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EFFECTS OF BENSERAZIDE AND CARBIDOPA ON THE METABOLISM OF L-TRYPTOPHAN BY ISOLATED RAT LIVER CELLS

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Abstract—(1) The effects of two inhibitors of aromatic amino acid decarboxylase, benserazide and carbidopa, on the metabolism of L-tryptophan by liver cells prepared from 48 hr starved rats was studied. Tryptophan 2,3-dioxygenase (pyrrolase) and kynureninase activities were determined simultaneously using L-[ring 2-^{14}C], L-[carboxyl-^{14}C] and L-[methylene-^{14}C]tryptophan. (2) In hepatocytes incubated with a low physiological concentration (0.1 mM) of tryptophan, carbidopa (5×10^{-5} M) produced a significant inhibiton of kynureninase activity. Inhibition of tryptophan dioxygenase flux occurred only at concentrations of carbidopa of 10^{-4} M and above. Benserazide was less potent; the concentrations required for significant inhibition of tryptophan dioxygenase and kynureninase fluxes were 2.5×10^{-4} M and 10^{-4} M respectively. (3) Rates of $^{14}CO_2$ production from the two side chain ^{14}C-labelled tryptophan radioisomers were inhibited by benserazide and carbidopa to much greater extents than were rates of [1-^{14}C]alanine and [3-^{14}C]alanine release. This phenomenon is consistent with the finding that both drugs block oxidation of alanine by inhibiting transamination. Neither compound influences pyruvate oxidation. The implications of these observations with reference to the interpretation of data from previous investigations of the effect of benserazide and carbidopa on tryptophan oxidation *in vivo* are discussed.

Benserazide (RO-4-4602, *N'*-(DL-seryl)-*N*^{2}-(2,3,4) trihydroxybenzyl hydrazine) and carbidopa (MK 486, α-hydrazino-3,4-dihydroxyphenyl-α-methyl-propionic acid) are two inhibitors of L-aromatic amino acid decarboxylase (EC 4.1.1.28) that are used in conjunction with L-DOPA for the treatment of Parkinson's disease [1]. Recent studies have indicated that these two compounds also influence the kynurenine pathway of tryptophan metabolism [2–4].

Benserazide and carbidopa are both potent inhibitors of tryptophan 2,3-dioxygenase (EC 1.13.11.11) and kynureninase (EC 3.7.1.2.) activities of crude extracts of rat liver [4]. Evidence for the inhibition of hepatic tryptophan oxidation by these two drugs *in vivo* has largely been derived from experiments involving determination of respiratory $^{14}CO_2$ production of animals injected with radiolabelled tryptophan. Administration to rats of benserazide or carbidopa inhibits $^{14}CO_2$ production from DL-[ring 2-^{14}C] and L-[carboxyl-^{14}C]tryptophan [3, 5].

However, these isotopic data cannot be interpreted unequivocally in terms of inhibition of tryptophan oxidation as metabolism of each tryptophan radioisomer by the kynurenine pathway does not generate $^{14}CO_2$ directly; in each case ^{14}C-labelled intermediates are formed that must undergo further metabolism before $^{14}CO_2$ is released (see Fig. 1).

After oxidative cleavage of the pyrrole moiety by tryptophan 2,3-dioxygenase, carbon at position 2 of L-[ring 2-^{14}C]tryptophan appears in the formyl group of *N*-formylkynurenine. This compound is rapidly deformylated by a high activity formamidase (EC 3.5.1.9) [6]. Only a fraction of [^{14}C]formate is oxidized directly to $^{14}CO_2$ [7], so that changes in $^{14}CO_2$ production from [ring 2-^{14}C]tryptophan may not solely be a reflection of changes in tryptophan dioxygenase flux but also of changes in formate metabolism.

^{14}C from [carboxyl-^{14}C]tryptophan is eliminated as [1-^{14}C]alanine by the action of kynureninase on kynurenine or 3-hydroxykynurenine. $^{14}CO_2$ is released by oxidative decarboxylation of [1-^{14}C]pyruvate, the transamination product of [1-^{14}C]alanine. (It is now well established that direct decarboxylation of tryptophan to tryptamine is not a quantitatively important pathway of tryptophan catabolism [3]). Since benserazide and carbidopa are hydrazine derivatives and can therefore inhibit, in addition to kynureninase, other pyridoxal phosphate-dependent enzymes [8, 9], changes in $^{14}CO_2$ production from [carboxyl-^{14}C]tryptophan may not only be a consequence of inhibition of tryptophan dioxygenase and kynureninase but also of inhibition of alanine metabolism.

To establish conclusively that benserazide and carbidopa inhibit tryptophan oxidation in the intact liver cell it is clearly essential that all ^{14}C-labelled intermediates (and not just $^{14}CO_2$) derived from ^{14}C-labelled tryptophan are quantified. In the present study we have investigated the effects of benserazide and carbidopa on the oxidation of tryptophan by isolated rat liver cells. The total flux of carbon through tryptophan dioxygenase and kynureninase was determined from *total* rates of isotope release by liver cells incubated with three radioisomers of

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tryptophan. To eliminate the problems inherent with DL-radiolabelled tryptophan [10] care was taken to ensure that the L-isomer alone was used.

MATERIALS AND METHODS

Chemicals

DL-[Ring 2-^{14}C]tryptophan (sp. act. 30 Ci/mole) was obtained from Schwarz-Mann, Orangeburg, New York, U.S.A. DL-[carboxyl-^{14}C]tryptophan (sp. act. 50 Ci/mole), DL-[methylene-^{14}C]tryptophan (sp. act. 50 Ci/mole) and other radiochemicals came from the Radiochemical Centre. DL-Radiolabelled tryptophan was resolved optically by affinity chromatography on bovine serum albumin–Sepharose 4B [11]. [1-^{14}C]Pyruvate (sp. act. 9.9 Ci/mole) was repurified by the paper chromatographic method of Halestrap [12]. The sources of all other materials are detailed elsewhere [10]. Carbidopa and benserazide were kindly given by Merck, Sharp and Dohme Research Laboratories and Roche Products respectively.

Isolated liver cell incubations

(i) Measurement of isotope release from radioisomers of L-tryptophan. Portions (0.3 ml) of liver cell suspensions isolated from 48 hr-starved rats [13] (containing 4–6 mg dry wt of tissue) were added to 1.7 ml of buffer [14] supplemented with 2% (w/v) fatty acid-free bovine serum albumin, 11.8 mM-L-glutamine and saturated with O_2/CO_2 (19:1), in 10 ml silicone-treated conical glass flasks. The flasks were closed with rubber stoppers from which were suspended disposable polypropylene centre wells. Incubations, in triplicate were carried out under an atmosphere of O_2/CO_2 (19:1) at 37°; the vials were shaken at 100 oscillations/min. Carbidopa and benserazide, as solutions in 0.9% NaCl, were added at the times and concentrations specified in the text. Radiolabelled L-tryptophan (sp. act. 0.05 μCi/μmole, final concentration 0.1 mM) was added by syringe injection after a preincubation period of 40 min. Incubations were terminated by injection of 0.2 ml 2M-$HClO_4$ into each flask. Collection and quanti-

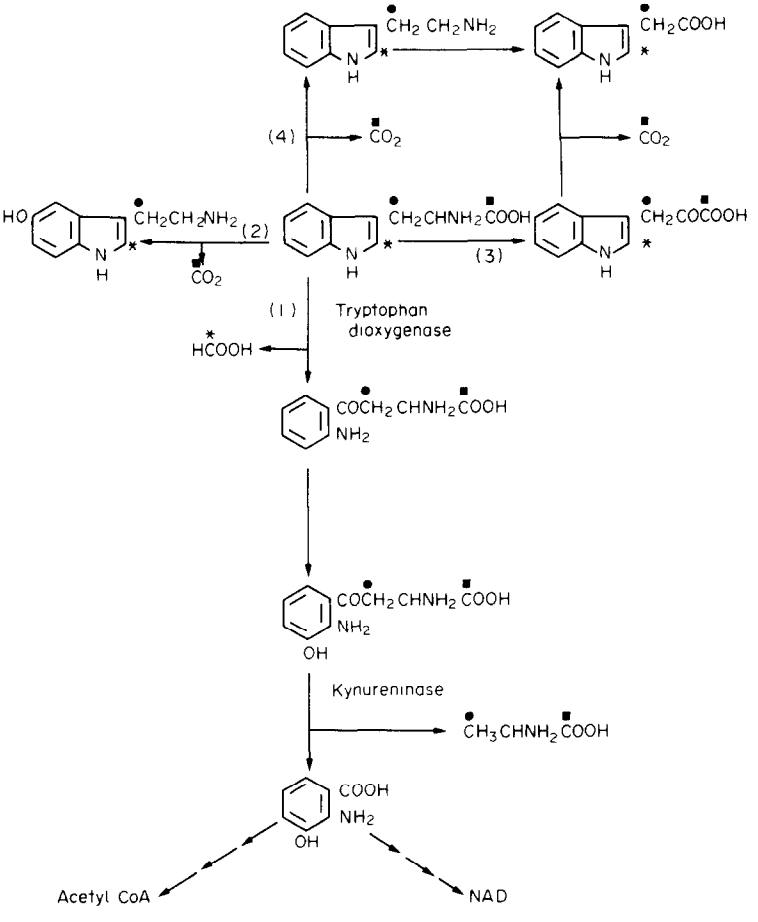
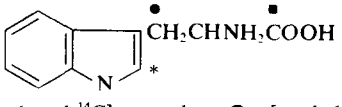


Fig. 1. Metabolic fate of isotope from specifically radiolabelled L-tryptophan. Key to radioisotope nomenclature:



* L-[ring 2-^{14}C]tryptophan; ■ L-[carboxyl-^{14}C]tryptophan; ● L-[methylene-^{14}C]tryptophan. The metabolic pathways: (1) kynurenine-glutarate pathway; (2) 5-hydroxytryptamine pathway; (3) indole pathway; (4) tryptamine pathway.

tation of metabolic $^{14}CO_2$ was determined as described previously [10].

The radioactivity of non-aromatic metabolites of tryptophan was measured as follows. Precipitated protein was removed by centrifugation (1200 *g*, 5 min) and 1 ml portions of supernatant were treated with 0.2 ml of a 50 mg/ml aqueous suspension of Norit GSX charcoal. After thorough mixing the charcoal was removed by centrifugation (10,000 *g*, 2 min). 0.5 ml of each supernatant was counted for ^{14}C radioactivity in PCS scintillator cocktail. Under these conditions, binding of unmetabolized L-tryptophan is quantitative [10]. There is no detectable binding of the major non-aromatic metabolites of tryptophan catabolism ([^{14}C]formate and [^{14}C]alanine) by charcoal [15]. To determine non-enzymic rates of isotope release, appropriate control incubations, in which tryptophan was added following $HClO_4$, were included for each radioisomer used. Results presented in the text have been corrected for non-enzymic isotope release. Rates of isotope release were linear with time throughout the incubation.

(ii) Determination of $^{14}CO_2$ production from [1-^{14}C]pyruvate and [1-^{14}C]alanine. Incubation conditions were essentially as described in (i) with the exception that glutamine was omitted from the incubation medium. [1-^{14}C]Pyruvate (final concentration 1 mM; sp. act. 5 nCi/μmole) or [1-^{14}C]alanine (final concentration 1 mM; sp. act. 5 nCi/μmole) were added 20 min after the start of the incubation. Metabolic $^{14}CO_2$ was quantified after the acidification of incubations with $HClO_4$ [10]. Rates of $^{14}CO_2$ production from each substrate have been corrected for non-enzymic isotope release.

RESULTS

Determination of tryptophan dioxygenase and kynureninase fluxes in isolated liver cells with radiolabelled L-tryptophan

In the experiments described, three radioisomers of L-tryptophan were used to determine carbon fluxes through tryptophan dioxygenase and kynureninase. The rate of release of [^{14}C]formate from L-[ring 2-^{14}C]tryptophan is a reliable, quantitative index of tryptophan dioxygenase activity [18]. Under the incubation conditions specified, total hepatocyte tryptophan dioxygenase activity is given by the sum of $^{14}CO_2$ and non-charcoal precipitable radioactivity (which represents the sum of [^{14}C]formate and ^{14}C-labelled amino acids derived therefrom) production from L-[ring 2-^{14}C]tryptophan [10, 15].

Kynureninase activity was measured with both L-[carboxyl-^{14}C] and L-[methylene-^{14}C]tryptophan. Non-charcoal precipitable radioactivity in extracts of liver cells incubated with either of these two radioisomers is produced only by cleavage of [1-^{14}C]alanine or [3-^{14}C]alanine by kynureninase from L-kynurenine or 3-hydroxyl L-kynurenine (Fig. 1). $^{14}CO_2$ is released during oxidation of [^{14}C]alanine. Kynureninase flux, therefore, is represented by the sum of the rates of $^{14}CO_2$ and non-charcoal precipitable ^{14}C radioactivity production by hepatocytes incubated either with L-[carboxyl-^{14}C] or L-[methylene-^{14}C]tryptophan [15].

Effects of carbidopa in the oxidation of 0.1 mM L-*tryptophan by isolated rat liver cells*

The effects of carbidopa on the tryptophan dioxygenase and kynureninase activities of isolated liver cells incubated with 0.1 mM radiolabelled L-tryptophan are shown in Table 1. Low concentrations (10^{-6} M) were without effect on either tryptophan dioxygenase or kynureninase activities. At concentrations of 10^{-4} M and above, carbidopa produced a significant inhibition of tryptophan dioxygenase flux. A maximal 40 per cent inhibition was observed in the presence of 10^{-3} M carbidopa. In all the experiments reported hepatocyte tryptophan dioxygenase activities are expressed as total rates of isotope release from L-[ring 2-^{14}C]tryptophan. Radioactivity released from this low, physiological concentration of tryptophan is distributed approximately equally between $^{14}CO_2$ and ^{14}C-non-aromatic metabolites [15]. Carbidopa produced an identical inhibition of the rates of release of isotope into each of these two component fractions (data not shown). This observation suggests that the reduction of rates of isotope release from L-[ring 2-^{14}C]tryptophan caused by carbidopa results from inhibition of tryptophan dioxygenase; there is no significant inhibition of the further metabolism of formate.

Hepatocyte kynureninase activity was more sensitive than tryptophan dioxygenase to carbidopa. 5×10^{-5} M Carbidopa produced a 35 per cent inhibition of kynureninase activity (measured as total rates of isotope release from either 0.1 mM L-[carboxyl-^{14}C] or L-[methylene-^{14}C]tryptophan. This inhibition rose to about 70 per cent with 10^{-3} M carbidopa. In contrast to the situation noted for L-[ring 2-^{14}C]tryptophan radioactivity released from [carboxyl-^{14}C] and [methylene-^{14}C]tryptophan was not distributed equally between the $^{14}CO_2$ and non-charcoal precipitable fractions. Table 1 shows that of the total ^{14}C released from each radioisomer only about 26 per cent ([carboxyl-^{14}C]tryptophan) and 6 per cent ([methylene-^{14}C]tryptophan) appeared as metabolic CO_2. Thus measurements of $^{14}CO_2$ production from [carboxyl-^{14}C]tryptophan that have been used to monitor rates of tryptophan oxidation *in vivo* will have grossly underestimated net carbon flux through the kynurenine pathway [2, 3, 5].

Carbidopa produced a differential inhibition of the rates of $^{14}CO_2$ and ^{14}C-labelled non-aromatic metabolite production from the two side-chain labelled radioisomers; $^{14}CO_2$ production was always inhibited to a much greater extent. 10^{-5} M Carbidopa inhibited $^{14}CO_2$ production by 15–20 per cent but because of the small contribution of the CO_2 fraction to total kynureninase flux, there was no significant effect on kynureninase activity. Similarly, at the highest concentration used, carbidopa inhibited $^{14}CO_2$ production by 80–90 per cent whereas [^{14}C]alanine release was inhibited by about 60 per cent.

Effects of benserazide on the oxidation of 0.1 mM-*tryptophan by isolated rat liver cells*

In a parallel series of experiments, the effects of benserazide on the oxidation of radiolabelled 0.1 mM-L-tryptophan by isolated liver cells were

Table 1. Effect of carbidopa on metabolism of 0.1 mM L-tryptophan by isolated rat liver cells

	Enzymic flux (as % of rate in absence of added drug)						
	L-[Ring 2-^{14}C] tryptophan	L-[Carboxyl-^{14}C]tryptophan			L-[Methylene-^{14}C]tryptophan		
Carbidopa concn (M)	Total ^{14}C release (tryptophan dioxygenase)	Total ^{14}C release (kynureninase)	$^{14}CO_2$	^{14}C Non-aromatic metabolites	Total ^{14}C release (kynureninase)	$^{14}CO_2$	^{14}C Non-aromatic metabolites
0	100 (5.92 ± 0.24)	100 (5.37 ± 0.46)	100 (1.38 ± 0.25)	100 (4.06 ± 0.52)	100 (4.62 ± 0.42)	100 (0.26 ± 0.06)	100 (4.36 ± 0.38)
10^{-6}	100	106	109	105	107	106	107
10^{-5}	107 ± 4	93 ± 4	81 ± 5*	95 ± 1	94 ± 7	84 ± 7	96 ± 6
5×10^{-5}	81 ± 6	66 ± 3‡	55 ± 4‡	71 ± 3‡	64 ± 6†	51 ± 8†	65 ± 6†
10^{-4}	77 ± 5†	50 ± 2‡	46 ± 6‡	53 ± 3‡	48 ± 3‡	41 ± 7‡	49 ± 3‡
5×10^{-4}	64 ± 7‡	30 ± 1‡	23 ± 5‡	34 ± 3‡	31 ± 4‡	23 ± 5‡	32 ± 3‡
10^{-3}	32 ± 3‡	27 ± 4‡	17 ± 3‡	36 ± 11‡	35 ± 5‡	12 ± 4‡	40 ± 7‡

Hepatocytes were incubated with various concentrations of carbidopa for 40 min before addition of 0.1 mM-radiolabelled L-tryptophan. Rates of $^{14}CO_2$ production and formation of ^{14}C-labelled non-aromatic products were determined between 30 and 90 min after addition of tryptophan. Absolute rates of enzymic flux (given as nmoles of tryptophan/hr/mg dry wt of cells) are shown in parentheses. Values are the means (± S.E.M.) from 3 independent experiments. P (versus corresponding controls: * <0.05; † <0.01; ‡ <0.001 by paired *t*-test.

Table 2. Effect of benserazide on metabolism of 0.1 mM L-tryptophan by isolated rat liver cells

	Enzymic flux (as % of rate in absence of added drug)						
	L-[Ring 2-^{14}C] tryptophan	L-[Carboxyl-^{14}C]tryptophan			L-[Methylene-^{14}C]tryptophan		
Benserazide concn (M)	Total ^{14}C release (tryptophan dioxygenase)	Total ^{14}C release (kynureninase)	$^{14}CO_2$	^{14}C-Non aromatic metabolites	Total ^{14}C release (kynureninase)	$^{14}CO_2$	^{14}C-Non aromatic metabolites
0	100 (6.56 ± 1.15)	100 (5.17 ± 0.70)	100 (1.40 ± 0.13)	100 (4.38 ± 0.46)	100 (5.03 ± 0.48)	100 (0.40 ± 0.04)	100 (4.60 ± 0.40)
10^{-5}	98 ± 3	93 ± 3	80 ± 6*	94 ± 2	94 ± 10	83 ± 4*	99 ± 2
5×10^{-5}	95 ± 2	90 ± 4	74 ± 1‡	92 ± 3	94 ± 10	82 ± 1‡	103 ± 7
10^{-4}	92 ± 4	86 ± 2*	74 ± 3‡	90 ± 6	83 ± 3*	75 ± 3‡	93 ± 2
5×10^{-4}	78 ± 6*	78 ± 6*	61 ± 7‡	87 ± 3*	86 ± 5*	57 ± 4‡	88 ± 4*
10^{-3}	45 ± 11‡	45 ± 6‡	24 ± 3‡	60 ± 9‡	48 ± 4‡	19 ± 2‡	57 ± 6‡

Hepatocytes were incubated with various concentrations of benserazide for 40 min before addition of radiolabelled 0.1 mM L-tryptophan. Rates of $^{14}CO_2$ production and formation of ^{14}C-labelled non-aromatic products were determined between 30 and 90 min after addition of tryptophan. Absolute rates of enzymic flux (given as nmoles of tryptophan/hr/mg dry wt of cells) are shown in parentheses. Values are the means (± S.E.M.) from 3 independent experiments. P (versus corresponding controls): * <0.05; ‡ <0.01; ‡ <0.001 by paired *t*-test.

determined and compared with those obtained with carbidopa. The results are presented in Table 2.

Benserazide decreased tryptophan dioxygenase flux although this inhibitor was not as potent as carbidopa; only concentrations of benserazide of 2.5×10^{-4} M and above produced significant inhibition. Paradoxically however, 10^{-3} M benserazide produced a greater inhibition of tryptophan dioxygenase than did 10^{-3} M carbidopa.

Benserazide decreased tryptophan dioxygenase inhibition of $^{14}CO_2$ and ^{14}C-labelled non-aromatic metabolites from the two side chain-^{14}C-labelled radioisomers; $^{14}CO_2$ release again was inhibited to a much greater extent. Hepatocyte kynureninase activity, like tryptophan dioxygenase activity, was less sensitive to inhibition by benserazide than by carbidopa. Significant inhibition of kynureninase activity was only observed with concentrations of benserazide of 10^{-4} M and above.

The lower ability of benserazide to inhibit tryptophan oxidation by isolated liver cells may be partially attributable to the fact that metabolism of the parent compound to form the active inhibitory species is a necessary prerequisite. Cleavage of the terminal seryl group to yield the free hydrazide is required for inhibition of L-aromatic amino acid decarboxylase [16]. Benserazide only inhibits highly purified rat liver kynureninase at concentrations above 10^{-3} M (i.e. under conditions that preclude seryl group cleavage) whereas carbidopa (which itself is the free hydrazide) is inhibitory at 10^{-5} M (S. A. Smith, unpublished work).

Effects of carbidopa and benserazide on [1-^{14}C]alanine and [1-^{14}C]pyruvate oxidation

The differential extents to which $^{14}CO_2$ and [^{14}C]alanine production from side chain ^{14}C-labelled tryptophan are inhibited by both benserazide and carbidopa in hepatocyte incubations strongly indicates that in addition to inhibition of kynureninase alanine oxidation may also be impaired. This possibility was examined by determining the effects of carbidopa and benserazide on $^{14}CO_2$ production by liver cells incubated with 1 mM [1-^{14}C]alanine (Table 3). Both compounds produced a concentration-dependent inhibition of $^{14}CO_2$ production from 1 mM-[1-^{14}C]alanine. The inhibition of alanine oxidation by carbidopa was considerably greater than that observed with benserazide; this may again by a reflection of the relatively slow conversion of benserazide to the active inhibitor. In this series of experiments liver cells were incubated with inhibitor for only 20 min (compared with 40 min for the tryptophan oxidation experiments) before substrate addition.

Metabolic $^{14}CO_2$ is produced from [1-^{14}C]alanine largely by a two step process—transamination to produce [1-^{14}C]pyruvate followed by oxidative decarboxylation of [1-^{14}C]pyruvate to yield $^{14}CO_2$ and acetyl CoA. The locus of inhibition of alanine oxidation by these two compounds was determined using 1 mM-[^{14}C]pyruvate as substrate. Over the range of concentrations used neither benserazide nor carbidopa had any significant effect on $^{14}CO_2$ production from [1-^{14}C]pyruvate. This indicates that pyruvate oxidation is unimpaired; the reduction of alanine oxidation is probably mediated by inhibition of the pyridoxal phosphate-dependent alanine aminotransferase.

DISCUSSION

This study clearly demonstrates that benserazide and carbidopa both inhibit the oxidation of L-tryptophan by isolated rat liver cells. Evidence obtained with different radioisomers of L-tryptophan indicates that the two compounds inhibit at two distinct enzymic steps of the kynurenine pathway, namely tryptophan dioxygenase and kynureninase.

Inhibition of tryptophan dioxygenase by inhibitors of aromatic amino acid decarboxylase has previously been demonstrated in extracts of rat liver [2, 3]. The observations that benserazide potentiates the rise in plasma tryptophan but decreases the rise in kynurenine formation after a tryptophan load in rats [3] and that carbidopa inhibits respiratory $^{14}CO_2$ production from [ring 2-^{14}C]tryptophan [5] suggests that this inhibition can occur *in vivo*. However, the pre-

Table 3. Effect of benserazide and carbidopa on $^{14}CO_2$ production from 1 mM [1-^{14}C]alanine and 1 mM [1-^{14}C]pyruvate by isolated rat hepatocytes

Drug concn (M)	Inhibition of $^{14}CO_2$ production (%)			
	Benserazide		Carbidopa	
	[1-^{14}C]Alanine	[1-^{14}C]Pyruvate	[1-^{14}C]Alanine	[1-^{14}C]Pyruvate
0	0 (101 ± 5)	0 (67 ± 20)	0 (95 ± 11)	0 (60 ± 22)
10^{-5}	1 ± 2	0 ± 3	15 ± 2*	1 ± 1
5×10^{-5}	4 ± 3	1 ± 3	58 ± 8‡	1 ± 0
10^{-4}	11 ± 7	3 ± 4	71 ± 9‡	1 ± 1
2.5×10^{-4}	13 ± 6	7 ± 3	88 ± 4‡	6 ± 3
10^{-3}	35 ± 7†	12 ± 9	96 ± 1‡	6 ± 4

Hepatocytes were incubated with various concentrations of benserazide or carbidopa for 20 min before addition of [1-^{14}C]alanine or [1-^{14}C]pyruvate (1 mM, final). Incubations were terminated 1 hr after substrate addition. Rates of $^{14}CO_2$ production in the absence of added drug (expressed as nmoles $^{14}CO_2$/hr/mg dry wt of cells) are given in parentheses. All values are means ± S.E.M. from 3 independent experiments. P (versus appropriate control): * <0.05; † <0.01; ‡ < 0.001 by paired *t*-test.

cise mechanism of inhibition of tryptophan dioxygenase is unknown. In addition to inhibition of the normally catalytically active holoenzyme species [3], it may involve a shift in the equilibrium of apo- and holoenzyme forms of the enzyme in favour of the inactive apoenzyme. Evidence from studies *in vitro* suggests that these compounds may interfere with binding of the haem cofactor to the enzyme [3].

The inhibition of kynureninase activity probably results primarily from Schiff-base formation between the hydrazine derivative and the pyridoxal phosphate cofactor of the enzyme [4]. Tissue concentrations of pyridoxal phosphate are severely depleted after administration to rats of carbidopa [17]. The potency of these compounds as inhibitors of kynuyreninase is increased compared to other hydrazides because of their structural similarities to the physiological substrates of the enzyme [4].

Our studies have shown that, in the intact hepatocyte, tryptophan dioxygenase and kynureninase display differential sensitivities to inhibition by benserazide and carbidopa; kynureninase is the more sensitive. 5×10^{-5} M Carbidopa and 10^{-4} M benserazide produced a significant reduction of carbon flux through kynureninase in the absence of any change in tryptophan dioxygenase activity. This finding is consistent with values obtained from measurements *in vitro*. The K_i values of tryptophan dioxygenase for carbidopa and benserazide are 2.6×10^{-5} M and 4.2×10^{-5} M respectively, whilst those of kynureninase for carbidopa and benserazide are 0.47×10^{-5} M and 2.6×10^{-5} M respectively [4].

At high drug concentrations ($>2.5 \times 10^{-4}$ M) two factors will contribute to the inhibition of kynureninase flux—first, a direct reduction of catalytic activity of the enzyme and, second, a decreased substrate supply, consequent upon the inhibition of the rate-limiting enzyme of the pathway, tryptophan dioxygenase.

In addition to demonstrating that benserazide and carbidopa inhibit tryptophan dioxygenase and kynureninase activities of hepatocyte suspensions, these experiments have established that the two compounds also inhibit alanine oxidation. Inhibition of alanine oxidation by hydrazides is not unexpected—alanine aminotransferase is a pyridoxal phosphate-dependent enzyme. Benserazide and carbidopa are known to be potent inhibitors of other transaminases [2, 8, 9]. Carbidopa also inhibits gluconeogenesis from lactate in isolated rat liver cells, a process involving pyridoxal-dependent transaminases [18].

More importantly perhaps, these findings highlight the hazards involved in the interpretation of experiments involving measurement of $^{14}CO_2$ production by whole animals from side-chain-[^{14}C]labelled tryptophan [3, 5]. Inhibition of $^{14}CO_2$ production has been attributed solely to inhibition of tryptophan oxidation. Our studies have shown that low concentrations of aromatic amino decarboxylase inhibitors inhibit $^{14}CO_2$ production from [carboxyl-^{14}C] and [methylene-^{14}C]tryptophan without inhibition of net carbon flux down the kynurenine pathway; this apparent paradox can be reconciled simply in terms of inhibition of alanine oxidation. Similarly, at higher drug concentrations, if $^{14}CO_2$ production alone is measured, the "apparent" inhibition of tryptophan oxidation will always be greater than the "real" inhibition.

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