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INTERACTION BETWEEN SEROTONIN AND MORPHINE IN THE GUINEA-PIG ILEUM[1, 2, 3]

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ABSTRACT

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Inhibition of the electrically induced contractions of the guinea-pig ileum has been shown to be a reliable index of the relative potency of various narcotic analgesics. This property suggests that this preparation might be useful in attempts to elucidate the mechanism by which morphine induces analgesia in the central nervous system. In light of the inhibitory effects of 5-hydroxytryptamine (5-HT) on the ileum and its interactions with morphine in other tissues, the effect of 5-HT on the inhibitory response of the ileum to morphine was studied. Concentrations of 5-HT ranging from 2×10^{-5} to 2×10^{-7} M can potentiate the inhibitory effects of morphine, and *vice versa;* small concentrations of morphine (10^{-8} M) can potentiate the inhibitory response to 5-HT. Both of these effects can be abolished by washing. This potentiation is also observed with both nalorphine and methadone and seems to be due to a specific interaction between 5-HT and narcotic analgesics. Similar concentrations of 5-HT have no effect on the inhibition produced by norepinephrine, and norepinephrine itself has no effect on the inhibition produced by morphine. Lysergic acid diethylamide (0.1 μg/ml) can antagonize the inhibitory effects of 5-HT but can also produce a marked potentiation of the response to morphine. The above suggests that 5-HT may play an essential role in the mechanism by which morphine induces analgesia in the central nervous system.

Inhibition of electrically induced contractions of the guinea pig ileum has been repeatedly shown to be a reliable index of the relative potency of a variety of narcotic analgesics (Schaumann *et al.*, 1952; Schaumann, 1954; Gyang *et al.*, 1964). This property suggests that this preparation may be useful in attempts to elucidate the mechanism by which morphine induces analgesia in the central nervous system (CNS). Characteristics of this preparation which support its use as a model system for studying analgesia are 1) the extremely small concentrations of morphine required to produce effects (Cox and Weinstock, 1960), 2)

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[3] A summary of this work was presented at the American Society for Pharmacology and Experimental Therapeutics fall meeting, August 19 to 24, 1973 (Pharmacologist **15:** 202, 1973).

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reversibility by naloxone (Kosterlitz and Watt, 1968), 3) steric properties required for inhibitory activity (Cox and Weinstock, 1966) and finally the direct parallel between the order of potency of drugs in causing analgesia and their potency in inhibiting ileal activity (Schaumann, 1954; Gyang *et al.*, 1964).

Ever since the work of Paton (1957) attempts have been made to explain the analgesic effects of morphine through some interaction with neurotransmitters thought to be present in the CNS. Indeed, it has been well established that morphine in pharmacological concentrations can cause a modest, but nevertheless, significant reduction in both the spontaneous (Paton and Zar, 1968) and electrically evoked release of acetylcholine (ACh) from the guinea-pig ileum (Paton, 1957; Cox and Weinstock, 1966) and the spontaneous release of ACh from the cat cerebral cortex (Beleslin and Polak, 1965; Jhamandas *et al.*, 1971). Norepinephrine (NE) and 5-hydroxytryptamine (5-HT), two putative CNS neurotransmitters, have also received much attention. The evidence, however, suggesting their involvement in the analgesic response is more indirect. Studies have indicated that morphine pretreatment can alter turnover rates of both NE (Clouet and Ratner, 1970; Smith *et al.*, 1972) and 5-HT, although there are several contradictory reports concerning the latter (Way, 1972). In all of these instances, however, it is difficult to say whether these changes are due to a primary effect of morphine which mediates the narcotic effects or to secondary or tertiary effects of the drug.

Since it has been well established that NE has inhibitory effects on the gut (McDougal and West, 1952, 1954; Schaumann, 1958), there have been many attempts to explain the effects of morphine on this tissue through some adrenergic mechanism (Schaumann, 1958). Despite such attempts, most of the evidence is to the contrary (Kosterlitz and Watt, 1968).

Serotonin has potent inhibitory effects on the gut (Kosterlitz and Robinson, 1957; Bulbring and Gershon, 1967) and has been shown to be an endogenous constituent, with most of it located in neurons (Ross and Gershon, 1972). These observations and the reported interactions between morphine and 5-HT in the CNS (Way, 1972) prompted us to investigate whether 5-HT was involved in the response of the gut to morphine.

Methods

Guinea pigs weighing from 300 to 450 g were killed by a blow on the head and bled by cutting the main arteries in the neck. The terminal portion of the ileum was removed and the last 20 cm were discarded. The gut was stimulated by transmural electrical stimulation essentially as described by Paton (1957); the intraluminal electrode was made the anode. Rectangular current pulses of 0.2 msec duration and of sufficient strength to produce a maximal response to a single shock were applied to the electrodes once every 10 seconds with a Grass model S 88 stimulator. Stimuli were monitored on an oscilloscope which was connected across the electrodes. It was initially found that there was a large voltage drop across the electrodes, *i.e.*, the voltage indicated on the stimulator was not the same voltage that was measured across the electrodes. To circumvent this problem, stimuli were first fed into a Crown DC300 audio amplifier before being applied across the electrodes. With the use of this amplifier, a voltage of 2.0 V applied across the electrodes was sufficient to produce maximal responses. The isometric contractions of the gut were recorded *via* a Grass FT 03 force transducer on a Grass polygraph which was calibrated before every experiment such that −2 mV fed into the preamplifier produced a pen displacement of 2 cm. The resting tension was fixed at 1 g. The temperature of the 50-ml organ bath was kept constant at 37°C. All of the experiments were made on pieces of ileum suspended in Krebs' solution of the following composition (in millimolar concentrations): NaCl, 118; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; $MgCl_2$, 1.2; $NaH_2PO_4 \cdot H_2O$, 1.2; $NaHCO_3$, 25; glucose, 11; choline chloride, 0.29. The buffer was bubbled with 95% O_2 and 5% CO_2 which maintained the pH at 7.40. All drugs used in the experiment were added directly to the organ bath in 10 to 100 μl of glass-distilled water. The respective concentrations are expressed in terms of the final molarity. Morphine was allowed to remain in contact with the gut for only 2 minutes to ensure consistent responses and to prevent deterioration of the preparation as suggested by Cox and Weinstock (1966). In our initial experiments with LSD a 90-minute preincubation time was used. This time period was based on the work of Cerletti and Doepfner (1958). In subsequent experiments it was found that a 15-minute preincubation time was sufficient to obtain maximum effects.

All of the chemicals used to prepare the buffer were of the highest purity obtainable. The following were the drugs used: morphine sulfate, 5-HT creatine sulfate water complex, *l*-norepinephrine bitartrate, *d*-lysergic acid diethylamide (LSD),

methadone hydrochloride and nalorphine hydrochloride.

Results

Effects of 5-HT on the response to transmural electrical stimulation. Figure 1 shows typical polygraph recordings obtained when 5-HT is added to the organ bath during electrical stimulation. The response consists of three phases. The first is characterized by a transient increase in the basal tone of the gut that lasts for almost 1 minute with the doses of 5-HT used. This is in keeping with the results of Gaddum and Picarelli (1957) who showed that 5-HT can cause the gut to contract both through the release of ACh and *via* a direct effect on the muscle. The second phase, characterized by a decrease in the height of the electrically induced contractions, usually reaches its maximum within 3 to 5 minutes. This observation is in agreement with the work of Bulbring and Gershon (1967) and Drakontides and Gershon (1972) who have given evidence that 5-HT is inhibitory on several segments of the gastrointestinal tract and functions *in vivo* as an inhibitory transmitter. The third phase is characterized by a recovery from this inhibition. This extent of recovery is found to vary from preparation to preparation and even varies within a single experiment when several additions of 5-HT are made. Figure 1 also indicates that the relative magnitude of each phase is a function of the concentration of the 5-HT added. As one increases the concentration of 5-HT added, not only is the initial inhibition more pronounced but recovery is either greatly reduced or nonexistent. In order to compare the response to morphine in the presence and absence of 5-HT and to keep the experimental contractions as similar to the control contractions as possible, minimal doses of 5-HT were used and the gut was allowed to recover from the inhibitory effects of 5-HT as much as possible prior to the addition of morphine.

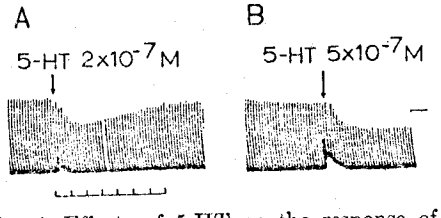


FIG. 1. Effects of 5-HT on the response of the guinea-pig ileum to transmural electrical stimulation as described in "Methods." 5-HT was added to the organ bath up to a final concentration of 2×10^{-7} M (fig. 1A) and 5×10^{-7} M (fig. 1B). Horizontal calibration is 1 min/division.

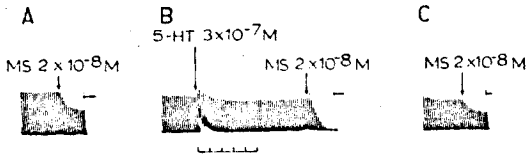


FIG. 2. Effect of 5-HT pretreatment on the response of the electrically stimulated guinea-pig ileum to morphine. Morpine (MS) and 5-HT were added such that the final concentration was as indicated in the figures. The preparation was washed three times 2 minutes after the addition of morphine and it was allowed to rest for 12 minutes before the next addition. The order shown represents the actual experimental sequence. The horizontal calibration is 1 min/division.

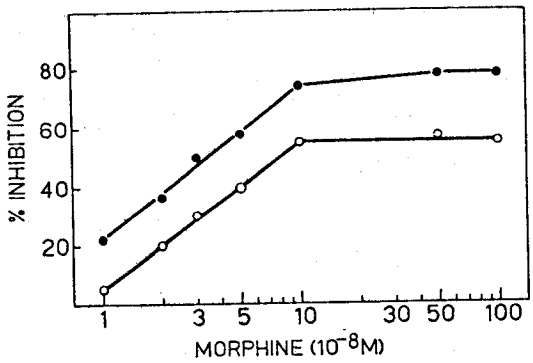


FIG. 3. Dose-response curve to morphine in the presence (●) and absence (○) of 5-HT. The inhibitory response of the electrically stimulated ileum to morphine was determined first in the absence and then in the presence of 2×10^{-8} M (10 ng/ml) 5-HT. The percent inhibitions indicated are the values obtained after a 2-minute exposure to morphine. The values represent single observations from one of two complete dose responses performed on separate days.

Interaction between 5-HT and morphine. The effect of 5-HT on the response to morphine is illustrated in figure 2. Figure 2A represents a control response to morphine; figure 2B represents the response to morphine after the preparation was allowed to recover from the inhibitory effects of 3×10^{-7} M (150 ng/ml) 5-HT. The inhibition produced by morphine increased from 45% in the control to 100% in the presence of 5-HT. After three washes and a 10-minute rest period, the response to morphine returned to the control level as is shown in figure 2C. In seven experiments, 10^{-8} M morphine produced a

mean inhibition of 18.8 ± 2.73% and after pretreatment with 5-HT (2×10^{-7} M) the same concentration of morphine produced an inhibition of 54.3 ± 6.43%. This potentiation of the inhibitory effects of morphine was highly significant with a P value of <.001. This interaction between 5-HT and morphine is manifest over a wide range of morphine concentrations as demonstrated by a seven-point dose-response curve to morphine determined in both the presence and absence of 2×10^{-8} M (10 ng/ml) 5-HT (fig. 3). It is important to emphasize that in this experiment the concentration of 5-HT was reduced approximately 10-fold relative to the previous experiment in order to avoid the large and persistent inhibitory effects of 5-HT which are frequently observed with larger doses. As expected, the potentiation of morphine is less pronounced with smaller doses of 5-HT. Conversely, morphine in very small concentrations (5×10^{-9} to 10^{-8} M) can potentiate the inhibitory response of the gut to 5-HT, as is demonstrated in figure 4. Since even under normal conditions the response to the inhibitory effects of 5-HT tended to vary considerably, it was not possible to obtain a reproducible dose-response curve to 5-HT in both the presence and absence of morphine. In two experiments, the mean inhibition produced by 1 to 2×10^{-7} M 5-HT was 14.5 ± 3.79% and, after pretreatment with 3 to 5×10^{-9} M morphine, the same concentrations produced a mean inhibition of 45.0 ± 6.86%. The P value was less than .02.

It should be noted that 5-HT pretreatment also potentiated the inhibitory effects of nalorphine (10^{-8} M) from an initial inhibition of 7% to an inhibition of 32%. The same was observed for methadone hydrochloride (10^{-8} M) which first produced an inhibition of 25% and after pretreatment with 5-HT (3×10^{-7} M) produced an inhibition of 50%. The latter is particularly significant since it is an analgesic which is structurally dissimiliar to morphine. Although the studies with nalorphine and methadone were not as extensive as those with morphine, they suggest that potentiation by 5-HT is a characteristic common to narcotic analgesics. In addition, the inhibition by morphine while in the presence of 5-HT was completely reversed by naloxone (10^{-7} M) but not the inhibition that was due to 5-HT. This implies that all of the increased response to morphine was narcotic in nature.

Interaction between 5-HT and norepinephrine; norepinephrine and morphine. In order to see if the effect of 5-HT on the response to morphine were specific, the interaction between 5-HT and NE was investigated. As can be seen in table 1, 2×10^{-7} M (0.1 μg/ml) 5-HT

TABLE 1

Effect of 5-HT pretreatment on the response of the electrically stimulated ileum to morphine (MS) and to NE[a]

Concentration of Drug[b]	Inhibition without 5-HT Pretreatment	Inhibition with 5-HT Pretreatment[c]	P Value
	%	%	
MS, 10^{-8} M	16.0 ± 1.04 (5)	45.3 ± 3.52 (3)	<.001
NE, 10^{-8} M	16.8 ± 2.52 (4)	21.5 ± 0.5 (2)	N.S.

[a] The results are the mean values ± S.E.M. from separate experiments performed on different days and the number of determinations is indicated in parentheses. Percent reduction in contraction height was determined after NE or morphine had been in contact with the gut for 2 minutes.

[b] Represents final molar concentration of the drug in the organ bath.

[c] 5-HT was added in 20 μl of water such that the final concentration was as indicated. Preincubation with 5-HT proceeded for 5 minutes after which NE or morphine was added.

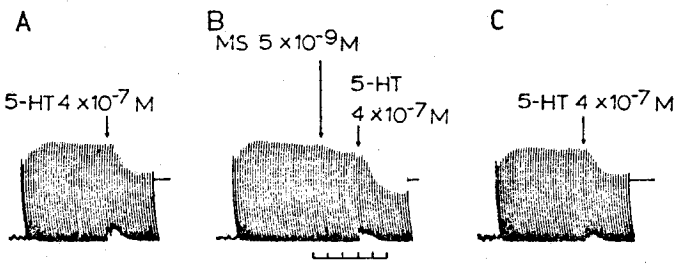


FIG. 4. Effect of morphine pretreatment on the inhibitory response of the electrically stimulated ileum to 5-HT. The inhibitory response to 4×10^{-7} M (0.2 μg/ml) 5-HT was determined first before and then after a 2-minute pretreatment with the indicated concentration of morphine. Horizontal calibration is 1 min/division.

had no appreciable effect on the inhibitory response to NE. In similar fashion, the interaction between NE and morphine was studied. As can be seen in table 2 and in figure 5, a concentration of NE as high as 4×10^{-6} M did not potentiate the response to morphine. Figure 5 also illustrates that the inhibition of the contractions *per se*, prior to the addition of morphine, does not necessarily result in an increased response to morphine. However, figure 5D shows that 2×10^{-7} M 5-HT was effective in causing an increase from 15 to 40% inhibition in response to morphine.

Interaction between LSD and morphine; LSD and 5-HT. If 5-HT is necessary for the action of morphine, then blockade of 5-HT receptors should diminish markedly the response to morphine. Since LSD has been reported to be a potent anti-5-HT agent peripherally (Cerletti and Doepfner 1958), its effects on the response to morphine were studied. Contrary to our expectations, LSD greatly potentiated the response to morphine. These results are summarized in table 3 and in figure 6. In two experiments, five separate exposures to 10^{-6} M morphine produced a mean inhibition of 53.6 ± 6.21% and, after pretreatment with LSD (0.1 μg/ml), the same concentration produced a mean inhibition of 96.0 ± 4.0%. The P value was less than .01.

The interaction between 5-HT and LSD was also investigated. The data in table 4 illustrate

TABLE 2

Effect of NE and 5-HT pretreatment on the response of the electrically stimulated ileum to morphine

Pretreatment		Inhibition of Morphine[c]
5-HT[a]	NE[b]	
M		%
	None	20
	5×10^{-9}	17
	1×10^{-8}	15
	4×10^{-8}	23
None		17
2×10^{-7}		37
2×10^{-7}		40

[a] 5-HT was added, as described in table 1, 4 minutes prior to the addition of morphine.

[b] NE was added in 40 to 100 μl of water such that the final concentration was as indicated. Preincubation time was 4 minutes after which morphine was added.

[c] Percent inhibition of contraction height was determined 2 minutes after the addition of morphine, such that the final concentration was 10^{-8} M. The values represent single observations from one of three experiments.

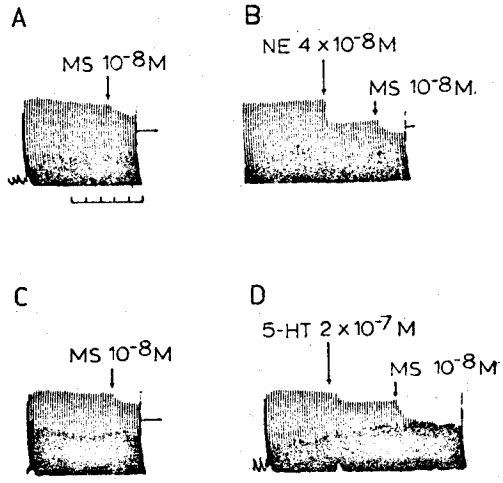


FIG. 5. Effect of NE and 5-HT pretreatment on the response of the electrically stimulated ileum to morphine (MS). NE and 5-HT were added up to the concentrations indicated in the figure, 2 and 4 minutes, respectively, before the addition of morphine. The ileum was washed three times after each 2-minute exposure to morphine and it was allowed to rest for 12 minutes. The results illustrated were obtained in the same experiment and they are shown in the actual experimental sequence.

TABLE 3

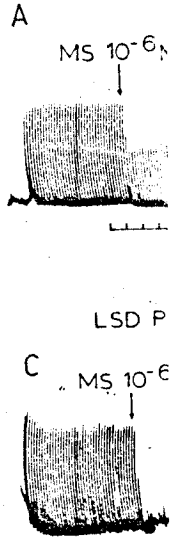
Effect of LSD pretreatment on the response of the electrically stimulated ileum to morphine (MS)[a]

MS Concentration[b]	Inhibition before LSD Pretreatment	Inhibition after LSD Pretreatment[c]	P Value
M	%	%	
10^{-6} M	53.6 ± 6.21 (5)	96.0 ± 4.0 (3)	<.01
10^{-7} M	54.3 ± 9.3 (4)	84.2 ± 3.6 (6)	<.05

[a] The results are the mean values ± S.E.M. from two experiments and the number of determinations is indicated in parentheses. Percent reduction in contraction height was determined after morphine had been in contact with the gut for 2 minutes.

[b] Final molar concentration of morphine in the organ bath.

[c] The ileum was preincubated with LSD (0.1 μg/ml) for 90 minutes after which time the response to morphine was tested.



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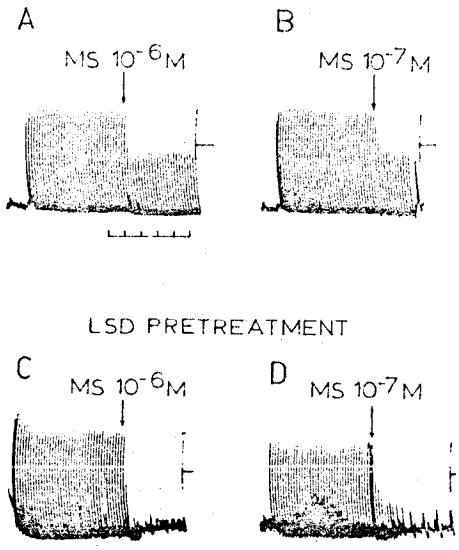

FIG. 6. Effect of LSD pretreatment on the response of the electrically stimulated ileum to morphine (MS). Responses to the indicated concentrations of morphine were determined first in the absence and then in the presence of LSD (0.1 μg/ml) after a 90-minute preincubation with the latter. Horizontal calibration is 1 min/division except in figure 6D where 2 minutes after the addition of morphine the chart speed was increased 5-fold in order to facilitate the measurement of the individual contractions.

that the response of the resting gut to the excitatory effects of 5-HT (1–2 × 10^{-7} M) was not affected by LSD (0.1 μg/ml), even if the gut was preincubated with LSD for 90 minutes. On the other hand, after only a 15-minute preincubation period, the same concentration of LSD blocked by 80% the inhibitory effects of a concentration of 5-HT (4 × 10^{-7} M) which was twice the amount used to produce the excitatory effects. This antagonism of the inhibitory effects of 5-HT by LSD is shown in figures 7 and 8. Since other 5-HT antagonists such as cyproheptadine, cinanserin and methysergide interfered with the response to electrical stimulation, their effect on the response of the ileum to morphine and 5-HT could not be investigated.

The above experiments suggest that the excitatory effects on the resting gut and the inhibitory effects of 5-HT on the response to transmural electrical stimulation are mediated *via* receptors which are pharmacologically distinguishable.

Discussion

Since 5-HT is an endogenous constituent of the ileum and its localization in this tissue has been shown to be mostly neuronal (Ross and

TABLE 4

Effect of LSD pretreatment on the response of the resting ileum to the excitatory effects of 5-HT

Concentration of LSD[a]	Concentration of 5-HT[b]	Height Recorded Contraction[c]
μg/ml	M	cm
	2×10^{-7}	3.5
	1×10^{-7}	2.6
	2×10^{-7}	3.2
0.1	2×10^{-7}	3.2
0.1	1×10^{-7}	2.2
0.1	2×10^{-7}	2.7
0.1	1×10^{-7}	2.4

[a] The resting ileum was incubated with the indicated concentration of LSD for 90 minutes after which the response to 5-HT, while still in the presence of LSD, was again tested.

[b] Final molar concentration of 5-HT in the organ bath.

[c] 5-HT was left in contact with the ileum only long enough for each concentration to exert its maximal effect. The heights of contraction indicated were determined by direct measurement of the pen displacement.

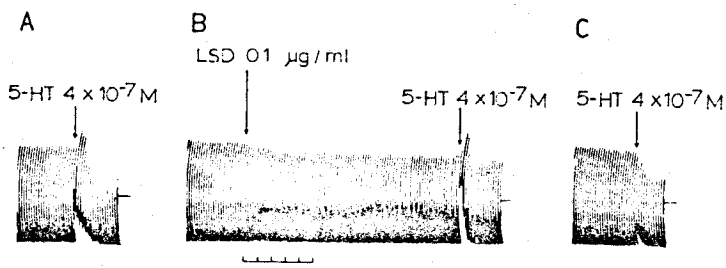

FIG. 7. Effect of LSD pretreatment on the response of the electrically stimulated ileum to the inhibitory effects of 5-HT. Inhibitory responses to the indicated concentrations of 5-HT were determined before and after a 15-minute incubation with 0.1 μg/ml of LSD. Horizontal calibration is 1 min/division.

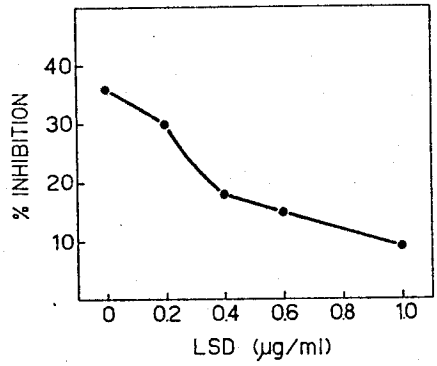

FIG. 8. Effect of increasing concentrations of LSD on the inhibitory response of the electrically stimulated ileum to 4×10^{-7} M 5-HT. Each point represents the degree of inhibition of the response to electrical stimulation produced by the addition of 5-HT (4×10^{-7} M) after a 15-minute incubation with the indicated concentration of LSD. 5-HT was left in contact with the ileum only long enough to produce its maximum effect after which the gut was washed several times and rested for 12 minutes.

Gershon, 1972), it is tempting to speculate that some interaction with the 5-HT system may play an essential role in the response of the ileum to morphine.

This paper demonstrates that 5-HT in concentration ranging from 2×10^{-8} to 3×10^{-7} M can significantly alter the response of the electrically stimulated guinea-pig ileum to morphine. This interaction between 5-HT and morphine appears to be specific since the inhibitory response to NE is not similarly affected and NE itself has no effect on the inhibitory response to morphine. This is particularly significant since it has been shown that NE, like morphine, is a potent inhibitor of both the spontaneous and electrically evoked release of ACh from the guinea-pig ileum (Cowie *et al.*, 1968; Paton and Vizi, 1969). Figure 5 illustrates that partial inhibition of the gut in itself does not potentiate the inhibitory effects of morphine. Thus, the inhibition of the response to electrical stimulation that remains after the addition of 5-HT cannot be the basis for the potentiation.

The effects of LSD in both peripheral tissue (Cerletti and Doepfner, 1958) and the CNS (Aghajanian *et al.*, 1972) have often been correlated with the drug's ability to act either as an antagonist or as an agonist of 5-HT. The above strongly suggested that the interaction between LSD and morphine in the guinea-pig ileum was mediated *via* the 5-HT system. It remained to be demonstrated, however, that LSD could function as an agonist and/or antagonist of 5-HT on this tissue. Although LSD, in concentrations up to 0.1 μg/ml, had no detectable influence on the excitatory effects of 5-HT, these same concentrations were able to produce a marked antagonism of the inhibitory effects of 5-HT on the response to transmural electrical stimulation. In the guinea-pig ileum, therefore, LSD appears to act as an antagonist of the inhibitory effects and as an agonist of the potentiating effects of 5-HT. These observations, coupled with the known interactions between 5-HT and LSD in other tissues, strongly imply that the LSD-morphine interaction is mediated *via* the 5-HT system.

The implications of this interaction with the 5-HT system in terms of the ultimate mechanism by which morphine acts are not very clear. One possibility immediately suggests itself: morphine may act on the gut and in the CNS by increasing the availability of 5-HT and, in doing so, potentiate its inhibitory effects. Morphine could do this by causing the release of 5-HT or by preventing its reuptake and/or inactivation. While this possibility is attractive, there are several observations which tend to argue against it. First, on a number of occasions, the ileum was very insensitive to the inhibitory effects of 5-HT and still showed the usual sensitivity to the inhibitory effects of morphine. If morphine were acting purely by making more 5-HT available, one would expect that the response to morphine would also be attenuated. Second, it has been found that the ileum can be made refractory to the inhibitory effects of 5-HT by successively adding the drug while still in the presence of the previous addition. When the gut is made refractory to 5-HT, as just described, it still shows an augmented response to morphine. If morphine acted *via* the release of 5-HT or *via* the blockade of its reuptake, one would expect that, when the gut no longer responded to the inhibitory effects of 5-HT, there would be no response to morphine. Instead, we still get potentiation.

In addition to our findings, the interaction between 5-HT and morphine is supported by the work of several investigators. Teuen (1967) found that *p*-chlorophenylalanine (PCPA) antagonized the effects of morphine and suggested that the antagonism might be attributable

to a deficiency of 5-HT induced by PCPA. In support of his postulate are additional findings (Tenen, 1968) wherein PCPA was found to increase the animal's sensitivity to pain. This implies that painful stimuli might be attenuated through 5-HT-dependent mechanisms. Consistent with these results, Harvey and Lints (1971) and Harvey *et al.* (1968) noted that a decrease in brain 5-HT, induced by lesions in the medial forebrain bundle or by PCPA in the rat, resulted in a decreased threshold to electrical foot shock. The increased pain sensitivity in the lesioned animals was associated with a decrease in 5-HT in the telencephalon. Injection of 75 mg/kg of 5-HT i.p. returned both telencephalic 5-HT and pain threshold to normal values whereas dopa had no effect. In similar fashion Samanin *et al.* (1970) found that the decrease of brain 5-HT obtained by lesions of the nucleus raphe medianus resulted in antagonism of morphine analgesia measured by the hot plate procedure. More recently, Schulz and Goldstein (1973) have shown that resting intestinal muscle strips from morphine-tolerant guinea pigs exhibited a 10 times higher sensitivity to the excitatory effects of 5-HT than strips from nontolerant guinea pigs. They postulated that this supersensitivity to 5-HT could be caused by a feedback mechanism in the excitatory pathway of the myenteric plexus and that it could account for the morphine tolerance.

All of these reports and our findings indicate that there is a definite interaction between 5-HT and morphine, but they do not demonstrate that 5-HT is directly involved in the mechanism of action of narcotic analgesics. It is suggested, however, that studies concerning the mechanism of action of 5-HT on the guinea-pig ileum could conceivably shed much light on the mechanism by which serotonin can potentiate the response to morphine, and, possibly, on the mechanism of action of morphine itself.

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