole)	5′-AMP recovered as ATP	
	Enzyme absent (pmole)	Enzyme present (pmole)
13.9	15.8 ± 2.3 (114%)	13.8 ± 1.5 (99%)
27.8	27.4 ± 1.2 (99%)	26.3 ± 2.1 (95%)

Islet extract (enzyme present) or buffer (enzyme absent) was incubated for 60 min at 37° with the listed amounts of 5′-AMP. Recoveries of 5′-AMP as ATP are given as mean values ± S.E. for 8 observations representing duplicate determinations of 4 different incubates. The islet extract used split 3′,5′-AMP at a rate of 6.7 pmole/islet per hr at 2.4 μM cyclic 3′,5′-AMP and at a rate of 9.2 pmole/islet per hr at 19.3 μM cyclic 3′,5′-AMP. These enzyme activities correspond to a total production of 36.2 and 50.2 pmole of 5′-AMP.

3. Results

The assay that we have used for the measurement of islet phosphodiesterase activity is based in principle on a method described [7] for assay of cyclic 3′,5′-AMP. Control experiments showed that the rate of formation of 5′-AMP from cyclic 3′,5′-AMP was constant for at least 1 hr under the conditions used and was linearly related to the concentration of islet extract added: the extent of breakdown of cyclic 3′,5′-AMP was less than 10% of the amount initially present. It was particularly important to verify that the islet extract did not catalyse significant loss of 5′-AMP [3, 8]. Table 1 shows that the mouse islet extracts did not significantly degrade 5′-AMP at concentrations similar to those arising from phosphodiesterase activity. The data given in table 1 also demonstrate that there was a quantitative conversion of 5′-AMP to ATP in the second stage of the assay procedure.

Rates of hydrolysis (v) of varying concentrations of cyclic 3′,5′-AMP (S) are shown in fig. 1 plotted in the form v against v/S. Such plots were curvilinear suggesting the presence in the extracts of two forms of phosphodiesterase with different K_m -values for cyclic 3′,5′-AMP. Estimates of the apparent K_m derived from four such plots yielded mean values of 10 and 500 μM: mean values of V_{max} for the two phosphodiesterase activities were 11 and 39 pmole/islet/hr equivalent to 0.37 and 1.33 μmole/min/g dry islet, assuming a mean islet weight of 0.5 μg [9].

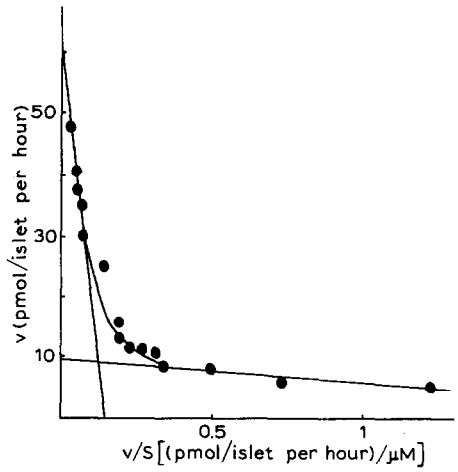

Fig. 1. Dependence of cyclic nucleotide phosphodiesterase activity (pmole of 3′,5′-AMP split/islet per hr) on cyclic 3′,5′-AMP concentration. The intercepts of the straight lines with the ordinate and the abscissa were used to estimate kinetic parameters, assuming the presence of two enzymes. K_{m_1} = 3.5 μM; V_{max_1} = 9.5 pmole/islet per hr; K_{m_2} = 0.5 mM; V_{max_2} = 53 pmole/islet per hr.

Over the range of cyclic 3′,5′-AMP concentrations from 1 to 25 μM the low K_m activity is the predominant contributor to the reaction rate. The effect of caffeine (5.3 mM) on the low K_m activity is shown in fig. 2. Caffeine was a competitive inhibitor with an apparent K_i of 1 mM.

The effects of some other agents on the phosphodiesterase activity at a cyclic 3′,5′-AMP concentration of 2 μM are shown in table 2. Glucose (16.5 mM), arginine (9 mM) and $CaCl_2$ (1.7 mM) had no significant effects ($P > 0.01$). 3-Isobutyl-1-methylxanthine was an extremely potent inhibitor of mouse islet phosphodiesterase activity, producing 89% inhibition at a concentration of 0.8 mM. Phosphodiesterase activity was also inhibited by glibenclamide (8.7 μg/ml) and by cyclic 3′,5′-GMP (8 μM). Imidazole (2.6 mM) significantly stimulated phosphodiesterase activity.

4. Discussion

The probable importance of cyclic 3′,5′-AMP in potentiating effects of glucose and other secretagogues on insulin release suggests the need for information on

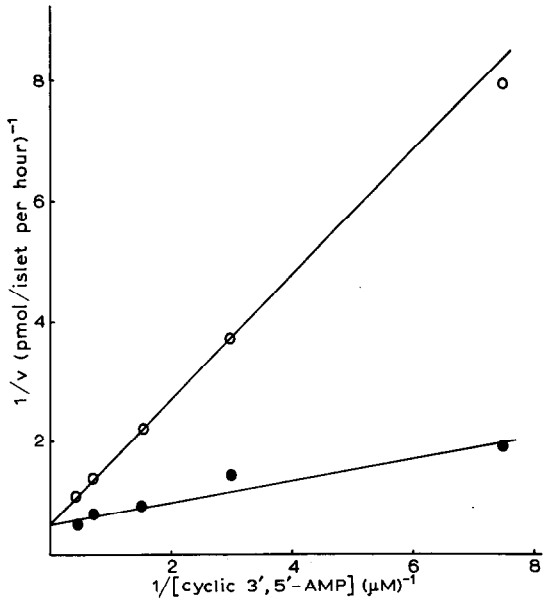

Fig. 2. Inhibition of cyclic nucleotide phosphodiesterase activity (pmole of 3',5'-AMP split/islet per hr) by caffeine ((●–●–●): zero; (o–o–o): 5.3 mM) at low cyclic 3',5'-AMP concentrations. Results are plotted in double-reciprocal form.

Table 2
Effects of various compounds on cyclic nucleotide phosphodiesterase activity.

Compound	Cyclic 3',5'-AMP split/islet per hr (pmole)	Change (%)
None (control)	2.41 ± 0.07	–
$CaCl_2$, 1.7 mM	2.16 ± 0.05	−10
Glucose, 16.5 mM	2.36 ± 0.05	− 2
Arginine, 9.1 mM	2.51 ± 0.06	+ 4
Cyclic 3',5'-GMP, 7.9 μM	1.28 ± 0.05	−47***
3-Isobutyl-1-methyl-xanthine, 0.8 mM	0.26 ± 0.08	−89***
Glibenclamide, 8.7 μg/ml	1.93 ± 0.04	−20***
Imidazole, 26 mM	2.79 ± 0.08	+16**

All tubes contained 2.0 μM cyclic 3',5'-AMP and enzyme extract corresponding to 5.5 islets. Results are given as mean values ± S.E. for 8 observations representing duplicate determinations of 4 different incubates.
** $P < 0.01$
*** $P < 0.001$

the enzymes involved in the formation and breakdown of this nucleotide in the β-cell. In the present study of cyclic 3',5'-AMP phosphodiesterase, the problem of assaying the kinetics of this activity in the small amounts of islet tissue isolable by current methods (< 1 mg wet tissue) has been overcome by the development of a sensitive assay for phosphodiesterase. A limitation of our results arises from the fact that mouse islets contain cell-types in addition to the β-cells; however the β-cells account for 80% of the mouse islet cells [10] and it seems reasonable to assume that the activity that we study is characteristic of the β-cell. Subject to this reservation, the properties of the islet phosphodiesterase show similarities to the properties of this activity described in other mammalian tissues. Thus inhibition of cyclic 3',5'-AMP hydrolysis by cyclic 3',5'-GMP has been observed for a low K_m phosphodiesterase prepared from thymic lymphocytes [11] and also for a purified phosphodiesterase prepared from rabbit skeletal muscle [12]. A stimulation of brain phosphodiesterase by imidazole has also been reported [13].

Of most significance for the problem of the control of insulin release are the effects of glibenclamide and methylxanthines. Inhibition of phosphodiesterase by sulfonylureas has previously been observed in preparations of phosphodiesterase from kidney and pancreas [14] and from an islet cell tumour from the hamster [4]. In the present study, glibenclamide was also found to inhibit phosphodiesterase activity in normal mouse islets. The question arises whether this effect may provide a basis for the insulin-secretory activity of the sulfonylureas. There are no published data on the effect of sulfonylureas on the concentration of cyclic 3',5'-AMP in islets and therefore direct evidence for this hypothesis is lacking. One objection to such a mechanism is the recent demonstration that sulfonylureas do not readily penetrate into the islet-cells of obese-hyperglycemic mice [15] suggesting that their locus of action may be the β-cell membrane; the intracellular localisation of phosphodiesterase in islets is not known.

Caffeine, which potentiates effects of glucose on insulin release from mouse islets [16] and elevates islet cyclic 3',5'-AMP concentration [2, 17] was found to be a competitive inhibitor of the low K_m

phosphodiesterase in islets with an apparent K_i of 1 mM. This result is consistent with the effect of caffeine on insulin release being mediated by cyclic 3′,5′-AMP, although additional or alternative modes of action can not be excluded. 3-Isobutyl-ethylxanthine was a more potent inhibitor of phosphodiesterase than was caffeine; this finding may explain the relative efficiency of these agents in elevating cyclic 3′,5′-AMP concentration in rat [2] or mouse [17] islets.

Kinetic analysis of the cyclic 3′,5′-AMP phosphodiesterase suggests the presence in mouse islets, as in a number of other mammalian tissues [18–20], of two forms of phosphodiesterase with widely different K_m values for cyclic 3′,5′-AMP. Since in mouse islets the concentration of cyclic 3′,5′-AMP is approximately 0.5 μM [14], the low K_m phosphodiesterase may be of most significance for the physiological regulation of islet cyclic 3′,5′-AMP concentration. Further characterisation of these activities may require separation and purification.

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References

[1] W. Malaisse, Etude de la secretion Insulinique in vitro (Editions Arscia S.A., Brussels, 1969).
[2] W. Montague and J.R. Cook, Biochem. J. 122 (1971) 115.
[3] T. Atkins and A.J. Matty, J. Endocrin. 51 (1971) 67.
[4] I.D. Goldfine, R. Perlman and J. Roth, Nature 234 (1971) 295.
[5] E. Coll-Garcia and J.R. Gill, Diabetologia 5 (1969) 61.
[6] P.E. Stanley and S.G. Williams, Anal. Biochem. 29 (1969) 381.
[7] R.A. Johnson, J.G. Hardman, A.E. Broadus and E.W. Sutherland, Anal. Biochem. 35 (1970) 91.
[8] P.F. Gulyassy and R.L. Oken, Proc. Soc. Exp. Biol. Med. 137 (1971) 361.
[9] S.J.H. Ashcroft, C.J. Hedeskov and P.J. Randle, Biochem. J. 118 (1970) 143.
[10] S.E. Brolin and C. Hellerstrom, Opuscula Med. 12 (1967) 261.
[11] D.J. Franks and J.P. MacManus, Biochem. Biophys. Res. Commun. 42 (1971) 844.
[12] Y-C. Huang and R.G. Kemp, Biochemistry 10 (1971) 2278.
[13] W.Y. Cheung, Biochim. Biophys. Acta 242 (1971) 395.
[14] G. Brooker and M. Fichman, Biochem. Biophys. Res. Commun. 42 (1971) 824.
[15] B. Hellman, J. Sehlin and I-B. Täljedal, Biochem. Biophys. Res. Commun. 45 (1971) 1384.
[16] S.J.H. Ashcroft, J.M. Bassett and P.J. Randle, Diabetes, in press.
[17] R.H. Cooper, personal communication.
[18] S. Kakiushi, R. Yamazoki and Y. Teshima, Biochem. Biophys. Res. Commun. 42 (1971) 968.
[19] E. Monn and R.O. Christianson, Science 173 (1971) 540.
[20] W.J. Thompson and M.M. Appleman, Biochemistry 10 (1971) 311.