ENHANCEMENT BY D_2O OF GLUCOSE-INDUCED CYCLIC AMP ACCUMULATION IN RAT ISLETS OF LANGERHANS

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1. Introduction

The importance of the microtubular apparatus for the process of exocytosis in secretory organs, including the pancreatic beta-cell, has been inferred from recent studies showing that agents which interfere with microtubular function also influence secretory responses. Such agents may either disrupt microtubules (colchicine, Vinca alcaloids) (for review see [1]), or 'stabilize' them, thus causing reversible functional impairments (D_2O) [2–5].

To investigate possible interrelationships between the adenylate cyclase-cyclic AMP system, microtubular function, and insulin secretion, the effects of deuterated water on cyclic AMP metabolism has been studied in isolated rat islets of Langerhans. Using a prelabeling method for assessing changes in cyclic AMP metabolism, it is shown here that D_2O, in addition to its well established inhibitory effect on insulin release, suppresses the efflux of cyclic nucleotide from the islets. On the other hand, the glucose-induced intra-islet accumulation of cyclic AMP is greatly enhanced. This latter finding is in contradistinction to the moderate effects of D_2O when tested together with a phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine, IBMX) or the adenylate cyclase activator cholera toxin. The results suggest that different compartments of cyclic AMP exist within the beta-cell, a possibly microtubule associated one being stimulated specifically by glucose.

2. Materials and methods

Pancreatic islets were isolated from fed male Sprague–Dawley rats by a modified collagenase technique [6]. Krebs–Henseleit bicarbonate buffer (KHB), containing 2 mg/ml bovine albumin, 10 mM Hepes and – when not otherwise indicated – 3.3 mM glucose was used throughout. All solutions containing the agents to be tested were adjusted to pH 7.4 (pH meter). Islets were pulse-labeled with [2-^{3}H]adenine (100 μCi/ml) for 60 min, washed and incubated in groups of 15 as described previously [7]. Incubations were terminated by boiling, and cyclic AMP purified basically according to the technique of Krishna [8]. Insulin was measured by radioimmunoassay [9,10].

For measurement of phosphodiesterase activity, 250 islets were collected (without prior preincubation) in a small homogenizer containing 0.5 ml of KHB and homogenized manually (10 strokes). 20 μl aliquots were added to incubation tubes containing 25 μl 100 mM Tris–HCl, pH 8.0 and 12 mM $MgCl_2$, 10 μl of [^{3}H]cyclic AMP (200 000 cpm) and 40 μl of cyclic AMP in various concentrations. The time between the onset of homogenization and the start of the incubations was kept constant to 1 min. Incubations were carried out for 20 min at 37°C and terminated by boiling. Control experiments showed the reaction rate to be linear for at least 30 min incubation. After cooling, 20 μl of the nucleotidase-containing *Crotalus atrox* venom (1 mg/ml) was added and incubations continued for 20 min. 100 μl

of 10^{-2} M adenosine was then added, followed by 1 ml of a resin slurry (50% H_2O, v/v) of Biorad AG 1-X2-400 mesh. After centrifugation, aliquots of the supernatant were counted for [^{3}H]adenosine. From parallel incubations, where labeled adenosine was measured before and after resin addition, it was possible to correct for the substantial (64.1%) but constant (range of triplicates 0.6%) binding of adenosine to the resin.

Crude collagenase was purchased from Worthington Biochemical Corporation, Freehold, N.J., USA; bovine albumin (Fraction V) from Armour Co, Eastbourne, UK; D_2O (99.8%) from Norsk Hydro, Oslo, Norway; 3-isobutyl-1-methylxanthine (IBMX) was obtained from Aldrich Co, Milwaukee, Wis., USA; cyclic AMP, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and *Crotalus atrox* venom from Sigma Chemicals, Saint Louis, Mo., USA; [2-^{3}H]adenine (specific activity 31.7 Ci/mmol) and cyclic [^{3}H]AMP (specific activity 37.7 Ci/mmol) from New England Nuclear, Dreiechenhain, West Germany. Rat insulin, used as a standard in the immunoassay, was a kind gift of Dr J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark.

3. Results

The stimulatory effects of glucose, tested together with a submaximal concentration (0.1 mM) of the phosphodiesterase inhibitor (IBMX), both on cyclic AMP accumulation and insulin release are evident from fig.1. In agreement with previous results [2], the glucose-induced intracellular rise in cyclic AMP was more marked after 15 than 60 min incubation, while the cyclic nucleotide and insulin continued to accumulate in the incubation medium throughout the incubation period. The addition of D_2O at a final

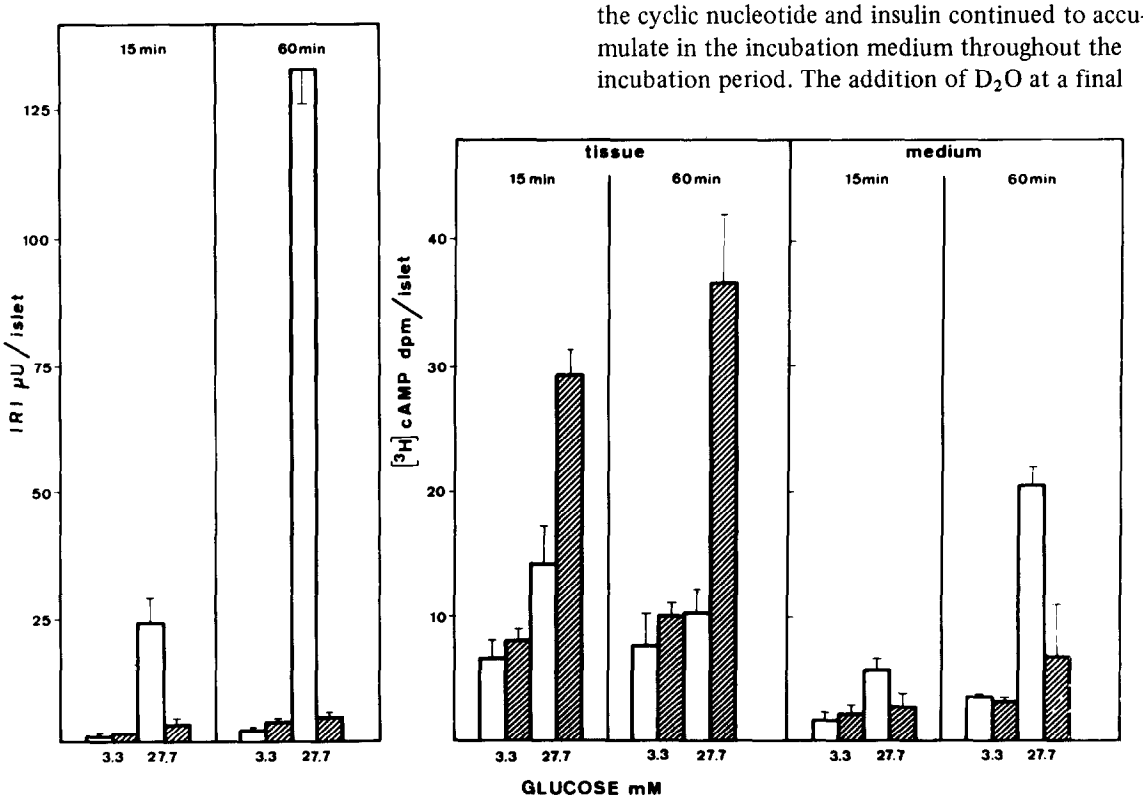

Fig.1. Effects of 50% D_2O (hatched columns) in the presence of 3.3 or 27.7 mM glucose on insulin release (IRI) (left panels) and cyclic [^{3}H]AMP accumulation in islets and incubation media (middle and right panels, respectively) after 15 and 60 min of incubation. 0.1 mM IBMX was included in all incubations. Mean ± S.E.M. of 4 complete experiments. Effects of D_2O together with 27.7 mM glucose were significant on IRI ($p < 0.02$ and < 0.001), on intra-islet ($p < 0.005$ and < 0.025) and medium cyclic [^{3}H]AMP ($p < 0.025$ and < 0.05) for 15 and 60 min of incubations, respectively.

Table 1

Effects of D_2O on cyclic [^{3}H]AMP accumulation and insulin release (IRI) induced by glucose, IBMX or cholera toxin. Incubation time was 30 min. Insulin was measured in 4 out of 5 otherwise complete experiments. Results are given as mean ± S.E.M.

Glucose (mM)	IBMX (1.0 mM)	D_2O (50%)	Cholera toxin (5 μg/ml)	c[^{3}H]AMP dpm/islet tissue	% effect of D_2O[c]	c[^{3}H]AMP dpm/islet medium	% effect of D_2O[c]	IRI μU/islet	% effect of D_2O[c]
3.3	–	–	–	3.06 ± 0.60		1.46 ± 0.24		1.05 ± 0.12	
27.7	–	–	–	6.82 ± 1.10		3.48 ± 0.56		23.22 ± 3.57	
3.3	–	+	–	3.49 ± 0.51	+ 14	2.00 ± 1.27	+ 37	1.02 ± 0.13	– 3
27.7	–	+	–	10.70 ± 1.98[a]	+ 92[c]	2.02 ± 0.98	– 99[c]	4.4 ± 2.70[b]	– 85[c]
3.3	+	–	–	21.84 ± 2.45		5.02 ± 0.94		2.12 ± 0.38	
27.7	+	–	–	35.02 ± 3.01		13.46 ± 0.94		56.45 ± 7.31	
3.3	+	+	–	34.98 ± 3.92[b]	+ 60	0.46 ± 0.31[a]	– 92	1.17 ± 0.34	– 45
27.7	+	+	–	62.38 ± 8.60[b]	+ 108[c]	0.46 ± 0.20[b]	– 100[c]	1.05 ± 0.20[b]	– 100[c]
3.3	+	–	+	32.00 ± 3.72		5.80 ± 0.89		2.15 ± 0.46	
3.3	+	+	+	38.50 ± 5.02	+ 20	5.24 ± 0.93	– 10	2.75 ± 0.42	+ 28

[a] $p < 0.05$.

[b] $p < 0.01$, significance of paired differences (in absolute values) from the appropriate control.

[c] Calculated from the mean differences with and without glucose 27.7 mM.

concentration of 50% caused nearly complete inhibition of the glucose-induced insulin release as well as of the efflux of cyclic [^{3}H]AMP. In contrast to these inhibitory effects, D_2O enhanced markedly the glucose effect on intracellular cyclic AMP, this action augmenting with time (105% and 258% increment after 15 and 60 min incubation, respectively).

In table 1, the effects of D_2O on glucose are compared with those of two other stimulators of islet cyclic AMP, a high concentration of IBMX (1.0 mM) and cholera toxin. The efflux of cyclic [^{3}H AMP induced by the former two agents, as well as insulin release, were depressed by D_2O, while no significant changes were seen in the presence of cholera toxin. D_2O had a pronounced enhancing effect on glucose-induced intracellular accumulation of cyclic AMP, while it augmented the effects of IBMX to a modest extent, and did not change significantly that of cholera toxin. Thus, in the presence of 1.0 mM IBMX, D_2O increased the intracellular radioactive nucleotide by 60%, while the effect of cholera toxin was augmented by 20% (not significant), and the action of 27.7 mM glucose was amplified 2-fold.

The effect of D_2O on the phosphodiesterase activity of islet homogenates is shown in fig.2. At all substrate concentrations tested (2.1–0.26 μM cyclic AMP) a small inhibition (mean 14%) was noted in the presence of 50% of D_2O.

4. Discussion

It is a common finding to all tissues tested so far that stimulation of intracellular cyclic AMP levels is accompanied by release of the nucleotide to the extracellular space. The present report adds deuterated water to a number of agents shown in other biological systems to inhibit this release [11–14]. As documented for D_2O, some of these agents also increase intracellular levels of nucleotide. In addition, without any obvious parallelism to other studies, we report that the intracellular augmentation of cyclic AMP by D_2O

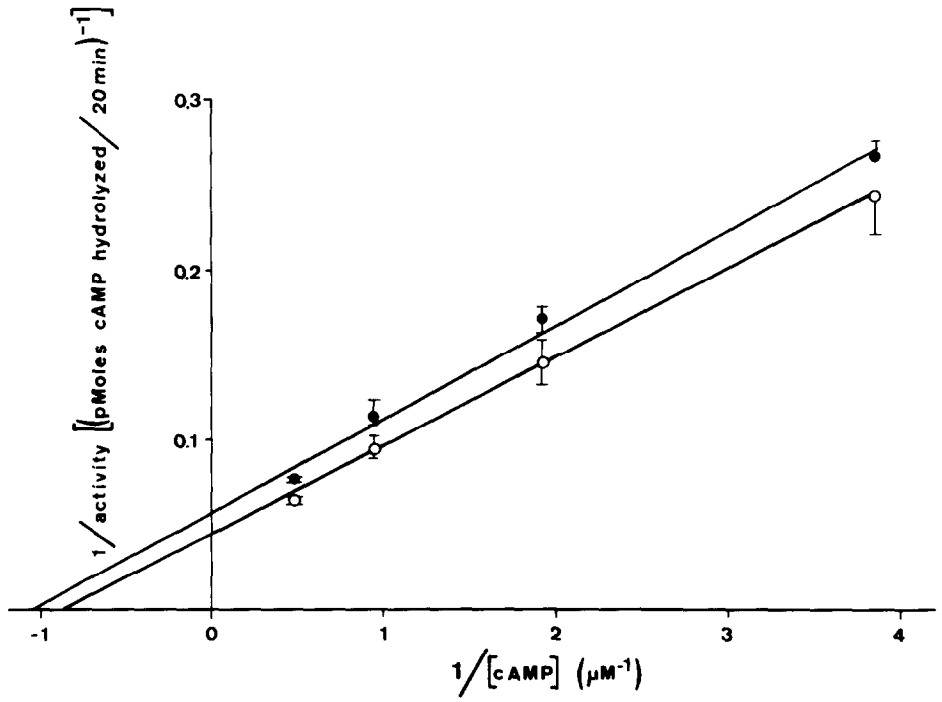

Fig.2. Effects of 50% D_2O (closed circles) on the phosphodiesterase activity in islet homogenates. Control values are denoted by open circles. Results are presented as a double reciprocal plot where the mean ± S.E.M. (of 3 complete experiments) are indicated together with the lines of best fit, obtained by the method of least squares.

depends on the initial stimulus of a cyclic nucleotide response in the islets, the glucose response being amplified to a much higher degree than that of IBMX.

Our findings with cholera toxin should be interpreted with caution. Deuterated water neither influenced the effect of cholera toxin on release nor on intra-islet cyclic AMP. This lack of effect, which is in contradistinction to the findings in the presence of 1.0 mM IBMX and a low glucose concentration, may reflect different actions of D_2O when adenylate cyclase activity is basal (IBMX) or stimulated (cholera toxin). However, it should be emphasized that the islets were incubated with cholera toxin for only 30 min, which did not allow the full toxin effect to express itself. Thus, the lag period of toxin activation, together with the time-dependency of the D_2O effect (fig.1) may have rendered our incubation conditions unfavourable for detecting possible effects of D_2O.

The question arises whether intracellular cyclic AMP elevation in response to D_2O and other agents occurs secondarily to inhibition of extruded nucleotide. Although such a possibility cannot be excluded it is not supported by available data indicating that the turnover of cyclic AMP is probably very high in all tissues tested and also in the islet [7]. This would suggest that only a minute fraction of the produced nucleotide per time unit is secreted, the inhibition of which would not appreciably change the intracellular content of cyclic AMP.

The present results render unlikely one obvious possibility of D_2O action on islet cyclic AMP metabolism, namely inhibition of the phosphodiesterase enzymes, the activity of which was inhibited by only about 15% in islet homogenates. Apart from these findings, a tentative explanation of the D_2O effects relies on circumstantial evidence as follows: (1) Although it is conceivable that D_2O interferes with multiple enzymatic processes, the stabilizing effect on microtubules is the most striking and well established one (2,15). (2) Cyclic AMP may interact with microtubules to regulate the turn-over between polymerized and soluble subunits [16]. (3) In islets, obtained under similar conditions as in the present experiments, glucose increases polymerized microtubular subunits [17]. As a working hypothesis we thus suggest that the glucose action on microtubules involves its effect on cyclic AMP, the binding of which may be increased as a result of the decreased turnover between polymerized and soluble microtubular units brought about by D_2O.

Acknowledgements

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