6. Hatefi, Y., Lester, R. L., *Biochim. et Biophys. Acta,* 1958, v27, 83.

7. Linnane, A. W., Ziegler, D. M., *ibid.,* 1958, v29, 630.

8. Kennedy, E. P., *J. Biol. Chem.,* 1953, v201, 399.

9. Zetterström, R., Ernster, L., *Nature,* 1956, v178, 1335.

10. Dianzani, M. U., Scuro, S., *Biochem. J.,* 1956, v62, 205.

11. Borst, P., Slater, E. C., *Biochim. et Biophys. Acta,* 1961, v48, 362.

12. Crane, R. K., Lipmann, F., *J. Biol. Chem.,* 1953, v201, 235.

13. Lardy, H. A., Johnson, D., McMurry, W. C., *Arch. Biochem. Biophys.,* 1958, v78, 587.

14. Huijing, F., Slater, E. C., *J. Biochem.,* 1961, v49, 493.

15. Cornatzer, W. E., Sarosi, G. A., Newland, J. R., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 463.

16. Marinetti, G. V., Erbland, J., Albrecht, M., Stotz, Elmer, *Biochim. et Biophys. Acta,* 1958, v30, 543.

17. Artom, C., *J. Biol. Chem.,* 1941, v139, 953.

18. Fiske, C. H., Subbarow, Y., *ibid.,* 1925, v66, 375.

19. Hogeboom, G. H., Schneider, W. C., Pallade, G. E., *ibid.,* 1948, v172, 619; Schneider, W. C., Hogeboom, G. H., *ibid.,* 1950, v183, 123.

20. Cornatzer, W. E., Sandstrom, W. A., Reiter, J. H., *Biochim. et Biophys. Acta,* 1962, v57, 568.

21. Hevesy, G., *Enzymologia,* 1938, v5, 138.

22. Zilversmit, D. B., Entenman, C., Fishler, M. C., *J. Gen. Physiol.,* 1943, v26, 325.

23. Chambers, E. G., *Statistical Calculation for Beginners,* Cambridge Univ. Press, N. Y., 1952, 2nd edition.

24. Strickland, K. P., *Canadian J. Biochem. and Phy.,* 1954, v32, 50.

25. Lehninger, A. L., *Pediatrics,* 1959, v26, 466.

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Centrally Mediated Inhibition of Gastrointestinal Propulsive Motility By Morphine over a Non-Neural Pathway. (28026)

S. MARGOLIN*

Schering Corp., Bloomfield, N. J.

The constipating action of morphine in man and in laboratory species is well known, but the mechanism of this action upon the intestine has remained obscure(1,2,3). A generally accepted view attributes this phenomenon primarily to a local direct action upon the intestine(4) and derives from the observation that morphine causes an inhibition of intestinal propulsive motility of both normal and extensively denervated dog intestine *in situ*(5,6). Nevertheless, Krueger (1) suggested "a central effect on gastrointestinal motility might be unmasked by craniad injections into the carotid artery . . . and related devices." Recent reviews(3,7) have reemphasized the possibility of an inhibitory action of morphine upon intestinal propulsion mediated centrally. Margolin(8) inferred and Green subsequently confirmed (9), that a primary route by which morphine causes a suppression of intestinal propulsive activity is indirect *via* the central nervous system. The present study based upon experiments with over 2,000 mice was designed to consider the possibility that morphine was causing a suppression of intestinal propulsive activity over a non-neural pathway.

Methods. The bioassay of drugs by intracerebral injections into mice has been reported(10), and our laboratory applied this technic to studies on the mechanism of the action of morphine and other drugs upon the central nervous system. The procedures for administering the drugs intracerebrally to mice, rats and guinea pigs and assaying effects upon intestinal propulsion according to the method of Macht-Barba-Gose(11) have been presented(8,12).

Results. Intracerebral injection of morphine sulfate into unanesthetized albino mice (22 g) reduced the distance traversed by a charcoal suspension through the small intestine. By bioassay in 50 experiments, a 50% reduction of the distance traveled by the

* Present address: Wallace Laboratories, Cranbury, N. J.

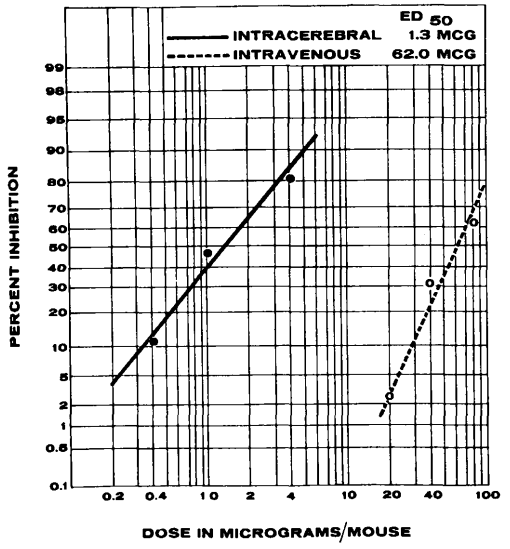

FIG. 1. Inhibition of gastrointestinal propulsive activity in mice following intracerebral or intravenous morphine sulfate.

charcoal was obtained with an intracerebral dose of 1.36 ± 0.08 μg per mouse (approx. 60 μg/kg body weight). By the intravenous route, 61.5 ± 12.0 μg per mouse (approx. 3075 μg/kg body weight) were required to induce a 50% decrease in small intestine charcoal movement. The 50-times greater dosage of morphine sulfate required to produce a 50% inhibition of charcoal transit by intravenous injection as compared to the intracerebral route (Fig. 1) indicates that the central nervous system is the primary route by which morphine causes inhibition of intestinal propulsive activity in mice. Intracerebrally, methadone at 5 μg per mouse, and codeine phosphate at 25 μg per mouse also inhibited propulsive activity by 50%. Meperidine and nalorphine by intracerebral injection were less certain in their effects, and depressed intestinal propulsion by less than 35% at maximal tolerated doses. The inhibitory action of intracerebral injection of morphine upon the gut has been confirmed in rat and guinea pig. The median effective doses per animal were 60 μg and 32 μg, respectively.

Attempts to inhibit significantly or completely block the inhibiting effect of intracerebral morphine upon intestinal propulsive activity by maximal tolerated subcutaneous, intraperitoneal or intracerebral doses of the diverse drugs listed in Table I were not successful.

Surgical transections of the mouse spinal cord at several levels ranging from C_4 to L_1 failed to block the suppression of motility by morphine (Table II). However, it did indicate that intracerebral morphine acted above C_4 in effecting an action upon the gut. Adrenalectomy blocked additional sympathetic discharge, but failed to modify the inhibition of propulsion by morphine. If the vagus outflow from the central nervous system to the gastrointestinal tract was severed just below the diaphragm, or when subdiaphramatic vagotomy was carried out concurrently with the spinal cord transection (C_4) the action of morphine was not modified.

The only successful reduction of the action of intracerebral morphine upon the gut was obtained by intravenous (400 μg per mouse) or intracerebral (16 μg per mouse) injection of nalorphine. The mutually antagonistic action of nalorphine and morphine upon the small intestine after systemic injection of both drugs has been described(13). The interaction of intracerebral morphine with intracerebral nalorphine affords further evidence of the centrally mediated action of morphine in inhibiting gastrointestinal propulsive activity.

Discussion. Since the intracerebrally administered morphine continued to exert its action upon the gastrointestinal tract in the absence of an intact sympathetic or parasympathetic outflow, the data suggest that the centrally injected morphine may be inhibiting the propulsive activity by inducing a neurohumoral discharge in the central nervous system which reaches the gut *via* the circulatory system. The frequently cited experiments of Magnus(5) and Plant and Mills (6) do not differentiate, because of their design, between a direct action upon the gut and a potential humoral discharge carried in the blood stream. Since vasopressin, oxytocin, serotonin, histamine, acetylcholin adrenalin or noradrenaline do not simulate the inhibitory effect of morphine upon gut motility, the postulated humoral agent must differ from these.

The relationship of the intestinal effects to the analgesic activity of morphine also was investigated in mice in our laboratory. Inhibition of the tail flick response caused by exposure to radiant heat occurred in 50% of the mice following intracerebral injection of

TABLE I. Ineffectiveness of Drugs with Diverse Classifications Against Inhibitory Action of Intracerebral Morphine Upon Gastrointestinal Propulsive Activity in Mice.

Exp No.	Drug tested as antagonist	Dose, mg/kg	Injection Route	Intracerebral morphine sulfate, μg/mouse	Intestinal traverse of charcoal marker, cm
1	Aminophylline	50	s.c.	2.5	2.8 ± .3‡
	Isotonic saline	—	"	2.5	2.4 ± .6
	Aminophylline	50	"	none*	10.4 ± .6
	Isotonic saline	—	"	"	10.7 ± .2
2	Papaverine	100	"	2.5	1.9 ± .4
	Isotonic saline	—	"	2.5	2.7 ± .3
	Papaverine	100	"	none	10.0 ± .8
	Isotonic saline	—	"	"	10.4 ± .7
3	TEA	40	"	2.5	2.5 ± .3
	Isotonic saline	—	"	2.5	2.9 ± .5
	TEA	40	"	none	13.9 ± 1.4
	Isotonic saline	—	"	"	12.9 ± .6
4	Atropine sulfate	40	"	2.5	2.1 ± .5
	Isotonic saline	—	"	2.5	4.4 ± .6
	Atropine sulfate	40	"	none	10.5 ± .9
	Isotonic saline	—	"	"	10.3 ± .9
5	Dibenamine	5	"	2.5	4.9 ± .9
	Isotonic saline	—	"	2.5	2.5 ± .4
	Dibenamine	5	"	none	10.3 ± 1.0
	Isotonic saline	—	"	"	11.2 ± 1.1
6	Physostigmine	.25	"	2.5	3.6 ± .5
	Isotonic saline	—	"	2.5	4.5 ± .5
	Physostigmine	.25	"	none	11.9 ± .5
	Isotonic saline	—	"	"	12.9 ± .7
7	Hexobarbital	100	i.p.	2.5	3.3 ± 1.0
	Isotonic saline	—	"	2.5	4.4 ± 1.7
	Hexobarbital	100	"	none	11.3 ± .7
	Isotonic saline	—	"	"	11.1 ± 1.4
8	Mephenesin	100	s.c.	2.5	3.0 ± .3
	Isotonic saline	—	"	2.5	3.6 ± .5
	Mephenesin	100	"	none	10.1 ± .4
	Isotonic saline	—	"	"	11.7 ± 1.0
9	Strychnine	.5	"	2.5	4.8 ± .3
	Isotonic saline	—	"	2.5	4.8 ± .7
	Strychnine	.5	"	none	12.2 ± .8
	Isotonic saline	—	"	"	10.3 ± .7
10	Vasopressin	2.5	"	2.5	2.2 ± .7
	Isotonic saline	—	"	2.5	4.4 ± 1.7
	Vasopressin	2.5	"	none	14.3 ± 1.3
	Isotonic saline	—	"	"	11.1 ± 1.4
11	*C. botulin* Toxin A	7.5 mld†	i.p.	2.5	4.3 ± .8
	Isotonic saline	—	"	2.5	4.4 ± .7
	C. botulin Toxin A	7.5 mld†	"	none	15.0 ± .7
	Isotonic saline	—	"	"	12.9 ± .7
12	Procaine	.4	i.c.	2.5	2.3 ± .4
	Isotonic saline	—	"	2.5	2.7 ± .7
	Procaine	.4	"	none	12.6 ± .7
	Isotonic saline	—	"	"	12.1 ± .9

* Isotonic saline injected intracerebrally instead of morphine.
† Minimum lethal dose (mld) per mouse.
‡ Mean with standard error.

TABLE II. Surgical Procedures Ineffective Against Inhibiting Action of Intracerebral Morphine Upon Gastrointestinal Propulsive Activity in Mice.

Exp	Surgical alteration	Intracerebral morphine SO_4, μg/mouse	Intestinal traverse of charcoal marker, cm
A	Bilateral adrenalec.	2.5	3.6 ± .6
	None	2.5	2.6 ± .4
	Bilateral adrenalec.	none	11.8 ± .9
	None	"	12.6 ± .6
B	Spinal cord transection at C_4	2.5	2.6 ± .4
	Sham operated	2.5	3.5 ± .3
	None	2.5	2.4 ± .5
	Spinal cord transection at C_4	none	9.8 ± .1
	Sham operated	"	10.1 ± .9
	None	"	10.1 ± .6
C	Subdiaphragmatic vagotomy	2.5	4.5 ± .8
	Sham operated	2.5	2.9 ± .4
	None	2.5	3.8 ± .5
	Subdiaphragmatic vagotomy	none	10.9 ± .6
	Sham operated	"	12.9 ± 1.1
	None	"	11.0 ± 1.0
D	Cord transection (C_4) plus subdiaphragmatic vagotomy	2.5	2.8 ± .9
	Sham operated	2.5	4.1 ± .7
	None	2.5	3.0 ± .5
	Cord transection (C_4) plus subdiaphragmatic vagotomy	none	10.1 ± 1.4
	Sham operated	"	11.4 ± .8
	None	"	11.8 ± 1.1
E	Cord transection (T_{10})	2.5	3.4 ± .4
	Sham operated	2.5	2.4 ± .6
	None	2.5	3.3 ± .8
	Cord transection (T_{10})	none	11.2 ± .7
	Sham operated	"	9.9 ± .5
	None	"	10.6 ± .6
F	Cord transection (L_4)	2.5	3.4 ± .6
	Sham operated	2.5	2.9 ± .2
	None	2.5	3.3 ± .4
	Cord transection (L_4)	none	11.1 ± .9
	Sham operated	"	9.7 ± .7
	None	"	11.9 ± .8

* Mean with standard error.

6 μg of morphine sulfate per mouse. The intravenous ED_{50} by this test was approximately 400 μg per mouse (18 mg/kg). These values are 5 to 7 times those required to induce a 50% inhibition of the propulsive activity of the intestine. Although a direct relationship between analgesic properties and depression of gastrointestinal activity for the morphine-type analgesics has been proposed (14,15), a direct relationship was not immediately apparent in our studies. The identification of a neurohumoral discharge from the central nervous system initiated by morphine, as inferred from these findings, possibly would clarify the multiple actions of morphine and related analgesics, and perhaps lead to an elucidation of a specific biochemical mechanism associated with narcotic drug addiction. The report by Kornetsky and Kiplinger(16) that sera from dogs tolerant to morphine sulfate potentiate the effect of morphine on motor behavior of the rat appears to lend further support to such an hypothesis.

Summary. 1. Intracerebral injection of small amounts (0.4 to 4.0 μg) of morphine sulfate in unanesthetized mice causes marked inhibition of gastrointestinal propulsive activity. Since a 50-times greater dose is required intravenously for an equivalent ef-

fect, this action of morphine upon the gut must be mediated through the central nervous system. Similar actions have also been observed in rats and guinea pigs. 2. Pharmacological inhibition or blockade of this morphine action upon the gut could not be demonstrated with a wide variety of autonomic blocking agents or central nervous system depressants. Concurrent, surgical interruption of the sympathetic and parasympathetic outflows from the central nervous system to the gastrointestinal tract also failed to block the activity. 3. The findings suggest that morphine may be causing inhibition of gut propulsive action by initiating a neurohumoral discharge from the central nervous system into all the circulating blood of the body.

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1. Krueger, H., *Physiol. Rev.,* 1937, v17, 618.

2. Krueger, H., Eddy, N. B., Sumwalt, M., *Pharmacology of the Opium Alkaloids,* Public Health Report, U. S. Govt. Printing Office, Washington, D.C., 1941, Part 1, Suppl. 165.

3. Vaughn-Williams, E. M., *Pharmacol. Rev.,* 1954, v6, 159.

4. Goodman, L., Gilman, A., *The Pharmacological Basis of Therapeutics,* Macmillan Co., New York, 1955.

5. Magnus, R., *Pfluger's Arch. ges. Physiol.,* 1926, v115, 316.

6. Plant, O. H., Miller, G. H., *J. Pharm. Exp. Therap.,* 1926, v27, 361.

7. Schaumann, O., *Brit. Med. J.,* 1956, v2, 1091.

8. Margolin, S., *Fed. Proc.,* 1954, v13, 383.

9. Green, A. F., *Brit. J. Pharmacol.,* 1959, v14, 26.

10. Blume, W., *Arch. exp. Path. u. Pharmakol.,* 1949, v208, 40.

11. Macht, D. I., Barba-Gose, J. J., *J. Am. Pharm. Assn.,* Sci. Ed., 1931, v20, 558.

12. Makovsky, A., Margolin, S., *Fed. Proc.,* 1954, v13, 382.

13. Hart, E. R., McCawley, E. L., *J. Pharm. Exp. Therap.,* 1944, v82, 339.

14. Schaumann, W., *Arch. exp. Path. u. Pharmakol.,* 1954, v223, 348.

15. Schaumann, O., Giovanni, M., Jochum, K., *ibid.,* 1952, v215, 460.

16. Kornetsky, C., Kiplinger, G. F., *Fed. Proc.,* 1962, v21, 418.

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Influence of Tumor Fractions on Incidence of Spontaneous Mammary Tumors in C3H/HeJ Mice.* (28027)

LEO G. NUTINI, JOHN E. PRINCE, ALEJANDRO G. DUARTE, SISTER RODERICK JUHASZ AND ELTON S. COOK

Divisions of Experimental Medicine and Biochemistry, Institutum Divi Thomae, Cincinnati, Ohio

Relatively few experiments have been reported on attempts to decrease the incidence of spontaneous tumors in mice, in part, no doubt, because of the considerable duration of such experiments. Isojima *et al.*(1) reported that injection of a homogenate of spontaneous tumors from C3H mice lowered the incidence of spontaneous tumors in such mice. Hirsch and Iversen(2), on the other hand, found that pretreatment with C3H tumor tissue had no significant effect in lowering tumor incidence and, indeed, significantly reduced age of tumor onset and survival time. These experiments were attempts to immunize. Our previous partial success in inducing resistance to transplantable tumors by use of an alcohol-soluble, protein-free tumor fraction(3,4) which seems unlikely to act as an antigen, led us to conduct a pilot experiment to determine the effects of a similar fraction from spontaneous tumors on the incidence of spontaneous tumor.

Materials and methods. Virgin female C3H/HeJ mice, originally obtained from the Roscoe B. Jackson Memorial Laboratory, were used as the experimental animals and as sources of spontaneous mammary tumors. The dbrB adenocarcinoma from DBA/1 mice

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