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CHANGES IN LIVER TRYPTOPHAN AND TRYPTOPHAN PYRROLASE ACTIVITY AFTER ADMINISTRATION OF SALICYLATE AND TRYPTOPHAN TO THE RAT

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Abstract—The tryptophan content and tryptophan pyrrolase activity of rat liver were measured after the intraperitoneal administration of a range of doses of L-tryptophan and salicylate. The injection of salicylate increased both the total and the free concentration of tryptophan in the liver. It is suggested that salicylate induces tryptophan pyrrolase by releasing tryptophan from its binding sites on serum and liver proteins.

SALICYLATE displaces tryptophan from its binding sites on bovine serum albumin *in vitro*[1] and from circulating human proteins *in vivo*.[2] The intraperitoneal administration of the drug (400 mg/kg body wt.) in the rat causes a 2-fold increase in the liver tryptophan concentration after 15 min which is followed at 5 hr by a 6-fold rise in tryptophan pyrrolase activity.[3,4] The present report provides evidence suggesting that salicylate enhances the substrate induction of the enzyme by displacing tryptophan from serum and liver protein binding sites.

METHODS

Animals and injections

Male Wistar rats (200–300 g) fed water and standard M.R.C. cube diet 41B *ad lib.* were killed by stunning and cervical dislocation and their livers were removed within 30 sec of death. All the chemicals were given intraperitoneally in 0·9% (w/v) NaCl at pH 7·3 and the animals were sacrificed either after 30 min (for liver and serum tryptophan determination) or after 3 hr (for measurement of liver tryptophan pyrrolase activity). Sodium salicylate (200 or 400 mg/kg body wt.) was dissolved in 1 ml of 0·9% NaCl. Tryptophan (5–20 mg/100 g) was injected as a 2% (w/v) solution prepared by dissolving 40 mg of the amino acid in the minimum amount of M NaOH, adding 1 ml of 0·9% NaCl, neutralising with M HCl and finally diluting to 2 ml with the saline solution. The 100 mg/100 g dose of tryptophan was administered as a neutralised suspension containing 100 mg/ml.

Preparation of homogenates. The liver was removed within 30 sec of the death of the animal and was homogenized for 1 min at 1100 rev/min in 7 vol. of a solution containing 140 mM KCl and 2·5 mM NaOH at 0° in a glass homogenizer with a loose-fitting Teflon pestle. The homogenates were used within 10 min of preparation.

Determination of tryptophan pyrrolase activity. The activity of holoenzyme, that

measured in the absence of added haematin, in the liver homogenates was determined by measuring the conversion of L-tryptophan into kynurenine.[5] Samples (15 ml) of the homogenate were added to a solution containing 15 ml of 0·2 M sodium phosphate buffer, pH 7·0, 5 ml of 0·03 M L-tryptophan and 25 ml of water at 0°. Samples (3 ml) of the mixture were incubated with shaking at 37° in glass-stoppered 25 ml conical flasks in an atmosphere of O_2 for appropriate time-intervals up to 105 min. The reaction was stopped by the addition of 2 ml of 15% (w/v) metaphosphoric acid; the flasks and contents were shaken for a further 3 min and then filtered with Whatman No. 1 filter paper. To a measured portion (2·5 ml) of the filtrate was added 1·5 ml of 1M NaOH and the kynurenine present determined by measuring the E_{365} with a Unicam SP 800 spectrophotometer. Tryptophan pyrrolase activity was obtained from the slope of the linear phase of a plot of μmole of kynurenine produced per gram wet wt. of liver against time of incubation.

Determination of serum and liver tryptophan. This was performed fluorometrically[6] on acid extracts (total) and ultrafiltrates (free) of serum and liver.

(a) *Total tryptophan.* One gram of liver (wet wt.) was heated with 3·2 ml of water in a boiling-water bath for 2 min, cooled, homogenized with the addition of 2 ml of 20% (w/v) trichloroacetic acid and centrifuged at 5000 *g* for 10 min. One millilitre of serum was treated with 3 ml of water and 2 ml of 20% trichloroacetic acid, homogenized and centrifuged as above. Samples of the supernatants were analyzed for total (free + protein-bound) tryptophan.

(b) *Free tryptophan.* This was determined by analysing ultrafiltrates prepared by centrifuging 1 ml of serum or 2 ml of a 50% (w/v) liver homogenate (in 0·14 M KCl, pH 7·0) in dialysis tubings (8/32 in. inflated diameter, Scientific Instruments Centre Ltd., London, W.C.1, U.K.) at 3000 *g* for 1·5–2 hr at 6–10°.

The protein-bound tryptophan was determined by difference. The results (means of four animals per group) were expressed in micrograms of tryptophan per gram of liver (wet wt.) or per millilitre of serum ± S.E.M.

RESULTS

The intraperitoneal administration of 5 mg/100 g body weight of L-tryptophan did not affect the activity of rat liver tryptophan pyrrolase (Table 1). There was a 3-fold increase in the enzyme activity 3 hr after administration of 10 mg/100 g and an approximately 6-fold increase with the 20 mg/100 g dose of the amino acid. A non-inducing (200 mg/kg) dose of sodium salicylate given shortly after tryptophan enhanced the substrate induction of the hepatic pyrrolase, the most pronounced increase occurring with the non-inducing dose (5 mg/100 g) of the amino acid. Measurement of the liver tryptophan concentration (Table 2) showed that it increased in proportion to the dose of tryptophan. Salicylate (200 mg/kg) alone exerted no effect on the liver tryptophan concentration but when administered 10 min after the injection of tryptophan, significantly increased the effect due to the amino acid itself. The effects of salicylate on the free and total tryptophan concentrations in sera and livers of control and tryptophan treated rats are shown in Table 3. Salicylate (400 mg/kg) increased the free and total amino acid concentrations in the liver and decreased its total concentration in serum. The non-inducing (200 mg/kg) dose of salicylate caused a 40 per cent increase in the free tryptophan concentration in the liver. The injection of salicylate (200 mg/kg)

TABLE 1. EFFECT OF SALICYLATE ON THE INDUCTION OF RAT LIVER TRYPTOPHAN PYRROLASE BY TRYPTOPHAN

Dose of tryptophan injected (mg/100 g body wt.)	Pyrrolase activity	
	Tryptophan	Tryptophan + Salicylate
0	1·89 ± 0.03	1·87 ± 0·08
5	1·74 ± 0·05	4·39 ± 0·15*
10	6·00 ± 0·41	8·56 ± 0·36*
20	10·22 ± 0·18	10·42 ± 0·55
100	11·86 ± 0·92	11·38 ± 1·01

Animals were injected at 0 min with either tryptophan in dose levels given above or with tryptophan followed after 10 min by 200 mg/kg body wt. of sodium salicylate and killed at 3 hr. Tryptophan pyrrolase activity was measured in liver homogenates as described in the experimental section. Each value is expressed as micromoles of kynurenine formed per hour per gram wet wt. of liver and represents the mean ± S.E.M. of four animals.

* Denotes a significant difference ($P < 0·005$) between effects of tryptophan alone and tryptophan plus salicylate.

TABLE 2. EFFECT OF SALICYLATE WITH OR WITHOUT TRYPTOPHAN ON LIVER TRYPTOPHAN CONCENTRATIONS IN THE RAT

Dose of tryptophan injected (mg/100 g body wt)	Liver tryptophan conc.	
	Tryptophan	Tryptophan + Salicylate
0	10·7 ± 0·3	10·8 ± 0·2
5	32·3 ± 1·0	53·6 ± 1·3*
10	63·4 ± 2·5	105·9 ± 4·8*
20	137·7 ± 3·3	211·2 ± 4·7*

Animals were injected at 0 min with either tryptophan alone or tryptophan followed after 10 min by 200 mg/kg body wt. of sodium salicylate and killed at 30 min. Each value is expressed as micrograms of tryptophan per gram wet wt. of liver and represents the mean ± S.E.M. of four animals.

* Denotes a significant difference ($P < 0·001$) between the effects of tryptophan alone and tryptophan plus salicylate.

TABLE 3. EFFECTS OF SALICYLATE AND TRYPTOPHAN ON THE FREE AND TOTAL TRYPTOPHAN CONCENTRATIONS IN RAT SERUM AND LIVER

Injection	Dose (mg/kg)	Liver		Serum	
		Free	Total	Free	Total
Saline		6·2 ± 0·6	10·2 ± 0·3	4·7 ± 0·2	15·6 ± 0·7
Salicylate	400	16·0 ± 0·8	17·4 ± 0·8	3·9 ± 0·2	5·0 ± 0·2
Salicylate	200	8·7 ± 0·3	10·8 ± 0·2	4·0 ± 0·4	13·6 ± 1·5
Tryptophan	50	14·4 ± 1·0	31·7 ± 2·8	29·6 ± 2·1	71·1 ± 3·4
Tryptophan + Salicylate	50 + 200	43·8 ± 1·4	52·4 ± 1·0	52·1 ± 2·2	61·0 ± 2·8

Experimental conditions as in Table 2. Results expressed as micrograms of tryptophan either per millilitre of serum or per gram wet wt. of liver and represent means ± S.E.M. of four animals.

shortly after tryptophan (5 mg/100 g) increased the free tryptophan concentrations in serum and liver and the total amino acid concentration in the liver.

DISCUSSION

The effects of the intraperitoneal injection of various doses of L-tryptophan on the activity of rat liver tryptophan pyrrolase (Table 1) confirm previous findings[7] after oral and parenteral tryptophan administration and suggest that maximum induction of the hepatic pyrrolase can be achieved with doses of the substrate which are considerably lower than those employed by other workers.[8,9] It has been suggested[10] that a small increase in the liver-cell tryptophan concentration may be sufficient to allow activation of apotryptophan pyrrolase to the reduced holoenzyme, sequestration of the enzyme by tryptophan and the subsequent stabilization of pyrrolase activity. Measurement of the liver tryptophan concentration showed that it was increased by threefold after the injection of a small (5 mg/100 g) dose of tryptophan (Table 2) which had no effect on pyrrolase activity (Table 1). A 2-fold increase in the liver tryptophan concentration brought about by the intraperitoneal administration of 400 mg of sodium salicylate/kg body wt., was associated with a marked elevation of liver tryptophan pyrrolase activity.[3] Previous work has shown that the induction of hepatic tryptophan pyrrolase activity by salicylate is not mediated by a mechanism involving adrenocortical steroids.[4]

Salicylate binds to serum proteins[11] and displaces protein-bound tryptophan.[1,2] The finding that a non-inducing, 200 mg/kg, dose of salicylate does not affect the liver tryptophan concentration (Table 2) may be due to the tryptophan binding sites on rat serum proteins not being sufficiently saturated to allow displacement by salicylate whereas after tryptophan administration the drug becomes capable of acting on the larger number of saturated sites. Parallel to the increase in liver tryptophan observed when salicylate (200 mg/kg) was administered shortly after tryptophan (Table 2), the drug enhanced the substrate induction of the hepatic pyrrolase (Table 1), the most pronounced effect occurring with the non-inducing, 5 mg/100 g, dose of the amino acid. The above findings suggest that salicylate, in non-inducing doses, increases the extent of pyrrolase induction by the administered tryptophan by increasing its concentration in the liver.

The absence of pyrrolase induction following the administration of 5 mg of tryptophan/100 g body wt. (Table 1) may be due to the ability of serum and liver to bind large amounts of tryptophan (Table 3), and the enhancing effect of salicylate may be the result of the marked elevation of the free tryptophan concentration in liver and serum of tryptophan treated rats. The inducing dose of salicylate (400 mg/kg) also markedly increased the free tryptophan concentration in the liver (Table 3). One possible explanation of the present results is that salicylate induces tryptophan pyrrolase, and enhances the substrate induction of the enzyme activity, in rat liver by displacing tryptophan from its binding sites on rat serum and liver proteins and that the capacity of serum and liver to bind the amino acid appears to be an important factor in determining the extent of the substrate induction of the hepatic pyrrolase.

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