Pharmacology Biochemistry & Behavior, Vol. 11, pp. 111–114. Printed in the U.S.A.

Toxic Interactions of Ethanol with Other Central Depressants: Antagonism by Naloxone to Narcosis and Lethality

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Received 27 December 1978

HO, A. K. S. AND C. C. HO. *Toxic interactions of ethanol with other central depressants: antagonism by naloxone to narcosis and lethality.* PHARMAC. BIOCHEM. BEHAV. 11(1) 111–114, 1979.—The effects of naloxone on narcosis and/or lethality induced by diazepam, lithium, methaqualone and phenobarbital either alone or in combination with ethanol were studied in mice. Interaction toxicities between ethanol and the various psychotropic drugs were dose-dependent and so was the degree of antagonism by naloxone. Treatment with phenobarbital (10 mg/kg) or methaqualone (50 mg/kg) or lithium (4 meq/kg) prolonged the narcosis induced by ethanol (5 g/kg) by 45, 269 and 107% respectively. Naloxone (10 mg/kg) shortened the ethanol (5 g/kg) induced narcosis by 38%. Naloxone (10 mg/kg) also shortened narcosis induced by ethanol (5 g/kg) in combination with phenobarbital (10 mg/kg) or methaqualone (50 mg/kg) or lithium (2 meq/kg) by 31, 12 and 38% respectively. At 10 mg/kg of naloxone, the LD_{50} due to methaqualone was increased from 240 mg/kg to 416 mg/kg, and the LD_{50} due to ethanol was increased from 9.2 g/kg to 10.8 g/kg. Multiple injections of naloxone significantly ($p<0.01$) protected against the lethality of phenobarbital but not that of lithium. These findings provide further evidence of naloxone antagonism towards various CNS depressants.

Interaction toxicity Narcosis and lethality Ethanol and central depressant combinations
Antagonism by naloxone Lithium Methaqualone Phenobarbital Diazepam

NALOXONE, an N-substituted derivative of oxymorphine, is a narcotic antagonist with little agonistic properties [7,14]. Recent studies suggest that naloxone also antagonizes the effects of some central nervous system (CNS) depressants other than the opiates. For example, Blum *et al.*, [2,3] showed antagonism of ethanol narcosis and withdrawal convulsions by naloxone and Ross *et al.*, [19] reported antagonism to the calcium depleting effects induced by ethanol. Naloxone antagonizes the anesthesia induced by barbiturates [8,13] and general anesthetics [6]. There are also case reports on the possible use of naloxone in the treatment of overdoses of CNS depressants other than the opiates. Two groups of investigators reported naloxone antagonism of ethanol intoxication and opiate effects in humans [16,20]. Moss [18] reported naloxone reversal of apnea in a patient who had ingested large doses of barbiturate, diazepam and ethanol. Bell [1] reported a case of diazepam overdose reversed by naloxone. Despite these studies, information is still lacking on the potential usefulness of naloxone in the treatment of intoxication and overdoses especially in the combined effects of ethanol and other CNS depressants.

METHOD

Male Swiss-Webster white mice weighing 25 to 30 g were housed in groups of 8 in standard plastic cages (11×7×5 in.) with food and water available ad lib and maintained in humidity and temperature controlled room with light-dark cycle of 12–12 hr. The drugs used were phenobarbital sodium (Merck Chemical Co.): methaqualone (MTQ) hydrochloride (Parke, Davis and Co.): diazepam (injectable, Hoffman-LaRoche Inc.): lithium carbonate (Li^+) (J. T. Baker Chemical Co.); naloxone hydrochloride (Endo Lab.), and ethanol 95% diluted to 20% with distilled water (Scientific Products Co.). MTQ was prepared fresh as a suspension in 5% Tween 80 and saline solution. Li^+ was dissolved in distilled water. Other drugs were either dissolved in, or diluted with, isotonic saline. The drugs were injected IP with the exception of naloxone which was administered SC. Control mice were similarly injected with an equivalent volume of saline (0.2–0.25 ml). The time between injection of drugs and loss of the righting reflex was taken as the onset of narcosis: and the time between the loss and regain of righting reflex was recorded as the duration of sleep. Drugs were injected immediately after administration of naloxone, with the exception of Li^+ which was injected 1 hr prior to naloxone.

In studying the effects of naloxone on the toxicity of various drugs, the dose producing 50% lethality (LD_{50}) by each drug was first determined. Groups of 8 mice each were injected with varying doses of drug using at least 6 different doses of each drug. The dose ranges of the various drugs used for LD_{50} determinations were: ethanol, 5.0–11.0 g/kg: MTQ, 100–400 mg/kg: phenobarbital, 180–300 mg/kg: and Li^+, 7–13 meq/kg. To determine the effects of naloxone on

TABLE 1

EFFECTS OF NALOXONE ON NARCOSIS INDUCED BY ETHANOL, METHAQUALONE, PHENOBARBITAL AND LITHIUM IN SWISS-WEBSTER MICE

Treatment*	Onset of sleep Mean ± S.E.	Significance	Duration of sleep Mean ± S.E.	Significance
	(Sec)		(Min)	
Methaqualone + Saline + Saline				
100 mg/kg (N=7)	96.4 ± 8.5		51.4 ± 3.3	
150 mg/kg (N=13)	75.7 ± 1.8		98.0 ± 4.3	
Ethanol + Saline + Saline				
4 g/kg (N=9)	100.0 ± 26.5§		12.3 ± 3.3	
5 g/kg (N=12)	117.9 ± 3.0		46.3 ± 1.2	
Methaqualone + Ethanol 5 g/kg + Saline				
20 mg/kg (N=7)	120.0 ± 5.7		58.7 ± 4.5†	$p<0.05$
35 mg/kg (N=6)	100.8 ± 10.3		128.5 ± 5.7†	$p<0.0005$
50 mg/kg (N=7)	80.7 ± 4.8†	$p<0.005$	171.1 ± 8.7†	$p<0.0005$
Lithium +Ethanol 5 g/kg+ Saline				
2 meq/kg (N=9)	123.9 ± 7.8		48.1 ± 4.1	
4 meq/kg (N=10)	137.9 ± 8.3†	$p<0.01$	96.1 ± 6.8†	$p<0.0005$
6 meq/kg (N=7)	182.0 ± 8.3†	$p<0.0005$	134.0 ± 12.8†	$p<0.0005$
Phenobarbital + Ethanol 5 g/kg + Saline				
10 mg/kg (N=6)	130.0 ± 7.4		67.0 ± 6.8†	$p<0.0005$
20 mg/kg (N=6)	102.5 ± 4.2		146.0 ± 10.5†	$p<0.0005$
Naloxone + Ethanol 5 g/kg + Saline				
1 mg/kg (N=8)	105.8 ± 6.8		46.0 ± 3.9	
2.5 mg/kg (N=8)	109.1 ± 5.4		33.6 ± 2.9†	$p<0.01$
10 mg/kg (N=8)	113.3 ± 4.8		28.5 ± 2.1†	$p<0.01$
Naloxone + Methaqualone 150 mg/kg + Saline				
2.5 mg/kg (N=6)	84.2 ± 8.5		96.8 ± 4.6	
5 mg/kg (N=9)	75.0 ± 2.2		79.0 ± 4.6†	$p<0.005$
20 mg/kg (N=6)	80.3 ± 3.4		68.3 ± 3.0†	$p<0.0005$
Methaqualone + Naloxone 10 mg/kg + Ethanol 5g/kg				
20 mg/kg (N=7)	132.8 ± 12.8		48.5 ± 4.7‡	$p<0.005$
35 mg/kg (N=6)	123.3 ± 12.0		97.3 ± 4.6‡	$p<0.005$
50 mg/kg (N=7)	112.1 ± 10.4‡	$p<0.01$	151.0 ± 5.1‡	$p<0.01$
Naloxone + Ethanol 5 g/kg + Phenobarbital 10 mg/kg				
1 mg/kg (N=6)	130.0 ± 6.3		50.0 ± 5.2‡	$p<0.002$
2.5 mg/kg (N=6)	159.2 ± 12.1		38.0 ± 4.9‡	$p<0.0005$
10 mg/kg (N=6)	148.3 ± 9.5		32.0 ± 3.8‡	$p<0.0005$
Naloxone + Ethanol 5 g/kg + Lithium 2 meq/kg				
1 mg/kg (N=8)	141.3 ± 10.7		35.0 ± 4.7‡	$p<0.05$
2.5 mg/kg (N=6)	142.5 ± 17.4		28.2 ± 2.3‡	$p<0.005$
10 mg/kg (N=6)	127.5 ± 5.1		29.6 ± 3.3‡	$p<0.005$
	(Min)		(Min)	
Phenobarbital + Saline				
130 mg/kg (N=11)	38.0 ± 2.0		118.0 ± 8.0	
Naloxone + Phenobarbital 130 mg/kg				
1 mg/kg (N=8)	71.0 ± 14.0†	$p<0.0005$	95.0 ± 13.0	
2.5 mg/kg (N=8)	78.0 ± 13.0†	$p<0.0005$	81.0 ± 13.0†	$p<0.02$
10 mg/kg (N=8)	85.0 ± 13.0†	$p<0.0005$	74.0 ± 17.0†	$p<0.005$

* Drugs were administered (IP) immediately after naloxone (SC) except lithium which was injected 1 hr before naloxone.

†Compared to corresponding drug alone.

‡Compared to corresponding drug combinations.

§33% of the mice failed to sleep at 4 g/kg of ethanol.

TABLE 2

EFFECTS OF NALOXONE ON LD_{50} OF PHENOBARBITAL, METHAQUALONE, LITHIUM, OR ETHANOL IN SWISS-WEBSTER MICE

Drug and Dose	LD_{50}*	95% C.L.	Significance
Phenobarbital	240mg/kg	254–226	
Phenobarbital + Naloxone 30 mg/kg†	266 mg/kg	278–254	$p<0.01$
Methaqualone	240 mg/kg	263–219	
Methaqualone + Naloxone			
2.5 mg/kg	337 mg/kg	358–318	$p<0.001$
10 mg/kg	416 mg/kg	448–386	$p<0.001$
Lithium carbonate	10.3 meq/kg	11.3–9.5	
Lithium carbonate + Naloxone 30 mg/kg	11.3 meq/kg	12.1–10.5	N.S.
Ethanol	9.2 g/kg	8.9–9.4	
Ethanol + Naloxone			
10 mg/kg	10.8 g/kg	10.6–11.1	$p<0.001$
30 mg/kg	11.2 g/kg	10.9–11.5	$p<0.001$

*LD_{50}value was determined by the method of Litchfield and Wilcoxon.
†3 multiple doses of naloxone each at 30 mg/kg given 12 hours apart.

the lethality of various drugs, the following treatment protocols were used: (i) naloxone (single injection at 10–30 mg/kg or 90 mg/kg in three divided doses of 30 mg/kg each given 12 hr apart) and phenobarbital ranging from 200 to 320 mg/kg: (ii) naloxone (10 or 30 mg/kg) and Li^+ ranging from 8 to 13 meq/kg: (iii) naloxone (2.5 or 10 mg/kg) and MTQ ranging from 220 to 500 mg/kg: and (iv) naloxone (5, 10 or 30 mg/kg) and ethanol ranging from 7 to 13 g/kg. The test drug was injected immediately after administration of naloxone. The LD_{50} was determined over a period of 7 days from the dose-lethality curve based on the procedure of Litchfield and Wilcoxon [15].

RESULTS

The effects of naloxone on narcosis induced by either drug (MTQ, phenobarbital, Li^+) alone or in combination with ethanol are summarized in Table 1. MTQ by itself produced a decrease in onset and increase in sleep time. Ethanol in combination with MTQ potentiated ethanol narcosis and the synergistic effect was dose dependent on either drug. Naloxone antagonized narcosis induced by MTQ and ethanol either alone or in combinations. The degree of antagonism by naloxone on the combined effects of MTQ and ethanol on sleep was significantly less than those observed using either MTQ or ethanol alone. Administration of naloxone (1.0 to 10 mg/kg) to mice treated with a combination of ethanol and phenobarbital significantly shortened the duration of sleep but slightly delayed the onset of narcosis as compared to mice treated only with the ethanol-phenobarbital combination. Naloxone shortened the duration of sleep but had no effect on the onset of narcosis induced by diazepam. Mean sleeping time for diazepam (36 mg/kg) alone was 255.2 ± 14.3 min whereas the values for mice treated with diazepam (36 mg/kg) in combination with naloxone at varying doses of 2.5, 20 and 40 mg/kg were 224 ± 12.9: 185 ± 13.3 and 189.1 ± 11.8 min respectively.

Pretreatment with Li^+ for 1 hr significantly prolonged both the onset and duration of narcosis induced by ethanol. Administration of naloxone to mice treated with ethanol-Li^+ combination significantly ($p<0.05$) decreased the duration of sleep, but not the onset of narcosis.

Data obtained showing the effects of naloxone on the lethality of ethanol, lithium carbonate, phenobarbital and methaqualone are summarized in Table 2. Administration of naloxone at 10 or 30 mg/kg produced either no change or increased slightly but not significantly the LD_{50} due to Li^+. Further increase in dosage (80 mg/kg) or repeated injections (90 mg/kg in three divided doses of 30 mg/kg each given 12 hr apart) of naloxone failed to enhance the protection of lithium toxicity. The mean values of the LD_{50} due to MTQ and ethanol were found to increase significantly ($p<0.001$) in the mice treated with naloxone. Multiple doses of naloxone, but not single dose, treatment in mice significantly ($p<0.01$) increased LD_{50} due to phenobarbital.

DISCUSSION

The results of this study provide further evidence of naloxone antagonism towards CNS depressants other than the opiates with respect to narcosis and lethality. These observations extend our recent findings showing interaction toxicities between ethanol and the opiates [9], ethanol and MTQ [12], and ethanol and lithium [11]. The mechanism of naloxone-CNS depressants interaction is obscure. The fact that naloxone antagonized narcosis induced by the various CNS depressants as well as the opiates in a dose-related manner further raises the possibilities that naloxone may be acting on sites other than the opiate receptors. Of interest is the finding that the dose of naloxone required to elicit significant reversal of narcosis and especially in the case of ethanol in combination with other CNS depressants, exceeds by many folds the usual agonist-antagonist interactions between naloxone and the opiates [10]. These findings are consistent with the report that naloxone is a GABA antagonist at high doses [4]. On the other hand CNS depressants may interact with endogenous opoid peptides in producing toxicities and higher doses of naloxone may be required for the antagonism to such interactions.

Lithium, unlike the other CNS depressants, penetrates the brain relatively slowly and was given one hr prior to treatment with ethanol. This may explain our findings on the potentiation of ethanol induced sleep by lithium at higher doses (4 to 6 meq/kg) whereas Messiha [17] reported a prolongation of ethanol narcosis by lithium given 20 min before ethanol only in mice previously treated with lithium for 7 days. The attenuation of lithium induced ethanol narcosis by naloxone but not the calcium-induced ethanol narcosis [5] suggests an interesting possible mechanism of lithium-ethanol-naloxone interaction. Li^+ is known to alter electrolyte (Na^+/K^+) and water balance and to interact with ethanol [11].

The potential that naloxone may be of therapeutic value in the treatment of intoxication and overdoses of ethanol and other CNS depressants is worthy of further exploration.

ACKNOWLEDGMENT

This work was supported in part by a Contract ADM-45-74-220 and a Career Teachers in Substance Abuse Grant TOIDA 00218-02 (A.K.S. Ho) from National Institute on Drug Abuse. We wish to thank Dr. Monique Braude for her interest and Mrs. Veronika Burmeister for technical assistance and Mrs. Evelyn Hodgkins for typing the manuscript.

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