Br. J. Pharmac. (1981), **74**, 514–516

THE MECHANISM OF THE ANTAGONISM BY NALOXONE OF ACUTE ALCOHOL INTOXICATION

A.A.-B. BADAWY & M. EVANS

South Glamorgan Health Authority, Addiction Unit Research Laboratory, Whitchurch Hospital, Cardiff CF4 7XB

Naloxone lowers blood-ethanol concentration and causes a simultaneous reversal of the disturbances in the redox states of the hepatic nicotinamide-adenine dinucleotide (phosphate) couples in acutely-ethanol-intoxicated rats. It is suggested that these effects of naloxone form the basis of its antagonism of acute alcohol intoxication.

Introduction Naloxone antagonizes a number of behavioural and pharmacological effects of ethanol, such as withdrawal, depletion of brain Ca^{2+} (Blum, Futterman, Wallace & Schwertner, 1977), acute intoxication or narcosis and the impairment of behaviour by intoxication (Sørensen & Mattisson, 1978; Jeffcoate, Herbert, Cullen, Hastings & Walder, 1979; Jefferys, Flanagan & Volans, 1980; Kimball, Huang, Torget & Houck, 1980). None of these actions of naloxone has, however, been satisfactorily explained.

One possible mechanism by which naloxone may antagonize acute alcohol intoxication and its behavioural effects is that of acceleration of ethanol metabolism by reversal of the drug-induced disturbance of the redox states of the hepatic nicotinamide-adenine dinucleotide (phosphate) couples. The following is a brief account of findings which led us to propose this hypothesis. Naloxone reverses the chronic morphine-induced inhibition of rat liver tryptophan pyrrolase activity by blocking the drug elevation of hepatic [NADPH] (Badawy, Punjani & Evans, 1981a). The latter concentration is also increased by chronic administration of ethanol, nicotine and phenobarbitone (Badawy & Evans, 1975a,b) and we found that naloxone is also capable of reversing the associated inhibition of liver pyrrolase activity (Badawy, Evans & Punjani, 1981b). Liver [NADPH] and, more significantly, [NADH] are increased in acute alcohol intoxication as a result of ethanol's own metabolism by NAD^+-dependent alcohol dehydrogenase (see e.g. Slater, 1972). Under these conditions, the availability of NAD^+ in the hepatic cytosol becomes an important rate-limiting factor for further ethanol metabolism (see Badawy, 1978 and references cited therein). A possible reversal by naloxone of these redox disturbances may therefore lead to an acceleration of ethanol metabolism and a consequent lowering of its blood concentration.

In the present communication, we show that naloxone administration to acutely ethanol-intoxicated rats causes, within 30 min, a full reversal of the above redox disturbances and a simultaneous lowering by 31% of blood-ethanol concentration from a mean intoxication value of 133 mg/dl.

Methods Locally bred male Wistar rats (326 g ± 2%) were housed three per cage and were maintained on cube diet 41B and water. The sources of various chemicals have been described previously (Badawy *et al.*, 1981a,b). Naloxone hydrochloride (1 mg/kg) or an equal volume (2.5 ml/kg) of 0.9% w/v NaCl solution (saline) was injected intraperitoneally at 1.5 h after a similar injection of either ethanol (2 g/kg) or an equal volume (20 ml/kg) of saline, and the animals were killed at 30 min after the second injection (usually between 12 h 00 min and 12 h 30 min).

Blood-ethanol concentration was determined with an Alcolmeter (model AE-D1, Lion Laboratories Ltd., Pearl Street, Cardiff CF2 1PP), whereas those of liver nicotinamide-adenine dinucleotides (phosphates) were determined in pieces of tissue frozen by a locally manufactured freeze-clamp by a modification (Punjani, Badawy & Evans, 1979) of the direct procedure of Slater, Sawyer & Sträuli (1964). Statistical analysis of results was performed by use of Student's *t* test.

Results As shown in Table 1, ethanol caused, at 2 h, a 59% decrease in liver [NAD^+], a 106% increase in that of NADH and a consequent 80% decrease in the [NAD^+]/[NADH] ratio, but did not alter significantly the sum of concentrations of the NAD couple. The NADP couple was also disturbed by ethanol, but to a lesser extent. Thus [NADPH] was elevated by only 15%, whereas the 13% decrease in [$NADP^+$] was not significant. However, the [NADPH]/[$NADP^+$] ratio was significantly increased by ethanol (by 32%), as was the sum of concentrations of the NADP couple.

0007-1188/81/110514-03 $01.00

Table 1 Effects of naloxone on blood-ethanol concentration and on the redox states of the hepatic nicotinamide-adenine dinucleotide (phosphate) couples in acutely-ethanol-intoxicated rats

Treatment ...	*Saline*		*Naloxone*	
Pretreatment ...	*Control*	*Ethanol*	*Control*	*Ethanol*
Determination				
Liver dinucleotides:	(1)	(2)	(3)	(4)
[NAD^+]	318±11	130±11***	296± 7	308± 8
[NADH]	161± 3	332± 4***	181±11	160± 8
Sum	479±12	462±14	477±14	468±14
[$NADP^+$]	46± 4	40± 2	63± 1**	53± 3
[NADPH]	269± 5	309± 7***	253± 5†	269± 9
Sum	315± 9	349± 9††	316± 8	322±10
Overall sum	794±17	811±22	793±16	790±18
[NAD^+]/[NADH]	1.98±0.07	0.39±0.03***	1.63±0.11††	1.93±0.08
[NADPH]/[$NADP^+$]	5.85±0.57	7.73±0.41††	4.02±0.12*	5.08±0.27
Blood ethanol	0	133.3±2.3	0	91.8±7.3***

Experimental details are as described in the text. Blood-ethanol concentration is expressed in mg/dl, whereas all other determinations (except the ratios) are in μg/g wet wt. of liver. Values for blood-ethanol concentrations are means ± s.e. for each group of 10 rats, whereas all other expressions are means ± s.e. for each group of six rats. The values in columns 2, 3 and 4 are compared with those in column 1, whereas blood-ethanol concentration in column 4 is compared with that in column 2. The significance of differences is indicated as follows: † $P<0.05$; †† $P<0.025$; * $P<0.02$; ** $P<0.005$; *** $P<0.001$.

Naloxone administration to ethanol-pretreated rats reversed not only the drug-induced narcosis (within 5 min), but also all the above disturbances in concentrations and ratios of the NAD(P) couples (Table 1); the values thus obtained were not significantly different from those observed in saline-treated control rats. Naloxone administration to control rats, however, caused a 37% increase in liver [$NADP^+$], a 6% decrease in that of NADPH and a 31% decrease in the [NADPH]/[$NADP^+$] ratio. Although naloxone also caused an 18% decrease in the [NAD^+]/[NADH] ratio in livers of control rats, the small changes in [NAD(H)] causing this decrease were not significant.

The results in Table 1 also show that the 133.3 mg/dl concentration of ethanol in blood of ethanol-intoxicated rats was decreased by 31% at 30 min after naloxone administration.

Discussion We have demonstrated the ability of naloxone to lower blood-ethanol concentration in acutely-ethanol-intoxicated rats (Table 1). Naloxone could thus be regarded as an 'amethystic agent' (Alkana & Noble, 1979) capable of antagonizing acute alcohol intoxication by a pharmacokinetic mechanism, and this provides a reasonable explanation of the actions of this opiate antagonist in this condition. Although the 31% decrease in blood-ethanol concentration obtained at 30 min after naloxone administration is remarkable, it is possible that a stronger effect may occur at earlier (or possibly also subsequent) time intervals. A detailed time course study is therefore required.

Pharmacokinetic antagonism of acute alcohol intoxication can be achieved by a variety of mechanisms (Alkana & Noble, 1979) but it is most likely that naloxone lowers blood-ethanol concentration by regenerating liver NAD^+, whose concentration in the cytosol becomes limiting for further ethanol metabolism. This NAD^+ regeneration is demonstrated by the observed (Table 1) reversal by naloxone of the ethanol-induced dramatic changes in the redox state of the NAD couple. Since the sum of concentrations of the NAD(P) couples was not significantly altered by naloxone (Table 1), it may be suggested that the NAD^+ regeneration is achieved by NADH oxidation, possibly through activation of the transdehydrogenase reaction of the latter with $NADP^+$, whose concentration is elevated by naloxone, thus creating a condition favouring transdehydrogenation. The mechanism(s) by which naloxone increases [$NADP^+$] in control rats may be related to interaction with the NADPH-dependent microsomal mixed-function oxidase, but further work is required in this context.

We thank the Welsh Office for a project grant, Mr M. Edwards of Llandough Hospital, Cardiff for blood-ethanol determinations, Mr A. Dacey for animal maintenance and Mr C.J. Morgan and Miss Nazeera F. Punjani for skilful assistance.

References

ALKANA, R.L. & NOBLE, E.P. (1979). Amethystic agents – reversal of acute ethanol intoxication in humans. In *Biochemistry and Pharmacology of Ethanol.* ed. Majchrowicz, E. & Noble, E.P. Vol. **2,** pp. 349–374. New York: Plenum Press.

BADAWY, A.A.-B. (1978). The metabolism of alcohol. *Clin. Endocrinol. Metab.*, **7,** 247–271.

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. & PUNJANI, N.F. (1981b). Reversal by naloxone of the effects of chronic administration of drugs of dependence on rat liver and brain tryptophan metabolism. *Br. J. Pharmac.*, **74,** 489–494.

BADAWY, A.A.-B., PUNJANI, N.F. & EVANS, M. (1981a). 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.

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

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.

JEFFERYS, D.B., FLANAGAN, R.J. & VOLANS, G.N. (1980). Reversal of ethanol-induced coma with naloxone. *Lancet,* **i,** 308–309.

KIMBALL, C.D., HUANG, S.M., TORGET, C.E. & HOUCK, J.C. (1980). Plasma ethanol, endorphin, and glucose experiment. *Lancet,* **ii,** 418–419.

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.

SLATER, T.F. (1972). Hepatotoxic effects of alcohol. In *Free Radical Mechanisms in Tissue Injury.* ed. Slater, T.F. pp. 171–197. London: Pion Limited.

SLATER, T.F., SAWYER, B. & STRÄULI, U. (1964). An assay procedure for nicotinamide-adenine dinucleotides in rat liver and other tissues. *Archs int. Physiol. Biochim.*, **72,** 427–447.

SØRENSEN, S.C. & MATTISSON, K. (1978). Naloxone as an antagonist in severe alcohol intoxication. *Lancet,* **ii,** 688.

(Received August 19, 1981.)