

Analgesic Effects of Sazetidine-A, a New Nicotinic Cholinergic Drug

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Background: **The use of nicotinic agonists for analgesia is limited by their unacceptable side effects. *Sazetidine-A* is a new partial agonist nicotinic ligand that has very high selectivity for $\beta 2$-containing nicotinic acetylcholine receptors. It potently and selectively desensitizes $\alpha 4\beta 2$ nicotinic acetylcholine receptors without measurable effects on $\alpha 3\beta 4$ receptors. The authors investigated the analgesic effects of Sazetidine-A using the formalin model of chronic inflammatory pain.**

Methods: **The formalin test was conducted after rats received intraperitoneal saline, Sazetidine-A (0.125, 0.25, 0.5, 1, 2 mg/kg), or subcutaneous epibatidine (2.5–5–10 μg/kg). In other experiments, Sazetidine-A was preceded by naloxone (0.5 mg/kg) or mecamylamine (10 mg). Effects of Sazetidine-A and epibatidine on locomotor were tested in an open field, and seizure activity was measured using the Racine scale. Locus coeruleus neuron extracellular single-unit spontaneous discharge was recorded in anesthetized animals after Sazetidine-A and epibatidine.**

Results: **Higher doses of Sazetidine-A (0.5, 1, or 2 mg/kg) induced analgesia, with pain scores significantly lower than those seen after saline, lower doses of Sazetidine-A, and epibatidine ($P < 0.001$). Naloxone did not antagonize the effects of Sazetidine-A, and mecamylamine had partial, dose-dependent antagonistic effects. Epibatidine excited locus coeruleus neurons, whereas Sazetidine-A had no effect on these neurons. Epibatidine and Sazetidine-A affected animals' locomotor activity for the initial 20 min. While analgesic doses of epibatidine caused seizures, no seizure activity or other neurologic complications were seen in animals that received as much as four times the minimum analgesic dose of Sazetidine-A.**

Conclusions: **Sazetidine-A seems to be a potent analgesic without causing neurologic side effects.**

THE analgesic properties of nicotine have been suspected for more than 75 yr and were well documented by the 1980s.[1–4] The discovery of epibatidine, a potent nicotinic acetylcholine receptor (nAChR) agonist[5–7] approximately 200 times greater than morphine, rekindled interest in nAChRs as targets for analgesics. Since then, other nicotinic ligands have been found to possess analgesic properties, and several have advanced to clinical trials.[8,9] However, an unacceptable level of side effects has limited their further development.

The $\alpha 4\beta 2$ nAChR subtype is the most common heteromeric nAChR in rat brain,[10,11] and the presence of these receptors in pain pathways suggests that they may be involved in the analgesic effects of nicotinic ligands.[1,12–15] Studies with knock-out[16] and knock-in mice[14] strongly support this possibility. Thus, an effective and useful nicotinic analgesic agent might be expected to act preferentially at $\alpha 4\beta 2$ nAChRs and have low affinity for receptor subtypes that are associated with side effects mediated by autonomic ganglia, such as the $\alpha 3\beta 4$ subtype. Consistent with this, epibatidine cannot be used clinically because of severe neurologic and hemodynamic side effects,[17] probably because it has high affinity for virtually all heteromeric nAChR subtypes, including those in ganglia and brain stem autonomic centers.

Sazetidine-A is a new nicotinic ligand with very high affinity and selectivity for $\beta 2$-containing nAChRs[18] in equilibrium binding assays. In previous studies, Sazetidine-A did not seem to act as either an agonist or a competitive antagonist in assays that measured $^{86}Rb^+$ efflux through the nAChR channel, but it did markedly and selectively desensitize $\alpha 4\beta 2$ nAChRs in a time-dependent manner.[18] Since that report, however, others have found that Sazetidine-A does activate rat $\alpha 4\beta 2$ nAChRs, possibly depending on the stoichiometry of the subunits.[19] Moreover, using patch clamp methods, we recently found that Sazetidine-A does elicit whole cell currents in transfected cells expressing $\alpha 4\beta 2$ nAChRs but not in $\alpha 3\beta 4$ nAChRs (Yingxian Xiao, Ph.D., Associate Professor; Robert Yasuda, Ph.D., Assistant Professor; Niaz Sahibzada, Ph.D., Associate Professor; Barry Wolfe, Ph.D., Professor; and Kenneth J. Kellar, Ph.D., Professor; Department of Pharmacology, Georgetown University School of Medicine, Washington, D.C.; unpublished data, May 2008). Those studies thus showed that Sazetidine-A is a highly selective partial agonist at $\alpha 4\beta 2$ nAChRs.

Based on its *in vitro* pharmacologic profile, we hypothesized that Sazetidine-A and drugs with similar potent and selective desensitizing actions at $\alpha 4\beta 2$ nAChRs would produce some effects *in vivo* similar to those of nicotine and other potent nicotinic agonists, but with potentially fewer undesirable side effects.[18] In this study, we investigated the analgesic effects of Sazetidine-A using the formalin model of persistent pain in the rat.

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Materials and Methods

Male Sprague-Dawley rats (250–300 g) were housed in pairs under a 12:12-h light/dark cycle with water and food available *ad libitum*. The protocols were in accordance with the animal care guidelines at the University of Pennsylvania and The Children's Hospital of Philadelphia (Philadelphia, Pennsylvania) and followed the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Sazetidine-A was synthesized by Alan P. Kozikowski, Ph.D. (Professor, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois), Sheela K. Chellappan, Ph.D., and Krishna Mohan Bajjuri, Ph.D. (Postdoctoral Fellows, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago), by previously described methods.[18] Epibatidine and mecamylamine were purchased from Sigma-Aldridge (St. Louis, MO).

Formalin Test

To habituate animals to the formalin test environment, rats were placed singly in the test chamber for 10–15 min on each of 3 days. The testing room was maintained at 22°C, under normal lighting conditions. The formalin test was conducted in a 60 × 30 × 40-cm clear glass chamber with a mirror under the floor to allow a complete view of the animal and paws. To score, the behavior of each rat was rated for 60 min after the injection of formalin into a rear paw. Rats were scored every 20 s for pain response based on four mutually exclusive categories of behavior.[20] These consisted of the following: (1) normal behavior (equal weight bearing on both hind paws), (2) favoring (injected paw resting on the floor without pressure on the foot pad), (3) lifting (injected paw elevated without touching the floor), and (4) licking (injected paw licked or bitten).[21,22] The observer who evaluated the rats' behavior was not blinded to the type of drug infused or concentration used.

Locomotor Activity

Locomotor activity was measured in a 60-min session. Animals were placed in 70 × 70-cm black Plexiglas opaque platform with exterior walls 30 cm high. Immediately after the injection of the test drug(s), rats were placed in a corner of the open field, and behavior was videotaped for 1 h for later analysis with the Ethovision (Noldus, VA) video tracking system. The total distanced traveled inside the chamber was analyzed over a period of an hour using 10-min bins.

Seizure Activity

Seizure activity was measured in control as well as in animals that received epibatidine and Sazetidine-A. We used the standard Racine five-stage scale: stage 1, facial movements; stage 2, rhythmic head movements and head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling and clonic convulsion.[23]

Recording from Locus Coeruleus Neurons

The firing rate of locus coeruleus (LC) neurons was measured by procedures similar to those described in Curtis *et al.*[24] Rats were anesthetized with 2% halothane-in-air mixture administered through a nose cone. The anesthetic was maintained at 1.0–1.5% throughout the experiment, and body temperature was maintained at 37.5° by a feedback-controlled heating pad. Rats were positioned in a stereotaxic frame with the head oriented at an 11° angle to the horizontal plane (nose down). The skull was exposed, and a hole, centered at 1.2 mm lateral to the midline and 2.8–3.7 mm caudal to lambda, was drilled over the cerebellum to reach the LC. The dura over the cerebellum was carefully removed with fine iridectomy scissors and tweezers to facilitate introduction of the recording micropipette. Single-barreled glass micropipettes (2- to 4-μm-diameter tip, 4–7 MΩ) filled with 0.5 M sodium acetate buffer saturated with Pontamine sky blue dye were used to record single-unit LC discharge. Neuronal signals were amplified and filtered. Impulse activity was monitored with an oscilloscope and loudspeaker to aid in finding the LC. LC neurons were tentatively identified during recording by their spontaneous discharge rates (0.5–5 Hz), entirely positive, notched waveforms (2–3 ms duration), and biphasic excitation–inhibition responses to tail pinch. When stable, unitary action potentials were isolated, a window discriminator was used to convert the occurrence of a single action potential into a digital pulse, which was led into a Windows-based computer *via* a CED 1401 interface using Spike 2 software for on-line visualization and storage and off-line analysis (Cambridge Electronic Design, Cambridge, United Kingdom).

Extracellular single-unit LC spontaneous discharge was recorded until it became stable (3–5 min). Study drugs were then injected. At the end of the experiment, the site of recording was labeled by iontophoresis (−15 μA, 25 min) of Pontamine sky blue, and the rats were killed with halothane overdose. Brains were removed and frozen, after which 30-μm-thick coronal sections were cut on a cryostat, mounted on glass slides, and stained with neutral red for localization of the Pontamine sky blue mark. Data presented are from neurons that were histologically identified, under the microscope, as being inside the LC.

Experimental Design Formalin Test

1. Formalin test. Eight groups of rats received intraperitoneal saline (control group, n = 7); 0.125, 0.25, 0.5,

1, or 2 mg/kg Sazetidine-A (n = 7 per group); or 2.5 and 5 μg/kg subcutaneous epibatidine (n = 9). Five minutes later, 5% formalin (50 μl) was injected subcutaneously into the plantar surface of one rear paw.
2. Antagonists experiments. Six additional group of rats received intraperitoneal mecamylamine followed by saline, naloxone, or mecamylamine 5 min before the intraperitoneal administration of Sazetidine-A. In the naloxone group, rats received 0.5 mg/kg naloxone followed by either 0.5 (n = 5) or 2 mg/kg (n = 5) Sazetidine-A (the lowest and highest tested analgesic doses, respectively). In the mecamylamine group, animals received 10 mg/kg mecamylamine alone (n = 5) or 10 mg/kg mecamylamine followed by 0.5 (n = 6) or 2 mg/kg (n = 6) Sazetidine-A. Five minutes after the administration of the drugs, 5% formalin (50 μl) was injected subcutaneously into the plantar surface of one rear paw.
3. Locomotor activity. Four different groups of animals received intraperitoneal saline (control group, n = 8), 2.5 μg/kg epibatidine (n = 8), or 0.5–2 mg/kg Sazetidine-A (n = 8 per group). We chose a dose of 2.5 μg/kg epibatidine because higher doses have been shown to cause significant neurologic impairments that could affect the data interpretation.
4. Seizure activity. Three groups of animals received intraperitoneal Sazetidine-A (2 mg/kg; n = 5) or subcutaneous epibatidine (2.5–5–10 μg/kg; n = 5 per group).
5. LC neuron recording. After identification of an LC neuron, three groups of rats received a subcutaneous injection of saline (n = 7); 0.5 or 2 mg/kg intraperitoneal Sazetidine-A (n = 4 and 3, respectively); or 10 μg/kg subcutaneous epibatidine (n = 5).

Statistical Analysis

The pain behavioral data from each rat were analyzed from a composite pain score. Behavior was rated for 1 h calculating the time spent in four mutually exclusive categories of behavior. The timed behaviors were those originally described by Dubuisson and Dennis[25]: 0 = normal weight bearing on the injected paw; 1 = limping during locomotion or resting the paw lightly on the floor; 2 = elevation of the injected paw so that at most the nails touch the floor; and 3 = licking, biting, or grooming the injected paw. The scores were binned into 5-min epochs, and for each epoch a pain score was calculated by the amount of time an animal spent in each behavioral category by the category weight (1, 2, or 3), summed, and then divided by the test session period.

The pain scores were compared among groups at different time points using one-way analysis of variance for repeated measures to examine responses over time, followed by the Tukey multiple-comparison test when a difference between mean values was observed. The area under the curve was calculated as a measure of global pain score. Paired data were analyzed with a *t* test. Statistical analyses were performed with Stata (StataCorp LP, College Station, TX).

Results

Effects of Sazetidine-A in the Formalin Test

Intraplantar injection of formalin induces a biphasic pattern of pain-related behavior, with an early acute period (phase 1; 0–9 min), which corresponds to an acute pain response, a brief quiescent period, and a second phase of sustained "tonic" pain behavior (phase 2; 10–60 min), which represents a chronic inflammatory condition.[26]

As shown in figure 1A, control rats, which received an injection of saline, exhibited the typical biphasic time course. The pain behavior decreased after the initial 5-min peak (phase 1) but then increased again after approximately 10 min and reached a second peak at 25–30 min (phase 2). The two lower doses of Sazetidine-A (0.125 and 0.25 mg/kg) resulted in pain scores similar to that of the control rats. In contrast, the three higher doses of Sazetidine-A (0.5, 1, and 2 mg/kg) ($P < 0.0001$) and both doses of epibatidine (2.5 and 5 μg/kg) ($P < 0.001$) (fig. 1B) resulted in significantly lower initial pain scores in phase 1.

During phase 2 of the reaction to the formalin injection, the pain scores of the two lower doses Sazetidine-A were still similar to that of saline. But again, the three higher doses of Sazetidine-A markedly attenuated the pain scores through the remaining 50-min observation period ($P < 0.0001$) (fig. 1A). Figure 1B also shows pain scores from rats that were injected with epibatidine (2.5–5 μg/kg). Treatment with epibatidine significantly reduced the pain score compared with animals treated with saline or the two lowest doses of Sazetidine-A ($P < 0.001$). However, epibatidine did not reduce the pain score to the same degree as the three highest doses of Sazetidine-A (0.5–2 mg/kg) ($P < 0.01$).

The area under the curve was calculated as a measure of the global pain score over time and to assess the ability of drugs to lower that score. The global pain score was reduced by approximately 75% in rats that received the three highest doses of Sazetidine-A compared with that observed in control rats or rats that received the two lower doses of Sazetidine-A (* $P < 0.0002$; fig. 2). The analgesic effects of 2.5 and 5 μg/kg epibatidine were similar, each reducing the overall pain score by approximately 45% (fig. 2). Epibatidine (2.5–5 μg/kg) reduced the pain score significantly compared with saline or the two lowest doses of Sazetidine-A ($P < 0.003$), but it did not reduce the pain score to the same extent as the three higher doses of Sazetidine-A ($P < 0.006$). We did not check pain scores after 10 μg/kg epibatidine because of significant seizure activity.[27]

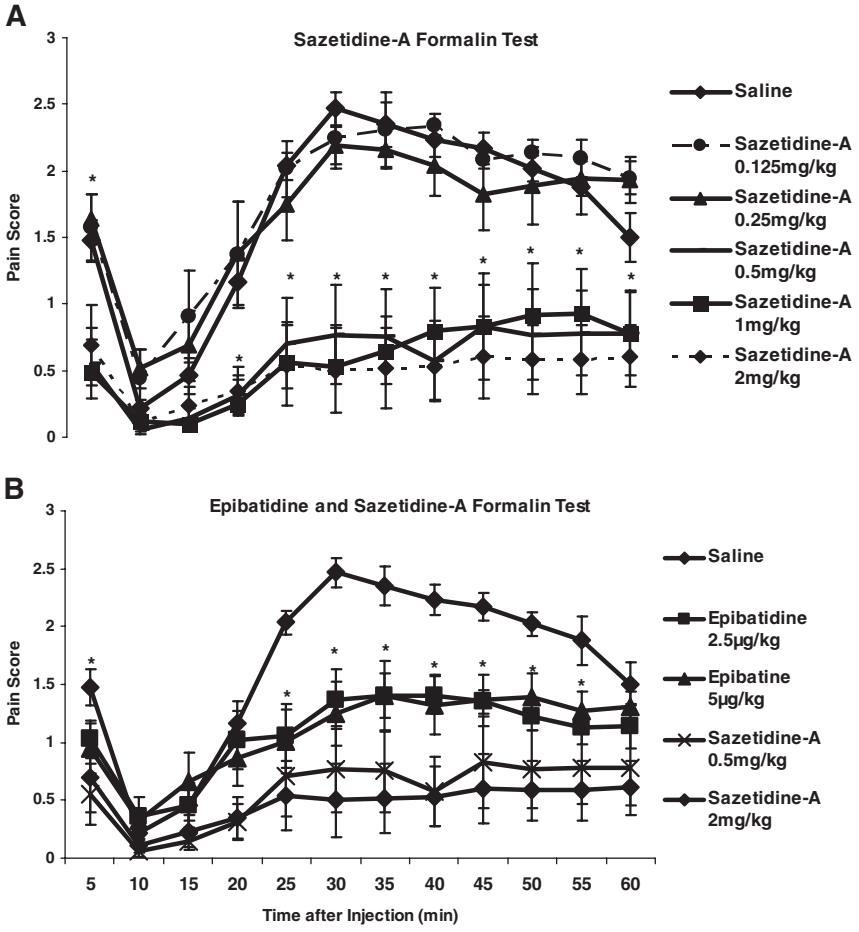

Fig. 1. (*A*) Pain behavior scores in rats that received intraperitoneal saline or Sazetidine-A (mean ± SEM). Scores after administration of the lowest doses of Sazetidine-A (0.125–0.25 mg/kg) were similar to those observed after saline. Pain scores after administration of the highest doses of Sazetidine-A (0.5–1–2 mg/kg) were significantly lower than after saline or the 0.125- to 0.25-mg/kg doses in phases 1 and 2 of the formalin pain test (* $P < 0.0001$). (*B*) Pain behavior scores in rats that received intraperitoneal saline or Sazetidine-A (mean ± SEM). Pain scores after epibatidine (2.5–5 µg/kg) were significantly lower than those after saline (* $P < 0.001$) and significantly higher than those after Sazetidine-A (0.5–2 mg/kg) (* $P < 0.01$) in phases 1 and 2 of the formalin pain test.

Effects of Antagonists on the Sazetidine-A Response

The noncompetitive nAChR antagonist mecamylamine (10 mg/kg, intraperitoneal) did not itself significantly affect the pain response to formalin, but pretreatment with it 5 min before injection of Sazetidine-A nearly completely blocked the analgesic response to 0.5 mg/kg Sazetidine-A and partially blocked the response to the 2-mg/kg dose of Sazetidine-A (fig. 3). In contrast, pretreatment with the opiate antagonist naloxone (0.5 mg/kg, intraperitoneal) did not significantly affect the analgesic response to either dose of Sazetidine-A (fig. 3).

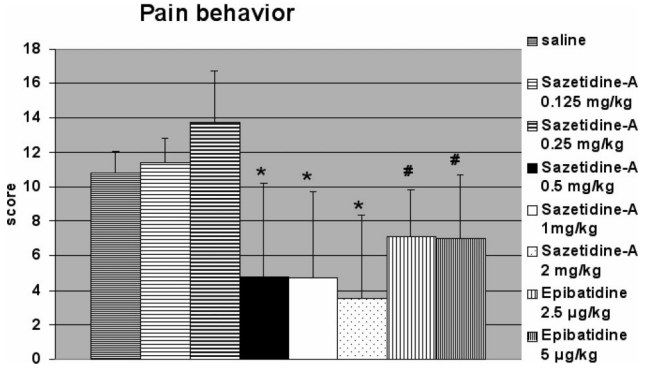

Fig. 2. Area under the curve (AUC) of pain behavior scores after administration of intraperitoneal saline, intraperitoneal Sazetidine-A (at 0.125, 0.25, 0.5, 1, and 2 mg/kg doses), or subcutaneous epibatidine (at 2.5 and 5 µg/kg doses). The AUC after the three higher doses of Sazetidine-A (at 0.5, 1, or 2 mg/kg) was significantly lower (* $P < 0.0002$) than what was observed in rats receiving saline or the two lower doses of Sazetidine-A (at 0.125 or 0.25 mg/kg). The AUC after doses of epibatidine (at 2.5 or 5 µg/kg) was significantly lower (# $P < 0.003$) than that seen after saline or the two lower doses of Sazetidine-A (at 0.125 or 0.25 mg/kg) but significantly higher (# $P < 0.006$) than observed after the three higher doses of Sazetidine-A (at 0.5, 1, or 2 mg/kg).

Open Field Activity

As shown in figure 4, locomotor activity during the first 20 min after injection was significantly decreased by analgesic doses of both epibatidine and Sazetidine-A, compared with saline ($P < 0.007$), but the locomotor activity during the following 40 min was similar in the treatment and saline groups. There were no significant differences in locomotion between the three treatment groups at any time after the administration of the drugs.

Seizure Activity

Animals that received analgesic doses of Sazetidine-A did not develop any observable seizure activity (fig. 5). Similarly, epibatidine at 2.5 µg/kg did not show seizure activity (data not shown). In contrast, higher doses of epibatidine did induce seizures. Thus, 60% of rats that received epibatidine at a dose of 5 µg/kg reached a stage

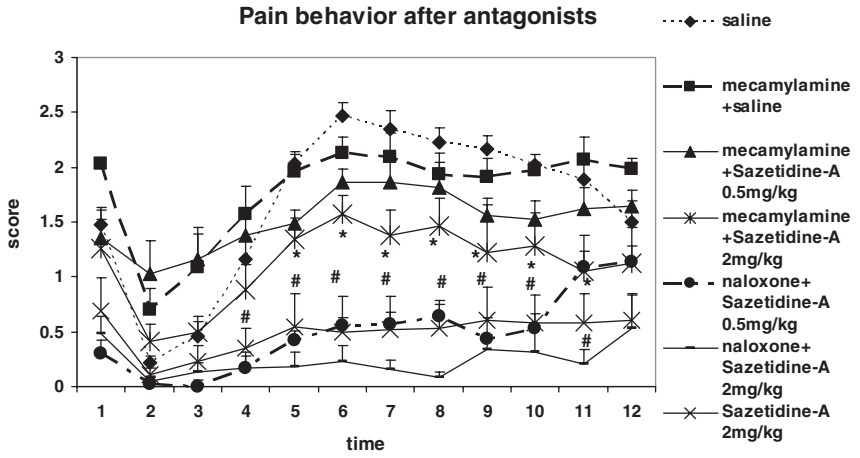

Fig. 3. Pain behavior scores in rats that received intraperitoneal saline, Sazetidine-A (2 mg/kg), mecamylamine (10 mg/kg) followed by saline, mecamylamine (10 mg/kg) followed by Sazetidine-A (0.5–2 mg/kg), naloxone (0.5 mg/kg) followed by Sazetidine-A (0.5–2 mg/kg) (mean ± SEM). Naloxone did not reverse the analgesic effects of Sazetidine-A with scores similar to those of animals that received Sazetidine-A alone and significantly lower compared with those of animals that received saline or mecamylamine and Sazetidine-A (# $P <$ 0.0002). The scores of animals that received mecamylamine and saline or mecamylamine and 0.5 mg/kg Sazetidine-A were similar to those of animals that received saline. The scores of animals that received mecamylamine followed by 2 mg/kg Sazetidine-A were significantly lower than those of animals that received saline, mecamylamine and saline, or mecamylamine and 0.5 mg/kg Sazetidine-A (* $P <$ 0.03).

1 seizure within 2 min after the administration of the drug, but seizure activity disappeared within 16 min (fig. 5). After epibatidine at a dose of 10 μg/kg, 66% of animals reached stage 5 seizure scores within 4 min (fig. 4), and 20% of these animals died. Seizure activity disappeared in the surviving animals within 26 min from the administration of epibatidine (fig. 5).

Locus Coeruleus Recording

The subcutaneous administration of Sazetidine-A (2 mg/kg) slightly reduced neuronal discharge rate of LC neurons, though this did not reach statistical significance (fig. 6 and table 1). In contrast, the administration of subcutaneous epibatidine (10 μg/kg) resulted in a significant increase in the discharge of LC neurons from 1.4 ± 0.6 to 5.3 ± 3.9 Hz (fig. 6 and table 1).

Discussion

Although the nAChR subtypes involved in the analgesic activity of nicotinic agonists are not known with certainty, there is very good evidence for the involvement of $\alpha4\beta2$ subtypes.[14] Nicotinic agonists have dual actions on nAChRs: first to activate them and then to desensitize them.[28] It is widely believed that activation of nAChRs plays the essential role in initiating analgesic effects of nicotinic agonists, but the role of receptor desensitization in sustaining these effects is unknown.

Sazetidine-A has very high affinity and selectivity for $\alpha4\beta2$ compared with $\alpha3\beta4$ nAChRs.[18] Interestingly, in measurements of receptor function with [^{86}Rb] ion efflux assays, Sazetidine-A does not display measurable agonist or antagonist activity at $\alpha4\beta2$ nAChRs, but it does potently and selectively desensitize these receptors for sustained periods of time, resulting in markedly diminished responses.[18] Similarly, in patch clamp studies, when Sazetidine-A is introduced to cells *via* the slow bath application method, it shows very little if any agonist activity at concentrations that completely desensi-

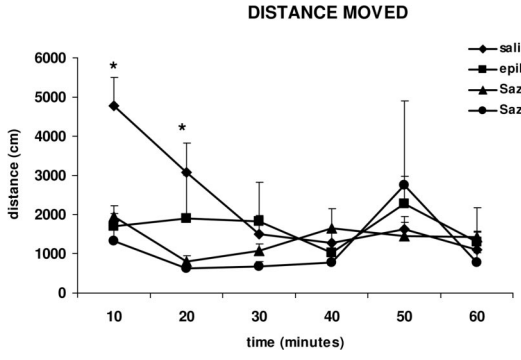

Fig. 4. Average distance moved in the whole arena expressed in centimeters after intraperitoneal administration of saline, epibatidine, and Sazetidine-A. The difference between saline and the three treatment groups was significantly different only in the first 20 min of the experiments (* $P <$ 0.007). Data are presented as mean ± SEM.

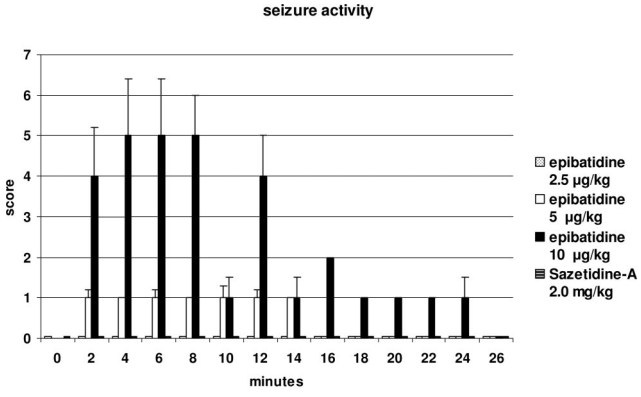

Fig. 5. Changes over time in the severity of seizure activity in animals that experienced seizures after administration of Sazetidine-A (2 mg/kg; 0%) and epibatidine (5–10 μg/kg; 60% and 66%, respectively). Seizure scores were calculated based on the Racine scale: stage 1, facial movements; stage 2, rhythmic head movements, head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling and clonic convulsion. Data are presented as mean ± SEM.

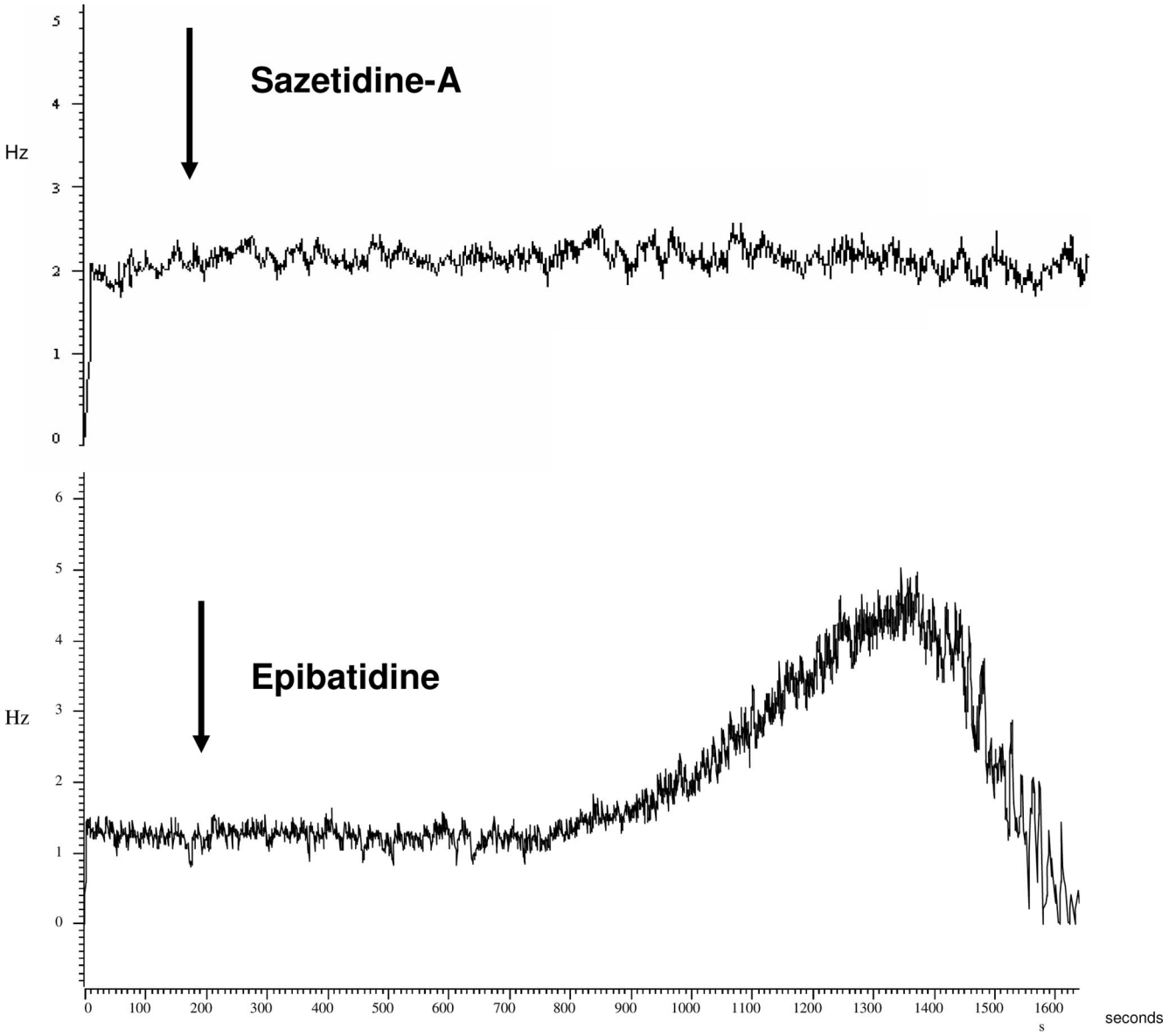

Fig. 6. Effects of Sazetidine-A (2 mg/kg) and epibatidine (10 μg/kg) on the mean frequency (Hz) of action potentials of single locus coeruleus neurons. After a baseline recording of 200 s, Sazetidine-A or epibatidine was injected subcutaneously. A slight decrease in firing rate after Sazetidine-A was observed. In contrast, a marked increase in firing rate after epibatidine was observed 200 s after injection, which peaked 1,000 s after injection.

tize $\alpha4\beta2$ receptors (Drs. Yingxian Xiao, Robert Yasuda, Niaz Sahibzada, Barry Wolfe, and Kenneth J. Kellar, Department of Pharmacology, Georgetown University School of Medicine; unpublished data, May 2008). In contrast, however, when Sazetidine-A is introduced to $\alpha4\beta2$ receptors *via* rapid application methods using Y-tubing or a picospritzer, it shows clear partial agonist activity in patch clamp studies.

Table 1. Effects of Subcutaneous Administration of Saline, Sazetidine-A (0.5 and 2 mg/kg), and Epibatidine (10 μg/kg) on LC Neuron Discharge Rate

Infusion Location and Treatment	Baseline Firing Rate, Hz	Maximum Firing Rate Change per 100-s Block, Hz	Percent Change	*P* Value
Saline, n = 7	1.4 ± 0.7	1.4 ± 0.5	0	NS
0.5 mg/kg Sazetidine-A, n = 4	2.20 ± 0.24	1.69 ± 0.21	−23	NS
2 mg/kg Sazetidine-A, n = 3	1.96 ± 0.63	1.67 ± 0.53	−15	NS
10 μg/kg Epibatidine, n = 5	1.4 ± 0.6	5.3 ± 3.9	+278	< 0.001

Data are presented as mean ± SD.

LC = locus coeruleus; NS = not significant.

In the current studies, Sazetidine-A produced marked and sustained analgesia in the formalin test, a well-established model of persistent chemical pain. Because the $\alpha4\beta2$ nAChRs in the central nervous system would probably be desensitized for all but a few seconds after systemically administered Sazetidine-A, the observation of sustained analgesia raises the interesting possibility that desensitization of $\alpha4\beta2$ nAChRs plays the crucial role in the analgesic effects of nicotinic drugs.

The analgesic effects of Sazetidine-A are attenuated by pretreatment with mecamylamine, a broad spectrum nAChR noncompetitive antagonist. This suggests that even the partial agonist activity of Sazetidine-A is important to its analgesic activity; indeed, it may be required to initiate the desensitization of the receptors. In contrast, pretreatment with naloxone, a μ-opioid competitive antagonist, did not inhibit the analgesic effects of Sazetidine-A, suggesting that antinociceptive effects of Sazetidine-A do not require activation of μ receptors.

Sazetidine-A produced a higher level of analgesia than epibatidine, as indicated by significantly lower pain scores after effective doses of Sazetidine-A compared with the highest tested dose of epibatidine used here that did not result in overt seizure activity. In fact, Sazetidine-A did not cause obvious seizure activity at doses at least four times higher than its effective analgesic dose. This is in marked contrast to the seizures observed after epibatidine in this study and to what has been reported previously.[27,29] The difference in the side effect profile between Sazetidine-A and previously tested nicotinic agonists may be related to its very high selectivity for $\alpha4\beta2$ receptors compared with $\alpha3\beta4$ receptors, which are thought to mediate several important side effects of nicotinic agonists.[9]

Both Sazetidine-A and epibatidine reduced spontaneous locomotor activity of rats during the first 20 min after injection. The mechanism related to these decreased locomotor effects after Sazetidine-A is unclear, although it resembles other nicotinic agonists in this respect, and a brief sedative-like effect similar to that of opioids cannot be excluded.

Early research on the mechanisms mediating the analgesic effects of nicotinic agonists suggested a potentially important role of midbrain nuclei.[30] In particular, the monoaminergic nucleus raphe magnus, dorsal raphe, LC, and A5 and A7 nuclear groups seem to be important components of the antinociceptive systems originating from midbrain nuclei.[31,32] Stimulation of these nuclei results in activation of neurons that project to the spinal cord and may inhibit pain pathways at the level of the dorsal horn. However, the involvement of these midbrain nuclei in the marked analgesia produced by Sazetidine-A is unclear. For example, both epibatidine and ABT-594, a more selective $\alpha4\beta2$ nAChR agonist, have been shown to, at least initially, activate midbrain nuclei located in descending pathways associated with analgesia,[21,22,33] and a similar effect of systemically administered epibatidine on LC neurons was seen in the current study. In contrast, the systemic administration of Sazetidine-A did not excite these neurons and may have actually caused a modest inhibition of their firing rate, though this did not reach statistical significance.

After systemic administration, drugs enter the central nervous system in a manner that is probably much more similar to slow bath application than to rapid application *via* Y-tubing or picospritzer. Under these conditions, Sazetidine-A potently and selectively induces sustained desensitization of $\alpha4\beta2$ nAChRs.[18] Therefore, the sustained analgesic effects of Sazetidine-A, and indeed possibly of all nicotinic agonists, are likely due much more to desensitization of $\alpha4\beta2$ nAChRs than to their activation.

In conclusion, this study shows that Sazetidine-A is a potent and efficacious analgesic in a rat model of formalin-induced pain. Sazetidine-A is highly selective for $\beta2$-containing nAChRs and, in these early *in vivo* studies, seems to have a much better analgesic and side effect profile compared with epibatidine.

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