Independence of Sedative and Analgetic Antagonizing Effects of Two Reserpine Esters.*† (24947)

L. B. WITKIN, M. MAGGIO AND W. E. BARRETT (Introduced by R. Gaunt)
Research Dept., CIBA Pharmaceutical Products, Summit, N. J.

Schneider(1), Sigg and Schneider(2) and Gross (personal communication) demontrated that reserpine has the property of antagonizing the analgetic effects of morphine. The fact that some stimulants including amphetamine(3) and certain other amines(2) can potentiate morphine analgesia suggests that sedative or stimulant properties of a compound may determine in part whether that compound will antagonize or potentiate analgetics. Recently Barrett and Plummer(4) described 2 reserpine esters which may be useful tools in elucidating this point since one of these (SU-5171) possesses a degree of sedation comparable to reserpine with minimal hypotensive effects while the other (syrosingopine) produces only about 1/20th the sedative effects of reserpine but yet retains hypotensive activity.

Materials and methods. Prolongation of reaction time (tail flick) to a constant intensity heat stimulus, according to the method of D'Amour-Smith(5) was used as criteria of analgesia. Mice (male CF-1, 19-21 g) were housed individually in stainless steel containers with only their tails protruding. Racks were used to hold 10 cages at a time in a line and were provided with shallow grooves into which the animal's tail could be laid. This rack was then placed on a flat surface which allowed it to be moved back and forth for alignment with the focal point of heat source as mounted above. This source consists of a DC powered end view 100 watt concentrated-arc bulb (Zenith) mounted vertically in an appropriate housing. Light from this source was then focused to a beam of approximately 2 mm diameter and adjusted to elicit a control reaction time of about 4 sec. A foot switch operated both a shutter blade attached to rotary solenoid (G. H. Leland, Inc., Dayton, Ohio) and a precision timer with a complete sweep of 10 sec. (Standard Electric Timer Co., Springfield, Mass.). Because of the high intensity of the emitted light, a double polaroid shield was placed between operator and light path and was rotated to the dark position when the light was on. Although this dampened the light intensity considerably, it still allowed the operator a full view of animal's reaction. The AD_{50} of morphine sulfate either alone or in combination with other agents was defined as that dose of morphine producing significant analgesia in half of the animals and was determined as follows: Control reaction time (duration of application of heat necessary to elicit a tail flick) was measured in duplicate in each of 10 mice before and every 15 minutes for 2 hours after morphine administration. Significant analgesia was said to occur in any animal when its duplicate reaction time exceeded the control mean of the 10 animals plus 2.3 (S.D.) at any time during the 2 hour period. AD_{50} values were calculated according to the graphic method of Miller and Tainter(6) using a minimum of 10 animals at each of at least 3 doses falling between probits 3.75 and 6.25. All drugs were administered subcutaneously. Reserpine (lyophilized Serpasil phosphate, CIBA), 2.5 mg/kg, syrosingopine, the carbethoxy syringate ester of methyl reserpate, 2.5 mg/kg and SU-5171 (methyl-18-0(dimethylamino benzoyl) reserpate), 2.5 mg/kg were administered 2 hours before administration of morphine. Both syrosingopine and SU-5171 were dissolved in N, N′-dimethylacetamide to give an injection volume of .04 ml/20 g of body weight. Methylphenidate was dissolved in water and administered in doses of 5 mg/kg at the same time as the morphine. Control studies were performed in which reserpine, syrosingopine, SU-5171, methylphenidate and N,N′-dimethylacetamide were given

* A portion of this work presented before Fed. Am. Soc. Exp. Biol., Apr., 1959, Atlantic City, N. J.

† The authors are grateful to H. Sylwestrowicz for statistical consultation, and to Mr. R. Wolf for suggesting heat source used.

TABLE I. Effects of Reserpine, Two Reserpine Esters, Methylphenidate and Solvent on Anal-

Compound	Control ± S.E., 95% confidence limits	Reaction time in sec. Post-inj. 15′	45′	90′	120′
Reserpine	4.11 ± .08				
	3.92–4.30	3.44‡	3.61‡	3.49‡	3.52‡
Syrosingopine	3.99 ± .08				
	3.82–4.16	3.95	3.87	4.44	4.43
SU-5171	4.01 ± .09				
	3.81–4.21	4.20	4.67	4.07	4.14
Methylphenidate	3.99 ± .06				
	3.85–4.13	4.72	4.31	3.83	4.18
ADMA†	4.06 ± .12				
	3.80–4.32	4.13	3.53	3.69	3.85

* Reserpine (lyophilized Serpasil phosphate, CIBA), 2.5 mg/kg (dissolved in water), syrosingopine, 2.5 mg/kg (dissolved in ADMA), and Su-5171, 2.5 mg/kg (dissolved in ADMA) as well as solvent control given in volumes of .04 ml/20 g body wt. All of above compounds administered 2 hr before post-inj. analgetic determinations. Methylphenidate dissolved in water and administered in doses of 5 mg/kg immediately before analgetic determination.

† N, N′-dimethylacetamide.

‡ Indicates significant difference from control ($p < .05$).

alone in doses and time sequence listed above to determine if they alone produced significant alteration of reaction time.

Results. Control studies (Table I) reveal that reserpine alone produced a significant shortening of the reaction time ($p < .05$) for the 2 hour test period when the drug was administered 2 hours before testing. Syrosingopine, SU-5171, methylphenidate and N, N′-dimethylacetamide caused no alteration in reaction time when given alone.

Methylphenidate in doses of 5 mg/kg produced a very significant potentiation of the analgetic effects of morphine as indicated by the fact that when combined with methylphenidate, the dose morphine could be reduced by almost a factor of 3 to achieve the same analgetic activity as morphine alone.

TABLE II. Interaction of Methylphenidate, Reserpine and 2 Reserpine Esters on Morphine Analgesia.

Compound	Dose, mg/kg	AD_{50} ± S.E.	95% confidence limits
Morphine sulfate		2.3 ± .26	1.7 - 2.8
Idem + methylphenidate	5.0	.87 ± .09	.69–1.05
Morphine sulfate 2 hr after:			
Reserpine	2.5	5.6 ± .30	5.0 –6.1
SU-5171	"	5.7 ± .13	5.5 –6.0
Syrosingopine	"	4.7 ± .67	3.4 –6.0
Morphine sulfate + methylphenidate 2 hr after:	5.0		
Reserpine	2.5	2.6 ± .31	2.0 –3.2
SU-5171	"	2.4 ± .44	1.5 –3.2
Syrosingopine	"	2.9 ± .35	2.2 –3.5

Reserpine, SU-5171 and syrosingopine administered in doses of 2.5 mg/kg 2 hours before testing all antagonized morphine analgesia to a very significant degree ($p < .05$). Prior administration of these compounds required that 2 to 2.5 times as much morphine was needed to effect the same degree of analgesia as compared to morphine alone. These compounds were equally potent in producing this antagonism since the AD_{50}'s for morphine in animals pretreated with these 3 compounds were not significantly different from one another ($p > .05$).

AD_{50} determinations following administration of methylphenidate (5 mg/kg) immediately before analgesic testing with morphine 2 hours after administration of reserpine, SU-5171 and syrosingopine (2.5 mg/kg) were not different ($p > .05$) from the AD_{50} for morphine alone.

Discussion. Significant reduction in reaction time caused by reserpine, about 18%, cannot explain the antagonism that this compound exerts against morphine analgesia which amounts to 250%. In addition, neither of the 2 esters caused any alteration in reaction time of themselves, yet both antagonized

morphine with the same order of potency as did reserpine. This shortening of reaction time may well be another manifestation of 'stimulant' effects of reserpine, reflected as a lowering of electroshock threshold(7) and prolongation of rhinencephalic seizures(8) and would suggest that neither SU-5171 nor syrosingopine have these properties to the degree that reserpine does.

That syrosingopine, with a potency of 1/20 that of reserpine in causing sedation, produces the same degree of antagonism to morphine analgesia, rules out the possibility that the sedative effect *per se* is a causal factor in producing this phenomenon.

Summary. The sedative properties of reserpine or esters or reserpine seems to bear no relationship to ability of these compounds to antagonize morphine analgesia since one such agent (syrosingopine) possesses 1/20 the sedative properties of reserpine yet is equally potent in its ability to antagonize morphine analgesia.

1. Schneider, J. A., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 614.

2. Sigg, E. B., Caprio, G., Schneider, J. A., *ibid.*, 1958, v97, 97.

3. Goetzl, F. R., Burrill, D. Y., Ivy, A. C., *ibid.*, 1944, v55, 248.

4. Plumer, A. J., Barrett, W. E., Maxwell, R. A., Finocchio, D., Lucas, R. A., Earl, A. E., *Arch. Int. de Pharmacodyn, et de Therap.*, 1959, v119, 245.

5. D'Amour, F. E., Smith, D. L., *J. Pharmacol. and Exp. Therap.*, 1941, v72, 74.

6. Miller, L. C., Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1954, v56, 261.

7. Chen, G., Bohner, B., Ensor, C. R., *ibid.*, 1954, v87, 602.

8. Sigg, E. B., Schneider, J. A., *EEG Clin. Neurophysiol.*, 1957, v9, 419.

Received April 9, 1959. P.S.E.B.M., 1959, v101.

Effect of Molybdenum on Fluoride Retention in the Rat.* (24948)

GEORGE K. STOOKEY AND JOSEPH C. MUHLER
Indiana University, Dept. of Chemistry, Bloomington

Adler(1) has shown that 0.1 μg Mo/ml (as ammonium molybdenate) reduces the incidence of dental caries in the rat. His studies were prompted by the observation that 2 communities in Hungary had low dental caries rates and low fluoride levels, thus suggesting that low caries incidence was not the result of fluoride in water. Spectrographic analysis of these waters showed that molybdenum was present in excess of that found in similar communities in which the incidence of dental caries was higher. Since these data have considerable practical importance it was of interest to learn more about the relationship between skeletal retention of fluoride in presence of molybdenum.

Methods. 24 weanling Sprague-Dawley strain rats, bred and raised in our laboratory, were divided into 4 groups according to initial body weight. Group 1 received fluoride-low drinking water (F = 0.01 μg/ml) *ad lib.* Group 2 received 50 μg/ml Mo (as $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) in drinking water. Group 3 received 50 μg/ml F (as NaF) in drinking water, and group 4 received Mo and F at same concentration as animals in Groups 2 and 3. All animals received the same fluoride low stock corn diet (F = 0.5 μg/g). Animals were housed individually in raised screen cages in temperature controlled room. After 30 days the animals were sacrified, the femur removed from each animal and the carcass prepared for fluoride analysis by methods previously described(2).

Results. The data obtained are in Table I. During the 30 day experimental period animals receiving fluoride-low drinking water and 50 ppm Mo gained on the average of 87 g. Those which received 50 ppm F gained less weight (70 g), but those receiving both Mo and F gained (98 g). No animals died during experimental period.

* This study supported in part by grant from U.S.P.H.S.