Sch 50971, an Orally Active Histamine H₃ Receptor Agonist, Inhibits Central Neurogenic Vascular Inflammation and Produces Sedation in the Guinea Pig

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ABSTRACT
We studied the actions of Sch 50971, a novel histamine H₃ receptor agonist, in an experimental neurogenic model of migraine and characterized its sedative and respiratory actions. Sch 50971 (i.v. and p.o) inhibited plasma protein extravasation in the dura mater of guinea pigs after electrical stimulation of the trigeminal ganglia. The minimum effective doses of Sch 50971 were 3.0 mg/kg i.v. and 10 mg/kg p.o., which produced a 40% and 42% decrease in plasma protein extravasation, respectively. The effects of Sch 50971 (3.0 mg/kg i.v.) were blocked by the histamine H₃ antagonist thioperamide (3.0 mg/kg i.v.). The 5-HT₁D agonist, sumatriptan (0.3 mg/kg i.v.), and the histamine H₃ agonist, (R)-α-methylhistamine (0.3 mg/kg), also inhibited plasma extravasation by 40 and 46%, respectively. In sedation studies, Sch 50971 (1–100 mg/kg p.o.) potentiated pentobarbital-induced sleep. The ED₄₀ min for Sch 50971, the benzodiazepines triazolam and diazepam, the histamine H₁ antagonist diphenhydramine and the H₃ receptor agonist (R)-α-methylhistamine were 7.0, 0.5, 2.3, 14.1 and 23.4 mg/kg p.o., respectively. The sedative effects of oral Sch 50971 was blocked by thioperamide (10 µg i.c.v.). The sedative activity of Sch 50971 was also examined using EEG activity, locomotor activity and sleep. In conscious guinea pigs, Sch 50971 (10 mg/kg p.o.) depressed locomotor activity, increased total sleep time and produced EEG patterns consistent with physiological sleep. Sch 50971 decreased beta wave activity but had no effects on delta wave activity, theta activity or alpha wave activity. In contrast, triazolam (1.0 mg/kg p.o.) depressed delta and theta wave activity and produced large increases in alpha and beta wave activity. In conclusion, Sch 50971 is an orally active, potent and selective agonist of histamine H₃ receptors that may act to ameliorate the sequelae of migraine headaches, where activation of histamine H₃ receptors may be beneficial. Sch 50971 also decreases motor activity and promotes EEG activity consistent with physiological sleep.

Current drug treatment for migraine headaches includes NSAIDs like ibuprofen, the ergot DHE and the 5-HT₁D agonist SUMA. NSAIDs inhibit prostaglandin synthesis and attenuate neurogenic inflammation in the trigeminovascular system (Buzzi et al., 1988). However, NSAIDs are not effective for many migraine patients and are associated with the risk of dyspepsia and gastrointestinal hemorrhage (Welch, 1993). DHE and SUMA are currently the most efficacious drugs available for migraine. DHE is associated with nausea, vomiting, abdominal pain, diarrhea and cerebral vasocostriction (Welch, 1993). Side effects associated with SUMA are coronary vasospasm and chest heaviness (Welch, 1993). Furthermore, both DHE and SUMA are significantly less active in humans after oral administration. Newer 5-HT₁D agonists may soon be available. These drugs are orally active and have been reported to be more potent than SUMA; however, the pharmacology of these second-generation “SUMA-like drugs” does not exclude potential cardiovascular effects. In light of these therapeutic limitations, an antimigraine drug that demonstrates oral efficacy without the cardiovascular liability of SUMA or DHE represents an improvement over current therapies.

Markowitz et al. (1987) proposed that migraine pain can occur as a result of trigeminal nerve stimulation. Factors and mediators that initiate activation of trigeminal C-fibers are unknown. However, the end result of trigeminal stimulation is a neurogenic inflammation within cephalic tissue. Neurogenic inflammation is mediated by the release of vasoactive peptides like substance P, calcitonin gene-related peptide and neurokinin A from sensory nerve terminals that innervate blood vessels in the dura mater (Buzzi et al., 1988; Markowitz et al., 1987). Once released, these neuropeptides activate postsynaptic receptors to cause vasodilation and enhance vascular permeability.

Recent evidence has demonstrated that neurogenic inflammation of the dura mater in rats and guinea pigs can be

ABBREVIATIONS: CNS, central nervous system; i.c.v, intracerebroventricular; s.c, subcutaneous; SUMA, sumatriptan; NSAID, nonsteroid anti-inflammatory drug; DHE, dihydroergotamine.
inhibited by a prejunctional histaminergic H₃ receptor mechanism (Matsubara et al., 1992). In an animal model of migraine that involves the stimulation of the trigeminal ganglia to elicit neurogenic inflammation, activation of presynaptic histamine H₃ receptors attenuated neurogenic vasculitis by inhibiting the release of neuropeptides from sensory nerve endings (Matsubara et al., 1992).

In addition, pharmacological studies have indicated that the CNS presynaptic histamine H₃ receptor may be an important regulator of arousal/sleep patterns (Tasaka et al., 1989; Lin et al., 1990; Sakai et al., 1991; Monti et al., 1991). It is well established that the classic antihistamines induce sedation in humans (Roth et al., 1987) by blockade of CNS histamine H₁ receptors (Levander et al., 1985). Conversely, studies in animals demonstrate that histamine H₁ receptor activation in the CNS produces an increase in arousal and a concomitant decrease in EEG sleep patterns (Wolf and Monnier, 1973). Previous studies have shown that activation of central histamine H₃ receptors produces locomotor hypoactivity in rats and mice (Sakai et al., 1991; Bristow and Bennet, 1993) and an increase in slow wave sleep activity and total sleep time in cats (Lin et al., 1990). However, the role of histamine H₃ receptors on sleep/arousal studies remains to be elucidated.

In light of the observations of the effects of histaminergic H₃ receptor activation on neurogenic inflammation within the dura mater and of actions on wakefulness, we characterized the CNS pharmacology of Sch 50971 (fig. 1), a selective, high affinity, orally active agonist of histamine H₃ receptors (Hey et al., 1998). It is concluded that the preclinical activity profile shows potential as a novel antimigraine and sedative agent.

Materials and Methods

General. Sch 50971 [(+)-trans-4-[4(R)-methyl-3(R)-pyrrolidinyl]-1H-imidazole dihydrochloride] (fig. 1) was prepared by the Chemistry Department of Schering-Plough Research Institute. Pyrillamine hydrochloride, ipratropium bromide, triazolam, gallamin triethiodide, atropine sulfate from Sigma; thioperamide maleate, was obtained from Research Biochemicals. (R)-α-Methylhistamine HCl, diphenhydramine HCl, cimetidine, histamine HCl and diazepam were obtained from Schering-Plough Research Institute (Compound Distribution Center). Sumatriptan was a gift from Glaxo Research. Other compounds were obtained from standard suppliers. All drug doses were calculated as their free base.

Electrical trigeminal stimulation and assessment of plasma protein extravasation within the dura mater. The assessment of Sch 50971 on plasma protein extravasation in the dura mater produced by electrical trigeminal stimulation was determined using the methods of Markowitz et al. (1987). Male Hartley guinea pigs (400–600 g; Charles River Laboratories, Wilmington, MA) were anesthetized with urethane (1.5 g/kg) The left jugular vein was cannulated for i.v. administration of drugs. Guinea pigs were mechanically ventilated (volume, 4 ml; rate, 45 breaths/min) via a tracheal cannula. Animals were placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), and a bipolar stainless steel electrode (Rhodes Medical Instruments, Woodland Hills, CA) was lowered –10.0 mm from the dura mater into the trigeminal ganglia (4.0 mm lateral, –10.0 mm posterior relative to bregma). [(R)-α-Methylhistamine (0.3 mg/kg), sumatriptan (0.5 mg/kg) and Sch 50971 (0.3, 3.0 and 30 mg/kg) administered i.v. were given 10 min before trigeminal electrical stimulation (1.2 mA, 5 Hz, 5 msec duration for 5 min). Sch 50971 (3, 10, 30 mg/kg) given p.o. was given 30 min before stimulation. Evans blue (50 mg/kg i.v.), a fluorescent dye that complexes with plasma proteins functioned as an index of plasma protein extravasation, was given 5 min before trigeminal stimulation. After electrical trigeminal stimulation, guinea pigs were perfused with 200 ml of saline via the left cardiac ventricle. The dura mater was removed from the left and right hemispheres. Dissected tissue were incubated in 1 ml formamide at 37°C for 18 hr. To quantify the amount of dye in each sample colorimetric measurements were performed using a SLT Lab instruments SLT-340 ATTC plate reader (Grodig, Salzburg). Extravasated Evans Blue was quantified by interpolation on a standard curve of dye concentration in the range of 0.3 to 30 μg/ml. The data are expressed as the ratio of the concentration of Evans blue on the stimulated/unstimulated side of the dura mater after unilateral electrical stimulation of the trigeminal ganglia.

Potentiation of pentobarbital-induced loss of righting reflex in guinea pigs. Sch 50971 and a series of standard sedating drugs were studied to determine their effects on pentobarbital-induced narcosis in guinea pigs, defined as loss of the righting reflex. In this model, sedation is defined as a potentiation of the loss of the righting reflex to a standard dose of sodium pentobarbital. Fasted male Hartley guinea pigs (400–600 g, Charles River) were orally pretreated with Sch 50971 (1–100 mg/kg), triazolam (0.3–3 mg/kg), diphenhydramine (3–30 mg/kg), diazepam (1–10 mg/kg), (R)-α-methylhistamine (3–100 mg/kg) or saline 30 min before receiving 18 mg/kg of sodium pentobarbital (i.p.). The onset of narcosis was defined as the time (in min) between i.p. pentobarbital dosing and the loss of the righting reflex. The duration of narcosis (sleep) was defined as the period (min) between the loss and recovery of the righting reflex. Return of the righting reflex was noted when the animal righted itself 3 times in a 1-min period. Potentiation of the loss of the righting reflex was determined by subtracting the total sleep time of the vehicle group from the experimental group.

In some animals, i.e.v. cannulas were implanted 1 week earlier according to McLeod et al. (1991). The stereotaxic coordinates used were anterior 0.5 mm, lateral 2.0 mm and ventral 4.5 mm in relation to the bregma. The i.e.v. studies were performed as described above with the modification that 10 min before i.p. pentobarbital, 10 μg of triperamide maleate in 10 μl of artificial CSF, or CSF alone, was given i.e.v.

EEG activity, spontaneous locomotor activity and sleep assessment studies in conscious guinea pigs. Adult male Hartley guinea pigs (400–500 g, Charles River) were anesthetized with a combination of xylazine (10 mg/kg s.c.) and ketamine (60 mg/kg s.c.). The animals were placed in a stereotoxic apparatus, and the surface of the skull was surgically exposed. Two gold-plated surface electrodes were placed in direct contact with the dura, above the parietal cortex, in the general somatosensory area of the brain. The exact placement used was posterior 2 to 3 mm, lateral ±2 mm (on both sides of the skull) with respect to bregma. The animals were allowed to recover for 1 week after surgery.

EEG analog activity (EEG amplitude) was transmitted from an extracorporeal T4C two-channel telemetric transmitter to a TR7–1 PC telemetry receiver (Konisberg Instruments). Differential EEG
activity was amplified (3 k) and processed through a low/high band pass filter at 30/0.3 Hz. The activity was recorded as a raw analog signal (0.1–100 Hz sampling rate). Also, a 10-sec integration of the EEG amplitude and moving frequency average was determined. The analog wave forms were displayed and recorded on a Mi2 Data Acquisition System (M-3000). The EEG amplitude and frequency values for each 10-sec bin were also recorded. The integrated activity was expressed as mV × sec, and frequency was expressed as Hz. Further processing of the integrated EEG signal for determination of specific components of cortical activity (power spectrum analysis) was done by fast Fourier transform. The power spectrum analysis was done for each bin on total cortical EEG activity over the course of the experiment.

Two guinea pigs were orally dosed on a given day (8:30 a.m.) with either Sch 50971 (10 mg/kg), triazolam (1.0 mg/kg), diphenhydramine (30 mg/kg) or saline vehicle. EEG measurements were recorded continuously for 6 hr. All animals that received one of the test drugs were also run as controls on a separate day. No animal was used more than once per week.

The average relative power (percent of activity for any given EEG wave band) was calculated for each hr. The EEG activity wave bands were divided as follows: 0 to 4 Hz delta wave, 4 to 8 Hz theta wave, 8 to 14 Hz alpha wave and 14 to 30 Hz beta wave (Scott, 1976).

The effect of these drugs on spontaneous locomotor activity was simultaneously measured in the guinea pigs undergoing EEG recording. Spontaneous locomotor activity (SLA) was measured using Digiscan activity monitors. These instruments measure movement using an eight-beam array of infrared sensors that, when crossed, transmit a digital pulse to a PC. The SLA of two animals was measured continuously for 6 hr while simultaneously recording EEG. Animals were housed separately in 100 × 100 × 60 cm activity cages. The average total activity and total distance moved for hr 1 through 6 was determined.

Concomitant to the EEG measurements and SLA recording the animals were also videotaped using a camcorder to assess sleep time and patterns of activity during the course of the experiment. The total time (min) an animal spent in each of the following categories was recorded: sleep (S), awake-quiet (AQ) and awake-moving (AM). Sleep time is defined as eyes closed with no voluntary movement. Awake-quiet is defined as eyes open with no movement. Awake moving is defined as eyes open with horizontal or vertical movement. All experiments were analyzed in a double-blind fashion.

Statistical analysis. Statistically significant (P < .05) differences between treatments were determined by Student’s t test (for either paired or unpaired observations) or by one-way analysis of variance (ANOVA) using a Dunnett’s t test for multiple comparisons. For plasma protein extravasation experiments significance (P < .05)
Effects of Sch 50971 on neurally induced Evans blue extravasation in the dura mater of guinea pigs. Electrical stimulation of the left trigeminal ganglia in control guinea pigs produced an increase in the concentration of Evans blue found on the ipsilateral side of the dura mater (ratio > 2.0) compared to the contralateral side (fig. 2A). In a sham group of guinea pigs (n = 8) in which the stimulating electrode was lowered to the level of the trigeminal ganglia but not stimulated a ratio of 0.71 ± 0.18 was observed. In guinea pigs given Sch 50971 at doses of 0.3, 3, and 30 mg/kg i.v. the ratios were 2.58 ± 1.21, 1.27 ± 0.06 and 1.58 ± 0.20 respectively, compared with a control group ratio of 2.10 ± 0.28. The minimum effective i.v. dose of Sch 50971 was 3 mg/kg. (R)-o-methylhistamine (0.3 mg/kg i.v.) and sumatriptan (0.3 mg/kg i.v.) inhibited Evans blue extravasation produced by trigeminal stimulation (fig. 2A). Neither treatment had any effects on the concentration of dye in the contralateral side (data not shown). The effects of Sch 50971 were mediated by histamine H3 receptors because the H3 antagonist thioperamide (3 mg/kg i.v.) blocked the inhibitory actions of Sch 50971 on plasma extravasation (fig. 2B). Figure 2C shows the effects of orally administered Sch 50971 (3, 10 and 30 mg/kg p.o.) on plasma leakage due to electrical trigeminal stimulation. Sch 50971 (10 and 30 mg/kg p.o.) inhibited Evans blue extravasation.

### Results

#### Effect of Sch 50971 on the pentobarbital-induced loss of righting reflex in guinea pigs

Sch 50971 was compared with a number of agents with established sedative activity in man for effects on pentobarbital-induced loss of righting reflex in guinea pigs. Oral Sch 50971 caused a dose-dependent enhancement of the narcosis induced by pentobarbital as indicated by the potentiation of the pentobarbital-induced loss of the righting reflex (fig. 3). The ED40 min is defined as the dose needed to increase the loss of righting reflex 40 min beyond the time of recovery of the righting reflex for the group given only pentobarbital. The ED40 min for Sch 50971 was 7.0 mg/kg. Triazolam and diazepam also produced dose-dependent increases in pentobarbital narcosis (fig. 3). The ED40 min for triazolam was 0.5 mg/kg and for diazepam was 2.5 mg/kg. The maximum responses with the benzodiazepine sedatives were greater than for Sch 50971 (data not shown). The sedating H1 antagonist diphenhydramine potentiated the responses to pentobarbital. The ED40 min for diphenhydramine was 14.1 mg/kg (fig. 3). Doses of diphenhydramine greater than 30 mg/kg produced convulsions in 4 of 11 animals. In contrast, the nonselecting antihistamine, terfenadine (100–300 mg/kg) did not potentiate the loss of the righting reflex induced by pentobarbital (fig. 3). The histamine H1 agonist, (R)-o-methylhistamine, also potentiated pentobarbital-induced loss of righting reflex.

### Table 1

Effect of intracerebroventricular thioperamide on the effect of Sch 50971 on the pentobarbital-induced loss of righting reflex in guinea pigs

| Compound* | Oral dose mg/kg | Potentiation of loss of righting reflex ± S.E.M. | Thioperamide | Thioperamide
|---|---|---|---|---
| Sch 50971 | 10 | +53 ± 19 | +5 ± 7
| (R)-o-Methylhistamine | 30 | +25 ± 10 | +4 ± 8
| Triazolam | 30 | +145 ± 27 | +27 ± 20
| Diazepam | 3 | +109 ± 42 | +164 ± 37

* Drugs or vehicle were given p.o. 30 min before pentobarbital administration (18 mg/kg i.p.). Thioperamide maleate (10 μg in 10 μl artificial CSF vehicle) or artificial CSF was given i.c.v. 10 min before pentobarbital (n = 4–6).

### Table 2

Duration of action of Sch 50971 on potentiation of pentobarbital-induced loss of righting reflex in guinea pigs

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Oral dose mg/kg</th>
<th>0.5 hr Pretreatment</th>
<th>2 hr Pretreatment</th>
<th>4 hr Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sch 50971</td>
<td>10</td>
<td>+43 ± 11</td>
<td>+44 ± 10</td>
<td>+13 ± 12</td>
</tr>
<tr>
<td>(R)-o-Methylhistamine</td>
<td>30</td>
<td>+42 ± 6</td>
<td>+77 ± 21</td>
<td>+32 ± 12</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>30</td>
<td>+52 ± 9</td>
<td>+88 ± 14</td>
<td>+59 ± 7</td>
</tr>
<tr>
<td>Triazolam</td>
<td>1</td>
<td>+89 ± 24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diazepam</td>
<td>3</td>
<td>+64 ± 19</td>
<td>—</td>
<td>+18 ± 10</td>
</tr>
</tbody>
</table>

* Drugs or vehicle were given p.o. 30 min, 2 hr or 4 hr before pentobarbital administration (18 mg/kg i.p.).

* Potentiation of loss of righting reflex is the increase in time when the animal is unable to right itself beyond the recovery of the righting reflex for the respective control groups (i.e., animals receiving saline p.o.). Values are mean ± S.E.M. (n = 5–6 per group).

Significantly different from vehicle group at respective pretreatment time (P < .05).
To determine the duration of action of Sch 50971, the drug was administered orally at 0.5, 2 hr and 4 hr before pentobarbital. The peramide (table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Loss of righting reflex&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Increase in sleep over group I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle 5 days</td>
<td>I</td>
<td>89 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>Vehicle 4 days, then Sch 50971 (10 mg/kg)</td>
<td>II</td>
<td>116 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+26</td>
</tr>
<tr>
<td>Sch 50971 (10 mg/kg) twice daily for 4 days, then one dose on day 5</td>
<td>III</td>
<td>118 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+29</td>
</tr>
<tr>
<td>Vehicle 4 days, then diphenhydramine (30 mg/kg) 1 dose</td>
<td>IV</td>
<td>159 ± 15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+63</td>
</tr>
<tr>
<td>Diphenhydramine (30 mg/kg) twice daily for 4 days, then one dose on day 5</td>
<td>V</td>
<td>141 ± 16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+46</td>
</tr>
<tr>
<td>Vehicle 4 days, then triazolam (1 mg/kg) 1 dose</td>
<td>VI</td>
<td>213 ± 22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+118</td>
</tr>
<tr>
<td>Triazolam (1 mg/kg) twice daily for 4 days, then one dose on day 5</td>
<td>VII</td>
<td>153 ± 15&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>+57</td>
</tr>
</tbody>
</table>

<sup>a</sup>Animals were dosed orally twice each day (first dose 8:00 a.m., second dose 3:30 p.m.) for 4 days. On the fifth day, groups II and III received a single dose of Sch 50971 10 mg/kg p.o.; groups IV and V received a single dose of diphenhydramine 30 mg/kg p.o.; groups VI and VII received a single dose of triazolam 1 mg/kg p.o. Group I received oral methylcellulose vehicle twice a day, and once on the 5th day.

<sup>b</sup>Loss of righting reflex is defined as time when animal is unable to right itself three times in a 1-min period. Values are the mean ± S.E.M. (n = 5–6).

<sup>c</sup>Statistically different from group I (P < .05).

<sup>d</sup>Statistically different from group VI (P < .05).

The ED<sub>40</sub> for (R)-α-methylhistamine was 23.4 mg/kg (data not shown).

Diphenhydramine maleate 10 µg (in 10 µl of artificial CSF) given i.c.v. completely blocked the effects of oral Sch 50971 and (R)-α-methylhistamine on the pentobarbital-induced loss of the righting reflex (table 1). In contrast, thioperamide did not attenuate the effect of diazepam. The effect of diphenhydramine was also blocked by thioperamide (table 1).

To determine the duration of action of Sch 50971, the drug was administered orally at 0.5, 2 hr and 4 hr before pentobarbital. The dose chosen for the duration studies was a dose that approximated the ED<sub>40</sub> for the respective drugs. At 10 mg/kg, Sch 50971 showed a significant effect at 0.5 and 2 hr but not at 4 hr pretreatment (table 2). Also, (R)-α-methylhistamine (30 mg/kg), and diazepam (3 mg/kg) were inactive at 4 hr. Both triazolam (1 mg/kg) and diphenhydramine (30 mg/kg) maintained activity at 4 hr.

In subacute studies with Sch 50971, there was no development of tolerance to its sedative effects after oral administration (10 mg/kg twice daily for 4.5 days) compared to animals that received a single dose of Sch 50971 (table 3). Also, in these subacute tolerance studies, diphenhydramine (30 mg/kg) did not show tolerance (table 3). In contrast, there was a significant development of tolerance to the effect of triazolam (1 mg/kg) (table 3).

**Effects of Sch 50971 on EEG, spontaneous locomotor activity and sleep in conscious guinea pigs.** To determine the effect on CNS function and assess the sedative activity of Sch 50971, its effects on EEG, spontaneous locomotor activity and sleep time were studied concurrently in the same animals. Oral Sch 50971 (10 mg/kg) produced a slight but not statistically significant increase in relative delta EEG power during the first 3 hr after dosing (fig. 4). Oral triazolam (1 mg/kg) caused a significant decrease in relative delta power during the 6 hr of the experiment. Sch 50971 (10 mg/kg) did not significantly alter theta or alpha relative EEG power (fig. 4). In contrast, triazolam (1 mg/kg) produced significant disruption in relative EEG power by reducing theta activity (fig. 4) and increasing alpha activity. The overall effect of the Sch 50971 EEG relative power spectra profile is to increase slow-wave EEG activity (increased delta wave) an action which is consistent with a sedative effect and increase in total sleep time. This action occurred without altering theta activity (component of REM) and alpha wave activity. Sch 50971 also produced a small but significant decrease of beta wave activity (associated with wakefulness) 2 to 4 hr after treatment (fig. 4). In contrast, triazolam produced significant disruptions of the overall EEG spectral profile, with large increases in alpha (component of light sleep) and beta activity and concomitant decreases in delta and theta activity. Oral diphenhydramine (30 mg/kg) produced disruptions in the overall EEG spectral profile, producing a decrease in delta and theta wave activity (data not shown).

Sch 50971 (10 mg/kg p.o.) and triazolam (1 mg/kg p.o.) produced comparable decreases in spontaneous locomotor activity and total distance moved (fig. 5). On the sleep assessment analysis, Sch 50971 produced significant increases in sleep time from 2 to 4 hr and triazolam produced significant increases over 6 hr compared to controls (fig. 6). Conversely, diphenhydramine (30 mg/kg p.o.) depressed spontaneous locomotor activity without significantly increasing total sleep time (data not shown).

**Discussion**

In the current study, we confirm the observations of Matsubara et al. (1992), who demonstrated that activation of histamine H<sub>3</sub> receptors inhibited plasma protein extravasation in the dura mater of rats after electrical stimulation of the trigeminal ganglia. This action has been reported to be due to prejunctional inhibition of neuropeptide release from sensory axons by activation of histamine H<sub>3</sub> receptors in the dura mater (Ishikawa and Sperelakis, 1987; Matsubara et al., 1992). Recently, it has been demonstrated that prejunctional histamine H<sub>3</sub> receptors may regulate tachykinin release in response to nonadrenergic, noncholinergic sensory nerve excitation (Ohkubo et al., 1995). We found that Sch 50971 inhibits neurogenic dura mater protein extravasation in the guinea pig. The effects of Sch 50971 are mediated by histamine H<sub>3</sub> receptors because pretreatment with thioperamide blocks the inhibitory actions of Sch 50971 on protein leakage into the dura mater. It is proposed that activation of prejunctional H<sub>3</sub> receptors with (R)-α-methylhistamine or Sch 50971 in the current study inhibited neuropeptide release from trigeminal nerve terminals; however, this issue was not directly addressed in the present study. Nevertheless, the present study shows that Sch 50971 given by either the i.v. or oral route of administration inhibits neurogenic inflammation in an experimental model of migraine.

Sumatriptan is a 5-HT<sub>1D</sub> receptor agonist that is currently available for the treatment of migraine. Sumatriptan activates serotonin receptors on the heart and in the vasculature to produce moderate to severe cardiovascular side effects (Welsh, 1993). The cardiovascular effects of central and peripheral histamine H<sub>3</sub> receptor activation have been well characterized (Malinowska and Schlicker, 1991, 1993; Hey et al., 1992; McLeod et al., 1991, 1993, 1996; Coruzzi et al., 1995). Activation of peripheral histamine H<sub>3</sub> receptors with (R)-α-methylhistamine produces a decrease in total peripheral resistance (McLeod et al., 1993). These findings indicate that activation of prejunctional histamine H<sub>3</sub> receptors maybe potential antimigraine agents without the vasocostriction liability of 5-HT<sub>1D</sub> receptor agonists. Further studies are needed to address these issues, especially those determining the actions of histamine H<sub>3</sub> receptor activation on coronary and cerebral blood flow.
It is well established that the classical antihistamines induce sedation in man (Roth et al., 1987) by blockade of CNS H1 receptors (Levander et al., 1985). Conversely, studies in animals demonstrate that H1 receptor activation in the CNS produces an increase in arousal and a concomitant decrease in EEG sleep patterns (Wolf and Monnier, 1973; Monti et al., 1991). Activation of the CNS H3 receptors produces locomotor hypoactivity in rats and mice (Bristow and Bennet, 1993; Sakai et al., 1991) and an increase in slow wave sleep EEG activity and total sleep time in cats (Lin et al., 1990). These actions are blocked by the histamine H3 antagonist thioperamide. Interestingly, thioperamide given alone causes arousal effects that are blocked not only by an H3 agonist, but also they are reversed by a sedating H1 antagonist. These studies show that sleep/wakefulness is intimately controlled by a balance of H3 and H1 receptor activation in the CNS, with H3 receptor activation promoting sleep.

Based on the evidence that CNS H3 mechanisms may be important in the regulation of sleep/arousal, we studied the potential for Sch 50971 to exhibit sedative properties. We selected the guinea pig to perform the EEG/sleep studies due to the known sensitivity of this species to histamine and histaminergic mechanisms that closely resemble those in humans (Levi et al., 1982). The effects of Sch 50971 were compared to the effects of diazepam and triazolam, standard benzodiazepine sedatives, and diphenhydramine, a sedating H1 antihistamine in man. Sch 50971 penetrated the CNS and potentiated the loss of righting reflex induced by pentobarbital, in a dose-related manner by activation of central histamine H3 receptors. In contrast i.c.v. thioperamide did not block the effects in this model of the benzodiazepine sedative, diazepam. Sch 50971 was short acting (between 2 and 4 hr) and, in a subacute tolerance study, no tolerance developed to the sedative effect of Sch 50971. Although triazolam was ~14-fold more potent than Sch 50971, tolerance developed to its sedative effects.

The sleep promoting action and EEG effects of Sch 50971 and triazolam were also compared. The EEG spectral activity
bands were divided into delta waves (0–4 Hz; associated with slow wave deep sleep), theta waves (4–8 Hz; associated with REM sleep), alpha waves (8–14 Hz; associated with light sleep) and beta waves (14–30 Hz). In these studies, Sch 50971 effects on EEG spectral activity were dramatically different from the effects observed with triazolam, and were more consistent with physiological sleep patterns. Sch 50971 produce a small but nonsignificant increase in relative delta

Fig. 5. Effect of oral Sch 50971 (■, n = 9) and triazolam (□, n = 10) on spontaneous locomotor activity and on total distance moved in conscious free roaming guinea pigs over a 6-hr period. Control animals (□, n = 35) received methylcellulose. Values represent mean ± S.E.M. *Statistical difference compared with control group was *P < .05.

Fig. 6. Effect of oral Sch 50971 (■, n = 9) and triazolam (□, n = 10) on total sleep time in guinea pigs over a 6-hr period. Control animals (□, n = 35) received methylcellulose. Values represent mean ± S.E.M. *Statistical difference compared with control group was *P < .05.
wave activity during the first 4 hr without affecting relative theta wave or alpha wave activity. Sch 50971 also produced a decrease in relative beta wave activity. Consistent with these effects on EEG patterns, Monti et al. (1996) showed that the H₃ agonist BP 2.94 produced a similar profile in rats characterized by an increase in delta slow wave sleep after oral dosing. In contrast to Sch 50971, triazolam produced significant disruptions in EEG patterns characterized by increases in alpha and beta wave activity, along with concomitant decreases in delta and theta wave activity. In these studies, both Sch 50971 (10 mg/kg) and triazolam (1 mg/kg) significantly increased sleep time over the 6-hr study period. It is important to note that at doses of Sch 50971 and triazolam that produced similar decreases of locomotor activity and total distance moved, Sch 50971 increased sleep time while maintaining a physiological EEG profile. In contrast, triazolam produced significant disruption of EEG patterns, indicating that the sleep was not physiological in nature. No triazolam produced significant disruption of EEG patterns, while maintaining a physiological EEG profile. In contrast, and total distance moved, Sch 50971 increased sleep time significantly over the 6-hr study period. In these studies, both Sch 50971 (10 mg/kg) and triazolam (1 mg/kg) significantly increased sleep time over the 6-hr study period. It should be noted that at doses of Sch 50971 and triazolam that produced similar decreases of locomotor activity and total distance moved, Sch 50971 increased sleep time while maintaining a physiological EEG profile. In contrast, triazolam produced significant disruption of EEG patterns, indicating that the sleep was not physiological in nature. No definitive EEG profile indicative of sedation was observed with diphenhydramine at doses of 30 mg/kg p.o.

The present findings also suggest that there is a difference between the relative rank order potency of Sch 50971 and (R)-α-methylhistamine in the migraine model vs. the sleep induction study. It should be noted that a potency comparison between Sch 50971 and (R)-α-methylhistamine in these models is complicated by the fact that in the drugs were given by two different routes (i.e. in the migraine model and p.o. in the sleep studies). Pharmacokinetic differences could also explain activity differences between Sch 50971 and (R)-α-methylhistamine. Specifically, the experimental migraine model has been proposed to involve a perivascular action and activity may not necessitate a drug crossing the blood brain barrier. In contrast, for sedation to occur it is most likely that CNS penetration is necessary. Thus, it is possible that Sch 50971 displays greater potency in the sleep studies because of its greater relative ability to penetrate the blood brain barrier faster.

In summary, Sch 50971 is an orally active, potent and selective agonist of histamine H₃ receptors. Sch 50971 may act to ameliorate the sequelae of migraine and related vascular headaches, where activation of histamine H₃ receptors are potentially beneficial. Furthermore, Sch 50971 decreases activity and promotes EEG activity consistent with physiologic sleep. Sch 50971 is well tolerated and is devoid of the side effects liability such as tolerance and respiratory depressant activity associated with some sedating drugs.

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References


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