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Profound, Non-Opioid Analgesia Produced by the High-Efficacy 5-HT_{1A} Agonist F 13640 in the Formalin Model of Tonic Nociceptive Pain

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Key Words
5-HT_{1A} receptors · Analgesic effect · Pain · Formalin test, rats

Abstract
Previously, we have reported that in rat models of chronic pain, in particular, the very-high-efficacy 5-HT_{1A} agonist F 13640 induces unprecedented pain relief by novel neuroadaptative mechanisms that involve inverse tolerance and cooperation with nociceptive stimulation in producing analgesia. The present studies detailed the actions of F 13640 and other compounds in the formalin model of tonic nociceptive pain. Intraperitoneal injection of F 13640 (0.01–2.5 mg/kg; t –15 min) caused a dose-dependent and complete inhibition of the paw elevation and paw licking that occurred both early (0–5 min) and late (22.5–27.5 min) after the intraplantar injection of diluted formaldehyde (2.5%) in the rat. The extent to which F 13640 and other 5-HT_{1A} receptor ligands inhibited these pain behaviors correlated ($p < 0.05$) with the extent to which they activated 5-HT_{1A} receptors. Under similar conditions, some inhibitory effects were also observed with various agents that are known to produce analgesia by different peripheral and/or central mechanisms (e.g., opioids, NA/5-HT reuptake inhibitors, COX-2 inhibitors and other nonsteroidal anti-inflammatory drugs, gabapentin, and ABT-594). However, with the possible exception of morphine, the effects of all of these agents at nontoxic doses were lower than those of F 13640, in particular in inhibition of early paw elevation. The 5-HT_{1A} antagonist WAY 100635, but not naloxone, antagonized the actions of F 13640. These results help to establish large-magnitude 5-HT_{1A} receptor activation as a new molecular mechanism of profound, central analgesia and suggest that F 13640 may be particularly effective against pain arising from severe tonic nociceptive stimulation.

Introduction

Considerable research has been conducted over the past decades to identify new, nonopioid, central mechanisms of pain relief [1–3]. In our laboratory, this research has been guided by a concept of signal transduction in nociceptive systems [4–6]. The concept specifies that any

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input to the nociceptive systems causes not a single, but dual effects that are bidirectional, or opposite in sign. Thus, morphine produces both analgesia as a so-called 1st-order effect and also hyperalgesia as a 2nd-order effect. Upon chronic opioid treatment, the 2nd-order hyperalgesia increases and neutralizes the 1st-order effect. This concept thus provides a powerful account of the dynamic, time-dependent neuroadaptative changes that ensue upon μ-opioid receptor activation [for a review see 5, 6]. Also according to this concept, nociceptive stimulation should similarly produce dual, bidirectional, effects which should amount to the mirror opposite of those produced by opioid receptor activation. Some molecular principle could exist that mimics the actions of nociceptive stimulation.

We have recently discovered [7] that very-high-efficacy 5-HT_{1A} receptor activation realizes this principle. The single injection of the very-high-efficacy selective 5-HT_{1A} agonist F 13640 produced dual and bidirectional hyper- and hypoalgesic effects on the vocalization threshold to mechanical stimulation in the rat. Upon chronic treatment, F 13640 produced an initial hyperalgesia that then decayed, while hypoalgesia became amplified, thus demonstrating the development of inverse tolerance. Continuous infusion of F 13640 produced an analgesia in rat models of chronic nociceptive pain and of chronic allodynia that surpassed that of morphine or other mechanisms of central analgesia. These data thus identify large-amplitude activation of 5-HT_{1A} receptors as a new molecular mechanism of analgesia, the neuroadaptive mechanisms of which consist of inverse tolerance and, also, of the cooperation with nociceptive stimulation in producing analgesia [7].

The studies reported here provide a pharmacological characterization of F-13640-induced analgesia. In the first series of experiments we examined the relationship between the magnitude of the receptor activation produced by different 5-HT_{1A} receptor ligands and that of analgesia as measured by different behavioral parameters. F 13640 was studied along with a series of prototypical as well as recent selective 5-HT_{1A} ligands that vary considerably in terms of their intrinsic activity [8, 9]. A second series of experiments aimed at determining the magnitude of F-13640-induced analgesia relative to that afforded by other mechanisms of analgesic drug action. Here, F 13640 was compared with chemically and mechanistically diverse agents that have previously been shown to exert analgesic effects by different central and peripheral mechanisms. In a third series of experiments we sought to further substantiate the involvement of 5-HT_{1A} receptors in F-13640-produced analgesia by determining the effects of pretreatment with WAY 100635, a selective 5-HT_{1A} antagonist [10].

Complementing data obtained in acute and chronic conditions [7], the present studies were carried out with the formalin model of tonic nociceptive pain. The formalin test was developed as a model of persistent pain that may be more relevant to clinical pain than threshold tests involving nociceptive stimulations of much shorter duration [11]. The localized injection of formalin into the hindpaw produces a characteristic biphasic electrophysiological [12] and behavioral [13] response. The first phase reflects a direct activation of sensory afferents, while the second phase presumably involves ongoing peripheral activity, inflammation, and central sensitization [14]. In addition, previous data have indicated that the formalin procedure is responsive to the actions of 5-HT_{1A} ligands [15, 16] in a manner that is behaviorally specific [17].

Materials and Methods

Animals

Male Sprague-Dawley rats [Ico: OFA SD (IOPS)] (Iffa Credo, l'Arbresle, France), weighing 160–180 g on arrival, were housed in groups (5 animals/cage) in an environmentally controlled room (temperature 21 ± 1°C; relative humidity 55 ± 5%) under a 12-hour light-dark cycle (lights on at 07.00 h) with food and water freely available. A 5-day acclimatization period was allowed before the animals were used in experiments. The animals were transferred to the laboratory room on the day before the experiment and were maintained under the same conditions as during quarantine. The animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) and the Guidelines of the Ethics Committee of the International Association for the Study of Pain (1983), and procedures were approved by the local Ethics Committee for animal research. Animals were used only once and were sacrificed immediately after the experiment.

Formalin Test

All experiments were performed in a blind fashion in a quiet room, between 09.00 and 16.00 h, by a single experimenter. The formalin test was carried out as described [17] in a clear plastic chamber with a mirror placed under the floor at a 45° angle to allow an unobstructed view of the paws. Each animal was placed in the chamber for a 30-min habituation period. Thereafter, the rats received a 50-μl subcutaneous injection of diluted (2.5%) formaldehyde into the plantar surface of the right hindpaw. Following this, the rat was returned to the chamber. The recording of behavior started immediately and lasted for 5 min ('early phase'). The recording of the 'late phase' started 22.5 min after formalin injection and lasted 5 min. During each of these two 5-min periods, observations were made in the following way. Every 30 s, rats were observed for the presence or absence of spontaneous pain behaviors, i.e.: the injected paw is elevated and not in contact with any surface, and the injected paw is

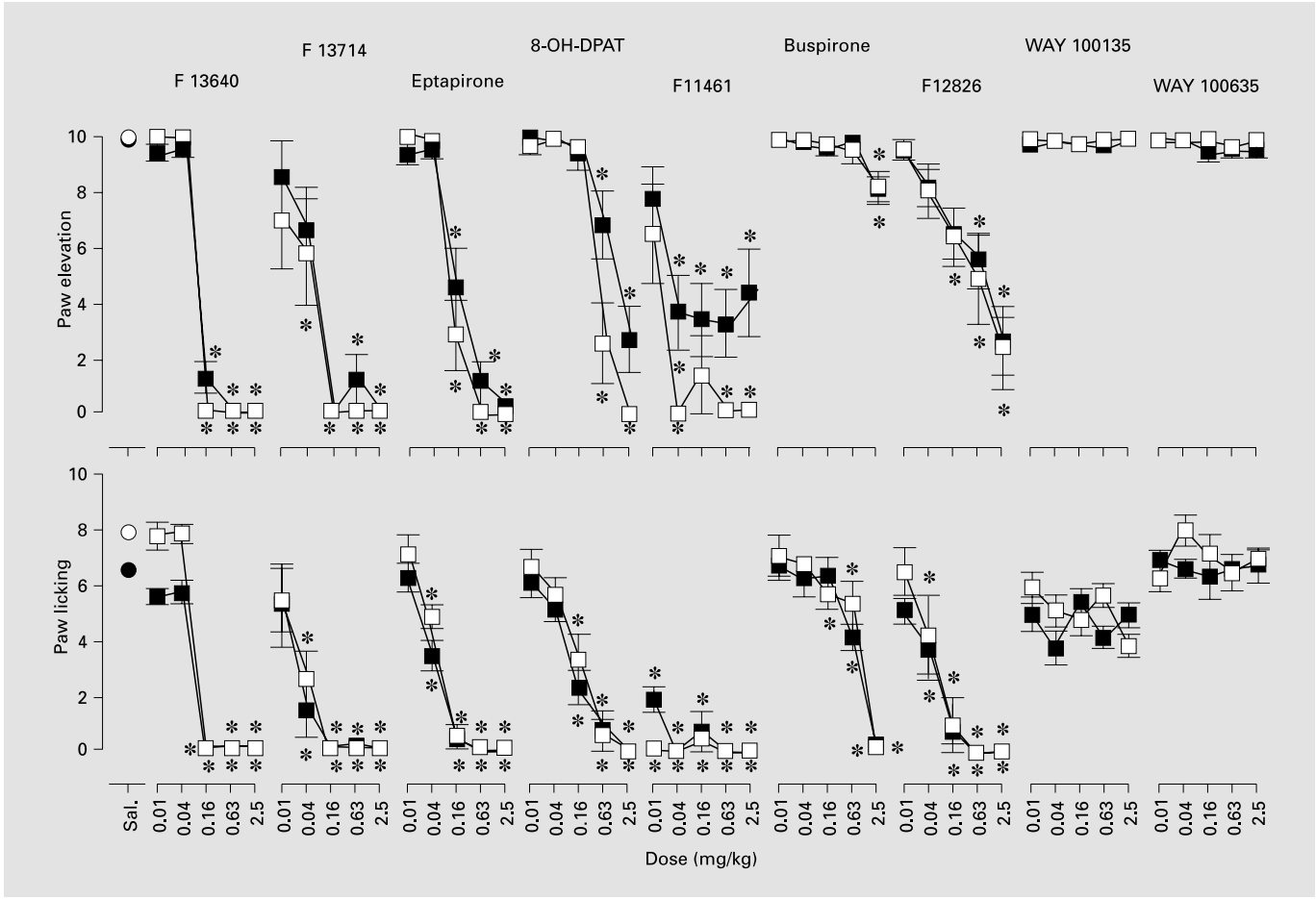

Fig. 1. Effects of 0.01- to 2.5-mg/kg doses of various 5-HT_{1A} ligands on paw elevation and paw licking that occur during the early (i.e., 0.5 min; solid symbols) and the late phase (i.e., 22.5–27.5 min; open symbols) after intraplantar injection of formalin (50 µl, 2.5%) in rats. Compounds or saline (Sal.) were administered intraperitoneally 15 min before formalin injection. Values represent mean ± SEM scores (maximal score = 10) of 7 animals. * $p < 0.05$ as compared with saline-treated controls (Dunnett's test).

licked. This observation cycle was repeated ten times during the 5-min period; thus, the incidence of a particular behavior could vary from 0 to 10 for each of the two observation periods.

Pharmacological Experiments

Drugs or vehicle was administered intraperitoneally 15 min before the injection of formalin. Any motor or other conspicuous effects observed 15 min after the intraperitoneal injection and before the injection of formalin were also recorded. In interaction studies, the rats were pretreated subcutaneously with WAY 100635 (0.63 mg/kg), naloxone (0.63 mg/kg), or saline 45 min before the intraperitoneal injection of F 13640 (0.63 mg/kg), morphine (10 mg/kg), or saline.

Drugs

The compounds used were: 8-OH-DPAT hydrobromide, imipramine hydrochloride, and U-50488 transmethane sulfate (Research Biochemicals, Natick, Mass., USA); acetylsalicylic acid, amitriptyline hydrochloride, baclofen, buspirone hydrochloride, buprenorphine chlorhydrate, carbamazepine, diclofenac sodium, ketamine chlorhydrate, and naloxone hydrochloride (Sigma Chemical, St. Louis, Mo., USA); morphine chlorhydrate (Cooperation Pharmaceutique Française, Melun, France); ABT-594 tosylate, BW-4030W92 hydrochloride, celecoxib, gabapentin, paroxetine hydrochloride, rofecoxib, and sumatriptan hydrochloride (synthesized by Centre de Recherche Pierre-Fabre); 4-acetamidophenol (Acros Organics/Janssen Pharmaceuticals, Geel, Belgium); 3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-pyridine-2-ylmethyl)-amino]-methyl}-piperidine-1-yl)-methanone (F 13640; MW = 511), 3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-6-methylamino-pyridine-2-ylmethyl)-amino]-methyl}-piperidine-1-yl)-methanone fumaric acid salt (F 13714; MW = 539), 4-methyl-2-[4-(4-pyrimidine-2-yl-piperazine-1-yl)-butyl]-2H[1,2,4]triazine-3,5-dione maleic acid salt (eptapirone, maleic acid salt of F 11440; MW = 461), 2-[4-[4-(7-methoxy-1-naph-

Table 1. Quantification of the ability of F 13640 and other 5-HT_{1A} receptor ligands to inhibit paw elevation and paw licking during the early and late phases after intraplantar formalin injection in rats

Drugs	Early phase				Late phase			
	potency			maximum effect (LS)	potency			maximum effect (LS)
	ED_{50}	95% CL	MSD		ED_{50}	95% CL	MSD	
Inhibition of formalin-induced paw elevation								
F 13640	0.029	0.0075–0.11	0.16	0 ± 0 (0.63)	0.081	0.051–0.13	0.16	0 ± 0 (0.16)
F 13714	0.032	0.010–0.098	0.16	0 ± 0 (0.16)	0.022	0.0051–0.093	0.04	0 ± 0 (0.16)
Eptapirone	0.038	0.015–0.092	0.16	0.29 ± 0.18 (2.5)	0.095	0.048–0.19	0.16	0 ± 0 (0.63)
8-OH-DPAT	0.43	0.19–0.98	0.63	2.7 ± 1.2 (2.5)	0.21	0.076–0.59	0.63	0 ± 0 (2.5)
F 11461	<0.01	–	0.04	3.3 ± 1.2 (0.63)	0.0082	0.0017–0.037	0.04	0 ± 0 (0.04)
Buspirone	0.95	0.29–3.11	2.5	8.1 ± 0.51 (2.5)	1.6	0.46–5.80	2.5	8.3 ± 0.57 (2.5)
F 12826	0.024	0.0077–0.079	0.16	2.6 ± 1.1 (2.5)	0.059	0.016–0.21	0.63	2.3 ± 1.5 (2.5)
WAY 100135	–	–	–	9.8 ± 0.14 (0.01)	–	–	–	9.8 ± 0.14 (0.16)
WAY 100635	–	–	–	9.6 ± 0.30 (0.16)	–	–	–	9.8 ± 0.14 (0.63)
Inhibition of formalin-induced paw licking								
F 13640	0.081	0.051–0.13	0.16	0 ± 0 (0.16)	0.081	0.051–0.13	0.16	0 ± 0 (0.16)
F 13714	0.018	0.0075–0.043	0.04	0 ± 0 (0.16)	0.0074	0.0014–0.038	0.04	0 ± 0 (0.16)
Eptapirone	0.029	0.011–0.072	0.04	0 ± 0 (0.63)	0.018	0.0086–0.041	0.04	0 ± 0 (0.63)
8-OH-DPAT	0.10	0.029–0.35	0.16	0 ± 0 (2.5)	0.051	0.018–0.15	0.16	0 ± 0 (2.5)
F 11461	<0.01	–	0.01	0 ± 0 (0.04)	<0.01	–	0.01	0 ± 0 (0.01)
Buspirone	0.45	0.19–1.0	0.63	0.29 ± 0.18 (2.5)	0.26	0.082–0.86	0.16	0.14 ± 0.14 (2.5)
F 12826	0.058	0.025–0.13	0.04	0 ± 0 (0.63)	0.034	0.012–0.92	0.04	0 ± 0 (0.63)
WAY 100135	–	–	–	3.9 ± 0.63 (0.04)	–	–	–	4.0 ± 0.45 (2.5)
WAY 100635	–	–	–	6.6 ± 0.89 (0.16)	–	–	–	6.6 ± 0.59 (0.01)

All compounds were administered intraperitoneally 15 min before formalin injection.

Analyses are based on the data shown in figure 1. All compounds were tested at doses ranging from 0.01 to 2.5 mg/kg. ED_{50} values (and 95% CL) are expressed as milligrams per kilogram and represent doses producing an inhibitory effect in 50% of the animals. MSD = Minimum significant dose (i.e., the lowest dose that is significantly different from saline-treated controls; $p < 0.05$); LS = lowest average score (between parentheses: dose at which LS was found).

thyl)piperazino]-butyl]-4-methyl-2H,4H-1,2,4-triazine-3,5-dione, maleic acid salt (F 11461; MW = 540), 2-[4-[4-(7-benzofuranyl)piperazino]butyl]-4-methyl-2H,4H-1,2,4-triazine-3,5-dione (F 12826; MW = 383), (s)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazine-1-yl)-2-phenylpropanamide hydrochloride [(s)-WAY 100135], and N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide dihydrochloride (WAY 100635; synthesized by Centre de Recherche Pierre-Fabre).

The drugs were dissolved in distilled water or suspended in distilled water by adding Tween 80 (2 drops/10 ml) and injected intraperitoneally or subcutaneously in a volume of 10 ml/kg. Vehicle controls received either physiological saline (0.9% NACl) or physiological saline plus Tween 80 (2 drops/10 ml), as appropriate. Doses refer to the free base.

Part of the data (i.e., inhibition of early-phase paw elevation by 5-HT_{1A} ligands) has been reported elsewhere [7].

Statistics

Data were analyzed by one- or two-way analyses of variance (Anova) followed, when the F value was significant, by Dunnett's test. $p < 0.05$ was considered to be statistically significant. To calculate ED_{50} values, the results were expressed as the percentage of animals showing significant inhibition of paw elevation and paw licking (i.e., scores lower than 9 for paw elevation and lower than 3 or 5 for paw licking during early or late phase, respectively; such scores occurred in less than 5% of all formalin-treated vehicle controls). Using this percentage, ED_{50} values and 95% confidence limits were calculated with the Litchfield and Wilcoxon probit analysis procedure, using the program described by Tallarida and Murray [18]. When less than two intermediate data points were obtained, 0% and/or 100% effects were transformed by means of Berkson's adjustment to permit the use of the Litchfield and Wilcoxon procedure. Relations between the rank orders of the maximal effects observed here with the 5-HT_{1A} ligands and the previously reported rank order of their intrinsic activities at 5-HT_{1A} receptors were examined by calculating Spearman's rank correlation (r_s) [19].

Results

Effects of 5-HT_{1A} Ligands

With the exception of the low-efficacy compound WAY 100135 and WAY 100635, all 5-HT_{1A} ligands decreased both paw elevation and paw licking during both the early and the late phases (fig. 1). In particular, F 13640 produced dose-dependent suppression of each of these four behavioral parameters (paw elevation, early phase: $F[5,36] = 312.47$, $p < 0.001$; paw elevation, late phase: $F[5,36] = 1{,}000$, $p < 0.001$; paw licking, early phase:

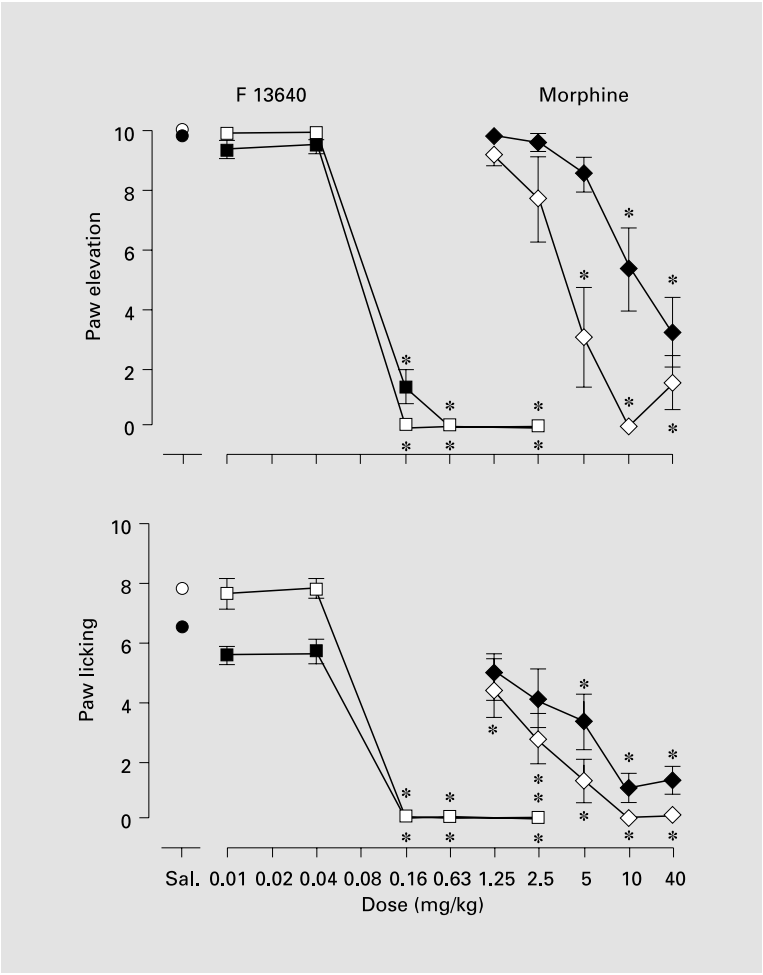

Fig. 2. Effects of F 13640 in comparison with those of morphine on the paw elevation and paw licking that occur during the early (i.e., 0–5 min; solid symbols) and the late phase (i.e., 22.5–27.5 min; open symbols) after intraplantar injection of formalin (50 µl, 2.5%) in rats. F 13640 data are replotted from figure 1. See also legend to figure 1.

Table 2. Elements of the 5-HT syndrome observed with 5-HT_{1A} receptor ligands, 15 min after their intraperitoneal administration

Drugs	Maximal percentage incidence		
	forepaw treading	irregular respiration	tremor
F 13640	100 (0.16)	–	–
F 13714	100 (0.16)	–	28 (2.5)
Eptapirone	100 (0.63)	28 (0.63)	–
8-OH-DPAT	85 (2.5)	43 (2.5)	–
F 11461	71 (0.04)	85 (2.5)	–
Buspirone	–	28 (0.01)	–
F 12826	29 (0.16)	28 (0.04)	–
WAY 100135	–	–	–
WAY 100635	–	14 (0.63)	–

All compounds were tested at doses ranging from 0.01 to 2.5 mg/kg; in parentheses: the dose at which the maximum effect was observed.

$F[5,36] = 118.47$, $p < 0.001$; paw licking, late phase: $F[5,36] = 240.90$, $p < 0.001$). Anova similarly yielded a significant ($p < 0.05$) main effect of dose for each of the other 5-HT_{1A} ligands, except WAY 100135 and WAY 100635.

The data shown in figure 1 and their further analysis (table 1) indicate that the ligands differed in potency, the most and least potent compounds being F 13714 and buspirone, respectively. More importantly, only F 13640 and F 13714 produced a complete suppression of all four parameters, the magnitude of the maximal effects of other ligands being somewhat (e.g., eptapirone) or considerably (e.g., buspirone) smaller. Although all four parameters were affected by the 5-HT_{1A} ligands, paw elevation was the behavior that was least readily suppressed, particularly during the early phase; in contrast, paw licking scores were readily reduced to zero, both in the early and in the late phase.

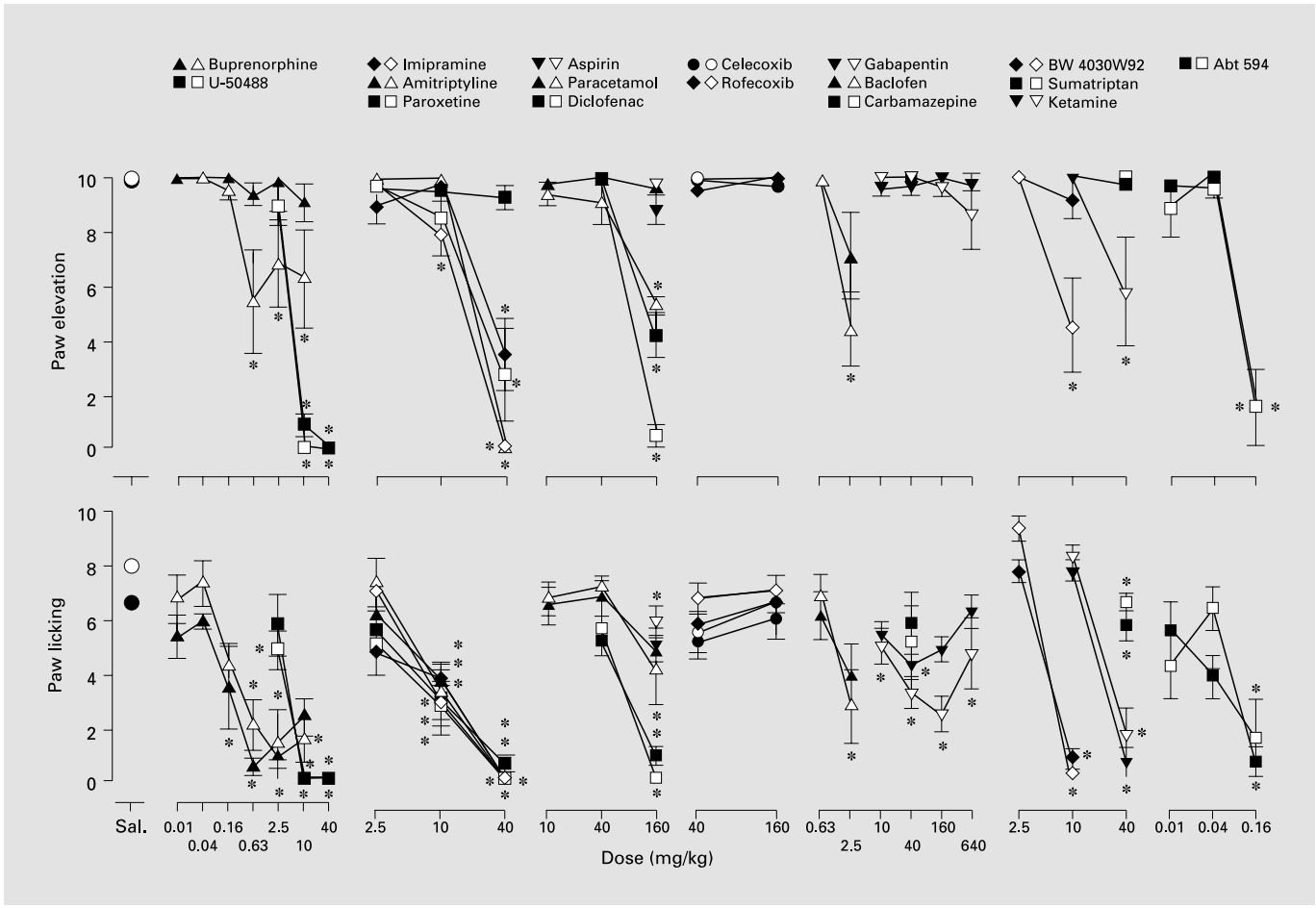

Fig. 3. Effects of various classes of analgesic drugs on the paw elevation and paw licking that occur during the early (i.e., 0–5 min; solid symbols) and the late phase (i.e., 22.5–27.5 min; open symbols) after intraplantar injection of formalin (50 µl, 2.5%) in rats. See also legend to figure 1.

Previous studies [7, 9] have characterized the efficacy at 5-HT_{1A} receptors of the ligands examined here and found their intrinsic activities to differ according to the rank order with which they are presented in figure 1 (i.e., highest intrinsic activity: F 13640; lowest intrinsic activity: WAY 100635). This rank order, then, appears to correlate positively with the rank order of maximal magnitude of effect (i.e., with the lowest score; table 1) which the ligands exerted on the four behavioral parameters (Spearman's rank correlation: paw elevation, early phase: $r_s = 0.89$, $p < 0.001$; paw licking, early phase: $r_s = 0.77$, $p < 0.05$; paw elevation, late phase: $r_s = 0.89$, $p < 0.001$; paw licking, late phase: $r_s = 0.73$, $p < 0.05$).

As shown in table 2, most of the 5-HT_{1A} receptor ligands also produced elements of the 5-HT syndrome. These generally occurred at doses equal to or higher than those that inhibited pain parameters. All of the compounds with 5-HT_{1A} receptor agonist properties, except buspirone, produced forepaw treading. In addition, all 5-HT_{1A} ligands induced irregular respiration, except F 13640, F 13714, and WAY 100135. At higher doses, F 13714 also produced some tremor (in 2 of 7 animals).

Effects of Other Agents

The results obtained are summarized in figures 2 and 3 and tables 3 and 4. Antinociception varied among the drug classes, between the early and the late phase, and also according to the pain behavior measured.

Morphine reduced dose dependently all parameters (fig. 2), but inhibited paw elevation in the early phase only at high doses (i.e., 10–40 mg/kg) producing adverse effects

Table 3. Quantification of the ability of various analgesic compounds to inhibit formalin-induced paw elevation during the early and late phases after intraplantar formalin injection in rats

Drugs	Dose range	Inhibition of formalin-induced paw elevation							
		early phase				late phase			
		potency			maximum effect (LS)	potency			maximum effect (LS)
		ED_{50}	95% CL	MSD		ED_{50}	95% CL	MSD	
Morphine	1.25–40	4.2	2.2–7.8	10	3.1 ± 1.2 (40)	2.1	1.1–3.7	10	0 ± 0 (10)
Buprenorphine	0.01–10	>10	–	>10	9.1 ± 0.70 (10)	5.8	0.51–70	0.63	5.4 ± 1.9 (0.63)
U-50488	2.5–40	4.6	2.1–9.9	10	0 ± 0 (40)	3.6	1.5–8.6	10	0 ± 0 (10)
Imipramine	2.5–40	14	3.9–48	40	3.4 ± 1.3 (40)	7.2	2.8–19	10	0 ± 0 (40)
Amitriptyline	2.5–40	18	9.2–36	40	0 ± 0 (40)	18	9.2–36	40	0 ± 0 (40)
Paroxetine	2.5–40	>40	–	>40	9.3 ± 0.47 (40)	16	6.1–41	40	2.7 ± 1.8 (40)
Aspirin	160	>160	–	>160	8.8 ± 0.55 (160)	>160	–	>160	9.8 ± 0.14 (160)
Diclofenac	40–160		–	160	4.1 ± 0.77 (160)		–	160	0.43 ± 0.43 (160)
Paracetamol	10–160	>160	–	>160	9.7 ± 0.29 (160)	55	16–192	160	5.2 ± 1.5 (160)
Rofecoxib	40–160	>160	–	>160	9.6 ± 0.20 (160)	>160	–	>160	9.4 ± 0.43 (160)
Celecoxib	40–160	>160	–	>160	9.7 ± 0.18 (160)	>160	–	>160	10 ± 0 (160)
Gabapentin	10–640	>640	–	>640	9.5 ± 0.30 (10)	>640	–	>640	8.5 ± 1.2 (640)
Baclofen	0.63–2.5	>2.5	–	>2.5	7.0 ± 1.6 (2.5)			2.5	4.3 ± 1.4 (2.5)
Carbamazepine	40	>40	–	>40	10 ± 0 (40)	>40	–	>40	10 ± 0 (40)
Ketamine	10–40	>40	–	>40	9.7 ± 0.29 (40)	>40	–	40	5.7 ± 2 (40)
Sumatriptan	40	>40	–	>40	9.7 ± 0.29 (40)	>40	–	>40	10 ± 0 (40)
BW 4030W92	2.5–10	>10	–	>10	9.1 ± 0.70 (10)	6.6[a]	ND	10	4.4 ± 1.7 (10)
ABT 594	0.01–0.16	0.041	0.014–0.12	0.16	1.4 ± 1.4 (0.16)	0.068	0.027–0.18	0.16	1.4 ± 1.4 (0.16)

All compounds were administered intraperitoneally 15 min before formalin injection. Abbreviations as in table 1. Doses and ED_{50} values are expressed as milligrams per kilogram. ND = Not determined due to insufficient data. Analyses are based on the data shown in figures 2 and 3.

[a] Obtained by interpolation.

(table 5). Note that the effects of F 13640 were at least equal to those of morphine at morphine doses (i.e. ≤ 5 mg/kg) that did not induce akinesia.

The partial μ-opioid agonist buprenorphine inhibited paw licking in a dose-dependent manner, but exerted no or only partial effects on paw elevation (fig. 3), even at the 10-mg/kg dose at which it induced akinesia (table 5).

The κ-opioid agonist U-50488 exerted little effect at 2.5 mg/kg; only at the 10- to 40-mg/kg doses, at which it compromised respiration and induced akinesia, did the compound suppress the different behaviors.

At 10 mg/kg, the NA/5-HT reuptake inhibitors imipramine, amitriptyline, and paroxetine exerted significant, partial effects on paw licking, but more intense effects on this behavior and any substantial effect on paw elevation required a dose (i.e., 40 mg/kg), at which the agents appeared toxic (table 5).

The nonsteroidal anti-inflammatory drugs aspirin and paracetamol exerted only little effects at doses as high as 160 mg/kg. Diclofenac at this latter dose exerted larger effects, but also apparent toxicity (table 5). The COX-2 inhibitors rofecoxib and celecoxib were inactive.

The anticonvulsant carbamazepine was ineffective at 40 mg/kg. Gabapentin partially inhibited paw licking from a dose as low as 10 mg/kg onward, but failed to significantly affect paw elevation at doses up to 640 mg/kg. At 2.5 mg/kg, baclofen significantly reduced both late-phase paw licking and late-phase paw elevation (fig. 3), but also induced akinesia (table 5).

Only at a dose (i.e., 40 mg/kg) at which it induced akinesia, did the NMDA antagonist ketamine exert significant effects.

The $5\text{-}HT_{1B/D}$ receptor agonist and antimigraine agent sumatriptan slightly diminished paw licking and failed to affect paw elevation.

At 10 mg/kg, the sodium channel blocker BW-4030W92 reduced all parameters except early paw elevation and induced ataxia (table 5).

Finally, the nicotinic acetylcholine receptor agonist ABT-594 was ineffective at 0.01 and 0.04 mg/kg; at

Table 4. Quantification of the ability of various analgesic compounds to inhibit formalin-induced paw licking during the early and late phases after intraplantar formalin injection in rats

Drugs	Dose range	Inhibition of formalin-induced paw licking							
		early phase				late phase			
		potency			maximum effect (LS)	potency			maximum effect (LS)
		ED_{50}	95% CL	MSD		ED_{50}	95% CL	MSD	
Morphine	1.25–40	2.4	1.1–5.4	10	1.0 ± 0.49 (10)	<1.25	–	1.25	0 ± 0 (10)
Buprenorphine	0.01–10	0.25	0.051–1.1	0.63	0.86 ± 0.46 (2.5)	0.051	0.0075–0.35	0.16	0.43 ± 0.30 (0.63)
U-50488	2.5–40	3.6	1.5–8.6	10	0 ± 0 (10)	3.6	1.5–8.6	2.5	0 ± 0 (10)
Imipramine	2.5–40	7.6	2.5–23	10	0 ± 0 (40)	3.6	1.5–8.6	10	0 ± 0 (40)
Amitriptyline	2.5–40	15	7.5–30	10	0 ± 0 (40)	5.1	3–8.7	10	0 ± 0 (40)
Paroxetine	2.5–40	13	6–28	10	0.57 ± 0.29 (40)	4.4	1.2–16	10	0 ± 0 (40)
Aspirin	160	>160	–	>160	5.0 ± 0.62 (160)	>160	–	160	5.8 ± 0.59 (160)
Diclofenac	40–160		–	160	0.86 ± 0.34 (160)		–	160	0 ± 0 (160)
Paracetamol	10–160	>160	–	>160	4.7 ± 0.63 (160)	93.1[a]	9.9–872	160	4.0 ± 1.2 (160)
Rofecoxib	40–160	>160	–	>160	5.7 ± 0.42 (40)	>160	–	>160	6.7 ± 0.57 (40)
Celecoxib	40–160	>160	–	>160	5.1 ± 0.70 (40)	>160	–	>160	5.4 ± 0.78 (40)
Gabapentin	10–640	>640	–	40	4.1 ± 0.86 (40)	<10	–	10	2.3 ± 0.68 (160)
Baclofen	0.63–2.5	>2.5	–	>2.5	3.1 ± 1.3 (2.5)			2.5	2.6 ± 1.3 (2.5)
Carbamazepine	40	>40	–	>40	5.7 ± 1.1 (40)	<40	–	>40	5 ± 1.4 (40)
Ketamine	10–40	22[a]	ND	40	0.57 ± 0.57 (40)	22[a]	ND	40	1.6 ± 1.0 (40)
Sumatriptan	40	>40	–	40	5.7 ± 0.64 (40)	>40	–	40	6.5 ± 0.30 (40)
BW 4030W92	2.5–10	5.0[a]	ND	10	0.71 ± 0.36 (10)	5.0[a]	ND	10	0.14 ± 0.14 (10)
ABT 594	0.01–0.16	0.033	0.012–0.092	0.16	0.57 ± 0.57 (0.16)	0.013	0.0021–0.086	0.16	1.4 ± 1.4 (0.16)

All compounds were administered intraperitoneally 15 min before formalin injection. Abbreviations as in table 1. Doses and ED_{50} values are expressed as milligrams per kilogram. ND = Not determined due to insufficient data. Analyses are based on the data shown in figures 2 and 3.

[a] Obtained by interpolation.

0.16 mg/kg, it had effects on all four parameters, but appeared also toxic (table 5).

The results summarized in tables 3 and 4 indicate that most of the analgesic agents reduced formalin-induced paw licking during both phases. In contrast, it was more difficult for these agents to inhibit formalin-induced paw elevation, notably in the early phase. Thus, for 11 of the 18 analgesics tested, the lowest score obtained for paw elevation in the early phase was higher than the lowest scores for all other parameters. The compounds also differed in terms of the relative doses, at which they reduced early-phase paw elevation. For example, the opioid analgesics (morphine, buprenorphine, and U-50488) reduced paw licking behavior at doses that were about fourfold lower than those needed to inhibit partially paw elevation during the early phase. All other compounds tested failed to affect paw elevation in the early phase or exerted only modest effects, like morphine and buprenorphine. These latter effects, however, occurred only at doses that also produced apparent toxicity. In all instances, paw elevation constituted the pain parameter that was most resistant to inhibition.

Effects of WAY 100635 and Naloxone on F 13640-Induced Analgesia

Pretreatment with WAY 100635 at a dose of 0.63 mg/kg, 45 min before intraperitoneal injection of 0.63 mg/kg of F 13640, completely blocked the antinociceptive effects of F 13640 (fig. 4). Two-way Anova found that during each phase, the pretreatment ($F[1,24] \geq 16.50$; $p < 0.001$) and the treatment ($F[1,24] \geq 23.75$; $p < 0.001$) interacted significantly ($F[1,24] \geq 13.36$; $p < 0.001$). Subsequent multiple comparisons showed that F 13640 significantly inhibited paw elevation and paw licking after vehicle pretreatment, but not after pretreatment with WAY 100635. Pretreatment with 0.63 mg/kg of naloxone failed to modify the antinociceptive effects of F 13640 (pretreatment·treatment interaction: $F[1,24] \leq 1.83$; $p > 0.05$).

Table 5. Adverse effects observed in rats 15 min after intraperitoneal administration of various analgesic compounds

Drugs	Dose range	Maximal percentage incidence				
		akinesia	ataxia	irregular respiration	convulsions	writhing
Morphine	1.25–40	100 (10)		71 (40)	14 (2.5)	
Buprenorphine	0.01–10	85 (10)				
U-50488	2.5–40	100 (40)		57 (10)		
Imipramine	2.5–40	100 (40)		100 (40)	29 (40)	
Amitriptyline	2.5–40	100 (40)		29 (40)		
Paroxetine	2.5–40	43 (40)	14 (40)	43 (10)		
Aspirin	160					
Diclofenac	40–160	100 (160)	100 (160)	100 (160)		
Paracetamol	10–160					
Rofecoxib	40–160			100 (160)		43 (160)
Celecoxib	40–160			29 (40)		43 (160)
Gabapentin	10–640	14 (640)				
Baclofen	0.63–2.5	100 (2.5)				
Carbamazepine	40					
Ketamine	10–40		85 (40)			
Sumatriptan	40			14 (40)		
BW 4030W92	2.5–10		43 (10)			
ABT 594	0.01–0.16	100 (0.16)	57 (0.04)	100 (0.16)	14 (0.16)	

Doses are expressed as milligrams per kilogram; in parentheses: the dose at which the maximum effect was observed.

Effects of Naloxone and WAY 100635 on Morphine-Induced Analgesia

Pretreatment with naloxone at a dose of 0.63 mg/kg, 45 min before intraperitoneal injection of 10 mg/kg of morphine, completely blocked the antinociceptive effects of morphine in the formalin test (fig. 5). Two-way Anova showed that in each phase, the pretreatment ($F[1,24] \geq 14.92$; $p < 0.001$) and the treatment ($F[1,24] \geq 37.01$; $p < 0.001$) interacted significantly ($F[1,24] \geq 21.73$; $p < 0.001$). Subsequent multiple comparisons showed that morphine significantly inhibited paw elevation and paw licking after vehicle pretreatment, but not after pretreatment with naloxone. Pretreatment with 0.63 mg/kg of WAY 100635 failed to modify the effects of morphine (pretreatment · treatment interaction: $F[1,24] \leq 1.33$; $p > 0.05$).

Discussion

Based on a concept of signal transduction in nociceptive systems [4, 5], we have recently proposed that large-amplitude 5-HT_{1A} receptor activation constitutes a novel mechanism of central analgesia [7]. The discovery of F 13640, a very-high-efficacy 5-HT_{1A} receptor agonist inducing complex neuroadaptive actions that present the mirror opposite of those of morphine [7], supports this concept. The research reported here characterized the analgesic activity of F 13640 in the formalin model of tonic nociceptive pain; for this purpose, the compound was compared both with a series of 5-HT_{1A} ligands and with a wide array of known analgesic agents which exemplify a wide diversity of chemical structures and of site and mode of action.

With the exception of WAY 100135 and WAY 100635, all 5-HT_{1A} ligands produced significant analgesic effects 15 min after their intraperitoneal injection (fig. 1). With F 13640, in particular, this analgesia occurred along the steep dose-response curve that is characteristic of

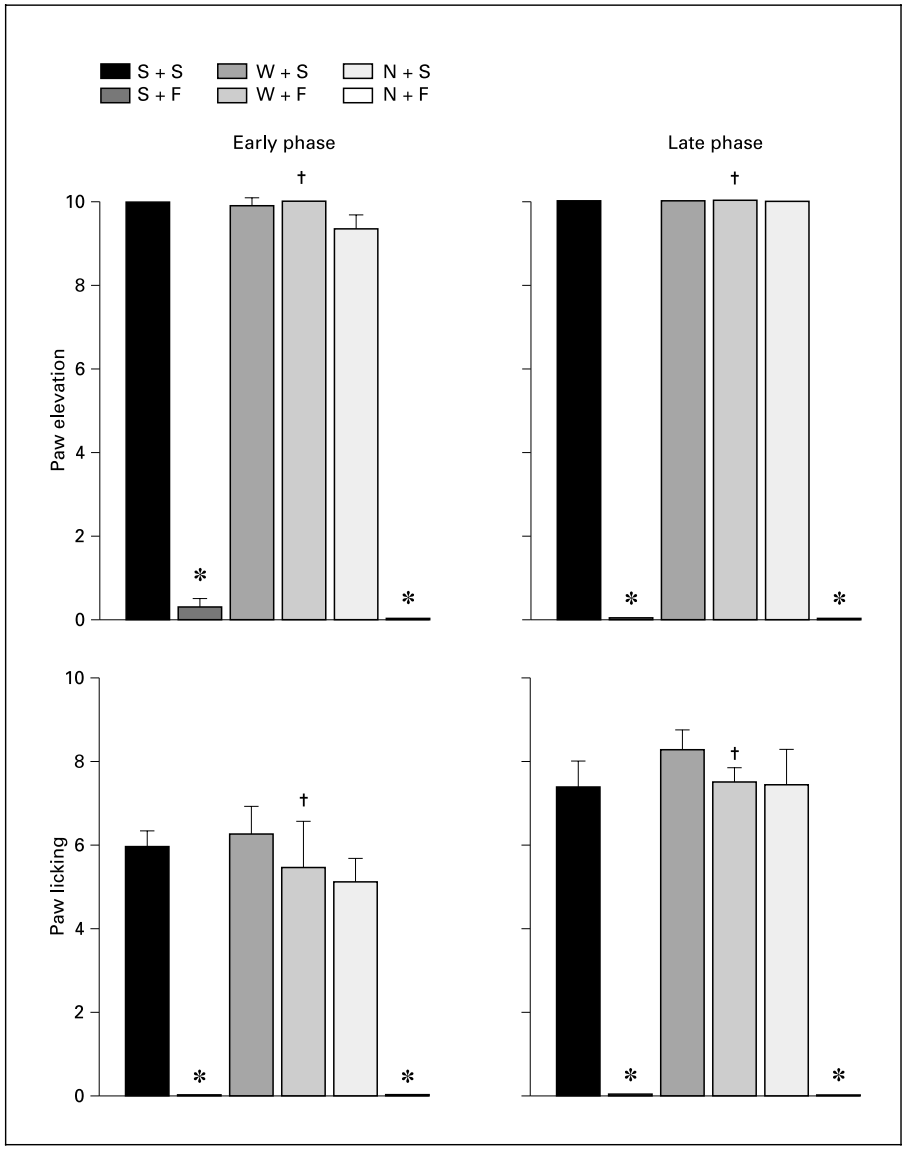

Fig. 4. Effects of pretreatment with WAY 100635 (W; 0.63 mg/kg s.c.) or naloxone (N; 0.63 mg/kg s.c.) on the ability of F 13640 (F; 0.63 mg/kg i.p.) to inhibit paw licking and paw elevation during the early (left panels) and the late (right panels) phase after formalin injection. Values represent the mean ± SEM scores of 7 animals. * $p < 0.05$ as compared with the saline + saline group (S + S); ** $p < 0.05$ as compared with the saline + F 13640 (S + F) group.

high-efficacy agonists. Regardless of whether it was assessed by the elevation or the licking of the formalin-injected paw during either the early or the late phase, the magnitude of this analgesia correlated positively ($p < 0.05$) with the magnitude with which the different ligands can activate 5-HT_{1A} receptors [9, 7]. Remarkable as it may seem, this latter efficacy rank order also correlates positively with the magnitude with which the same ligands, paradoxically, lower the threshold for mechanically elicited vocalization (Randall-Selitto test; also measured 15 min after their intraperitoneal injection) [7]. These outcomes are consistent, however, with the concept [5, 7] that 5-HT_{1A} receptor activation produces analgesia by mimicking the central neuroadaptive actions that ensue upon nociceptive stimulation. Specifically, the concept implies that 5-HT_{1A} receptor activation should, in a both intensity- and duration-dependent manner, cooperate with nociceptive stimulation in producing analgesia. Thus, and unlike the case of the Randall-Selito procedure when carried out in normal rats, formalin-induced nociception may reach the requirements of intensity and duration that allow this cooperative analgesia to become apparent. This is in accordance with evidence that, in the formalin procedure, the 'interphase' separating the immediate and the late phases of pain behaviors is due to an active inhibition induced by formalin nociception [20].

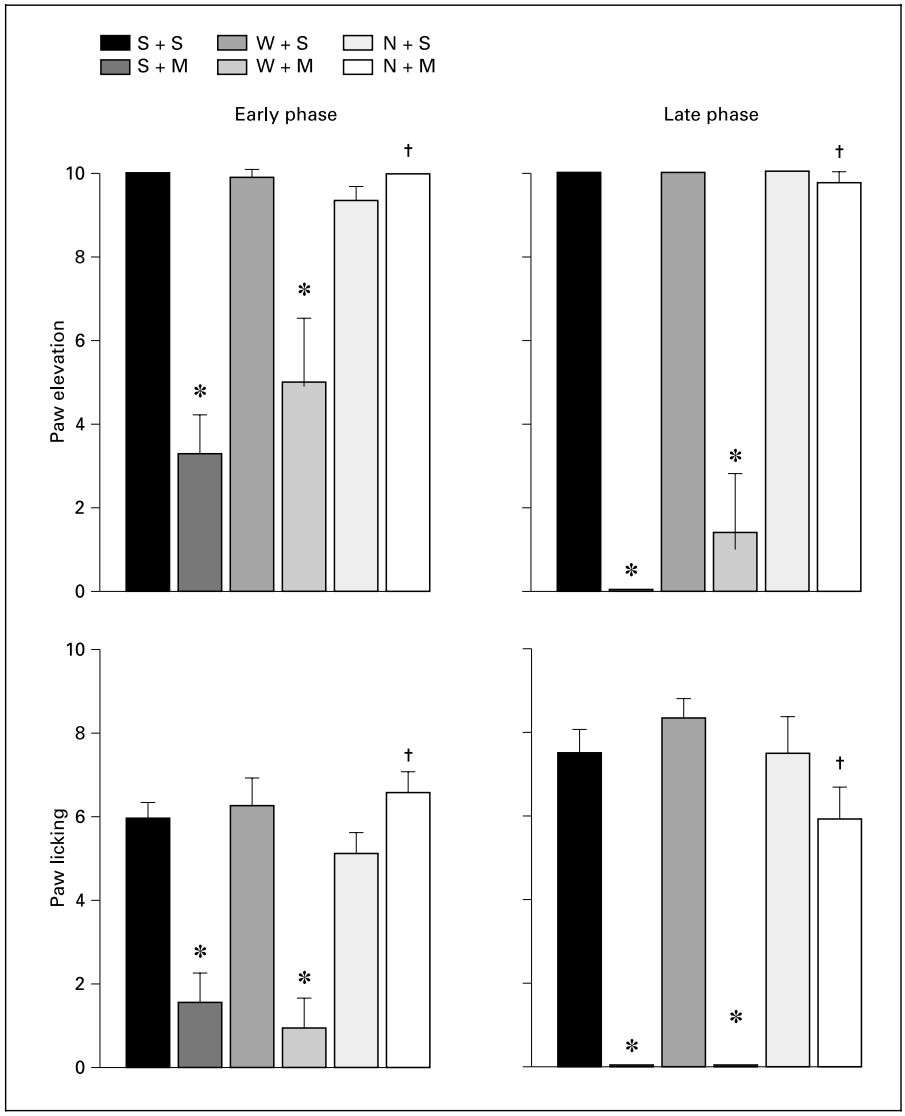

Fig. 5. Effects of pretreatment with naloxone (N; 0.63 mg/kg s.c.) or WAY 100635 (W; 0.63 mg/kg s.c.) on the ability of morphine (M; 10 mg/kg i.p.) to inhibit paw licking and paw elevation during the early (left panels) and the late (right panels) phase after formalin injection. Values represent the mean ± SEM scores of 7 animals. * $p < 0.05$ as compared with the saline + saline group (S + S); ** $p < 0.05$ as compared with the saline + morphine (S + M) group.

Other evidence [21, 22] indicates that formalin injection recruits descending, serotoninergic, pain-modulating systems; the possible role of 5-HT_{1A} receptors in these systems remains to be specified.

In spite of the two phases being allegedly mediated by partly different mechanisms [11] and responding differently to some analgesic treatments [23], the inhibitory actions of 5-HT_{1A} agonists were similar during the two phases (fig. 1, table 1). Along with previous evidence demonstrating the analgesic actions of F 13640 in a wide range of pain models [7], this outcome would be consistent with a central site of action of the 5-HT_{1A} ligands in producing analgesia. The finding that the low-efficacy ligand WAY 100635 [24], but not naloxone, antagonizes the inhibitory effects of F 13640 (fig. 4) is further evidence that its actions in the model are mediated by 5-HT_{1A} receptors.

The data obtained with agents exerting a diversity of other molecular mechanisms of analgesic action (fig. 2, 3; tables 3, 4) confirm that many of these agents exert significant effects in the formalin model [23, 25–28]. However, and again confirming the earlier findings, the magnitude of these effects was often limited, in particular with early-phase paw elevation. Imipramine, diclofenac, and ABT 594 produced substantial inhibition of paw elevation (fig. 3), but did so only at doses that appeared toxic (table 5). Substantial inhibition of paw elevation was also observed with the opioid ligands morphine (fig. 2) and U-50488 (fig. 3), but this at doses that induced akinesia and

compromised respiration (table 5). In keeping with previous data [23, 29], the doses of μ-opioids required here to inhibit early-phase behaviors were higher than those inhibiting late-phase behaviors (tables 3, 4). In fact, and unlike morphine (fig. 1, 3; tables 1, 3, 4), only the highest-efficacy 5-HT_{1A} ligands (i.e., F 13640 and F 13714) were capable of inhibiting completely (i.e., reducing to zero) each of the two pain behaviors, as they were monitored during the two phases. The latter outcome would seem to indicate that large-amplitude 5-HT_{1A} receptor activation produces an exceptionally powerful analgesia in the formalin model of tonic pain. This is consistent with evidence that in several models of chronic nociceptive and neuropathic pain, the chronic subcutaneous infusion of F 13640 produces a greater analgesic effect than that, if any, observed in these conditions with morphine or with other central mechanisms of analgesic drug actions (i.e., as exemplified by ketamine, imipramine, and gabapentin) [7]. Inasmuch as F 13640 effectively mimics the central neuroadaptive effects of nociceptive stimulation [7], the magnitude of its analgesia is also consistent with data demonstrating the extent to which such stimulation may engender analgesic effects. Thus, the chronic nociception that occurs in rats with adjuvant arthritis [30] prolongs the tail-flick latency to an extent that otherwise can be achieved only with opioids [4]. Equally, ischemic muscle exercise in man [31] as well as intraplantar formalin [20] or subdermal capsaicin injection [32] in rats result in a similarly powerful analgesia [for a review see 5].

Expectedly, the 5-HT_{1A} ligands studies here, like other ligands at this receptor [33], induced forepaw treading, a prominent, productive sign of the so-called 5-HT syndrome; little other intrinsic, conspicuous effects were observed (table 2). The co-occurrence of forepaw treading with inhibition of pain behaviors in the formalin model does not mean that they are related. That is, the forepaw treading induced by the prototypical 5-HT_{1A} agonist 8-OH-DPAT, but not its inhibition of formalin-induced paw elevation and paw licking, can be blocked by prazosin [17]. Also, repeated 8-OH-DPAT injection causes tachyphylaxis to forepaw treading [34], but not to its ability to inhibit formalin-induced pain behaviors [17]; similar results have been obtained with F 13640 [unpubl. data]. Thus, the analgesic action of 5-HT_{1A} agonists in the formalin model appears to be behaviorally specific [17]. Further, confirming reports that 8-OH-DPAT may increase the respiratory rate in rats [35] and other animal species [36], some 5-HT_{1A} agonists in the present studies also compromised respiration (table 2). Interestingly, the greatest respiratory effects were observed with the intermediate-efficacy agonist F 11461, whilst both lower- and higher-efficacy ligands produced a smaller effect. That the ability to produce a given action may be confined to ligands activating the receptor to within a particular range was first demonstrated with μ-opioid receptor ligands [37–39]. Some evidence of this effect has now been obtained with the anxiolytic-like action of 5-HT_{1A} ligands in a pigeon conflict procedure [9]. The molecular mechanisms of this confinement can perhaps be understood from recent data [40] indicting that 8-OH-DPAT displays a bell-shaped concentration-response curve in activating $G\alpha_{i3}$ subunits through h5-HT_{1A} receptors in CHO cells, possibly reflecting changes in receptor conformation or agonist-directed trafficking of receptor signaling. The present observational data (table 2) would seem to suggest that the respiratory action of 5-HT_{1A} ligands in rats is confined to those ligands activating the 5-HT_{1A} receptor to a particular range. However, more detailed studies are required to substantiate this possibility and, in particular, to confirm the relative inability of the exceptionally high-efficacy agonist F 13640 to affect respiration.

In summary, the present data indicate that 5-HT_{1A} receptor ligands inhibit both the paw licking and paw elevation that occur during both the early and late phases that appear upon intraplantar injection of formalin in the rat. In all instances the analgesic effects of these ligands covaried with the maximal extent to which they activate 5-HT_{1A} receptors; the high-efficacy agonist F 13640 produced a complete inhibition. The analgesic action of F 13640 was at least equivalent to that of morphine at doses where the opioid produced akinesia and compromised respiration. Also, F 13640-induced analgesia surpassed that observed at nontoxic doses of any of various agents that are known to exert peripheral or central analgesic effects by a wide diversity of molecular mechanisms. The data add to previous evidence [7] in identifying large-amplitude 5-HT_{1A} receptor activation as a nonopioid molecular mechanism of central analgesia. The magnitude of analgesia produced by F 13640 in this model of tonic nociceptive pain suggests that F 13640 may be particularly effective with pain that arises from severe, lasting nociceptive stimulation. Further studies are needed to identify the neurophysiological pathways that are involved in this apparently powerful analgesia.

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