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Tolerance and inverse tolerance to the hyperalgesic and analgesic actions, respectively, of the novel analgesic, F 13640

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Abstract

5-HT_{1A} receptor activation by the very-high-efficacy, selective 5-HT_{1A} receptor agonist F 13640 [(3-Chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone] was recently discovered to constitute a novel central mechanism of broad-spectrum analgesia that, remarkably, grows rather than decays with chronicity. However, in rodents not exposed to nociception, F 13640 induces its analgesic effect only after having initially induced hyperalgesia. Numerical simulations implementing a signal transduction theory here show that the progressive increase in the intensity of nociceptive stimulation which F 13640 presumably mimics should eventually produce a large analgesic effect without initially causing marked pain. In vivo studies examined the effects of progressively increasing doses of F 13640 on the threshold of mechanically induced vocalization and, also, on the 5-HT syndrome in rats. The infusion of increasing (0.04–0.63 mg/rat/day) doses of F 13640 over a 5-week period induced a large analgesia preceded by a hyperalgesic effect that was small and comparable to that induced by initial exposure to a low, 0.04 mg/rat/day dose. Furthermore, increasing the dose of F 13640 induced tachyphylaxis to the 5-HT syndrome. Producing the mirror opposite of morphine's neuroadaptive actions, F 13640 causes an analgesia that becomes more powerful with chronic administration, and this at the expense of the initial hyperalgesia which it may also produce.

Keywords: Analgesia; Hyperalgesia; Pain; 5-HT_{1A} receptor; Tolerance

1. Introduction

A concept of signal transduction (Colpaert, 1978, 1996) specifies that any input to nociceptive systems causes not one effect but two effects that are paradoxical, or opposite in sign. Accordingly, opioid receptor activation produces both analgesia as a so-called *first-order* effect and hyperalgesia as a *second-order* effect (Bruins Slot and Colpaert, 1999; Colpaert, 1996). As opioid treatment is continued, the *second-order* hyperalgesia grows and neutralizes the *first-order* effect, offering an unprecedented account of the neuroadaptive tolerance and sensitization that develop with opioids (Colpaert, 1996; Colpaert and Frégnac, 2001). Intriguingly, this concept suggests that a central mechanism entirely different from opioid receptor activation may exist whereby analgesia can be produced; a molecular action mimicking the central effects of nociceptive stimulation should initially produce pain as a *first-order* effect, but also analgesia (Colpaert, 1978). With chronicity, this *second-order* analgesia should grow, neutralize the *first-order* hyperalgesia and cause the development of inverse tolerance to the analgesia (i.e., incremental pain relief).

We have recently discovered that large-amplitude activation of 5-HT_{1A} receptors induces precisely such effects; in rat, the uniquely high-efficacy and selective 5-HT_{1A} receptor agonist F 13640 caused hyperalgesia followed by analgesia (Colpaert et al., 2002), thus mirroring morphine as it produced analgesia followed by hyperalgesia. Again producing the mirror opposite of morphine, chronic infusion of F 13640 caused an increasingly powerful analgesia. As a result, at a dose of 0.63 mg/rat/day, F 13640 was effective in models of chronic nociceptive pain and of neuropathic allodynia, its effects surpassing those produced by morphine and by other available mechanisms of central analgesia (i.e., as exemplified by imipramine, ketamine and gabapentin).

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Thus, large-amplitude 5-HT_{1A} receptor activation constitutes a novel central mechanism of broad-spectrum analgesia that grows rather than decays with chronicity (Colpaert et al., 2002).

A concern that arises with the possible clinical use of 5-HT_{1A} receptor activation for the treatment of pain is the dose-dependent hyperalgesia, if not pain, which such activation may produce initially. The initial hyperalgesia should be of little concern with organisms that are exposed to lasting, intense nociceptive stimulation. This is because, as F 13640 presumably mimics the central effects of such stimulation, it should actually cooperate with nociception in producing, paradoxically, analgesia (Colpaert et al., 2002). Indeed, 15 min after its intraperitoneal (i.p.) injection, 0.63 mg/kg of F 13640 produces a powerful analgesia when rats are then challenged with an intraplantar injection of formalin, a challenge that induces a tonic (i.e., some 60 min), relatively intense nociceptive stimulation (Bardin et al., 2003; Colpaert et al., 2002). The initial hyperalgesia is of concern, however, with organisms that are exposed to no or perhaps only to short-lived, mild-intensity nociceptive stimulation; probing the pain sensitivity of rats by means of the Randall and Selitto's (1957) technique 15 min upon the i.p. injection of 0.63 mg/kg of F 13640 reveals a hyperalgesia such that the animals may vocalize even in the absence of the hind-paw mechanical stimulation which the technique implements (Colpaert et al., 2002).

The studies reported here sought to devise an administration mode allowing to attenuate the hyperalgesia that F 13640 may produce. To this end, firstly, numerical stimulations were carried out to determine how pain sensitivity would evolve in normal organisms that are exposed to increasingly high doses of this agent that presumably mimics the central effects of nociceptive stimulation. These simulations implemented a signal transduction algorithm that formalizes the concept referred to above and that was found previously to be adequate in predicting several of the dynamic, neuroadaptive actions of opioids (Bruins Slot and Colpaert, 1999; Bruins Slot et al., 2001, 2002; Colpaert, 1996). The simulations found that, following a progressive increase of dose, the hyperalgesia which the highest dose would otherwise produce was considerably blunted, whereas its analgesic effect was considerably amplified. Those predictions were tested, secondly, by in vivo experiments in rats with which we examined the effects of continuous exposure to progressively increasing doses of F 13640 on the hyperalgesic and analgesic actions of a final, 0.63 mg/rat/day dose of F 13640. For this, osmotic mini-pumps were implanted and replaced every week, the pumps releasing from 0.04 up to 0.63 mg/rat/day of F 13640. Rats with this previous exposure to the compound were compared with animals that received only one of these doses without having been exposed previously to F 13640. Pain sensitivity was assessed by measuring the threshold of vocalization in response to increasing paw pressure (Randall and Selitto, 1957). In addition, and since 5-HT_{1A} receptor agonists induce a so-called 5-HT syndrome in rats (Tricklebank et al., 1984; Berendsen et al., 1989) to which tachyphylaxis may develop (Berendsen and Broekkamp, 1991; De Vry, 1995), body temperature and several behavioral signs of the 5-HT syndrome in rats were also monitored.

2. Materials and methods

2.1. Numerical simulations

The simulations used the general equation $\delta = \varphi_0 - \iota_\tau$ (Colpaert, 1996; Bruins Slot et al., 2001), a practical account of which has been presented elsewhere (Bruins Slot and Colpaert, 1999). This algorithm specifies how any input (φ_0) to pain-processing systems (such as nociceptive stimulation) is transduced so as to generate an output (δ, such as pain). That is, the instantaneous input φ_0 (the input that is present at any given moment in time) is appreciated by determining the extent to which it differs from the input that prevailed in the recent past (ι_τ). The latter is found as the moving average of the recent input. The raw data resulting from these simulations are available upon request.

The present simulations determined the outcome δ (i.e., the response or effect) to a (positive) input φ_0 that mimics the nociceptive stimulation presumably induced by F 13640 in the in vivo experiments. Thus, the equation was fed with values of φ_0 (i.e., 4, 8, 16, 31 and 63 arbitrary units; A.U.) that corresponded to the F 13640 doses used in the empirical experiments. Also, the simulations applied these φ_0 values for 168 A.U. of time, corresponding with the time (i.e., 168 h or 7 days) during which F 13640 was to be administered in empirical experiments.

A number of simulations were run so as to correspond with the experimental design specified in Table 1. That is, the effect (δ) of the exposure during 168 A.U. to the different values of φ_0 were examined both when this exposure was preceded by a (''saline'') history of no antecedent exposure to any input (corresponding to groups 2–5) and when the stimulations were implemented progressively (corresponding to group 6). A (control) simulation corresponding to group 1 was also run.

Outcomes obtained with the general equation $\delta = \varphi_0 - \iota_\tau$ can vary depending on the relative weights of φ_0 and ι_τ, and these weights are not known a priori. In particular, the sign of the outcome δ reverses, even with a constant input φ_0, if the weight (w) that is attributed to ι_τ is larger than unity (Bruins Slot et al., 2002). The latter study having successfully predicted the original finding that continuous exposure to negative input (i.e., as with opioids) causes a reversal of effect (i.e., initial analgesia followed by hyperalgesia), and since the algorithm should apply regardless of the input's sign, we here implemented the equation $\delta = \varphi_0 - \iota_\tau w$ where $w = 4$ as in that study (Bruins Slot et al., 2002). Finally, the computation of ι_τ requires the definition of the length of time

Table 1
Experimental design of in vivo studies and numerical simulations that examined dual, hyperalgesic and analgesic actions of incremental doses of F 13640

Group	Week 1	Week 2	Week 3	Week 4	Week 5
1	saline	saline	saline	saline	saline
2	saline	F 13640 0.08			
3	saline	saline	F 13640 0.16		
4	saline	saline	saline	F 13640 0.31	
5	saline	saline	saline	saline	F 13640 0.63
6	F 13640 0.04	F 13640 0.08	F 13640 0.16	F 13640 0.31	F 13640 0.63

On the first day of the experiment, animals were implanted with an osmotic mini-pump that was replaced every week for 5 weeks. Rats in the previous exposure group (i.e., group 6) received incremental doses of F 13640 over the 5-week period starting with 0.04 mg/rat/day infused during week 1 and ending with 0.63 mg/rat/day infused during week 5. Non-pre-exposed animals (i.e., groups 2–5) received only a single dose of F 13640 (i.e., either 0.08, 0.16, 0.31 or 0.63 mg/rat/day) for a 1-week time period and were run simultaneously with the previous exposure group. Saline control animals (i.e., group 1) received 0.12 ml/day of saline throughout the 5-week period.
Numerical simulations of these experiments were also conducted using a similar experimental design. In these simulations, the 1-week time (168 h) time period corresponded to 168 A.U. of time; the doses of F 13640 corresponded to (4–63 A.U.) intensities of nociceptive stimulation (φ_0).

over which this moving average is determined; this length of time (the "sample period"; Colpaert, 1996) was arbitrarily set at 400 A.U.

Note that the numerical values obtained in these simulations do not necessarily predict the absolute values that empirical studies provide. This is because the simulations assume all input/output relationships to be simply linear. Therefore, the simulation outcomes may accurately reflect relative values, but not necessarily absolute values (Colpaert, 1996).

2.2. Animals

After a 10-day quarantine period, male Sprague–Dawley rats (Iffa Credo, Lyon, France) weighing 140–160 g on arrival were transferred to an environmentally controlled room (ambient temperature, 21 ± 1 °C; relative humidity; 55 ± 5%; 12:12-h light/dark cycle, lights on at 7 a.m.) and housed in individual cages with standard laboratory food and water freely available. The studies were carried out in accordance with the European Community guidelines for the use of experimental animals and were approved by the institutional Ethical Review Committee.

2.3. Drug treatments

Treatments were delivered by means of an osmotic mini-pump (model 2ML2; nominal pump rate: 5 μl/h; Alza®, Palo Alto, CA, USA) that was implanted subcutaneously as described elsewhere (Colpaert et al., 2001). The pump was inserted through a transversal incision in the skin of the lower middle part of the back, its aperture directed towards the head. Pumps were replaced every week for 5 weeks. For pump re-implantation, the transversal incision was made approximately 1.5 cm from the previous incision. The site of pump emplacement was massaged daily to avoid tissue adherence.

In-house synthesized F 13640 [(3-Chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone] was dissolved in distilled water and administered at doses from 0.04 to 0.63 mg/rat/day (dose expressed as free base). Saline pumps delivered 0.12 ml/day of 0.9% NaCl.

2.4. Experiments

Rats were randomly assigned to one of six experimental groups ($n = 13$ per group; see Table 1 for treatment schedule). The first pump implantation was carried out on day 0 of the experiment; subsequently, pumps were replaced on days 7, 14, 21 and 28. Measurements, described below, were made immediately before pump implantation (i.e., on days 0, 7, 14, 21 and 28) as well as 0.5, 1, 2, 4 and 8 h after implantation. Measurements comprised (1) behavioral observations, (2) nociceptive threshold and (3) rectal body temperature, in that order. The behavioral observations consisted of determining the presence (score 1) or absence (score 2) of forepaw treading, flat body posture and lower lip retraction during a 10-s period by an observer who was unaware of the treatment conditions (see Colpaert et al., 1992; Kleven et al., 1995). Nociceptive thresholds were determined using the Randall and Selitto's (1957) method in which increasing pressure was applied to the left hindpaw of rats until a squeak (vocalization threshold) was obtained. Results are expressed in grams. A 750-g cutoff value was used to prevent tissue damage. Rectal body temperature was measured to the nearest 0.1 °C by means of a thermal probe (Ellab model RM6, Carrieri Instruments, Paris, France).

Note that unlike the numerical simulations, measurements here were made for only 8 h after pump implantation, and were not continued thereafter. Earlier data (Colpaert et al., 2002) indicate that a sign-reversal of F 13640's effects may occur within 8 h of its administration; this limitation also was to avoid the possibly confounding (e.g., conditioning, inflammatory) effects that further tests may produce.

2.5. Data analyses

For nociceptive thresholds, the areas below (i.e., hyperalgesic effect) and above (i.e., analgesic effect) the grouped saline control time-course curves were determined. For behavioral observations, data are expressed

as the number of times per animal that a particular sign was observed at the different times after implantation (i.e., 0.5, 1, 2, 4 and 8 h). For rectal body temperature assessments, the differences between values (in °C) measured before and at the different times after pump implantation were determined, and data are expressed as the number of times per animal that a temperature difference greater than −1.1 °C (i.e., a criterion value derived from temperature differences observed in saline control animals; see Results) was observed at different times after implantation.

Mean values were considered significantly different from control if their 95% confidence limits did not include zero (for hyperalgesia, analgesia and the behavioral signs) or the saline control mean (for hypothermia). Differences between two experimental groups were analyzed, per dose, using the one-tailed Student's *t*-test; statistical significance was defined as $P < 0.05$.

3. Results

3.1. Numerical simulations

The outcomes δ of the different simulations are presented in Fig. 1A. In simulations that implemented different intensities of input φ_0 during 168 A.U. of time, without a history of previous exposure, the response δ was initially positive (i.e., hyperalgesic). Regardless of the magnitude of φ_0, this effect was always followed by a negative value of δ, even in the continued presence of φ_0. Thus, during the continuous input φ_0, a sign-reversal of hyper-to-hypoalgesic effect was obtained. The magnitude of both the initial hyperalgesic and the subsequent contra-directional, hypoalgesic effect was proportional to the magnitude of input φ_0.

In the simulation where exposure to input increased progressively from 4 to 63 A.U., a similar sign-reversal of effect occurred; however, the latency with which sign-rever-

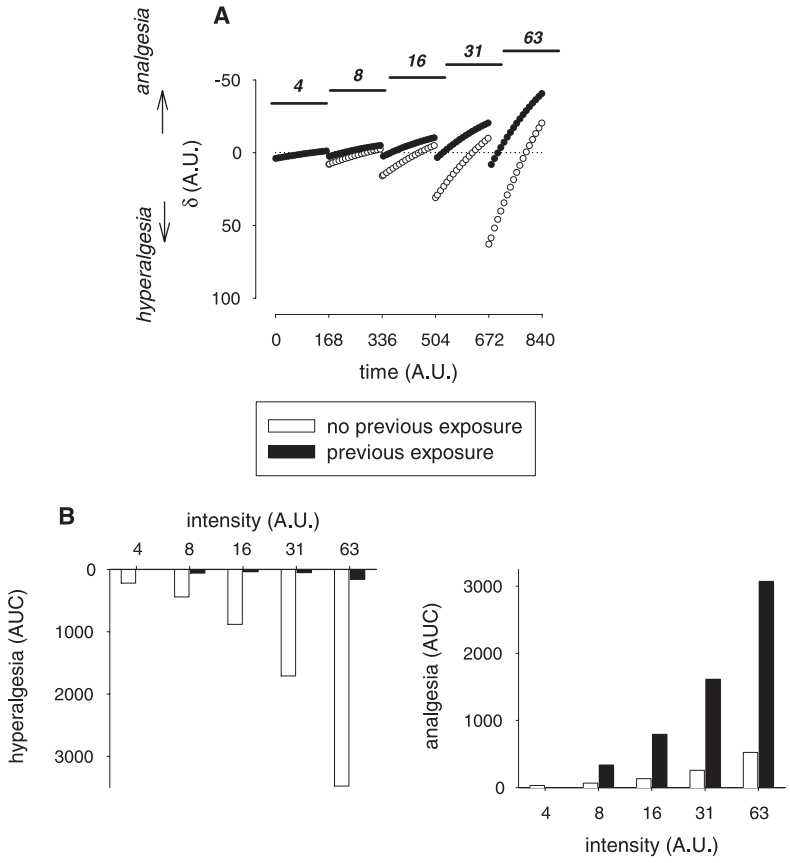

Fig. 1. Numerical simulations that applied a signal transduction algorithm to specify the effects on pain sensitivity of the chronic exposure to nociceptive stimulation applied either in the absence of previous nociceptive stimulation or as the intensity of the stimulation was progressively increased. In A, data points represent the outcome δ (ordinate; in A.U.) as a function of input intensity (φ_0) that varied from 4 to 63 A.U. and that lasted for 168 A.U. of time (abscissa). See also legend to Table 1. Open symbols: results from simulations corresponding to groups 2–5, where the exposure to the specified intensity of stimulation was not preceded by any previous stimulation. Closed symbols: results from the simulation corresponding to group 6, where the exposure to 63 A.U. input was preceded by stimulations that increased progressively from 4 to 31 A.U. Note that positive values of δ point downward and reflect hyperalgesic effects; negative values point upward and reflect analgesic effects. The dotted line represents the zero value of δ obtained in a control simulation where no input was presented. In B, the areas below (i.e., AUC hyperalgesia; left panel) and above (i.e., AUC analgesia; right panel) the zero value of δ are presented for each intensity of stimulation (abscissa; in A.U.).

sal took place was shorter than in non-pre-exposed simulations. Also, the magnitude of both the initial hyperalgesic and subsequent hypoalgesic effects was proportional to the magnitude of input. However, at each of the 8–63 A.U. values of φ_0, the peak magnitude of the initial hyperalgesic effect was smaller than that found in the simulations where there was no previous exposure to input. Equally, the peak magnitude of the subsequent analgesic effect was larger.

As a result of the differences obtained in terms of both the peak magnitudes of the two effects and the latency with which the sign-reversal occurred, area under the curve (AUC) values for the two effects differed considerably between the non-pre-exposed and the pre-exposed simulations (Fig. 1B). In particular, for the 63 A.U. intensity of φ_0, the AUC for the hyperalgesic effect was 3475 A.U. in the previously non-exposed simulation, and only 162 A.U. in the previously exposed simulation (Fig. 1B, left panel). Conversely, the AUC of the analgesic effect was only 524 A.U. in the previously non-exposed simulation, but reached as high a value as 3070 A.U. in the previously exposed simulation (Fig. 1B, right panel). Thus, previous exposure to incremental values of input made it possible for the 63 A.U. intensity of φ_0 to generate a hypoalgesic effect that was much larger than that achieved in any other condition. Also, this large hypoalgesic effect was preceded by an initial hyperalgesic effect that was smaller than that produced by acute exposure to an intensity of φ_0 as low as 4 A.U. (i.e., AUC: 161 and 221 A.U., respectively).

3.2. In vivo experiments

3.2.1. Pain responses

Fig. 2A depicts vocalization thresholds as a function of time after pump implantation. Pooled threshold values [mean (± S.E.M.)] in controls that received saline throughout the 5-week period were 288.5 (11.9), 236.3 (12.1), 233.5 (6.3), 235.1 (6.6) and 245.5 (8.7) g for weeks 1 through 5, respectively. Note that the week 1 control threshold was somewhat higher than that found during weeks 2 through 5, reflecting, perhaps, that some conditioning occurred. In animals that received only a single dose of F 13640 without having been exposed previously to the compound, the initial threshold was always lower than that observed in saline controls. This initial hyperalgesic effect was followed, with all doses of the compound, by an increase in the threshold above saline control values (i.e., by analgesia).

In animals that were exposed to progressively increasing doses of F 13640, a similar sign-reversal of hyperalgesic to analgesic effect was observed. With the 0.16 and 0.63 mg/

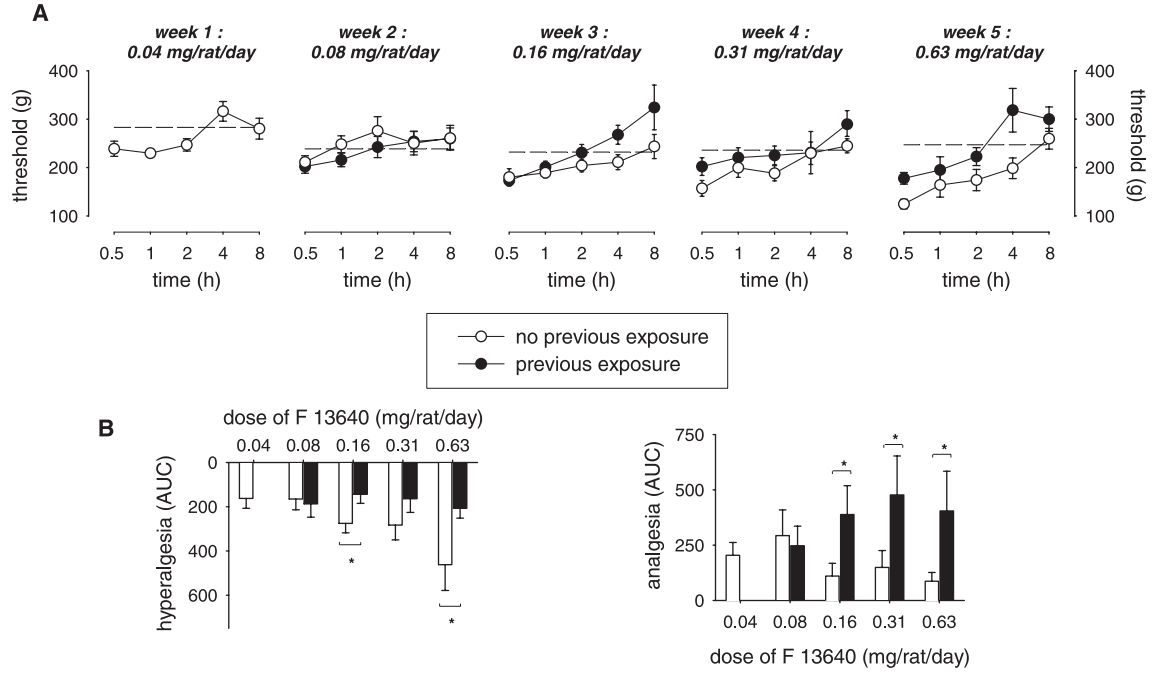

Fig. 2. Effects of progressively increasing dose of F 13640 on pain sensitivity in rats. On the first day of the experiment, animals were implanted with an osmotic mini-pump that was replaced every week for 5 weeks. Rats in the "previous exposure" group received incremental doses (i.e., 0.04–0.63 mg/rat/day) of F 13640 over the 5-week experimental period while rats in the "no previous exposure" groups received only a single dose of F 13640 (i.e., either 0.04, 0.08, 0.16, 0.31 or 0.63 mg/rat/day) for a 1-week period. See also legend to Table 1. In A, pain responses were examined by measuring the threshold of vocalization in response to increasing paw pressure (nociceptive thresholds in grams, mean ± S.E.M.; $n = 13$) at different time intervals (i.e., 0.5, 1, 2, 4 and 8 h) after pump implantation. A 750-g cutoff was used to prevent tissue damage. Broken lines represent the mean nociceptive thresholds of saline controls, the results of which were pooled for each week. In B, the data from A are expressed for each dose as the areas below (i.e., hyperalgesia, left panel) and above (i.e., analgesia, right panel) the saline control values (AUC; means ± S.E.M.; $n = 13$). Student's t-test: $^*P < 0.05$.

rat/day doses of F 13640, this sign-reversal appeared to occur earlier in time than in non-pre-exposed animals. Furthermore, the peak magnitude of the hyperalgesic effect was smaller than that observed in non-pre-exposed animals for the 0.31 and 0.63 mg/rat/day doses of F 13640. Similarly, at the 0.16–0.63 mg/rat/day doses, the peak magnitude of the subsequent analgesic effect was larger in the pre-exposed animals.

For each individual, drug-treated animal, the areas below (i.e., hyperalgesia; Fig. 2B, left panel) and above (i.e., analgesia; Fig. 2B, right panel) the saline control value (grouped per week) were determined. In animals that received only a single dose of F 13640 without having been exposed previously to the compound, hyperalgesia was larger as the dose of F 13640 administered was higher; a significant hyperalgesic effect was observed at all doses compared to controls (95% C.L.; $P<0.05$). In comparison, animals exposed to progressively increasing doses of F 13640 demonstrated little hyperalgesia and this small, but significant ($P<0.05$), hyperalgesic effect grew little, if at all, as the F 13640 dose was higher. In previously exposed animals, the highest, 0.63 mg/rat/day dose of F 13640 induced a hyperalgesic effect comparable to that produced by acute exposure to the 0.04 mg/rat/day dose (i.e., AUC: 203 and 179, respectively). Statistical analysis revealed a significant difference between animals not previously exposed and those that were previously exposed, for the 0.16 and 0.63 mg/rat/day doses (Fig. 2B, left panel; Student's *t*-test, $P<0.05$).

Non-pre-exposed animals that received only a single dose of the compound showed a relatively small analgesic effect, the relationship of which to dose was not clear; significant analgesia was apparent at the 0.04 and 0.08 mg/rat/day doses of F 13640 compared to controls (95% C.L.; $P<0.05$). In contrast, previous exposure to progressively increasing doses of F 13640 resulted in an analgesic effect that was significantly different from controls for each of the 0.08–0.63 mg/rat/day doses of the compound ($P<0.05$). The magnitude of this latter effect increased dose-dependently with doses up to 0.31 mg/rat/day. Statistical analysis showed a significantly greater analgesic effect in pre-exposed animals at all but the 0.08 mg/rat/day dose of F 13640 (Fig. 2B, right panel; Student's *t*-test, $P<0.05$ for the 0.16–0.63 mg/rat/day doses of F 13640).

3.2.2. 5-HT syndrome

In controls that received saline throughout, decreases in body temperature by more than 1.1 °C occurred in less than 5% of all observations. A decrease greater than 1.1 °C was therefore considered significant. In saline controls, the mean (± S.E.M.) number of times that a decrease in body temperature greater than 1.1 °C was observed at the different times after implantation was 0 (0), 0.3 (0.1), 0.1 (0.1), 0.5 (0.2) and 0.2 (0.1) for weeks 1–5, respectively. In non-pre-exposed animals, F 13640 induced a dose-dependent hypothermia (Fig. 3D) that was significantly different from controls at

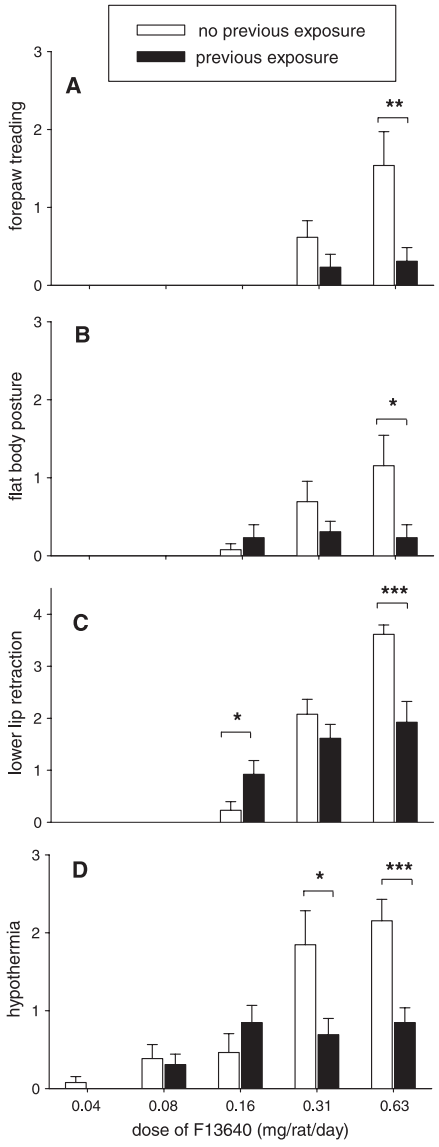

Fig. 3. Effects of progressively increasing dose of F 13640 on behavioral signs of the 5-HT syndrome and rectal body temperature in rats. On the first day of the experiment, animals were implanted with an osmotic mini-pump that was replaced every week for 5 weeks. Rats in the "previous exposure" group received incremental doses (i.e., 0.04–0.63 mg/rat/day) of F 13640 over the 5-week experimental period while rats in the "no previous exposure" groups received only a single dose of F 13640 (i.e., either 0.04, 0.08, 0.16, 0.31 or 0.63 mg/rat/day) for a 1-week period. See also legend to Table 1. Behavioral observations were made at different time intervals (i.e., 0.5, 1, 2, 4 and 8 h) after pump implantation and consisted of determining the presence or absence of forepaw treading (A), flat body posture (B) and lower lip retraction (C). Data are expressed as the number of times per animal that a particular sign was observed at the different times after implantation (means ± S.E.M.; $n=13$). For rectal body temperature assessments, the differences between values (in °C) measured before and at different times (i.e., 0.5, 1, 2, 4 and 8 h) after pump implantation were determined. Data are expressed (D) as the number of times per animal that a decrease in body temperature greater than 1.1 °C was observed at the different times after implantation (means ± S.E.M.; $n=13$) (a decrease >1.1 °C occurred in less than 5% of all saline control observations, and was therefore considered significant). Student's *t*-test : $*P<0.05$, $**P<0.01$ and $***P<0.001$.

0.31 and 0.63 mg/rat/day (95% C.L.; $P<0.05$). In contrast, animals previously exposed to F 13640 demonstrated hypothermia that, although statistically significant at the 0.16 and 0.63 mg/rat/day doses compared to controls ($P<0.05$), was considerably less than observed in non-pre-exposed animals. Statistical analysis demonstrated a significantly greater hypothermic effect for the 0.31 and 0.63 mg/rat/day doses of F 13640 in animals with no prior F 13640 history (Fig. 3D; Student's *t*-test, $P<0.05$ and $P<0.001$, respectively).

Forepaw treading, flat body posture and lower lip retraction were not observed at any time after implantation in control animals that received saline throughout the 5-week experimental period. In non-pre-exposed animals, F 13640 induced a dose-dependent increase in each of these three signs (Fig. 3A–C), with significant effects occurring at the 0.31 and 0.63 mg/rat/day doses compared to controls ($P<0.05$). In pre-exposed animals, only a small effect was seen on forepaw treading and flat body posture; this effect remained small despite increasing the dose of F 13640 and was significantly different from controls for flat body posture only ($P<0.05$ for 0.31 mg/rat/day). Only for lower lip retraction did the effect in pre-exposed rats increase with dose of F 13640; compared to controls, significant lower lip retraction was observed for the 0.16–0.63 mg/rat/day doses of the compound ($P<0.05$). Statistical analysis demonstrated a significantly smaller effect at the highest, 0.63 mg/rat/day dose of F 13640 for forepaw treading (Student's *t*-test, $P<0.05$), flat body posture ($P<0.05$) and lower lip retraction ($P<0.01$) in pre-exposed animals compared to those with no previous F 13640 history (Fig. 3A–C).

4. Discussion

The studies presented here examined the effects of progressively increasing doses of F 13640, a novel central analgesic (Colpaert et al., 2002), on both pain sensation and the 5-HT syndrome in rats. Specifically, the work addressed the concern that in normal organisms F 13640 induces its analgesic effect only after having initially induced hyperalgesia (Colpaert et al., 2002), and shows that by gradually stepping up the dose of F 13640 the initial hyperalgesia remains modest while the subsequent analgesia grows considerably (by the neuroadaptive mechanism of ''inverse tolerance''; see Colpaert, 1996).

This approach being based on predictions stemming from a signal transduction concept (Colpaert, 1996), the first series of studies presented here used numerical simulations implementing the transduction algorithm to specify the expected outcomes. The results (Fig. 1) demonstrate that in both the previously exposed simulations (i.e., where the intensity of nociceptive stimulation increased progressively over time) and in the non-previously exposed simulations (i.e., that implemented different intensities of nociception without a previous history of exposure), a sign-reversal of hyperalgesic-to-analgesic effect occurs. That is, the response was initially positive, translating a hyperalgesic effect, and this hyperalgesia was always followed, in the continued presence of the stimulation, by a switch to a negative, analgesic effect. While this sign-reversal occurred in both pre-exposed and non-pre-exposed conditions, the latencies with which sign-reversal took place and the magnitude of both the initial hyperalgesic and subsequent analgesic effect differed between the two conditions. As a result, previous exposure to incremental values of nociceptive input made it possible for the highest, 63 A.U., intensity to generate an analgesic effect—expressed in AUC—that was considerably larger than that produced by any other condition. Furthermore, this large analgesic effect was preceded by an initial hyperalgesia that was smaller than that produced by acute exposure to a low-intensity stimulation (Fig. 1).

We then investigated, in actual in vivo experiments, whether the outcome generated by the simulations could be verified empirically. Specifically, we determined whether the administration of progressively increasing doses of F 13640 could similarly induce a high-magnitude analgesia without it inducing a large hyperalgesia initially. Chronic administration of F 13640 was carried out using subcutaneously implanted osmotic mini-pumps that were replaced every week for 5 weeks. Animals in which the dose of F 13640 was gradually increased over the 5-week period demonstrated an increased analgesic effect as the dose of F 13640 was augmented while controls without previous F 13640 history showed significantly less analgesia (Fig. 2). At the same time, hyperalgesia was marked in controls that received only a single dose of F 13640, while this effect was significantly smaller and remained small throughout the 5-week period in animals receiving incremental doses of the compound (Fig. 2). Thus, and in accordance with theory-guided simulations, the progressive increase of the dose of F 13640 over time established a large analgesia that was preceded by a hyperalgesic effect that was small and comparable to that induced by low doses of the compound.

In the course of these experiments, we also monitored the 5-HT syndrome which F 13640, like other 5-HT_{1A} receptor agonists, is expected to produce. Specifically, we determined whether by gradually increasing the dose of F 13640 over time, tachyphylaxis would develop progressively to the syndrome-inducing action of the compound so that eventually the 0.63 mg/rat/day dose could be reached without ever inducing a frank 5-HT syndrome. For this purpose, body temperature and behavioral elements of the 5-HT syndrome (i.e., forepaw treading, flat body posture and lower lip retraction) were measured over the 5-week experimental period. Animals without a previous history of F 13640 treatment showed a significant hypothermic effect when infused with 0.31 or 0.63 mg/rat/day; the administration of progressively increasing doses of F 13640 significantly attenuated this hypothermia (Fig. 3D). The

chronic administration of incremental doses of F 13640 also appeared to induce tachyphylaxis to each of the three behavioral elements of the 5-HT syndrome that were monitored; this was most evident, and statistically significant, at the 0.63 mg/rat/day dose (Fig. 3A–C). These results are consistent with earlier evidence (for review, see De Vry, 1995) demonstrating repeated injections of 5-HT_{1A} receptor agonists to induce tachyphylaxis to the 5-HT syndrome in rats. Thus, the findings reported here demonstrate gradually increasing doses of F 13640 to induce a large analgesic effect on a mechanically induced pain response in the relative absence of the 5-HT syndrome. The findings also are consistent with earlier data (Bardin et al., 2001) demonstrating that 5-HT_{1A} receptor activation induces a behaviorally specific effect on formalin-induced pain.

The signal transduction concept that guided this work provides a formal mechanism of transduction which has been successful here as elsewhere (e.g., Bruins Slot et al., 2002) in predicting input/output relationships; the actual molecular, intracellular and neurophysiological signaling pathways that are involved in the dual and paradoxical effects of 5-HT_{1A} receptor activation remain to be identified. Of note, serotonin exerts dual and paradoxical electrophysiological effects on afferent synaptic transmission to the lateral giant neurons of crayfish (Teshiba et al., 2001). It would be of interest to investigate, for instance, whether the dual effects which the activation of G-protein-coupled 5-HT_{1A} receptors produces on nociceptive systems in mammals may be mediated by a shift in the coupling of the receptor from one (e.g., stimulatory) to another (e.g., inhibitory) G-protein subunit. Indeed, such shifts have been suggested to mediate dual effects of G-protein-coupled opioid receptors (e.g., Chakrabarti et al., 2001).

In conclusion, the studies presented here examined the effects of progressively increasing doses of F 13640, a novel central 5-HT_{1A} analgesic, on a mechanically induced pain response and on the 5-HT syndrome in rats. Chronic administration via subcutaneously implanted mini-pumps of progressively increasing doses of F 13640 over a 5-week period induced a large analgesia preceded by an only small hyperalgesic effect. Furthermore, incrementing the dose of F 13640 induced tachyphylaxis to the hypothermia and behavioral 5-HT syndrome that was otherwise induced by F 13640. Mirroring the (analgesia-to-hyperalgesia) reversal of the sign of the effects of continuous opioid exposure (Bruins Slot et al., 2002), the present data indicate that continuous infusion of F 13640 induces a hyperalgesia-to-analgesia sign-reversal of its effects on nociceptive systems. The date further indicate that even in the absence of ongoing nociception, a progressive increase in dose allows this new mechanism of pain relief to produce a large analgesia without initially causing marked hyperalgesia. Further research is required to elucidate the molecular and cellular pathways of the signal transduction mechanisms underlying these novel neuroadaptive actions.

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