Counteraction of Narcotic Antagonist Analgesics by the Narcotic Antagonist Naloxone. (31595)

HAROLD BLUMBERG, HYMAN B. DAYTON, AND PETER S. WOLF
Endo Laboratories, Garden City, N. Y.

The work of Lasagna and Beecher(1) and others has demonstrated that some narcotic antagonists are themselves potent analgesics in man. Although this analgesic activity of narcotic antagonists was never detected in animals by the usual tests for potent analgesics, such as the mouse hot-plate or rat tail-flick tests, Taber, Greenhouse and Irwin (2) and Blumberg, Wolf and Dayton(3) found that some narcotic antagonists did show analgesic activity on the phenylquinone mouse writhing test of Siegmund, Cadmus and Lu(4). Furthermore, it was demonstrated that similar analgesic activity was shown on the phenylquinone writhing test in rats, and that the writhing test analgesic potencies appeared to correlate roughly with analgesic potencies found in man, but not with the narcotic antagonist potencies(3).

Naloxone, or N-allylnoroxymorphone, is a highly potent narcotic antagonist both in animals(5) and in man(6). Although it was by far the most potent narcotic antagonist of 6 studied, it showed essentially no analgesic activity on either the mouse or rat writhing test(3). Also, naloxone does not appear to show satisfactory dose-response results in analgesic studies in man thus far(6,7). Furthermore, unlike the currently used narcotic antagonists for counteracting overdosage, *e.g.*, nalorphine, naloxone does not seem to cause respiratory depression or psychotomimetic side-effects in man(7,8). Because of these apparent differences between the properties of naloxone and those of other narcotic antagonists, it occurred to us to determine if naloxone would not only antagonize narcotic analgesics but would also antagonize the analgesic effect of narcotic antagonist analgesics. This was indeed found to be the case, as will be described below.

Materials and methods. Six narcotic antagonists were used: cyclazocine* (2-cyclopropylmethyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan), and the corresponding 2-(3,3-dimethylallyl) derivative, pentazocine* (9), cyclorphan or (-)-3-hydroxy-N-cyclopropylmethylmorphinan hydrochloride† (10), levallorphan tartrate (Lorfan Tartrate, Hoffmann-La Roche), nalorphine (Nalline) hydrochloride (Merck), and naloxone (Narcan) hydrochloride (Endo). The cyclazocine and pentazocine bases were dissolved in minimal amounts of dilute HCl at pH 4-5. The other compounds were water-soluble powders. The phenylquinone was phenyl-p-benzoquinone (Eastman). The mice used were CF #1 male albinos, weighing 20-25 g; the rats were CFN male albinos, weighing 100-160 g.

The writhing tests in both mice and rats were carried out by the previously described method(3), except that the observation period was reduced from 60 to 30 minutes. For the 4 potent narcotic antagonist analgesics—pentazocine, nalorphine, cyclazocine, and cyclorphan—dosages were selected that produced analgesia in 87-93% of the animals by subcutaneous injection. Along with each analgesic naloxone was injected simultaneously on the opposite side of the animal. The naloxone was used at 4 dose levels with each analgesic, and there was a saline group as a control. Ten animals were used on each naloxone group or level, with 15-20 animals in each control group. For quantal treatment the ED_{50} and 95% confidence limits(11) were calculated in terms of the naloxone dose required to effect a 50% reduction of the analgesia, that is, to reduce the analgesia from about 87-93% to about 44-47%, respectively.

Levallorphan tartrate shows such weak analgesic activity(3) that an 87-93% analgesic dose subcutaneously in mice could be attained only in the toxic range of 80-100 mg/kg. Consequently, levallorphan tartrate

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TABLE I. Counteraction of Narcotic Antagonist Writhing Test Analgesia by Naloxone.

	Mouse—S.C. injection				Rat—S.C. injection			
ıg	Drug dosage, mg/kg	Naloxone dosage, mg/kg	Analgesia, %	Naloxone ED_{50} (95% C.L.), mg/kg	Drug dosage, mg/kg	Naloxone dosage, mg/kg	Analgesia, %	Naloxo (95% mg
ıne	10	0	87		2	0	90	
	10	.1	70		2	.05	70	
	10	.2	50	.20	2	.1	40	.0
	10	.4	10	(.13–.30)	2	.2	20	(.05
	10	.8	0		2	.4	0	
ıe HCl	1	0	87		1	0	93	
	1	.1	80		1	.1	80	
	1	.2	50	.21	1	.2	60	.2
	1	.4	10	(.14–.33)	1	.4	20	(.15
	1	.8	0		1	.8	0	
ne	.2	0	87		.05	0	90	
	.2	.1	70		.05	.05	80	
	.2	.2	50	.20	.05	.1	60	.1
	.2	.4	10	(.13–.30)	.05	.2	10	(.07
	.2	.8	0		.05	.4	0	
ın HCl	.1	0	93		.05	0	93	
	.1	.1	70		.05	.05	80	
	.1	.2	50	.22	.05	.1	60	.1
	.1	.4	30	(.12–.40)	.05	.2	20	(.08
	.1	.8	0		.05	.4	0	
han tartrate	60	0	67		6	0	80	
	60	.8	60		6	.4	50	
	60	1.6	40	1.7	6	.8	30	.5
	60	3.2	10	(1.1–2.7)	6	1.6	10	(.31

was studied at 60 mg/kg, which produced only 67% analgesia. In rats also the writhing test analgesic action is weak, with a plateau effect at about 80% analgesia at 6 mg/kg. Therefore, the ED_{50} values for levallorphan tartrate were calculated in terms of the naloxone dose required to effect a 50% reduction of analgesia, that is, from 67% to 34% in mice and from 80% to 40% in rats.

Results. In Table I are shown the writhing test analgesia results with pentazocine, nalorphine, cyclazocine, and cyclorphan. It may be seen that naloxone effectively counteracted the analgesic activity of all of these narcotic antagonist analgesics. In fact, at the highest naloxone dose levels the analgesic activity was completely blocked. The values for the ED_{50} indicate that the naloxone HCl dosage required to effect a 50% decrease in analgesia was in approximately the same range for all 4 of the compounds, *i.e.*, .092-.24 mg/kg, despite the fact that there was at least a 40-fold difference in the analgesic potencies of the drugs, as between pentazocine and cyclorphan. The ED_{50} for naloxone counteraction of analgesia was roughly the same in both mice and rats, although tending to be slightly higher in the mice.

Naloxone also antagonized the weak analgesic action of levallorphan, as shown in Table I. The ED_{50} values for the naloxone counteraction of levallorphan analgesia were 1.7 mg/kg in mice and 0.56 mg/kg in rats. The mouse value is about 8 times and the rat value is about 4 times the ED_{50} values found for counteraction of the potent narcotic antagonist analgesics. However, it may be noted that these dosages of naloxone had to counteract the comparatively high dosages of levallorphan tartrate, namely, 60 mg/kg in mice and 6 mg/kg in rats, that were required to achieve the limited 67-80% writhing test analgesia.

Discussion. The relative narcotic antagonist potencies of the drugs used, as determined previously by counteraction of oxymorphone narcosis in rats(3), were: levallorphan 3.4, pentazocine .05, nalorphine 1.0, cyclazocine 1.8, cyclorphan 2.0, and naloxone 19. It was rather surprising to find that although naloxone and the other drugs behave similarly in antagonizing narcotics, naloxone was able to counteract the writhing test analgesic activity of the other narcotic antagonists. This is consistent with the fact that naloxone itself does not exhibit significant analgesic activity on the writhing test(3). This is also additional evidence of the fact that the analgesic potency of narcotic antagonists does not correlate with narcotic antagonist potency. Thus, naloxone appears to have the capability of displacing both narcotics and narcotic antagonists at receptor sites. It should be of interest to determine whether or not naloxone could counteract the undesirable clinical side-effects of nalorphine and other narcotic antagonist analgesics, such as respiratory depression and psychotomimetic reactions, when given to man.

It was mentioned that the ED_{50} of naloxone was approximately the same for counteraction of the writhing test analgesia of pentazocine, nalorphine, cyclazocine, and cyclorphan, despite the 40-fold or greater difference in the analgesic potencies of the drugs, such as between pentazocine and cyclorphan. In this connection it may be noted that Grumbach and Chernov(12) reported a similar type of finding, and evidence of a competitive type of inhibition, in the counteraction of various narcotics by nalorphine, *i.e.*, that the same dosage of nalorphine antagonized the analgesic dosages of various narcotics of widely varying potencies when tested by the rat tail-flick method. Likewise, a constant dose of levallorphan antagonized all of the narcotics. Other workers have found that the antagonist dose of naloxone also was approximately constant in counteracting different narcotics of varying potencies when tested against narcotic depression in rats and respiratory depression in rabbits(13) and against narcotic-induced respiratory depression in man(14).

Summary. When the potent narcotic antagonist, naloxone, was injected subcutaneously into mice and rats simultaneously with narcotic antagonist analgesics, naloxone counteracted the writhing test analgesic activity of the antagonists. Naloxone completely antagonized the potent analgesic activity of pentazocine, nalorphine, cyclazocine, and cyclorphan, and also antagonized the weak analgesic activity of levallorphan.

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Demonstration of Rubella Complement-Fixing Antigens of Two Distinct Particle Sizes by Gel Filtration on Sephadex G-200.* (31596)

NATHALIE J. SCHMIDT, EDWIN H. LENNETTE, AND PINKIE S. GEE
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

Previous studies on rubella complement-fixing (CF) antigens derived from the fluid phases of infected RK-13 rabbit kidney cells (1) and the GMK-AH 1 line of green monkey kidney cells(2) have shown virtually all of the CF activity to be "soluble" in nature, *i.e.*, separable from the infectious viral particle by high-speed centrifugation(1) or by filtration(2).

Recently we have found(3) that high-titered rubella CF antigens can be prepared from the fluid phase of infected BHK-21 hamster kidney cultures, and using such higher-titered preparations it has been possible to demonstrate, by gel filtration on Sephadex G-200, the occurrence of two distinct CF antigens. One of the antigens is associated with the infectious viral particle and is sedimentable by centrifugation at 80,000 $\times$ *g* for 3 hours, and the other is smaller than the infectious particle and is not sedimented under these conditions of centrifugation. This report describes the separation of the two antigens.

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Materials and methods. Rubella virus preparations. Rubella virus (RV strain†) was propagated in cultures of the BHK-21 line of hamster kidney cells as recently described(3). Fluids from infected cultures were clarified by centrifugation at 1500 rpm for 15 minutes and then concentrated 100-fold by dialysis against polyethylene glycol (Carbowax 20 M‡). For certain experiments the fluid concentrates were treated with either(3) prior to Sephadex gel filtration.

Complement fixation tests. Materials were assayed for rubella CF antigen activity in block titrations against known positive human sera using our standard procedure adapted to use in the microtiter system(4).

Infectivity titrations. Infectivity titrations were performed in tube cultures of the BS-C-1 line of grivet monkey kidney cells. After 7 days incubation at 36°C inoculated cultures were challenged with 100 TCD_{50} of echovirus type 11, and results were read 2 to 3 days later. The presence of rubella virus in the

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