receptor-destroying-enzyme of *V. cholerae*. This is consonant with our observation(3) that the glycoprotein obtained by the previously described procedure from red cells pre-treated with influenza viruses is devoid of hemagglutination inhibitory activity. The similarity in chemical composition and in sensitivity to neuraminidase between the stromal hemagglutination inhibitor and the M-N blood group mucoids has led us to suspect that these may be the same substance. However, Klenk and Uhlenbruck(5,7,8) have suggested that they are different because M-N blood group active mucoids can be isolated from the supernatant solutions of erythrocytes treated with proteolytic enzymes such as trypsin, papain, ficin, or bromelin at which time the erythrocytes were still agglutinable by influenza viruses. The soluble mucoids were not inhibitors of viral hemagglutination. The latter observation can be explained by the studies of Gottschalk(9) as well as our own observations, which indicate that a certain minimum moleculer weight is essential for a glycopeptide to function as a viral hemagglutination inhibitor irrespective of the structural aspects of the oligosaccharide moiety with which the viruses would interact. Their observation that erythrocytes can be agglutinated even after proteolytic removal of M-N specific mucoids may be due to incomplete removal of sialic acid-containing substances. Mäkelä *et al*(10) showed that only about half of the sialic acid is lost from red cells by trypsin. The remainder of the sialo-mucoids may continue to function as sites for viral attachment.

We are now preparing stromal hemagglutination inhibitory glycoprotein from pure M or pure N human red cells to look for differences between them. No obvious differences have been found in carbohydrate composition.

Summary. The data reported indicate that a stromal glycoprotein, homogeneous by several criteria, is an active inhibitor of viral hemagglutination and has M-N blood group specificity. Both properties decrease in activity when the protein is removed from solution by mixing with influenza virus and centrifuging the mixture or when it is reacted with anti-M-N serum. It has always been observed that the disappearance of the one property of the protein is accompanied by the disappearance of the other. These results are interpreted as an indication that a single protein possesses both of these properties.

1. Kathan, R. H., Johnson, C. A., Winzler, R. J., *Acta Soc. Med. Upsaliensis,* 1959, v64, 290.

2. Kathan, R. H., Winzler, R. J., Johnson, C. A., *J. Exp. Med.,* 1961, v113, 37.

3. Kathan, R. H., Winzler, R. J., *J. Biol. Chem.,* 1963, v238, 21.

4. Romanowska, E., *Naturwiss.,* 1960, v47, 66.

5. Klenk, E., Uhlenbruck, G., *Z. physiol. Chem.,* 1960, v319, 151.

6. Springer, G. F., Ansell, N. J., *Proc. Nat. Acad. Sci.,* 1958, v44, 182.

7. Uhlenbruck, G., *Z. Bakt., Parasit. Infekt. Krankh. u. Hyg. Ref.* 1960-61, v177, 197.

8. ———, *Nature,* 1961, v190, 181.

9. Gottschalk, A., in *The Chemistry and Biology of Sialic Acids and Related Substances,* Cambridge Univ. Press, London, 1960.

10. Mäkelä, O., Miettinen, T., Pesola, R., *Vox Sang.,* 1960, v5, 492.

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Antagonism of Morphine and of Reserpine by Levorotatory and Dextrorotatory Isomers of 2,2-Diphenyl-4-(2-Piperidyl)-1,3-Dioxolane HCl. (28595)

JOHN HIDALGO AND CHARLES R. THOMPSON
Research Division, Cutter Laboratories, Berkeley, Calif.

The compound, 2,2-diphenyl-4-(2-piperidyl)-1,3-dioxolane HCl, has 2 asymmetric carbon atoms and is composed of 2 racemates of different melting points. The lower melting racemate, dioxadrol*, was separated into the *d* isomer, dexoxadrol*, and the *l* isomer, levoxadrol*(1).

* Generic names.

The *d* and *l* isomers were found to have significantly different pharmacologic properties(2). One of the differences between the two optical isomers is that the *d* isomer, but not the *l* isomer, exhibited the unusual property of shortening the response time of mice subjected to a thermal stimulus. This was characterized as "hyperalgesia" or "hyperacuity." The two optical isomers were, therefore, studied for their effects on morphine analgesia and reserpine antagonism. Reserpine antagonism was investigated because it has been shown(3) that reserpine, like morphine, prolongs the reaction time of mice subjected to a thermal stimulus.

Methods. 1. Determination of "hyperalgesic" effect and morphine antagonsim. Two methods were employed. a. The reaction times of mice were determined by the hot plate method(4) before and after drug administration. Levoxadrol and dexoxadrol were administered in single oral doses alone and in combination with morphine sulfate subcutaneously.

The effects of repeated administration of dexoxadrol on the reaction times of mice were also determined. The reaction times of 2 groups of 10 mice each were determined on day 1 at 0800. Dexoxadrol, 50 mg/kg orally, was then administered to one group and saline to the other group. Thirty minutes later, the reaction times were redetermined and the mice which had been fasted overnight were returned to their cage and allowed access to food. At 1600, they were administered a second identical dose of dexoxadrol or saline. Dosage was repeated on days 2 and 3. After the second dose on day 3, food was withdrawn. On day 4, both groups were dosed in the A.M. only and tested 30 minutes later. b. The effect of levoxadrol and dexoxadrol on the number of writhes induced by phenylquinone in mice(5) was determined alone and in combination with the subcutaneous dose of morphine sulfate that decreased the number of writhes by 50%.

2. Reserpine antagonism. Two methods were employed. a. The effects of levoxadrol and dexoxadrol on reserpine potentiated sleep time induced by hexobarbital was determined in mice administered 5 mg/kg of reserpine (Serpasil, Ciba) intraperitoneally one hour before hexobarbital sodium, 100 mg/kg intraperitoneally. Levoxadrol and dexoxadrol were administered orally as soon as the mice lost the righting reflex. Other groups of mice were administered hexobarbital alone, hexobarbital and levoxadrol or dexoxadrol, and reserpine and hexobarbital. Control groups were run simultaneously with each dose response experiment. The normal variability of the sleep time was reduced to a minimum by employing male Swiss-Webster mice, weighing 14-18 g, from the same storage cages and fasted for the same period. b. The effect of levoxadrol, dexoxadrol and amphetamine on the reaction time of reserpine treated mice to a thermal stimulus was determined. Reserpine was administered intraperitoneally in doses of 1 mg/kg just after control reaction times were obtained. Thirty minutes later, levoxadrol, dexoxadrol, or amphetamine was administered orally and reaction times redetermined 1, 2, 3, and 6 hours after reserpine. One group of mice administered only reserpine served as a control.

3. Statistical methods. Significance of values was determined by the Student "T" test(6). All p values $< .05$ were considered significant. ED_{50}'s and standard error were estimated graphically(7).

Results. 1. "Hyperalgesic" and morphine antagonism studies. a. Thermal stimulus. The effects of dexoxadrol and levoxadrol on the reaction time of mice (exposed to a theral stimulus on the hot plate) are shown in Table I. Dexoxadrol alone caused a highly significant decrease in reaction time and in doses of 50 mg/kg completely prevented the prolongation in reaction time induced by morphine, 5 mg/kg subcutaneously. Levoxadrol had no effect in both procedures.

No tachyphylaxis to the effects of dexoxadrol could be demonstrated by the hot plate method. The reaction times 30 minutes after dexoxadrol were always significantly shorter and varied little from day to day. The control group of mice injected with saline showed a mean difference of 0.5 second or less (range 6.3-6.8). The only finding of questionable significance was the slight daily increase in reaction times of the mice adminis-

TABLE I. Hyperalgesic Effect and Morphine Antagonism of Dexoxadrol and Levoxadrol in Mice Exposed to a Thermal Stimulus.

Treatment	Oral dose, mg/kg	No. mice	Mean response time: Control	Mean response time: 30 min after dosage	% change from control	t	p
Dexoxadrol	25	10	6.4	4.5	− 30	2.6	<.05
"	50	10	6.2	3.2	− 48	5.9	<.01
"	100	10	6.3	3.1	− 51	7.0	<.01
Morphine	5(SQ)	10	6.7	12.2	+ 82	3.4	<.05
"	5	10	7.5	15.5	+107	5.0	<.01
Morphine* + Dexoxadrol	5(SQ) 50	10	8.5	8.6	0	—	—
Levoxadrol	75	10	5.8	6.0	+ 3	—	—
"	150	10	6.4	6.7	+ 5	—	—
Morphine* + Levoxadrol	5(SQ) 50	10	6.9	13.5	+ 95	6.0	<.01

* Morphine and levoxadrol or dexoxadrol were administered in quick succession.

tered dexoxadrol. The mean starting control value on day 1 for this group of mice was 6.6 seconds; on day 4, it was 7.7 seconds and the increase was in equal increments of about 0.4 second/day.

b. Writhing test. Dexoxadrol increased the number of writhes induced by intraperitoneal phenylquinone in mice (Table II). Levoxadrol had no effect.

The subcutaneous ED_{50} (dose reducing the number of writhes by 50%) of morphine against phenylquinone induced writhing was found to be 0.75 ± .13 mg/kg. The ED_{50} of morphine in the presence of dexoxadrol, 12.5 mg/kg orally, was 0.53 ± 0.09 mg/kg.

Even though dexoxadrol antagonized the analgesic activity of morphine in the hot plate method, it did not have any significant effect on morphine analgesia in the writhing test.

2. Anti-reserpine studies. a. Effect on reserpine potentiated hexobarbital hypnosis.

Dexoxadrol, 6.25 and 12.5 mg/kg orally, and levoxadrol, 12.5 mg/kg orally, antagonized the reserpine induced prolongation of hexobarbital hypnosis, Table III. A U-shaped curve was obtained because oral doses of 50 mk/kg of either compound significantly prolonged the hypnosis induced by hexobarbital alone, thus masking the reserpine antagonism. The doses of levoxadrol and dexoxadrol which antagonize the reserpine induced potentiation of hexobarbital had no effect on the duration of hypnosis induced by hexobarbital alone, indicating that the antagonism was to the effect of reserpine and not to that of the barbiturate. This is in contrast to amphetamine which in oral doses of 5 mg/kg antagonized both the reserpine induced potentiation of hexobarbital hypnosis and the duration of hexobarbital hypnosis. b. Effect on reserpine induced changes in reaction time to a thermal stimulus (Fig. 1). Reserpine, 1 mg/kg intraperitoneally, significantly shortened the reaction time of the mice to a thermal stimulus ($p < 0.05$) 30 and 60 minutes after dosage. Two hours after dosage, reaction times were significantly prolonged, began to plateau at about 3 hours and were still prolonged 6 hours later. Dexoxadrol, 12.5 and 25 mg/kg orally, administered 30 minutes after reserpine and tested 30 minutes after administration, decreased significantly the reaction time of the mice more than reserpine alone. Levoxadrol, 50 mg/kg, did not alter the decreased reaction time induced by reserpine. Two and 3 hours after administration, both levoxadrol and dexoxadrol significantly antagonized the

TABLE II. Effect of Dexoxadrol on Number of Writhes Induced by Phenylquinone in Mice.

Dexoxadrol, mg/kg orally	No. mice	Mean No. of writhes	t	p
6.25	10	188		
Controls	10	169	1.5	>.05
12.5	25	235		
Controls	25	159	6	<.01
25	25	231		
Controls	25	173	2	0.05

TABLE III. Effect of Oral Dexoxadrol, Levoxadrol and Amphetamine on Duration of Hexobarbital Hypnosis and on Reserpine-Induced Prolongation of Hexobarbital Hypnosis in Mice.

Treatment	Dexoxadrol, mg/kg	No. mice	Mean sleep time (range), min	% of controls	p
Reserpine + hexobarbital*	—	10	46 (27-65)	100	—
Idem	6.25	10	28 (19-40)	61	<.05
"	12.5	10	25 (15-34)	50	<.05
Hexobarbital†	50	10	56 (40-70)	278	<.05 *vs* hexobarb. controls
	Levoxadrol, mg/kg				
Reserpine + hexobarbital	—	10	41 (26-60)	100	—
Idem	12.5	10	25 (15-33)	60	<.05 *vs* reserpine + hexobarb.
Hexobarbital	50	10	56 (40-65)	255	<.05 *vs* hexobarb. controls
	Amphetamine, mg/kg				
Reserpine + hexobarbital	—	10	37.5 (17-52)	100	—
Idem	5	10	20.6 (19-30)	55	<.05
Hexobarbital	5	10	13.2 (7-20)	70	<.05

* Reserpine, 5 mg/kg intraperitoneally, was administered 1 hr before hexobarbital, 100 mg/kg intraperitoneally. Levoxadrol and dexoxadrol were administered orally immediately after hexobarbital.

† Hexobarbital control groups of 10 mice were run with each dose response curve for a total of 6 groups. Mean sleep times varied from 20 to 28 min; range was 14 to 35 min. Percentage calculations were based on the respective controls for each compound.

prolonged reaction times induced by reserpine.

Amphetamine alone, 10 mg/kg orally, had no significant effect on the reaction time of the mice. Oral doses of 5 and 10 mg/kg administered 30 minutes after reserpine and tested 30 minutes after administration did not significantly modify the effect of reserpine nor decrease reaction time. Two and 3 hours after administration, amphetamine significantly prevented the increase in reaction time induced by reserpine.

Discussion and summary. The *d* (dexoxadrol and the *l* (levoxadrol) isomers of the lower melting racemic modification of 2,2-diphenyl-4-(2-piperidyl)-1,3-dioxolane HCl have surprisingly different pharmacologic properties.

Dexoxadrol, but not levoxadrol, produces "hyperalgesia" or "hyperacuity" as evidenced by shortening of the reaction time of mice to a thermal stimulus and increasing the number of writhes following the intraperitoneal administration of phenylquinone. Tachyphylaxis does not occur to the "hyperalgesia" produced by dexoxadrol.

Dexoxadrol, but not levoxadrol, antagonizes the analgesic activity of morphine in

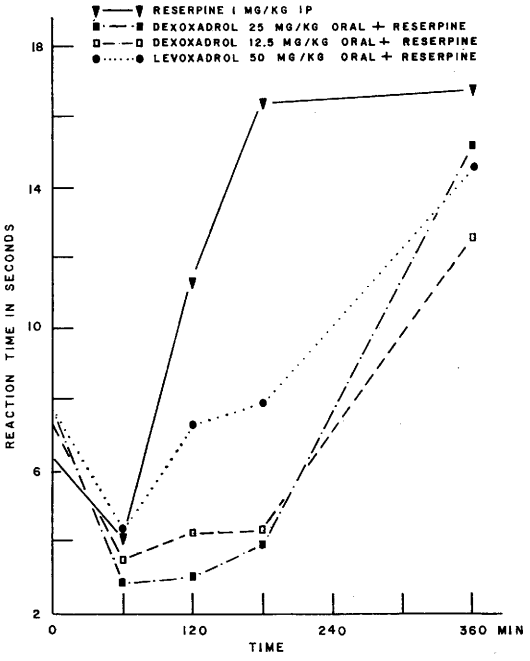

FIG. 1. Effect of levoxadrol and dexoxadrol on reaction time to a thermal stimulus of reserpine-treated mice. Levoxadrol and dexoxadrol were administered 30 min after reserpine. Control mice administered saline at 0 hr and tested at same time intervals varied from 7.6 to 8.2 sec during 6 hr test period.

mice subjected to a thermal stimulus. Both are ineffective in those administered phenylquinone. This difference is possibly related to differences between visceral and somatic pain.

Both isomers antagonize the effects of reserpine as measured by the reversal of the reserpine induced prolongation of hexobarbital hypnosis and the reserpine induced prolongation of the reaction time of mice exposed to a thermal stimulus.

This unusual "hyperalgesia" or "hyperacuity" property of dexoxadrol suggests that it may be an agent which will increase awareness.

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1. Hardie, W. R., Halverstadt, I. F., Allen, R. E., Abst. of Papers 141st Meeting, Am. Chem. Soc., March, 1962, p. 3N.

2. Hidalgo, J., Gallin, J., Williams, B., Thompson, C. R., *Pharmacologist,* 1961, v3, 69, Fall.

3. Garcia Leme, J., Rocha e Silva, M., *J. Pharm. Pharmacol.,* 1961, v13, 734.

4. Eddy, N. B., Leimbach, D. J., *J. Pharmacol.,* 1953, v107, 385.

5. Hendershot, L. C., Forsaith, J., *ibid.,* 1959, v125, 237.

6. Snedecor, G. W., *Statistical Methods,* 5th Ed., Iowa State Coll. Press, Ames, 1956.

7. Miller, L. C., Tainter, M. L., PROC. SOC. EXP. BIOL. MED., 1944, v57, 216.

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Effect of Environmental Temperature on Tetanus Intoxication in Mice.* (28596)

M. F. SHEFF, M. PERRY AND S. I. ZACKS
(Introduced by W. E. Ehrich)
Department of Neurology and Ayer Clinical Laboratory, Pennsylvania Hospital, Philadelphia

While studying acute tetanus poisoning in mice we observed that body temperature began to fall at an early stage in intoxication. Attempts to maintain body temperature by keeping the poisoned animals at an ambient temperature of 35°C resulted in a considerable decrease in survival time. More complete studies were therefore undertaken to determine the effect of environmental temperature and dosage on survival time.

Method. The toxin used was a sterile filtrate from cultures of *Cl. tetani* prepared by Wyeth Labs., Radnor, Pa. The effective toxin content, estimated by nitrogen determinations and electrophoretic analysis, was 180 μg/ml.

The animals used were white female Swiss mice weighing from 23 to 30 g. They had been kept in an animal house at temperatures of 15° to 22°C, and were thus acclimated to one of the experimental temperatures (21°C).

Groups of 21 mice were injected via the intraperitoneal route with 1 of 6 graded doses (0.01 to 0.8 ml of the toxin filtrate, equivalent to 1.8 to 144 μg of toxin). These groups were then divided into 3 sub-groups of 7 animals each. Each sub-group was then kept at either 5°C, 21°C or 35°C and closely observed until the animals died. Survival times of the individual animals were recorded in minutes. For the 72 μg dose, 2 sets of 21 animals were used. Groups of control animals were also kept at these 3 temperatures. None of these died during the experiments.

To quantitate the original observation on the fall of body temperature noticed in the animals kept at room temperature (21°C) after poisoning, the internal and surface temperatures of control and poisoned animals were measured with thermistor probes. Yellow Springs Instruments probe #402 was used for measurements of rectal temperature and YSI probe #409 was used for measuring the surface temperature. This latter probe was taped to the unshaven abdomen of the animal. The probes were kept in place by a small harness

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