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IMPORTANCE OF TRYPTOPHAN PYRROLASE AND AROMATIC AMINO ACID DECARBOXYLASE IN THE CATABOLISM OF TRYPTOPHAN

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Abstract—Benserazide, an inhibitor of aromatic amino acid decarboxylase, potentiates the rise of plasma tryptophan in rats given a tryptophan load. It also inhibits $^{14}CO_2$ release from animals given carboxyl-labeled tryptophan. These results are explained by the ability of benserazide to inhibit tryptophan pyrrolase, the most important enzyme catabolizing tryptophan. Direct decarboxylation is not a quantitatively important pathway of tryptophan catabolism and carboxyl-labeled tryptophan is metabolized to $^{14}CO_2$ primarily by the pyrrolase pathway. These data have implications for the clinical use of tryptophan as an antidepressant. Pyridoxine, which is often given with tryptophan in clinical use, can activate aromatic amino acid decarboxylase. However, pyridoxine does not inhibit the rise of plasma tryptophan in rats given a tryptophan load and is unlikely to antagonize the therapeutic effect of tryptophan. It may be possible to potentiate the therapeutic effect of tryptophan by administering it with benserazide to inhibit its peripheral catabolism through the pyrrolase pathway.

Two recent studies suggest that decarboxylation is a quantitatively important pathway of tryptophan catabolism. The aromatic-L-amino acid decarboxylase (AADC) inhibitor benserazide [Ro-4-4602, N^1-(DL-seryl)-N^2-(2,3,4)-trihydroxybenzylhydrazine] potentiated the rise in plasma tryptophan concentration after a tryptophan load [1] and inhibited the release of respiratory $^{14}CO_2$ in mice given carboxyl-labeled [^{14}C]tryptophan [2]. However, this conclusion is surprising in view of other data. Clinical chemical studies show that in man the urinary excretion of tryptamine is about 100 μg/day [3] and that of indoleacetic acid is no more than 6 mg/day [4]. Thus, the daily excretion of the two major products of the decarboxylation pathway represents a tiny fraction of the normal daily dietary intake of tryptophan, which is in the range of 0.5 to 1.5 g.

Experimental work in this laboratory has shown that in adrenalectomized rats respiratory $^{14}CO_2$ is released almost as fast from [side-chain-3-^{14}C] tryptophan as from the carboxyl-labeled compound [5]. The side-chain carbons are released as CO_2 in a catabolic route initiated by tryptophan pyrrolase. After the side chain is split from kynurenine and 3-hydroxykynurenine as alanine, it undergoes transamination to pyruvate, and the pyruvate passes through the Krebs cycle. This pathway liberates CO_2 from the carboxyl group of tryptophan on decarboxylation of pyruvate. The C-3 carbon is liberated later, after one and a half turns of the Krebs cycle. Thus, the pyrrolase pathway must give as much or more CO_2 from the carboxyl group of the side chain as from the 3 position. The fact that respiratory $^{14}CO_2$ appears almost as quickly from [side-chain-3-^{14}C]tryptophan as from the carboxyl-labeled compound [5] indicates that the carboxyl-carbon of tryptophan is released primarily through the action of the pyrrolase pathway and not of AADC.

Inhibition of $^{14}CO_2$ release from [^{14}COOH]tryptophan by benserazide could indicate that this decarboxylase inhibitor is also a pyrrolase inhibitor. The ability of benserazide to potentiate the rise in plasma tryptophan concentration after a tryptophan load [1] is also consistent with inhibition of tryptophan pyrrolase. Therefore, we have looked at the effect of benserazide on the breakdown of tryptophan by AADC and tryptophan pyrrolase, in order to re-assess the quantitative significance of these two enzymes in tryptophan catabolism.

MATERIALS AND METHODS

Chemicals. Benserazide was a gift from Hoffmann-LaRoche, Inc. DL-[Ring-2-^{14}C]tryptophan was from International Chemical and Nuclear Corp. and L-[side-chain-1-^{14}C]tryptophan was from New England Nuclear Corp. L-[side-chain-1-^{14}C]α-Methyldopa was a gift from Merck, Sharp & Dohme Research Laboratories.

Animals. Male Sprague–Dawley rats (110–160 g) were obtained from Canadian Breeding Farms and Laboratories Ltd., St. Constant, Quebec. Rats were deprived of food but not water, beginning at 9:00 a.m. on the day of an experiment. Five to 6 hr after the food had been removed the rats were either killed or an assay *in vivo* was performed.

Measurements in vivo. Tryptophan breakdown *in vivo* was measured by the method of Madras and Sourkes [5] with modifications described previously [6]. Rats were injected intraperitoneally with 5 μCi/kg of DL-[ring-2-^{14}C]tryptophan or L-[side-chain-1-^{14}C]tryptophan. Both compounds were injected with 50 mg/kg of cold L-tryptophan. To measure AADC, rats were injected with a solution containing 10 μCi/kg of L-[side-chain-1-^{14}C]α-methyldopa and 10 mg/kg of cold L-α-methyldopa. Respiratory $^{14}CO_2$

was trapped in ethanolamine–ethylene glycol monomethyl ether and estimated by liquid scintillation counting.

Assays in vitro. Tryptophan pyrrolase activity was assayed in the fraction obtained from a 25% liver homogenate after centrifuging at 100,000 *g* [7, 8]. Preincubation of this enzyme solution with methemoglobin (to conjugate the apoenzyme with its coenzyme) permitted the measurement of total tryptophan pyrrolase (apoenzyme + holoenzyme) [7]. Preincubation without methemoglobin provided holoenzyme alone [8]. The appearance of kynurenine was measured spectrophotometrically. Pyrrolase activity is expressed in the following units: μmoles kynurenine formed/hr/g of liver. In experiments in which benserazide was added to liver homogenates *in vitro*, the rats were pretreated with tryptophan to ensure high tryptophan pyrrolase activity.

Formamidase was measured by the method of Knox [9]; the unit of activity is μmoles kynurenine formed/hr/g of liver. To measure the effect of benserazide added *in vitro*, it was necessary to add ascorbic acid at a concentration of 5 mg/ml to the assay mixture in order to prevent the formation of an oxidation product of benserazide, which absorbs at the same wavelengths as kynurenine.

Liver radioactivity was measured as described previously [6] by precipitating proteins from a liver homogenate with ethanol.

Plasma tryptophan was measured by the method of Denckla and Dewey [10] and plasma kynurenine by the method of Joseph and Risby [11].

RESULTS

α-Methyldopa cannot be transaminated like dopa and, therefore, release of $^{14}CO_2$ from carboxyl-labeled α-methyldopa gives a good index of "whole body" AADC activity [12]. With the small amount of [^{14}C]α-methyldopa available, we showed that 400 mg/kg of benserazide inhibited whole body AADC greatly. Rats were injected with saline or benserazide (400 mg/kg) and 30 min later with the labeled α-methyldopa. Between 30 and 60 min after the α-methyldopa, two control rats were expiring $^{14}CO_2$ at rates of 0.62 and 0.58 per cent of the injected ^{14}C per hr, while for two treated rats values were 0.12 and 0.08 per cent. This represents about 85 per cent inhibition.

The results in Fig. 1 show that the same dose of benserazide inhibits $^{14}CO_2$ release from carboxyl-labeled tryptophan to a smaller extent. However, Fig. 2 reveals that benserazide decreases $^{14}CO_2$ release from [ring-2-^{14}C]tryptophan more than from the carboxyl-labeled tryptophan. Tryptophan pyrrolase is the rate-limiting enzyme in the short pathway of the C_2 of the ring-labeled tryptophan to $^{14}CO_2$ [6, 13, 14]. Therefore, an alteration in respiratory $^{14}CO_2$ derived from the pyrrole ring-labeled tryptophan indicates either that there is a change in the uptake of radioactive tryptophan into the liver, the site of tryptophan pyrrolase, or that there is a change in tryptophan pyrrolase activity. The data of Table 1 show that benserazide does not influence the amount of [^{14}C]tryptophan found in the liver 30 min after injection of the labeled tryptophan. At this time after labeled tryptophan injection about 85 per cent of the ^{14}C in liver is still part of the tryptophan molecule (S. N. Young, unpublished data). Thus, benserazide does not influence the amount of labeled or unlabeled tryptophan in the liver and, in this situation, $^{14}CO_2$ release is a good index of the rate of tryptophan catabolism by tryptophan pyrrolase *in vivo*. Thus, the dose of benserazide given inhibits tryptophan pyrrolase at least as effectively as it inhibits AADC.

Another method that can give a good index of tryptophan catabolism by tryptophan pyrrolase in the living animal is measurement of plasma kynurenine [15], the second metabolite on the pyrrolase pathway. The data in Table 2 confirm that benserazide potentiates the rise in plasma tryptophan after a trypto-

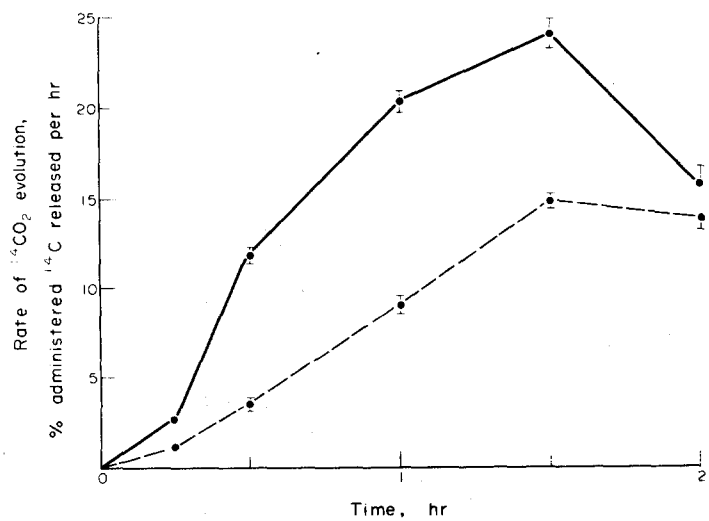

Fig. 1. Effect of benserazide on $^{14}CO_2$ release from L[side-chain-1-^{14}C]tryptophan. Rats were injected intraperitoneally with saline (——) or 400 mg/kg of benserazide (----) 15 min before receiving carboxyl-labeled tryptophan. Rates of respiratory $^{14}CO_2$ production are shown for various times after administration of the labeled tryptophan. Each point represents the mean for six rats ± S. E.

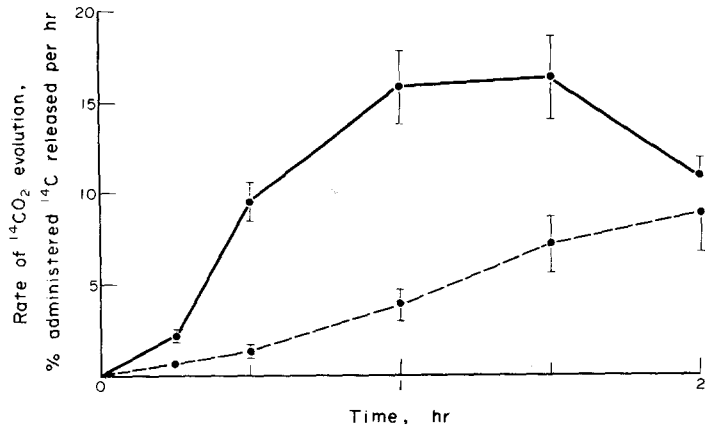

Fig. 2. Effect of benserazide on $^{14}CO_2$ release from DL[ring-2-^{14}C]tryptophan. Rats were injected intraperitoneally with saline (——) or 400 mg/kg of benserazide (----) 15 min before receiving pyrrole ring-labeled tryptophan. Rates of respiratory $^{14}CO_2$ production are shown for various times after administration of the labeled tryptophan. Each point represents the mean for six rats ± S. E.

phan load [1] and demonstrate that this drug can decrease the rise in plasma kynurenine. These results also indicate that the inhibition of tryptophan catabolism occurs by way of the tryptophan pyrrolase pathway.

The conclusion that benserazide inhibits tryptophan pyrrolase is consistent with the results obtained by measuring tryptophan pyrrolase *in vitro*; these are shown in Table 1. Thus, when benserazide is administered to rats, less tryptophan pyrrolase activity is found *in vitro*. The inhibition is greater for the holoenzyme than for the total (i.e. holo- plus apo-) enzyme activity. This result suggests that addition of heme *in vitro* may partially overcome the inhibition. Benserazide is also capable of inhibiting tryptophan pyrrolase when added *in vitro* (Table 3). The lowest concentration tested, 0.1 mM, is less than the concentration that would occur in the liver if a load of 50 mg/kg were evenly distributed throughout the animal. The tryptophan pyrrolase assay, which measures the appearance of kynurenine (and not formylkynurenine), depends on the presence of an excess of formamidase. Therefore, we undertook to test the action of benserazide on formamidase. When the drug was administered to rats before removing the liver for enzymic assay, there was no effect (Table 1). When benserazide was added *in vitro* at 5 mM, there was a slight inhibition of formamidase (Table 3). As formamidase activity in the liver is several hundred times greater than that of tryptophan pyrrolase [9], the slight inhibition seen would have had no effect on the tryptophan pyrrolase assays.

For reasons described in the Discussion, tryptophan is often given clinically with a pyridoxine supplement. If decarboxylation were important in the catabolism of tryptophan, it would be expected that pyridoxine would speed the catabolism of a tryptophan load through AADC, a pyridoxal phosphate-dependent enzyme. The results set out in Table 2 show that pyridoxine has no effect on the plasma tryptophan concentration after a tryptophan load, although, under the same conditions, benserazide potentiates the rise of plasma tryptophan.

DISCUSSION

As mentioned in the introduction, the urinary content of tryptamine and indoleacetic acid suggests that less than 1 per cent of the daily dietary intake of tryptophan is metabolized by decarboxylation. Decar-

Table 1. Effect of benserazide administration on ^{14}C, tryptophan, tryptophan pyrrolase and formamidase in the liver*

	N	Control	Benserazide
First experiment			
Per cent of ^{14}C administered/g liver	6	0.44 ± 0.01	0.42 ± 0.03
Tryptophan (μg/g liver)	5	16.9 ± 1.5	14.7 ± 1.8
Second experiment			
Tryptophan pyrrolase (units)			
Holoenzyme	6	0.79 ± 0.06	0.32 ± 0.04†
Total enzyme	6	2.10 ± 0.12	1.75 ± 0.13
Formamidase (units)	6	735 ± 33	750 ± 20

* In the first experiment, rats were injected intraperitoneally with saline or benserazide (400 mg/kg) 15 min before injection of DL[ring-2-^{14}C]tryptophan (5 μCi/kg) with cold L-tryptophan (50 mg/kg), and killed 30 min later. ^{14}C and tryptophan were measured in the livers. In the second experiment, rats were injected with saline or benserazide (400 mg/kg) and killed 30 min later. Tryptophan pyrrolase and formamidase were measured in the livers. Results are given as mean ± S.E.

† $P < 0.001$, compared with control.

Table 2. Effect of benserazide on plasma tryptophan and kynurenine and the effect of pyridoxine on plasma tryptophan after a tryptophan load*

	N	Tryptophan (μg/ml)	Kynurenine (μg/ml)
First experiment			
Control	7	79.3 ± 3.5	1.25 ± 0.03
Benserazide	7	90.9 ± 7.0	1.06 ± 0.07†
Second experiment			
Control	6	129 ± 4	
Benserazide	6	165 ± 4‡	
Pyridoxine	6	111 ± 20	

* In the first experiment, rats were injected intraperitoneally with benserazide (400 mg/kg) or saline 15 min before an injection of L-tryptophan (100 mg/kg). They were killed 30 min after the L-tryptophan. In the second experiment, the pyridoxine-treated group was given three daily injections of pyridoxine hydrochloride (2 mg/kg) with the last one given 15 min before L-tryptophan (200 mg/kg). The other two groups were given a single injection of benserazide (400 mg/kg) or saline 15 min before the tryptophan (200 mg/kg). All three groups were killed 45 min after tryptophan administration. All values are given as mean ± S. E.

† $P < 0.05$, compared with control.

‡ $P < 0.001$, compared with control.

boxylation could still be a quantitatively important pathway of tryptophan breakdown if tryptamine or indoleacetic acid were metabolized through unknown pathways. This is very unlikely. When tryptophan labeled in the benzene ring is administered to mice, metabolites along the pyrrolase and 5-hydroxytryptamine pathways accounted for all the urinary components which contained ^{14}C in appreciable amounts [16]. This was so even up to 6 hr after administration of the labeled tryptophan or when the radioactive compound was given with a dose of tryptophan as high as 1 g/kg. It must be concluded from this that decarboxylation is not a quantitatively important pathway of tryptophan catabolism even after large tryptophan loads.*

The ability of benserazide to potentiate the rise of plasma tryptophan after a tryptophan load is best explained by its ability to inhibit tryptophan breakdown by tryptophan pyrrolase. In support of this idea, it is known that changes in tryptophan pyrrolase activity can influence plasma tryptophan concentration after administration of tryptophan to rats [15].

The action of benserazide on tryptophan pyrrolase also explains why the drug inhibits $^{14}CO_2$ release from carboxyl-labeled tryptophan. The evidence described in the introduction indicates that little if any of the $^{14}CO_2$ from that labeled tryptophan is released by action of AADC, so inhibition of that enzyme is not relevant in this situation.

The ability of benserazide to inhibit catabolism of tryptophan by tryptophan pyrrolase is shared by another AADC inhibitor, carbidopa (MK 486, α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid). Studies in this laboratory have shown that carbidopa can inhibit $^{14}CO_2$ production in rats injected with both DL[ring-2-^{14}C]tryptophan and DL[side-chain-3-^{14}C]tryptophan [17]. Inhibition of tryptophan pyrrolase by both benserazide and carbidopa may be due, in part, to the hydrazino group which they both contain, as other hydrazino derivatives are also tryptophan pyrrolase inhibitors [17].

Benserazide can potentiate the rise in plasma tyrosine after a tyrosine load as well as the rise in tryptophan after a tryptophan load [1]. This effect is not due to the importance of decarboxylation in the catabolism of tyrosine as was originally suggested [1], but to the ability of benserazide to inhibit the major enzyme catabolizing tyrosine, tyrosine transaminase [18]. Thus, benserazide, which first became of pharmacologic interest because of its ability to inhibit synthesis of both catecholamines and indoleamines by its action on AADC, can also inhibit the major enzymes catabolizing the catecholamine and indolea-

* When this work was essentially complete, one of us (T. L. S.) received a personal communication (March 1977) from W. Dairman and S. Udenfriend. These investigators, in their more recent work, have administered to mice by the oral route 1.5 g/kg of L-tryptophan labeled with ^{14}C in the carboxyl position. During the following 6 hr, they observed that 21.7 per cent of the administered radioactivity was recoverable as expired carbon dioxide. When benserazide was administered (75 mg/kg, intraperitoneally) 45 min prior to the administration of 1.5 g/kg of L-tryptophan, only a slight (6 per cent) decrease of expired radioactive carbon dioxide was observed. Based on these data, they have concluded that even under conditions of high L-tryptophan intake decarboxylation could have accounted for no more than 1 per cent of the metabolism of the loading dose.

Table 3. Effect of benserazide on tryptophan pyrrolase and formamidase *in vitro**

Concn of benserazide (mM)	Tryptophan pyrrolase		Formamidase	
	Activity (units)	% Inhibition	Activity (units)	% Inhibition
0	2.81		820	
0.1	2.05	27		
0.5	1.43	49		
5	0.70	75	710	13

* Tryptophan pyrrolase and formamidase were measured in a liver homogenate from a rat treated with tryptophan to ensure high tryptophan pyrrolase activity. The concentration of benserazide shown was added to the assay mixture. Tryptophan pyrrolase activity was measured without addition of heme and, therefore, was holoenzyme activity.

mine precursors, tyrosine transaminase and tryptophan pyrrolase.

The work described in this paper has implications for the use of tryptophan in neuropsychiatric disorders. The therapeutic efficacy of levodopa is decreased when it is given with pyridoxine [19] because pyridoxine activates AADC and, therefore, speeds catabolism of levodopa in the liver and kidney [20]. Tryptophan is often given clinically with pyridoxine because several of the enzymes related to the pyrrolase pathway use pyridoxal phosphate as coenzyme. If AADC were a quantitatively important pathway of tryptophan breakdown, pyridoxine might antagonize the therapeutic action of this amino acid in the same way that it counteracts the clinical effect of levodopa. Thus, the equivocal action of tryptophan as an antidepressant might be explained in part by the variable use of pyridoxine with tryptophan [21] if decarboxylation of tryptophan were quantitatively significant. However, our results demonstrate that the rise in plasma tryptophan after a tryptophan load is not affected by pyridoxine administration. Therefore, the data available at the present time suggest that pyridoxine should be given with tryptophan in clinical studies in order to prevent accumulation of metabolites produced along the pyrrolase pathway.

The ability of benserazide to inhibit tryptophan pyrrolase may also have clinical significance. Although catabolism of tryptophan by AADC in the periphery will not reduce the availability of tryptophan to the brain after tryptophan loads, catabolism by tryptophan pyrrolase can do so [15]. This effect of tryptophan pyrrolase could be overcome by inhibiting the enzyme. Allopurinol has been suggested for this purpose, in situations where tryptophan is used clinically [22]. However, although allopurinol inhibits tryptophan pyrrolase *in vitro*, it has no influence on the enzyme *in vivo* [15]. We have suggested the use of nicotinamide, which is a feedback inhibitor of tryptophan pyrrolase [23]. In a preliminary study of the action of a tryptophan–nicotinamide combination in depressed patients, we have obtained evidence which is consistent with the idea that the variable action of tryptophan as an antidepressant might be explained in terms of its high rate of catabolism through the pyrrolase pathway [24]. Our present study suggests that benserazide, which is already in clinical use in conjunction with levodopa as a peripheral AADC inhibitor, may also be useful clinically as a tryptophan pyrrolase inhibitor. The use of benserazide might potentiate and prolong the rise of brain tryptophan and, therefore, also the rise of brain 5-hydroxytryptamine that occurs on tryptophan administration.

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