The role of liver tryptophan pyrrolase in the opposite effects of chronic administration and subsequent withdrawal of drugs of dependence on rat brain tryptophan metabolism

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1. Chronic administration of morphine, nicotine or phenobarbitone has previously been shown to inhibit rat liver tryptophan pyrrolase activity by increasing hepatic [NADPH], whereas subsequent withdrawal enhances pyrrolase activity by a hormonal-type mechanism. 2. It is now shown that this enhancement is associated with an increase in the concentration of serum corticosterone. 3. Chronic administration of the above drugs enhances, whereas subsequent withdrawal inhibits, brain 5-hydroxytryptamine synthesis. Under both conditions, tryptophan availability to the brain is altered in the appropriate direction. 4. The chronic drug-induced enhancement of brain tryptophan metabolism is reversed by phenazine methosulphate, whereas the withdrawal-induced inhibition is prevented by nicotinamide. 5. The chronic morphine-induced changes in liver [NADPH], pyrrolase activity, tryptophan availability to the brain and brain 5-hydroxytryptamine synthesis are all reversed by the opiate antagonist naloxone. 6. It is suggested that the opposite effects on brain tryptophan metabolism of chronic administration and subsequent withdrawal of the above drugs of dependence are mediated by the changes in liver tryptophan pyrrolase activity. 6. Similar conclusions based on similar findings have previously been made in relation to chronic administration and subsequent withdrawal of ethanol. These findings with all four drugs are briefly discussed in relation to previous work and the mechanism(s) of drug dependence.

We have previously reported (Badawy & Evans, 1973a) that chronic ethanol administration to rats inhibits the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) by increasing hepatic [NAD(P)H], whereas subsequent withdrawal causes a hormonal-type enhancement of pyrrolase activity that has been shown (Badawy *et al.*, 1980) to be associated with an increase in serum corticosterone concentration. Liver tryptophan pyrrolase is one of the peripheral factors that influence brain 5-hydroxytryptamine synthesis by altering the availability of circulating tryptophan to the brain, and we presented evidence (Badawy *et al.*, 1979, 1980; Punjani *et al.*, 1979) strongly suggesting that the above opposite changes in pyrrolase activity mediate the correspondingly opposite effects of chronic administration and subsequent withdrawal of ethanol on brain 5-hydroxytryptamine synthesis. Chronic administration of three other drugs of dependence (morphine, nicotine and pheno-

barbitone) also inhibits liver pyrrolase activity by a mechanism not involving a defective apoenzyme synthesis nor a decreased cofactor (haem) availability, but associated with an increased liver [NADPH] (Badawy & Evans, 1973b, 1975a,b). That NADPH is the cause of the above inhibition is suggested by the then observed reversal of the inhibition by addition *in vitro* of NAD(P)⁺ or by administration *in vivo* of the regenerator of these oxidized dinucleotides phenazine methosulphate. Moreover, the above inhibition of pyrrolase activity by morphine, nicotine or phenobarbitone was not caused by NADH, because of the failure of the NAD⁺ regenerators fructose and pyruvate to reverse it. These latter compounds are capable of reversing the pyrrolase inhibition by chronic administration of ethanol, glucose or nicotinamide, all of which increase liver [NAD(P)H] (Badawy & Evans, 1975a, 1976a). It was also shown (Badawy & Evans, 1973b, 1975a,b) that withdrawal of

morphine, nicotine or phenobarbitone enhances liver tryptophan pyrrolase activity by a hormonal-type mechanism similar to that exerted by ethanol withdrawal. It was therefore decided to find out if the opposite effects of chronic administration and subsequent withdrawal of morphine, nicotine and phenobarbitone on liver pyrrolase activity are also involved in any likely changes in brain 5-hydroxytryptamine synthesis. Such investigation, which is also of interest in relation to the possible involvement of 5-hydroxytryptamine in drug dependence, forms the subject of the present paper.

Materials and methods

Chemicals

Morphine sulphate was purchased from Macarthys Ltd. (Trecenydd Industrial Estate, Caerphilly, Mid Glam., Wales, U.K.), whereas the hydrochloride of naloxone (17-allyl-4,5 α -epoxy-3,14-dihydroxynormorphinan-6-one) was from Winthrop Laboratories (Surrey, Surrey, U.K.) and was in ampoules, each containing 0.4 mg/ml. All other chemicals were from BDH Chemicals and Sigma (London) Chemical Co. (both of Poole, Dorset, U.K.) and were of the purest commercially available grades.

Animals and treatments

Male Wistar rats (150 g $\pm$ 10% at the start of experiments) were either locally bred (those used in experiments with naloxone) or purchased from Hacking and Churchill, Wyton, Huntingdon, Cambs., U.K. (those used in all other experiments). All animals were housed three per cage and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water (purchased rats were so maintained for at least 1 week before the start of experiments). Rats used for the determination of liver tryptophan pyrrolase activity were killed by stunning and cervical dislocation between 13:00 and 15:00h, whereas those used for all other determinations were killed by decapitation between 11:00 and 12:00h.

Drugs of dependence were freely administered in drinking water for 3 weeks. Nicotine hydrogen (+)-tartrate was given at a constant concentration of 3.08 mg (1 mg of the free base) per 100 ml of drinking water. Morphine sulphate was given in increasing concentrations (48 h apart) of 0.1, 0.15, 0.2, 0.3 and finally 0.4 mg/ml, and the animals were then maintained on the latter concentration till the end of the 3-week experimental period. Similarly, phenobarbitone sodium was given in increasing concentrations (48 h apart) of 1, 1.5, 2, 2.5 and finally 3 mg/ml, and the animals were then maintained on the latter concentration till the end of the experimental period.

Some chronic drug-treated rats and their respective controls received, 2h before death, an intraperitoneal injection of either phenazine methosulphate (10 mg/kg body wt.) or an equal volume (2 ml/kg body wt.) of 0.9% (w/v) NaCl. Additionally, some chronic morphine-treated rats and their controls received, at various times before death, an intraperitoneal injection of either naloxone hydrochloride (1 mg/kg body wt.) or an equal volume (2.5 ml/kg) of 0.9% NaCl.

Withdrawal of drugs of dependence was achieved by replacing the drug solution with drinking water. The effects of withdrawal of morphine, phenobarbitone and nicotine were studied at 1, 2 and 3 days respectively after withdrawal. Other rats received during these withdrawal periods a solution of nicotinamide in drinking water (5 g/litre) to find out if this agent is capable of reversing the withdrawal effects.

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in fresh liver homogenates (Badawy & Evans, 1975c) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μ M) haematin. The apoenzyme activity was obtained by difference. Concentrations of liver nicotinamide-adenine dinucleotides (phosphates) were determined in pieces obtained by a locally manufactured freeze-clamp by the direct method of Slater *et al.* (1964) with the minor modifications described by Punjani *et al.* (1979).

Free (ultrafiltrable) serum, total (acid-soluble) serum liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations were determined by standard procedures (for references, see Badawy *et al.*, 1979). Analyses were performed with livers that had been obtained by the freeze-clamp and with brains that had been rapidly removed (within 30 s of the death of the animals), immersed in liquid N₂ for 3 min and stored (along with livers) at -20°C for 24 h.

Fluorimetric determination of concentrations of serum glucose and corticosterone was performed by the methods of Slein (1963) and Glick *et al.* (1964) respectively. The naloxone-induced opiate-withdrawal syndrome was assessed by the method of Collier *et al.* (1974). Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

Enhancement of rat brain tryptophan metabolism by chronic administration of drugs of dependence

The effects on brain tryptophan metabolism, and other related aspects, of chronic administration (with or without a single injection of phenazine methosulphate) of morphine, nicotine and phenobarbitone

are shown in Tables 1, 2 and 3 respectively. None of these three drugs of dependence exerted any significant effects on the holo-(tryptophan pyrrolase) activity. By contrast, the total pyrrolase activity was decreased by morphine, nicotine and phenobarbitone by 46, 61 and 44% respectively. The corresponding decreases in the apoenzyme activity were 92, 87 and 89% respectively. The concen-

trations of free serum, total serum, liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were increased by morphine by 25, 21, 18, 14, 50 and 18% respectively. Nicotine increased these concentrations by 23, 21, 26, 23, 18 and 29% respectively, whereas phenobarbitone caused increases of 33, 23, 26, 34, 24 and 25% respectively. Binding of tryptophan to serum

Table 1. *Effects of chronic morphine administration on rat liver and brain tryptophan metabolism and their reversal by phenazine methosulphate*

Morphine was administered in drinking water for 3 weeks as described in the Materials and methods section. Both control and morphine-treated rats received, at 2h before death, an intraperitoneal injection of either phenazine methosulphate (10mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% (w/v) NaCl. Liver tryptophan pyrrolase activity is in μmol of kynurenine formed/h per g wet wt., whereas all other determinations (except the percentage of free serum tryptophan) are in $\mu\text{g}/\text{ml}$ of serum or in $\mu\text{g}/\text{g}$ wet wt. of tissue. Values are means $\pm$ S.E.M. for each group of four (pyrrolase activities) or of six rats (all other determinations). The values in columns 2 and 3 are compared with those in column 1, whereas those in column 4 are compared with those in column 3. Significance of differences is indicated as follows: *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ***** $P < 0.001$.

Determination	Treatment ...	Tryptophan metabolism parameters			
		0.9% NaCl		Phenazine methosulphate	
	Pretreatment ... Column no. ...	Nil (1)	Morphine (2)	Nil (3)	Morphine (4)
Holo-(tryptophan pyrrolase)		1.20 $\pm$ 0.06	1.20 $\pm$ 0.05	1.30 $\pm$ 0.05	1.30 $\pm$ 0.06
Total tryptophan pyrrolase		2.40 $\pm$ 0.14	1.30 $\pm$ 0.02*****	2.60 $\pm$ 0.04	2.80 $\pm$ 0.16
Apo-(tryptophan pyrrolase)		1.20 $\pm$ 0.08	0.10 $\pm$ 0.02*****	1.30 $\pm$ 0.06	1.50 $\pm$ 0.16
Liver tryptophan		5.70 $\pm$ 0.26	6.72 $\pm$ 0.20***	6.17 $\pm$ 0.17	6.12 $\pm$ 0.26
Free serum tryptophan		1.38 $\pm$ 0.06	1.69 $\pm$ 0.06*****	1.31 $\pm$ 0.04	1.36 $\pm$ 0.04
Total serum tryptophan		31.75 $\pm$ 0.53	38.55 $\pm$ 0.48*****	30.20 $\pm$ 1.15	29.83 $\pm$ 1.14
Free serum tryptophan (%)		4.35 $\pm$ 0.18	4.38 $\pm$ 0.11	4.64 $\pm$ 0.32	4.56 $\pm$ 0.43
Brain tryptophan		2.22 $\pm$ 0.02	2.54 $\pm$ 0.07*****	2.23 $\pm$ 0.06	2.22 $\pm$ 0.03
Brain 5-hydroxytryptamine		0.66 $\pm$ 0.02	0.99 $\pm$ 0.03*****	0.70 $\pm$ 0.03	0.69 $\pm$ 0.02
Brain 5-hydroxyindol-3-ylacetic acid		0.39 $\pm$ 0.01	0.46 $\pm$ 0.01*****	0.38 $\pm$ 0.01	0.33 $\pm$ 0.01****

Table 2. *Effects of chronic nicotine administration on rat liver and brain tryptophan metabolism and their reversal by phenazine methosulphate*

Experimental details and comparisons, expressions and mean values of results are as described in Table 1, except that the rats were given nicotine in drinking water for 3 weeks as described in the Materials and methods section. The significance of differences is indicated as follows: **** $P < 0.01$; ***** $P < 0.005$; ***** $P < 0.001$.

Determination	Treatment ...	Tryptophan metabolism parameters			
		0.9% NaCl		Phenazine methosulphate	
	Pretreatment ... Column no. ...	Nil (1)	Nicotine (2)	Nil (3)	Nicotine (4)
Holo-(tryptophan pyrrolase)		1.30 $\pm$ 0.11	1.30 $\pm$ 0.06	1.30 $\pm$ 0.04	1.30 $\pm$ 0.04
Total tryptophan pyrrolase		4.40 $\pm$ 0.49	1.70 $\pm$ 0.15*****	4.60 $\pm$ 0.38	4.60 $\pm$ 0.36
Apo-(tryptophan pyrrolase)		3.10 $\pm$ 0.39	0.40 $\pm$ 0.09*****	3.30 $\pm$ 0.39	3.30 $\pm$ 0.35
Liver tryptophan		4.37 $\pm$ 0.08	5.53 $\pm$ 0.08*****	4.56 $\pm$ 0.08	4.65 $\pm$ 0.14
Free serum tryptophan		1.66 $\pm$ 0.06	2.05 $\pm$ 0.07*****	1.61 $\pm$ 0.04	1.59 $\pm$ 0.03
Total serum tryptophan		26.72 $\pm$ 1.52	32.25 $\pm$ 0.51****	25.77 $\pm$ 1.22	25.00 $\pm$ 1.43
Free serum tryptophan (%)		6.21 $\pm$ 0.58	6.36 $\pm$ 0.32	6.25 $\pm$ 0.29	6.36 $\pm$ 0.37
Brain tryptophan		2.09 $\pm$ 0.03	2.58 $\pm$ 0.06*****	2.20 $\pm$ 0.04	2.26 $\pm$ 0.03
Brain 5-hydroxytryptamine		0.73 $\pm$ 0.027	0.90 $\pm$ 0.016*****	0.77 $\pm$ 0.009	0.75 $\pm$ 0.024
Brain 5-hydroxyindol-3-ylacetic acid		0.38 $\pm$ 0.011	0.49 $\pm$ 0.011*****	0.38 $\pm$ 0.012	0.40 $\pm$ 0.008

Table 3. *Effects of chronic phenobarbitone administration on rat liver and brain tryptophan metabolism and their reversal by phenazine methosulphate*

Experimental details and comparisons, expressions and mean values of results are as described in Table 1, except that the rats were given phenobarbitone in drinking water for 3 weeks as described in the Materials and methods section. The significance of differences is indicated as follows: ** $P < 0.025$; ***** $P < 0.005$; ***** $P < 0.001$. The free serum tryptophan concentration in phenazine methosulphate-treated control rats was not determined (N.D.) because of a methodological error.

Determination	Treatment ... Pretreatment ... Column no. ...	Tryptophan metabolism parameters			
		0.9% NaCl		Phenazine methosulphate	
		Nil (1)	Phenobarbitone (2)	Nil (3)	Phenobarbitone (4)
Holo-(tryptophan pyrrolase)		1.40 ± 0.03	1.60 ± 0.02*****	1.60 ± 0.11	1.60 ± 0.08
Total tryptophan pyrrolase		3.20 ± 0.06	1.80 ± 0.08*****	3.40 ± 0.09	3.60 ± 0.15
Apo-(tryptophan pyrrolase)		1.80 ± 0.07	0.20 ± 0.06*****	1.80 ± 0.11	2.00 ± 0.11
Liver tryptophan		6.15 ± 0.17	7.73 ± 0.16*****	6.12 ± 0.11	5.58 ± 0.22
Free serum tryptophan		1.43 ± 0.04	1.90 ± 0.04*****	N.D.	1.47 ± 0.07
Total serum tryptophan		22.58 ± 0.69	27.84 ± 0.51*****	22.94 ± 0.92	22.02 ± 0.70
Free serum tryptophan (%)		6.33 ± 0.09	6.82 ± 0.15**	N.D.	6.67 ± 0.32
Brain tryptophan		1.98 ± 0.05	2.65 ± 0.08*****	2.07 ± 0.05	1.95 ± 0.05
Brain 5-hydroxytryptamine		0.67 ± 0.009	0.83 ± 0.008*****	0.67 ± 0.011	0.69 ± 0.015
Brain 5-hydroxyindol-3-ylacetic acid		0.36 ± 0.004	0.45 ± 0.011*****	0.36 ± 0.010	0.35 ± 0.009

proteins (expressed as a percentage of free serum tryptophan) was not significantly altered by morphine or nicotine, and was only slightly altered (by 8%; $P < 0.025$) by phenobarbitone.

The results in Tables 1–3 also show that phenazine methosulphate did not exert any significant effects on the various aspects of tryptophan metabolism examined in control rats, but was capable of reversing all the changes produced by chronic administration of the three drugs of dependence. The effects of these drugs and of phenazine methosulphate on serum glucose and corticosterone concentrations were also studied (results not shown). It was found that serum glucose concentration was not significantly altered ($P > 0.10$) by any of these treatments, whereas that of serum corticosterone was increased by phenobarbitone (by only 7%; $P < 0.01$) and by phenazine methosulphate administration to control or chronic drug-treated rats (by 19–29%; $P = 0.005$ – 0.001).

Brain tryptophan concentration plays an important role in 5-hydroxytryptamine synthesis (Curzon & Knott, 1974, and references cited therein; Badawy & Evans, 1976b; Badawy *et al.*, 1979, 1980), and the increases in brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations caused by chronic administration of morphine, nicotine and phenobarbitone are therefore consistent with these three drugs enhancing brain 5-hydroxytryptamine synthesis by increasing brain tryptophan concentration. That this latter increase is not insulin-mediated or lipolysis-depen-

dent is suggested by the absence of changes in serum glucose concentration (see above) or in tryptophan binding to serum proteins (see Tables 1–3). It is therefore likely that the chronic drug-induced increase in brain tryptophan concentration is caused by the increase in the availability of circulating tryptophan to the brain, because of the proportionate increases in free serum and total serum tryptophan concentrations. That this increased availability of tryptophan may be secondary to the inhibition of liver tryptophan pyrrolase activity leading to a decreased hepatic tryptophan catabolism is suggested by the observed increase in liver tryptophan concentration (Tables 1–3). Similar results have been reported under conditions involving the inhibition of liver tryptophan pyrrolase activity by chronic administration of ethanol or nicotinamide (Badawy *et al.*, 1980). More direct evidence for the possible involvement of the inhibition of liver pyrrolase activity by chronic administration of drugs of dependence in the increased availability of circulating tryptophan to the brain is provided by the findings (Tables 1–3) that reversal by phenazine methosulphate of the former effect also reverses the chronic drug-induced accumulation of tryptophan in the liver and the increased concentrations of the amino acid in serum and brain. Phenazine methosulphate (and the liver NAD⁺ regenerators fructose, Methylene Blue and pyruvate) have previously been shown (Badawy *et al.*, 1979) to reverse the effects of chronic ethanol administration on liver pyrrolase activity and on

tryptophan availability to the brain. It may therefore be suggested that chronic administration of the above four drugs of dependence enhances brain 5-hydroxytryptamine synthesis by increasing tryptophan availability to the brain secondarily to the inhibition of liver pyrrolase activity.

Reversal by naloxone of the effects of chronic morphine administration on liver and brain tryptophan metabolism

Shen *et al.* (1970) reported that the opiate antagonist naloxone reverses the chronic morphine-induced enhancement of brain 5-hydroxytryptamine synthesis. In view of the present findings with morphine (Table 1), it was considered important to find out if naloxone is also capable of reversing the morphine effects on tryptophan availability to the brain and possibly also on liver tryptophan pyrrolase activity. The results in Table 4 show that the chronic morphine-induced increases in free serum, total serum, liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid concentrations (22, 26, 24, 31, 20 and 24% respectively) were all reversed at 2 h after administration of naloxone. This antagonist did not exert any significant effects on these parameters in control rats. These findings with naloxone therefore provide further evidence that the chronic morphine-induced enhancement of brain 5-hydroxytryptamine synthesis is caused by the increase in tryptophan availability to the brain. Messing *et al.* (1978) also reported that chronic morphine administration increases brain tryptophan concentration, but they

also found that this increase was potentiated by naloxone. This latter effect contrasts with that observed in the present work, and one possible explanation of this may be the different conditions under which the authors performed their experiments.

In experiments not shown here, it was found that neither acute naloxone injection nor chronic morphine administration caused any significant changes in serum glucose or corticosterone concentration, but that only the latter was increased by 23% ($P < 0.01$) when naloxone was administered to morphine-pretreated rats. Under these conditions, naloxone produced the so-called opiate-withdrawal syndrome (C. M. Evans & A. A.-B. Badawy, unpublished work), despite the fact that our rats received doses of morphine considerably smaller than those used by other workers.

The reversal by naloxone of the chronic effects of morphine on tryptophan availability to the brain described above suggests that this antagonist may also reverse the drug-induced inhibition of liver tryptophan pyrrolase activity. That this is so is shown in Table 5. The chronic morphine-induced decreases in activities of the total enzyme (44–67%; $P = 0.01$ – 0.001) and apoenzyme (71–91%; $P < 0.001$) were no longer observed after naloxone administration. Moreover, this reversal by naloxone was a rapid phenomenon, occurring as early as 15 min after antagonist administration. These results therefore provide additional support to the suggestion that the chronic effects of morphine on brain tryptophan metabolism are mediated by the inhibition of liver tryptophan pyrrolase activity.

Table 4. *Reversal of naloxone of the chronic morphine-induced increases in concentrations of tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid in rat serum, liver and/or brain*

Morphine was administered in drinking water for 3 weeks as described in the Materials and methods section. Both control and morphine-treated rats received, at 2 h before death, an intraperitoneal injection of either naloxone hydrochloride (1 mg/kg body wt.) or an equal volume (2.5 ml/kg body wt.) of 0.9% NaCl. Values are means $\pm$ S.E.M. for each group of six rats, and all (except the percentage of free serum tryptophan) are expressed in $\mu\text{g/ml}$ of serum or in $\mu\text{g/g}$ wet wt. of tissue. The values in columns 2 and 3 are compared with those in column 1, whereas those in column 4 are compared with those in column 3. The significance of differences is indicated as follows: * $P < 0.05$; *** $P < 0.01$; ***** $P < 0.005$; ***** $P < 0.001$.

Determination	Treatment ...	Tryptophan metabolism parameters			
		0.9% NaCl		Naloxone	
	Pretreatment ...	Nil (1)	Morphine (2)	Nil (3)	Morphine (4)
Liver tryptophan		5.64 ± 0.48	$6.98 \pm 0.22^*$	6.17 ± 0.20	5.48 ± 0.20
Free serum tryptophan		1.56 ± 0.09	$1.91 \pm 0.03^{*****}$	1.56 ± 0.11	1.45 ± 0.08
Total serum tryptophan		28.17 ± 1.28	$35.58 \pm 1.67^{*****}$	28.37 ± 1.02	28.45 ± 1.09
Free serum tryptophan (%)		5.54 ± 0.32	5.37 ± 0.21	5.50 ± 0.49	5.10 ± 0.33
Brain tryptophan		2.61 ± 0.15	$3.41 \pm 0.14^{*****}$	2.66 ± 0.09	2.55 ± 0.08
Brain 5-hydroxytryptamine		0.51 ± 0.009	$0.61 \pm 0.018^{*****}$	0.50 ± 0.011	0.50 ± 0.004
Brain 5-hydroxyindol-3-ylacetic acid		0.29 ± 0.014	$0.36 \pm 0.006^{*****}$	0.27 ± 0.006	0.26 ± 0.008

Table 5. *Reversal by naloxone of the chronic morphine-induced inhibition of rat liver tryptophan pyrrolase activity*
Experimental details and comparison of results are as described in Table 4, except that the injections were performed at various times, values are means $\pm$ S.E.M. for each group of four rats and pyrrolase activities are in μ mol of kynurenine formed/h per g wet wt. of liver. Significance of differences is indicated as follows: **** $P < 0.01$; ***** $P < 0.001$.

Time after injection (h)	Pyrrolase	Treatment ...	Liver tryptophan pyrrolase activity			
			0.9% NaCl		Naloxone	
		Pretreatment ... Column no. ...	Nil (1)	Morphine (2)	Nil (3)	Morphine (4)
0.25	Holoenzyme		1.3 $\pm$ 0.05	1.2 $\pm$ 0.14	1.5 $\pm$ 0.18	1.4 $\pm$ 0.10
	Total enzyme		4.6 $\pm$ 0.12	1.5 $\pm$ 0.10*****	4.6 $\pm$ 0.40	4.6 $\pm$ 0.48
	Apoenzyme		3.3 $\pm$ 0.11	0.3 $\pm$ 0.05*****	3.1 $\pm$ 0.27	3.2 $\pm$ 0.40
0.5	Holoenzyme		1.5 $\pm$ 0.14	1.3 $\pm$ 0.05	1.3 $\pm$ 0.07	1.4 $\pm$ 0.05
	Total enzyme		4.1 $\pm$ 0.58	1.8 $\pm$ 0.06****	4.0 $\pm$ 0.29	4.2 $\pm$ 0.31
	Apoenzyme		2.6 $\pm$ 0.54	0.5 $\pm$ 0.07****	2.7 $\pm$ 0.24	2.8 $\pm$ 0.30
1	Holoenzyme		1.7 $\pm$ 0.11	1.4 $\pm$ 0.14	1.6 $\pm$ 0.09	1.7 $\pm$ 0.14
	Total enzyme		4.3 $\pm$ 0.36	1.8 $\pm$ 0.11*****	4.1 $\pm$ 0.28	4.4 $\pm$ 0.48
	Apoenzyme		2.6 $\pm$ 0.34	0.4 $\pm$ 0.11*****	2.5 $\pm$ 0.31	2.7 $\pm$ 0.39
2	Holoenzyme		1.5 $\pm$ 0.05	1.3 $\pm$ 0.14	1.5 $\pm$ 0.10	1.3 $\pm$ 0.09
	Total enzyme		3.2 $\pm$ 0.05	1.8 $\pm$ 0.10*****	3.3 $\pm$ 0.14	3.1 $\pm$ 0.31
	Apoenzyme		1.7 $\pm$ 0.09	0.5 $\pm$ 0.13*****	1.8 $\pm$ 0.14	1.8 $\pm$ 0.23

Table 6. *Reversal by naloxone of the chronic morphine-induced disturbance in the redox states of the rat hepatic nicotinamide-adenine dinucleotide (phosphate) couples*

Experimental details and comparison of results are as described in Table 4. Values are means $\pm$ S.E.M. for each group of six rats, and all (except the ratios) are in μ g/g wet wt. of liver. The significance of difference is indicated as follows: ***** $P < 0.001$.

Test	Treatment ...	Liver dinucleotide concentrations or ratios			
		0.9% NaCl		Naloxone	
	Pretreatment ... Column no. ...	Nil (1)	Morphine (2)	Nil (3)	Morphine (4)
[NADP ⁺]		44 $\pm$ 2	40 $\pm$ 1	40 $\pm$ 2	43 $\pm$ 2
[NADPH]		193 $\pm$ 1	230 $\pm$ 6*****	194 $\pm$ 2	191 $\pm$ 4
[NAD ⁺]		332 $\pm$ 10	284 $\pm$ 3*****	339 $\pm$ 12	336 $\pm$ 7
[NADH]		148 $\pm$ 1	152 $\pm$ 3	144 $\pm$ 2	142 $\pm$ 3
Sum		717 $\pm$ 13	706 $\pm$ 6	717 $\pm$ 13	712 $\pm$ 8
[NADPH]/[NADP ⁺]		4.39 $\pm$ 0.21	5.75 $\pm$ 0.19*****	4.85 $\pm$ 0.22	4.44 $\pm$ 0.17
[NAD ⁺]/[NADH]		2.24 $\pm$ 0.05	1.87 $\pm$ 0.03*****	2.35 $\pm$ 0.07	2.37 $\pm$ 0.05

As mentioned in the introduction, Badawy & Evans (1975a) suggested that chronic morphine administration inhibits liver tryptophan pyrrolase activity by increasing [NADPH]. The results in Table 6 show that the drug increased [NADPH] by 19% and also decreased [NAD⁺] by 14%, thus confirming the findings by the above authors in unfrozen livers of older rats, and that naloxone is capable of reversing these effects of morphine. These latter results and those in Table 5 therefore provide support for the above suggestion.

The mechanism(s) by which morphine increases [NADPH] is not clearly understood at present. Because the sum of concentrations of all four dinucleotides was not altered by the drug (Table 6),

it may be suggested that morphine increases [NADPH] at the expense of [NAD⁺], but further work is clearly required to examine the possibility. It is also not clear how naloxone reverses the morphine-induced changes in [NADPH] and [NAD⁺]. Direct oxidation of NADPH is ruled out (results not shown), and further work is therefore required.

Inhibition of rat brain tryptophan metabolism by withdrawal of drugs of dependence

The effects on brain tryptophan metabolism, and other metabolic systems, of withdrawal of morphine, nicotine and phenobarbitone (with or without nicotinamide administration during the withdrawal

phases) are shown in Tables 7, 8 and 9 respectively. These effects were examined at 1, 3 and 2 days respectively after withdrawal of the above drugs in view of the previous findings (Badawy & Evans, 1975*a,b*) that these are the time intervals after withdrawal of the drugs at which maximum enhancement of liver tryptophan pyrrolase activity occurs. In support of these previous findings, the

results in Tables 7–9 show that the activities of the pyrrolase holoenzyme, total enzyme and apo-enzyme were enhanced during morphine withdrawal by 163, 150 and 139% respectively (Table 7). The corresponding increases observed during nicotine withdrawal (Table 8) were 160, 144 and 129% respectively, whereas those caused by phenobarbitone withdrawal (Table 9) were 150, 151, and 153%

Table 7. *Effects of morphine withdrawal on rat liver and brain tryptophan metabolism and on serum corticosterone concentration and their prevention by nicotinamide*

Morphine was administered in drinking water for 3 weeks as described in the Materials and methods section. Experiments were performed at 1 day after withdrawal of morphine. Some rats withdrawn from morphine also received a solution of nicotinamide in drinking water (5 g/litre) for the 24 h period. Liver tryptophan pyrrolase activity is in μmol of kynurenine formed/h per g wet wt., serum corticosterone concentration is in $\mu\text{g/litre}$ and all other values (except the percentage of free serum tryptophan) are in $\mu\text{g/ml}$ of serum or in $\mu\text{g/g}$ wet wt. of tissue. Values are means $\pm$ S.E.M. for each group of four (pyrrolase activities) or six rats (all other determinations). The effects of morphine withdrawal with or without nicotinamide administration are compared with results in control rats. The significance of difference is indicated as follows: **** $P < 0.01$; ***** $P < 0.001$.

Determination	Serum corticosterone and tryptophan metabolism parameters		
	Treatment ...	Control	Nicotinamide during morphine withdrawal
Holo-(tryptophan pyrrolase)		1.90 $\pm$ 0.04	5.00 $\pm$ 0.27*****
Total tryptophan pyrrolase		4.20 $\pm$ 0.12	10.50 $\pm$ 0.22*****
Apo-(tryptophan pyrrolase)		2.30 $\pm$ 0.16	5.50 $\pm$ 0.17*****
Liver tryptophan		5.38 $\pm$ 0.06	4.10 $\pm$ 0.14*****
Serum corticosterone		65.30 $\pm$ 2.20	107.50 $\pm$ 2.90*****
Free serum tryptophan		1.51 $\pm$ 0.04	1.17 $\pm$ 0.02*****
Total serum tryptophan		29.30 $\pm$ 0.23	23.92 $\pm$ 0.37*****
Free serum tryptophan (%)		5.15 $\pm$ 0.19	4.89 $\pm$ 0.09
Brain tryptophan		2.07 $\pm$ 0.03	1.66 $\pm$ 0.03*****
Brain 5-hydroxytryptamine		0.61 $\pm$ 0.009	0.51 $\pm$ 0.006*****
Brain 5-hydroxyindol-3-ylacetic acid		0.35 $\pm$ 0.008	0.27 $\pm$ 0.010*****

Table 8. *Effects of nicotine withdrawal on rat liver and brain tryptophan metabolism and on serum corticosterone concentration and their prevention by nicotinamide*

Experimental details and comparisons, expressions and mean values of results are as described in Table 7, except that the rats were given nicotine in drinking water for 3 weeks as described in the Materials and methods section, the experiments were performed at 3 days after withdrawal and nicotinamide was administered for the 3-day withdrawal period. The significance of difference is indicated as follows: *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ***** $P < 0.001$.

Determination	Serum corticosterone and tryptophan metabolism parameters		
	Treatment ...	Control	Nicotinamide during nicotine withdrawal
Holo-(tryptophan pyrrolase)		1.50 $\pm$ 0.06	3.90 $\pm$ 0.06*****
Total tryptophan pyrrolase		3.20 $\pm$ 0.31	7.80 $\pm$ 0.51*****
Apo-(tryptophan pyrrolase)		1.70 $\pm$ 0.27	3.90 $\pm$ 0.45****
Liver tryptophan		6.07 $\pm$ 0.10	4.53 $\pm$ 0.10*****
Serum corticosterone		64.70 $\pm$ 5.90	105.50 $\pm$ 3.80*****
Free serum tryptophan		1.21 $\pm$ 0.02	0.88 $\pm$ 0.02*****
Total serum tryptophan		30.40 $\pm$ 0.86	24.28 $\pm$ 0.90*****
Free serum tryptophan (%)		3.98 $\pm$ 0.11	3.62 $\pm$ 0.20
Brain tryptophan		2.17 $\pm$ 0.03	1.78 $\pm$ 0.07*****
Brain 5-hydroxytryptamine		0.67 $\pm$ 0.019	0.55 $\pm$ 0.012*****
Brain 5-hydroxyindol-3-ylacetic acid		0.41 $\pm$ 0.012	0.32 $\pm$ 0.004*****

Table 9. *Effects of phenobarbitone withdrawal on rat liver and brain tryptophan metabolism and serum corticosterone concentration and their prevention by nicotinamide*

Experimental details and comparisons, expressions and mean values of results are as described in Table 7, except that the rats were given phenobarbitone in drinking water for 3 weeks as described in the Materials and methods section, the experiments were performed at 2 days after phenobarbitone withdrawal and nicotinamide was administered for the 2-day withdrawal period. The significance of difference is indicated as follows: ***** $P < 0.001$.

Determination	Treatment ...	Serum corticosterone and tryptophan metabolism parameters		
		Control	Phenobarbitone withdrawal	Nicotinamide during phenobarbitone withdrawal
Holo-(tryptophan pyrrolase)		1.80 ± 0.07	4.50 ± 0.22*****	1.80 ± 0.05
Total tryptophan pyrrolase		3.50 ± 0.14	8.80 ± 0.31*****	1.80 ± 0.05*****
Apo-(tryptophan pyrrolase)		1.70 ± 0.07	4.30 ± 0.15*****	0.00 ± 0.00*****
Liver tryptophan		6.74 ± 0.22	5.24 ± 0.09*****	8.27 ± 0.16*****
Serum corticosterone		64.50 ± 1.50	118.30 ± 4.60*****	65.70 ± 1.70
Free serum tryptophan		1.73 ± 0.03	1.39 ± 0.04*****	2.12 ± 0.04*****
Total serum tryptophan		27.47 ± 0.42	21.90 ± 0.41*****	31.67 ± 0.49*****
Free serum tryptophan (%)		6.30 ± 0.18	6.35 ± 0.20	6.69 ± 0.14
Brain tryptophan		2.57 ± 0.05	2.05 ± 0.06*****	3.03 ± 0.07*****
Brain 5-hydroxytryptamine		0.59 ± 0.007	0.50 ± 0.008*****	0.69 ± 0.008*****
Brain 5-hydroxyindol-3-ylacetic acid		0.39 ± 0.008	0.31 ± 0.006*****	0.48 ± 0.007*****

respectively. These proportionate increases in pyrrolase activities characterize the hormonal induction mechanism (see Badawy & Evans, 1975c), and this is supported by the observed (Tables 7–9) increases in serum corticosterone concentration (of 63–85%) during withdrawal of the above three drugs. Similar findings have been reported during ethanol withdrawal (Badawy *et al.*, 1980). Plasma corticosterone concentration has also been shown to be increased during withdrawal of opiates (Kokka *et al.*, 1973) and nicotine (Benwell & Balfour, 1979).

The concentrations of free serum, total serum, liver and brain tryptophan and those of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were decreased during withdrawal of morphine, nicotine and phenobarbitone. Morphine withdrawal (Table 7) decreased the above concentrations by 22, 18, 24, 20, 16 and 23% respectively. The corresponding decreases by nicotine withdrawal (Table 8) were 27, 20, 25, 18, 18 and 22% respectively, whereas those by phenobarbitone withdrawal (Table 9) were 20, 20, 22, 20, 15 and 20% respectively. Tryptophan binding to serum proteins (expressed as a percentage of free serum tryptophan) was not significantly altered by withdrawal of any of these three drugs.

In experiments not shown here, it was found that serum glucose concentration was not significantly altered by withdrawal of the above drugs.

These results therefore suggest that withdrawal of morphine, nicotine and phenobarbitone inhibits brain 5-hydroxytryptamine synthesis by decreasing tryptophan availability to the brain secondarily to the enhancement of liver tryptophan pyrrolase activity

and the associated increase in hepatic tryptophan catabolism. Similar conclusions have been reached on the basis of similar findings from experiments in which pyrrolase activity was enhanced by ethanol withdrawal (Badawy *et al.*, 1980) or by acute administration of cortisol (Green *et al.*, 1975) or ethanol (Badawy & Evans, 1976b). More direct evidence for the involvement of the enhanced liver pyrrolase activity in the drug-withdrawal-induced inhibition of brain tryptophan metabolism is provided by the findings (Tables 7–9) that nicotinamide [a tryptophan pyrrolase inhibitor (Badawy & Evans, 1976a)] administration during the withdrawal phases not only prevented the enhancement of liver pyrrolase activity and the decreases in concentrations of tryptophan and hydroxyindoles in liver, serum and/or brain, but also caused inhibition of the total pyrrolase and apoenzyme activities (of 57–100%) and increases in tryptophan and hydroxyindole concentrations (of 10–37%), in comparison with values observed in control rats. The effects of nicotinamide on these parameters in these latter rats have previously been reported. It was then found (Badawy *et al.*, 1980) that nicotinamide administration for 8 days inhibited the pyrrolase total enzyme and apoenzyme activities by 49 and 77% respectively, and increased the concentrations of free serum, total serum, liver and brain tryptophan and brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid by 26, 26, 26, 30, 22 and 29% respectively. All these effects of nicotinamide in control rats are not dissimilar to those observed when it was administered during drug withdrawal (Tables 7–9) and also during ethanol

withdrawal (Badawy *et al.*, 1980). This suggests that the ability of nicotinamide to exert its effects in control rats is not impaired by the opposite effects of withdrawal of drugs of dependence.

The results in Tables 7–9 also show that nicotinamide prevented the increases in serum corticosterone concentration caused by withdrawal of nicotine or phenobarbitone, but that it was only partially effective in preventing that caused by morphine withdrawal. In experiments not shown here, it was found that neither drug withdrawal nor nicotinamide administration during withdrawal caused any significant changes in serum glucose concentration.

Previous studies of the effects of chronic administration and subsequent withdrawal of drugs of dependence on brain tryptophan metabolism

Such studies with ethanol have previously been discussed (Badawy *et al.*, 1979, 1980). Chronic phenobarbitone administration does not alter brain 5-hydroxytryptamine concentration, whereas subsequent withdrawal (at 3 days) causes a decrease (Bantutova *et al.*, 1978). This latter finding is similar to that of the present work (Table 9). Chronic administration and subsequent withdrawal of nicotine have been reported (Benwell & Balfour, 1979) to decrease brain hippocampal 5-hydroxytryptamine concentration. This finding, however, cannot be compared directly with those in the present work (Tables 2 and 8) because of differences in drug administration procedures.

By contrast, numerous reports on the effects of chronic morphine administration on brain 5-hydroxytryptamine synthesis and turnover have been published. Many of the findings are contradictory (for references, see Way, 1972; Way *et al.*, 1974), and this may be explained by differences in strains and/or species of animals used, routes of administration of, and duration of treatment with, morphine, and methods of assessment of rates of synthesis and turnover of 5-hydroxytryptamine. However, the view (Way, 1972) that morphine enhances these rates is supported by the present findings (Tables 1 and 4). One additional possible explanation of the failure of some workers to demonstrate the above enhancement is that of choice of the experimental time interval after morphine administration. In a number of investigations (see, e.g., Bhargava & Matwyshyn, 1977; Bhargava, 1979), tests were carried out many hours after the last of the daily morphine injection(s) or after removal of the implanted drug. The results in Table 7 in the present work showing that brain tryptophan metabolism is inhibited at 24 h after morphine withdrawal suggest that, at an earlier time interval, brain tryptophan metabolism might have returned from the stimulated state to the normal one.

Mechanism(s) of drug dependence

The biochemical pathology of drug dependence is more likely to be advanced by examining what such lesion-producing drugs exert in common, rather than by trying to differentiate between them. In addition to providing considerable support to the concept of the inverse relationship between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine synthesis, the present findings are the first to establish that four drugs of dependence of different chemical classes share, other than depression of the central nervous system and the production of physical dependence, the single property of influencing brain 5-hydroxytryptamine synthesis in similar fashions, by acting on liver tryptophan pyrrolase activity. Moreover, the ability of naloxone to reverse the chronic effects of morphine on liver tryptophan and dinucleotide metabolism illustrates a new and unique aspect of the actions of this opiate antagonist. In view of the possible involvement of 5-hydroxytryptamine in mood regulation and in depressive illness (Curzon, 1969; Lapin & Oxenkrug, 1969; van Praag, 1978 and references cited therein), it is not unreasonable to suggest that the actions of drugs of dependence on tryptophan metabolism reported here may be related to the mechanism(s) of drug dependence. It may therefore be of interest to find out if tryptophan metabolism is similarly disturbed in drug-dependent patients, and, if so, whether the withdrawal symptoms exhibited by such patients could be prevented by therapy with agents such as nicotinamide.

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