

Role of 5-hydroxytryptamine receptors in narcotic-induced reduction in gastrointestinal transit in rats

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ABSTRACT Narcotic drugs are unique in inducing constipation. Morphine is known to induce a reduction in gastrointestinal transit through release of 5-hydroxytryptamine. A number of 5-HT antagonists, ketanserin, methysergide and MDL 72222 were used to investigate the role of 5-HT receptors in the morphine-induced reduction in intestinal propulsion. It was observed that ketanserin, at a dose of 5 mg/kg given subcutaneously, antagonized the effect of morphine on intestinal transit. Since ketanserin is a specific 5-HT₂ receptor blocker, it appears that morphine produces this effect via activating the 5-HT₂ receptors. The reduction in intestinal transit caused by the intrathecal administration of sufentanil appears to be mediated by 5-HT₂ receptors because this effect is also antagonized by pretreatment with ketanserin. Thus this study indicates that 5-HT₂ receptors in the intestine are responsible for the reduction in gastrointestinal transit.

KEY WORDS morphine; naloxone; ketanserin; serotonin receptors; gastrointestinal motility; rats; intrathecal injections; methysergide; MDL 72222

Opioids have been utilized for centuries in the treatment of diarrhea and pain⁽¹⁾. The effects of opioids on gastrointestinal motility have been investigated by a number of workers^(2,3). The inhibition of gastrointestinal transit by morphine has been shown to be produced by both

central^(4,5) and peripheral mechanisms⁽³⁾. In addition, morphine can influence storage and release of acetylcholine from parasympathetic nerve endings in the intestine. On the other hand, morphine may increase the gastrointestinal contractility by releasing 5-HT. This, in turn, then stimulates the release of acetylcholine⁽⁶⁾.

The purpose of this study was to identify the types of 5-HT receptors involved in morphine-induced decrease in gastrointestinal transit in rats. This was accomplished by using a number of 5-HT antagonists. In order to prevent the direct action of opiates on the gastrointestinal tract, this work was further carried out using a model in which drugs could be injected directly into the subarachnoid space.

METHODS

Wistar rats (319 ± SD 18 g) of either sex were used. The catheterization of the subarachnoid space was performed using the technique of Yaksh and Rudy⁽⁷⁾. A thin polyethylene catheter (od 0.61 mm) was introduced under halothane, N₂O and O₂ anaesthesia, into the subarachnoid space via the cisterna magna. It was advanced caudally for 8 cm so that the tip of the catheter was located in the lumbar region. The catheter was filled with sterile saline and sealed. The rats were allowed to recover and only those rats showing no neurological abnormality were used in the subsequent experiments.

Drugs were administered intrathecally to overnight fasted rats in a volume of 5-10 µl and the catheter was then flushed

with 10 μ l of saline (the internal volume of the catheter). A charcoal meal test⁽⁸⁾ was used to determine the gastrointestinal transit. Each rat received 2 ml of the charcoal meal (containing charcoal, flour and water in the ratio of 1:2:6) by intragastric gavage via a blunt metal cannula. After 20 min the rats were killed by cervical dislocation. The distance that the charcoal had travelled from the pyloric sphincter was measured and expressed as a percentage of the total distance from the pyloric sphincter to the ileocecal junction. One group of rats received only saline intrathecally. Another group of rats received the 5-HT₂ blocker ketanserin (5 mg/kg subcutaneously) and 10 min later they were challenged with sufentanil (0.5 μ g) intrathecally. Another group received only sufentanil (0.5 μ g) through the catheter into the subarachnoid space.

Other groups of rats received subcutaneous doses of morphine, either alone or 10 min after the subcutaneous administration of naloxone (1 and 2 mg/kg) or a 5-HT antagonist, ketanserin (5 mg/kg), methysergide (5 mg/kg) or MDL 72222 (5 mg/kg). In these groups a charcoal meal was given 25 min after the morphine (1, 3 and 10 mg/kg). In view of the day to day variation in transit speed of the charcoal meal in saline control rats, a separate

saline control group of rats which received saline 1 ml/kg sc was used for each day on which experiments were performed.

RESULTS

The effects of various doses of subcutaneous morphine on gastrointestinal transit are presented in Table 1. As can readily be seen, these doses produced a dose-dependent reduction in the transit of the charcoal meal.

Tab 1. Effect of morphine and pretreatment of naloxone on gastrointestinal transit (%). 6 rats/group. $\bar{x} \pm SD$.

Treatment sc	No pretreatment	Subcutaneous naloxone	
		1 mg/kg	2 mg/kg
Saline	77.3 \pm 8.3	74.2 \pm 9.8	54.4 \pm 4.4
Morphine 1 mg/kg	32.2 \pm 3.7***	54.2 \pm 5.6***	72.0 \pm 6.1**
Morphine 3 mg/kg	29.9 \pm 10.1**	53.5 \pm 9.0***	73.9 \pm 9.3**
Morphine 10 mg/kg	22.6 \pm 3.7***	42.5 \pm 4.9***	76.1 \pm 3.9**

** $p < 0.05$, *** $p < 0.01$ in comparison to saline control.

Naloxone pretreatment was administered at two dose levels, 1 and 2 mg/kg. The results, which are also presented in Table 1, showed that naloxone 1 mg/kg

Tab 2. Effect of pretreatment with 5-HT antagonists on morphine-induced reduction in gastrointestinal transit (%). 6 rats/group. $\bar{x} \pm SD$

Treatment sc	No pretreatment	Ketanserin 5 mg/kg	Methysergide 5 mg/kg	MDL 72222 5 mg/kg
Saline	77.3 \pm 8.3	54.4 \pm 4.4	63.6 \pm 4.2	64.3 \pm 5.6
Morphine 1 mg/kg	32.2 \pm 3.7***	59.2 \pm 7.3*	53.8 \pm 11.3*	54.5 \pm 11.5*
Morphine 3 mg/kg	29.9 \pm 10.1***	51.6 \pm 10.0*	19.5 \pm 11.3***	32.5 \pm 8.6***
Morphine 10 mg/kg	22.6 \pm 3.7***	35.1 \pm 7.6***	18.2 \pm 5.4***	32.0 \pm 5.7***

* $p > 0.05$, *** $p < 0.01$ in comparison to saline control.

caused partial antagonism of the morphine-induced decrease in intestinal transit. A dose of 2 mg/kg, on the other hand, not only antagonised the morphine-induced reduction in gastrointestinal transit but significantly increased the rate of passage of the charcoal meal. Naloxone (2 mg/kg) was administered subcutaneously to another group of rats. Significant increases in transit ($p < 0.05$) were also observed in this group compared with a saline control group: 75.0% (± 14.5 SD) compared with 64.4% (± 9.5 SD).

The effects of 5-HT antagonists on morphine-induced reduction in gastrointestinal transit are presented in Table 2. The 5-HT₂ antagonist ketanserin significantly antagonized the morphine effect on the transit of the charcoal meal except that of the highest dose (10 mg/kg) of morphine. On the other hand, subcutaneous methysergide (5 mg/kg) and MDL 72222 (5 mg/kg) were only able to antagonise the effects of morphine at the lowest dose (1 mg/kg).

Sufentanil (0.5 μ g) reduced the gastrointestinal transit by 31.6% \pm 13.5 and in ketanserin pretreated group sufentanil decreased the intestinal transit by 53.10% \pm 16. In saline and ketanserin pretreated group the gastrointestinal transit was 70.3% \pm 5.1. Therefore sufentanil in ketanserin pretreated group of rats significantly ($p < 0.05$) increased the gastrointestinal transit.

DISCUSSION

The present study has confirmed the observation of many other investigators, that there is a dose-dependent reduction in gut transit of a charcoal meal following treatment with morphine. The effect of morphine on intestinal transit was significantly antagonized by pretreatment with naloxone and this confirms the presence of mu receptors in the intestinal tract of the

rat^(4,5).

Apart from its effect on mu receptors, morphine also releases 5-HT^(3,6) and inhibits the release of acetylcholine from cholinergic endings in the gastrointestinal tract. 5-HT is stored partly in the mucosal enterochromaffin cells of the gut and partly in intramural nerve plexuses. Some workers⁽⁹⁾ have shown that it plays the role of neurotransmitter in the regulation of normal gastrointestinal motility.

In order to investigate the role of 5-HT receptors in morphine-induced reduction in intestinal transit in rats, a number of serotonin antagonists were used. These included ketanserin, a selective 5-HT₂ receptor antagonist, which has a very high affinity for 5-HT₂ binding sites but not for 5-HT₁ sites⁽¹⁰⁾. These 5-HT₂ binding sites in the rat appear to be identical to the previously described D-type of 5-HT receptors⁽¹¹⁾ which have been found in the ileum of the guinea pig⁽¹²⁾.

The present investigation has shown that pretreatment with ketanserin antagonises morphine-induced reduction in gastrointestinal transit. The fact that this is ineffective at the highest dose of morphine (10 mg/kg) suggests that morphine overcomes the blockade of 5-HT₂ receptors by ketanserin and thus suggests that the blockade is surmountable in nature.

Intrathecal morphine is also effective in freshly prepared rats with implanted intrathecal catheter, but sufentanil is effective in chronically implanted catheterized rats. The effect of sufentanil was significantly antagonized by prior treatment with ketanserin which indicates involvement of 5-HT₂ receptor, probably at peripheral levels.

Methysergide has been shown to possess both antagonistic, and agonistic⁽¹³⁾ actions with regard to 5-HT. Furthermore, it has recently been shown that methysergide binds to 5-HT₁ and 5-HT₂ sites^(10,14)

and blocks 5-HT₁ receptors at doses equal to, or higher than, those which effect 5-HT₂ receptors. The present investigation seems to indicate that methysergide is not as active a blocker as ketanserin with respect to 5-HT₂ receptors, or that higher doses are required than those used in this study.

The third 5-HT antagonist used in these experiments was MDL 72222. This has been shown to block 5-HT-induced reflex bradycardia, the Bezold-Jarish reflex⁽¹⁵⁾, but it was unable to completely antagonize the morphine-induced reduction in gastrointestinal transit in the present investigation. As with methysergide it was only able to antagonize the lowest dose of morphine.

To summarise, this study has demonstrated that morphine-induced reduction in gastrointestinal transit in the rat is mediated through 5-HT₂ receptors in the intestine. However, more work is necessary to define any minor role played by other 5-HT receptors.

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5-羟色胺受体在大鼠的麻醉品致胃肠转运减缓中的作用

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提要 麻醉药品能特别地引起便秘。吗啡便是已知通过释放 5-羟色胺而减缓胃肠道转运。Ketanserin, 美舍吉特, MDL 72222 等很多 5-HT 拮抗剂用以观察 5-HT 受体对吗啡引起的肠推进延缓的作用。皮下注射 ketanserin 5 mg/kg 可以看到拮抗吗啡对肠运转的影响。由于 ketanserin 是 5-HT₂ 受体的特异阻断剂, 可见吗啡是通过激活 5-HT₂ 受体而起此作用的。由于

经 ketanserin 预处理可以拮抗鞘内注射舒芬太尼引起的肠转运减缓, 可见此作用也是通过 5-HT₂ 受体产生的。因而本实验提出, 肠内 5-HT₂ 受体负责胃肠转运减缓功能。

关键词 吗啡; 纳洛酮; ketanserin; 血清素受体; 胃肠活动; 大鼠; 鞘内注射; 美吉舍特; MDL 72222

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