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European Journal of Pharmacology 522 (2005) 38–46
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Antinociceptive, brain-penetrating derivatives related to improgan, a non-opioid analgesic

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Received 19 May 2005; received in revised form 16 August 2005; accepted 26 August 2005
Available online 10 October 2005

Abstract

The antinociceptive profile of selected histamine H_2 and histamine H_3 receptor antagonists led to the discovery of improgan, a non-brain-penetrating analgesic agent which does not act on known histamine receptors. Because no chemical congener of improgan has yet been discovered which has both antinociceptive and brain-penetrating properties, the present study investigated the antinociceptive effects of a series of chemical compounds related to zolantidine, a brain-penetrating histamine H_2 receptor antagonist. The drugs studied presently contain the piperidinomethylphenoxy (PMPO) moiety, hypothesized to introduce brain-penetrating characteristics. Following intracerebroventricular (i.c.v.) dosing in rats, six of eight drugs produced dose- and time-related antinociception on both the tail flick and hot plate tests over a nearly eight-fold range of potencies. Ataxia and other motor side effects were observed after high doses of these drugs, but two of the compounds (SKF94674 and loxtidine) produced maximal antinociception at doses which were completely devoid of these motor effects. Consistent with the hypothesis that PMPO-containing drugs are brain-penetrating analgesics, SKF94674 and another derivative (JB-9322) showed dose-dependent antinociceptive activity 15 to 30 min after systemic dosing in mice, but these effects were accompanied by seizures and death beginning 45 min after dosing. Other drugs showed a similar pattern of antinociceptive and toxic effects. In addition, loxtidine produced seizures without antinociception, whereas zolantidine produced neither effect after systemic dosing in mice. Although several of the drugs tested have histamine H_2 receptor antagonist activity, neither the antinociception nor the toxicity was correlated with histamine H_2 receptor activity. The present results are the first to demonstrate the existence of brain-penetrating antinociceptive agents chemically related to zolantidine and improgan, but further studies are needed to understand the mechanisms of both the pain relief and toxicity produced by these agents.

Keywords: Histamine; H_2 receptor; H_2 receptor antagonist; Zolantidine; Improgan

1. Introduction

Li et al. (1996) reported antinociceptive (i.e. pain-relieving) effects of SKF92374 (later renamed improgan, see Fig. 1) after i.c.v. injection into the rodent central nervous system (CNS). This compound, a chemical congener of the histamine H_2 receptor antagonist cimetidine (Fig. 1), was first described as a "chemical control" for the histamine H_2 receptor antagonist properties of cimetidine (Ganellin, 1982). Several studies found that improgan has the pre-clinical profile of a highly effective, non-opioid analgesic which lacks motor side effects (Hough et al., 2001a). Because of improgan's negligible affinity for histamine H_2 receptors, improgan antinociception is not mediated by the histamine H_2 receptor, nor by any other known histamine or opioid receptor (Mobarakeh et al., 2003). Despite extensive in vivo and in vitro characterization (Mobarakeh et al., 2003; Hough et al., 2002, 2001b; Svokos et al., 2001; Nalwalk et al., 2004, 2005), the mechanism of the analgesic action of improgan remains unknown. Although structure–activity studies of improgan have not been extensive, analgesic activity has been found for many (but not all) compounds related to histamine H_2 and histamine H_3 receptor antagonists (Hough et al., 1997, 2000b).

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doi:10.1016/j.ejphar.2005.08.040

A significant impediment to both pre-clinical study and clinical development of improgan-like drugs is their lack of brain-penetration. Thus, among histamine H_2 receptor antagonists, the non-penetrating agents cimetidine, ranitidine and metiamide produce antinociception after i.c.v. administration, but zolantidine, the first brain-penetrating histamine H_2 receptor antagonist (Fig. 1), was reported to be inactive after either i.c.v. or systemic dosing (Hough et al., 1997). Similarly, the non-penetrating histamine H_3 receptor antagonist burimamide and several of its analogs induce antinociception after i.c.v. administration, but the closely related brain-penetrating histamine receptor H_3 antagonists thioperamide and clobenpropit were reported to be inactive by either route (Hough et al., 1997). Thus, no chemical congener of improgan has yet been discovered which has both antinociceptive and brain-penetrating properties.

Young et al. described the synthesis and testing of a series of piperidinomethylphenoxy-(PMPO) alkylamine derivatives which were developed as the first brain-penetrating histamine H_2 receptor antagonists (Young et al., 1988). The prototype, zolantidine (Fig. 1, Table 1), was characterized in animals (Calcutt et al., 1988), but was never given to humans. Even though zolantidine was found to lack antinociceptive properties (Hough et al., 1997), the introduction of the piperidinomethylphenoxy (PMPO) moiety in place of more hydrogen-bonding heterocycles (e.g. imidazole in cimetidine) was seen as significant in attaining brain-penetration (Young et al., 1988). Several other histamine H_2 receptor antagonists have been developed which contain the PMPO group (Table 1), and are thus likely to have significant brain penetration. Presently, we have investigated the properties of a number of PMPO-containing drugs, and report for the first time the identification of brain-penetrating congeners of improgan and zolantidine with antinociceptive properties.

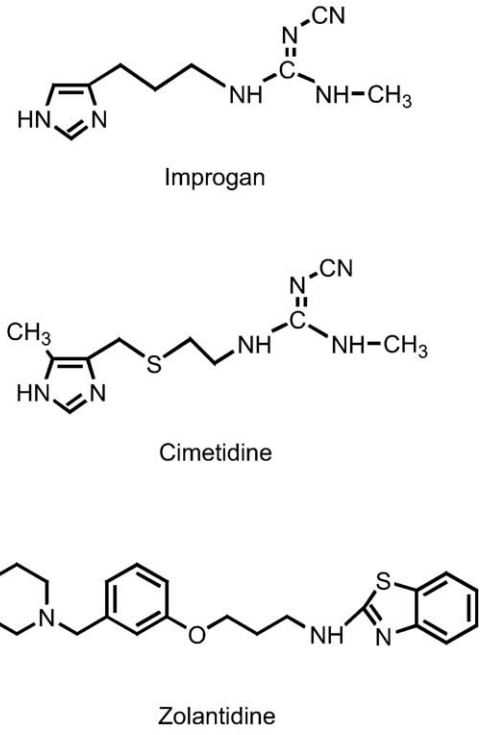
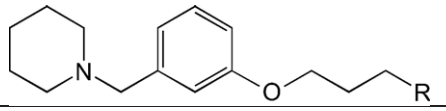
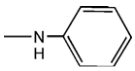
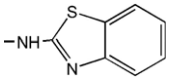
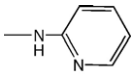
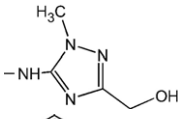
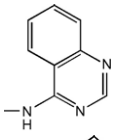
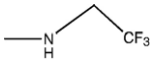
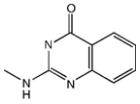
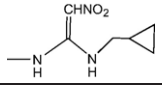


Fig. 1. Chemical structures of improgan, cimetidine and zolantidine.

Table 1
Chemical structures and histamine H_2 receptor potencies of compounds tested for brain-penetrating antinociceptive activity



Drug	R	H_2 K_d (μM)	References
SKF95299		2.0	Gogas and Hough, 1989; Young et al., 1988
Zolantidine (SKF95282)		0.04	Calcutt et al., 1988; Young et al., 1988; Gogas and Hough, 1989
SKF94674		0.81	Gogas and Hough, 1989; Young et al., 1988
Loxtidine (AH23844A)		0.013 [a]	Brittain et al., 1985
SKF95456		0.02	Gogas and Hough, 1989; Young et al., 1988
SKF95123		50.1	Gogas and Hough, 1989; Young et al., 1988
NO-794		0.029 [a]	Oshita et al., 1986
JB-9322		0.017	Palacios et al., 1995

[a] Calculated from pA_2, but reported to be an irreversible histamine H_2 receptor antagonist.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–350 g) and male Swiss-Webster mice (25–40 g, both from Taconic Farms, Germantown, NY) were maintained on a 12-h light/dark cycle (lights on from 0700 to 1900) with food and water ad libitum. Rats were housed in groups of three or four until the time of surgery and were housed separately thereafter. Mice were housed in groups of 4–8. All animal experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

2.2. Drugs

Loxtidine hemisuccinate (AH23844, (Brittain et al., 1985)), SKF95456 base and NO-794 base were kindly provided by Dr. D.E. Bays (formerly of Glaxo Group Research, Hertsfordshire, UK), Dr. R. Gannelin (formerly of SmithKlineBeecham, Hertsfordshire, UK) and Dr. M. Oshita (formerly of Hokuriku

Seiyaku, Ltd., Fukui, JP) respectively. Zolantidine dimaleate (Young et al., 1988), SKF95299 dihydrochloride (Young et al., 1988), SKF94674 dimaleate (Young et al., 1988), SKF95123 base (Young et al., 1988) and JB-9322 trihydrochloride (Palacios et al., 1995) were synthesized as described. For i.c.v. experiments, specified doses are equivalent to the base form of the drug, except for loxtidine (doses of which are given as the hemisuccinate salt). For systemic administration of drugs in mice, doses specified represent amounts of the available form of the drug.

2.3. Intracerebral surgery

Rats were anesthetized with pentobarbital (25 mg/kg, i.p.) and supplemented with isofluorane. Chronic cannulas were stereotaxically implanted into the left lateral ventricle and anchored to the skull using three stainless steel screws and dental cement (Crane and Glick, 1979). Stereotaxic coordinates (AP, ML and DV, mm from bregma) for placements of the guide cannulas were: −0.8 AP, 1.5 ML, −3.3 DV (Paxinos and Watson, 1986). Following surgery, the animals were individually housed and allowed to recover for a minimum of 5 days before testing. Each animal was used for a single experiment.

2.4. Injections and nociceptive testing in rats

Two nociceptive tests were used. For the hot plate test (Eddy and Leimbach, 1953), animals were placed on a 52 °C surface and the latency to hind paw lift or lick was recorded with a maximal exposure of 60 s. Baseline latencies were 10 to 15 s. For the tail flick test (D'Amour and Smith, 1941), the ventral surface of the tail (a randomly selected location 2–5 cm from the tip) was exposed to radiant heat, and the latency for tail movement was recorded. The heat source was set so that baseline latencies were generally between 3 and 4 s with a 15-s cutoff; the heat source was not adjusted for individual animals. Subjects were tested with a single, baseline hot plate test, followed by three tail flick tests performed at one min intervals, with the third test used as the baseline score. Animals were then gently secured by wrapping with a laboratory pad and received an i.c.v. injection. The guide stylet was removed, and the injection cannula (which extended 1 mm beyond the guide to penetrate the ventricle) was inserted. I.c.v. injections were delivered in a volume of 5 µl over 5 min unless noted otherwise. One min after the end of the i.c.v. injection, wire cutters were used to cut off and seal the injection cannula approximately 2 mm above the juncture with the guide cannula. Successful i.c.v. injections were assured by following the movement of an air bubble in the tubing between the syringe and the cannula and by the absence of leakage. At the end of testing, animals received an overdose of pentobarbital sodium (100 mg/kg, i.p.) followed by i.c.v. injection (5 µl) of India Ink in order to verify cannula placement in the lateral ventricle. Brains were removed and distribution of the ink in the ventricular space was used to confirm successful cannulation. Data from animals with unsuccessful injections were excluded.

2.5. Mouse nociceptive testing

Nociceptive testing was performed with the hot water tail immersion test (Li et al., 1997a). Animals were restrained in a conical polypropylene tube, and the tail (2–3 cm) was immersed into a 55 °C water bath and the latency to sudden movement (flick) or removal of the tail was recorded. Cutoff latencies were 8 s. Subjects were baseline tested once, received a single subcutaneous injection, and were re-tested at 15 min intervals for 90 min.

2.6. Data treatment and curve-fitting

Antinociception results are expressed as latencies (s, mean±S.E.M.). Data for all doses of each drug were fitted by use of iterative non-linear regression methods (Graphpad Prism 4.0, San Diego, CA) to the following equation:

$$E = \mathrm{BL} + (\mathrm{Top}-\mathrm{BL}) - \frac{(\mathrm{Top}-\mathrm{BL})}{\left(1+\frac{D}{\mathrm{ED}_{50}}\right)^{n}}$$

where E is latency (s), D is the dose of drug injected (µg), BL is the baseline latency, Top is the cutoff latency, n is the slope

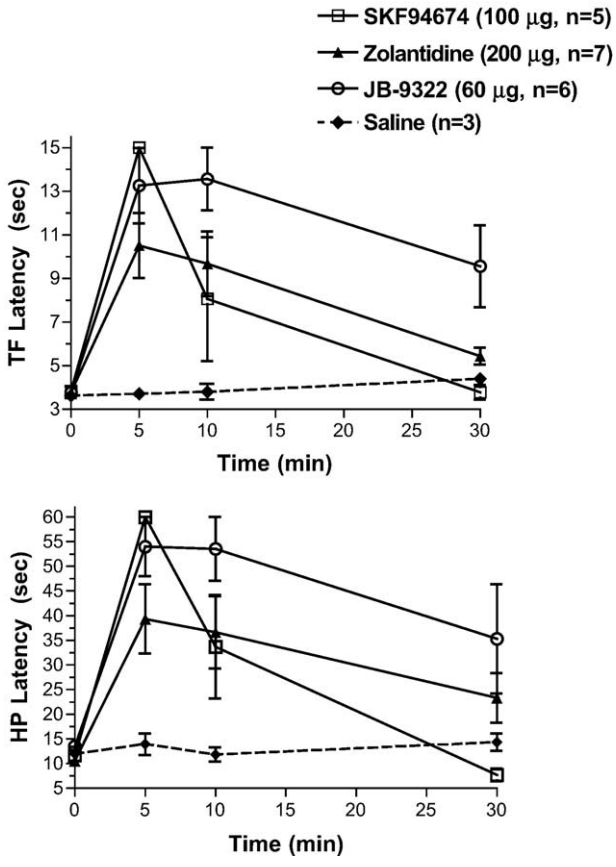


Fig. 2. Time course of antinociceptive actions of zolantidine-like drugs on the tail flick (TF, top) and hot plate (HP, bottom) tests in rats. Conscious, chronically cannulated rats were baseline tested (0 time), received i.c.v. injections of the identified drugs, and were re-tested at the times shown after the end of the infusion (abscissa, min). Nociceptive response latencies (ordinate, s, mean±S.E.M. for n values shown) are given for each drug.

function, and ED_{50} is the dose of drug inducing a 50% of maximum effect (μg). Robust fits to ED_{50} were obtained by constraint of the following variables: Top (15 and 60 s for tail flick and hot plate results, respectively), BL (3.5 and 11.0 s, respectively) and *n* (slope, 8.0 and 10.0, respectively). All fits converged with statistically significant ($P<0.05$) regression parameters. For each fitted ED_{50} a 95% confidence interval was also obtained. The same equation was used to obtain estimates

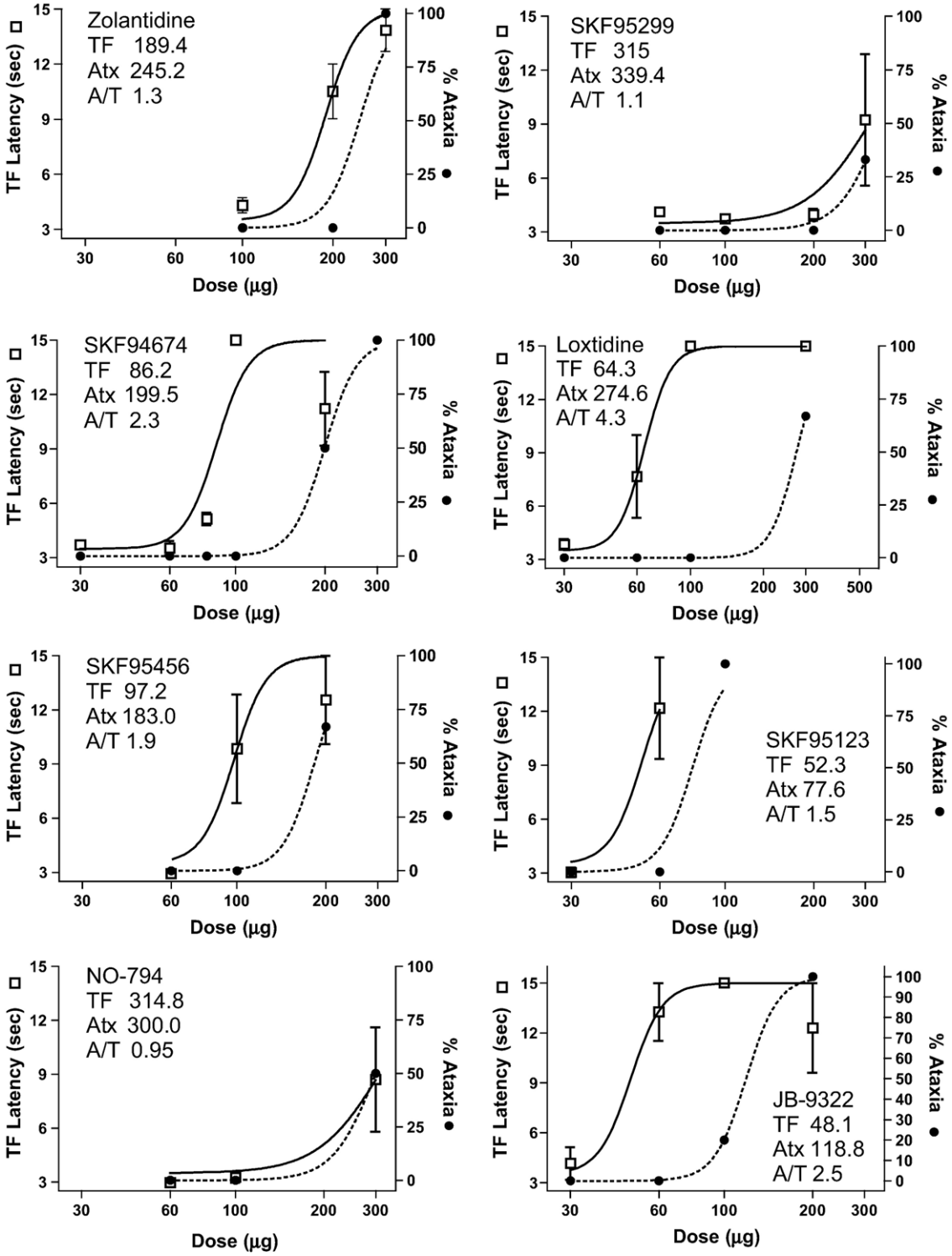


Fig. 3. Dose-response curves for antinociceptive and motor-impairing effects of the compounds in Table 1. Subjects received the specified dose of drug (μg, abscissa), and were tested as in Fig. 2. Tail flick latencies (TF, left ordinate, s, mean±S.E.M.) recorded five min after the end of drug infusions are shown. For each dose of drug, the right ordinate gives the percent of subjects showing ataxia (i.e., postural or motor abnormalities) at any time during the experiment. Antinociceptive (TF) and ataxia (Atx) ED_{50} values (μg) were estimated by non-linear regression, and are given in the inset of each graph. *A*/*T* represents the estimated therapeutic ratio of each drug, computed as the ataxia ED_{50} divided by the antinociceptive ED_{50}. See Table 2 for additional details.

Table 2
Antinociceptive and toxicity characteristics of zolantidine-like drugs in rats

Drug	No. of doses	No. of subjects	TF ED_{50} (μg±S.E.M.)	TF ED_{50} (nmol±S.E.M.)	Atx ED_{50} (μg±S.E.M.)	Atx ED_{50} (nmol±S.E.M.)	Atx ED_{50}/ TF ED_{50}
SKF95299	4	12	315.0±40.4	971.6±124.6	339.4±2.0	1046.9±6.2	1.1
Zolantidine	3	15	189.4±9.4	495.8±24.6	245.2±25.9	641.9±67.8	1.3
SKF94674	5	24 [a]	86.2±5.3	264.8±16.4	199.5±1.7	612.9±4.9	2.3
Loxtidine	4	17 [a]	64.3±4.0	178.9±11.1	274.6±0.1	763.9±0.3	4.3
SKF95456	3	11 [a]	97.2±8.3	258.2±22.0	183.0±0.6	486.1±1.6	1.9
SKF95123	3	9 [a]	52.3 ± 5.7	158.3±17.3	77.6±7.7	234.9±23.3	1.5
NO-794	3	7	314.8±56.9	802.0±145.0	300.0±0.1	764.3±0.3	0.95
JB-9322	4	18 [a]	48.1±5.0	123.8±12.9	118.8±0.8	305.8±2.1	2.5

Antinociceptive (tail flick, TF) and motor-impairing (ataxia, Atx) potencies of the compounds in Table 1 (given by i.c.v. injection) are summarized. The data of Fig. 3 were fitted by non-linear regression to estimate ED_{50} values±S.E.M. The estimated therapeutic index of each drug is given in the far right column.
[a] At the highest dose tested, motor-impairment prevented tail flick testing in one subject.

of toxicity, where *E* was the fraction of subjects showing the toxicity, BL, Top, and *n* were set to 0, 100, and 8.0 respectively.

3. Results

3.1. Rat i.c.v. dosing studies

Although the goal of the present study was to discover brain-penetrating antinociceptive agents, candidate compounds were first studied by i.c.v. injection in rats to verify activity. I.c.v. administration of the compounds in Table 1 resulted in antinociceptive activity on both the tail flick and hot plate tests. In all cases, nociceptive latencies peaked 5–10 min after drug treatment; lower or normal scores were observed after 30 min (Fig. 2). Testing these compounds over a range of doses yielded dose-dependent antinociception for most of the agents (Fig. 3). On the tail flick test, maximal or near-maximal latencies were produced by six of the eight drugs tested. Antinociceptive tail flick ED_{50} values, estimated by non-linear regression, ranged from 124 to 972 nmol (Table 2). Simultaneous testing on the hot plate test produced results which were very similar to those from the tail flick test (Fig. 4). The estimated antinociceptive hot plate ED_{50} values were highly correlated with the tail flick ED_{50} values (Fig. 4).

In the same experiments, large i.c.v. doses of the drugs in Table 1 (usually 200 μg or higher) produced postural

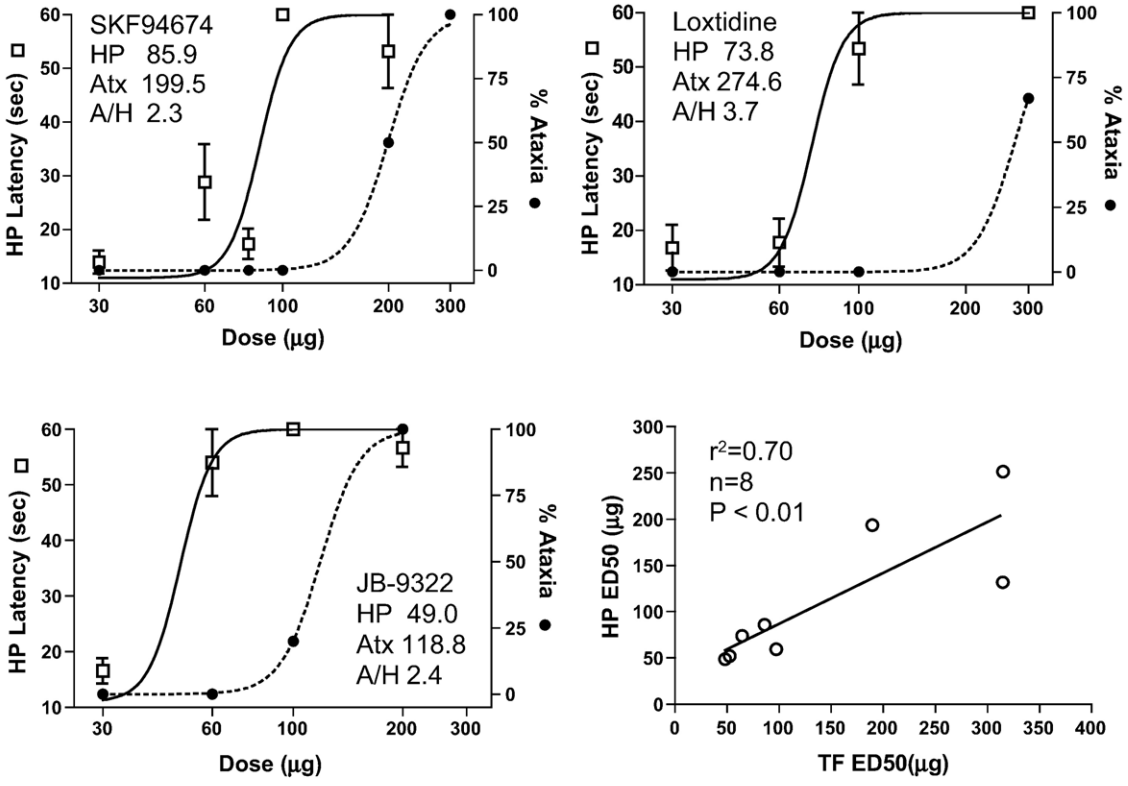


Fig. 4. Hot plate (HP) antinociceptive actions of compounds selected from Table 1. Latencies (5 min after end of infusion) are shown from the experiment described in Fig. 3 for three drugs. Abbreviations and format of the graphs are exactly as in Fig. 3. In the lower right panel, the estimated tail flick ED_{50} for each compound in Table 1 (taken from Fig. 3) is plotted against the estimated hot plate ED_{50} for that drug.

abnormalities and/or unusual locomotor behavior. These effects were occasionally as subtle as tilting the head or body to one side at rest, but could also be more pronounced (e.g. circling, "barrel rolling", or other abnormal movement). These motor changes nearly always occurred between 30 and 60 min after drug injection, and thus did not usually interfere with nociceptive latency measurements (see Table 2). None of the i.c.v. treatments of Fig. 3 resulted in loss of righting reflex, seizures, or death. Following drug treatment and throughout the nociceptive test period, the incidence of these motor abnormalities (collectively termed "ataxia") was recorded (Fig. 3). Ataxia ED_{50} values, estimated by non-linear regression, ranged from 235 to 1047 nmol (Table 2). Since the experiments yielded both an estimate of antinociceptive potency (tail flick ED_{50}) and toxicity (ataxia ED_{50}) for each drug, the ratio of these (latter/former) was calculated as an estimate of therapeutic index. These values ranged from 0.95 to 4.3 (Table 2). SKF94674, JB-9322 and loxtidine had the highest therapeutic indices (2.3, 2.5 and 4.3, respectively). Consistent with this, maximal antinociceptive scores for these drugs were obtained in the complete absence of motor side effects (Fig. 3). Thus, these drugs showed the greatest potential as brain-penetrating analgesic agents related to improgan.

3.2. Mouse systemic dosing studies

In order to evaluate the hypothesis that these drugs are brain-penetrating analgesics, they were tested after systemic (subcutaneous) dosing in mice. Consistent with predicted properties, both SKF94674 and JB-9322 showed dose-dependent antinociceptive activity 15 to 30 min after systemic dosing in mice (Fig. 5). Maximal doses produced near-maximal antinociceptive latencies. However, unlike the i.c.v. effects observed in rats, tonic-clonic seizures and death were produced in mice after systemic antinociceptive doses of these two drugs beginning 45 min after drug administration (Fig. 5). The estimated LD_{50} values of SKF94674 and JB-9322 in these experiments were similar to the respective antinociceptive ED_{50} values, yielding therapeutic indices only slightly greater than 1.0 (Fig. 5). Systemic dosing in mice with four of the other drugs from Table 1 (SKF95299, SKF95456, SKF95123, and NO-794) also produced significant antinociception and toxicity, but with therapeutic indices of approximately 1.0 (data not shown). Interestingly, systemic administration of loxtidine produced toxicity similar to that seen with the other drugs, but failed to produce dose-related antinociception (Fig. 5), suggesting that the phenomena are separable. The only drug from Table 1 which produced neither toxicity nor antinociception after systemic dosing in mice was zolantidine (Fig. 5).

4. Discussion

All of the clinically available histamine H_2 receptor antagonists (including cimetidine) lack significant brain penetration. Drug levels in brain or cerebrospinal fluid following

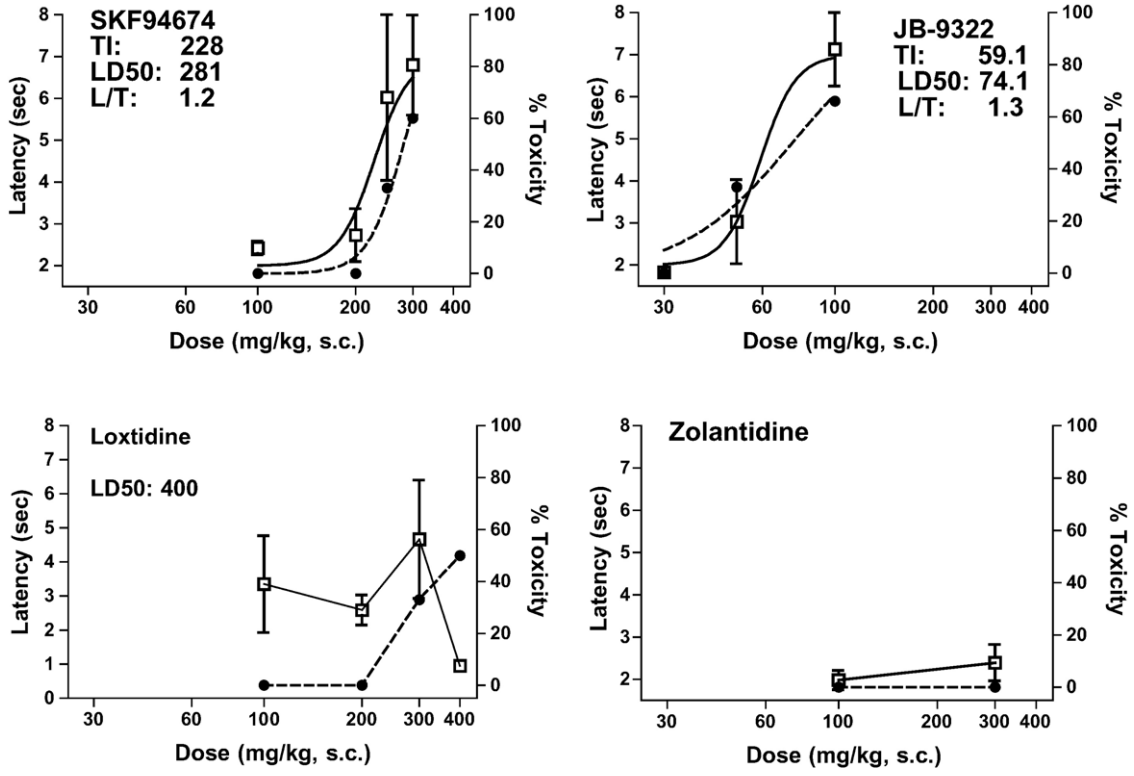


Fig. 5. Antinociceptive activity and therapeutic index of PMPO-containing drugs after systemic dosing in mice. Subjects were tested for baseline responses, received a subcutaneous injection of drug, and were re-tested 15, 30, 60, and 90 min later on the tail immersion (TI) nociceptive test. Antinociceptive latencies (left ordinate, s, mean±S.E.M., $n=3–6$) are shown for SKF94674 (top left, 30 min after injection), loxtidine (lower left, 15 min), JB-9322 (upper right, 30 min), and zolantidine (lower right, 30 min). Right ordinate shows the toxicity of each treatment expressed as percent of subjects which died (death occurred 45 min after injection or later). Insets show the fitted values for potency (TI is the antinociceptive ED_{50} on the tail immersion test), toxicity (LD_{50}) and therapeutic index (L/T).

systemic administration of cimetidine to animals or healthy humans are only a fraction of the plasma levels (Schentag et al., 1981; Hough et al., 1986). In rats, very large systemic doses of cimetidine can produce effects consistent with blockade of brain histamine H_2 receptors (Hough et al., 1986). However, such large systemic doses of cimetidine or improgan do not change nociceptive thresholds, even though cimetidine produces antinociception after i.c.v. administration (Netti et al., 1984; Li et al., 1997a). Therefore, chemical modification of improgan was needed to achieve significant brain penetration and to retain antinociceptive properties. The hypothesis that PMPO-containing derivatives might have both properties was tested presently because 1) the brain-penetrating characteristics of PMPO-containing derivatives have been documented (Young et al., 1988), and 2) zolantidine, a PMPO-containing derivative of cimetidine, was shown to penetrate the brain and to resemble cimetidine sufficiently to retain histamine H_2 receptor-blocking properties (Calcutt et al., 1988; Gogas and Hough, 1988). Present results, which confirm this hypothesis, are the first to report the existence of drugs related to improgan, cimetidine and zolantidine which show both brain-penetrating and antinociceptive properties.

Because nociceptive assays measure motor reflexes, it is essential to consider the possibility that motor impairment (not analgesia) accounts for the observed antinociceptive effects, especially when toxicity has been observed. Evidence that the presently observed antinociceptive effects are related to analgesia, and not motor toxicity includes: 1) antinociceptive potencies were highly correlated across two different nociceptive tests (hot plate vs. tail flick, Fig. 4); these tests are known to depend on different reflexes (supraspinal vs segmental, respectively). 2) Antinociceptive effects disappeared over time, showing that irreversible effects were not produced (Fig. 2); this time course is identical with that seen after other i.c.v.-injected analgesics such as improgan, which lacks toxic effects (Li et al., 1997a). 3) For several compounds, maximal antinociception was observed after doses which produced no observable motor effects (Fig. 3); when toxicity was observed, it occurred much later (30–60 min) than the antinociceptive effects (5–10 min). 4) In both rats (Fig. 3) and mice (Fig. 5), some of the drugs produced toxicity without significant antinociception (NO-794 and loxtidine, respectively), showing that antinociception is not a necessary consequence of the motor effects.

Following i.c.v. administration, the compounds in Table 1 demonstrated a nearly eight-fold range of antinociceptive potencies. Two of the lower-potency drugs (SKF95299 and zolantidine) were previously reported to be inactive because large enough doses were not studied (Hough et al., 1997). Consistent with this low antinociceptive potency, systemically administered zolantidine has previously been shown to block brain histamine H_2 receptors after doses that have no effect on nociceptive latencies (Gogas and Hough, 1988). The antinociceptive activity of the other compounds in Table 1 has not been previously reported. Three of these (JB-9322, loxtidine, and SKF94674) showed antinociceptive potencies significantly greater those that of improgan and cimetidine (Table 2 and (Li et al., 1996)). These three drugs also showed the highest therapeutic index after i.c.v. dosing.

The mechanism of antinociceptive action of the compounds in Table 1 is unknown. However, similar to the characteristics of improgan (Li et al., 1996, 1997b; Mobarakeh et al., 2003), the activity of the presently studied drugs seems unrelated to the histamine H_2 receptor. For example, SKF94674 is twice as potent as zolantidine on the tail flick test (Table 2), whereas the former is 20-fold less active than the latter on the histamine H_2 receptor (Table 1). For all of the drugs tested presently, the lack of correlation between antinociceptive potencies and histamine H_2 receptor affinities (Fig. 6) solidifies this conclusion.

The presently studied compounds produced two patterns of unexpected side effects: ataxia in rats (after i.c.v. dosing) and seizure-like activity in mice (after subcutaneous dosing). It is not known if these effects share the same mechanism. Improgan and its congeners do not show ataxia even after large i.c.v. doses in rats (Li et al., 1997a). Large intracerebral doses of improgan sometimes produce excitement and hyperreflexia (Nalwalk et al., 2004), but the motor abnormalities seen presently after PMPO-containing drugs have not been observed following administration of improgan or histamine H_2 receptor antagonists. Because imidazole-containing congeners (e.g. cimetidine, improgan, burimamide) and substituted furans (e.g. ranitidine) induce antinociception without ataxia after i.c.v. dosing (Hough et al., 1997), it is tempting to speculate that the ataxic effects produced by the present drugs are related to the replacement of the imidazolyl or furanyl groups with the PMPO moiety. Further studies are needed to verify this. However, ataxia was not observed in rats following systemic or i.c.v. administration of histamine H_2 receptor-blocking doses of the PMPO-containing zolantidine (Gogas and Hough, 1988, 1989), showing that this drug's potency on the histamine H_2 receptor exceeds its potency to produce ataxia. These findings also argue that the ataxia is unrelated to histamine H_2 receptor potency, which is confirmed by the lack of significant correlation between these potencies (Fig. 5).

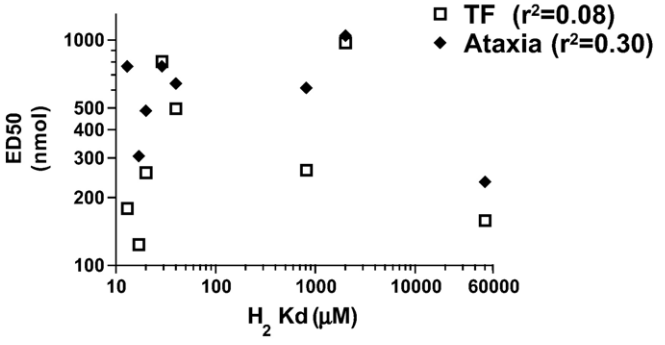


Fig. 6. Pharmacological analysis of the activity of PMPO-containing drugs. The histamine H_2 receptor potency of each of the drugs in Table 1 (abscissa) is plotted against the ED_{50} values of that drug on both antinociceptive (TF, open squares) and ataxic (solid diamonds) effects after i.c.v. administration in rats (ordinate values are taken from Table 3). The figure shows that the histamine H_2 receptor activity of these drugs does not account for the antinociceptive or toxic effects seen in Fig. 3.

Table 3
Synopsis of pharmacological characteristics of zolantidine-like drugs

Drug	Antinociceptive activity? (i.c.v. rat)	Selective antinociception? (i.c.v. rat)[a]	Antinociceptive activity? (systemic, mouse)[b]	CNS toxicity? (systemic, mouse)	Brain-penetrating characteristics?[c]
SKF95299	Y	N	Y	Y	Likely
Zolantidine	Y	N	N	N	Y
SKF94674	Y	Y	Y	Y	Y
Loxtidine	Y	Y	N	Y	Unlikely
SKF95456	Y	N	Y	Y	Likely
SKF95123	Y	N	Y	Y	Likely
NO-794	Y	N	Y	Y	Likely
JB-9322	Y	Y	Y	Y	Likely

[a] Defined as having a therapeutic index >2.0 (see Table 2).
[b] Defined as producing a maximal (i.e. cutoff) response latencies.
[c] Two compounds ("Y") have documented brain-penetrating characteristics (Young et al., 1988). Drugs identified as "Likely" to penetrate the brain are those showing antinociceptive activity after systemic dosing.

Even though both the antinociception and the ataxia produced by these drugs in rats have unknown mechanisms, it is essential to understand any relationship that might exist between these effects. When the estimated antinociceptive (tail flick) ED_{50} value for each drug was plotted vs the ataxia ED_{50} of that drug after i.c.v. dosing in rats (Table 2), a significant correlation was obtained ($R^2=0.64$, $P<0.02$, $n=8$, data not shown). One explanation for this finding is that the analgesic and ataxic mechanisms are identical. Confirmation of such a conclusion would be critical, since further searches for non-toxic PMPO-containing analgesics would be fruitless. However, other explanations are possible. For example, if the same brain area contained both ataxic and analgesic receptors for these drugs, then pharmacokinetic variables (e.g. the ability of a particular drug to reach that structure) might account for the correlation. This would be consistent with findings showing that lipid/water solubility plays a role in the brain distribution of drugs after i.c.v. dosing (Kutter, 1970).

The present results in mice (Fig. 5) demonstrate that most of the drugs in Table 1 reached the brain following subcutaneous dosing. This conclusion is consistent with earlier work, which found brain/blood ratios of 4.9 and 1.4 in rats after systemic dosing with SKF94674 and zolantidine, respectively (Young et al., 1988). However in the present study, fatal seizures were produced by 6 of the 8 drugs in mice. Unlike the case of ataxia in rats (which was completely unexpected), seizures have been observed in animals and humans following treatment with histamine H_2 receptor antagonists (Shimokawa et al., 1996; von Einsiedel et al., 2002; Gerald and Richter, 1976; Amabeoku and Chikuni, 1993). Speculation has pointed to a role for GABAergic mechanisms (either directly or indirectly) in histamine H_2 receptor antagonist-induced seizures, but a recent study found no support for this hypothesis (Cannon et al., 2004). Shimokawa et al. (1996) reported a significant correlation between convulsive potency in mice and histamine H_2 receptor affinity for three histamine H_2 receptor antagonists (famotidine, ranitidine, and cimetidine), which led the authors to suggest a mechanistic role for histamine H_2 receptors in seizure production. This is clearly not the case, since zolantidine is a potent histamine H_2 receptor antagonist ($K_d=25$ nM) that penetrates the blood–brain barrier and does not produce seizures when administered systemically (Gogas and Hough, 1988) or intraventricularly in rats ((Hough et al., 1997) and present results), or systemically in mice (Fig. 5). Although the receptors mediating the presently observed seizures are unknown, it is interesting to note that subcutaneous loxtidine produced these seizures without antinociception (Fig. 5). This suggests that the receptors for toxicity and analgesia are either pharmacologically or anatomically distinct. Brain penetration of loxtidine has not been measured, but, based on its structure, it is likely that the CNS penetration of this drug is more limited than that of the other compounds of Table 1. Even though loxtidine contains the PMPO group, the presence of the hydroxylated triazole suggests many opportunities for hydrogen bonding, which would be likely to reduce brain penetration (Young et al., 1988). It does not appear that the seizures are caused simply by the presence of the PMPO structure, since zolantidine contains this moiety, penetrates the brain, and does not produce seizures (Fig. 5).

The goal of this study was to discover improgan-like analgesics with brain-penetrating properties. The presently studied compounds chemically resemble improgan and cimetidine, and have both brain-penetrating and antinociceptive properties. However, further studies are needed to establish if these drugs share improgan's analgesic mechanism. The extensive in vivo characterization of improgan's antinociceptive profile will make such studies possible (Li et al., 1997b; Svokos et al., 2001; Hough et al., 2000a, 2001b, 2002; Nalwalk et al., 2005). It is also essential to understand the mechanism of toxicity produced by these new analgesic drugs.

Acknowledgement

This work was supported by a grant (DA-015915) from the National Institute on Drug Abuse. We also thank Yang Chen for excellent technical assistance.

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