The Regulation of Rat Liver Tryptophan Pyrrolase Activity by Reduced Nicotinamide-Adenine Dinucleotide (Phosphate)

EXPERIMENTS WITH GLUCOSE AND NICOTINAMIDE

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1. Chronic administration of glucose or nicotinamide in drinking water inhibits the activity of rat liver tryptophan pyrrolase, and subsequent withdrawal causes an enhancement. The enzyme activity is also inhibited by administration in drinking water of sucrose, but not fructose, which is capable of preventing the glucose effect. 2. The inhibition by glucose or nicotinamide is not due to a defective apoenzyme synthesis nor a decreased cofactor availability. 3. The inhibition by nicotinamide is reversed by regeneration of liver NAD^+ and NADP^+ *in vivo* by administration of fructose, pyruvate or phenazine methosulphate. Inhibition by glucose is also reversed by the above agents and by NH_4Cl . Reversal of inhibition by glucose or nicotinamide is also achieved *in vitro* by addition of NAD^+ or NADP^+ . 4. Glucose or nicotinamide increases liver $[\text{NADPH}]$. $[\text{NADP}^+]$ is also increased by nicotinamide. $[\text{NADPH}]$ is also increased by sucrose, but not by fructose, which prevents the glucose effect. Phenazine methosulphate prevents the increase in $[\text{NADPH}]$ caused by both glucose and nicotinamide. 5. It is suggested that the inhibition of tryptophan pyrrolase activity by glucose or nicotinamide is mediated by both NADPH and NADH .

The activity of rat liver tryptophan pyrrolase [L-tryptophan-oxygen 2',3'-oxidoreductase (de-cyclizing), EC 1.13.11.11] is regulated by three mechanisms: hormonal induction by glucocorticoids, substrate activation by tryptophan and cofactor activation by haem (Knox, 1966; Schimke, 1969; Badawy & Evans, 1973, 1975c). Cho-Chung & Pitot (1967) reported that the activity of the purified enzyme is allosterically inhibited by NADPH and also by NADH . Since these two reduced nucleotides are the end products of tryptophan metabolism via the kynurenine pathway, Cho-Chung & Pitot (1967) suggested that the pyrrolase activity may be regulated by a feedback mechanism. That such a mechanism may operate in the rat *in vivo*, at least under certain conditions, is suggested by the results of experiments with drugs of dependence. Chronic administration of ethanol, phenobarbitone, morphine or nicotine (Badawy & Evans, 1975a,b) inhibits apo-(tryptophan pyrrolase) activity and increases liver $[\text{NADPH}]$. Ethanol treatment also increases $[\text{NADH}]$. Evidence suggesting the involvement of these two nucleotides in the above inhibition is provided by the findings that regeneration of liver NADP^+ *in vivo* (by phenazine methosulphate administration) or *in vitro* (by the addition of NADP^+ or NAD^+) reverses the pyrrolase inhibition by all four drugs of dependence,

whereas NAD^+ regeneration (by administration of fructose or pyruvate) only reverses that by ethanol.

In a further attempt to study the possible involvement of NADPH and NADH in the regulation of rat liver tryptophan pyrrolase activity, we have examined the effects on the latter of the two agents that are most physiologically relevant to the former, namely the precursor, nicotinamide, and glucose (metabolism), which can be assumed to be one of the major factors in the utilization of the redox couples constituting nicotinamide-adenine dinucleotides (phosphates). An acute large dose of nicotinamide has previously been shown to inhibit the pyrrolase activity in hypophysectomized or adrenalectomized, but not in intact, rats; the latter show induction, presumably by a non-specific corticosteroid-mediated mechanism (Yamaguchi *et al.*, 1967; Cho-Chung & Pitot, 1968). Glucose administration also inhibits the enzyme activity, and the inhibition has been suggested (Wetterberg *et al.*, 1970; Yuwiler *et al.*, 1970; Greengard & Dewey, 1973) to involve a metabolite(s) but not the sugar itself.

In the present paper, the inhibition of rat liver tryptophan pyrrolase activity by chronic nicotinamide administration is demonstrated. The nature of this inhibition and of the previously reported glucose effect are examined. The results suggest that the

pyrrolase inhibition by these two agents is associated with, and may be caused by, an increased liver [NADPH] and possibly also [NADH].

Materials and Methods

Chemicals

Enzymes and reagents used for the direct determination of liver nicotinamide-adenine dinucleotides (phosphates) have previously been described (Badawy & Evans, 1975a). 5-Aminolaevulinic acid hydrochloride, cortisol 21-acetate, haemin (haematin hydrochloride), nicotinamide, NAD⁺, NADP⁺ and L-tryptophan were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Ethanol (99.7–100%) and all other chemicals (from BDH Chemicals Ltd., Poole, Dorset, U.K.) were of AnalaR grade, except for dimethylformamide, D(-)-fructose, phenazine methosulphate (*N*-methylphenazonium methosulphate), 2,6-dichlorophenol-indophenol and sodium pyruvate, which were of reagent grade.

Animals and injections

Locally bred male Wistar rats (150–210g), maintained on cube diet 41B (Oxoid Ltd., Basingstoke, Hants., U.K.) and water (or solutions of compounds in water) *ad libitum*, were killed between 12:00 and 15:00h by stunning and cervical dislocation. Rats given nicotinamide chronically (body weight at the start of the experiment was 150g $\pm$ 10g) were purchased from Anglia Laboratory Animals, Alconbury, Huntingdon, U.K. Groups of rats received an oral intubation of water, glucose (15g/kg), fructose (15g/kg) or sucrose (30g/kg), all in volumes of 3ml/100g body wt., 3h before being killed. All other chemicals used in acute experiments were given intraperitoneally at various times before death as indicated in the Results section. Cortisol acetate (20mg/kg) and haematin hydrochloride (35mg/kg) were dissolved in dimethylformamide; each rat received 0.1ml/100g body wt. Control rats received an equal volume of the solvent which resembled 0.9% (w/v) NaCl in not significantly affecting tryptophan pyrrolase activity. 5-Aminolaevulinic acid hydrochloride (dissolved in 0.9% NaCl) was given in four injections at 1h intervals (15mg/kg body wt. each) and the animals were killed at 1h after the last injection. Fructose (400mg/kg), phenazine methosulphate (10mg/kg) and sodium pyruvate (200mg/kg) were dissolved in 0.9% NaCl; each rat received 0.1–0.2ml/100g body wt. Tryptophan (500mg) was dissolved in the minimum amount of 4M-NaOH and diluted to 20ml with 0.9% NaCl after the pH had been adjusted to 7.3 with 2M-HCl; each rat received 2ml/100g body wt. Some normal and glucose-treated rats received intraperitoneal doses of 5ml/kg body wt. of either

1M-NH₄Cl (267.5mg/kg) or 1M-NaCl (292.5mg/kg) 15min before being killed.

Chronic administration of nicotinamide, glucose, fructose and sucrose, and withdrawal of nicotinamide and glucose

Nicotinamide was freely administered to fed and individually housed rats in concentrations of 0.01–1.0g/litre of drinking water for up to 5 days. Additional rats (four per cage) were maintained on a 5g/litre solution of nicotinamide for up to 19 days. In this latter experiment, withdrawal was started by replacing the nicotinamide solution with drinking water. Fluid intake was similar in all rats irrespective of the dose of nicotinamide. Thus rats receiving the 0.01g/litre dose consumed 1–1.5mg of nicotinamide/kg body wt. per day, whereas those given the 5g/litre solution drank 0.5–0.75g/kg per day.

Glucose was freely administered to fed rats (four per cage) as a 15% (w/v) solution for up to 19 days, and, for withdrawal, the glucose solution was replaced with drinking water. Some individually housed rats received, for 1 day or 2 days, a (w/v) solution of either glucose (2.5–15%), fructose (10%), sucrose (20%) or a mixture of fructose (10%) plus glucose (5 or 10%). Sugar consumption varied between 55 and 90ml per rat per day, and a tendency to drink larger volumes was observed with rats receiving the weaker glucose solutions.

Chemical and enzymic determinations

Tryptophan pyrrolase activity was determined in liver homogenates by measuring the formation of kynurenine from L-tryptophan (Feigelson & Greengard, 1961) in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M) as detailed previously (Badawy & Evans, 1975a). The apoenzyme activity was calculated by difference. The concentrations of NAD⁺, NADH, NADP⁺ and NADPH were determined in pieces of liver (0.5g each) by the method of Slater & Sawyer (1962) and Slater *et al.* (1964) with minor modifications as described previously (Badawy & Evans, 1975a). Rats used for these direct determinations were of the same age, strain, sex (male) and body-weight range (150–160g).

Results

Effects of glucose, sucrose and fructose on rat liver tryptophan pyrrolase activity

When glucose was administered in drinking water (15g/litre) (Fig. 1), the holotryptophan pyrrolase activity was not significantly altered ($P > 0.10$) at any time during the 19 days of treatment. The total

Fig. 1. Effects of chronic glucose administration and subsequent withdrawal on the activity of rat liver tryptophan pyrrolase

Fed rats (four per cage) were allowed free access to a 15% (w/v) solution of glucose in drinking water. Withdrawal (the start of which is indicated by the arrow) was performed by replacing the glucose solution with drinking water. The enzyme activity was determined, as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin ($2\mu\text{M}$). The apoenzyme activity was calculated by difference. Each point represents the mean value for four rats. ●, Holoenzyme activity; ○, total enzyme activity; —, activities during chronic treatment; ----, activities during withdrawal.

pyrrolase activity, on the other hand, was significantly ($P < 0.001$) decreased (by 41%) after 1 day, and by 51–54% after 2–5 days of glucose consumption. The apoenzyme activity, calculated by difference, was also significantly ($P < 0.001$) decreased by 64% after 1 day, and by 86–91% after 2–5 days of treatment. The total enzyme activity then recovered slightly (on day 6), but remained significantly ($P = 0.05–0.001$) decreased (by 28–36%) until the end of the experimental period. When withdrawal was started after 10 days of treatment, both holoenzyme and total pyrrolase activities rose to a maximum 1.8- and 2.3-fold increase respectively ($P = 0.005–0.001$) 2 days, and returned to the basal values 5 days, after withdrawal. The ratio of holoenzyme/apoenzyme activity never exceeded 1 (range 0.61–0.91) during withdrawal, as compared with a basal ratio of 0.77. In experiments not reported here, it was found that doses of glucose of 5% (w/v) and above in drinking water significantly ($P = 0.005–0.001$) decreased the total pyrrolase activity by 45–69%. The holoenzyme activity was not significantly affected by any of the doses of glucose tested.

The effects on the enzyme activity of administration in drinking water of glucose, sucrose, fructose or mixtures of fructose plus glucose are shown in Table 1. The inhibition of the total pyrrolase activity by glucose (5 or 10%) is as described above. Sucrose (20%) also significantly ($P = 0.005–0.001$) inhibited the total enzyme activity (by 58 and 64%) and that of the apoenzyme (by 91 and 96%) respectively after 1 and 2 days of treatment. Fructose, on the other hand, did not significantly affect the pyrrolase

Table 1. Effects of administration of glucose, sucrose, fructose or mixtures of fructose plus glucose on rat liver tryptophan pyrrolase activity

Individually housed rats were allowed free access to food and drinking water. Groups of rats received either glucose, sucrose, fructose or mixtures of fructose plus glucose for either 1 or 2 days before being killed. The doses of sugars are expressed in concentrations (w/v) in drinking water. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin ($2\mu\text{M}$). The apoenzyme activity was calculated by difference. Values are means $\pm$ S.E.M. for each group of four rats.

Kynurenine formed ($\mu\text{mol/h per g wet wt. of liver}$)

Treatment	Dose (g/100ml)	1 day treatment		2 days treatment	
		Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
Nil	—	1.6 ± 0.1	3.8 ± 0.4	1.7 ± 0.1	4.4 ± 0.1
Glucose	5	1.8 ± 0.1	2.5 ± 0.2	1.6 ± 0.2	1.9 ± 0.3
Glucose	10	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
Sucrose	20	1.5 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Fructose	10	1.4 ± 0.1	3.1 ± 0.1	1.4 ± 0.2	3.2 ± 0.3
Fructose + glucose	10	1.6 ± 0.1	4.0 ± 0.5	1.4 ± 0.2	3.3 ± 0.2
Fructose + glucose	5				
Fructose + glucose	10	1.4 ± 0.0	2.9 ± 0.1	1.5 ± 0.2	3.3 ± 0.3

activities after 1 day, but after 2 days it moderately inhibited both total enzyme (by 27%; $P < 0.02$) and apoenzyme (by 33%; $P < 0.025$) activities. The inhibition of the total enzyme activity observed at 1 day after administration of glucose (5%) was reversed by fructose administration at the same time. The inhibition caused by the 10% glucose solution (after 1 or 2 days) and that observed at 2 days after treatment with the 5% glucose dose were partially reversed by fructose, the mixtures giving values similar to those observed in fructose-treated rats.

The effects of oral intubation of equimolar doses of glucose, fructose and sucrose on the pyrrolase activity were also studied. All three sugars significantly ($P = 0.005-0.001$) inhibited the total enzyme activity (by 36, 36 and 52% respectively), but exerted no significant effects ($P > 0.10$) on that of the holoenzyme.

Effects of nicotinamide on rat liver tryptophan pyrrolase activity

The effects of chronic administration and subsequent withdrawal of nicotinamide (5 g/litre of drinking water) on rat liver tryptophan pyrrolase activity are shown in Fig. 2. The holoenzyme activity was not

Fig. 2. Effects of chronic nicotinamide administration and subsequent withdrawal on the activity of rat liver tryptophan pyrrolase

Fed rats (four per cage) were allowed free access to a 5 g/litre solution of nicotinamide in drinking water. Withdrawal (the start of which is indicated by the arrow) was performed by replacing the nicotinamide solution with drinking water. The enzyme activity was determined, as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. The apoenzyme activity was calculated by difference. Each point represents the mean value for four rats. ●, Holoenzyme activity; ○, total enzyme activity; —, activities during chronic treatment; ---, activities during withdrawal.

significantly altered by nicotinamide treatment. The total activity was significantly ($P < 0.05$) decreased (by 36%) after 1 day, and by 37–56% ($P = 0.02-0.001$) during the remaining period of treatment. The apoenzyme activity, calculated by difference, was therefore decreased (by 76–96%; $P = 0.005-0.001$) for the same length of time. When withdrawal of nicotinamide was started after 11 days of treatment, both holoenzyme and total pyrrolase activities rose to a maximum 1.7- and 1.9-fold respectively ($P = 0.05-0.025$) 1 day, and returned to the basal values 6 days, after withdrawal.

In experiments not reported here, it was found that the inhibition of the total pyrrolase activity was significant ($P = 0.05-0.001$) as early as 1 day after administration of a dose of nicotinamide as little as 0.01 g/litre of drinking water. Also not reported here are the effects of an intraperitoneal injection of nicotinamide (500 mg/kg) on the enzyme activity. The total activity was significantly ($P < 0.05$) decreased by 35 and 37% respectively at 0.5 and 1 h after the injection, whereas that of the holoenzyme was not altered. The total activity then returned to normal at 1.5 h, and this was followed by rises in both holoenzyme and total pyrrolase activities of 22 and 80% respectively at 4 h. Both activities returned to the basal values at 8 h after the injection.

Nature of the inhibition of rat liver tryptophan pyrrolase activity by administration of glucose and nicotinamide

Tryptophan pyrrolase activity in liver homogenates from rats treated with glucose (10 g/100 ml of drinking water) for 48 h was compared with that from untreated rats after various treatments *in vivo* (Table 2). Tryptophan or haematin was equally effective in activating the pyrrolase in glucose-treated and control rats, whereas cortisol or 5-aminolaevulinate was not. The glucose-induced inhibition of the total enzyme activity was reversed by an injection of fructose, pyruvate, phenazine methosulphate or NH_4Cl . None of these agents exerted any significant effect on the enzyme activity in control rats.

Cortisol was equally effective in increasing the pyrrolase activities in nicotinamide-treated (5.0 g/litre of drinking water for up to 22 days) and control rats (Table 3), whereas tryptophan, haematin or 5-aminolaevulinate was not. The nicotinamide-induced inhibition of the total enzyme activity was reversed by the injection of fructose, pyruvate or phenazine methosulphate.

The inhibition of the total pyrrolase activity in liver homogenates from rats treated with glucose or nicotinamide (in the doses and for the durations listed above) was also reversed by the addition *in vitro* of either NAD^+ or NADP^+ (Table 4). Neither nucleotide exerted any significant effect on the basal pyrrolase activities in normal rats. Table 4 also shows that

Table 2. Comparison of tryptophan pyrrolase activity in control and glucose-treated rats after various treatments *in vivo*

Glucose was administered to fed rats in drinking water (10 g/100 ml) for 48 h before death. Each control and glucose-treated rat received, 4 h before being killed, an intraperitoneal injection of either 0.9% (w/v) NaCl, dimethylformamide (1 ml/kg), cortisol acetate (20 mg/kg), tryptophan (500 mg/kg), haematin hydrochloride (35 mg/kg), fructose (400 mg/kg) or sodium pyruvate (200 mg/kg). 5-Aminolaevulinate hydrochloride was given in four doses at 1 h intervals (15 mg/kg each) and the animals were killed at 1 h after the last injection. Rats from both groups also received an intraperitoneal injection of either 0.9% NaCl or phenazine methosulphate (10 mg/kg) 1 h, or of 1 M-NaCl (292.2 mg/kg) or 1 M-NH₄Cl (267.5 mg/kg) 15 min, before death. The enzyme activity was determined, as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M). The apoenzyme activity was calculated by difference. Values are means $\pm$ S.E.M. of each group of four rats.

Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	Control rats		Glucose-treated rats	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
Nil	1.7 $\pm$ 0.1	4.4 $\pm$ 0.1	1.8 $\pm$ 0.1	1.9 $\pm$ 0.1
0.9% NaCl	1.8 $\pm$ 0.2	4.4 $\pm$ 0.5	1.7 $\pm$ 0.1	2.6 $\pm$ 0.3
Dimethylformamide	1.9 $\pm$ 0.2	4.4 $\pm$ 0.5	1.8 $\pm$ 0.2	2.6 $\pm$ 0.2
Cortisol	9.3 $\pm$ 1.0	19.7 $\pm$ 0.2	4.7 $\pm$ 0.4	11.6 $\pm$ 1.2
Tryptophan	6.1 $\pm$ 0.8	11.0 $\pm$ 1.1	5.8 $\pm$ 0.4	11.0 $\pm$ 0.6
Haematin	4.4 $\pm$ 0.4	5.1 $\pm$ 0.2	4.1 $\pm$ 0.2	4.9 $\pm$ 0.8
5-Aminolaevulinate	4.8 $\pm$ 0.2	5.7 $\pm$ 0.5	1.8 $\pm$ 0.1	2.3 $\pm$ 0.2
Fructose	1.5 $\pm$ 0.1	4.0 $\pm$ 0.1	1.6 $\pm$ 0.2	3.9 $\pm$ 0.3
Pyruvate	1.8 $\pm$ 0.2	4.1 $\pm$ 0.4	1.6 $\pm$ 0.1	4.2 $\pm$ 0.5
0.9% NaCl	1.6 $\pm$ 0.1	3.7 $\pm$ 0.3	1.8 $\pm$ 0.1	2.6 $\pm$ 0.1
Phenazine methosulphate	1.7 $\pm$ 0.1	3.9 $\pm$ 0.2	1.6 $\pm$ 0.1	3.8 $\pm$ 0.3
1 M-NaCl	1.4 $\pm$ 0.0	3.1 $\pm$ 0.2	1.4 $\pm$ 0.1	2.1 $\pm$ 0.1
1 M-NH ₄ Cl	1.4 $\pm$ 0.1	2.9 $\pm$ 0.1	1.4 $\pm$ 0.0	3.6 $\pm$ 0.2

Table 3. Comparison of tryptophan pyrrolase activity in control and nicotinamide-treated rats after various treatments *in vivo*

Nicotinamide was administered to fed rats in drinking water (0.5 g/100 ml) for up to 22 days. Each control and nicotinamide-treated rat received an intraperitoneal injection of either 0.9% NaCl, cortisol, tryptophan, haematin hydrochloride, fructose, sodium pyruvate, phenazine methosulphate or repeated injections of 5-aminolaevulinate hydrochloride in the doses and for the durations listed in Table 2. The enzyme activity was determined, as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M). The apoenzyme activity was calculated by difference. Values are means $\pm$ S.E.M. for each group of four rats, except for the untreated control and nicotinamide-treated groups, which had means for ten animals each.

Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	Control rats		Nicotinamide-treated rats	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
Nil	1.7 $\pm$ 0.1	3.8 $\pm$ 0.3	1.8 $\pm$ 0.0	2.2 $\pm$ 0.1
0.9% NaCl	1.7 $\pm$ 0.1	3.8 $\pm$ 0.3	1.6 $\pm$ 0.1	2.7 $\pm$ 0.2
Cortisol	5.3 $\pm$ 0.1	12.2 $\pm$ 0.2	5.2 $\pm$ 0.3	12.0 $\pm$ 1.0
Tryptophan	15.0 $\pm$ 0.7	21.7 $\pm$ 0.2	3.1 $\pm$ 0.5	5.6 $\pm$ 0.8
Haematin	4.5 $\pm$ 0.2	4.5 $\pm$ 0.1	2.4 $\pm$ 0.1	3.9 $\pm$ 0.3
5-Aminolaevulinate	3.9 $\pm$ 0.5	5.1 $\pm$ 0.6	2.7 $\pm$ 0.3	3.2 $\pm$ 0.2
Fructose	1.5 $\pm$ 0.1	4.0 $\pm$ 0.1	1.6 $\pm$ 0.2	4.5 $\pm$ 0.4
Pyruvate	1.8 $\pm$ 0.2	4.1 $\pm$ 0.4	1.5 $\pm$ 0.2	5.0 $\pm$ 0.3
0.9% NaCl	1.6 $\pm$ 0.1	3.7 $\pm$ 0.3	1.6 $\pm$ 0.0	2.5 $\pm$ 0.2
Phenazine methosulphate	1.7 $\pm$ 0.1	3.9 $\pm$ 0.2	2.0 $\pm$ 0.2	4.9 $\pm$ 0.0

Table 4. *Effects of NAD⁺ and NADP⁺ in vitro on liver tryptophan pyrrolase activities of normal, glucose- or nicotinamide-treated rats, and of normal or glucose-treated rats given cortisol*

Glucose or nicotinamide were administered in drinking water to fed rats as described in Tables 6 and 7 respectively. Some normal and glucose-treated rats were given an intraperitoneal injection of cortisol acetate (20 mg/kg) 4 h before being killed. The enzyme activity was determined, as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μ M), with or without the addition of NAD⁺ or NADP⁺ (2 mM each). Values are means $\pm$ S.E.M. for each group of four rats.

Treatment	Addition	Tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt. of liver)	
		Holoenzyme activity	Total enzyme activity
Nil	Nil	1.6 $\pm$ 0.1	5.9 $\pm$ 0.4
	NAD ⁺	1.8 $\pm$ 0.1	5.3 $\pm$ 0.4
	NADP ⁺	1.9 $\pm$ 0.2	6.0 $\pm$ 0.0
Nicotinamide	Nil	1.9 $\pm$ 0.2	2.3 $\pm$ 0.1
	NAD ⁺	1.7 $\pm$ 0.1	5.7 $\pm$ 0.6
	NADP ⁺	2.8 $\pm$ 0.2	6.2 $\pm$ 0.6
Glucose	Nil	1.8 $\pm$ 0.1	2.2 $\pm$ 0.2
	NAD ⁺	1.8 $\pm$ 0.2	5.5 $\pm$ 0.1
	NADP ⁺	2.1 $\pm$ 0.2	5.7 $\pm$ 0.2
Nil (+cortisol)	Nil	7.5 $\pm$ 0.8	24.4 $\pm$ 2.4
	NAD ⁺	8.3 $\pm$ 1.4	23.0 $\pm$ 1.4
	NADP ⁺	6.7 $\pm$ 0.4	21.9 $\pm$ 3.2
Glucose (+cortisol)	Nil	5.9 $\pm$ 0.7	15.1 $\pm$ 0.9
	NAD ⁺	7.9 $\pm$ 0.2	21.7 $\pm$ 1.1
	NADP ⁺	8.3 $\pm$ 0.6	21.6 $\pm$ 1.1

Table 5. *Effects of administration of glucose and nicotinamide on the concentrations of rat liver NADP⁺ and NADPH*

Rats were allowed free access to a solution of either glucose (10 g/100 ml) for up to 4 days or nicotinamide (10 mg/100 ml) for up to 5 days before being killed. The concentrations of liver NADP⁺ and NADPH were determined directly as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each group of six rats.

Duration of treatment (days)	Nucleotide concentration (μ g/g wet wt. of liver)			
	Glucose-treated rats		Nicotinamide-treated rats	
	NADP ⁺	NADPH	NADP ⁺	NADPH
0	39 $\pm$ 2	203 $\pm$ 8	45 $\pm$ 1	206 $\pm$ 3
1	41 $\pm$ 1	245 $\pm$ 13	64 $\pm$ 1	264 $\pm$ 7
2	43 $\pm$ 1	254 $\pm$ 11	70 $\pm$ 3	268 $\pm$ 6
3	49 $\pm$ 1	249 $\pm$ 8	69 $\pm$ 1	261 $\pm$ 8
4	48 $\pm$ 4	278 $\pm$ 18	71 $\pm$ 2	260 $\pm$ 9
5	—	—	72 $\pm$ 3	261 $\pm$ 7

the weaker response of glucose-treated rats to pyrrolase induction by cortisol was enhanced by the addition *in vitro* of NAD⁺ or NADP⁺, whereas no further increase in enzyme activities occurred when either nucleotide was added to homogenates from livers of normal rats treated with cortisol only. In experiments not shown here, it was also found that the tryptophan activation of the pyrrolase in glucose-treated rats, which was similar to that in normal animals, was not further enhanced by NAD⁺ or NADP⁺ *in vitro*.

The inhibition of the total pyrrolase activity by administration of glucose or nicotinamide was not reversed by the addition *in vitro* of excess of haematin. The enzyme activity in the absence or presence of 2, 4, 6 and 8 μ M-haematin in glucose-treated rats (in μ mol of kynurenine formed/h per g wet wt. of liver, $\pm$ S.E.M. of four rats) was 1.6 $\pm$ 0.1, 1.9 $\pm$ 0.1, 1.9 $\pm$ 0.2, 1.9 $\pm$ 0.1 and 2.0 $\pm$ 0.2 respectively. The corresponding values (expressed as above) for the activities in nicotinamide-treated rats were 1.9 $\pm$ 0.1, 2.3 $\pm$ 0.1, 2.4 $\pm$ 0.2, 2.3 $\pm$ 0.2 and 2.0 $\pm$ 0.1 respectively.

Table 6. *Effects of administration of sucrose, glucose, fructose or a mixture of fructose plus glucose on the concentrations of rat liver NADP⁺ and NADPH*

Rats were allowed free access to a solution of either sucrose (20g/100ml), glucose (10g/100ml), fructose (10g/100ml) or fructose plus glucose (10g/100ml each) for 1 or 2 days before being killed. The concentrations of liver NADP⁺ and NADPH were determined directly as described in the Materials and Methods section. Values are means $\pm$ S.E.M. for each group of five or six rats.

Treatment	Duration (days)	Liver nucleotide concentration (μ g/g wet wt.)	
		NADP ⁺	NADPH
Nil	—	51 $\pm$ 1	213 $\pm$ 7
Sucrose	1	48 $\pm$ 2	267 $\pm$ 6
	2	49 $\pm$ 3	262 $\pm$ 10
Nil	—	39 $\pm$ 2	203 $\pm$ 8
Glucose	1	41 $\pm$ 1	245 $\pm$ 13
	2	43 $\pm$ 1	254 $\pm$ 11
Fructose	1	45 $\pm$ 3	204 $\pm$ 4
	2	43 $\pm$ 3	210 $\pm$ 3
Fructose + glucose	1	41 $\pm$ 2	211 $\pm$ 5
	2	44 $\pm$ 1	216 $\pm$ 9

Table 7. *Effects of phenazine methosulphate on the concentrations of liver NADP⁺ and NADPH in normal rats and in those treated with glucose or nicotinamide*

Rats were allowed free access to either water or a solution of glucose (10g/100ml) for 2 days, or of nicotinamide (10mg/100ml) for 3 days, before being killed. Some control (nil) and treated rats also received, 1 h before death, an intraperitoneal injection of phenazine methosulphate (10mg/kg). The concentrations of NADP⁺ and NADPH were determined directly as described in the Materials and Methods section. Values are means $\pm$ S.E.M. of each group of six rats.

Treatment	Liver nucleotide concentration (μ g/g wet wt.)	
	NADP ⁺	NADPH
Nil	45 $\pm$ 1	206 $\pm$ 3
Phenazine methosulphate	96 $\pm$ 1	184 $\pm$ 3
Nil	39 $\pm$ 2	203 $\pm$ 8
Glucose	43 $\pm$ 1	254 $\pm$ 11
Glucose + phenazine methosulphate	55 $\pm$ 1	201 $\pm$ 5
Nil	45 $\pm$ 1	206 $\pm$ 3
Nicotinamide	69 $\pm$ 1	261 $\pm$ 8
Nicotinamide + phenazine methosulphate	137 $\pm$ 3	205 $\pm$ 7

Effects of glucose, other sugars and nicotinamide on the concentration of rat liver NADP⁺ and NADPH

The effects of administration in drinking water of glucose and nicotinamide on the concentrations of

the above nucleotides are shown in Table 5. Glucose treatment for 1–4 days significantly ($P = 0.025$ – 0.005) increased [NADPH] by 21–37%, whereas [NADP⁺] was slightly increased after 3 and 4 days of treatment. Nicotinamide administration for 1–5 days increased [NADPH] (by 26–30%; $P < 0.001$) and [NADP⁺] (by 42–60%; $P < 0.001$).

The effects of glucose, sucrose, fructose and a mixture of fructose plus glucose on [NADP⁺] and [NADPH] are shown in Table 6. Sucrose administration for 1 or 2 days significantly ($P < 0.001$) increased [NADPH] but did not alter [NADP⁺]. Fructose by itself did not exert any significant effects, but when administered with glucose, it prevented the increase in [NADPH] caused by the latter sugar.

The effects of phenazine methosulphate on [NADP⁺] and [NADPH] in livers of normal, glucose- or nicotinamide-treated rats are shown in Table 7. This compound increased [NADP⁺] in all three groups of rats, and prevented the increase in [NADPH] caused by glucose or nicotinamide administration. The increase in [NADP⁺] caused by phenazine methosulphate administration did not solely result from the re-oxidation of NADPH; it also involved NADH re-oxidation (results not shown here).

Discussion

Effects of nicotinamide on rat liver tryptophan pyrrolase activity

Chronic administration of nicotinamide inhibits the activity of rat liver tryptophan pyrrolase and subsequent withdrawal causes an enhancement (Fig. 2). The withdrawal-induced increase is not primarily cofactor-dependent, since the ratio of holoenzyme/apoenzyme activity (which indicates the extent of haem saturation of the apoenzyme; see also Badawy & Evans, 1973) is less than 1. The above ratio resembles that of the basal enzyme and the enzyme induced by cortisol. It is therefore possible that the above withdrawal-induced enhancement is caused by a corticosteroid-mediated mechanism.

Since the holoenzyme activity is not affected by chronic nicotinamide administration, it may be concluded that the above inhibition is specific to the apoenzyme. The inhibition is not due to an interference with the synthesis, availability or utilization of the haem activator, since excess of haematin *in vitro* does not reverse it (see the text). The above inhibition is also not due to a defective apoenzyme synthesis, since cortisol is equally effective in inducing the pyrrolase activity in nicotinamide-treated and control rats (Table 3). This conclusion supports the finding (Cho-Chung & Pitot, 1968) that pretreatment of adrenalectomized rats with a single dose of nicotinamide does not inhibit the cortisol-induced increase in synthesis or activity of the enzyme.

Activation of pre-existing apoenzyme by acute administration of tryptophan (Knox, 1966; Schimke 1969), 5-aminolaevulinate or haematin (Badawy & Evans, 1973, 1975c) is strongly decreased in nicotinamide-treated rats (Table 3), presumably because the apoenzyme there is already inactive (Fig. 2). The nicotinamide inhibition of the tryptophan activation of the enzyme has been reported in adrenalectomized rats (Cho-Chung & Pitot, 1968), and these authors have suggested that nicotinamide acts in this instance by inhibiting genetic translation because, in their rats, tryptophan has also been shown to increase the synthesis of the apoenzyme, an effect that was inhibited by nicotinamide but not by actinomycin D. However, this explanation may be unlikely for two reasons: (1) inhibitors of translation, such as puromycin or cycloheximide, inhibit the enhancement of the pyrrolase activity by both tryptophan and cortisol (Badawy & Evans, 1975c); (2) tryptophan does not increase pyrrolase synthesis in adrenalectomized rats maintained on adequate diets (Schimke *et al.*, 1965). The finding by Cho-Chung & Pitot (1968) that tryptophan enhances pyrrolase synthesis may be related to the fact that their adrenalectomized rats were maintained on a protein-free diet for 6 days before the experiment; this might have altered liver protein synthesis and/or the response to administered tryptophan.

Effects of glucose on rat liver tryptophan pyrrolase activity

Chronic administration of glucose inhibits the activity of rat liver tryptophan pyrrolase, and subsequent withdrawal causes an enhancement (Fig. 1). The withdrawal-induced increase resembles that by nicotinamide withdrawal (Fig. 2), and may therefore be caused by the same mechanism. The effects of chronic administration and subsequent withdrawal of glucose, nicotinamide and four drugs of dependence (ethanol, phenobarbitone, morphine and nicotine; Badawy & Evans, 1975a,b) are remarkably similar.

Sucrose is as effective as glucose in inhibiting apo-(tryptophan pyrrolase) activity, whereas fructose causes only a moderate degree of inhibition after 2 days of administration in drinking water (Table 1). This suggests that sucrose may act via glucose, and that the moderate inhibition by fructose may be caused by the slow and partial conversion of this sugar into glucose under conditions of drinking *ad libitum*. A more rapid conversion of fructose into glucose, as would be expected to occur after oral intubation of a larger dose, may explain the ability of fructose to inhibit as effectively as glucose (see the text).

The glucose-induced inhibition of the total pyrrolase activity is not due to shortage of the haem

activator, since excess of haematin *in vitro* does not reverse it (see the text). Glucose administration has previously been shown to inhibit the activity of the basal enzyme in intact, adrenalectomized or hypophysectomized rats, the cortisol-induced enzyme in intact or adrenalectomized rats and the tryptophan-activated enzyme in starved intact but not in starved adrenalectomized rats (Wetterberg *et al.*, 1970; Yuwiler *et al.*, 1970; Greengard & Dewey, 1973). In the present work it was found that, whereas the tryptophan activation of the enzyme in intact rats is not affected by glucose treatment, the activity of the cortisol-induced enzyme is 41–46% lower than in control rats (Table 2). This effect of glucose has been suggested (Yuwiler *et al.*, 1970) to be due to inhibition of the RNA-mediated cortisol induction of apo-(tryptophan pyrrolase) synthesis, a suggestion similar to that proposed by Marver *et al.* (1966) in experiments in which glucose, like actinomycin D, has been shown to inhibit the induction of 5-aminolaevulinate synthetase and the increase in 5-aminolaevulinate dehydratase activities in livers of rats treated with the prophyrogen 2-allyl-2-isopropylacetamide. Indeed, the ability of glucose to prevent the pyrrolase activation by 5-aminolaevulinate itself (Table 2) would support this suggestion as far as dehydratase is concerned. However, an alternative and more likely explanation is that 5-aminolaevulinate, which acts by activating pre-existing apo-(tryptophan pyrrolase) (Badawy & Evans, 1973, 1975c) fails to enhance the pyrrolase in glucose-treated rats simply because the apoenzyme there is already inhibited (Table 2). The effect of glucose on the cortisol-induced enzyme may not necessarily involve inhibition of synthesis. It may simply be due to inhibition of activity of some of the newly synthesized apoenzyme, as is suggested by the results in Table 4, which show that whereas the cortisol-induced enzyme activities in normal rats are not further increased by the addition *in vitro* of NAD⁺ or NADP⁺, those of glucose-treated rats given cortisol are raised to the values observed in the above normal rats.

Whereas the pyrrolase inhibition by chronic nicotinamide administration prevents the activation by tryptophan or haematin (Table 3), that by glucose does not (Table 2). No satisfactory explanation can as yet be provided to interpret these two unexpected results in glucose-treated rats.

Possible involvement of NADPH and NADH in the inhibition of rat liver tryptophan pyrrolase activity by chronic administration of glucose or nicotinamide

The above experiments leave us with the question of whether the known physiologically occurring pyrrolase inhibitors NADPH and NADH are

involved in the above inhibition. A similar situation has been encountered in experiments with drugs of dependence (Badawy & Evans, 1975a,b). It was then shown that the inhibition by these drugs was associated with an increased liver [NADPH] and, for ethanol, also [NADH]. For these nucleotides to be involved in the inhibition observed in the present work, it is necessary to show that their concentrations are increased during inhibition, and that agents known to prevent such increases should reverse the inhibition.

NADPH (and possibly also NADH) allosterically inhibits the activity of the purified enzyme (Cho-Chung & Pitot, 1967), which is located in the cytosol compartment of the liver cell. The concentrations of these nucleotides, which are important in relation to the inhibition, were therefore determined by a direct method (Slater & Sawyer, 1962; Slater *et al.*, 1964). However, direct determinations were not attempted in the cytosol compartment, because tissue fractionation procedures cause redistribution of nucleotides between subcellular fractions. Instead, nucleotide concentrations were determined in whole liver, although this does not measure their distribution in subcellular compartments, which is known to be uneven (Borst, 1963). Also, although the concentrations of NAD⁺, NADH, NADP⁺ and NADPH have been determined, only the data on the latter two are presented in the present paper, because they are less sensitive to anoxia (see Slater *et al.*, 1964). It should also be pointed out that whereas the [NADPH] measured is both free and bound, NADP⁺ may represent the free fraction, i.e. that which is not acid-labile (see Burch *et al.*, 1963; Slater *et al.*, 1964).

Glucose administration increases [NADPH] (Table 5). This may be explained by the pentose phosphate pathway being enhanced. Sucrose also increases [NADPH], whereas fructose does not (Table 6). The increases in [NADP⁺] and [NADPH] caused by nicotinamide administration (Table 5) may be explained by increased NAD⁺ synthesis resulting from precursor administration. That the inhibition of the pyrrolase activity by glucose or nicotinamide may be due to an increased liver [NADPH] is suggested by the findings that phenazine methosulphate, which rapidly re-oxidizes NADPH (Katz & Wals, 1970), prevents the increase in [NADPH] (Table 7) and reverses the pyrrolase inhibition by the above two treatments (Tables 2 and 3). Addition of NADP⁺ *in vitro* also reverses the inhibition by both glucose and nicotinamide (Table 4). Whether this effect of NADP⁺ is due to a change in the [NADPH]/[NADP⁺] ratio or is related to other phenomena remains to be investigated.

Administration of glucose or nicotinamide also increases the liver [NADH]. Although these changes cannot be satisfactorily interpreted for the reasons mentioned above, the present evidence suggests that

NADH is also involved in the pyrrolase inhibition by the above two agents. Thus the addition of NAD⁺ *in vitro* reverses the inhibition (Table 4). Also, acute administration of agents such as fructose or pyruvate reverses the inhibition in rats treated with either glucose (Table 2) or nicotinamide (Table 3). The effects of fructose and pyruvate are consistent with their ability to regenerate liver NAD⁺ (see, e.g., Slater, 1972). Fructose administration in drinking water also reverses the pyrrolase inhibition (Table 1) and prevents the increase in [NADPH] (Table 6) caused by glucose administration. These effects of fructose may, however, be explained by prevention of glucose metabolism by competition for phosphorylation by ATP. The ability of NH₄Cl to reverse the glucose-induced inhibition of the pyrrolase activity (Table 2) may be of interest in that prevention of changes in tryptophan pyrrolase activity can be achieved by altering the redox state of the free NAD⁺ couple. NH₄Cl is known (Krebs, 1967) to alter the concentrations of the reactants of the β -hydroxybutyrate dehydrogenase and glutamate dehydrogenase systems by increasing the [NH₄⁺] in the liver, and its action on the pyrrolase activity is a further evidence in favour of NADH involvement in the inhibition.

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