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Additional Evidence for the Participation of 5-Hydroxytryptamine in The Intestinal Response to Morphine.* (32055)

T. F. BURKS† AND J. P. LONG

Department of Pharmacology, College of Medicine, University of Iowa, Iowa City

The means by which morphine produces its profound effects on the mammalian gastrointestinal tract has been the subject of a great deal of scientific controversy and even more speculation. Since the early work of Slaughter and Gross(1) on the dog intestine it has generally been thought that cholinergic mechanisms are involved in the pathway mediating the actions of morphine on the small intestine. This concept was supported by the report of Vaughan Williams and Streeten(2) that atropine antagonized the intestinal actions of moderate doses of morphine. Plant and Miller(3), however, found that the effect of morphine on the dog intestine was not modified by atropine and considered that morphine could exert its actions directly on the intestinal smooth muscle. More recently Margolin(4) and Margolin and Plekss(5) have obtained evidence to indicate that in certain species, inhibition of the intestinal propulsive activity is accomplished indirectly *via* the central nervous system.

Burks and Long(6) have presented evidence that some of the effects of morphine on tone of the dog small intestine may be due to local release of 5-hydroxytryptamine (5-HT) by morphine. Since there is evidence which indicates that the intestinal actions of 5-HT may be antagonized by atropine and enhanced by anticholinesterase agents(7), such a hypothesis would be consistent with the finding that eserine may potentiate the action of morphine on the dog intestine(1) and that atropine may antagonize morphine effects (2). Treatment with reserpine has been shown(6) to diminish the spasmogenic effect of morphine on the dog small intestine, but no study of the influence of prior treatment with reserpine on the actions of morphine on propulsive activity of the intestine has been made. The purpose of this investigation therefore was to compare the effects of atropine and physostigmine on morphine and 5-HT actions on propulsive activity of the small intestine of the mouse, to evaluate the effects of reserpine treatment on the response to morphine and to determine whether a 5-HT antagonist, methysergide (UML), would alter intestinal responses to morphine.

Materials and methods. A total of 2470 young adult male Swiss Webster white mice were randomly divided into groups of 10 animals each and the mean values for each group were employed in the analysis of the data. All chemical agents and saline were administered intraperitoneally to the animals in volumes of 0.01 ml/g and their effects on gastrointestinal propulsion assayed by the method of Macht and Barba-Gose(8). Powdered charcoal was suspended in glycerine and administered p.o. to the animals in volumes of 1 ml. At the time of sacrifice by cervical fracture, the entire small intestine from the stomach to the cecum was removed and the distance traversed in cm by the charcoal

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† USPHS Fellow.

meal was divided by the total length of the small intestine. This value was expressed as percent passage of the meal. The animals were sacrificed 20 minutes after they received the charcoal meal. The morphine (in doses of 1.2-5.0 mg/kg), physostigmine (0.1 mg/kg), 5-HT (10 mg/kg) and saline were administered 10 min prior to charcoal; UML (10 mg/kg) and atropine (0.2 mg/kg) were given 30 min before the charcoal; and reserpine (5.0 mg/kg) was given 16 hours before the charcoal.

Drugs and chemicals employed were morphine sulfate, methysergide (UML),‡ reserpine, (Serpasil, Ciba), physostigmine sulfate, 5-hydroxytryptamine (5-HT, serotonin) creatinine sulfate and atropine methylbromide (AMB). All doses were calculated as the salts employed.

The data were statistically analyzed by analysis of variance(9), randomized complete block design or 2 × 2 factorial design with days on which experiments were performed representing the blocks. Values of P equal to or less than 0.05 were considered significant. Means were compared by use of Duncan's New Multiple Range Test(9) at the 5% special protection level.

Results. Morphine and UML. Analysis of variance of the data indicated that the treatments applied had an effect ($P<0.01$) and that the F ratio for the blocks was significant ($P<0.05$). In Table I it can be seen that administration of UML antagonized but did not completely block the morphine-induced delay of charcoal meal passage. The UML alone had no effect on passage of the meal.

Reserpine pretreatment. As can be seen in Table II, both the reserpine pretreatment and the administration of morphine affected passage of the meal, but these effects were independent of one another. The action of morphine on delay of intestinal passage of charcoal meal was not altered by prior treatment of the animals with reserpine. The mortality resulting from the reserpine administration (5.0 mg/kg) was about 2% of the animals treated.

‡ Kindly supplied by Dr. J. A. Harris, Sandoz Pharmaceuticals, Hanover, N. J.

TABLE I. Effects of UML and Morphine on Percent Passage of Charcoal Meal in Mice. (Each value = mean ± S.E. of 13 groups of 10 mice each.)

Saline	UML	Morphine	UML + morphine
50 ± 1.3	51 ± 2.0	34 ± 1.1	39 ± 1.5*

* Any two means not underscored by the same line differ significantly, protection level = 0.05.

TABLE II. Effects of Reserpine on Intestinal Responses to Morphine. (Each value = mean ± S.E. of 9 groups of 10 mice each.)

Percent passage of charcoal meal			
Saline treated		Reserpine treated	
Saline	Morphine	Saline	Morphine
47 ± 1.9	38 ± 1.7	56 ± 2.2	41 ± 1.3

Analysis of variance

Source of variance	df	SS	MS	F	P
(Treatments)	(3)	1710	—	—	—
Reserpine	1	342	342	14.1	<.01
Morphine	1	1284	1284	52.8	<.01
Interaction	1	84	84	3.5	N.S.
Blocks (days)	8	373	46.6	1.9	N.S.
Error	24	584	24.3	—	—
Total	35	2667	—	—	—

Comparison of means: 56 47 41 38*

* Any two means not underscored by the same line differ significantly, protection level = 0.05.

Treatment with physostigmine. Despite the prevalent impression that the gastrointestinal actions of morphine are enhanced or even potentiated by physostigmine, no evidence that this occurs in mice could be obtained in these experiments. The results obtained with 3 different doses of morphine and 3 different doses of 5-HT were presented in Table III. It can be seen that the delay in transit produced by 1.2 mg/kg morphine was enhanced (not statistically significant) by physostigmine in Experiment 3 (percent passage decreased from 47 ± 2.7% to 42 ± 1.4%), but also that these results were not duplicated in Experiments 4 and 5. The delay in passage due to 5-HT was significantly enhanced by physostigmine in Experiment 1 but results were variable in the remainder of the experiments. It cannot be concluded from these data that the delay in meal passage through the mouse small intestine produced by either morphine or 5-HT was enhanced by physostigmine.

TABLE III. Influence of Physostigmine on Intestinal Actions of Morphine and 5-HT.

Exp	Agent	Dose (per kg)	No. of groups (10 mice each)	Percent passage of charcoal meal (means ± S.E.) Physostigmine Absent	Physostigmine Present	P*
1	Saline	10 ml	6	48 ± 2.3	55 ± 1.2	N.S.
1	Morphine	5 mg	6	40 ± 1.7	41 ± 2.3	"
1	5-HT	10 mg	6	42 ± 2.6	39 ± 2.0	<0.05
2	Saline	10 ml	4	54 ± 2.6	54 ± 2.6	N.S.
2	Morphine	2.5 mg	4	40 ± 1.5	42 ± 2.3	"
2	5-HT	10 mg	4	46 ± 2.2	40 ± 3.2	"
3	Saline	10 ml	5	54 ± 3.1	51 ± 2.7	"
3	Morphine	1.2 mg	5	47 ± 2.7	42 ± 1.4	"
3	5-HT	10 mg	5	38 ± 1.1	40 ± 1.9	"
4	Saline	10 ml	12	50 ± 1.4	50 ± 1.3	"
4	Morphine	1.2 mg	12	43 ± 1.6	44 ± 1.5	"
4	5-HT	5 mg	12	48 ± 2.3	47 ± 1.4	"
5	Saline	10 ml	4	50 ± 2.1	50 ± 2.1	"
5	Morphine	1.2 ml	4	41 ± 1.3	44 ± 1.9	"
5	5-HT	2.5 mg	4	50 ± 2.1	48 ± 3.0	"

* Level of significance, analysis of variance, randomized complete block design.

TABLE IV. Effects of Atropine Methylbromide (AMB) on Intestinal Responses to Morphine and 5-HT. (Each value = mean ± S.E. of 11 groups of 10 mice each.)

Saline	AMB	Morphine	5-HT	AMB + morphine	AMB + 5-HT
50 ± 1.9	54 ± 1.8	45 ± 2.3	44 ± 1.4	38 ± 1.8	38 ± 1.4*

* Any two means not underscored by the same line differ significantly, protection level = 0.05.

Treatment with atropine methylbromide. The data presented in Table IV show that AMB in the dose employed (0.2 mg/kg) did not significantly alter the meal passage when administered alone, but the mean transit through the small intestine was slightly greater than that observed after saline. When administered with morphine or 5-HT, however, the AMB evidently enhanced the ability of these agents to delay meal passage. It is also interesting to note that the 10 mg/kg dose of 5-HT used in this experiment produced effects quite similar to those seen with 1.25 mg/kg morphine employed, despite the fact that the primary effect of 5-HT in similar experiments has been said to be on the pylorus(7). The two agents were equally enhancd by the AMB.

Discussion. The ability of UML to antagonize the delay in passage of a charcoal meal in the mouse small intestine produced by morphine lends support to the hypothesis that 5-HT is somehow involved in the mediation of the gastrointestinal actions of morphine. The effect of UML appeared to be a true pharmacological antagonism of morphine and not merely a physiological antagonism since the UML alone had little or no effect on passage of a meal. The failure of physostigmine to enhance the actions of morphine and 5-HT was surprizing since the ability of the former to potentiate these agents *in vitro* has often been reported(1,3) and since the dose employed should have been adequate to provide anticholinesterase actions(10,11). Similarly, the inability of reserpine treatment to alter the morphine effect on propulsion was unexpected since it has been shown(6) that reserpine treatment reduces the intestinal response of dogs to morphine. The dose of reserpine employed (5 mg/kg) would appear to be adequate to ensure fairly good depletion of 5-HT stores and has been found to produce maximal depletion about 90% of intestinal 5-HT(12). It should be noted, however, that the gastrointestinal tract is notoriously difficult to completely deplete of its stores of 5-HT.

The ability of atropine methylbromide (AMB) to enhance the actions of 5-HT and morphine was unexpected and could suggest the addition of its anticholinergic effects to

the effects of 5-HT and morphine in delaying meal passage. The quanternary form of atropine was deliberately chosen to eliminate or reduce the central effects that could have resulted from use of the tertiary amine. Since AMB did not antagonize the actions of 5-HT and morphine, this could be considered as support for the hypothesis that the effects of morphine on intestinal propulsion are centrally mediated, but more likely simply indicates that cholinergic, atropine-sensitive pathways are not necessary for the morphine-induced delay of intestinal propulsion in mice. The failure of physostigmine to potentiate the actions of 5-HT and morphine may be similarly interpreted. These data would agree with findings that morphine may in fact reduce acetylcholine liberation from the intestine(13).

The primary purpose of these investigations was to demonstrate similarities between the actions of morphine and 5-HT in order to further the hypothesis that 5-HT participates in the mediation of the responses of the small intestine to morphine. The 5-HT and morphine do behave in parallel manners and that a 5-HT antagonist, UML, can antagonize the intestinal actions of morphine has been demonstrated.

Summary. The delay of passage of a charcoal meal through the mouse small intestine by morphine was antagonized by methysergide (UML), a potent inhibitor of 5-hydroxytryptamine (5-HT). Treatment of mice 16 hours prior to use with 5.0 mg/kg reserpine failed to modify the intestinal response to morphine. The effects of atropine methylbromide (AMB) on morphine and 5-HT were similar; treatment with AMB enhanced the delay in intestinal transit produced by these agents. Treatment of mice with physostigmine failed to alter the intestinal effects of either 5-HT or morphine. The data are interpreted as adding support to the hypothesis that some of the actions of morphine on the mammalian small intestine may be mediated by 5-HT.

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