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REVERSAL BY NALOXONE OF THE EFFECTS OF CHRONIC ADMINISTRATION OF DRUGS OF DEPENDENCE ON RAT LIVER AND BRAIN TRYPTOPHAN METABOLISM

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1 Chronic administration of ethanol, morphine, nicotine or phenobarbitone has previously been shown to enhance rat brain 5-hydroxytryptamine (5-HT) synthesis by increasing the availability of circulating tryptophan to the brain secondarily to the NADPH-mediated inhibition of liver tryptophan pyrrolase activity.

2 Naloxone reverses the above enhancement of 5-HT synthesis and the accompanying increase in tryptophan availability to the brain and the inhibition of liver tryptophan pyrrolase activity.

3 It is suggested that naloxone exerts these effects by antagonizing the chronic drug-induced increase in liver [NADPH].

4 Naloxone increases serum corticosterone concentration in rats chronically treated with the above four drugs of dependence. Possible explanations of this effect are discussed.

Introduction

We have shown that chronic administration to rats of ethanol, morphine, nicotine or phenobarbitone enhances brain 5-hydroxytryptamine (5-HT) synthesis by increasing the availability of circulating tryptophan to the brain (Badawy, Punjani & Evans, 1979; 1981) secondarily to the NADPH-mediated inhibition of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) activity (Badawy & Evans, 1975a, b). These conclusions were reached in part from the results of experiments demonstrating the ability of phenazine methosulphate (an agent that rapidly re-oxidizes liver NAD(P)H) to reverse the above chronic drug-induced changes in liver and brain tryptophan metabolism. The opiate antagonist, naloxone, is known to reverse the chronic morphine-induced enhancement of brain 5-HT synthesis (see e.g. Shen, Loh & Way, 1970), and we (Badawy *et al.*, 1981) found that it is also capable of reversing the morphine effects on tryptophan availability to the brain, liver tryptophan pyrrolase activity and liver [NADPH].

Because hepatic [NADPH] is also increased by chronic administration of ethanol, nicotine or phenobarbitone (Badawy & Evans, 1975a, b), it was considered of interest to find out whether naloxone can reverse the effects of these three drugs of dependence on liver and brain tryptophan metabolism. The results of the experiments described and discussed in the present paper show that this is so.

Methods

Animals

Locally bred male Wistar rats (150 g ± 6% at the start of experiments) were housed three per cage and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants) and water. The animals were killed between 13 h 00 min and 14 h 00 min either by stunning and cervical dislocation (for the determination of tryptophan pyrrolase activity in fresh liver homogenates) or by decapitation (for all other determinations).

Treatments

Drugs of dependence were freely administered in drinking water for 3 weeks as described previously (Badawy & Evans, 1975a, b). Briefly, ethanol was administered in concentrations (v/v) of 5% for 2 days, 7.5% for 2 days more and finally 10% for the remainder of the experimental period. Nicotine hydrogen (+)-tartrate was administered at a constant concentration throughout, of 3.08 mg (1 mg of the free base) per 100 ml of drinking water. Phenobarbitone sodium was given in increasing concentrations (48 h apart) of 1, 1.5, 2, 2.5 and finally 3 mg/ml of drinking water, and the animals were then maintained on the latter concentration till the end of the experimental period.

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Both control and chronic drug-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) of 0.9% (w/v) NaCl solution.

Chemical, enzymatic 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 μM) haematin. The apoenzyme activity was obtained by difference. The holoenzyme and apoenzyme are respectively the haem-containing and haem-free forms of tryptophan pyrrolase in rat liver. The total enzyme activity is the sum of activities of the above two forms.

Serum glucose and corticosterone concentrations were determined by the fluorimetric methods of Slein (1963) and Glick, Von Redlich & Levine (1964) respectively.

Liver, free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan concentrations were determined by a modification (Bloxam & Warren, 1974) of the method of Denckla & Dewey (1967) as described previously (Badawy & Evans, 1976b). Brain tryptophan was present in the extract containing 5-HT. This and the extract containing 5-hydroxyindol-3-ylacetic acid (5-HIAA) were prepared and analysed by the method of Curzon & Green (1970).

Statistical analysis of results was performed by use of Student's *t* test.

Chemicals

Naloxone hydrochloride (in ampoules, each containing 0.4 mg/ml) was from Winthrop Laboratories, Surbiton-upon-Thames, Surrey; all other chemicals were from BDH Chemicals and the Sigma Chemical Co. (both of Poole, Dorset) and were of the purest commercially available grades.

Results

Effects of chronic ethanol administration on rat liver and brain tryptophan metabolism and their reversal by naloxone

As shown in Table 1, chronic ethanol administration did not alter the holoenzyme activity of liver tryptophan pyrrolase, but caused a 43% decrease in that of the total enzyme. The apoenzyme was therefore the form specifically inactivated by the drug treatment, by 72%. Ethanol increased the concentrations of liver, free serum, total serum and brain tryptophan and those of brain 5-HT and 5-HIAA by 24, 27, 27, 23, 21 and 20% respectively ($P < 0.001$). Neither tryptophan binding to serum proteins (expressed as the percentage free serum tryptophan) nor serum corticosterone concentration were significantly altered by chronic ethanol administration.

Table 1 Effects of chronic ethanol administration with or without a single dose of naloxone on rat liver and brain tryptophan metabolism and on serum corticosterone concentration

	Saline		*Naloxone*	
Determination	*Control*	*Ethanol*	*Control*	*Ethanol*
Pyrrolase activity	(1)	(2)	(3)	(4)
Holoenzyme	1.70 ± 0.06	1.70 ± 0.09	1.80 ± 0.05	2.30 ± 0.08**
Total enzyme	4.20 ± 0.26	2.40 ± 0.13***	5.40 ± 0.21†	5.70 ± 0.20
Apoenzyme	2.50 ± 0.23	0.70 ± 0.10***	3.60 ± 0.18*	3.40 ± 0.15
Liver Trp	6.22 ± 0.17	7.73 ± 0.11***	5.98 ± 0.07	6.02 ± 0.12
Free serum Trp	1.29 ± 0.03	1.64 ± 0.07***	1.30 ± 0.02	1.26 ± 0.01
Total serum Trp	21.70 ± 0.29	27.50 ± 1.22***	22.08 ± 0.22	22.23 ± 0.34
Free serum Trp (%)	5.94 ± 0.09	5.96 ± 0.48	5.89 ± 0.14	5.67 ± 0.09
Brain Trp	2.23 ± 0.05	2.74 ± 0.02***	2.23 ± 0.05	2.15 ± 0.05
Brain 5-HT	0.63 ± 0.011	0.76 ± 0.007***	0.63 ± 0.011	0.62 ± 0.012
Brain 5-HIAA	0.40 ± 0.007	0.48 ± 0.009***	0.38 ± 0.007	0.39 ± 0.007
Serum corticosterone	57.73 ± 2.09	57.53 ± 1.89	55.98 ± 1.02	69.43 ± 1.74***

Ethanol was administered in drinking water for 3 weeks as described in the Methods section. Both control and ethanol-treated rats received, at 2 h before death, an intraperitoneal injection of either naloxone hydrochloride (1 mg/kg) or an equal volume (2.5 ml/kg) of saline. Values are means ± s.e. of each group of four (pyrrolase activities) or six rats (all other determinations). Pyrrolase activity is in μmol of kynurenine formed h^{-1} g^{-1} wet wt. of liver, serum corticosterone concentration is in μg/litre, and all other determinations (except the percentage free serum tryptophan (Trp)) are in μg/ml of serum or per 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: †$P < 0.02$; *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

Table 2 Effects of chronic nicotine administration with or without a single dose of naloxone on rat liver and brain tryptophan (Trp) metabolism and on serum corticosterone concentration

	Saline		*Naloxone*	
Determination	*Control*	*Nicotine*	*Control*	*Nicotine*
Pyrrolase activity	(1)	(2)	(3)	(4)
Holoenzyme	2.40 ± 0.12	2.80 ± 0.18	2.40 ± 0.13	2.80 ± 0.16
Total enzyme	6.60 ± 0.71	4.10 ± 0.16†	6.10 ± 0.28	6.70 ± 0.22
Apoenzyme	4.20 ± 0.61	1.30 ± 0.15**	3.70 ± 0.22	3.90 ± 0.27
Liver Trp	6.47 ± 0.14	8.10 ± 0.14***	6.18 ± 0.08	6.17 ± 0.07
Free serum Trp	1.19 ± 0.05	1.46 ± 0.07†	1.13 ± 0.06	1.15 ± 0.05
Total serum Trp	24.17 ± 0.21	30.00 ± 0.78***	24.25 ± 1.16	23.50 ± 0.62
Free serum Trp (%)	4.92 ± 0.17	4.87 ± 0.33	4.66 ± 0.39	4.89 ± 0.20
Brain Trp	2.04 ± 0.02	2.54 ± 0.07***	1.92 ± 0.06	2.03 ± 0.03
Brain 5-HT	0.65 ± 0.020	0.79 ± 0.011***	0.64 ± 0.016	0.65 ± 0.009
Brain 5-HIAA	0.36 ± 0.007	0.45 ± 0.012***	0.36 ± 0.015	0.35 ± 0.011
Serum corticosterone	56.50 ± 0.50	57.50 ± 2.50	55.40 ± 2.70	67.00 ± 1.10**

Experimental details and comparisons, expressions and mean values of results are as described in Table 1, except that the experimental rats were given nicotine in drinking water for 3 weeks as described in the Methods section. The significance of differences is indicated as follows: †$P < 0.02$; **$P < 0.005$; ***$P < 0.001$.

The results in Table 1 also show that all the above effects of chronic ethanol administration were reversed by naloxone, which caused a 24% increase ($P < 0.001$) in serum corticosterone concentration in the ethanol-treated rats. However, in control rats naloxone did not exert any significant effects on the above parameters, except for the moderate increases (29–44%) in the total activity and apoenzyme activity of liver tryptophan pyrrolase. This latter effect in control rats should be viewed as an isolated incident, because it was not observed in comparable experiments in the present work (see Tables 2 and 3) or in experiments described in a previous publication (Badawy *et al.*, 1981).

Effects of chronic nicotine administration on rat liver and brain tryptophan metabolism and their reversal by naloxone

The results in Table 2 show that chronic nicotine administration decreased the total and apoenzyme activities of liver tryptophan pyrrolase by 38 and 69% respectively ($P = 0.02–0.005$), but exerted no significant effect on that of the holoenzyme. Nicotine increased the concentrations of liver, free serum, total serum and brain tryptophan and those of brain 5-HT and 5-HIAA by 25, 23, 24, 25, 21 and 25% respectively ($P = 0.02–0.001$). The drug treatment did not influence significantly the binding of tryptophan to serum proteins or the concentration of serum corticosterone.

Naloxone administration to chronically nicotine-treated rats (Table 2) reversed the nicotine-induced inhibition of liver tryptophan pyrrolase activity and the accompanying increases in tryptophan and hydroxyindole concentrations in liver, serum and/or brain. The concentration of serum corticosterone in nicotine-treated rats was increased by naloxone (by 21%; $P < 0.005$). In contrast, naloxone administration to control rats failed to cause any significant changes in all the parameters examined.

Effects of chronic phenobarbitone administration on rat liver and brain tryptophan metabolism and their reversal by naloxone

As shown in Table 3, chronic phenobarbitone administration inhibited the total pyrrolase and apoenzyme activities by 31 and 55% respectively ($P = 0.005–0.001$) but exerted no significant effect on that of the holoenzyme. Phenobarbitone increased the concentrations of liver, free serum, total serum and brain tryptophan and those of brain 5-HT and 5-HIAA by 25, 24, 23, 21, 22 and 23% respectively ($P < 0.001$), but exerted no significant effects on serum corticosterone concentration or tryptophan binding to serum proteins.

The above chronic phenobarbitone-induced changes in liver tryptophan pyrrolase activity and tryptophan and hydroxyindole concentrations were all reversed by naloxone (Table 3). Naloxone caused a 34% increase ($P < 0.005$) in serum corticosterone concentration in chronically phenobarbitone-treated rats. None of the above parameters was significantly altered by naloxone administration to control rats.

In experiments not shown here, it was found that serum glucose concentration was not significantly altered by chronic administration of drugs of dependence nor by acute administration of naloxone to either control or chronic drug-treated rats.

Table 3 Effects of chronic phenobarbitone administration with or without a single dose of naloxone on rat liver and brain tryptophan (Trp) metabolism and on serum corticosterone concentration

	Saline		*Naloxone*	
Determination	*Control*	*Phenobarbitone*	*Control*	*Phenobarbitone*
Pyrrolase activity	(1)	(2)	(3)	(4)
Holoenzyme	2.50 ± 0.11	2.60 ± 0.21	2.90 ± 0.17	3.10 ± 0.07
Total enzyme	5.80 ± 0.15	4.00 ± 0.22***	6.00 ± 0.47	6.10 ± 0.16
Apoenzyme	3.30 ± 0.11	1.40 ± 0.32**	3.10 ± 0.32	3.00 ± 0.11
Liver Trp	7.62 ± 0.27	9.53 ± 0.31***	7.42 ± 0.31	7.34 ± 0.40
Free serum Trp	1.25 ± 0.01	1.55 ± 0.04***	1.24 ± 0.06	1.18 ± 0.02
Total serum Trp	26.52 ± 0.53	32.70 ± 0.43***	24.33 ± 1.21	25.64 ± 0.45
Free serum Trp (%)	4.71 ± 0.09	4.74 ± 0.14	5.09 ± 0.32	4.60 ± 0.11
Brain Trp	2.14 ± 0.06	2.59 ± 0.06***	2.11 ± 0.06	2.01 ± 0.02
Brain 5-HT	0.60 ± 0.018	0.73 ± 0.009***	0.58 ± 0.017	0.63 ± 0.016
Brain 5-HIAA	0.39 ± 0.006	0.48 ± 0.008***	0.37 ± 0.010	0.36 ± 0.005
Serum corticosterone	61.30 ± 1.20	60.80 ± 1.80	61.30 ± 2.90	82.00 ± 4.20**

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

Discussion

Mechanism of enhancement of rat brain 5-hydroxytryptamine synthesis by chronic administration of drugs of dependence

The effects of chronic administration of ethanol, nicotine and phenobarbitone on liver and brain tryptophan metabolism shown in the present work (Tables 1–3) confirm those previously described and resemble those caused by chronic morphine administration (Badawy *et al.*, 1979; 1981). As is also suggested by the present results, it was then concluded that drugs of dependence enhance brain 5-HT synthesis by elevating brain tryptophan concentration (which plays an important role in 5-HT synthesis; see Curzon & Knott, 1974 and references cited therein; Badawy & Evans, 1976c) by increasing the availability of the circulating amino acid to the brain. This increased availability is not insulin-mediated or lipolysis-dependent (because of the absence of any changes in serum glucose concentration or tryptophan binding to serum proteins) but is most probably the result of the chronic drug-induced inhibition of liver tryptophan pyrrolase activity. This inhibition is caused by an increase in the hepatic concentration of the allosteric inhibitor NADPH (Badawy & Evans, 1975a, b), and its reversal by the $NAD(P)^+$ regenerator, phenazine methosulphate, leads to a reversal of the increase in tryptophan availability to the brain and the enhancement of brain 5-HT synthesis (Badawy *et al.*, 1979; 1981). Further evidence that chronic morphine administration enhances brain 5-HT synthesis by this pyrrolase-mediated mechanism was provided by the finding (Badawy *et al.*, 1981) that naloxone (which reverses the morphine effect on 5-HT synthesis, see e.g. Shen *et al.*, 1970) also reverses the accompanying changes in tryptophan availability to the brain, liver tryptophan pyrrolase activity and hepatic [NADPH]. In these respects, naloxone resembles phenazine methosulphate. The two agents differ in that only the latter oxidizes liver NADPH in control rats (see e.g. Badawy & Evans, 1976a).

Reversal by naloxone of the enhancement of rat brain 5-hydroxytryptamine synthesis by chronic administration of drugs and dependence

The above similarity of actions of naloxone and phenazine methosulphate has therefore led us to examine the effects of the former on the enhancement of 5-HT synthesis by chronic administration of ethanol, nicotine or phenobarbitone. As the results in Tables 1–3 show, naloxone was capable of reversing the above enhancement and also the accompanying increase in tryptophan availability to the brain and the inhibition of liver tryptophan pyrrolase activity. These findings therefore provide further support for the previous conclusions (Badawy *et al.*, 1979; 1981) concerning the mechanism of enhancement of brain 5-HT synthesis by chronic administration of drugs of dependence.

The mechanism by which naloxone reverses the inhibition of pyrrolase activity by chronic morphine administration involves reversal of the increase in liver [NADPH] caused by morphine (Badawy *et al.*, 1981). Since the other three drugs of dependence also increase liver [NADPH], it may be suggested that naloxone acts in rats treated with these latter

three drugs by the same mechanism, although this can only be ascertained by determination of concentrations of pyridine dinucleotides. Such a possible action of naloxone in chronic ethanol-treated rats may be of further interest, because a reversal by naloxone of the ethanol-induced increase in liver [NADPH], which is caused by the rise in [NADH] that is secondary to ethanol's own metabolism by alcohol dehydrogenase (Punjani, Badawy & Evans, 1979), may lead to an increased availability of NAD^+ for further ethanol metabolism. Naloxone is known to antagonize some pharmacological actions of ethanol including dependence and intoxication (see Blum, Futterman, Wallace & Schertner, 1977; Jeffcoate, Herbert, Cullen, Hastings & Walder, 1979) and it therefore remains to be seen whether this antagonism involves, at least in part, a possible acceleration of ethanol metabolism.

The present findings with naloxone deserve further comment. This opiate antagonist caused an increase in serum corticosterone concentration in rats chronically treated with ethanol, nicotine or phenobarbitone (Tables 1–3), and was shown (Badawy *et al.*, 1981) to exert a similar effect in chronic morphine-treated rats. As far as we could ascertain, this is the first description of such an effect of naloxone in experimental animals. In normal human volunteers, naloxone administration has very recently been shown (Jeffcoate, Platts, Ridout, Hastings, MacDonald & Selby, 1981) to elevate plasma corticotrophin, cortisol and prolactin concentrations in the presence of infused ethanol. The mechanism(s) of the above effect of naloxone in rats requires investigation, but, in view of the role of brain 5-HT in the regulation of corticotrophin and corticosteroid secretion (see Azmitia, 1978 and references cited therein), it is not unreasonable to suggest that the above naloxone effect may be caused by decrease in 5-HT concentration from an elevated level to the normal one. A similar increase in serum corticosterone concentration occurs after administration of phenazine methosulphate to chronically drug-treated rats (Badawy *et al.*, 1981). Alternatively, the increase in serum corticosterone concentration is a characteristic of withdrawal from drugs of dependence (Badawy *et al.*, 1981), and it is therefore possible that the above naloxone effect on this steroid may represent some aspect of withdrawal. It may be of interest to note in this respect that, whereas naloxone caused morphine-treated rats to exhibit the opiate-withdrawal syndrome under our experimental conditions (Badawy & Evans, 1981), it did not do so in rats chronically treated with ethanol, nicotine or phenobarbitone (unpublished observation).

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References

AZMITIA, E.C. (1978). The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. *Handb. Psychopharmac.*, **9**, 233–314.

BADAWY, A. A.-B. & EVANS, M. (1975a). The effects of ethanol on tryptophan pyrrolase activity and their comparison with those of phenobarbitone and morphine. *Adv. exp. Med. Biol.*, **59**, 229–251.

BADAWY, A. A.-B. & EVANS, M. (1975b). The effects of acute and chronic nicotine hydrogen (+)-tartrate administration and subsequent withdrawal on rat liver tryptophan pyrrolase activity and their comparison with those of morphine, phenobarbitone and ethanol. *Biochem. J.*, **148**, 425–432.

BADAWY, A. A.-B. & EVANS, M. (1975c). The regulation of rat liver tryptophan pyrrolase by its cofactor haem-experiments with haematin and 5-aminolaevulinate and comparison with the substrate and hormonal mechanisms. *Biochem. J.*, **150**, 511–520.

BADAWY, A. A.-B. & EVANS, M. (1976a). The regulation of rat liver tryptophan pyrrolase activity by reduced nicotinamide-adenine dinucleotide (phosphate)-experiments with glucose and nicotinamide. *Biochem. J.*, **156**, 381–390.

BADAWY, A. A.-B. & EVANS, M. (1976b). Animal liver tryptophan pyrrolases-absence of apoenzyme and of hormonal induction mechanism from species sensitive to tryptophan toxicity. *Biochem. J.*, **158**, 79–88.

BADAWY, A. A.-B. & EVANS, M. (1976c). The role of free serum tryptophan in the biphasic effect of acute ethanol administration on the concentrations of rat brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid. *Biochem. J.*, **160**, 315–324.

BADAWY, A. A.-B. & EVANS, C.M. (1981). Naloxone-precipitated withdrawal symptoms in rats chronically treated with small concentrations of morphine in drinking water. *Br. J. Pharmac.*, (in press).

BADAWY, A. A.-B., PUNJANI, N.F. & EVANS, M. (1979). Enhancement of rat brain tryptophan metabolism by chronic ethanol administration and possible involvement of decreased liver tryptophan pyrrolase activity. *Biochem. J.*, **178**, 575–580.

BADAWY, A. A.-B., PUNJANI, N.F. & EVANS, M. (1981). 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. *Biochem. J.*, **196**, 161–170.

BLOXAM, D.L. & WARREN, W.H. (1974). Error in the determination of tryptophan by the method of Denckla and Dewey. A revised procedure. *Anal. Biochem.*, **60**, 621–625.

BLUM, K., FUTTERMAN, S., WALLACE, J.E. & SCHWERTNER, H.A. (1977). Naloxone-induced inhibition of ethanol dependence in mice. *Nature, Lond.*, **265**, 49–51.

CURZON, G. & GREEN, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39,** 653–655.

CURZON, G. & KNOTT, P.J. (1974). Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma. *Br. J. Pharmac.*, **50,** 197–204.

DENCKLA, W.D. & DEWEY, H.K. (1967). The determination of tryptophan in plasma, liver and urine. *J. Lab. clin. Med.*, **69,** 160–169.

GLICK, D., VON REDLICH, D. & LEVINE, S. (1964). Fluorimetric determination of corticosterone and cortisol in 0.02–0.05 millilitres of plasma or submilligram samples of adrenal tissue. *Endocrinology*, **74,** 653–655.

JEFFCOATE, W.J., HERBERT, M., CULLEN, M.H., HASTINGS, A.G. & WALDER, C.P. (1979). Prevention of the effects of alcohol intoxication by naloxone. *Lancet*, **ii,** 1157–1159.

JEFFCOATE, W.J., PLATTS, P., RIDOUT, M., HASTINGS, A.G., MACDONALD, I. & SELBY, C. (1981). Endocrine effects of ethanol infusion in normal subjects: modification by naloxone. *Pharmac. Biochem. Behav.*, **13,** (Suppl. 1), 145–148.

PUNJANI, N.F., BADAWY, A. A.-B. & EVANS, M. (1979). Prevention by pyrazole of the effects of chronic ethanol administration on the redox states of the hepatic nicotinamide-adenine dinucleotide (phosphate) couples and on liver and brain tryptophan metabolism in the rat. *Biochem. J.*, **184,** 165–168.

SHEN, F.H., LOH, H.H. & WAY, E.L. (1970). Brain serotonin turnover in morphine tolerant-dependent mice. *J. Pharmac. exp. Ther.*, **175,** 427–434.

SLEIN, M.W. (1963). *D*-glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis.* ed. Bergmeyer, H.-U. pp. 117–123. New York: Academic Press.

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