liver cell plasma membrane ATPases. This kind of effect is reversible and it may be considered as having a possible marginal role in the pathogenesis of the liver lesion initiated by CCl_4.

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Effects of 5-diazoimidazole-4-carboxamide and 3-diazopyrazole-4-carboxamide and related thioazo compounds on xanthine oxidase, uricase, and hypoxanthine–guanine phosphoribosyltransferase

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RECENTLY, Iwata *et al.*[1] showed that 5-diazoimidazole-4-carboxamide (Diazo-ICA) and its thioazo compounds, but not triazeno compounds, were potent inhibitors of rat liver xanthine oxidase and milk xanthine oxidase, both *in vivo* and *in vitro*. This finding stimulated further investigations of the effects of these compounds on purine metabolism.

This communication describes the actions of Diazo-ICA and its ring analog, 3-diazopyrazole-4-carboxamide (Diazo-PCA), and related thioazo derivatives on xanthine oxidase, uricase and hypoxanthine–guanine phosphoribosyltransferase (HG–PRTase) *in vitro*.

Bovine milk xanthine oxidase was prepared by the method of Klenow and Emberland.[2] Xanthine oxidase activity was determined by a modification of the methods described by Plesner and Kalckar,[3] and by Della Corte and Stirpe.[4] Uricase activity was determined by the method of Fridovich.[5] A soluble powder of bovine kidney uricase (Type II) was used as the uricase preparation. The HG–PRTase activity of rat erythrocyte hemolysates was assayed isotopically by the method of Kelley *et al.*[6]

Table 1 shows the relative potencies of Diazo-ICA and Diazo-PCA and their thioazo derivatives as inhibitors of xanthine oxidase, uricase and HG–PRTase; for comparison the inhibition of xanthine oxidase by allopurinol, of uricase by oxonate, and of HG–PRTase by 6-mercaptopurine (6-MP) are included. In our previous paper,[1] Diazo-ICA and two related thioazo compounds, 5-(2-aminoethylthioazo)imidazole-4-carboxamide (Aminoethylthioazo-ICA) and 5-(2-amino-2-carboxyethylthioazo)-

TABLE 1. INHIBITION OF XANTHINE OXIDASE, URICASE AND HYPOXANTHINE–GUANINE PHOSPHORIBOSYLTRANSFERASE BY 5-DIAZOIMIDAZOLE-4-CARBOXAMIDE, 3-DIAZOPYRAZOLE-4-CARBOXAMIDE AND RELATED COMPOUNDS, *in vitro*

Compound	Enzyme (M)	Inhibition, % (A) Xanthine oxidase 10^{-6}	(A) Xanthine oxidase 10^{-5}	(B) Uricase 10^{-6}	(B) Uricase 10^{-5}	(C) HG-PRTase* 10^{-4}	(C) HG-PRTase* 10^{-3}
(a) AICA HC		0	0	0	0		4
(b) Aza–AICA		0	0	2	3		0
(c) APCA 1/2 H_2SO_4		2	9	0	3		0
(d) Diazo–ICA		79	96	40	81	0	0
(e) Diazo–PCA		21	96	0	4	6	−8
(f) Aminoethylthioazo–ICA HCl		66	98	50	88	0	0
(g) Aminocarboxyethylthioazo–ICA		42	96	32	81	−2	−3
(h) Hydroxyethylthiozao–ICA		67	97	40	91		−12
(i) Aminophenylthioazo–ICA		2	90	12	66		14
(j) Nitrophenylthioazo–ICA		5	78	11	63	0	9
(k) Aminoethylthioazo–PCA HCl		24	97	0	5	5	17
(l) Dimethyltriazeno–ICA HCl†		3	18	0	8	0	5
(m) Dipropyltriazeno–ICA HCl†		3	13	0	0		0
(n) Dibutyltriazeno–ICA HCl†		0	4	0	2		0
2-Azahypoxanthine H_2O		0	5	35	85	0	16
6-Mercaptopurine H_2O		2	5	0	0	9	37
Oxonic acid-K		0	0	25	75	0	0
Allopurinol		17	72	4	3	0	3
(o) 4-HPT H_2O		11	48	0	5	−2	2
Caffeine		0	0	0	0		0

* Hypoxanthine–guanine phosphoribosyltransferase.

† In the dark.

(a) 5-Aminoimidazole-4-carboxamide hydrochloride, (b) 5-Amino-v-triazole-4-carboxamide, (c) 3-Aminopyrazole-4-carboxamide hemisulfate, (d) 5-Diazoimidazole-4-carboxamide, (e) 3-Diazopyrazole-4-carboxamide, (f) 5-(2-Aminoethylthioazo)imidazole-4-carboxamide hydrochloride, (for chemical structure see, ref. 1), (g) 5-(2-Amino-2-carboxyethylthioazo)imidazole-4-carboxamide, (for chemical structure see, ref. 1), (h) 5-(2-Hydroxyethylthioazo)imidazole-4-carboxamide, (i) 5-(*o*-Aminophenylthioazo)imidazole-4-carboxamide, (j) 5-(*p*-Nitrophenylthioazo)imidazole-4-carboxamide, (k) 3-(2-Aminoethylthiozao)pyrazole-4-carboxamide hydrochloride, (l) 5-(3,3-Dimethyl-1-triazeno) imidazole-4-carboxamide hydrochloride, (m) 5-(3,3-Dipropyl-1-triazeno)imidazole-4-carboxamide hydrochloride, (n) 5-(3,3-Dibutyl-1-triazeno)imidazole-4-carboxamide hydrochloride, (o) 4-Hydroxypyrazolo(3,4-d)-v-triazine monohydrate.

(A) The assay mixture contained, in a final volume of 4·0 ml, 0·1 M Tris–HCl buffer, pH 7·5, 60 μM xanthine. 0·1 mM EDTA–2 Na, various concentrations of drugs or water and the enzyme. The enzyme activity was measured by the increase in E_{293} due to formation of uric acid from xanthine in 10 min at 25° in a Shimadzu QV–50 type spectrophotometer. Xanthine was omitted from blanks.

(B) Uricase activity was determined in 0·1 M borate buffer, pH 8·5, in terms of urate disappearance and was followed at 292 mμ for 2 hr at 29°. The assay mixture contained 0·6 ml of urate solution (uric acid 4 mg/100 ml of water), 1·0 ml of enzyme solution (17·5 mg of soluble powder of bovine kidney uricase Type II/100 ml of buffer), 0·3 ml of various concentrations of drugs or water and 1·6 ml of 0·138 M borate buffer, pH 8·5.

(C) Incubation mixture contained 55 mM Tris–HCl buffer, pH 7·4, 5·0 mM $MgCl_2$, 1·0 mM PRPP–2 Mg and 2·0 mg of protein from a dialyzed erythrocyte hemolysate of rats in a final volume of 100 μl. As purine base, 0·6 mM hypoxanthine-8-^{14}C (20·7 mc/mmole) was added to the reaction mixture. After incubation for 60 min at 38°, reactions were terminated by addition of 2 μmoles/20 μl of EDTA–2 Na and the mixture was immediately frozen in a dry-ice acetone bath. The reaction products (IMP + inosine) were separated from the substrate by high voltage electrophoresis in 0·05 M borate buffer, pH 9·0, containing 1 mM EDTA–2 Na, at 2000 volts for 30 min. The radioactivity was counted in a liquid scintillation counting system.

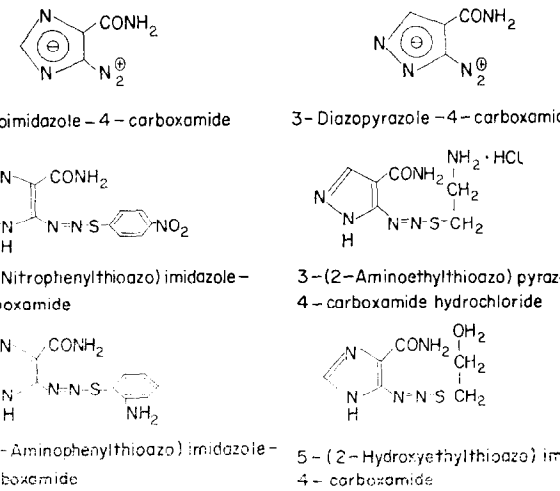

FIG. 1. Chemical structures of 5-diazoimidazole-4-carboxamide, 3-diazopyrazole-4-carboxamide and their thioazo derivatives.

imidazole-4-carboxamide (Aminocarboxyethylthioazo-ICA), were shown to be potent inhibitors of xanthine oxidase, *in vivo* and *in vitro*. In the present work, three new thioazoimidazoles, 5-(2-hydroxyethylthioazo)imidazole-4-carboxamide (Hydroxyethylthioazo-ICA), 5-(*o*-aminophenylthioazo)imidazole-4-carboxamide (Aminophenylthioazo-ICA) and 5-(*p*-nitrophenylthioazo)imidazole-4-carboxamide (Nitrophenylthioazo-ICA), and a related pyrazole derivative, 3-(2-aminoethylthioazo)pyrazole-4-carboxamide (Aminoethylthioazo-PCA) were also synthesized and their inhibitory effects on the enzymes were tested. These new thioazo compounds, like Diazo-ICA, were found to be even more effective inhibitors of milk xanthine oxidase than allopurinol and 4-hydroxypyrazolo (3,4-d)-v-triazine (4-HPT). Diazo-PCA itself was also a potent inhibitor of xanthine oxidase. Triazene derivatives of Diazo-ICA tested were only weak inhibitors of xanthine oxidase. Assay with triazene compounds was performed in the dark, because these compounds are known to be stable in the dark, but to decompose in the light to yield Diazo-ICA.[7] Little or no inhibition was observed with 5-aminoimidazole-4-barboxamide (AICA), 5-aminotriazole-4-carboxamide (Aza-AICA), 3-aminopyrazole-4-carboxamide (APCA), 2-azahypoxanthine, 6-MP, oxonate or caffeine.

Oxonate, which is an inhibitor of uricase, was prepared as described by Brandenberger.[8] It caused 75 and 25 per cent inhibition of uricase activity at concentrations of 1×10^{-5} and 1×10^{-6} M, respectively. Diazo-ICA and thioazoimidazoles were found to be potent inhibitors of uricase, being as, or more inhibitory than oxonate. The pyrazole compounds tested, Diazo-PCA, Aminoethylthioazo-PCA and 4-HPT did not inhibit uricase. Three triazenoimidazoles also had no effect on uricase in the dark. Moreover, allopurinol, AICA, Aza-AICA, APCA, 6-MP and caffeine had no affect on uricase activity. The effect of allopurinol on uricase had already been reported by Truscoe and Williams.[9] 2-Azahypoxanthine markedly inhibited uricase activity at a concentration of 1×10^{-5} M. Kinetic studies showed that it inhibited uricase by competing with the substrate (unpublished data). Iwata *et al.* showed that the thioazo compounds tested ultimately decompose, both *in vivo* and *in vitro*, to yield Diazo-ICA (unpublished data), which then cyclizes readily in aqueous solution to 2-azahypoxanthine.[10,11] Therefore, the inhibitory effect of Diazo-ICA and its thioazoimidazoles on uricase is thought to be largely due to the inhibitory effect of 2-azahypoxanthine.

We also examined the effect of Diazo-ICA and related compounds on the purine slavage enzyme, HG-PRTase of rat erythrocyte hemolysates. 6-MP at a concentration of 1×10^{-3} M inhibited this enzyme activity by 37 per cent. Neither Diazo-ICA, Diazo-PCA nor their thioazo- and triazeno compounds tested except three thioazo compounds inhibited HG-PRTase even at a high concentration of 1×10^{-3} M, while Aminophenylthioazo-ICA, Nitrophenylthioazo-ICA, Aminoethylthioazo-PCA and 2-azahypoxanthine were slightly inhibitory. AICA, Aza-AICA, APCA, oxonate, 4-HPT and caffeine also had no inhibitory effects. Kelley *et al.*[12] suggested, on the basis of indirect evidence, that in man HG-PRTase converts allopurinol to allopurinol ribonucleotide, and the enzymatic synthesis of the ribonucleotide was reported by McCollister *et al.*[13] However, allopurinol did not inhibit HG-PRTase activity under the present experimental conditions.

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Effects of peripheral aromatic L-amino acids decarboxylase inhibitor on L-[2-^{14}C]-3,4-dihydroxyphenylalanine metabolism in man

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ONE CURRENT approach to the treatment of Parkinson's disease is focused on the possible combination of L-3,4-dihydroxyphenylalanine (L-dopa) with an inhibitor of peripheral aromatic L-amino acids decarboxylase. This approach attempts to reduce[1–3] the therapeutic dose of L-dopa by inhibiting its extracerebral decarboxylation, thus minimizing certain dopa-induced side effects. It has been shown previously[1–3] that certain aromatic hydrazine-type compounds may interfere in the metabolism of exogenous L-dopa by inhibiting peripheral dopa-decarboxylase (DC). Examples of such compounds are: RP-4-4602 [*N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine], MK-485[DL-α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid] and NSD-1015 (*m*-hydroxybenzylhydrazine). Animal experiments have demonstrated that although MK-485 is a less potent inhibitor than an equimolar dose of RO-4-4602 or NSD-1015, it has the advantage of not penetrating into the brain,[4] hence cerebral DC remains unaffected.

The present investigation studied the effect of pretreatments with MK-486, the L-isomer of MK-485, on blood plasma and urinary excretion patterns of L-[2-^{14}C]-dopa and metabolites in three Parkinsonian patients free of renal, hepatic or cardiovascular disease. All medication was discontinued 1 week