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Uric acid may inhibit glucose-induced insulin secretion via binding to an essential arginine residue in rat pancreatic β-cells

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Abstract—Uric acid (**1a**) suppresses basal insulin release in isolated rat pancreatic islets and inhibition of glucose-stimulated insulin secretion (GSIS) occurs right at hyperuricaemic levels ($\geqslant$0.4 mM). Conversely, 1 mM guanidinium urate (**2a**) was completely ineffective, strongly suggesting that binding to an essential arginine residue triggers the inhibitory effect. A specific recognition of **1a** molecule at the crucial β-cell receptor is probably involved in the blocking glucose signal transduction.

Environmental and immunological factors are intertwined in the destruction of pancreatic β-cells, but the precise mechanisms and mediators of the initial attack are still unknown.[1] Selective destruction of the β-cells by alloxan (**3**) has been a useful tool in diabetes research and the idea that alloxan-like metabolites arising from oxidation of uric acid (**1a**) could conceivably play a part in the aetiology of diabetes has acquired much attention.[2–5] In humans and certain primates, uricase or urate oxidase [EC 1.7.3.3],[6] which catalyses the oxidation of **1a** to allantoin in most mammals, has been lost by some unknown mechanism.[7] Whether this sudden event is beneficial or not, the high serum urate level, in combination with the loss of biosynthesis of ascorbic acid,[8] puts emphasis on its primary antioxidant role in humans.[9,10] It has been proposed that allantoin can be used as a marker of oxidative stress.[11] A high incidence of diabetes has been observed in gout.[12] Although some studies hint at an important association between elevated serum urate and prediabetic state in humans,[13,14] the evidence to support such a hypothesis remains inconclusive. An interesting work by Griffiths[15] on lasting hyperglycaemia induced by **1a** in glutathione-depleted rabbits was difficult to reproduce in other animal models.[3] However, the uricase-inhibited rat model revealed a decrease in serum insulin accompanied by hyperglycaemia.[16] The rapid suppression of basal insulin release in vitro and reversal of the effect by removing **1a** (0.04–0.2 mM) had suggested an interference with insulin release machinery.

We aimed this study to explore the short-term effects of **1a**, its modified counterparts and uricase inhibitors on glucose-stimulated insulin secretion (GSIS) in isolated rat islets. We chose to focus on hyperuricaemic concentrations ($\geqslant$0.4 mM) in examining molecular mechanisms behind the inhibitory effects on insulin secretion.

The effects of exposure to **1a** (15 min at 0–1 mM) on pancreatic β-cell insulin secretion were evaluated. Three batches of five islets were incubated in Krebs bicarbonate buffer (1 ml, 2 mg/ml BSA) at 37 °C for 15 min in the absence (control) and in the presence of **1a** or its alkali and ammonium salts. Similar experiments were carried out with methyluric acids **1b–d**, guanidinium urate (**2a**), hypoxanthine (**4**), xanthine (**5**), 8-azaxanthine (**6**) and oxonic acid (**7**). The islets treated with alloxan (**3**) at the same concentrations were used as a positive control (Scheme 1).[17–20] Compounds were dissolved immediately before use and, if necessary, adjusted to pH 7.4 and 280 ± 10 mOsm/kg. The medium was then removed

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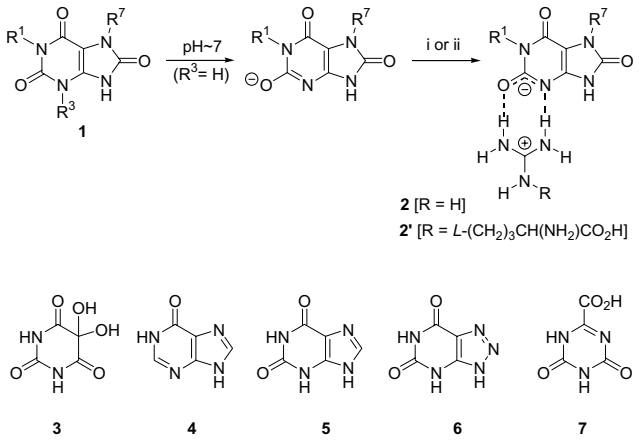

Scheme 1. Examples of compounds examined for the effect on insulin release in isolated rat pancreatic islets. a: $R^1 = R^3 = R^7 = H$; b: $R^1 = Me$, $R^3 = R^7 = H$; c: $R^1 = R^3 = H$, $R^7 = Me$; d: $R^1 = R^3 = Me$, $R^7 = H$. i, $C(NH_2)_3^+$ $[H_2O]$; ii, L-$(NH_2)_2C^+NH(CH_2)_3CH(NH_2)CO_2H$ (cf. Ref. 20).

and replaced by an equal volume of Krebs buffer containing either 3.3 or 20 mM glucose and incubated for 1 h. Samples were collected and stored (−20 °C) for basal and stimulated insulin release assays.[21] In rat islets, both basal and insulin secretion evoked by glucose (20 mM) were markedly blunted following exposure to **1a**. Of particular interest was the finding that the onset of inhibition of GSIS occurred at 0.4 mM levels (Fig. 1).

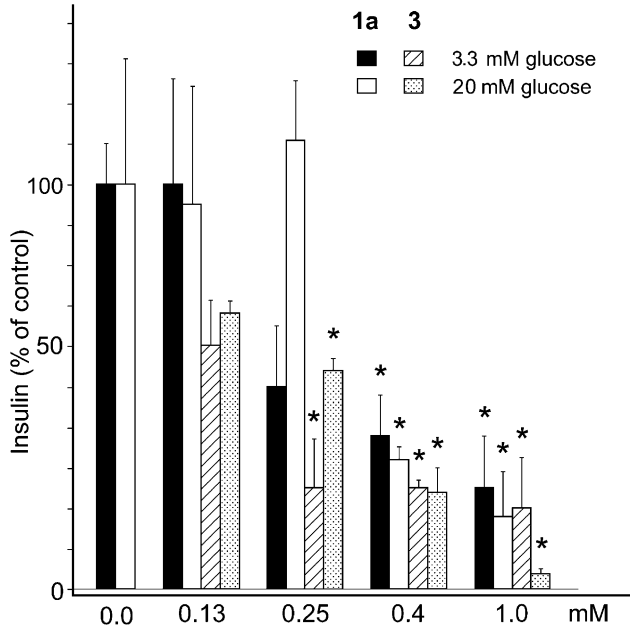

Figure 1. Effect of uric acid (**1a**) on insulin release from isolated rat pancreatic islets of Langerhans. Insulin released after a 15 min exposure to increasing concentrations of **1a** is expressed as a percentage of the basal and stimulated release (1.01 ± 0.12 and 10.88 ± 3.36 ng/islet/h, respectively) in control islets, with alloxan (**3**) as a positive control (basal insulin is subtracted from the stimulated values). Values shown are the means ± SEM for three experiments. Asterisks indicate significant inhibitory effect, $^*P < 0.01$.

The current findings confirm the earlier observations by Scott et al.[16] on substantial attenuation of basal insulin release by up to 0.2 mM **1a**, reaching levels 35–70% of control values at 60 min, which could be restored to normal GSIS response after the exchange of medium. A characteristic of the inhibition of GSIS is its sudden occurrence at a threshold of 0.4 mM, in contrast to the concentration-dependent effect of **3**. Moreover, **1a** did not alter glucokinase activity (data not shown),[22] which is an alloxan-sensitive glucose sensor of pancreatic β-cells.[23] The defective insulin release is probably due to impaired stimulus-secretion coupling rather than an adverse effect on β-cell viability,[24] and the significant drop of basal release may not be critical for GSIS at less than 0.4 mM urate levels (Fig. 1). Permissive concentrations, approaching those normally seen in human sera (0.25–0.35 mM), are thus fully counteracted by glucose and the onset of inhibition of GSIS follows when the inhibitory threshold is exceeded. One of the conceivable mechanisms that may be involved in the observed inhibitory effect is β-cell re-polarisation through binding on the cell surface.

While hypoxanthine (**4**) had no influence on GSIS, xanthine (**5**) showed an inhibitory effect at a high threshold dose (1 mM). A distinctive feature of **5** is that it could be removed from the islets by rinsing, with a concomitant return to a normal insulin response to glucose (Table 1), whereas the inhibition by **1a** persisted.[25] This is presumably due to the fact that **5** ($pK_a = 7.7$) is a much weaker acid while **1a** ($pK_a = 5.4$) exists as the monoanion at physiological pH and, consequently, has a quite different strength of binding. In an attempt to identify the site of interaction with **1a**, the effect of

Table 1. Short-time effects of 1 mM concentrations of compounds **1**–**7** on insulin release in rat pancreatic β-cells

Compds (1 mM/15 min)	Insulin release (ng/islet/h)[a]	
	Basal[b]	Stimulated[c]
Control	1.01 ± 0.26	8.68 ± 1.96
1a	0.24 ± 0.07*	1.65 ± 0.35*
K-salt of **1a**	0.26 ± 0.08*	1.68 ± 0.32*
Na-salt of **1a**	0.27 ± 0.09*	1.70 ± 0.39*
NH_4-salt of **1a**	0.28 ± 0.09*	1.66 ± 0.30*
1b	0.84 ± 0.32	7.92 ± 0.94
1c	0.92 ± 0.24	8.04 ± 0.92
1d	1.13 ± 0.09	8.48 ± 1.59
2a	1.04 ± 0.28	8.39 ± 1.59
2b	0.98 ± 0.22	8.75 ± 1.66
2c	0.89 ± 0.25	8.55 ± 1.71
3	0.20 ± 0.24*	0.39 ± 0.04*
4	1.02 ± 0.41	8.44 ± 0.51
5	1.25 ± 0.22	3.50 ± 0.51*
5[d]		7.72 ± 1.98
6	0.91 ± 0.30	7.92 ± 1.81
7	0.73 ± 0.29	8.26 ± 1.99

[a] Values are means of three experiments ± SEM. ANOVA and Duncan's post-hoc test were employed to indicate significant effects. The level of significance was set for $^*P < 0.01$.
[b] Nonstimulatory 3.3 mM glucose.
[c] 20 mM glucose-stimulated insulin secretion (GSIS).
[d] PBS-rinsing of the islets (cf. Ref. 25).

its guanidine salt was assessed. A salient characteristic of guanidinium urate (**2a**) is its stability, which is maintained both through a strong bidentate salt-bridge/hydrogen-bonding association.[19] Most surprisingly, only this one among a range of salts of **1a** was totally ineffective, strongly suggesting that binding to an essential arginine residue caused a loss of GSIS. The binding competition explains the absence of inhibition when high concentrations of **2a** are used (Table 1).[20] 8-Azaxanthine (**6**) and oxonic acid (**7**), which also have a high affinity for guanidinium ions but cannot participate in oxygen activation,[19] had no influence on insulin release. Therefore, modulation of cell surface potential through formation of a salt-bridge to fixed cations cannot by itself account for the effect of urate. A rather specific recognition of the **1a** molecule was denoted by the complete lack of effect of methyl analogues **1b–d** (Table 1). In any event, such a divergent response between **1a** and **1b–d** or **2a** in terms of insulin release, could not be accounted for simply by a reduced cell viability. Moreover, two different indicators of the islet viability, trypan blue exclusion[24a,b] and resorufin fluorescence,[24c,d] showed no impairment in the presence of **1a** (1 mM, 15 min) relative to the untreated control. Taken together, these data point to a buried arginine residue as the primary target for the specific binding of urate, which will eventually interrupt the transduction of signals normally coupling glucose metabolism to insulin secretion.

It was therefore important to set it in the perspective of known interactions of this sort. Three-dimensional structures of the archetypal guanidinium urate (**2a**)[19] and the active pocket of uricase[6,26] allowed deduction of the bonding network with the substrate. The formation of a salt-bridge with Arg 176, in conjunction with the stacking onto the Phe 159 residue, is responsible for the specific interaction with **1a**, which is functioning as an expendable prosthetic group in the enzymic reduction of oxygen. This binding motif may have far-reaching implications, since such stacking interactions provide a way to an enhanced reactivity toward oxidation. Whether an oxidation-assisted mechanism operates in inhibition of GSIS remains to be established.

In conclusion, these findings provide direct evidence for the involvement of uric acid in altering the primary β-cell function and suggest the presence of an essential arginine residue at the critical binding site. Moreover, the clear inhibition of GSIS is likely to play an important role in a variety of disease states in which hyperuricaemia is implicated. Studies revealing a similar inhibitory effect of uric acid on GSIS in human pancreatic islets are currently underway and the results will be published elsewhere.

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19. Poje, N.; Poje, M.; Vicković, I. *Z. Krist.—New Cryst. Struct.* **2000**, *215*, 583–584. The general procedure for guanidinium urates is illustrated for the preparation of **2a**. An equimolar amount of guanidine or L-arginine hydrochloride was added to an aqueous solution of the sodium salt of **1a–c** (10 mmol). The resultant crystalline precipitate was collected, washed with water and recrystallised to yield colourless prisms (90–95%) as monohydrates (±0.3% C, H, N). All these salts shrink at 110–115 °C and decompose above 270 °C without melting. UV spectra exhibited bands characteristic of a monoanionic chromophore, being identical in water and bicarbonate buffer (pH 7.5). λ_{max} ($\log\varepsilon$): **2a**, 235 (4.0), 292 (4.09); **2b**, 204 (4.36), 236 (3.92), 291 (4.08); **2c**, 237 (4.00), 293 (4.09) nm. Virtually identical spectra were obtained for the corresponding argininium urates **2′a–c**. It is, therefore, clear that N(9)H is undissociated under physiological conditions. Xanthinate (**5**), 8-azaxanthinate (**6**) and oxonate (**7**), which are competitive inhibitors of uricase, also show a high affinity for guanidinium ions, affording crystalline salts on mixing their aqueous solutions. While guanidinium salts of **1a–c** could be recovered from bicarbonate buffer, the ammonium salts are unstable under comparable conditions; as soon as they go into buffer solution they are destroyed.
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21. Insulin was measured using an enzyme immunoassay (High Range Rat Insulin ELISA, Mercodia, Uppsala, Sweden), calibrated with rat insulin (0.15–5.5 ng/L) both in the presence and absence of **1a**.
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