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The novel analgesic, F 13640, produces intra- and postoperative analgesia in a rat model of surgical pain

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Abstract

F 13640 is a newly discovered high-efficacy 5-HT_{1A} receptor agonist that produces exceptional analgesia in animal models of tonic and chronic, nociceptive and neuropathic pains by novel molecular and neuroadaptive mechanisms. Here we examined the effects of F 13640 and remifentanil (0.63 mg/kg with either compound) when injected i.p. either before or 15 min after rats underwent orthopedic surgery. Surgery consisted of the drilling of a hole in the calcaneus bone and of an incision of the skin, fascia and plantar muscle of one foot. During surgery, the concentration of volatile isoflurane was progressively incremented depending on the animal's response to surgical maneuvers. Other experiments examined the dose-dependent effects of F 13640 (0.04 to 0.63 mg/kg) on surgical pain as well as on the Minimum Alveolar Concentration of isoflurane. Both F 13640 and remifentanil markedly reduced the intra-operative isoflurane requirement. F 13640 also reduced measures of postoperative pain (i.e., paw elevation and flexion). With these postoperative measures, remifentanil produced short-lived analgesia followed by hyperalgesia. F 13640 significantly reduced both surgical pain and the isoflurane Minimum Alveolar Concentration from 0.16 mg/kg onward. F 13640 produced powerful intra- and postoperative analgesia in rats undergoing orthopedic surgery. Unlike the opioid, remifentanil, F 13640 caused no hyperalgesia with ongoing postoperative pain, and should remain effective with protracted postoperative use.

Keywords: Serotonin; 5-HT_{1A} receptor; Orthopedic surgery; Intra-operative pain; Postoperative pain; Opioid hyperalgesia

1. Introduction

We recently reported on the discovery of the central analgesic, (3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone (F 13640) that acts by novel molecular and neuroadaptive mechanisms (Colpaert et al., 2002). F 13640 is a new 5-hydroxytryptamine 1A (5-HT_{1A}) receptor ligand that uniquely associates receptor selectivity with the ability to activate 5-HT_{1A} receptors to a very high degree (for structure and receptor binding and efficacy data, see: Colpaert et al., 2002; Wurch et al., 2003). The discovery of F 13640 was guided by a theory of signal transduction in nociceptive systems which specifies that any input to such systems causes not one effect, but two, dual effects that are paradoxical, or opposite in sign (Colpaert, 1996). Thus, opioids produce not only ("1st order") analgesia, but also "2nd order" hyperalgesia. Accounting also for the dynamical, neuroadaptive actions of opioids, the proposed transduction mechanism further explains how, with chronic opioid use, the 2nd order hyperalgesia grows and makes 1st order analgesia decay (i.e., tolerance to opioid analgesia). F 13640 was identified as an agent that should produce the mirror inverse of the effects of opioids. Indeed, in normal rats, F 13640 causes an initial hyperalgesia followed by analgesia. In thus mimicking the effects of nociceptive stimulation (Colpaert et al., 2002; Buritova et al., 2003), F 13640 initiates two remarkable, unprecedented neuroadaptive actions. First, repeated or chronic F 13640 causes an analgesia that grows, rather than decays. Second, F 13640 cooperates with nociceptive stimulation in, paradoxically, causing analgesia. As the result of these actions, the chronic administration of F 13640 causes an analgesia in

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rodent models of chronic nociceptive pain and neuropathic allodynia, that is superior to that, if any, of morphine and of agents exemplifying further central mechanisms of pain relief (Colpaert et al., 2002; Deseure et al., 2003). In the formalin model of tonic nociceptive pain, F 13640 also produces an extent of analgesia that is rivaled only by opioids (Colpaert et al., 2002; Bardin et al., 2003). The possible action of F 13640 on severe, acute nociceptive pain has not been examined so far.

The research presented here was aimed at investigating F 13640's effects on both the intra- and the postoperative pain that is associated with surgery in the rat. To this end, rats underwent a combination of two surgical interventions that have been described earlier (i.e., incision of skin, fascia and plantar muscle of the foot and drilling of a hole in the calcaneus; Brennan et al., 1996; Houghton et al., 1997). The esterase-metabolised, short-acting μ-opioid remifentanil was also studied, as it is often implemented in human anesthesia (Bürkle et al., 1996; Glass et al., 1999). The experiments thus examined the effects of F 13640 and of remifentanil as the agents were administered either pre- or postoperatively. A further experiment determined F 13640's effects on the Minimum Alveolar Concentration of isoflurane in rats exposed to a standardized nociceptive stimulation.

2. Methods

2.1. Experimental animals

Male Sprague–Dawley rats (Iffa Credo, Lyon, France) weighing 180–200 g on arrival were used after a 4–5 day quarantine period; they were housed individually in wire bottom cages measuring 18×31×18 cm (ambient temperature 21±1 °C; relative humidity 55±5%; reversed 12:12 h dark–light cycle, lights on at 6 a.m.). The European Community guidelines for the use of experimental animals were adhered to; the protocol complies with these guidelines and was approved by the institutional Ethical Committee (n° 246). Throughout, experimenters were blinded to the independent variables.

2.2. Vocalization threshold: dose- and time-effect experiments

These two experiments were conducted to define the dose and the time at which F 13640 and remifentanil were to be implemented in the studies of surgical pain. To this end, the Randall and Selitto (Randall and Selitto, 1957) technique was used, as it can express the in vivo pharmacodynamic actions both of F 13640 (which can produce hyperalgesia in this assay; Colpaert et al., 2002) and of opioids (which can produce analgesia; Zhou et al., 1998). The technique determines the vocalization threshold to acute mechanical stimulation; using a Ugo Basile analgesia-meter (Apelex®; probe tip diameter: 1 mm; weight: 20 g; cut-off pressure: 600 *g*), progressively increasing pressure was applied to the left hind paw until a squeak (vocalization threshold, in g) was obtained.

In one experiment, rats (n=7/group) received an intraperitoneal (i.p.) injection of either saline or one of different doses of remifentanil (0.16, 0.31, 0.63, 2.5 and 10 mg/kg), and the vocalization threshold was determined immediately before as well as at stated intervals (Fig. 1A) for up to 60 min after the injection. In a second experiment, rats (n=6/group) received an i.p. injection of either saline or one of different doses of F 13640 (0.04, 0.16 and 0.63 mg/kg), and the threshold was determined before as well as at stated intervals (Fig. 1B) for up to 4 h after the injection.

2.3. Intra- and postoperative pain

2.3.1. Anesthesia and surgery

Anesthesia was induced by isoflurane 2 vol.% in 2 l/min oxygen delivered in a closed induction box. After 3 min, anesthesia was continued by isoflurane 1.5 vol.% provided via a facemask. At this point, surgery was started. In the case the animal made any movement on surgical stimulation, surgery was stopped, the concentration of isoflurane was increased by 0.5 vol.%, and, after 30 s, surgery was resumed whilst maintaining the rats under this new level of anesthesia. The same procedure was repeated and the anesthesia further increased every time a movement occurred. Note that this fixed, 30 s duration of exposure does not guarantee that equilibrium be

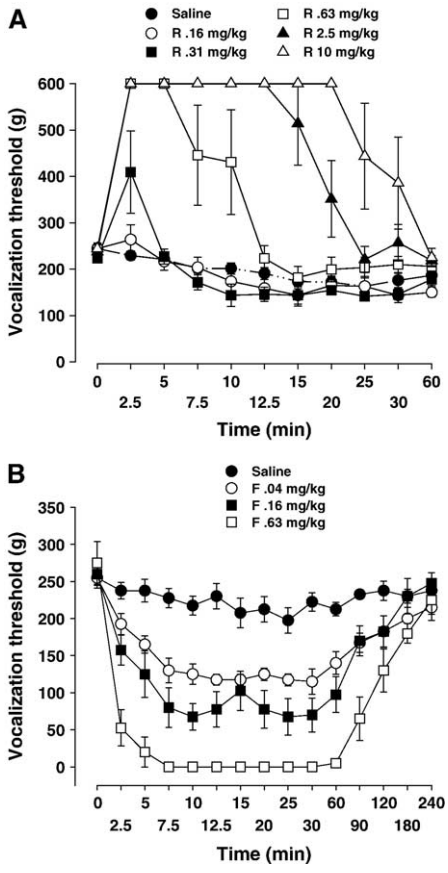

Fig. 1. Dose- and time-dependence of the effects of remifentanil and F 13640 on the mechanical threshold to induce vocalization in rats. In two separate experiments, animals received an i.p. injection of either saline, remifentanil (R; n=7/dose; A) or F 13640 (F; n=6/dose; B), and the threshold for mechanical stimulation (ordinate, in g) to induce vocalization was determined immediately before as well as at stated intervals after the injection. Data are mean±S.E.M.

reached between the alveolar concentration of isoflurane with that in the inspirate; it was implemented here, though, so as to avoid that any large differences would occur between control and drug-treated rats in terms of the overall duration of anesthesia and surgery.

Surgery was carried out under sterile conditions. Desinfection and antibiotic treatment included the topical use of Vetedine® (VETOQUINOL; betadine) preoperatively and local application of Totapen® (Bristol-Myers-Squibb; ampicillin, 0.5 g/5 ml; two drops) as well as Negerol® wound spray (Ceva Santé Animal; chloramphenicol) at the end of the surgery. Two previously described rat models of acute surgical pain were combined. First a perpendicular hole (diameter: 1 mm; 5 mm deep), was made percutaneously through the calcaneus bone of the left hind limb by means of an electric drill (Houghton et al., 1997). This was followed by a 1.5-cm-long incision of the plantar aspect of the skin and fascia of the paw on the same limb, starting 0.5 cm from the edge of the heel. The plantar muscle was elevated and cut 1.0 cm longitudinally (incision model; Brennan et al., 1996). Thus, while the surgery was essentially orthopedic, to more closely model human orthopedic interventions, the surgery also incorporated larger incisional maneuvers than the mere percutaneous penetration by the drill. Surgery was completed by the suture (silk; 4/0) of fascia and skin. Thereafter, rats were allowed to recover from isoflurane anesthesia for 15 min in a 28 °C incubator, and were then placed in an elevated Plexiglas cylinder with a wire mesh bottom. Postoperative observations began from the moment that the animals were placed in the incubator.

2.3.2. Effects of preoperative drug treatment

This experiment examined the effects of remifentanil and of F 13640 administered preoperatively, on intra- and postoperative pain. The 0.63 mg/kg dose was used with both agents, as it was the first dose at which remifentanil and F 13640 produced their ceiling effects in the dose- and time-finding experiments (see: Results. Thus, the agents matched each other in terms of potency, albeit not in terms of duration of action.). The latter also indicated these agents to reach peak effect immediately and 15 min after injection, respectively; therefore, remifentanil and F 13640 here were injected immediately and 15 min before the start of surgery, respectively. As these different pretreatment intervals otherwise would call for the ethically less desirable use of two rather than one saline control group, all animals received two i.p. injections, one given 15 min the other given immediately before the induction of anesthesia. Thus, three different groups (n=10/group) received injections of saline+remifentanil, F 13640+saline and saline+saline, respectively, prior to surgery; a fourth, sham, group, received two saline injections, was anaesthetized and disinfected, but did not undergo surgery.

Intra-operative pain was quantified by the isoflurane concentration that was reached at the end of surgery (i.e., when fascia and skin had been sutured).

Several measurements were made in an effort to assess postoperative pain. As in previous studies of painful surgical (Houghton et al., 1997) or chemical (Abbott et al., 1995) manipulations of the rat's foot, the position of the injured paw was monitored. The paw was considered to be elevated if the foot's plantar surface was not fully in contact with the floor while the animal was at rest. The presence/absence of this elevated paw position was determined every 5 min during the hour that followed (actual/sham) surgery (maximal response: 12). Further observations were made 120, 180 and 240 min after surgery, as well as once daily on postoperative days 1–4.

Limb flexion was monitored as preliminary experiments indicated this response to occur ipsilaterally in operated, but not contralaterally nor in sham-operated animals. Though this response has not been used before as a possible sign of postoperative pain its validity is supported by its prior use with other painful manipulations of the rat's hind limb (Colpaert, 1983; Roughan and Flecknell, 2001). Thus, the number of times per 5 min episode that hind limb flexion occurred was determined at the postoperative intervals specified above.

In preliminary experiments, electronic von Frey (Bioseb) testing was used as elsewhere (e.g., Houghton et al., 1997) to assess mechanical allodynia. During the 24 h post-surgery, the threshold for eliciting paw withdrawal was generally lower in operated as compared to sham animals. However, at the time (i.e., first 2 h) that the difference was most marked, variability was also large, perhaps because of the then-frequent elevation and flexion of the paw confounding the threshold's assessment. Therefore, von Frey testing was not conducted in the experiments reported below.

Additional measurements were the latency (in s) since surgery that the animals recovered the righting reflex and the daily determination of body weight.

2.3.3. Effects of postoperative drug treatment

This experiment determined the effects of remifentanil and F 13640 administered postoperatively on postoperative pain. All animals underwent surgery as described above and, 15 min after the end of surgery, received an i.p. injection of either saline, 0.63 mg/kg of remifentanil, or 0.63 mg/kg of F 13640. Measurements of postoperative pain (i.e., paw elevation and flexion response) were monitored as described above for up to 60 min after the injection.

2.3.4. F 13640 dose–response on intra- and postoperative pain

This experiment examined the dose-dependent effects of F 13640 administered preoperatively, on intra- and postoperative pain. Rats (n=7/group) received an i.p. injection of either saline or 0.04, 0.08, 0.16, 0.31 and 0.63 mg/kg of F 13640, 15 min before the induction of anesthesia. The animals then underwent surgery, and the intra-operative isoflurane requirement and latency to recovery were determined as described above. Postoperative pain behaviors were similarly monitored for up to 60 min after surgery.

2.3.5. Effects of the 5-HT$_{1A}$ receptor antagonist, WAY 100635

The experiment examined the effects of WAY 100635 when this selective 5-HT$_{1A}$ antagonist (Forster et al., 1995) was administered either alone or as a pretreatment before F 13640.

60 min before the induction of anesthesia, rats were injected subcutaneously (s.c.) with either saline or 0.63 mg/kg of WAY 100635 and 45 min later received an i.p. injection of either saline or 0.63 mg/kg of F 13640 ($n=7$/group). The animals then underwent surgery, and intra- and postoperative measurements were conducted as described above.

2.4. F 13640 Minimum Alveolar Concentration reduction study

This experiment used larger rats (320 g; CRIFFA, Barcelona, Spain) to determine F 13640's effects on the isoflurane Minimum Alveolar Concentration upon exposure to a standardized nociceptive stimulation as described elsewhere (Criado et al., 2000). Briefly, anesthesia was induced with isoflurane 5% (Ohmeda Isotec 3, BOC Health Care. Steeton, England) and maintained 2–3 min later at 2.5–3%. Endotracheal intubation was performed using a 14 gauge catheter connected to a small T piece of minimal dead space. Fresh gas flow to the T piece was adjusted to 1 l/min and the isoflurane concentration adjusted as required.

2.4.1. Monitoring

The carotid artery was catheterized for arterial blood sampling and blood pressure measurement. ECG, blood pressure, oxygen saturation of hemoglobin and rectal temperature were continuously monitored (CM-8, Schiller, Switzerland). Arterial blood gases (Blood gas analyzer, Statnova profile-1, Nova Biomedical, Waltham, MA, USA) were measured at the end of the experiment to ensure values were within normal limits of pH (7.34–7.45), PaO_2 (>90 mm Hg) and $PaCO_2$ (33–47 mm Hg). Rectal temperature was maintained between 37 and 38 °C by means of a circulating-water warming blanket (Heat Therapy Pump Model TP-220, Gaymar, Orchand Park, New York, USA) and, occasionally, a heating light.

2.4.2. Determination of the Minimum Alveolar Concentration

Intratracheal gas was sampled to measure the anesthetic gas concentration for the determination of the Minimum Alveolar Concentration. Samples were collected in triplicate by withdrawing 10 ml (Gastight #1010SL, Hamilton. 81656 Reno, Nevada, USA) through a fine catheter (0.9 mm external diameter) with the tip located at the level of the carina. After every step change in anesthetic concentration, at least 15 min were allowed for equilibration before samples were taken (Eger, 1965). Samples were assayed using gas chromatography (Datex-Ohmeda Capnomac Ultima, Helsinki, Finland).

A supramaximal noxious stimulus was applied by means of a long hemostat (9.5-in. Crafoord-Sellors Hemostatic Forceps; Martin, Tuttlingen, Germany) clamped to the first ratchet lock on the tail for 60 s (Eger et al., 1965) while the third gas sample was obtained from the lung. A positive response was considered when a gross purposeful movement of the head, extremities and/or body was observed whereas a negative response was the lack of movement or grimacing, swallowing, chewing, or tail flick. The isoflurane concentration was then reduced in decrements of 0.1% to 0.15% until the negative response became positive. The concentration midway between the highest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented movement was considered to be the Minimum Alveolar Concentration. Minimum Alveolar Concentration determination was performed in triplicate. Once the animals were anesthetized and instrumented, a basal isoflurane Minimum Alveolar Concentration was determined, each animal serving as its own control. Animals then received an i.p. injection of either saline or one of three doses of F 13640 (0.04, 0.16, or 0.63 mg/kg; $n\geq 7$/group), and the isoflurane Minimum Alveolar Concentration was redetermined 15 min after the reduction of the isoflurane concentration. The isoflurane Minimum Alveolar Concentration value was reevaluated continuously thereafter over the following 4 h to observe potential changes due to drug availability and effect.

In addition to the continuous monitoring of blood pressure and EDG, heart and respiratory rates were recorded immediately before each Minimum Alveolar Concentration step, as well as 30 min after the i.p. injection.

2.5. Statistical analysis

Time–response data were analyzed by two-way repeated measures (RM) analysis of variance (ANOVA) followed, where appropriate, by post hoc Student–Newman–Keuls test. Other data were analyzed by one-way ANOVA followed by Dunnett's test versus saline control or by Student–Newman–Keuls for multiple comparisons. $P<0.05$ was considered to be significant.

2.6. Drugs

Remifentanil was obtained from GlaxoWellcome; F 13640 was synthesized in-house. Both agents were dissolved in bi-distilled water. Drug solutions and saline (0.9% NaCl) were injected i.p. in a 1 ml/100 g body weight volume.

3. Results

3.1. Vocalization threshold: dose- and time-effect experiments

Remifentanil produced a dose- and time-dependent increase of the vocalization threshold, the peak of which was reached within 2.5 min (Fig. 1A); 0.63 mg/kg was the lowest dose at which the 600 g cut-off latency was attained. Two-way RM ANOVA indicated a significant effect of treatment [$F(5,360)=59.97$], of time [$F(10,360)=57.91$] and of the treatment * time interaction [$F(50,360)=12.21$; $P<.001$ in each case]. Post hoc analysis indicated 0.31 mg/kg to be the lowest dose exerting a significant effect ($P<.05$).

F 13640 produced a dose- and time-dependent lowering of the threshold, the peak of which appeared to be reached after some 15 min (Fig. 1B); 0.63 mg/kg was the lowest dose at which the threshold was lowered to zero. The effects of treatment [$F(3,280)=36.32$], of time [$F(14,280)=67.08$] and of their interaction [$F(42,280)=8.12$) were significant ($P<.001$ in each case); 0.04 mg/kg was the lowest dose exerting a significant effect.

3.2. Effects of preoperative drug treatment

3.2.1. Intra-operative pain

Fig. 2A shows the isoflurane concentration that was eventually delivered in order to maintain adequate anesthesia (i.e., immobility) throughout surgery. While all saline-pretreated, sham-operated remained at 1.5 vol.%, saline-pretreated operated (control) rats reached a concentration of 2.5±0.33 vol.% (mean±S.E.M.). Both remifentanil and F 13640 lowered this isoflurane requirement. One-way ANOVA revealed an effect of group [$F(3,36)=20.50$; $P<.001$]; post hoc tests indicated the effects of remifentanil and, in particular, of F 13640 to be highly significant (Fig. 2A).

After surgery which lasted for 5–8 min, operated saline-pretreated rats seemed to recover the righting reflex earlier than sham animals (Fig. 2B). However, one-way ANOVA did not reveal a significant group effect [$F(3,36)=1.85$; $P>.05$).

3.2.2. Postoperative pain

Saline-pretreated operated controls, but not sham animals, demonstrated paw elevation that was most prominent from 10 to 25 min postoperatively, and then gradually subsided. F 13640, but not remifentanil, blocked this response (Fig. 3A, left panel). Two-way RM ANOVA indicated a significant effect of time [$F(17,612)=8.28$], of group [$F(3,612)=9.08$] and of the time*group interaction [$F(51,612)=2.51$; $P<.001$ in each case]. One-way ANOVA of the paw elevation during the hour that followed surgery again showed a significant effect of group [$F(3,36)=9.08$; $P<.001$]; F 13640 was more effective than remifentanil (Fig. 3A, right panel).

Saline-pretreated operated controls, but not sham animals, demonstrated a highly frequent, ipsilateral flexion response that was prominent throughout the initial 60 min following surgery, and then subsided (Fig. 3 B, left panel). Remifentanil further enhanced flexion during the first 25 min. In contrast, F 13640 markedly inhibited flexion throughout the first hour. Two-way RM ANOVA indicated a significant effect of time [$F(17,612)=11.24$], of group [$F(3,612)=13.53$], and of the time*group interaction [$F(51,612)=4.26$; $P<.001$ in each case]. One-way ANOVA of the flexion that occurred during the first hour after surgery again indicated a significant group effect [$F(3,36)=13.53$; $P<.001$] with F 13640 again exerting a larger effect than remifentanil (Fig. 3B, right panel). Note

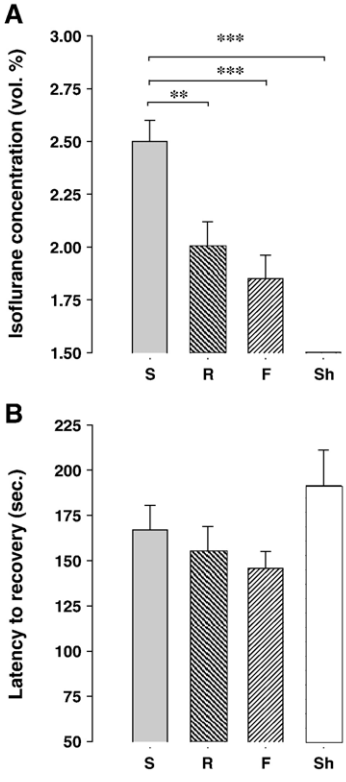

Fig. 2. Effects of preoperative remifentanil or F 13640 treatment on intra-operative pain and on hypnosis in rats undergoing orthopedic surgery. Animals received an i.p. injection of either saline (S), 0.63 mg/kg remifentanil (R), or 0.63 mg/kg F 13640 (F) before surgery; sham animals (Sh) were injected with saline and were not operated ($n=10$/group). (A) Effects of treatments on the isoflurane concentration that was eventually required to maintain immobility throughout surgery (ordinate, in vol.%). (B) Effects of treatments on the time after surgery (ordinate, in s) that animals required to recover from isoflurane-induced loss of the righting reflex. Asterisks denote results from post hoc Dunnett's test (*$P<.05$; **$P<.01$; ***$P<.001$).

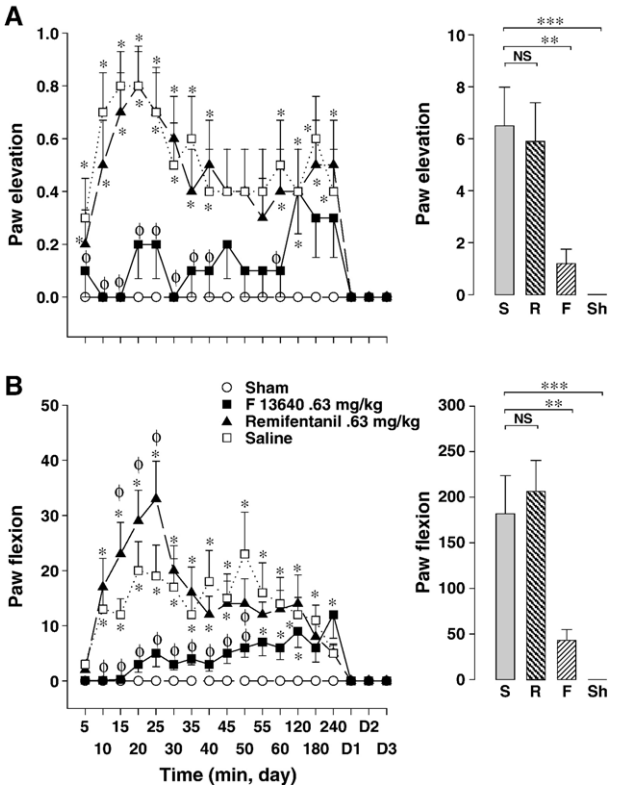

Fig. 3. Effects of preoperative remifentanil or F 13640 treatment on postoperative pain in rats undergoing orthopedic surgery. See also legend to Fig. 2. (A) Ipsilateral paw elevation was observed as being present or absent every 5 min for 60 min, and then at further intervals after surgery. (B) Number of times per 5 min episode that flexion of the ipsilateral paw occurred. Data are mean±S.E.M. The left panels represent the data recorded throughout the three days that followed surgery, and show results of post hoc analyses (Student–Newman–Keuls test; * and $^{\phi}P<.05$ versus sham and saline-treated, operated controls, respectively). The right panels represent the data obtained during the first hour following surgery; asterisks represent results from post hoc Dunnett's test (*$P<.05$; **$P<.01$; ***$P<.001$; NS: not significant).

that the hyperalgesia, which remifentanil produced with the flexion response during the first 25 min (Fig 3B, left panel), was no longer apparent when all data for the first hour were pooled (Fig. 3B, right panel).

Finally, two-way RM ANOVA of body weight data (not shown) indicated an effect of time [$F(3,108)=96.58$; $P<.001$] but neither of group [$F(3,108)=0.53$; $P>.05$] nor of the time * group interaction [$F(9,108)=0.99$; $P>.05$].

3.3. Effects of postoperative drug treatment

Relative to saline control animals, the injection of remifentanil 15 min postoperatively, decreased paw elevation during the first 5 min after the injection (Fig. 4A, left panel). This was followed by a period, between 10 and 25 min post-injection, during which paw elevation in remifentanil-treated rats was similar to that in saline controls. Thereafter, remifentanil caused an enhanced paw elevation that seemed to not entirely have subsided 60 min after its injection. In contrast, F 13640 caused an immediate inhibition of paw elevation that persisted throughout the observation period. Two-way RM ANOVA indicated a significant effect of time [$F(14,378)=1.96$; $P=.01$], of group [$F(2,378)=7.81$; $P<.01$] and of the group * time interaction [$F(28,378)=2.24$; $P<.001$]. One-way ANOVA of the paw elevation that occurred throughout the hour following injection indicated a significant treatment effect [$F(2,27)=8.52$; $P<.001$]. Post hoc analysis found remifentanil to actually increase this overall response; F 13640 produced a near-complete inhibition (Fig. 4A, right panel).

Relative to saline control animals, remifentanil initially inhibited, then enhanced the flexion response (Fig. 4B, left panel). F 13640 caused an immediate and near-complete inhibition of the response that persisted throughout. Two-way RM ANOVA indicated a significant effect of group [$F(2,378)=11.61$; $P<.001$], of time [$F(14,378)=2.52$; $P<.01$] and of the group * time interaction [$F(28,378)=3.85$; $P<.001$). Analysis of the overall incidence of the flexion response throughout the hour post-injection found a significant treatment effect [$F(2,27)=14.58$; $P<.001$]. Post hoc analysis showed no difference between remifentanil- and saline-treated animals, and confirmed F 13640's powerful effect (Fig. 4B, right panel).

3.4. F 13640 dose–response on intra- and postoperative pain

One-way ANOVA indicated F 13640 to decrease the isoflurane requirement in a dose-dependent manner [$F(5,36)=12.32$; $P<.001$], the effects being significant at 0.16 mg/kg and higher doses (Fig. 5A). The compound again exerted no effect on the latency to recover the righting reflex ($F=0.87$; $P>.05$; Fig. 5B), and decreased paw flexion ($F=8.71$; $P<.001$) and paw elevation ($F=4.87$: $P<.01$) in a dose-dependent manner. As with the intra-operative isoflurane requirement, F 13640's effects on both these postoperative pain behaviors were significant at 0.16 mg/kg and higher doses (Fig. 5C, D).

3.5. Effects of the 5-HT_{1A} antagonist, WAY 100635

One-way ANOVA indicated a significant treatment effect [$F(3,24)=7.71$; $P<.001$] on the isoflurane requirement. When given alone, F 13640, but not WAY 100635 again lowered this requirement; WAY 100635 did, however, fully antagonize F 13640's effect (Fig. 6A). The treatments did not affect the latency to recover the righting reflex ($F=2.14$; $P>.05$; Fig. 5B), but the ANOVA was significant both with postoperative paw flexion and paw elevation ($F=9.02$ and 13.78, respectively; $P<.001$ in either case). When given alone, F 13640, but not WAY 100635, again inhibited both behaviors; more importantly, WAY 100635 fully antagonized F 13640's effects (Fig. 6C, D).

3.6. F 13640 Minimum Alveolar Concentration reduction study

One-way ANOVA indicated a significant treatment effect with the Minimum Alveolar Concentration [$F(3,28)=14.42$; $P<.001$] and mean arterial pressure ($F=4.61$; $P=.01$), but not with heart rate ($F=1.63$; $P>.05$) nor respiratory rate ($F=0.04$; $P>.05$).

F13640-induced Minimum Alveolar Concentration reduction was significant at the 0.16 and 0.63 mg/kg doses, but not at 0.04 mg/kg (Table 1). F 13640's effects on arterial blood pressure were also significant at the 0.16 and 0.63 mg/kg doses, and were of limited magnitude. No changes in oxygen saturation of hemoglobin were observed and pH, PaO_2 and $PaCO_2$ values were within normal range throughout the study period (not shown).

4. Discussion

We here examined the effects of novel analgesic, F 13640, on the intra- and postoperative pain that is associated with orthopedic surgery in rats.

During surgery, the isoflurane concentration to which the animals were exposed was incrementally adapted so as to achieve a depth of anesthesia that ensured immobilization in all rats in the absence of a muscle relaxant. The (on average: 2.5 to 3.2 vol.%; Figs. 2A, 5A, 6A)) isoflurane concentration that was so reached in saline-treated animals is likely higher than the corresponding minimal alveolar concentration which is used in human clinical anesthesia to characterize a volatile anesthetic as it typically secures immobilization in 50% of patients during surgical intervention (Eger et al., 1965), and was about two-fold higher than the Minimum Alveolar Concentration of isoflurane that we here, typically, found to average 1.38 vol.% in saline-treated rats (Table 1). This difference most likely results from the fact that while the Minimum Alveolar Concentration experiments allowed ample time (i.e., at least 15 min) for equilibrium to be reached between the intratracheal concentration and that in the inspirate, in our studies of surgical pain, a standardized interval of only 30 s of exposure to the incremented isoflurane concentration in the inspirate was implemented. While certainly not permitting that equilibrium was always achieved,

the institution of this short interval avoided that any large differences would occur between control and drug-treated subjects in terms of the overall duration of both the anesthesia and the surgery.

F 13640 markedly reduced the isoflurane concentration that was eventually required to maintain adequate anesthesia throughout surgery, suggesting that it produced profound intra-operative analgesia (Fig. 2A). While the use of this parameter, to our knowledge, is unprecedented in animal experiments, the following findings support the validity of the isoflurane requirement as a measure of intra-operative nociception. The isoflurane concentration that was set at 1.5 vol.% at the beginning of surgery, remained at that same level in all, unoperated, sham animals, suggesting that isoflurane-induced immobility was insensitive to tachyphylaxis or other non-nociceptive variables. In contrast, the conduct of surgery in saline-pretreated rats did increase the isoflurane requirement in a highly significant manner. Further, the opioid remifentanil, which provides adequate intra-operative analgesia in humans (Bürkle et al., 1996; Glass et al., 1999), markedly lowered this requirement. These findings converge to validate the lowering by F 13640 of the isoflurane requirement as evidence that the compound produced an apparently powerful intra-operative analgesia. However, formal proof of this point awaits the formal definition of intra-operative pain, in laboratory animals in particular.

When administered preoperatively, F 13640 also produced a near-complete block of both the paw elevation and flexion that occurred postoperatively in control animals (Fig. 3). At this point, remifentanil produced no analgesia, most likely because of its short duration of action (Glass et al., 1999). Indeed, (0.63 mg/kg) remifentanil's effect in the Randall-Selitto assay in normal rats lasted just 10 min (Fig. 1A), while observations of postoperative pain behaviors began only about 8–11 min after its injection in this experiment (Fig. 3).

Earlier studies in which either bone injury was produced by drilling a hole in the calcaneous (Houghton et al., 1997) or a longitudinal incision was made through skin, fascia and muscle of the plantar aspect of the rat's foot (Brennan et al., 1996), reported a variable but significant increase in the response to von Frey filament stimulation that lasted up to several hours and days, respectively (see also: Field et al., 1997, 1999; Gonzales et al., 1998; Prado and Pontes, 2002). Here, the surgery similarly produced a decrease in the von Frey threshold to induce paw withdrawal, but the effect was considered to be insufficiently robust for use in the pharmacological experiments. One difference with these previous studies is that we used an automated rather than the original version of von Frey filaments; perhaps this resulted in an enhanced variability which in turn diminished statistical power. Another source of variability here were the possibly confounding effects on the von Frey threshold, of the spontaneous, highly frequent elevation and flexion of the injured paw (see: Methods). Also, we combined these interventions rather than implementing them separately; this may be relevant as, in another study, an incisional procedure that was implemented after allodynia had developed upon the constriction of the infra-orbital nerve, decreased the allodynic response to von Frey stimulation (Deseure et al., 2003).

While the data in Fig. 3 offer initial evidence of F 13640's action on postoperative pain, several considerations made us to also examine the effects of F 13640, and of remifentanil, on postoperative pain in a condition where the agents were administered 15 min after surgery, i.e., at a time when postoperative pain had begun to occur. Firstly, though the failure of preoperatively injected remifentanil to attenuate the postoperative pain behaviors is likely due to a short duration of action, the absence of any such opioid effect may question the validity of these behaviors as measures of pain. Secondly, F 13640 has been found to produce remarkably long-lasting preemptive analgesia, in rat models of trigeminal neuropathic and spinal cord injury pain in particular (Deseure et al., 2003; Wu et al., 2003). Hence the possibility arises that the effects that were found after F 13640 was administered preoperatively reflect a preemptive, rather than a direct, symptomatic, action on postoperative pain. Thirdly, and although F 13640 did not prolong isoflurane-induced loss of the righting reflex (Figs. 1B and 5B), the confirmation of the agent's effects when the latter is administered 15 min after isoflurane discontinuation would render unlikely any possibility that those effects are due to an interaction with the volatile anesthetic.

The data, then, indicate that the μ-opioid agonist remifentanil, when administered postoperatively, inhibited both paw elevation and flexion 5 min after its injection (Fig. 4); paw elevation and flexion are commonly used as endpoints in studies of nociception (Le Bars et al., 2001), and their inhibition by remifentanil contributes to the validation of these behaviors (Roughan and Flecknell, 2001) as measures of postoperative pain. Also, when administered postoperatively at a time when the animals had recovered from isoflurane-induced hypnosis, F 13640 produced an analgesia with both these measures that was profound and similar to that found after preoperative injection. The latter data indicate that F 13640's effects were not due to a possible interaction with isoflurane; along with the intra-operative results (Fig. 2A), they also extend to acute nociception earlier findings that F 13640 produces profound analgesia in rat models of tonic and chronic nociceptive pain (Colpaert et al., 2002; Bardin et al., 2003). The doses at which F 13640 alleviated intra- and postoperative pain (Fig. 5) are strikingly similar to those at which the agent suppresses the paw elevation that occurs early after intraplantar formalin injection (Bardin et al., 2003); only large morphine doses similarly inhibit this response and the present findings suggest that the mechanisms whereby F 13640 produces analgesia are similar with both these instances of nociceptive pain. There can be little doubt that these mechanisms are initiated at the molecular level by the activation of 5-HT_{1A} receptors; the extent to which 5-HT_{1A} receptor agonists produce analgesia in the formalin model correlates with the extent to which they activate the receptor (Colpaert et al., 2002), and F 13640-induced analgesia in this model is counteracted (Bardin et al., 2003) by the selective 5-HT_{1A} antagonist, WAY 100635 (Forster et al., 1995). Indeed, as in the formalin model, 0.63 mg/kg of WAY 100635 fully

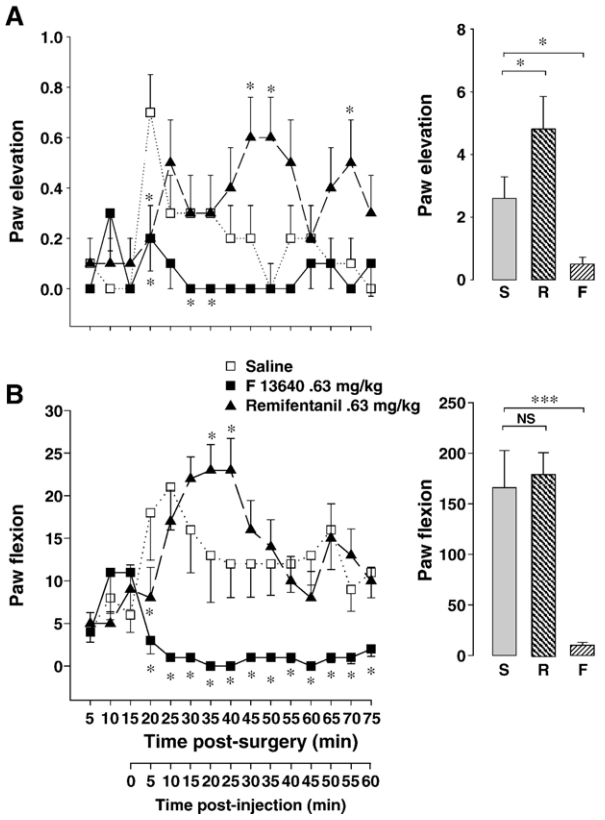

Fig. 4. Effects of remifentanil and F 13640 administered postoperatively, on postoperative pain in rats having undergone orthopedic surgery. Animals underwent surgery, and were injected i.p. with either saline (S), 0.63 mg/kg remifentanil (R), or 0.63 mg/kg F 13640 (F) 15 min after surgery (n=10/group). Observations were made during this 15 min before as well as for up to 60 min after injection. Panels A and B represent data on paw elevation and flexion, respectively (see also legend to Fig. 2). The left panels represent the data obtained throughout the 75 min observation period; the asterisk indicates P<.05 versus the saline control group (post hoc; Student–Newman–Keuls test). The right panels report the data obtained during the 60 min period following injection as well as the results from post hoc Dunnett's test (*P<.05; **P<.01; ***P<.001; NS: not significant).

antagonized the effects of 0.63 mg/kg of F 13640 in the present experiments (Fig. 6).

The brief analgesia followed by longer-lasting hyperalgesia that were found here (Fig. 4) with remifentanil express the paradoxical outcomes that are produced by opioid signal transduction in nociceptive systems (Colpaert, 1996; Colpaert and Frégnac, 2001; Colpaert et al., 2002). These dual, analgesic and hyperalgesic opioid actions are associated with tolerance to opioid analgesia and likely result from a baseline shift in pain sensitivity (Colpaert, 1996); they have been well demonstrated in both normal animals and healthy volunteers in whom pain sensitivity was assessed experimentally by means of short-lived probing stimulations (Vinik and Kissin, 1998; Luginbühl et al., 2003; for review, see: Colpaert, 1996). Of greater clinical import, however, the present data (Fig 4) confirm evidence (Li et al., 2001; Rivat et al., 2002) that this so-called (Colpaert, 1996) 2nd order hyperalgesia also occurs with ongoing pain; in fact, the same 0.63 mg/kg remifentanil injection in normal rats produced no detectable hyperalgesia in the Randall-Selitto assay (Fig. 1). Pretreatment with fentanyl in addition to bupivacaine in women undergoing Caesarian section reduced the analgesic requirement before delivery, but increased it between 6 and 23 h later (Cooper et al., 1997). Also, in patients undergoing major abdominal surgery, the intra-operative use of a high as opposed to a low dose of fentanyl or remifentanil increased postoperative pain intensity and patient-controlled opioid consumption (Chia et al., 1999; Guignard et al., 2000). The long-term sequellae, if any, of the 2nd order hyperalgesia that opioids may produce with ongoing pain have been studied only little (Perkins and Kehlet, 2000; Macrae, 2001), but can on theoretical grounds be expected to be long-lasting (Colpaert, 1996; Bruins Slot and Colpaert, 2003). Producing the mirror inverse of the long-term neuroadaptive actions of opioids (Colpaert et al., 2002), F 13640 can be expected to produce a long-term, 2nd order analgesia. Indeed, with the neuropathic allodynia that ensues upon ischemic spinal cord lesionning, F 13640 has been found to induce a preemptive and curative-like action that persisted for two months (Wu et al., 2003; Colpaert et al., 2004).

Upon formalin injection, pain behaviors occur in a biphasic manner (i.e., "early" and "late" after the injection), and F 13640 inhibits the behaviors in both phases (Bardin et al., 2003). Here, pain behaviors were prominent early upon surgery and then decayed, but did not quite disappear after 4 h; no paw elevation or flexion was apparent after 24 h (Fig. 3) though this does not

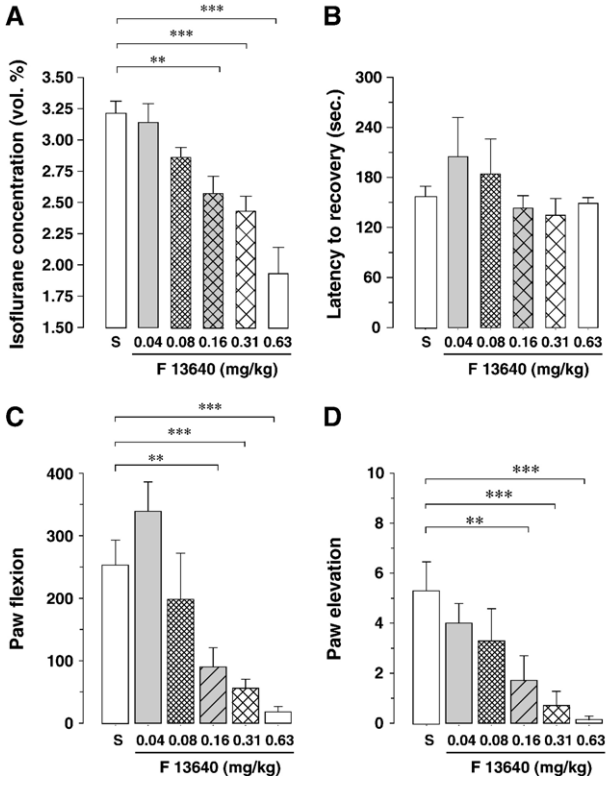

Fig. 5. Dose-dependent effects of F 13640 administered preoperatively, on intra-operative pain, loss of the righting reflex, and postoperative pain in rats undergoing orthopedic surgery. See also legend to Figs. 2 and 3. Postoperative pain behaviors (panels C, D) were monitored for 60 min after surgery. Data are mean±S.E.M. (n=7/group). Asterisks indicate differences compared with saline (control) animals (post hoc Dunnett's test: *P<.05; **P<.01, ***P<.001; NS: not significant).

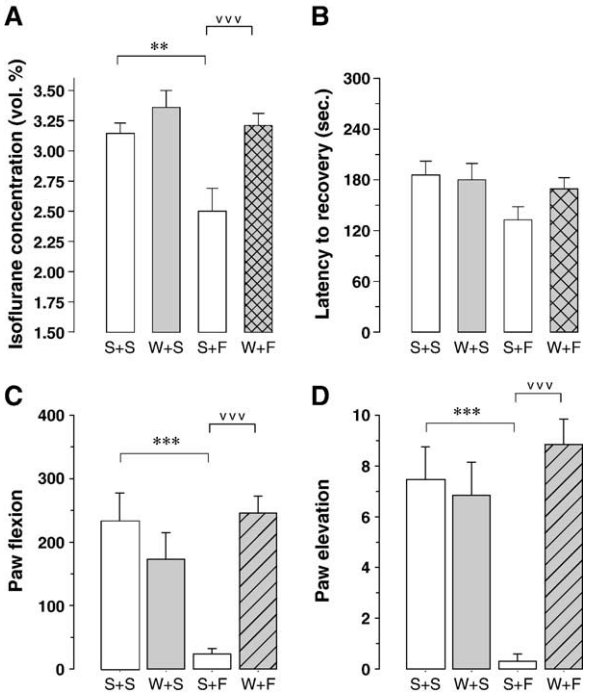

Fig. 6. Effects of the 5-HT_{1A} antagonist, WAY 100635, on the actions of F 13640. Rats were pretreated with either saline or WAY 100635 (W) before an injection of either saline or F 13640. See also legend to Figs. 2 and 3. Postoperative pain behaviors (panels C, D) were monitored for 60 min after surgery. Data are mean±S.E.M. (n=7/group). Asterisks indicate differences between treatment groups (post hoc Student–Newman–Keuls test; **P<.01; ***P<.001; other comparisons yielded no significance; P>.05).

imply that the animals were pain-free during and after the 4-to-24 h interval. It would be interesting for further work to determine whether the surgery, beyond the 4-h interval, may induce a "late" phase of pain behaviors and of inflammatory hyperalgesia; and how F 13640 and opioids may affect any such "late" responses. Indeed, fentanyl enhances carrageenan-induced inflammatory hyperalgesia (Rivat et al., 2002) and morphine pretreatment enhances post-incisional thermal hyperalgesia (Li et al., 2001). Note that, in rats, both opioid- and F 13640-induced hyperalgesia have been reported with the Randall-Selitto technique (e.g., Colpaert et al., 2002; Rivat et al., 2002); the extent to which the hyperalgesia induced by either agent may occur with a broad range of stimuli and responses remains to be determined.

In is noteworthy that the same 0.63 mg/kg dose of F 13640 produced profound intra- and postoperative analgesia (Figs. 2–4) at precisely the post-injection time at which in normal rats it produced hyperalgesia in the Randall-Selitto technique (Fig. 1). This can be explained by one of the remarkable, unprecedented neuroadaptive mechanisms which the compound initiates. Indeed, both a theory of signal transduction in nociceptive systems (Colpaert, 1996) and experimental evidence (Colpaert et al., 2002; Bardin et al., 2003; Deseure et al., 2003) indicate that high-efficacy 5-HT_{1A} receptor stimulation cooperates with nociceptive stimulation to produce analgesia. The cellular and neuroanatomical pathways that mediate this cooperation remain to be identified.

When administered during anesthesia, analgesic agents typically reduce the Minimum Alveolar Concentration of the inhalational anesthetic. The Minimum Alveolar Concentration reduction offers a useful measure of putative pain relief, albeit that it may also involve non-specific (e.g., sedative) effects (Criado et al., 2000) and interactions between the agents and the anesthetic (Docquier et al., 2003). Here, F 13640 reduced the isoflurane Minimum Alveolar Concentration in rats exposed to a nociceptive, standardized, 60 s hemostat clamping of the tail. Much as with intra- and postoperative (Fig. 5) and formalin-induced pain (Bardin et al., 2003), the lowest F 13640 dose reducing the isoflurane Minimum Alveolar Concentration was 0.16 mg/kg (Table 1). Though no comparative study was conducted here, the extent of the Minimum Alveolar Concentration reduction produced by this 0.16 and the 0.63 mg/kg dose (i.e., 10.6% and 12.3%, respectively) is comparable to that (i.e., 16%) which in similar conditions has been found with 1 mg/kg of intravenous morphine (Criado et al., 2000); this reduction is modest, however, compared with that (i.e., 55%) reported with 10 mg/kg of intravenous morphine. The co-operation mentioned above between 5-HT_{1A} receptor and nociceptive stimulation by which analgesia occurs is dependent on both the intensity and the duration of both stimulations (Colpaert, 1996; Colpaert et al., 2002; Deseure et al., 2003). Thus, the relative modesty of F 13640's Minimum Alveolar Concentration reducing effects suggests that the tail clamping used in the Minimum Alveolar Concentration experiments produced a nociceptive stimulation that was of smaller intensity and/or of shorter duration than that associated with either surgery (Figs. 2–4) or intraplantar (2.5%) formalin injection (Colpaert et al., 2002; Bardin et al., 2003), conditions in which pain behaviors persist for at least 1 h and 0.16–0.63 mg/kg doses of i.p. F 13640 produce profound analgesia. Indeed, upon formalin injection, 0.63 mg/kg of F 13640 produced an analgesia that is at least as powerful, if not more so, than that

Table 1
Differences in Minimum Alveolar Concentration of isoflurane (MACiso; %), heart rate (HR; beats/min), respiratory rate (RR; breaths/min) and mean arterial pressure (MAP; mm Hg) in rats before and after receiving an i.p. injection of either saline or one of different doses of F 13640

	Saline (n=7)	F 13640 0.04 mg/kg (n=7)	F 13640 0.16 mg/kg (n=9)	F 13640 0.63 mg/kg (n=9)
MACiso	1.38±0.07	1.42±0.03	1.48±0.02	1.41±0.03
MACiso+drug	1.38±0.06	1.39±0.03	1.32±0.03	1.23±0.02
Difference (%)	0.5±0.9	−1.9±1.1 ns	−10.6±2.1***	−12.3±1.7***
HR basal	390±8.3	381±10.5	379±12.3	380±19.6
HR 30 min	391±9.8	376±8.7	370±12	348±17.6
Difference (%)	0.5±2.2	−1.2±1.9 ns	−1.7±3.5 ns	−8.0 ± 3.1 ns
RR basal	81±3	63±2.2	68±2.6	72±1.6
RR 30 min	77±2.6	61±1.5	66±4.6	69±6
Difference (%)	−4.2±3.2	−3.5±3.1 ns	−1.6±6.4 ns	−4.0±7.5 ns
MAP basal	104±2.2	101±3.8	110±3.3	107±2.6
MAP 30 min	111±4.1	93±4.1	103±3.3	100±4.3
Difference (%)	6.5±4.8	−8±3.7	−5.4±3.2**	−6.3±2.7**

Data are mean±S.E.M. Asterisks denote significance compared to saline (post hoc Dunnett's test; *P<.05; **P<.01; ***P<.001; ns=not significant).

obtained with 10 mg/kg of (i.p.) morphine (Bardin et al., 2003). The limited intensity and/or duration of the nociceptive stimulation produced by the tail clamping may perhaps also account for the apparent shallowness of F 13640's dose–response relationship in reducing the Minimum Alveolar Concentration.

It also is useful to point out that here as elsewhere (Bruins Slot et al., 2003), and like other 5-HT_{1A} receptor agonists (De Vry, 1995), F 13640 induced signs of the 5-HT syndrome (e.g., fore paw treading; not shown). These signs do not, however, explain the analgesic effects that were observed. This is evident for the intra-operative and Minimum Alveolar Concentration effects, as those signs were not apparent during isoflurane anesthesia. Also, tachyphylaxis develops to these signs, and prazosin blocks them (Bardin et al., 2001; Bruins Slot et al., 2003), but the ability of 5-HT_{1A} agonists to inhibit (formalin-induced) paw elevation remains unaltered after that tachyphylaxis has developed; it also can not be blocked by prazosin (Bardin et al., 2001). Thus, here as in previous studies (Bardin et al., 2001; Colpaert et al., 2002; Deseure et al., 2002), the analgesic effects of 5-HT_{1A} receptor activation were behaviorally specific; only ethical considerations with the use of the present model have refrained us from again demonstrating this specificity here.

In conclusion, the present findings indicate that, in the rat, the new central analgesic, F 13640, produces powerful analgesia with the intra- and postoperative pain that is associated with orthopedic surgery. Unlike remifentanil, F 13640 induced no postoperative hyperalgesia, and, also unlike opioids, produces no tolerance. In fact, as shown elsewhere, the repeated or continuous administration of F 13640 acts to further increase analgesia (inverse tolerance), rather than to make it decay (Colpaert et al., 2002, 2004; Bruins Slot and Colpaert, 2003; Deseure et al., 2003). These findings warrant the study of F 13640's effects in human intra- and postoperative pain and open the perspective of this compound being able to maintain adequate postoperative pain relief for protracted periods of time.

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