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Antinociception in rat by sarpogrelate, a selective 5-HT_{2A} receptor antagonist, is peripheral

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Abstract

The antinociceptive effect of sarpogrelate, a new selective 5-hydroxytriptamine (5-$HT)_{2A}$ receptor antagonist, in the formalin test was examined in rats. Sarpogrelate was administered intraperitoneally, locally (subcutaneously at the formalin test site) or intrathecally 10 min before formalin injection. Intraperitoneal (1–100 mg/kg) and local (0.01–1 mg) administration of sarpogrelate suppressed flinching behavior in both phases 1 (0–9 min) and 2 (10–60 min) in a dose-dependent manner. Intraperitoneal (100 mg/kg) and local (1 mg) injection 7 min after formalin injection reduced phase 2 flinches to the same degree as with the pre-treatment. Intrathecal administration (1–100 μg) showed no antinociceptive action, and facilitated phase 2 flinches at 10 μg. The plasma concentration of sarpogrelate after local administration of 1 mg was lower than after intraperitoneal administration of 10 mg/kg, although local administration produced more potent antinociception. The data imply that the antinociceptive effect of sarpogrelate results mainly from an action at peripheral sites.

Keywords: Sarpogrelate; 5-HT_{2A} receptor antagonist; Antinociception; Formalin test; (Rat)

1. Introduction

The 5-hydroxytriptamine (5-HT) plays an important role in nociceptive transmission. Intrathecally administered 5-HT shows antinociceptive effects in acute pain models in rats (Yaksh and Wilson, 1979; Schmauss et al., 1983; Bardin et al., 1997). In contrast, peripheral injection of 5-HT facilitates pain responses (Hong and Abbott, 1994). The 5-HT receptors are divided into several subgroups. Among these receptor subtypes, the 5-HT_2 receptor is crucial for pain modulation. This subtype is widely distributed in peripheral tissues including platelets and is also present in the central nervous system (Hoyer et al., 1994). The 5-HT_2 receptor has a localization that subserves nociception and analgesia. Local administration of selective 5-HT_2 receptor antagonists produces antinociception in the rat formalin test (Abbott et al., 1996), and intradermal injection of a 5-HT_2 receptor agonist into the rat hindpaw produces hyperalgesia in the paw (Tokunaga et al., 1998). In contrast, intrathecal injection of 5-HT_2 receptor agonists mediates antinociception in rat acute pain models (Solomon and Gebhart, 1988; Danzebrink and Gebhart, 1991).

Sarpogrelate {MCI-9042, or (*R*,*S*)-1-[2-[2-(3-methoxyphenyl)ethyl]phenoxyl-3-(dimethylamino)-2-propyl hydrogen succinate hydrochloride] is a new 5-HT_2 receptor antagonist that was first introduced as a therapeutic agent for ischemia associated with thrombosis. In radioligand binding studies, this drug shows specificity for 5-HT_{2A} receptors (Maruyama et al., 1991; Nishio et al., 1996), a result confirmed by studies using in vitro functional assay systems (Pertz and Elz, 1995). Kikumoto et al. (1990) proved that the effect of sarpogrelate against platelet aggregation was mediated by its 5-HT_{2A} receptor-blocking properties. Furthermore, sarpogrelate inhibits 5-HT release accompanying collagen-induced platelet aggregation (Hara et al., 1991). The drug shares both these properties with the classical 5-HT_{2A} receptor antagonist, ketanserin (De-Clerck and Xhonneux, 1985), which also has weak activity

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for α_1-adrenoceptor blocking. A radioligand binding assay in rat brain showed that, while the displacement potency of sarpogrelate for the [^{3}H]ketanserin binding site was approximately 8% of that of ketanserin, the α_1-adrenoceptor-blocking activity of sarpogrelate was only 1% of that of ketanserin (Maruyama et al., 1991). Oral administration of sarpogrelate was reported to reduce chronic pain in patients with complex regional pain syndrome (Otake et al., 1998). However, the detailed mechanisms underlying the analgesic effects of this drug are not clear. We therefore investigated, using the formalin test, the antinociceptive effect of sarpogrelate administered to rats by different routes.

2. Materials and methods

The study was approved by the Animal Care and Use Committee at our institution. Male Wistar rats weighing 280–350 g were used.

2.1. Animal preparation

For intrathecal administration of drugs, an indwelling intrathecal catheter was inserted as previously described (Yaksh and Rudy, 1976). Under isoflurane anesthesia, this polyethylene catheter (PE-10) was advanced 8.5 cm caudally from the cisterna magna to the T12-L1 spinal cord

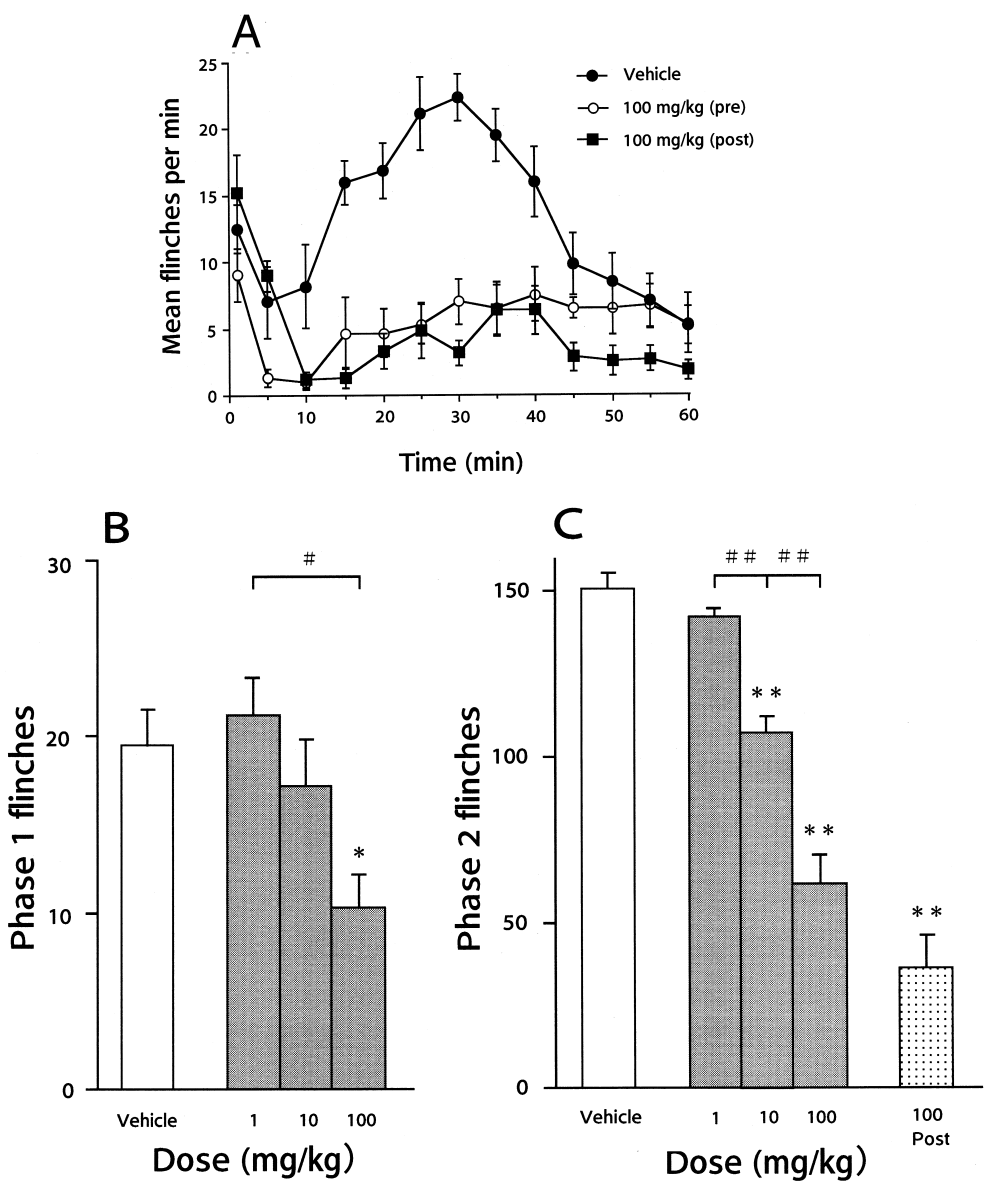

Fig. 1. Antinociceptive effect of intraperitoneal administration of sarpogrelate to rats. (A) Time course of effect of drug administered 10 min before (pre) or 7 min after (post) formalin injection. (B and C) Cumulative scores indicating dose-related antinociception produced by intraperitoneal administration of sarpogrelate as changes in phase 1 (B) and phase 2 (C) flinching. Data are expressed as the means ± S.E.M. ($n = 6$ for each group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle group. $\#P < 0.05$, $\#\#P < 0.01$ between two groups.

level at the upper portion of the lumbar enlargement. The catheter was externalized at the vertex and the wound was closed with 3-0 silk sutures. We used catheterised rats in which flaccid paralysis of the hind limbs was observed after intrathecal administration of lidocaine (500 μg in 10 μl). Rats showing neuromuscular dysfunction were killed immediately. The animals were allowed to recover for 7 days before experiments.

2.2. Behavioral analysis

The general behaviour of the rats was carefully monitored and scored as described previously (Malmberg et al., 1995; Hwang et al., 1999). Basically, motor function was evaluated with two tests. For the placing reflex, the dorsal surface of the hindpaws was brought into contact with the edge of a table. In normal rats, this stimulus elicits upward lifting of the paw onto the surface of the table. For the righting reflex, the rat was placed on its back on a flat table. A rat normally will show immediate rolling movements to regain its normal position. Sedation was also assessed in terms of spontaneous movement such as grooming, chewing and ambulation, as well as evoked movement (a startle reflex evoked by tapping on the cage). Behavioral changes were scored as follows: score 0, no change; score 1, mild change; score 2, moderate to severe change (Hwang et al., 1999).

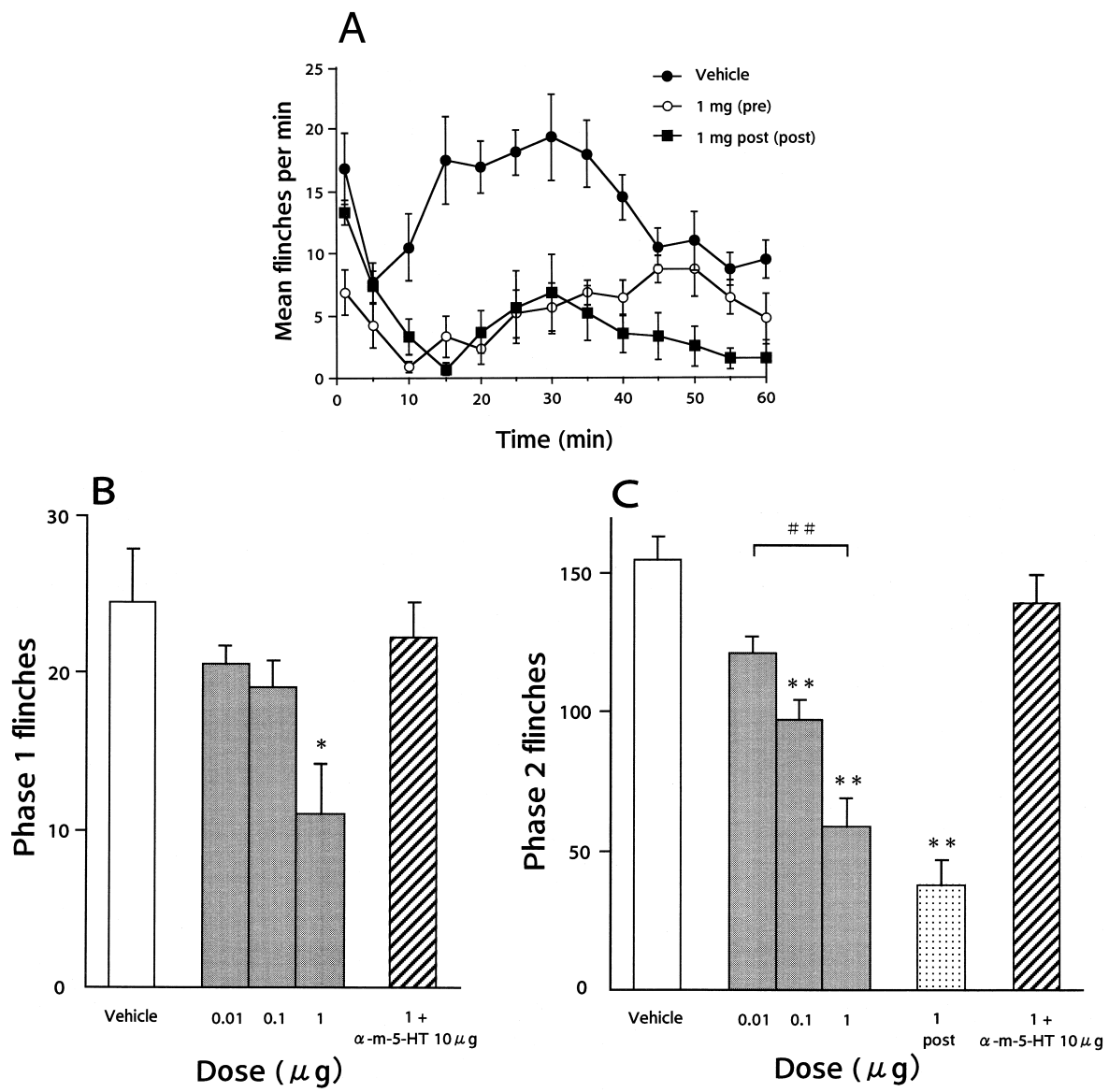

Fig. 2. Antinociceptive effect of local administration of sarpogrelate to rats. (A) Time course of effect of drug administered 10 min before (pre) or 7 min after (post) formalin injection. (B and C) Cumulative scores indicating dose-related antinociception produced by local administration of sarpogrelate as changes in phase 1 (B) and phase 2 (C) flinching. Columns filled by diagonal lines indicate coadministration of sarpogrelate (1 mg) with the 5-HT_2 receptor agonist α-methyl-5-HT (10 μg). Data are expressed as the means ± S.E.M. ($n = 6–7$ for each group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle group. $\#\#P < 0.01$ between two groups.

2.3. Formalin test

Rats were injected subcutaneously with 50 μl of 2.5% formalin in the plantar surface of the right hindpaw with a 27-gauge needle. Immediately after injection, the rat was placed in an open Plexiglas box (10 × 20 × 24 cm), which permitted observation. Within 1 min after formalin injection, spontaneous flinching of the injected paw could be observed. Flinching was readily identified and was characterized as rapid brief withdrawal by flexion of the injected paw. Pain perception was quantified as described previously (Yamamoto and Yaksh, 1992; Malmberg et al., 1995) by periodically counting occurrences of spontaneous flinching of the injected paw for 1-min periods at 1–2 and 5–6 min (phase 1) and then at 5-min intervals from 10 to 60 min after formalin injection (phase 2). After the observation period, the animals were immediately killed with an overdose of barbiturate.

2.4. Drugs and injections

In pre-treatment experiments (10 min before formalin injection), sarpogrelate (gift from Mitsubishi-Tokyo Pharmaceuticals, Tokyo, Japan) was administered intraperitoneally, locally (subcutaneously at the formalin test site) or intrathecally. For intraperitoneal administration, doses

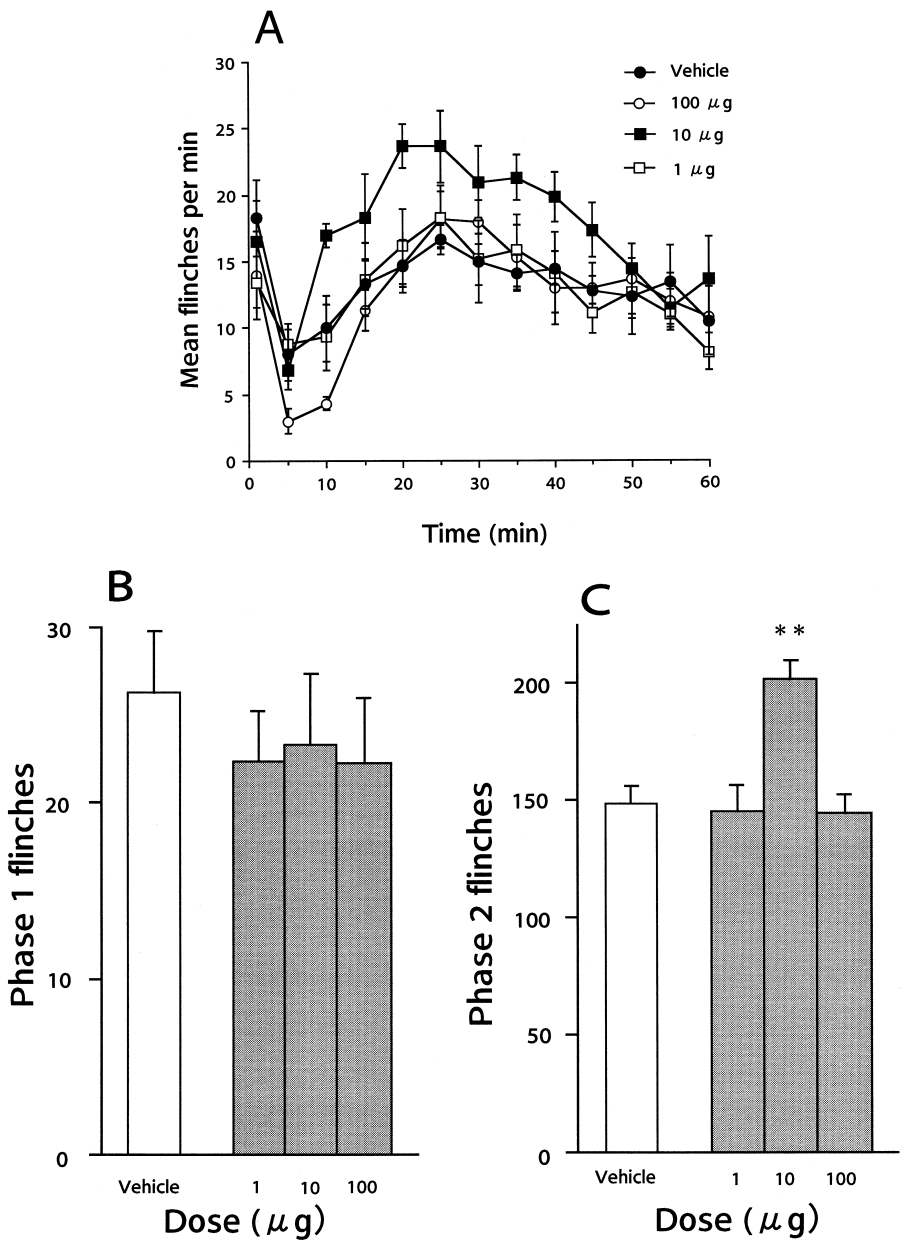

Fig. 3. Effect of intrathecal administration of sarpogrelate to rats. (A) Time course of effect of drug administered 10 min before formalin injection. (B and C) Cumulative scores indicating the effect produced by intrathecal administration of sarpogrelate as changes in phase 1 (B) and phase 2 (C) flinching. Data are expressed as the means ± S.E.M. ($n = 6$ for each group). $^{**}P < 0.01$ compared to vehicle group.

of 1, 10 or 100 mg/kg were given in a volume of 0.5 ml. For local administration, doses of 0.01, 0.1 or 1 mg were administered in a volume of 20 μl with a 30-gauge needle. For intrathecal administration, doses of 1, 10 or 100 μg were injected through the intrathecal catheter in a volume of 10 μl followed by 10 μl of saline to flush the catheter. For local administration, the drug was dissolved in 40% dimethylsulfoxide (DMSO) and 60% saline. For other routes, the drug was dissolved in 10% DMSO and 90% saline. If the drug showed antinociceptive action, post-treatment studies were performed 7 min after formalin injection with the highest dose used in the pre-treatment study. The opposing effect of a selective 5-HT_2 receptor agonist, α-methyl-5-hydroxytryptamine maleate (α-methyl-5-HT; Research Biomedical, Natick, MA, USA), was studied by coadministration with sarpogrelate. As a control, the vehicle was injected intraperitoneally, locally or intrathecally.

2.5. Plasma concentration assay of sarpogrelate

To evaluate the systemic absorption of locally administered sarpogrelate, we compared the plasma sarpogrelate concentration after local administration of 1 mg to that after intraperitoneal injection of 10 mg/kg. Blood samples were obtained before and at 10, 30 and 60 min after administration. These were centrifuged at 2000 rpm for 15 min, and plasma was frozen at −20°C. The plasma concentration of sarpogrelate were measured by high-performance liquid chromatography (HPLC; L-7100, Hitachi, Tokyo, Japan) after deproteinization.

2.6. Statistical analysis

Time–response data are expressed as the mean number of flinches (±S.E.M.) per minute for the period from 1 to 2 min, that from 5 to 6 min, and then for 1-min periods at 5-min intervals up to 60 min. For statistical analysis, data from phase 1 and phase 2 were considered independently. To compare the cumulative number of flinches between groups, one-way analyses of variance (ANOVA) with Scheffe's multiple comparison test were used. Plasma concentrations of sarpogrelate in each group were analyzed independently with the same method. Behavioral scores were analyzed with the Kruskal–Wallis test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Behavioral analysis

Intraperitoneal, local or intrathecal administration of sarpogrelate had no effect on the placing reflex or the righting reflex at the doses employed in this study. Intraperitoneal administration of 100 mg/kg of sarpogrelate had a minor sedative effect, which consisted of attenuated spontaneous movement. A normal brisk startle reflex still was elicited after drug administration. Each animal in the group showed score 1 level sedation ($n = 6$). This value was significantly different from those for the other groups (in the other groups, the scores were zero for all animals).

3.2. Formalin test

In rats receiving vehicle, subcutaneous injection of formalin into the hindpaw resulted in biphasic flinching behavior. In the pre-treatment study, intraperitoneally administered sarpogrelate showed significant antinociceptive effects in phase 1 at 100 mg/kg (Fig. 1B) and in phase 2 at 10 and 100 mg/kg (Fig. 1C) compared to the vehicle group. Higher doses of the drug had more potent antinociceptive effects (Fig. 1B and C). Post-treatment with the highest dose similarly decreased phase 2 responses (Fig. 1A and C). No significant difference was evident between pre- and post-treatment with 100 mg/kg of the drug.

Local pre-treatment with sarpogrelate suppressed both phases 1 and 2 flinches in a dose-dependent manner at doses of 0.01–1 mg (Fig. 2B and C). Significant suppressive effects were shown in phase 1 at 1 μg (Fig. 2B) and in phase 2 at 0.1 and 1 μg (Fig. 2C) against the vehicle. Post-treatment with the highest dose also reduced phase 2 flinching (Fig. 2A and C). No significant difference was found between pre-treatment and post-treatment with 1 mg locally. Antinociceptive effects of local injection of sarpogrelate 1 mg on phases 1 and 2 were countered by coadministration of 10 μg of the agonist, α-methyl-5-HT (Fig. 2B and C).

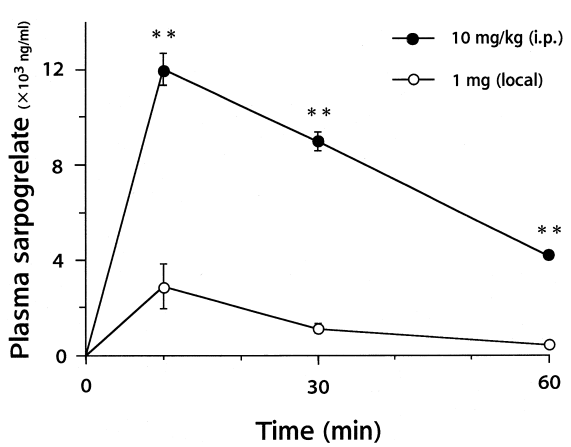

Fig. 4. Plasma levels of sarpogrelate at various times following intraperitoneal administration (10 mg/kg, filled circles) and local administration (1 mg, open circles). Data are expressed as the means ± S.E.M. values ($n = 3$ for each group). $^{**}P < 0.01$ between groups.

Intrathecal injection of sarpogrelate had no antinociceptive effect at doses of 1–100 μg, and it significantly facilitated phase 2 flinching at 10 μg (Fig. 3A to C).

3.3. Plasma concentration of sarpogrelate

The plasma concentration of sarpogrelate after local administration of 1 mg was lower than after intraperitoneal administration of 10 mg/kg at 10, 30 and 60 min after administration (Fig. 4).

4. Discussion

The data showed that systemic administration of sarpogrelate to rats produced antinociception in the formalin test. While intrathecal injection of the drug had no antinociceptive effect, local administration was followed by antinociception. This local antinociceptive effect was completely abolished by coadministration of a 5-HT_2 receptor agonist, indicating that the antinociceptive effect of sarpogrelate was mainly dependent on peripheral mechanisms.

In the periphery, 5-HT is a potent proinflammatory and nociceptive mediator in man and in rodents (Armstrong et al., 1953; Sufka et al., 1992). The peripheral source of 5-HT is platelets, which are known to play an important role in inflammatory processes in humans (Page, 1989). Intracutaneous injection of platelets provokes acute pain and hyperalgesia in humans (Schmelz et al., 1997). Hong and Abbott (1994) reported that, in rats, intraplantar injection of 5-HT induces dose-dependent pain-related behavior. These authors found that 5-HT produced a synergistic increase in pain behavior when combined with other mediators such as bradykinin, prostaglandin E_2, substance P, noradrenalin or histamine. Abbott et al. (1996) reported that pain behavior produced by intraplantar injection of 5-HT plus prostaglandin E_2 was attenuated by local pretreatment with ketanserin in rats. Furthermore, the second phase of the formalin test was suppressed by local pretreatment with 5-HT_2 receptor antagonists such as ketanserin, ritanserin and spiperone. Their results imply that 5-HT_2 receptor antagonists act in the periphery and should be effective against pain associated with 5-HT release from platelets. Our data obtained after local administration of a more selective 5-HT_2 receptor antagonist were consistent with their findings. One possibility is that sarpogrelate may mediate antinociception by inhibiting 5-HT release from aggregating platelets by binding at the 5-HT_{2A} receptor on the surface of platelets. Recently, Carlton and Coggeshall (1997) raised another possibility by demonstrating the presence of 5-HT_{2A} receptors on unmyelinated and large myelinated axons in the rat glabrous skin. Tokunaga et al. (1998) detected 5-HT_2 receptor mRNA by in situ hybridization in both large and small neurons of rat dorsal root ganglia. They suggested that 5-HT_{2A} receptors localized at the peripheral nerve terminals of these neurons mediated the hyperalgesia produced by intraplantar injection of α-methyl-5-HT. A second possibility is that sarpogrelate acts at 5-HT_{2A} receptors at peripheral nerve terminals of primary afferents and induces analgesia.

Supraspinal roles of the 5-HT_2 receptor in nociceptive transmission are not clear. Barber et al. (1989) found that a 5-HT_2 receptor antagonist, ritanserin, mediated analgesia in the writhing test when administered subcutaneously to rats. Alhaider (1991) reported that subcutaneously administered ketanserin produced dose-dependent antinociception in the writhing test and the hot-plate test in mice. These authors suggested that the antinociceptive effect of systemically administered 5-HT_2 receptor antagonists involved activation of descending monaminergic inhibitory neurons in the spinal cord. Several lines of evidence indicate that supraspinal 5-HT neurons exert an inhibitory action on noradrenergic neurons in the locus coeruleus (Segal, 1979; Leger and Descarries, 1978; McRae-Degueurce et al., 1985) via 5-HT_2 receptors (Rasmussen and Aghajanian, 1986; Gorea and Adrien, 1988). Antagonists at 5-HT_2 receptors are likely to activate cells in the locus coeruleus by blocking an inhibitory input, thereby mediating analgesia. In a preliminary study with the hot-plate test, used to assess supraspinal analgesia (Ramabadran and Bansinath, 1986), we observed that intraperitoneal administration of 100, but not 30 mg/kg of sarpogrelate produced antinociception at 30 and 60 min after drug injection (unpublished study). In the present study, the higher intraperitoneal doses also had a minor sedative effect. While these effects might be derived from a supraspinal antinociceptive action of the drug, sarpogrelate penetrates the blood–brain barrier only minimally. According to information supplied by the manufacturers, the brain tissue concentration of sarpogrelate was 0.25–0.5% of the plasma concentration, in a tracer experiment using [^{14}C]-labeled sarpogrelate (Mitsubishi Kasei, unpublished data). Therefore, antinociception from intraperitoneal administration of the drug probably involves a mainly peripheral action. This view is supported by our results for sarpogrelate in plasma; the plasma concentration after local administration of 1 mg was lower than that after intraperitoneal administration of 10 mg/kg, while the antinociceptive action of the former was greater than that of the latter.

In the dorsal horn of the spinal cord, activation of 5-HT_2 receptors has been reported to mediate antinociception (Solomon and Gebhart, 1988; Danzebrink and Gebhart, 1991). We now found that spinal administration of sarpogrelate did not mediate antinociception; instead, intrathecal administration of 10 μg of sarpogrelate increased flinching. This phenomenon is consistent with findings that 5-HT_2 receptor agonists mediate analgesia in the spinal cord. We do not know why an intrathecal dose of 10 μg facilitated the formalin response while 100 μg did not, but sarpogrelate may have been redistributed to peripheral tissues following the highest intrathecal dose. In this case,

any spinal nociceptive action of the antagonist could be countered by a peripheral antinociceptive action.

Abbott et al. (1997) found that local post-treatment with three 5-HT_2 receptor antagonists (ketanserin, ritanserin or spiperone) was less effective in the second phase of the formalin test. In our study, intraperitoneal or local post-treatment using the highest pre-treatment dose of sarpogrelate produced potent antinociception similar to that seen in pre-treatment experiments. One possible explanation for the conflicting results may involve the timing of drug administration. We administered sarpogrelate 7 min after formalin injection. In contrast, Abbott et al. injected the drugs 15 min after formalin injection, when the second phase of the formalin response already was in progress. This delay may have resulted in the difference in post-treatment effect.

In summary, the antinociceptive effect of sarpogrelate is mediated mainly by peripheral mechanisms, although some supraspinal action is likely at higher doses. Local application of a selective 5-HT_{2A} receptor antagonist may be effective to treat pain provoked by 5-HT release from platelets, which commonly occurs with injury and inflammation.

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