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# Effects of a 5-$HT_{2A}$ receptor antagonist, sarpogrelate on thermal or inflammatory pain

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**Abstract**

The effects of intrathecally and systemically administered 5-hydroxytriptamine (5-$HT)_{2A}$ receptor antagonist, sarpogrelate on acute thermal or formalin induced pain were examined. Male Sprague–Dawley rats with lumbar intrathecal catheters were tested with their tail withdrawal response to thermal stimulation (tail flick test) or their paw flinching and shaking response by subcutaneous formalin injection into the hind paw (formalin test) after intrathecal or intraperitoneal administration of sarpogrelate. 5-$HT_{2A}$ receptor agonist was used to antagonize the effects of sarpogrelate. In the tail flick test, only intraperitoneal administration induced analgesia. In the formalin test, both intrathecal and intraperitoneal administration were analgesic. The analgesic effects were inhibited by pretreatment with 5-$HT_{2A}$ receptor agonist. Motor disturbance and behavioral side effects were not observed. In conclusion, sarpogrelate might be analgesic on inflammatory induced acute and facilitated pain by intrathecal or systemic administration. However, only systemic administration could be effective on thermal induced acute pain.


*Keywords:* Analgesia; Acute pain; Inflammation; Spinal cord; 5-Hydroxytriptamine$_{2A}$ receptor antagonist

## 1. Introduction

Serotonin (5-hydroxytriptamine, 5-HT) plays an important role in nociceptive transmission. In the periphery, 5-HT is a potent proinflammatory and nociceptive mediator in man and in rodents (Sufka et al., 1992). However, intrathecally administered 5-HT was analgesic in the electrical current and tail flick latency tests in rats (Goodchild et al., 1997). Hylden and Wilcox (1983) reported that intrathecally administered low dose of 5-HT might be analgesic while high dose of 5-HT might be algesic. Thus, 5-HT would act as algesic or analgesic depending on the experimental situation. There are many subtypes in the 5-HT receptors. Therefore, these inconsistent results of a non-subtype specific 5-HT might be due to the different subtype of the receptors on which different stimuli worked.

The 5-$HT_{2A}$ receptor is involved in 5-HT induced hyperalgesia in acute injury and inflammation in the rat (Tokunaga et al., 1998). The 5-$HT_{2A}$ receptor antagonist, when peripherally administered, suppressed the expression of Fos-like immunoreactivity in the dorsal horn of the spinal cord caused by peripheral 5-HT (Doi-Saika et al., 1997). In contrast, intrathecally administered 5-$HT_2$ receptor agonist has been found to produce analgesic effects (Eide and Hole, 1991), while Xu et al. (1994) did not find evidence for spinal 5-$HT_2$ receptor involvement in analgesia. Thus, whether the 5-$HT_{2A}$ receptors are nociceptive or antinociceptive is still controversial. In the present study, to explore whether 5-$HT_{2A}$ receptors are algesic or analgesic, the effects of intrathecally and systemically administered 5-$HT_{2A}$ receptor antagonist, sarpogrelate on acute thermal or acute and facilitated formalin induced pain were examined with or without pretreatment of 5-$HT_{2A}$ receptor agonist using rats.

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## 2. Materials and methods

The protocol was approved by the Research Committee of the University of Tokyo. In each dose group, eight randomly selected rats were enrolled after exclusion. Each rat was used only once.

### 2.1. Animal preparation for intrathecal experiment

Male Sprague–Dawley rats (280–300 g, Nippon Bio-Supply, Tokyo, Japan) were implanted with chronic lumbar intrathecal catheters under halothane (2%) anesthesia. An 8.5 cm polyethylene (PE-10; Clay Adams, Parsippany, NJ, USA) catheter was advanced caudally through an incision in the atlanto-occipital membrane, to the thoracolumbar level of the spinal cord. The external part of the catheter was tunneled subcutaneously to exit on the top of the skull and plugged with a 28G stainless steel wire. After surgery, all rats were housed individually in a temperature- and light-controlled environment with free access to food and water. Only rats with normal motor function and behavior 7 days after surgery were used. The position of the catheter was confirmed to be in the intrathecal space at lumbar enlargement by exposing the lumbar spinal cord after sacrificing the animals at the end of the study. The data of the rats with the catheter was not in the proper place were excluded from the study.

### 2.2. Drugs for intrathecal experiment

Sarpogrelate (Mitsubishi Pharma, Co. Ltd, Tokyo, Japan) 1, 10, 100, and 1000 μg were dissolved in 10 μl distilled water and kept at 4 °C until just before administration. 1000 μg per 10 μl distilled water was the maximum soluble dose. Alpha-methyl-5-hydroxytryptamine (α-methyl-5-HT, 5-$HT_{2A}$ receptor agonist, Sigma, St. Louis, MO, USA) 100 μg was dissolved in 10 μl saline. Sarpogrelate 1, 10, 100, 1000 μg, distilled water (control), or α-methyl-5-HT 100 μg followed by sarpogrelate 1000 μg 5 min later was intrathecally administered. After intrathecal drug injection, the catheter was flushed with a subsequent injection of 10 μl of water to clear the dead space of the catheter (8±0.9 μl, mean±S.D.). Micro injector syringes were used for all injections.

### 2.3. Tail flick test

For the tail flick test, the rats were placed in a clear plastic cylindrical cage with their tail extending through a slot provided in the rear of the cylinder. Noxious stimulation was provided by a beam of high intensity light (Tail-flick Analgesia Meter MK-330A, Muromachi Kikai Co. Ltd., Tokyo, Japan) focused on the tail 2 to 3 cm proximal to the end. The response time was measured and defined as the interval between the onset of the thermal stimulation and the abrupt flick of the tail. The cut-off time in the absence of a response was set to 14 s, to prevent burn injury. The response time was measured before (control), 5, 10, 15, 30, 60, 90, and 120 min after the intrathecal administration of the agent, and thereafter every 1 h until the response time returned to near the baseline (control).

### 2.4. Formalin test

For the formalin test, 10 min after the intrathecal administration of the agent, 50 μl of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30 G needle. Immediately after injection, the rat was placed in an open Plexiglas chamber and observed for 60 min. Quantification of pain behavior was made by counting the incidence of spontaneous flinches/shaking of the injected paw for 1 min at 1–2 min, 5–6 min and at 5 min intervals during a period of 10–60 min after formalin injection. Two distinct phases were observed after formalin injection: phase 1, during 0–6 min interval after injection, and phase 2, beginning about 10 min after injection.

### 2.5. Intraperitoneal experiment

Male Sprague–Dawley rats (280–300 g) received intraperitoneal injection of sarpogrelate 0.3, 3, or 30 mg in 300 μl distilled water, 300 μl distilled water (control) or α-methyl-5-HT 3 mg in 300 μl saline followed by sarpogrelate 30 mg in 300 μl distilled water 5 min later using a plastic syringe with 30 G needle. The tail flick test and the formalin test were performed as intrathecal experiment.

### 2.6. Behavioral and motor function test

The general behavior (including agitation and allodynia-like behavior), motor function, flaccidity, pinna reflex, and corneal reflex were examined in the rats for the tail flick test in both intrathecal and intraperitoneal experiments. They were judged as present or absent. Agitation was judged as spontaneous irritable movement and/or vocalization. The presence of allodynia-like behavior was examined by looking for agitation (escape and/or vocalization) evoked by lightly stroking the flank of the rat with a small probe. Motor function was evaluated by the placing/stepping reflex and the righting reflex. The former was evoked by drawing the dorsum of either hind paw across the edge of the table. Normally rats try to put the paw ahead into a position to walk. The latter was assessed by placing the rat horizontally with its back on the table, which normally gives rise to an immediate, coordinated twisting of the body to an upright position. The disturbance of the righting reflex also shows impairment of function of central nervous system. Flaccidity was judged as muscle weakness by putting the fore paw 3 to 5 cm higher than the hind paw. Normally the rat will walk up. We judged the rat flaccid when rat did not move after positioning. Pinna and corneal reflexes were examined with a paper string. When a paper string was put into the ear canal or touched the cornea, rats normally shake their head or blink, respectively.

### 2.7. Data analysis

Data are expressed as mean±S.D. or 95% confidence interval (CI). In the tail flick test, the data were shown as the % maximum possible effect: = (post drug time − pre drug time) × 100/(cut off time − pre drug time). The 50% effective dose ($ED_{50}$) values were calculated using the % maximum possible effect in the tail flick test and using the area under the curve of the number of the flinches and time in the formalin test by the computer programs made in the Anesthesiology Laboratory of University of California, San Diego (Takano, personal communication). This program has already been used in other studies (Nishiyama and Hanaoka, 2001, 2004). The $ED_{50}$ was shown as mean with 95% confidence interval.

## 3. Results

### 3.1. Intrathecal experiment

No dose dependent effects on the tail withdrawal latency were observed in the tail flick test (Fig. 1). Even the maximum available dose (1000 μg/10 μl) had the effect of less than 50% maximum possible effect.

Dose dependent decreases of the flinch response in both phase 1 and 2 of the formalin test were observed (Fig. 1). The $ED_{50}$ doses were 94.4 μg (95% CI, 18.7–346.2 μg) in the phase 1 and 306.1 μg (95% CI, 12.0–750.0 μg) in the phase 2.

Pretreatment with α-methyl-5-HT inhibited the effects of sarpogrelate in both the tail flick test and the formalin test (Fig. 1).

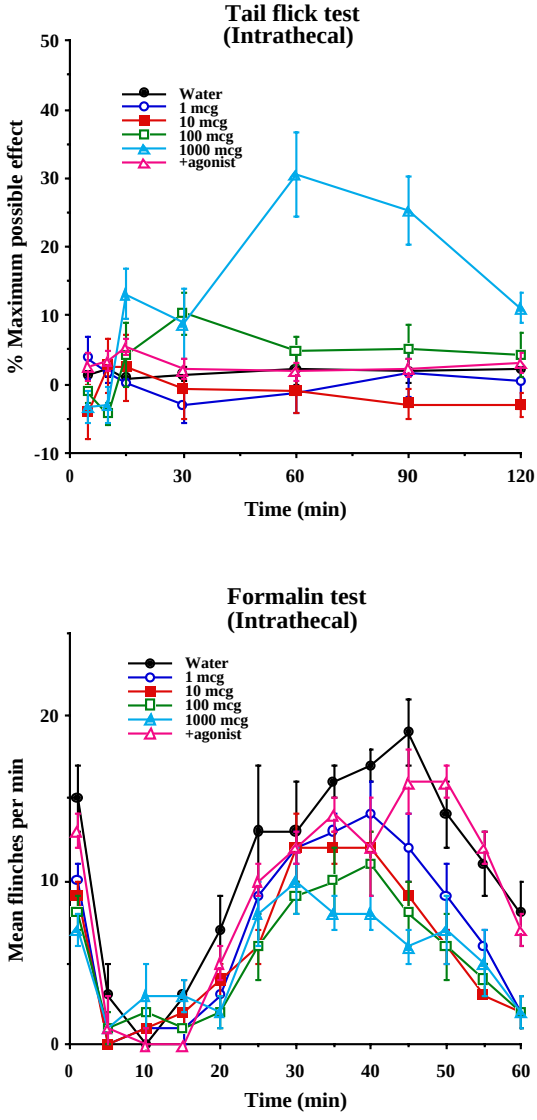


Fig. 1. Time course of the effects of intrathecal sarpogrelate on the tail flick test (upper) and the formalin test (lower). Each point represents mean±S.D. of eight animals. +agonist, α-methyl-5-HT 100 μg followed by sarpogrelate 1000 μg 5 min later.

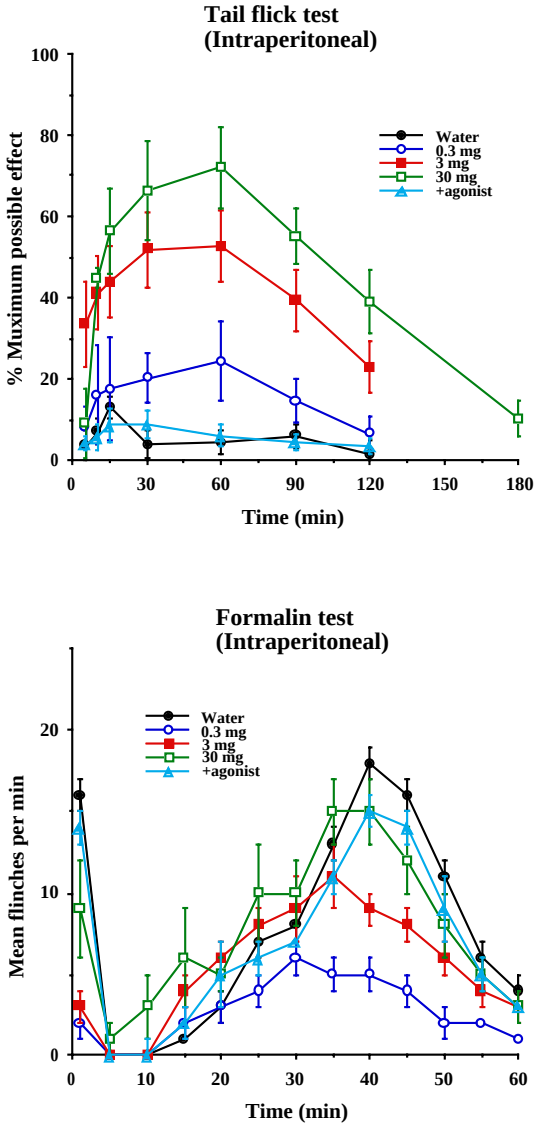


Fig. 2. Time course of the effects of intraperitoneal sarpogrelate on the tail flick test (upper) and the formalin test (lower). Each point represents mean±S.D. of eight animals. +agonist, α-methyl-5-HT 3 mg in 300 μl saline followed by sarpogrelate 30 mg in 300 μl distilled water 5 min later.

### 3.2. Intraperitoneal antinociceptive experiment

Dose dependent increase of the tail withdrawal latency was observed in the tail flick test (Fig. 2). The $ED_{50}$ dose was 2.0 mg (95% CI, 0.9–4.4 mg).

Dose dependent decreases of the flinch response in both phase 1 and 2 of the formalin test were observed (Fig. 1). The $ED_{50}$ doses were 0.49 mg (95% CI, 0.16–4.89 mg) in the phase 1 and 15.9 mg (95% CI, 3.7–37.6 mg) in the phase 2.

Pretreatment with α-methyl-5-HT inhibited the effects of sarpogrelate in both the tail flick test and the formalin test (Fig. 2).

### 3.3. Behavioral and motor function test

With the doses used in this study (up to 1000 μg in intrathecal and up to 30 mg in intraperitoneal administration), no observable motor disturbance or behavioral side effects were seen.

## 4. Discussion

In the present study, a 5-$HT_{2A}$ receptor antagonist, sarpogrelate had analgesic effects on both phase 1 and 2 of the formalin test by both intrathecal and intraperitoneal administration. However, only intraperitoneal (not intrathecal) administration was effective in the tail flick test. These analgesic effects were inhibited by pretreatment with a 5-$HT_{2A}$ receptor agonist. Therefore, these effects were mediated by 5-$HT_{2A}$ receptor.

The 5-HT by itself or together with other mediators produces pain behavior such as lifting and licking in rats, and that 5-$HT_{2A}$ receptors are responsible for it (Abbott et al., 1996). Immunologically, 5-$HT_{2A}$ receptor was found on the axon of primary afferent neurons (Carlton and Coggeshall, 1997) and dorsal horn of the spinal cord (Pierce et al., 1996). 5-HT binds to 5-$HT_{2A}$ receptors and activates adenyl cyclase to produce cyclic adenine monophosphate (cAMP). It activates protein kinase A and close $K^+$ channel which depolarizes nociceptors and increases pain sensation (Taiwo and Levine, 1991). 5-$HT_{2A}$ receptor exists also on vascular smooth muscles and platelets and induces vascular constriction and platelet aggregation (Hara et al., 1991). These also contribute to pain.

In the study by Obata et al. (2000), intrathecal administration of sarpogrelate in rats did not show analgesic action, while intraperitoneal and local administration decreased flinching response in both phase 1 and 2 of the formalin test. However, their doses used for intrathecal administration were 1–100 μg, which might be too small to induce sufficient analgesic effects considered from the present results. Systemic administration of sarpogrelate produced analgesic effects on thermal hyperalgesia caused by peripheral inflammation (Okamoto et al., 2002). In the formalin test, the first phase seems to be caused predominantly by direct activation of C fibers, while the second phase is dependent on the combination of an inflammatory reaction in the peripheral tissues and changes in the dorsal horn functions of the spinal cord (Shibata et al., 1989). Sarpogrelate might prevent the binding of 5-HT to 5-$HT_{2A}$ receptor in the peripheral sensory terminals and inhibits the excitation of primary sensory neurons. Therefore, it is suggested that sarpogrelate inhibits excitatory neurotransmission of the 5-HT axons at the injured tissue, then inhibits afferent inputs to the spinal cord (Nakanishi and Ishikawa, 2001). Sarpogrelate may also inhibit the release of 5-HT from aggregated platelets, a step that is involved in the cascade of inflammatory and hyperalgesic processes (Page, 1989). Interleukin 6 production by 5-HT in human vascular smooth muscle cells was significantly inhibited by sarpogrelate (Ito et al., 2000). These might be the mechanism of the analgesic effects of intrathecal and intraperitoneal administration of sarpogrelate in the formalin test observed in the present study.

Systemic administration of ketanserine, another selective 5-$HT_{2A}$ receptor antagonist, was shown to produce analgesia in the hot plate test in mice (Alhaider, 1991). Peripherally acting 5-$HT_{2A}$ antagonists may be effective in reducing pain of peripheral origin (Carlton and Coggeshall, 1997). These reports are consistent with the present results showing analgesic effects of intraperitoneal administration of sarpogrelate in the tail flick test. Intraperitoneally administered sarpogrelate would have its effect in the periphery. However, intrathecal administration of sarpogrelate did not induce analgesia in the tail flick test in the present study. Intrathecal injection of 5-$HT_2$ receptor agonists is reported to mediate analgesia in rat colorectal distension models (Danzebrink and Gebhart, 1991). 5-$HT_2$ receptor agonist activated γ-aminobutyric acid (GABA) ergic interneurons in the rat prefrontal cortex (Abi-Saab et al., 1999) and probably also in the spinal cord. Therefore, it might be possible that in some pain stimuli, 5-$HT_2$ receptor agonist is analgesic through this mechanism.

After oral administration of sarpogrelate to rats, the peak sarpogrelate concentration in the brain and spinal cord was about 2% of the plasma concentration (Komatsu et al., 1991). Thus, sarpogrelate has very low permeability of the blood–brain barrier. Therefore, intrathecally administered sarpogrelate acts only in the brain or spinal cord, where 5-$HT_2$ receptor agonists would be analgesic, and does not go into systemic circulation and periphery. These are the reason why only intraperitoneal not intrathecal administration of sarpogrelate had analgesic effects in the tail flick test.

In conclusion, sarpogrelate, a 5-$HT_{2A}$ receptor antagonist might be analgesic on inflammatory induced acute and facilitated nociception in the periphery by intrathecal or systemic administration. However, only systemic not intrathecal administration could be effective on thermal induced acute pain in the periphery. Motor disturbance or behavioral side effects would not occur with the doses of sarpogrelate used in the present study.

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