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Structure-activity relationships of alloxan-like compounds derived from uric acid

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1 The diabetogenic activity of a range of alloxan-like compounds derived from uric acid has been investigated.

2 The classes of derivatives were: 5-substituted-isouric acids; 4,5-disubstituted-4, 5-dihydrouric acids; 5-substituted-pseudouric acids; salts of dehydro-uramil hydrate; salts of dehydro-isouramil hydrate; alloxan derivatives.

3 Compounds were tested by intravenous injection into rats and diabetogenic activity assessed by production of persistent hyperglycaemia and glycosuria.

4 The only essential structural feature common to all active compounds was the presence of a quinonoid pyrimidine system or its hydrated equivalent. The presence of the five-membered ring of uric acid (or an opened form thereof) did not abolish and in some compounds enhanced diabetogenic activity.

Introduction

Since the discovery of alloxan diabetes the possible role of an alloxan-like compound derived from uric acid in the aetiology of diabetes mellitus has been extensively discussed (Cooperstein & Watkins, 1981). Alloxan-like derivatives of uric acid exerted strong cytotoxic effects upon β-cells of rat pancreatic islets of Langerhans, which revived interest in the plausible hypothesis postulating that aberrations in purine metabolism could have relevance to the aetiology of diabetes mellitus (Poje & Rocic, 1980; Poje *et al.*, 1983; Tait *et al.*, 1983; Dominis *et al.*, 1984) and re-opened the question of alternative uricolytic pathways. It is of interest in this connection that a report has appeared describing the occurrence of an unidentified urinary excretion product in animals and in man, which, on alkaline hydrolysis, yielded mesoxalic acid (Paley *et al.*, 1953). The possibility that alloxanic acid could be the source of mesoxalate has also been considered since administered alloxan or alloxanic acid caused a significant increase of urinary mesoxalate in animals. It has been shown that under physiological conditions alloxan rearranges rapidly into alloxanic acid; this suggested that the relationship of alloxan-like compounds to diabetes could be investigated by looking for more stable derivatives, such as alloxanic acid, rather than the labile alloxan.

Recent findings (Malaisse *et al.*, 1982) demonstrated that the selective cytotoxicity of alloxan to pancreatic β-cells may result from the conjunction of two features: a rapid cellular uptake of the drug and an exquisite sensitivity of the β-cell to peroxides, itself attributable to low peroxidase activity. The postulated intracellular site of action of alloxan gave an impetus to study the structure-activity relationships of alloxan-like compounds derived from uric acid, which may be regarded as potential precursors of endogenous alloxan. Although examples of alloxan homologues have been known for nearly forty years, diabetogenic compounds which have been tested were almost exclusively of the N-alkyl type; the only common variants are those produced by reduction into alloxantins or dialuric acids (Bruckmann & Wertheimer, 1945; Bruckmann & Wertheimer, 1949). These simple modifications of classical diabetogenic alloxan derivatives are not considered here (for review see Cooperstein & Watkins, 1981). Instead we studied the biological activities of those derivatives in which the bicyclic nucleus of uric acid is altered either by introduction of new functionalities or by ring-opening reactions and further modification of the side-chain.

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Methods

All experiments were performed with male Lewis rats weighing 180–220 g, reared on standard laboratory chow (Pliva, Zagreb) and fasted 24 h before injections. Freshly prepared 2% (w/v) solutions of drugs in saline were given intravenously via a tail vein unless otherwise indicated. Blood glucose concentrations were measured by the glucose oxidase method (Huggett & Nixon, 1957) and urine samples tested for glucose (Urokomb 8, Boehringer, Mannheim). A rat was considered diabetic if marked hyperglycaemia (15 mmol l^{-1}) and glucosuria (1%) occurred within 24 h after injection and persisted for 5 days. Five doses of each drug (with 12 rats at each dose) were used and the dose required to produce diabetes in 50% of animals (ED_{50}) was estimated graphically (Lithfield & Wilcoxon, 1949). Additional information was obtained by examination of histological changes and islet morphology (Dominis *et al.*, 1984); islets from each experimental group were analysed with respect to the normal appearance of the β-cells, their degree of degranulation, picnosis and necrosis.

Results

Table 1 shows the results obtained after a single administration of a range of alloxan-like compounds derived from uric acid: for completion, Table 1 includes data from previous (Bruckmann & Wertheimer, 1947; Poje & Rocic, 1980; Poje *et al.*, 1983) as well as the present study. The compound was considered inactive if a dose of 3 mmol kg^{-1} did not cause diabetes or, in the case of toxic substances, postmortem morphological examination of pancreata did not show significant β-cell injury. The histological changes in the islets as a consequence of injection of diabetogenic compounds are characteristic. These substances cause almost selective injury to the β-cells of the islets of Langerhans: definite picnosis of nuclei and degranulation are evident about 1 h after injection. After 24 h the β-cells become disintegrated and form an extensive necrotic mass in the islets. The disappearance of insulin-producing β-cells results in permanent hyperglycaemia and the islet eventually consists only of A-cells which show little regressive changes. Although a reliable comparison between ED_{50} values is doubtful on account of marked differences in stability of aqueous solutions, the data demonstrate the following features of the structure-activity relationships of alloxan-like compounds derived from uric acid.

Substitution at C-5

Although it has long been supposed that substitution in the 5-position results in loss of the diabetogenic activity of alloxan (Bruckmann & Wertheimer, 1947), it was found that 5-amino and related 5-ureido derivatives were more active than the parent alloxan. Replacement of both geminal hydroxyl groups resulted in an inactive compound. Thus, although a free hydroxyl in the 5-position is necessary for the diabetogenic activity, any group susceptible to hydrolytic substitution (e.g. chlorine in 5-chloro-isouric acids) or a grouping which readily forms covalent adducts with a molecule of water (e.g. the 5-amino group in dehydro-uramil (Poje *et al.*, 1983)) seems to contribute positively towards diabetogenic activity.

Substitution at nitrogens

An intact pyrimidine ring is required for diabetogenicity; substitution at one nitrogen diminishes and at both nitrogens abolishes the diabetogenic effect of alloxan (Bruckman & Wertheimer, 1945; Bruckman & Wertheimer, 1947). The same effect of substitution at pyrimidine nitrogens was found here for alloxan-like derivatives of uric acid; all 1,3-dimethyl derivatives were devoid of diabetogenic activity. Interestingly, compounds with substituents in 5-membered ring or side-chain urea equivalent of uric acid derivatives were lower in activity than parent compounds. It seems that substitution at N-7 and/or N-9 increases the tendency for rearrangement to occur yielding the inactive alloxanic acid ureides (Poje *et al.*, 1980), presumably through stabilization of the 5-membered ring in the product.

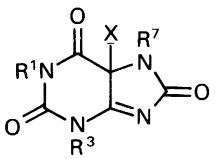
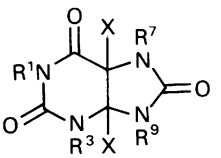
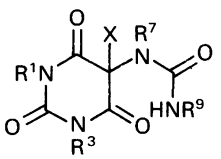
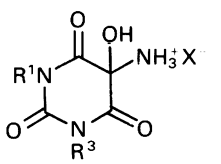
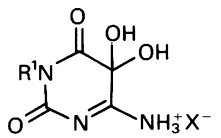
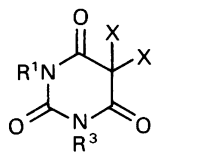
Substitution at C-4 or C-6

Dehydro-isouramil, a 4-imino analogue of alloxan, was more potent than alloxan. The bridgehead position in 5-substituted isouric acids bears an analogous imine grouping whilst that in 4,5-disubstituted dihydrouric acids represents the hydrated form. The substitution of oxygen for nitrogen in this series seems compatible with retention of diabetogenicity.

Discussion

We conclude from this investigation of alloxan-like compounds that the only essential common feature for diabetogenicity is the quinonoid system itself. The significant difference between quinonoid systems and their acyclic counterparts (or ring-contraction products such as alloxanic acid derivatives) is the ease of reduction, the driving force being the formation of the hetero-aromatic system. The ability to accept firstly one electron, forming the semiquinone-like anion radical, following by a further electron to give a dianion, is the dominant feature of quinonoid systems.

Table 1 Diabetogenic activity of alloxan-like compounds derived from uric acid

		X	R^1	R^3	R^7	R^9	ED_{50} (mmol kg^{-1})	*Ref.*
A		Cl	H	H	Me	—	0.25	1
		Cl	H	Me	H	—	0.30	1
		Cl	Me	H	Me	—	0.35	1
		Cl	Me	Me	H	—	inactive	1
		OMe	H	Me	Me	—	inactive	2
		OMe	Me	Me	H	—	inactive	3
		OMe	Me	Me	Me	—	inactive	4
B		OH	H	H	H	H	1.06 i.p.[a]	5
		OMe	H	H	H	H	inactive	6
		OEt	H	H	H	H	inactive	6
		OCMe(OMe)O	H	H	H	Oac	inactive	7
		$O(CH_2)_2O$	H	Me	H	Me	inactive	8
C		OH	H	H	H	H	0.15[a]	5
		OH	Me	H	H	H	0.32	8
		OH	H	H	Me	Me	0.92	8
		OH	Me	Me	H	H	inactive	8
		NH_2	H	H	H	H	inactive[b]	9
		OMe	H	H	H	H	inactive	6
D		Cl	H	H	—	—	0.14[b]	9
		Br	H	H	—	—	0.15[b]	9
		Cl	Me	Me	—	—	inactive	8
		Br	Me	Me	—	—	inactive	8
E		Cl	H	—	—	—	0.20[c]	10
		Br	H	—	—	—	0.25	8
F		OH	H	H	—	—	0.28[d]	11
		OH	Me	H	—	—	0.30[d]	11
		OH	Me	Me	—	—	inactive[d]	11
		OMe	H	H	—	—	inactive	12

The structures shown represent: (A) 5-substituted-isouric acids, (B) 4,5-disubstituted-4,5-dihydrouric acids, (C) 5-substituted-pseudouric acids, (D) dehydrouramil hydrate salts, (E) dehydro-isouramil hydrate salts, (F) alloxan derivatives. The drugs were synthesized according to the following references: 1, Biltz & Pardon (1934); 2, Biltz & Strufe (1916); 3, Biltz & Damm (1914); 4, Biltz (1910); 5, Poje & Rocic (1979); 6, Biltz & Heyn (1916); 7, Poje *et al.* (1982); 8, unpublished; 9, Poje *et al.* (1983); 10, Rocic *et al.* (1985); 11, Bruckman & Wertheimer (1945, 1947); 12, Regitz & Adolph (1969). They were administered to 620 fasted rats as freshly prepared 2% (w/v) solutions in saline intravenously via a tail vein (except where indicated 'i.p.' when the route of administration was intraperitoneal). The occurrence of hyperglycaemia and glucosuria which persisted for 5 days was used as the criterion of diabetogenicity; selective pancreatic β-cell cytotoxicity was substantiated by histological examination of β-cell injury in all experimental groups. ED_{50}s were estimated graphically using 60 rats for each active compound. Data from previous studies: [a]Poje & Rocic (1979, 1980), [b]Poje *et al.* (1983), [c]Rocic *et al.* (1985), [d]Bruckman & Wertheimer (1945, 1947).

It is the reversible redox process which accounts for the biological effects of quinonoid systems; the radicals possess a significant energy, above that of the precursor, which can contribute to the lowering of the free energy of an associated reaction. The existence of alloxan anion radicals in complexes with metal ions has been established by ESR measurements (Daul *et al.*, 1983).

Thus the active compounds may be regarded as isosteres of quinonoid dehydro-uric acid or its hydrated forms. The 5-membered ring of uric acid or fragments thereof may be present with retention of full or even enhanced diabetogenic activity. The occurrence of such transformations in the course of biological oxidations of uric acid could be relevant to the β-cell destruction found in Type I diabetes and merits further investigation.

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