Novel Qualitative Structure-Activity Relationships for the Antinociceptive Actions of H2 Antagonists, H3 Antagonists and Derivatives


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ABSTRACT

Recent studies have shown that cimetidine, burimamide and improgan (also known as SKF92374, a cimetidine congener lacking H2 antagonist activity) induce antinociception after intracerebroventricular administration in rodents. Because these substances closely resemble the structure of histamine (a known mediator of some endogenous analgesic responses), yet no role for known histamine receptors has been found in the analgesic actions of these agents, the structure-activity relationships for the antinociceptive effects of 21 compounds chemically related to H2 and H3 antagonists were investigated in this study. Antinociceptive activity was assessed on the hot-plate and tail-flick tests after intracerebroventricular administration in rats. Eleven compounds induced time-dependent (10-min peak) and dose-dependent antinociceptive activity with no observable behavioral impairment. ED50 values, estimated by nonlinear regression, were highly correlated across nociceptive assays (r2 = 0.98, n = 11). Antinociceptive potencies varied more than 6-fold (80–464 nmol), but were not correlated with activity on H1, H2 or H3 receptors. Although highly potent H3 antagonists such as thioperamide lacked antinociceptive activity, homologs of burimamide and thioperamide containing N-aromatic substituents retained H3 antagonist activity and also showed potent, effective analgesia. A literature review of the pharmacology of these agents did not find a basis for their antinociceptive effects. Taken with previous findings, the present results suggest: 1) these compounds act on the brain to activate powerful analgesic responses that are independent of known histamine receptors, 2) the structure-activity profile of these agents is novel and 3) brain-penetrating derivatives of these compounds could be clinically useful analgesics.

Several studies have established that the neuromodulator HA induces antinociception when directly administered into the CNS (Lamberti et al., 1996; Parolaro et al., 1989; Bhattacharya and Parmar, 1985; Onodera and Ogura, 1983; Glick and Crane, 1978). Although the pharmacology of this response is complex, both H1 and H2 antagonists have been reported to inhibit HA-induced antinociception (Thoburn et al., 1994; Parolaro et al., 1989; Netti et al., 1988; Bhattacharya and Parmar, 1985). However, other H2 antagonists (cimetidine and ranitidine) induce antinociception in the absence of exogenous HA when administered directly into the brain (Li et al., 1996; Leza et al., 1990; Oluymo and Hart, 1991; Netti et al., 1984, 1988).

In previous work from one of our laboratories (Li et al., 1996), cimetidine-induced antinociception was characterized by studying improgan, a chemical congener of cimetidine that lacks H2 antagonist activity (see table 1 for structure). Improgan, formerly known as SKF92374 (Li et al., 1996), induced a highly effective, reversible, dose-related and time-related inhibition of both supraspinally mediated (hot plate) and intraspinally mediated (tail flick or tail immersion) nociceptive responses in rats (Li et al., 1996) and mice (Li et al., 1997a) after ivt administration. The compound had a similar profile in rats when studied with a mechanical nociceptive test (Li et al., 1997a). Additional behavioral testing in rats showed that a large dose of improgan lacked effects on spontaneous locomotor activity (implying the absence of stimulant or depressant actions) and on an accelerated rotorod test (implying the absence of motor impairment, Li et al., 1997a). These results suggest that improgan-like compounds have selective analgesic properties after ivt administration. While the present work was in progress, the antinociceptive activity of burimamide, another closely related compound with both H2 and H3 blocking properties (table 1), was reported after ivt administration in mice (Lamberti et al., 1996).

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ABBREVIATIONS: CNS, central nervous system; HA, histamine; ivt, intracerebroventricular; SAR, structure-activity relationship.
The fact that several (but not all) H2 and H3 antagonists induce antinociception after CNS administration implies the possible existence of a novel class of analgesic agents. The close similarity in structure among cimetidine, improgan and burimamide (table 1) further suggests that these compounds could be acting by a similar (but unknown) mechanism. With respect to improgan, both in vitro (Li et al., 1996) and in vivo (Lamberti et al., 1997a,b) studies with agonists and antagonists suggest that the effects are not caused by an action on opiate receptors, on H1, H2 or H3 receptors or on HA metabolism. With respect to burimamide, detailed dose-response studies in mice suggest that neither H2 nor H3 receptors mediate burimamide-induced antinociception (Lamberti et al., 1996). An H3-related antinociceptive mechanism is also excluded by findings showing that: 1) improgan-induced antinociception is not opposed by the H3 agonist (R)-α-methylhistamine (Li et al., 1997b); 2) improgan and cimetidine are both very weak H3 antagonists (Li et al., 1996); and 3) burimamide-like effects (i.e., full activity against thermal tail-flick responses) are not produced by potent, selective H3 antagonists such as thioperamide (Lamberti et al., 1996; Li et al., 1996). With respect to the third point, however, the possibility of subtypes of H3 receptors should not be overlooked (Leurs et al., 1996; West et al., 1990). The suggestion (Lamberti et al., 1996) that burimamide antinociception might be related to its weak H1 antagonist properties seems unlikely because selective H1 antagonists are inactive on the tail-flick test (Dews and Graham, 1946; Li et al., 1997b).

Further study of the mechanism of antinociceptive action of improgan-like compounds is warranted because these agents bear obvious structural resemblance to that of HA (table 1), a substance strongly implicated in analgesic mechanisms, and known HA receptors do not seem to participate in the antinociceptive effects of these agents. To perform a detailed pharmacological investigation of the latter hypothesis, the antinociceptive actions of 21 structurally related agents have been investigated in this study. The results show compelling evidence for a novel pharmacological activity of these compounds. The discovery that certain derivatives of burimamide are potent, effective antinociceptive agents is also reported.

### Methods

**Animals.** Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY), weighing 210 to 320 g at the time of testing, were maintained on a reverse 12-hr light/dark cycle (lights on 7:00 p.m., lights off 7:00 a.m.) and used for nociceptive testing. The reverse cycle has been widely used for nociceptive testing in this and other laboratories, because rodents are nocturnal (Gogas et al., 1989, Li et al., 1996, 1997a,b). Adult male Dunkin-Hartley guinea pigs (350–450 g, Harlan CPB, Zeist, The Netherlands) were used for in vivo assays of H3 activity. All experiments were reviewed and approved by the appropriate Institutional Animal Care and Use Committees.

**Drugs and solutions.** Compounds assessed for antinociceptive activity are in table 1. (HA was not included in the present study.) Cimetidine, metiamide, improgan (SKF92374), burimamide, norburimamide (bases) and zolantidine dimaleate were kindly provided by Dr. Robin Ganellin, formerly of SmithKline Beecham, Herts, U.K. Thioperamide maleate was purchased from RBI (Natick, MA). Tioti-
dine base was kindly provided by Dr. David McCurdy, formerly of Stuart Pharmaceuticals (Wilmington, DE). Ranitidine dihydrochloride was kindly provided by Dr. D.E. Bays (Glaxo Group Res. Ltd., Ware, Herts, UK). Except for VUF5261 and VUF5262, VUF-prefixed compounds and R-α-methylhistamine dihydrochloride were available from laboratory stock. These include clobenpropl (also known as VUF9153, Van der Goot et al., 1992), VUF8298 (Sterk et al., 1987), VUF8299 (Sterk et al., 1987) and the remaining burimamide derivatives (Vollinga et al., 1995).

VUF5261 and VUF5262 (free bases) were synthesized from 4-(imidazol-4(5)-yl)piperidine according to Arrang et al. (1987) except that the corresponding isothiocyanates were used instead of cyclohexyl isothiocyanate (VUF5261: 67% yield, m.p. = 193.6°C; analysis: C = 53.42 [calc = 53.54]; H = 7.23 [calc = 7.19]; N = 24.87 [calc = 24.98]; VUF5262: 22% yield, m.p. = 127.1°C; analysis: C = 62.90 [calc = 62.91]; H = 6.26 [calc = 6.33]; N = 19.64 [calc = 19.56]). SKF95299 was synthesized by a modification of literature procedures (Fujisawa, 1983; Young et al., 1988). Reductive amination of 3-hydroxybenzaldehyde with piperidine and sodium cyanoborohydride gave 3-[3-(piperidin-1-ylmethyl)-phenoxy]propylamine by treatment with aniline ac-1chloride, which was converted to SKF95299 (N-phenyl-3-[3-(piperidin-1-ylmethyl) phenoxy]propylamine) by treatment with aniline ac-cording to the literature method. The crude product was purified by column chromatography.

Solutions were dissolved in isotonic saline. Bases were dissolved in HCl (1.0–1.2 N), titrated to a pH between 5.5 and 6.5 and diluted with column chromatography.

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Solutions were dissolved in isotonic saline. Bases were dissolved in HCl (1.0–1.2 N), titrated to a pH between 5.5 and 6.5 and diluted with saline. Vehicle injections consisted of either saline, or neutralized, di-luted HCl. VUF5298 and VUF5299 were dissolved in neutral or slightly acidic aqueous ethanol (0.46 mg/μL, pH 4.5–6); these solutions alone as vehicle controls had no effect on nociceptive thresholds.

Surgery for microinjections. The microinjection apparatus, consisting of a chronically implanted guide cannula along with a stylet and an injection cannula, has previously been described in detail (Crané and Glick, 1979). Rats were anesthetized with methohexital (50 mg/kg, i.p.) and supplemented with metoxyflurane. Unilateral guide cannulas were stereotaxically implanted into the brain and anchored to the skull with three stainless steel screws and dental cement. After surgery, animals were individually housed with food and water freely available for 1 week before testing. Guide cannulas were implanted such that injections were made into the left lateral ventricle. Coordinates (in millimeters from bregma, Paxinos and Watson, 1986) for the guide cannulas were: AP = −0.8, ML = 1.5, DV = −3.3, 0° angle. Injection cannulas were made to extend 1 mm ventrally beyond the tip of the guides. Each animal was only used for a single experiment.

Nociceptive testing. Two nociceptive tests were used, the radi-ant heat tail-flick test (D’Amour and Smith, 1941) and the hot-plate test (Eddy and Leimbach, 1953). For the tail-flick test, the radiant heat source was set such that base-line latencies were generally between 3 and 4 sec, with a 15-sec cutoff. The heat source was not adjusted for individual animals. The ventral surface of the tail (on a randomly selected location 2–5 cm from the tip) was exposed to radiant heat and the latency for tail movement was recorded. For the hot-plate test, animals were placed on a 52° surface and the latency to a hind paw lift or lick was recorded, with a maximal exposure of 60 sec. Base-line latencies were 8 to 14 sec. Three to seven hours into the dark portion of the diurnal cycle, animals were tested for base-line nociception (one hot-plate test followed by three tail-flick tests). Animals were then gently secured by wrapping with a laboratory pad, the stylet was removed and the injection cannula was inserted. Except for two compounds, drugs were injected manually in a total volume of 5 μL during 5 min. VUF8299 and its vehicle were injected in a volume of 2.5 μL. Some VUF8299 experiments were performed with 7.5-μL injection volumes. Successful injection was assured by following movement of an air bubble in the tubing between the syringe and the cannula and by the absence of leakage. One minute after the end of the infusion, the injection cannula was removed and the stylet replaced. Animals were retested with single hot-plate and tail-flick tests at 5, 10 and 30 min after the replacement of the stylet. Each tail-flick test was performed 1 min after a hot-plate test. At the end of each experiment, animals received i.p. pentobarbital (50 mg/ kg) and ivt (5 μL) injections of India Ink. Successful ivt injections were verified by observing the proper distribution of ink throughout the ventricular system. Data from animals whose injections were outside the lateral ventricle or who had unsuccessful injections were excluded.

Analysis of antinociceptive data. Antinociceptive scores for each animal were calculated as percent of maximum possible effect (%MPE), where

\[
\text{%MPE} = \frac{\text{Drug latency} - \text{base-line latency}}{\text{Cutoff latency} - \text{base-line latency}} \times 100
\]

For the tail-flick test, the third latency before the drug treatment was used as the base-line score, because the first two scores are higher than the subsequent latencies when no drug is given (Gogas et al., 1989; Hough and Nalwalk, 1992). Results for each treatment group are given as mean ± S.E.M. Data for all doses of each drug were fitted by use of iterative nonlinear regression methods (Graphpad Prism, San Diego, CA) to the following equation:

\[
E = 100 - \frac{100}{1 + \left(\frac{D}{ED_{50}}\right)^{-m}}
\]

where \(E\) is observed analgesic effect (% MPE), \(D\) is the dose of drug injected (μg), \(n\) is the slope function of the dose-response curve and \(ED_{50}\) is the dose of drug inducing a 50% of maximum effect (μg). All fits converged with statistically significant (P < .05) regression pa-rameters. For each parameter (ED_{50} and \(n\)), mean and S.E.M. values of the regression were obtained.

Assay of \(H_3\) antagonist activity. VUF5261 and VUF5262 were assessed for \(H_3\) antagonist activity on the guinea pig isolated jeju-num, as described previously (Vollinga et al., 1992; Leurs et al., 1996). The intestine was removed rapidly and kept in oxygenated (95% O_2-5% CO_2) Krebs’ buffer (composition in mM: NaCl, 118; KCl, 5.6; CaCl_2, 2.5; MgSO_4, 1.18; NaH_2PO_4, 1.28; NaHCO_3, 25; and glucose, 5.5). Jejunal segments (2 cm) were equilibrated at 37°C for 60 min, then stimulated maximally (15 V, 0.1 Hz, 0.5 msec duration), and isotonic contractions were recorded. After 30 min of stimulation, a cumulative dose-response curve for the \(H_3\) agonist R-α-methylhistamine was recorded. After wash-out, antagonists were preincubated 15 min during stimulation, and the \(H_3\) agonist dose-response curve was redetermined. Antagonists were studied at three concentrations, ranging from 3 mM to 1 μM. Four preparations were used for each compound.

Results

Base-line and vehicle antinociceptive scores. As documented previously (Li et al., 1996, 1997a, b), animals receiving ivt injections of saline vehicle showed no changes in nociceptive threshold on either test at any of the test times (not shown). In the present study, %MPE values for saline-injected animals were 5.0 ± 2.7 and −0.59 ± 2.7 for hot-plate and tail-flick tests, respectively (10 min, mean ± S.E.M., \(n = 6\)).

Overview of antinociceptive results. Of 21 compounds studied, 11 agents induced time- and dose-dependent antino-iceptive activity on both the hot-plate and tail-flick tests. Figure 1 shows the time course of antinociceptive activity on the hot-plate test for selected doses of many of the compounds. Results were similar for the 5- and 10-min groups.
after ivt administration, with slightly smaller variences at 10 min. Responses approached control levels by 30 min. The antinociceptive effects of cimetidine and imogran (time courses of which are not included in fig. 1) were previously shown to peak at 10 min (Li et al., 1996). The time courses of action of these agents on the tail-flick test (not shown) were similar to those of the hot-plate test. Thus, 10-min data were used for further analysis. ED$_{50}$ values (table 2) for these compounds were estimated from dose-response data from both the hot-plate (figs. 2–5) and tail-flick tests (fig. 6, legend). Hot-plate ED$_{50}$ values varied by approximately 6-fold (20.8–116.1 µg; 80.3–464.3 nmol); mean slope parameters for all but one of the dose-response curves (VUF5261) ranged between 2.0 and 5.4. For all substances in table 2, there was excellent agreement between hot-plate and tail-flick scores; ED$_{50}$ values were highly correlated across the tests ($r^2 = 0.98$, $n = 11$, fig. 6).

**Toxicity and dose dependence.** For most compounds (figs. 2–5), the highest doses tested induced maximum (i.e., cut-off) antinociception on both tests, with no observable behavioral or motor impairment. However, large doses of three compounds (metiamide, tiotidine and VUF4740) evoked responses suggestive of toxicity (e.g., abnormal posture, jumping, biting or vocalizing), and no antinociceptive data are reported for these treatment groups. Lower doses of metiamide and VUF4740 had no such effects and gave dose-dependent antinociceptive responses, permitting estimates of ED$_{50}$ for these agents (table 2). ED$_{50}$ values were not estimated for VUF4741 (fig. 3) or VUF5262 (fig. 5), which gave highly variable responses that were not dose-dependent. Responses to the highest dose of VUF4686 tested (60 µg) were smaller than the effects of the lower doses. Although the two lower doses showed dose-dependent responses (fig. 4), an ED$_{50}$ was not estimated (the 30-µg dose yielded an ED$_{75}$ effect, fig. 4, table 2).

**Antinociceptive activity of selected H$_2$ antagonists (fig. 2 and table 2).** Among H$_2$ antagonists and chemical congeners, ranitidine and burimamide showed the highest antinociceptive potency (ED$_{50} < 200$ nmol); metiamide, tiotidine, cimetidine and imogran (devoid of H$_2$ activity) showed considerably lower activity (ED$_{50} > 250$ nmol). Zolantidine and its analog SKF95299 were inactive at the highest dose tested (100 µg, table 2). VUF8299 (a cimetidine congener lacking H$_2$ activity, Sterk et al., 1987) was inactive at 100 µg. VUF8298 (an effective H$_2$ antagonist which is the 2-pyridyl homolog of cimetidine) was inactive at 150 µg. Data for cimetidine and imogran were reported previously (Li et al., 1996).

**Antinociceptive activity of burimamide derivatives.** Several structural analogs of burimamide were studied. Variations in the length of burimamide’s carbon side-chain (–(CH$_2$)$_Y$–) showed the analgesic potency of norburimamide (Y = 3) to be similar to that of burimamide (Y = 4). However, the longer chain analog VUF4740 (Y = 6) was approximately twice as potent as the other compounds (fig. 3, table 2). Variations in burimamide’s N-terminal substituents (where R = CH$_3$ in burimamide) produced several high-potency antinociceptive agents (ED$_{50}$ = 70–80 nmol): VUF4685 (R = phenyl), VUF4686 (R = benzyl) and VUF4687 (R = phenylethyl, fig. 4). The N-cyclohexyl congener of burimamide (VUF4684, fig. 4) was only weakly active as an antinociceptive agent, with only about one third of the activity of the aromatically substituted congeners (VUF4685, VUF4686 and VUF4687, table 2).

**Antinociceptive activity of clobenpropit, thioperamide and derivatives (fig. 5).** At a dose of 30 µg, the H$_3$ antagonist clobenpropit induced modest activity (40% MPE) on both nociceptive tests (not shown), but behavioral toxicity prevented the testing of higher doses. As observed previously (Li et al., 1996), thioperamide, the prototype H$_3$ antagonist, was nearly inactive when tested up to 100 µg. Chemically, thioperamide can be viewed as a burimamide derivative with two modifications (table 1): 1) a piperidyl bridging group reducing the flexibility of burimamide’s open chain, and 2) an N-cyclohexyl substituent replacing burimamide’s methyl group. Because the reduced activity of the burimamide derivative VUF4684 appeared to be caused by the N-cyclohexyl substituent, the hypothesis that thioperamide’s lack of antinociceptive activity might be related to its N-cyclohexyl substituent was tested. This was accomplished by the synthesis and testing of the N-methyl (VUF5261) and N-phenyl (VUF5262) analogs of thioperamide. These compounds can be also be viewed as rigid, piperidyl analogs of burimamide and VUF4685, respectively (table 1). The antinociceptive activity of VUF5261 was similar to that of burimamide, and nearly twice that of VUF4684, in support of the hypothesis. The N-phenyl substituent VUF5262, like the other N-phenyl substituent VUF4741, gave highly variable, non-dose-dependent responses.
The pA2 values were derived from Schild plots with slopes of estimated dose-response parameters and number of subjects. Omitidine, VUF8298 and VUF8299 showed negligible activity. See table 2 for dose-response curves estimated by nonlinear regression are shown. Time data from the hot-plate test. For compounds that were active, fitted antinociceptive (hot-plate) potencies of H2 and H3 antagonists after ivt administration

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug no.</th>
<th>Drug Name</th>
<th>Doses Fitted</th>
<th>Subjects Fitted</th>
<th>Fitted ED50 μM ± S.E.M.</th>
<th>Fitted ED50 μM ± S.E.M.</th>
<th>Fitted Slope μM</th>
<th>H1 Kd μM</th>
<th>H2 Kd μM</th>
<th>H3 Kd μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most potent</td>
<td>1</td>
<td>VUF4686</td>
<td>–</td>
<td>7c</td>
<td>30±</td>
<td>104.5±</td>
<td>–</td>
<td>0.079m</td>
<td>0.100m</td>
<td>0.025m</td>
</tr>
<tr>
<td>(ED50 = 70–110 nmol)</td>
<td>2</td>
<td>VUF4687</td>
<td>3</td>
<td>17</td>
<td>24.2 ± 2.7</td>
<td>80.3 ± 8.9</td>
<td>5.3 ± 2.3</td>
<td>0.100m</td>
<td>0.013m</td>
<td>0.195m</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>VUF4685</td>
<td>3</td>
<td>15</td>
<td>22.3 ± 1.9</td>
<td>81.4 ± 7.0</td>
<td>3.0 ± 0.6</td>
<td>0.025m</td>
<td>0.013m</td>
<td>0.039m</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>VUF4740</td>
<td>2h</td>
<td>13</td>
<td>20.8 ± 3.6</td>
<td>86.6 ± 15.1</td>
<td>3.4 ± 1.7</td>
<td>0.013m</td>
<td>0.013m</td>
<td>0.039m</td>
</tr>
<tr>
<td>5 Ranitidine</td>
<td>3</td>
<td>15</td>
<td>34.3 ± 5.0</td>
<td>109.3 ± 15.9</td>
<td>3.6 ± 2.9</td>
<td>&gt;100f</td>
<td>0.063g</td>
<td>50i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately potent</td>
<td>6 Burimamide</td>
<td>3</td>
<td>21</td>
<td>39.2 ± 3.3</td>
<td>184.4 ± 15.6</td>
<td>4.7 ± 1.3</td>
<td>–300m–m</td>
<td>7.8i</td>
<td>0.109m</td>
<td>0.018i</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>VUF5261</td>
<td>3</td>
<td>17</td>
<td>45.1 ± 1.9</td>
<td>200.8 ± 8.7</td>
<td>9.8 ± 6.5</td>
<td>0.044m</td>
<td>0.001m</td>
<td>0.020m</td>
</tr>
<tr>
<td>Least potent</td>
<td>8 Norburimamide</td>
<td>3</td>
<td>18</td>
<td>43.1 ± 5.7</td>
<td>217.3 ± 28.9</td>
<td>3.2 ± 1.1</td>
<td>115h</td>
<td>0.400m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ED50 &gt; 250 nmol)</td>
<td>9</td>
<td>VUF4684</td>
<td>3</td>
<td>15</td>
<td>79.9 ± 5.9</td>
<td>285.1 ± 21.0</td>
<td>5.4 ± 1.6</td>
<td>0.080m</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Improgan</td>
<td>4</td>
<td>25</td>
<td>68.0 ± 5.5</td>
<td>329.7 ± 26.6</td>
<td>5.4 ± 2.2</td>
<td>&gt;100f</td>
<td>340g</td>
<td>5.3i</td>
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<tr>
<td></td>
<td>11</td>
<td>Metiamide</td>
<td>2h</td>
<td>14</td>
<td>85.9 ± 9.2</td>
<td>370.1 ± 38.4</td>
<td>4.0 ± 1.9</td>
<td>0.9i</td>
<td>2.5j</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Cimetidine</td>
<td>4</td>
<td>21</td>
<td>116.2 ± 22.2</td>
<td>464.3 ± 88.6</td>
<td>2.0 ± 0.7</td>
<td>440–630j</td>
<td>0.8i</td>
<td>35j</td>
</tr>
<tr>
<td>3 Tiotidine</td>
<td>6h</td>
<td>&gt;100 μg</td>
<td>&gt;320.4</td>
<td>–</td>
<td>&gt;100f</td>
<td>0.02i</td>
<td>16j</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Thiopeperamide</td>
<td>6e</td>
<td>&gt;100 μg</td>
<td>&gt;342.0</td>
<td>–</td>
<td>&gt;100f</td>
<td>10j</td>
<td>0.001i</td>
<td></td>
</tr>
<tr>
<td>Zolantidine</td>
<td>4d</td>
<td>&gt;100 μg</td>
<td>&gt;262.1</td>
<td>–</td>
<td>–</td>
<td>0.04d</td>
<td>&gt;10j</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>SKF95299</td>
<td>3c</td>
<td>&gt;100 μg</td>
<td>&gt;308.2</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VUF8298</td>
<td>6</td>
<td>&gt;150 μg</td>
<td>&gt;601.6</td>
<td>–</td>
<td>–</td>
<td>0.93i</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VUF8299</td>
<td>7</td>
<td>&gt;100 μg</td>
<td>&gt;403.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

**H2 antagonist activity of VUF5261 and VUF5262.** As found previously (Leurs et al., 1996), Rα-methylhistamine induced dose-dependent inhibition of the neurogenic contractions of the guinea pig jejunum (pD2 = 7.9 ± 0.2, n = 8, not shown). In this preparation, both VUF5261 and VUF5262 behaved as competitive H3 antagonists, with pA2 values of 7.4 ± 0.1 and 8.7 ± 0.1, respectively (mean ± S.E.M., n = 4). The pA2 values were derived from Schild plots with slopes of 1.14 ± 0.14 and 0.85 ± 0.19, respectively, not significantly different from unity.

**Discussion**

The present results show that several compounds possessing H2 antagonist and/or H3 antagonist activity induce dose-related antinociception after ivt administration in rats. Under the conditions used (i.e., high temperature thermal stimuli), the cut-off or near cut-off latencies produced by most of the compounds show highly effective antinociception. Some differences in antinociceptive efficacy may exist, however, because not all of the compounds achieved 100% scores (e.g., VUF4684, VUF4686, VUF4741). In most cases, a large degree of antinociception was obtained without observable motor or behavioral impairment. Although motor or balance tests were not performed in the present study, previous experiments found that near-maximal antinociceptive doses of improgan do not change locomotor activity or rotorod performance (Li et al., 1997a). The reduction in nociceptive responses without impairment of motor function implies that these compounds act on the brain to reduce pain perception (i.e., produce analgesia).

The antinociception produced by these compounds was characterized in studies of time course (fig. 1) and dose (figs. 2–5). The former results, showing peak effects 5 to 10 min after ivt administration with a return to near base-line latencies at 30 min, demonstrate the reversibility of the drug effects. The dose-response relationships found for most of the compounds permitted estimates of in vivo antinociceptive potency. The reliability of these estimates is strengthened by the excellent agreement between ED50 values obtained from two independent nociceptive tests (fig. 6) and by the size of the estimated S.E.M. values (usually 10–20% of the corresponding ED50, table 2). For the tail-flick assays, S.E.M. values (fig. 6, legend) and slope values (not shown) were larger than for the corresponding hot-plate estimates (table 2). Although most potency estimates were based on three or more doses, ED50 values for VUF4740 were derived from two doses; these values should be interpreted with some caution.

**Fig. 2.** Antinociceptive dose-response curves for H2 antagonists and analogs after ivt administration. Injection and testing were performed as described in figure 1 and under “Materials and Methods.” Results are 10 min data from the hot-plate test. For compounds that were active, fitted dose-response curves estimated by nonlinear regression are shown. Thiopeperamide, VUF8298 and VUF8299 showed negligible activity. See table 2 for estimated dose-response parameters and number of subjects.
The results suggest that the present compounds can be classified into three potency groups (table 2). ED₅₀ values for the most potent group (70–110 nmol, table 3) show that these compounds have about one third of the analgesic potency of ivt morphine in rats (Appelbaum and Holtzman, 1985; Yeung and Rudy, 1980). The slope parameters of the presently obtained dose-response curves (usually 3–5) are somewhat steep; analysis of published morphine dose-response curves (Appelbaum and Holtzman, 1985; Yeung and Rudy, 1980) by the same methods used in this study yielded slope values of about 1.5, with confidence intervals between 0 and 3. The significance of these slope values is unclear.

A critical assumption in the analysis of the present findings is that the active compounds share a common antinociceptive mechanism. Although this seems likely based on the SARs found, confirmation of this assumption awaits the discovery of agents capable of selectively inhibiting the activity of these agents.

The analgesic potencies of the present compounds are important for understanding the mechanism of action of these agents. In general, antinociceptive doses of these drugs are larger than those needed to block H₂ or H₃ receptors. For example, ivt ranitidine inhibited footshock-induced antinociception in rats (an H₂ effect) with an IC₅₀ of 6.3 nmol (Gogas and Hough, 1989); this dose is approximately 17-fold lower than the hot-plate antinociceptive ED₅₀ (table 2). Although the brain concentrations achieved by the present treatments are unknown, they can be estimated. If a Kᵋ concentration of ranitidine (63 nM on the H₂ receptor, table 2) is achieved at brain receptors by an IC₅₀ ivt dose (6.3 nmol), then it can be crudely estimated that the antinociceptive activity of ivt ranitidine is achieved at brain concentrations in the range of 1 × 10⁻⁶ M (17 3 63 nM). It is not known whether the presently active compounds are behaving as receptor agonists or antagonists. However, the antinociceptive activity of these drugs seems unlikely to be caused by either action on H₁, H₂ or H₃ receptors. An H₁-antagonist mechanism can be excluded because selective H₁ antagonists like pyrilamine do not show antinociceptive activity on the tail-flick test (Dews and Graham, 1946; Li et al., 1997b), and at 100 μM, cimetidine, ranitidine, burimamide and improgan are either inactive or weakly active on the guinea pig ileum H₁ response (table 2). An H₁ agonist action is ruled out by results showing the inability of a large dose of pyrilamine to inhibit improgan...
antinociception, a treatment which blocked ivt HA antinociception (Li et al., 1997b). The H1 antagonist activity of many of the burimamide derivatives tested in this study has not yet been determined (table 2).

The antinociceptive effects of the compounds studied presently are also not attributable to an action at H3 receptors. Previous work showed that the analgesic properties of ivt cimetidine and ranitidine are not shared by the H3 antagonist famotidine (Netti et al., 1988). More recently, it was shown that the cimetidine analog improgan is a more potent analgesic than cimetidine after ivt administration, but is virtually inactive on H2 receptors (Li et al., 1996; fig. 2, table 2). The present results add further data to support this conclusion (fig. 2, table 2). Metiamide has analgesic activity greater than or equivalent to that of tiotidine and zolantidine, both of which are much more potent H2 blockers than metiamide. In addition, ranitidine, burimamide and norburimamide show similar analgesic potencies (figs. 2 and 3), but have H2 blocking properties that vary by more than 1000-fold (table 2). Furthermore, the cimetidine analog VUF8298, an H2 antagonist with potency similar to that of cimetidine (table 2), was inactive as an analgesic agent. The antinociceptive activity of these agents also seems not to depend on the activation of H2 receptors, because a large dose of the H2 antagonist zolantidine inhibited the analgesia produced by ivt HA, but not the analgesia produced by ivt improgan (Li et al., 1997b).

As mentioned in the introduction, many studies support the hypothesis that endogenous HA can mediate pain-relieving responses in animals. Because H3 antagonists block presynaptic autoreceptors and increase the release of neuronal HA (Barke and Hough, 1994; Tedford et al., 1995; Mochizuki et al., 1991; Itoh et al., 1991), these compounds were predicted to have analgesic properties. However, tested across a broad range of doses on thermal nociceptive tests (hot plate and tail flick), the H3 antagonists thioperamide and GT-2016 had little or no analgesic activity after systemic (L. B. Hough and J. W. Nalwalk, unpublished), intracerebral (Li et al., 1996) and ivt (Li et al., 1996, present results) routes of administration. In this study, the H3 antagonist clobenpropit had measurable, but submaximal activity after 30 µg ivt, but behavioral effects prevented assessment of larger doses. On nonthermal nociceptive tests, H3 antagonists showed slight, non-dose-dependent antinociceptive activity (Lamberti et al., 1996; Malmborg-Aiello et al., 1994).

Even though thioperamide showed little activity against thermal nociceptive responses, the discovery and character-

### Table 3

*In vitro* pharmacology of cimetidine, burimamide and derivatives

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug*</th>
<th>Active Concentration</th>
<th>Order of Potency</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-activated regulation of 5-HT uptake</td>
<td>tiot</td>
<td>0.2 nM</td>
<td>tiot &gt; ran &gt; cim &gt; norbur</td>
<td>“atypical” HA receptor</td>
<td>c</td>
</tr>
<tr>
<td>3H-cimetidine binding</td>
<td>cim</td>
<td>0.2 µM</td>
<td>cim &gt; bur &gt; ran</td>
<td>Ran and tiot (1 mM) inactive</td>
<td>d</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; receptor</td>
<td>cim</td>
<td>0.1–100 µM</td>
<td>cim &gt; met &gt; ran</td>
<td>Ran much less active</td>
<td>e</td>
</tr>
<tr>
<td>Imidazoline (l&lt;sub&gt;i&lt;/sub&gt;) sites</td>
<td>cim</td>
<td>0.1 µM</td>
<td>cim &gt; ran, tiot</td>
<td>Ran and tiot inactive (100 µM)</td>
<td>f,g</td>
</tr>
<tr>
<td>Imidazoline (l&lt;sub&gt;j&lt;/sub&gt;) sites</td>
<td>cim</td>
<td>&gt;1 mM</td>
<td></td>
<td>Very high concentrations</td>
<td>g</td>
</tr>
<tr>
<td>Alpha-1 receptor</td>
<td>bur</td>
<td>2 µM</td>
<td>bur &gt; cim &gt; met</td>
<td>Relevance to analgesia unclear</td>
<td>h</td>
</tr>
<tr>
<td>Alpha-2A receptor</td>
<td>cim</td>
<td>4 µM</td>
<td></td>
<td>Cim analgesia not clonidine-like</td>
<td>i</td>
</tr>
<tr>
<td>Alpha-2B receptor</td>
<td>cim</td>
<td>100 µM</td>
<td></td>
<td>High concentrations</td>
<td>j,g</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>cim</td>
<td>8 µM</td>
<td>cim &gt; met &gt; tiot</td>
<td>Tiotidine inactive</td>
<td>j</td>
</tr>
<tr>
<td>HA-activated Ca influx (human eosinophils)</td>
<td>cim</td>
<td>13 µM</td>
<td>thio &gt; bur &gt; cim</td>
<td>Novel H&lt;sub&gt;1&lt;/sub&gt; subtype?</td>
<td>k</td>
</tr>
<tr>
<td>Mu&lt;sub&gt;1&lt;/sub&gt; opiate receptor</td>
<td>cim</td>
<td>16–100 µM</td>
<td></td>
<td>Unconfirmed in unpublished studies</td>
<td>l,m</td>
</tr>
<tr>
<td>Diamine oxidase (DAO)</td>
<td>bur</td>
<td>20 µM</td>
<td>bur &gt; met, cim</td>
<td>Bur 20–× more active</td>
<td>n</td>
</tr>
<tr>
<td>TXA&lt;sub&gt;2&lt;/sub&gt; synthetase</td>
<td>bur</td>
<td>25 µM</td>
<td>bur &gt; met, cim</td>
<td>Relevance to analgesia unclear</td>
<td>o</td>
</tr>
<tr>
<td>Benzodiazepine binding</td>
<td>cim</td>
<td>70 µM</td>
<td>cim &gt; met</td>
<td>Met 50–× less active</td>
<td>i</td>
</tr>
<tr>
<td>Muscarinic receptor</td>
<td>cim</td>
<td>100–300 µM</td>
<td>cim &gt; tiot &gt; ran</td>
<td>Very high concentrations</td>
<td>p</td>
</tr>
<tr>
<td>HA methyltransferase (HMT)</td>
<td>bur</td>
<td>160–280 µM</td>
<td>bur &gt; met, cim</td>
<td>Bur 10–× more active, high</td>
<td>q</td>
</tr>
<tr>
<td>Beta receptor</td>
<td>cim</td>
<td>200 µM</td>
<td>cim &gt; met, bur</td>
<td>Very high concentrations</td>
<td>r</td>
</tr>
<tr>
<td>HA (&quot;Hic&quot;) inhibition of platelet aggregation</td>
<td>cim</td>
<td>0.1–1 mM</td>
<td>cim &gt; ran</td>
<td>Very high concentrations</td>
<td>r</td>
</tr>
<tr>
<td>Arachidonate cyclo-oxygenase</td>
<td>bur</td>
<td>2.3 mM</td>
<td></td>
<td>Very high concentrations</td>
<td>o</td>
</tr>
</tbody>
</table>

* Drug abbreviations: cim, cimetidine; tiot, tiotidine; bur, burimamide; ran, ranitidine; thio, thioperamide.
* IC<sub>50</sub> or K<sub>a</sub> value for the compound named. A range of concentrations indicates findings from more than one study. Where a receptor is given as the target, the compounds acted as antagonists.

References: * Launay et al., 1994; * Smith et al., 1980; * Trzeciakowski and Frye, 1986; Lakoski et al., 1983; * Emmsberger et al., 1987; * Emmsberger et al., 1995; * McCulloch et al., 1978; * Emmsberger et al., 1992; * Sivelle et al., 1982; * Raible et al., 1997; * Speeg et al., 1981; * Takayanagi et al., 1978; * Bieganski et al., 1980; * Allan et al., 1980; * Cavanagh et al., 1983; * Barth et al., 1973; Taylor, 1973; * Saxena et al., 1989.
ization of improgan antinociception prompted further investigation of the H₃ analgesia hypothesis. Although virtually inactive at H₁ and H₂ sites, this compound behaved as an H₃ antagonist in the micromolar range; cimetidine was slightly less potent on both analgesia and at the H₃ receptor (Li et al., 1996; table 2). However, ivt coadministration of the H₃ agonist (R)-α-methylhistamine failed to reduce improgan antinociception, lending no support to the H₃ antagonist hypothesis (Li et al., 1997b). Furthermore, coadministration of H₃ antagonists and improgan produced no evidence for an H₃ agonist analgesic mechanism (Li et al., 1997b). It was also considered possible that blockade of brain H₃ receptors could induce analgesia, but that thioperamide’s effects on other receptors (e.g., 5-HT₃, Leurs et al., 1995a) might prevent expression of this response. If this were the case, however, thioperamide also should have reduced improgan antinociception, an effect not observed (Li et al., 1997b).

The present experiments provide considerable additional data for evaluation of the H₃ analgesia hypothesis. Figure 7 shows the relationship between antinociceptive potency and H₃ receptor activity for 11 compounds. The data show that the H₃ receptor does not contribute to the antinociceptive responses measured presently. The correlation fails only because of ranitidine (a compound with moderate antinociceptive potency and very low H₃ activity, data point on bottom right of fig. 7), but because of the lack of analgesic activity of potent H₃ antagonists like thioperamide, GT-2016 and clobenpropit (none of which are included in fig. 7). Also note that the N-cyclohexyl congener of burimamide (VUF4684) is considerably less potent than burimamide as an analgesic, a pattern distinct from the respective H₃ activities of these agents (table 2). The scales of the axes of figure 7 also rule out a meaningful role for the H₃ receptor, because the “most potent” and “least potent” groups (table 2) differ by only 5- to 6-fold on analgesic activity, but vary by 300- to 1000-fold on H₃ receptor activity (table 2, fig. 7). Although the existence of H₃ receptor subtypes seems likely (Leurs et al., 1996; West et al., 1990), the possibility that one such subtype contributes to analgesic mechanisms would require much additional work to be verified. Thus, the present results confirm the hypothesis that H₁, H₂ and H₃ receptors seem to be excluded as the mechanism of antinociceptive action for the compounds studied presently.

Because the actions of burimamide-like compounds are not limited to HA receptors, the additional pharmacology of these agents must be considered in the search for the mechanism(s) of antinociceptive action (table 3). Some effects of these drugs (e.g., antagonism at muscarinic, beta adrenergic, benzodiazepine, HMT and “Hic” sites) occur only at high micromolar or low millimolar concentrations, which are probably not relevant to the present results. For other potential sites of action (e.g., labeled cimetidine binding, histaminergic regulation of 5-HT uptake, androgen receptors or imidazole I₁ sites), potencies of these drugs may be relevant, but the inactivity of ranitidine and/or tiotidine in these systems argues against the significance of these sites for analgesic responses (table 3). An unusual HA response in eosinophils is inhibited by appropriate doses of burimamide and cimetidine, but thioperamide was very active in this system (table 3), in contrast to the present results. Other potential targets are not excluded by pharmacological criteria. For example, the burimamide-induced inhibition of thromboxane A₂ synthetase (25 μM, table 3) might be achieved by the present ivt doses, but this effect would not be expected to induce analgesia. Inhibition of arachidonate cyclooxygenase, which does cause analgesia, was not observed below millimolar concentrations of these drugs (table 3). Older reports that cimetidine acts weakly at mu opioid receptors (table 3) may have possible relevance to the present results. However, unpublished studies in our laboratory show slight and no inhibition of mu opioid receptor binding by cimetidine and improgan, respectively, at 100 μM. Furthermore, because improgan antinociception was unaffected by large doses of naltrexone, opioid analgesic mechanisms are unlikely to account for the antinociception produced by these compounds (Li et al., 1997b). Also noteworthy may be the reported inhibition of diamine oxidase (DAO) by burimamide (IC₅₀ = 20 μM); metiamide and cimetidine were about 20-fold less active (Bieganski et al., 1980). Although only small amounts of DAO are present in the brain, and this enzyme is not thought to contribute substantially to brain HA metabolism, the product of this reaction, imidazole acetic acid, is pharmacologically active (Ernsberger et al., 1995; Thomas and Prill, 1995). Thus, this mechanism may be of further interest.

The present results could be related to brain catecholamine systems. Burimamide, metiamide and cimetidine (50–250 μg ivt) decreased rat hypothalamic norepinephrine levels with an order of potency similar to that found presently on analgesia (Nowak, 1980; Nowak et al., 1978). These effects may be related to the ability of burimamide to activate the peripheral sympathetic system after large systemic doses (Brimblecomb et al., 1976). An action on central alpha-2 receptors may also require further investigation, because information is limited, and the reported potencies of cimetidine on two alpha-2 subtypes are somewhat discrepant (table 3). Although blockade of alpha-2 receptors might account for the ability of these compounds to deplete brain norepinephrine levels, this effect would not be expected to cause analgesia, because alpha-2 agonists, not alpha-2 antagonists induce analgesia (Tasker and Melzack, 1989). Centrally adminis-

Fig. 7. Relationship between antinociceptive potency and H₃ antagonist affinity of 11 compounds. The antinociceptive ED₅₀ value (hot plate, abscissa, −log mol, ± transformed S.E.M.) for each drug is plotted against the pA₂ value (ordinate, −log Kd ± S.E.M.) of that compound for the H₃ receptor. No correlation is evident (also note differences in axis scales). See table 2 for details.
tered metiamide reverses ivt clonidine-induced hypotension (Karpapan, 1981), but this effect is thought to occur by blockade of imidazole (I1), not alpha-2 sites (Ernberger et al., 1995). However, ranitidine’s inactivity at I1 sites seems to exclude them as an analgesic target (table 3). Other potentially significant adrenergic sites include alpha-1 receptors, which are blocked by burimamide in the low micromolar range (table 3).

Spinal noradrenergic systems participate in pain-relieving mechanisms, and the alpha-2 receptor agonists clonidine and dexametomidine are thought to act at supraspinal and intraspinal sites to induce antinociceptive responses (Tasker and Melzack, 1989; Idänpää-Heikkilä et al., 1994). Because of overlapping structural and pharmacological profiles, the possibility that the presently observed antinociception results from a clonidine-like (i.e., alpha-2 agonist) effect requires further study. However, the following observations suggest that impropgan antinociception is not mediated by a clonidine-like mechanism: 1) studies in progress in our laboratory show that impropgan antinociception is not antagonized by yohimbine treatments that reverse clonidine analgesia (B. Y. Li and L. B. Hough, unpublished); and 2) unlike clonidine analgesia (which is accompanied by sedation and depression of locomotor activity, e.g., Smythe and Pappas, 1989), near-maximal impropgan antinociception was observed without inhibition of spontaneous locomotor activity (Li et al., 1997a).

Despite the pharmacological considerations above, the mechanism of impropgan antinociception remains unknown. However, the present results have revealed new information on the structural requirements for the analgesic activity of this class of compounds. For compounds closely related to cimetidine and impropgan (see table 1), there seems to be an imidazole requirement, because VUF8F892 (a pyridyl analog) and VUF8299 (a phenyl analog) were both inactive (table 2). However, imidazoles are not an absolute requirement for activity, because ranitidine (a substituted furan) was active. Netti et al. (1988) reported that ivt ranitidine induced analgesia, but no data were shown. With respect to other H2 antagonists, the inactivity of zolantidine and SKF95299 is particularly disappointing, because these agents are the only compounds studied presently which possess significant brain-penetrating characteristics (Gogas et al., 1989).

Variations in the structures of burimamide and thioperamides revealed significant analgesic profiles. Similar to the pattern found for antagonist activity on H2 receptors (Volinga et al., 1995), either elongation of the burimamide side-chain (VUF4740, fig. 3) or replacement of the N-methyl group with N-aromatic substituents (e.g., VUF4685, fig. 4) increased analgesic activity as compared with burimamide. The incorporation of both of these changes in the same molecule (VUF4741, fig. 3) did not further enhance potency on either the H3 receptor or in analgesic assays. If fact, in the latter tests, VUF4741 gave a highly variable result that was not amenable to pharmacological analysis. Although not understood, the same pattern was seen with another N-phenyl analog, VUF5262 (fig. 5). The discovery that the N-cyclohexyl burimamide homolog (VUF4684) has considerably lower analgesic potency as compared with the N-phenyl (VUF4685) or N-benzyl (VUF4686) analogs (table 2) was unexpected, especially considering the similarities in their respective H3 activities (table 3). Because thioperamide is also an N-cyclohexyl derivative, it was hypothesized that the cyclohexyl moiety of thioperamide contributed to its lack of analgesic activity. The analgesic activity of VUF5261, an N-methyl homolog of thioperamide, seems to support this hypothesis. The lack of analgesic activity of VUF5262, the N-phenyl homolog of thioperamide, shows that, unlike the case for burimamide, the open-chain and piperidine-bridging groups are not equivalent. That is, substitution of the N-methyl group with N-phenyl increased analgesic potency for burimamide, but not for the thioperamide analog VUF5261. Further work is required to discover the mechanism of action of these compounds and to develop congeners that could be used for the clinical relief of pain.

Acknowledgments

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References


