Biochemical Pharmacology 82 (2011) 967–976

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Potentiation of analgesic efficacy but not side effects: Co-administration of an α4β2 neuronal nicotinic acetylcholine receptor agonist and its positive allosteric modulator in experimental models of pain in rats

Chang Z. Zhu *, Chih-liang Chin, Nathan R. Rustay, Chengmin Zhong, Joe Mikusa, Prasant Chandran, Anita Salyers, Erica Gomez, Gricelda Simler, La Geisha Lewis, Donna Gauvin, Scott Baker, Madhavi Pai, Ann Tovcimak, Jordan Brown, Victoria Komater, Gerard B. Fox, Michael W. Decker, Peer B. Jacobson, Murali Gopalakrishnan, Chih-Hung Lee, Prisca Honore

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500, USA

ARTICLE INFO

Article history:
Received 30 March 2011
Accepted 10 May 2011
Available online 17 May 2011

Keywords:
Positive modulator
Analgesic profile
nAChR

ABSTRACT

Positive modulation of the neuronal nicotinic acetylcholine receptor (nAChR) α4β2 subtype by selective positive allosteric modulator NS-9283 has shown to potentiate the nAChR agonist ABT-594-induced anti-allodynic activity in preclinical neuropathic pain. To determine whether this benefit can be extended beyond neuropathic pain, the present study examined the analgesic activity and adverse effect profile of co-administered NS-9283 and ABT-594 in a variety of preclinical models in rats. The effect of the combined therapy on drug-induced brain activities was also determined using pharmacological magnetic resonance imaging. In carrageenan-induced thermal hyperalgesia, co-administration of NS-9283 (3.5 μmol/kg, i.p.) induced a 6-fold leftward shift of the dose–response of ABT-594 (ED_{50} = 26 vs. 160 nmol/kg, i.p.). In the paw skin incision model of post-operative pain, co-administration of NS-9283 similarly induced a 6-fold leftward shift of ABT-594 (ED_{50} = 26 vs. 153 nmol/kg). In monoiodo-acetate induced knee joint pain, co-administration of NS-9283 enhanced the potency of ABT-594 by 5-fold (ED_{50} = 1.0 vs. 4.6 nmol/kg). In pharmacological MRI, co-administration of NS-9283 was shown to lead to a leftward shift of ABT-594 dose–response for cortical activation. ABT-594 induced CNS-related adverse effects were not exacerbated in presence of an efficacious dose of NS-9283 (3.5 μmol/kg). Acute challenge of NS-9283 produced no cross sensitization in nicotine-conditioned animals. These results demonstrate that selective positive allosteric modulation at the α4β2 nAChR potentiates nAChR agonist-induced analgesic activity across neuropathic and nociceptive preclinical pain models without potentiating ABT-594-mediated adverse effects, suggesting that selective positive modulation of α4β2 nAChR by PAM may represent a novel analgesic approach.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels activated by the neurotransmitter acetylcholine. Multiple neuronal nAChR subunits (α2–α10 and β2–β4) are differentially expressed in neuronal tissues and combined to form diverse nAChR subtypes, characterized by a wide range of physiological and pharmacological profiles with distinct central nervous system (CNS) or ganglionic distribution [1,2]. The α4β2 nAChR, abundant in the CNS with high affinity for nicotine and cytisine but low affinity for α-bungarotoxin, has been demonstrated to play an important role in neuronal nociceptive transmission [3–5]. The α3β4 nAChR, which is expressed primarily in ganglia and also displays a high affinity for nicotine and cytisine, is generally regarded as responsible for many nAChR agonist-induced adverse effects [1].

We have demonstrated that the nAChR agonist ABT-594 produces a broad spectrum analgesia in diverse pain states including multiple forms of acute, chronic, inflammatory and neuropathic pain in rodents [1,3,6,7]. Compelling evidence collectively demonstrate that the antinociceptive effect of nAChR agonists are mediated via activation of α4β2 nAChR at multiple locations throughout the pain pathways [1,3–5,8,9]. More importantly, significant pain relief was also observed in humans, providing clinical validation for the α4β2 nAChR agonist as a novel mechanism for the treatment of pain [10]. However, these

* Corresponding author at: Neuroscience Research, Abbott Laboratories, R4N5, AP9A LL, 100 Abbott Park Road, Abbott Park, IL 60064, USA. Tel.: +1 847 938 0331; fax: +1 847 938 0072.
E-mail address: Chang.Z.Zhu@abbott.com (C.Z. Zhu).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.05.007

antinociceptive effects are accompanied by adverse effects such as nausea and vomiting, possibly mediated via the interactions with distinct ganglionic α3β4 nAChR, at or near the doses required for antinociceptive effect seen in the clinical trial [10]. These dose-limiting effects in humans have precluded the development of ABT-594 as an analgesic agent [10,11]. Therefore, one strategy would be to maximize the nAChR agonist effect at α4β2 nAChR, and/or avoid its activation of other nAChR subtypes, i.e., α3β4 [10].

Positive allosteric modulators (PAMs) are compounds that do not interact with the agonist binding sites or possess intrinsic activity at the receptor per se, but potentiate the effect of the agonist [12–14]. We have previously reported that NS-9283, a PAM for α4β2 nAChR, selectively modulates the α4β2 nAChR, but not the α3β4 nAChR in an *in vitro* assay [see Lee et al., accompanying article]. Moreover, in the rat spinal nerve ligation (SNL) model of neuropathic pain, co-administration of NS-9283 produces a leftward shift in the dose–response curve (DRC) of ABT-594, such that previously non-efficacious doses are now efficacious at reversing the mechanical allodynia observed in this model. In contrast, co-administration of NS-9283 produces no changes in ABT-594-induced emesis in ferret or exaggeration in ABT-594-induced cardiovascular effects in anesthetized dog, demonstrating an increased therapeutic index for the combination of NS-9283 with ABT-594 vs. ABT-594 alone [see Lee et al., accompanying article].

To investigate whether this leftward shift is specific to neuropathic pain, the present study was designed to examine the analgesic and CNS-related adverse effects of co-administered of NS-9283 and ABT-594 in a variety of preclinical models in rats. The effects of the combined therapy on drug-induced brain activities were also determined using pharmacological magnetic resonance imaging (MRI). Additionally, since nicotine addiction might be a concern for the clinical application of any nAChR agonists or modulators as analgesics [15], a locomotor sensitization assay was also used to explore the potential cross sensitization following the acute challenge of PAM to nicotine-conditioned animals.

2. Materials and methods

2.1. Animals and compounds

Adult male Sprague Dawley rats (200–250 g, Charles River Laboratories, Wilmington, MA, USA) were housed five per cage. Animals were in quarantine for 5–7 days before entering the study. All animals were kept in a temperature-regulated environment under a controlled 12-h light–dark cycle with lights on at 6:00 AM. Food and water were provided *ad libitum*. All procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facility and approved by the Institutional Animal Care and Use Committee (IACUC) at Abbott Laboratories.

NS-9283 [3-(3-(pyridine-3-yl)-1,2,4-oxadiazol-5-yl)benzonitrile] and ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine] were synthesized at Abbott Laboratories as previously described [see Lee et al., accompanying article] (molecular weight of 285, 370.08, respectively). Hydroxypropyl-β-cyclodextrin (HBC) and nicotine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). NS-9283, ABT-594, and the combination of NS-9283 with ABT-594 were dissolved in a solution of HBC/physiological saline (30:70, v/v) and were intraperitoneally (i.p.) administered in a final injection volume of 4.0 ml/kg, in all behavioral experiments except for the locomotor sensitization assay. In the locomotor sensitization assay, compounds were administered in a volume of 1.0 ml/kg (i.p.). In pharmacological MRI experiments, ABT-594 alone was intravenously (i.v.) administered in a volume of 1.0 ml/kg of physiological saline, NS-9283 alone was orally (p.o.) administered in a volume of 2.0 ml/kg in a solution of HBC/physiological saline (30:70, v/v).

2.2. In vivo pain models

2.2.1. Carrageenan model

Acute inflammatory thermal hyperalgesia was induced by injecting 100 μl of a 1% solution of λ-carrageenan (Sigma Chemical Co., St. Louis, MO, USA) in physiological saline into the plantar surface of the right hind paw. Thermal hyperalgesia measured as paw withdrawal latency (PWL) was determined using a commercially available thermal paw stimulator (UARDG, University of California, San Diego, CA, USA) as described by Hargreaves et al. [16]. Rats were placed into individual plastic cubicles mounted on a glass surface maintained at 30 °C, where a thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, was then applied to the plantar surface of each hind paw. The stimulus current was maintained at 4.50 ± 0.05 A, and the maximum time of exposure was set at 20.48 s to prevent possible tissue damage. The elapsed time until a brisk withdrawal of the hind paw from the thermal stimulus was recorded automatically using photodiode motion sensors. The right (carrageenan injected paw) and left hind paw of each rat was tested in three sequential trials at approximately 5 min intervals. Carrageenan-induced thermal hyperalgesia of paw withdrawal latency (PWL_{carra}) was calculated as the mean of the two shortest latencies. PWL was assessed 30 min following i.p. compound administration (2 h after carrageenan injection). PWL_{carra} of the vehicle control group was taken as 0%, whereas the PWL of the contralateral side (PWL_{contra}) of the vehicle control group was taken as 100%. The analgesic effect was expressed as the percentage return of PWL_{carra} to PWL_{contra}.

2.2.2. Skin incision model

Paw skin incision surgery was performed under isoflurane (2–3%) anesthesia, and followed procedures previously described [17]. Briefly, the plantar aspect of the left hindpaw was placed through a hole in a sterile plastic drape. A 1-cm longitudinal incision was made through the skin and fascia, starting 0.5 cm from the proximal edge of the heel and extending towards the toes; the plantar muscle was elevated and injured longitudinally leaving the muscle origin and insertion points intact. After homeostasis with gentle pressure, the skin was apposed with two mattress sutures of 5-0 nylon. Mechanical allodynia was measured using calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) as previously described [18] in all animals 2 h following surgery. Rats were placed into inverted individual plastic containers (20 cm × 12.5 cm × 20 cm) on top of a suspended wire mesh grid, and acclimated to the test chambers for 20 min. The von Frey filaments were presented perpendicularly to the plantar surface pointing towards the medial side of the incision [17], and then held in this position for approximately 8 s with enough force to cause a slight bend in the filament. Positive responses included an abrupt withdrawal of the hind paw from the stimulus, or flinching behavior immediately following removal of the stimulus. A 50% paw withdrawal threshold (PWT) was determined using an up–down procedure [19]. Mechanical allodynia was assessed 30 min following i.p. compound administration.

2.2.3. Monoiodoacetate-induced osteoarthritis (MIA-OA)

Under light isoflurane (2–3%) anesthesia, MIA (3 mg) was intra-articularly injected unilaterally into right hind knee joint in a volume of 50 μl as previously described [20]. Compressive grip force (CGF) was determined 20 days following MIA injection by recording the maximum compressive force exerted on the hind limb strain gauge setup, using a commercially available grip force

Table 1
Summary of NS-9283 alone in nociceptive and inflammatory models of pain, and in CNS-related side effect assays.

Test	Route of administration	Doses (μmol/kg)	Findings
Carrageenan hot box	i.p.	3, 10, 30	N.S.
Skin incision	i.p.	3, 10, 30	N.S.
MIA-OA	i.p.	1, 3, 10	50% effect by 10 μmol/kg
Locomotor	i.p.	3.0, 13, 35	N.S.
Rotarod	i.p.	3, 10, 30	N.S.
Body temperature	i.p.	3, 10, 30	N.S.
Edge performance	i.p.	3, 10, 30	N.S.

N.S., no significant effect.

measurement system (Columbus Instruments, Columbus, OH, USA). In this assay, each rat was gently restrained and the hind paw was allowed to grasp the wire mesh frame (10 cm × 12 cm) attached to the strain gauge. The animal was then moved in a rostral-to-caudal direction until the grip was broken, and CGF reading expressed as g was recorded. Each rat was sequentially tested twice at approximately 2–3 min intervals to obtain an average raw CGF. To account for the impact of body weight on grip force, CGF was converted into CGF_{body} following the formula: g (CGF)/kg (body weight). CGF was assessed 30 min following i.p. compound administration. The vehicle control group was taken as 0%, whereas an age-matched naïve group was designated as 100% (normal). The analgesic effect was expressed as the percentage return of CGF_{body} to normal (naïve group): [(treatment − vehicle)/(naïve − vehicle)] × 100%. Experiments were performed in animals 20 days following MIA knee joint injection.

2.3. Measurement of CNS effects

2.3.1. Edge-balance test and body temperature

Using an edge-balance assay developed in our laboratory [6], rats were acclimated to the test room for 30 min, and were then placed on the edge (0.25 in. wide) of a rectangular Plexiglas box (40 cm × 40 cm × 38 cm). The time to fall off the top of the vertical wall was determined and reported as fall latency, with a maximum cut-off point of 120 s. Edge-balance test was assessed 30 min following i.p. compound administration, and core body temperatures were recorded with a rectal thermometer (Physitemp Instruments, Inc., Clifton, NJ, USA) immediately upon the completion of the edge test.

2.3.2. Rotarod performance and horizontal exploratory behavior

Rotarod performance was measured using an accelerating rotarod apparatus (Omnitech Electronics, Inc., Columbus, OH, USA). Rats were placed onto a 9 cm diameter rod, which increased in speed from 0 to 20 rpm in one min. The time required for the rat to fall from the rod was automatically recorded, with a maximum cut-off of 60 s. Following three training sessions, rats were randomly assigned into treatment groups. Latency to fall from the rotarod was determined 30 min after i.p. compound administration. Horizontal exploratory behavior was measured in an open field using photobeam activity monitors (AccuScan Instruments, Columbus, OH, USA). Rats were placed into 42 cm × 42 cm × 30 cm activity chambers where photobeam

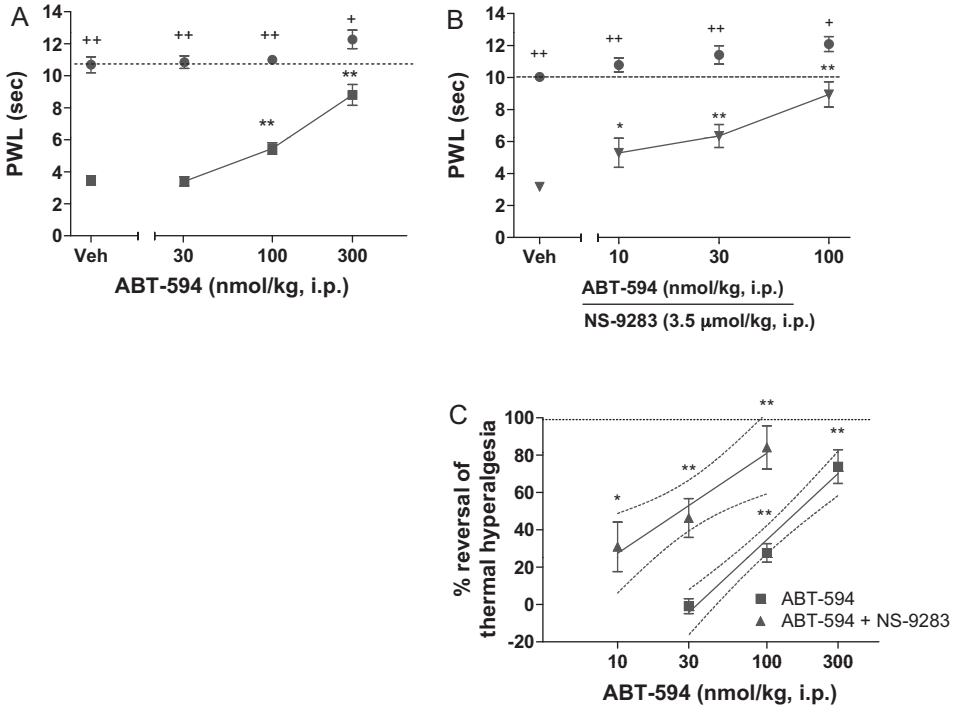

Fig. 1. Effect of ABT-594 (30–300 nmol/kg) or co-administration of NS-9283 (3.5 μmol/kg) and ABT-594 (10–100 nmol/kg) in carrageenan-induced acute inflammatory thermal hyperalgesia in rats. PWL was measured 30 min after intraperitoneal (i.p.) drug administration (2 h following carrageenan injection of the right hind paw). PWL data for ABT-594 (A), co-administration (B), and data represented as percent reversals for determination of ED_{50} (C) are shown. Data are mean ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle (Veh) treated group of PWL_{carra}, $^{+}p < 0.05$, $^{++}p < 0.01$ for the comparison between PWL_{carra} and PWL_{contra}, n = 12–18 per group. Dashed line: paw withdrawal latency of non-carrageenan injected paw for A and B, or 100% reversal of thermal hyperalgesia for C.

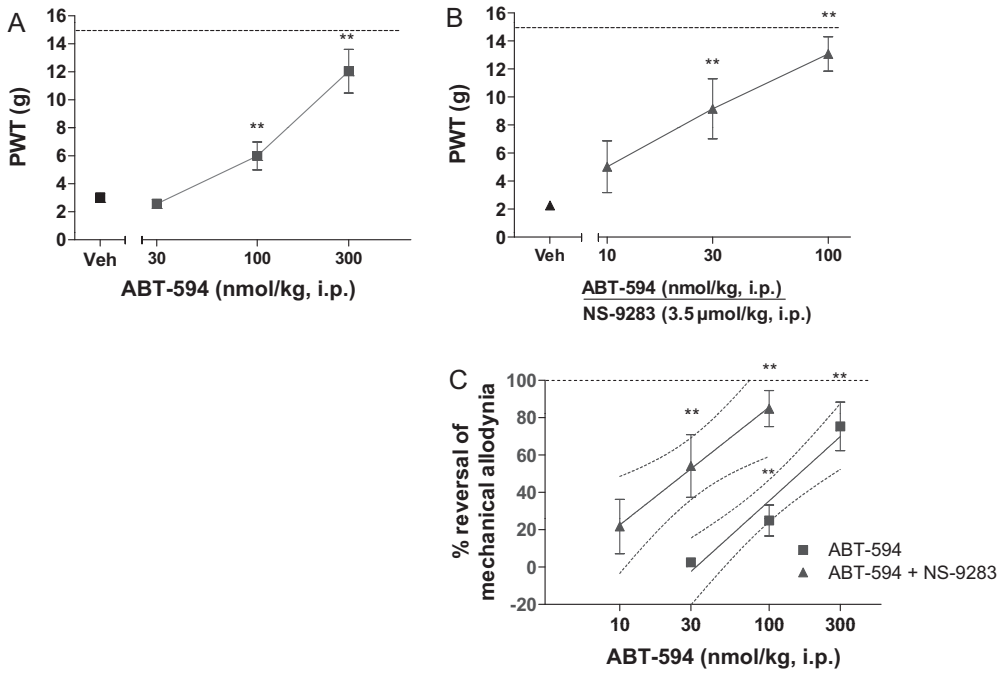

Fig. 2. Effect of ABT-594 (30–300 nmol/kg) or co-administration of NS-9283 (3.5 μmol/kg) and ABT-594 (10–100 nmol/kg) in skin incision-induced post-operative pain. PWT was assessed 30 min after intraperitoneal (i.p.) drug administration (2 h following paw injury). PWT data for ABT-594 (A), co-administration (B), and data represented as percent reversals for determination of ED_{50} (C) are shown. Data are mean ± S.E.M. $^{**}p < 0.01$ vs. vehicle (Veh) treated group, $n = 6$ per group. Dashed line: paw withdrawal threshold of non-injured paw for A and B, or 100% reversal of mechanical allodynia for C.

breaks were recorded for a period of 30 min. Horizontal exploratory behavior was assessed for 30 min, starting 30 min following i.p. compound administration.

2.3.3. Locomotor sensitization

The potential of NS-9283, ABT-594, and the combination of the two drugs to induce cross sensitization was examined in nicotine- and ABT-594-sensitized rats. During the sensitization-induction stage, animals were administered ABT-594 (100 nmol/kg, i.p.) or nicotine (2.5 μmol/kg, i.p.) daily on the first 4 consecutive days (Monday through Thursday) of each week for 3 weeks, and total distance traveled was recorded for 60 min period immediately following drug administration. A separate group of rats was treated daily with vehicle. Nicotine-sensitized animals were then challenged on Wednesdays of the fourth week, with NS-9283 (3.5 or 10 μmol/kg), fifth week with ABT-594 (10 or 100 nmol/kg), and sixth week with nicotine (2.5 μmol/kg) or the combination of NS-9283 (3.5 μmol/kg) with ABT-594 (10 nmol/kg). ABT-594-sensitized rats were challenged with NS-9283 (3.5 μmol/kg), ABT-594 (10 nmol/kg), or the combination of NS-9283 (3.5 μmol/kg) with ABT-594 (10 nmol/kg) following the same pattern as the nicotine-sensitized animals. Total distance traveled (cm) was recorded for a period of 60 min immediately following "challenge" treatment. On the "non-challenge" days of these weeks, rats were maintained on their induction treatment.

2.4. Pharmacological MRI

Pharmacological MRI was conducted following the cerebral blood volume (CBV)-based gradient-echo imaging protocol described previously [21,22]. Rats were imaged using a high-field MRI scanner (Bruker, Karlsruhe, Germany) under awake conditions. Prior to imaging, each animal was habituated inside a "mock scanner" using a training procedure to alleviate the stress during imaging [22]. To examine the effect of co-administration of NS-9283 with ABT-594 on the cortical neuronal activation pattern, rats

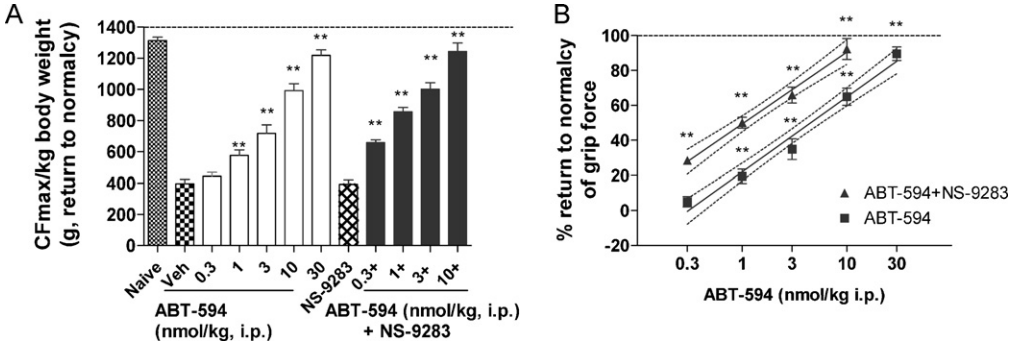

Fig. 3. Effect of ABT-594 (0.3–30 nmol/kg) or co-administration of NS-9283 (3.5 μmol/kg) and ABT-594 (0.3–10 nmol/kg) in MIA-induced osteoarthritis pain. Grip force was assessed 30 min after intraperitoneal (i.p.) drug administration (20 days following MIA knee joint injection). Returning normalcy of grip force to that of naïve rats (A), and data represented as percent reversals for determination of ED_{50} (B) are shown. Data are mean ± S.E.M. $^{**}p < 0.01$ vs. vehicle (Veh) treated group, $n = 7$ per group. Dashed line: 100% return to normal grip force.

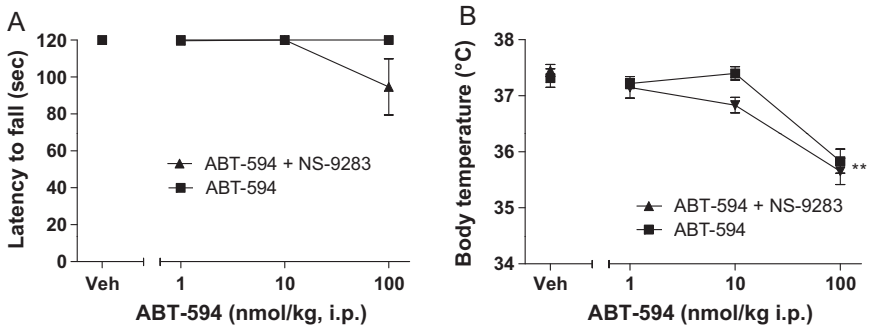

Fig. 4. Effect of ABT-594 (1–100 nmol/kg) or co-administration of NS-9283 (3.5 μmol/kg) and ABT-594 (1–100 nmol/kg) in edge performance and body temperature in naïve rats. Edge performance measured as the fall latency (A) and body temperature (B) was assessed 30 min following intraperitoneal (i.p.) drug administration. Data are mean ± S.E.M. $^{**}p < 0.01$ vs. vehicle (Veh) treated group, $n = 6–12$ per group.

were. infused i.v with ABT-594 (30, 100 and 300 nmol/kg, $n = 5$) alone, or animals were pretreated orally with NS-9283 (3.5 or 35 μmol/kg, $n = 3$) 40 min prior to ABT-594 i.v. infusion (30 nmol/kg). Saggital imaging slices were acquired with an in-plane resolution = 156 × 156 μm^2 and a slice thickness = 1.25 mm. Five continuous saggital slices were selected to cover the volume of whole brain, with the middle slice positioned along the center of the fourth ventricle. Functional data were acquired using the imaging protocol described previously [21] 20 min following ABT-594 administration. Data analysis was performed using AFNI software package and in-house IDL (Research Systems, Inc., Boulder, CO, USA) programs. To identify activated brain voxels, cross-correlation coefficients between the time-course data and a step function (OFF/ON ≡ pre-drug baseline/post-drug period) were calculated pixel-wisely for individual animals and corresponding *z*-scores were also derived. Group brain activation maps were obtained by averaging the co-registered *z* score maps retrieved from individual animals with a threshold of $z > 1.96$ ($p < 0.05$).

2.5. Statistics

Nociceptive and side effect behavioral data are presented as mean ± S.E.M., statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison. Two-way repeated-measure ANOVA (GraphPad Prism, version 4.03, San Diego, CA, USA) was performed for the induction of locomotor sensitization, and locomotor activity on drug challenge days was analyzed by one- or two-way ANOVA where appropriate. Bonferroni post hoc testing was done for significant main effects. $p < 0.05$ was considered to be significant, and ED_{50} values were defined as the dose of drug producing 50% of the maximum possible effect (MPE). All experiments were performed by experimenters unaware of the treatment received by the animals, and rats were randomly distributed into different experimental groups.

3. Results

3.1. Co-administration of NS-9283 and ABT-594 in pain models

3.1.1. Effect on carrageenan-induced acute thermal pain

The effect of ABT-594 or co-administration of NS-9283 with ABT-594 on carrageenan-induced acute inflammatory thermal hyperalgesia was examined in rats that underwent carrageenan injection into the hind paw. Hind paw injury resulted in the development of thermal hyperalgesia as indicated by a decreased PWL_{carra} to heat stimulus. NS-9283 alone produced no alteration of PWL_{carra} (up to 35 μmol/kg, i.p., $p > 0.05$), compared to vehicle treated animals (Table 1). ABT-594 at 100 and 300 nmol/kg increased PWL_{carra} to 5.46 ± 0.36 and 8.80 ± 0.65 s, compared to 3.46 ± 0.29 s in vehicle treated animals (Fig. 1A, $p < 0.01$, $n = 18$ per group). A significant increase of the PWL_{contra} was only observed in animals treated with ABT-594 at 300 nmol/kg, compared to that of vehicle treated animals (Fig. 1A, $p < 0.05$). In the presence of NS-9283 (3.5 μmol/kg), ABT-594 at 10, 30 and 100 nmol/kg increased PWL_{carra} to 5.29 ± 0.91, 6.35 ± 0.71 and 8.94 ± 0.79 s, respectively, compared to 3.17 ± 0.25 s in vehicle treated animals (Fig. 1B, $p < 0.01$, $n = 12$ per group). A significant increase of the PWL_{contra} was only observed in animals treated with the co-administration of ABT-594 at 100 nmol/kg, compared to that of vehicle treated animals (Fig. 1B, $p < 0.05$). Representing the raw data as a percent reversal of thermal hyperalgesia, ABT-594 at 100 and 300 nmol/kg demonstrated percent reversals of 28 ± 5 and 74 ± 9%, respectively, with an ED_{50} (95% CI) of 160 (123–218) nmol/kg; ABT-594 at 10, 30 and 100 nmol/kg combined with NS-9283 (3.5 μmol/kg), produced percent reversals

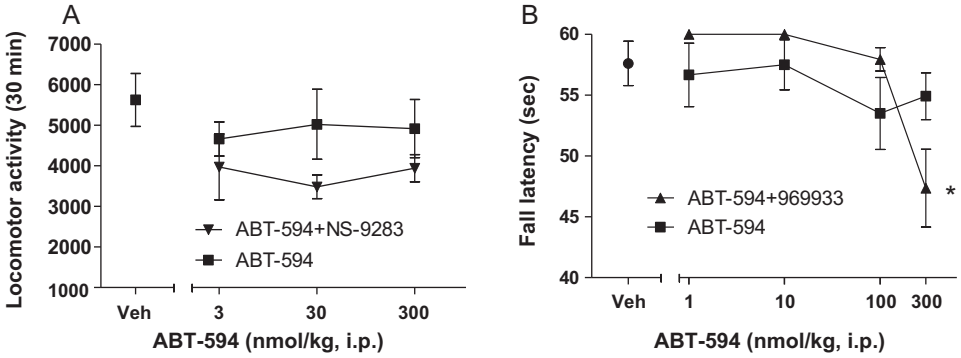

Fig. 5. Effect of ABT-594 (3–300 nmol/kg) or co-administration of NS-9283 (3.5 μmol/kg) and ABT-594 (3–300 nmol/kg) on spontaneous exploratory behavior and rotarod performance in naïve rats. Spontaneous exploratory behavior measured as the counts of horizontal activity (A) and rotarod performance measured as the fall latency from the rotarod (B) was assessed 30 min after intraperitoneal (i.p.) drug administration. Data are mean ± S.E.M. $^{*}p < 0.05$, vs. vehicle (Veh) treated group, $n = 6–8$ per group.

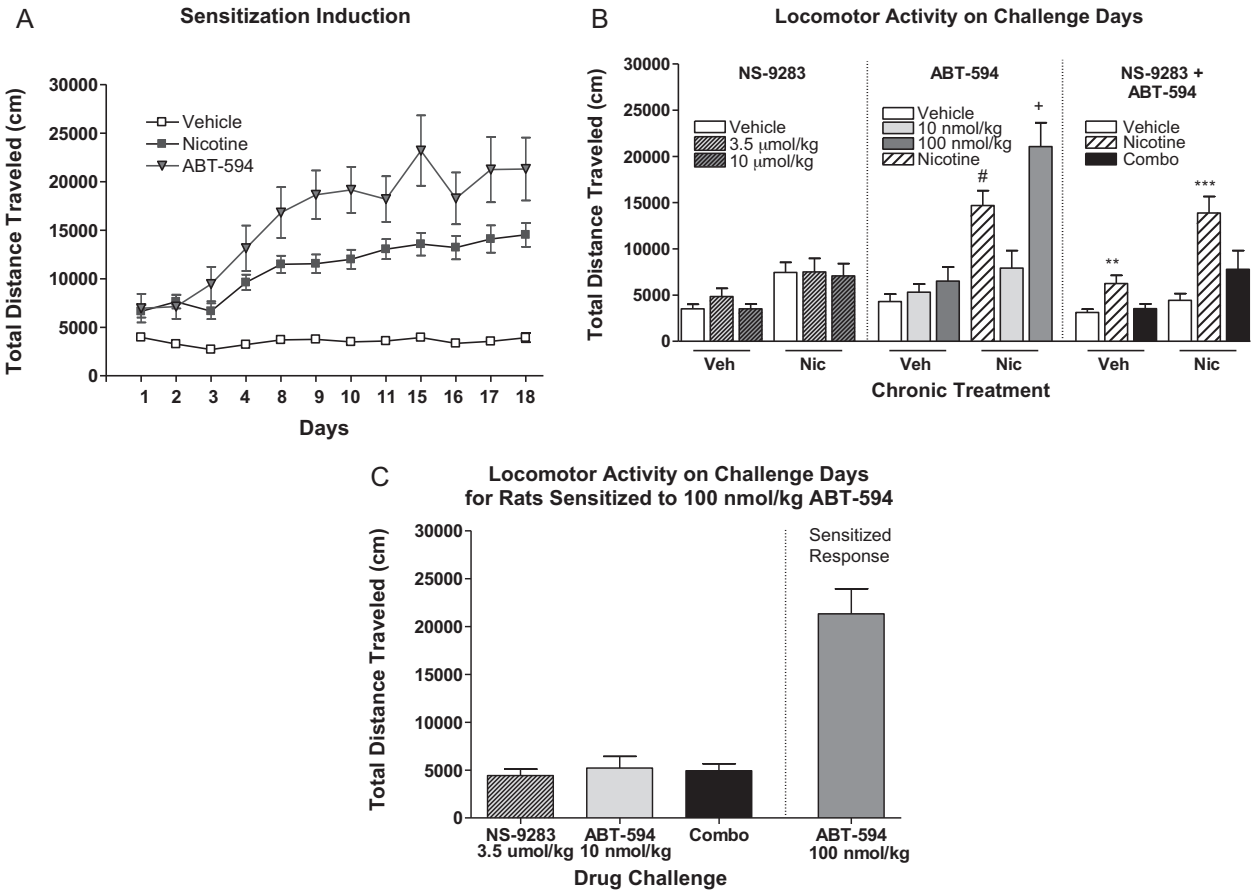

Fig. 6. Effect of NS-9283 on locomotor activity in nicotine or ABT-594 sensitized rats. During sensitization induction stage, rats were run in the locomotor activity boxes for a 60 min session following nicotine or ABT-594 administration on Monday–Thursdays for 3 weeks. Different compound challenges were applied to sensitized rats on the Wednesdays of subsequent weeks, and animals were maintained with their induction treatment for non-challenge days. At the initial sensitization stage, ABT-594 (100 nmol/kg), nicotine (2.5 μmol/kg) produced robust locomotor sensitization compared to vehicle treated rats (A). Animals were then challenged with NS-9283 at 3.5 or 10 μmol/kg, ABT-594 at 10 or 100 nmol/kg, and co-administration of NS-9283 at 3.5 μmol/kg with ABT-594 at 10 nmol/kg, or nicotine at 2.5 μmol/kg. No effect of challenge was seen in chronic vehicle treated rats; however, cross-sensitization to the high dose of ABT-594 was seen in the chronic nicotine treated rats (B). Rats sensitized to 100 nmol/kg ABT-594 show locomotor activity in response to the low dose of NS-9283, ABT-594, and the combination which are indistinguishable from vehicle treatment (C). The locomotor response to 100 nmol/kg of ABT-594 determined on the following day is also shown for comparison. Data are mean ± S.E.M. #, $p < 0.001$ vs. chronic vehicle challenged with vehicle, $^{+}p < 0.001$ vs. chronic vehicle challenged with 100 nmol/kg ABT-594, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. respective vehicle treated group.

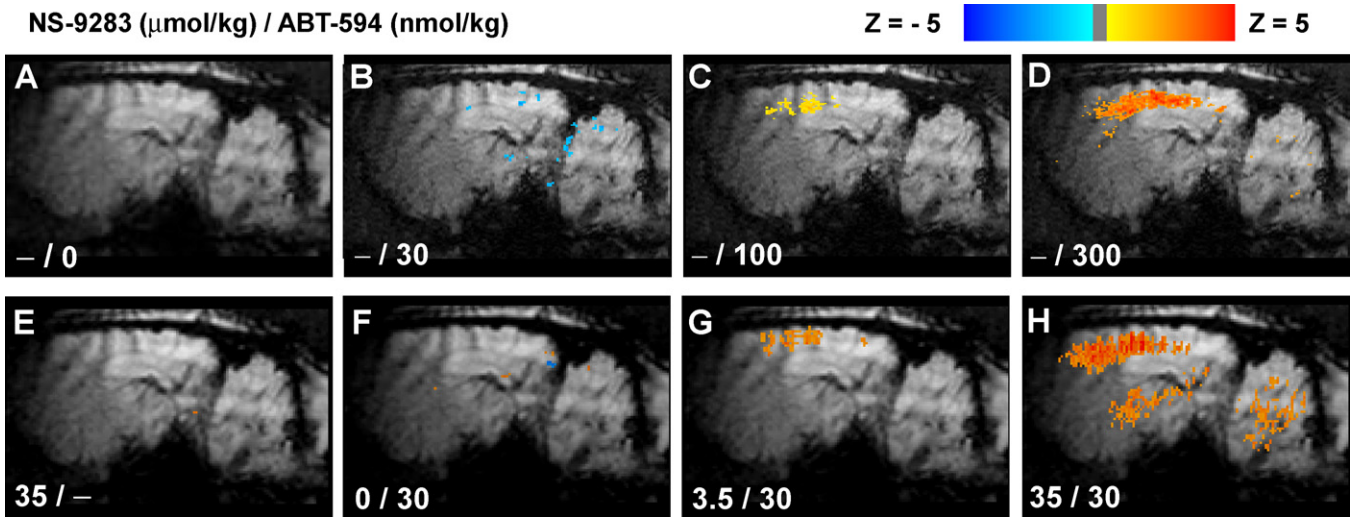

Fig. 7. Pharmacological MRI responses induced by ABT-594 or co-administration of NS-9283 with ABT-594 in cerebral cortex in naïve rats. Saggital group average brain activation patterns (z-score; threshold: $z > 1.96$, $p < 0.05$, location: lateral 2.9 mm) obtained from awake rats (n = 3–5 per group) following acute infusion of vehicle, ABT-594 at 30, 100, 300 nmol/kg, i.v. (A–D); co-administration of NS-9283 at 35 μmol/kg, or ABT-594 at 30 nmol/kg with vehicle (E and F), and co-administration of NS-9283 at 3.5 or 35 μmol/kg with ABT-594 at 30 nmol/kg (G and H). The regions ascribed to negative z-score values (blue) indicate a decrease in CBV after the drug administration, while an increase in CBV is represented in positive z-score values (red).

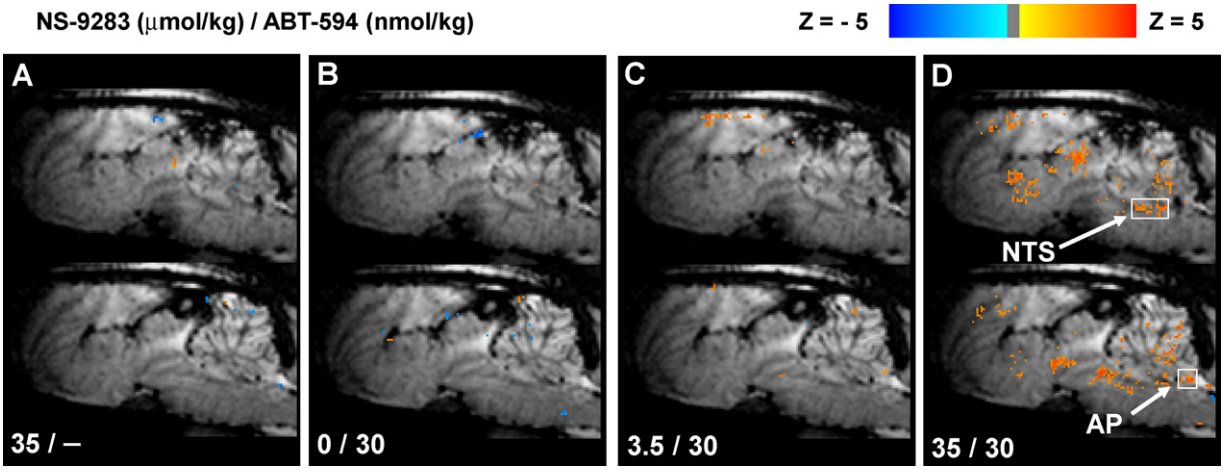

Fig. 8. Pharmacological MRI reposes induced by the co-administration of NS-9283 (3.5 or 35 μmol/kg) with ABT-594 (30 nmol/kg) in brain stem emetic center in naïve rats. Saggital group average brain activation patterns (*z*-score; threshold: $z > 1.96$, $p < 0.05$, slice location: lateral 0.9 and −0.1 mm) obtained from awake rats ($n = 3$–5 per group), co-administration of NS-9283 (35 μmol/kg, i.v.) with vehicle (A), vehicle with ABT-594 (B), NS-9283 at 3.5 or 35 μmol/kg ABT-594 (C and D). The regions ascribed to negative *z*-score values (blue) indicate a decrease in CBV after the drug administration, while an increase in CBV is represented in positive *z*-score values (red).

of 31 ± 13, 46 ± 10 and 84 ± 12%, respectively, with an ED_{50} (95% CI) of 26 (10–50) nmol/kg (Fig. 1C). Therefore, co-administration of NS-9283 revealed a leftward shift in the DRC of ABT-594 by 6-fold.

3.1.2. Effect on skin-incision induced post-operative pain

The effect of ABT-594 or co-administration of NS-9283 with ABT-594 on post-operative pain behaviors was examined in rats that underwent skin incision of the hind paw. Hind paw injury resulted in the development of mechanical allodynia as indicated by a decreased PWT to a series of mechanical stimuli of calibrated von Frey filament 2 h post-incision. NS-9283 (up to 35 μmol/kg, i.p.) alone produced no alteration of PWT of the injured paw, compared to vehicle treated animals (Table 1). ABT-594 at 100 and 300 nmol/kg increased PWT to 6.0 ± 1.0 and 12.0 ± 1.6 g, compared to vehicle-treated group of 3.0 ± 0.3 (Fig. 2A, $p < 0.01$, $n = 6$ per group); in the presence of NS-9283 (3.5 μmol/kg), ABT-594 at 10, 30 and 100 nmol/kg increased PWT to 5.0 ± 1.9, 9.2 ± 2.1 and 13.1 ± 1.2 g, compared to vehicle-treated group of 2.3 ± 0.2 g (Fig. 2B, $p < 0.01$, $n = 6$ per group). Representing the raw data as a percent reversal of mechanical allodynia, ABT-594 at 100 and 300 nmol/kg demonstrated percent reversals of 25 ± 8, and 75 ± 12%, respectively, with an ED_{50} (95% CI) of 153 (109–261) nmol/kg; ABT-594 at 10, 30 and 100 nmol/kg combined with NS-9283 (3.5 μmol/kg), produced percent reversals of 22 ± 14, 54 ± 16 and 85 ± 10%, respectively, with an ED_{50} (95% CI) of 26 (11–52) nmol/kg (Fig. 2C). Therefore, co-administration of NS-9283 revealed a leftward shift in the DRC of ABT-594 by 6-fold.

3.1.3. Effect on MIA induced knee joint pain

The effect of ABT-594 or co-administration of NS-9283 with ABT-594 on return of the grip force normalcy was investigated in rats injected with MIA in the right knee joint. CGF_{body} was reduced in animals assessed 20 days following MIA injection of the right hind knee joint. NS-9283 at 10 μmol/kg alone induced the return of grip force normalcy by 50%, however, NS-9283 at 1 or 3.5 μmol/kg alone produced no effect on grip force in OA rats (Table 1). NS-9283 at 3.5 μmol/kg was subsequently chosen for combination studies. ABT-594 at 1, 3, 10 and 30 nmol/kg increased CGF_{body} to 575 ± 37, 717 ± 64, 992 ± 45 and 1234 ± 37 g, respectively; in the presence of NS-9283 (3.5 μmol/kg), ABT-594 at 0.3, 1, 3, and 10 nmol/kg increased CGF_{body} to 657 ± 21, 855 ± 28, 971 ± 35 and 1243 ± 56 g, respectively, compared to 397 ± 29 g in vehicle treated animals (Fig. 3A, $p < 0.01$, $n = 7$ per group). Representing the raw data as a percent return of grip force to normalcy, ABT-594 at 1, 3, 10 and 30 nmol/kg demonstrated percent return of grip force to normalcy of 20 ± 4, and 35 ± 6, 65 ± 5, and 90 ± 4%, respectively, with ED_{50} (95% CI) = 4.6 (3.6–5.7) nmol/kg; ABT-594 at 0.3, 1, 3, and 10 nmol/kg, combined with NS-9283 (3.5 μmol/kg), produced percent return of grip force to normalcy of 29 ± 2, 50 ± 3, 66 ± 5 and 92 ± 6%, respectively, with ED_{50} (95% CI) = 1.0 (0.8–1.3) nmol/kg (Fig. 3B). Therefore, co-administration of NS-9283 revealed a leftward shift in the DRC of ABT-594 by 5-fold.

3.2. Co-administration of NS-9283 with ABT-594 on CNS side effect

3.2.1. Effect on edge performance or body temperature

The effect of ABT-594 or co-administration of NS-9283 with ABT-594 on edge-balance and body temperature was examined in naïve rats. NS-9283 (up to 35 μmol/kg, i.p., $n = 6$ per group) alone had no effect on rat edge performance or body temperature (Table 1). ABT-594 (up to 100 nmol/kg, i.p.) alone produced no significant edge performance deficit. This was not significantly altered in the presence of NS-9283 at 3.5 μmol/kg (Fig. 4A, $p > 0.05$, $n = 6$–12 per group), compared to that of vehicle-treated animals. ABT-594 alone at 100 nmol/kg reduced body temperature by 1.5 °C (35.83 ± 0.21 °C), and this was not exacerbated in the presence of NS-9283 at 3.5 μmol/kg (35.65 ± 0.24 °C, Fig. 4B, $p > 0.05$, $n = 6$–12 per group), compared to that of vehicle-treated animals (37.32 ± 0.17 °C).

3.2.2. Effect on exploratory horizontal behavior and rotarod performance

The effect of ABT-594 or co-administration of NS-9283 with ABT-594 on locomotor activity and rotarod performance was examined in naïve rats. ABT-594 (3, 30, 300 nmol/kg, i.p.) alone produced no significant reduction of spontaneous horizontal exploratory activities. This was not significantly altered in the presence of 3.5 μmol/kg NS-9283 (Fig. 5A, $p > 0.05$, $n = 8$ per group). NS-9283 (up to 35 μmol/kg, i.p., $p > 0.05$, $n = 8$ per group) alone produced no effect on locomotor activity (Table 1).

ABT-594 (up to 300 nmol/kg, i.p.) alone produced no deficit of rotarod performance. In the presence of NS-9283 (3.5 μmol/kg), ABT-594 at 300 nmol/kg reduced fall latency to 47 ± 3 s (18% ± 6% deficit, Fig. 5B, $p < 0.05$, $n = 6$–12 per group), compared to fall latency of 57 ± 6 s in vehicle-treated animals. NS-9283 (up to 35 μmol/kg, i.p., $p > 0.05$, $n = 6$ per group) alone produced no alteration of rotarod performance (Table 1).

3.2.3. Effect on locomotor activity sensitization

During the sensitization-induction stage, a main effect of treatment ($p < 0.001$) suggested that ABT-594 (100 nmol/kg) or nicotine (2.5 μmol/kg) administration increased locomotor activity, compared to that of vehicle treated animals (Fig. 6A, $p < 0.001$, $n = 8$–26 per group). Chronic ABT-594 (100 nmol/kg) or nicotine (2.5 μmol/kg) administration produced a progressive enhancement in locomotor activity, evidenced by a main effect of day ($p < 0.001$, i.e., behavioral sensitization), and chronic vehicle administration maintained the same level of activity over the course of 18-day sensitization period. Rats chronically treated with vehicle or nicotine did not show any change of locomotor activity when challenged acutely with NS-9283 (3.5 or 10 μmol/kg) when compared to rats from these respective chronic treatment groups challenged with vehicle (Fig. 6B, left panel, $p > 0.05$). However, on this acute treatment day, rats in the chronic nicotine group had significantly higher activity than rats in the chronic vehicle group, which is likely the result of "conditioned sensitization" in chronically nicotine treated animals compared to that of chronically vehicle treated rats (Fig. 6B, left panel, $p < 0.001$ for comparison of chronic nicotine and chronic vehicle groups collapsed across acute treatments, $n = 26$ per group). Acute challenge of ABT-594 (10 or 100 nmol/kg) had no effect in chronic vehicle treated animals, and acute challenge with ABT-594 at 10 nmol/kg to chronic nicotine treated animals induced no change of locomotor activity beyond that which can be attributed to "conditioned sensitization" (Fig. 6B, middle panel, $p > 0.05$, $n = 8$–9 per group), suggesting lack of cross-sensitization between nicotine and a low dose of ABT-594. Acute challenge of ABT-594 at 100 nmol/kg to chronic nicotine treated animals enhanced locomotor activity ($p < 0.001$ vs.100 nmol/kg challenge in chronic vehicle treated rats), suggesting cross-sensitization between nicotine and this high dose of ABT-594. Although higher levels of activity were seen in chronic nicotine treated rats in response to the high dose of ABT-594 compared to a nicotine challenge, this effect was not significant ($p > 0.05$). Finally, acute challenge with co-administered NS-9283 (3.5 μmol/kg) and ABT-594 (10 nmol/kg) to chronic vehicle or chronic nicotine treated animals produced no change of locomotor activity, compared to acute vehicle challenge (only nicotine challenge produced a significant increase over vehicle, $p < 0.01$), suggesting lack of cross-sensitization of an analgesic combination of NS-9283 and ABT-594 in nicotine-sensitized animals (Fig. 6B, right panel, $p > 0.05$, $n = 8$–9 per group). Neither NS-9283 at 3.5 μmol/kg, ABT-594 at 10 nmol/kg, or the combination produced a locomotor response greater than that seen with vehicle in rats chronically treated with 100 nmol/kg ABT-594 (Fig. 6C, $n = 8$ per group). This low response is in contrast to the high level of activity seen in response to the 100 nmol/kg dose given during sensitization induction and maintenance (Fig. 6C, right bar).

3.3. Co-administration of NS-9283 with ABT-594 on pharmacological MRI response

ABT-594 (30, 100 and 300 nmol/kg, i.v., $n = 5$ per group) activated brain parenchyma in a region-specific and dose-related manner (Fig. 7B–D). Restricted de-activation, or a decrease in CBV, was observed at 30 nmol/kg (Fig. 7B), and cortical activation was shown at 100 nmol/kg (Fig. 7C), with the most extensive at 300 nmol/kg (Fig. 7D), compared to vehicle treated animals (Fig. 7A). Co-administration of vehicle with NS-9283 (35 μmol/kg, p.o.) or ABT-594 (30 nmol/kg) produced no change of functional activity of the brain (Fig. 7E and F). Conversely, the co-administration of NS-9283 (3.5 or 35 μmol/kg, p.o.) with ABT-594 (30 nmol/kg) potentiated ABT-594-induced-brain functional activities (~5-fold, Fig. 7G and H), consistent with the potentiating effect of PAM characterized *in vitro* and *in vivo* behavioral studies. Fig. 8 highlights the neuronal activation pattern in area postrema and nucleus tractus solitarius (AP/NTS) in brain stem ($n = 3$), following co-administration of NS-9283 (3.5 or 35 μmol/kg, p.o.) and ABT-594 (30 nmol/kg, i.v.). NS-9283 (35 μmol/kg) alone produced no neuronal activation in AP/NTS (Fig. 8A), similar patterns were obtained from rats treated with co-administration of vehicle and ABT-594, or lower dose of NS-9283 (3.5 μmol/kg) and ABT-594 (Fig. 8B and C). In the contrary, co-administration of high dose of NS-9283 (35 μmol/kg) with ABT-594 significantly increased neuronal activity in AP/NTS (Fig. 8D). These observations agree with behavioral measures of emetic liability in ferrets [see Lee et al., accompanying article].

4. Discussion

Previously, we have demonstrated that α4β2 nAChR agonist ABT-594-induced anti-allodynic activity was potentiated by co-administration of PAM NS-9283 in a rodent model of neuropathic pain, without adversely affecting cardiovascular and gastrointestinal side effects [see Lee et al., accompanying article]. Here, we report that these effects are not limited to neuropathic pain but extend to several preclinical models of nociceptive/inflammatory pain. In the presence of the PAM NS-9283, a leftward shift of ABT-594 DRC was demonstrated in carrageenan-induced acute inflammatory thermal hyperalgesia, skin-incision induced post-operative pain and MIA-induced osteoarthritic pain. In contrast, in the presence of PAM NS-9283, ABT-594-induced CNS-related adverse effects, including rotarod/edge performance, horizontal exploratory activity and body temperature, were not affected, further demonstrating that the addition of a PAM does not further increase side effects being CNS, cardiovascular or gastrointestinal side effects. Additionally, acute challenge of NS-9283 produced no cross sensitization to nicotine-mediated locomotor sensitization, suggesting that co-administration of the PAM and nAChR agonist at analgesic doses could be devoid of locomotor deficits or long term liability. In agreement with the *in vivo* behavioral profile, pharmacological MRI analysis has showed that NS-9283 enhanced ABT-594 induced neuronal activity in cortical areas, without increasing the functional responses of the brainstem emetic center.

4.1. Co-administration of NS-9283 and ABT-594: anti-nociceptive activity

In the presence of NS-9283, the concentration–response curve to ABT-594 in the calcium flux assay is left-shifted in a concentration-dependent manner at the α4β2, but not at α3β4 in cells expressing nAChRs, whereas NS-9283 alone has no effects on α4β2, α3β4, or other nAChR subtypes [see Lee et al., accompanying article]. In agreement with *in vitro* findings, a lower dose of NS-9283 (<10 μmol/kg) alone produced no behavioral alteration in either pain or CNS side effect related animal models in the current investigation. Taken together, these data demonstrate that NS-9283 is indeed an α4β2 nAChR selective allosteric modulator, providing a basis for an improved therapeutic index relative to ABT-594 alone. Following systemic co-administration NS-9283 and ABT-594, NS-9283 potentiated ABT-594-induced analgesic response, with a leftward shift of ABT-594 DRC by 6-fold, in reversing the carrageenan-induced acute inflammatory thermal hyperalgesia of the injured paw in a dose-dependent manner. In the post-operative pain model, we have shown that ABT-594 alone attenuates mechanical allodynia in a dose-dependent manner, with full efficacy observed when ABT-594 was administered at 300 nmol/kg. These analgesic effects in post-surgical pain further expand the already broad antinociceptive profile of nAChR agonist in experimental pain models [3,23]. More

importantly, co-administration of NS-9283, which by itself had no analgesic activity, potentiated the effect of ABT-594, producing a leftward shift of the DRC of ABT-594 by 6-fold in the same model, providing widened safety margin for the potential advancement of nAChR agonist in the management of post-operative pain in humans. In the MIA-induced osteoarthritis pain, a dose of ABT-594 as low as 1 nmol/kg attenuated osteoarthritis pain, with full efficacy at 30 nmol/kg. These efficacious doses are well below those that induce CNS side effects [3,23]. Furthermore, co-administration of NS-9283 and ABT-594 demonstrated full efficacy, with a leftward shift of the ABT-594 DRC by 5-fold. The results from the current study, along with the substantial enhancement of ABT-594-induced anti-allodynic activity in a rat model of neuropathic pain in the presence of NS-9283 [see Lee et al., accompanying article], demonstrate that co-administration of a PAM can enhance nAChR agonist-induced analgesic activity across multiple preclinical nociceptive and neuropathic pain models. These ligands act at multiple locations throughout the pain pathway, and their antinociceptive effects through $\alpha4\beta2$ nAChRs have been attributed to enhancing descending inhibition (for detailed discussion, see Lee at al., the accompanying article). Taking the *in vitro* finding that the leftward shift of the ABT-594 induced calcium influx in the presence of NS-9283 into consideration, the increased analgesic activity of nAChR agonists is most likely due to the enhancement of the activation of $\alpha4\beta2$ nAChR, but not other subtypes. Additionally, NS-9283 (up to 35 μmol/kg) alone produced no analgesic activity in any of the nociceptive pain models tested, indicating that potentiation of the endogenous transmitter ACh alone via modulating synaptic activity or facilitating transmitter release is not sufficient to induce antinociception.

4.2. Co-administration of NS-9283 and ABT-594: pharmacological MRI

In the current study, pharmacological MRI analysis demonstrated that ABT-594 dose-dependently increased cortical activity, and co-administration of NS-9283 (3.5 μmol/kg) potentiated ABT-594 effects, which is consistent with the leftward shift of dose–responses obtained from behavioral pain models. Further, the results also indicate that significant changes in the activity in the area postrema and nucleus tractus solitarius were observed in rats pretreated with NS-9283 at 35 μmol/kg, but not 3.5 μmol/kg, suggesting that the dose of NS-9283 should also be well controlled in order to explore the maximum benefit of the combined therapy in pain management. Corresponding with the lack of behavioral alteration in either pain or CNS related assay, NS-9283 (up to 35 μmol/kg) alone elicited no effect on brain activity, which further supports the notion that both exogenous administration of PAM and nAChR agonists is necessary for the induction of antinociception. Previously, we have demonstrated the use of drug-induced neural activity in AP/NTS as a biomarker for assessing emetic liability, in which we found that ABT-594 at 30 nmol/kg did not significantly activate area postrema and nucleus tractus solitarius [21] Thus, adding NS-9283 to an inactive dose of ABT-594 selectively increased cortical activity, without alteration of activity in the brainstem emetic center, confirming that PAM could promote analgesic activity of $\alpha4\beta2$ nAChR agonist without inducing emetic side effect.

4.3. Co-administration of NS-9283 and ABT-594: CNS side effects

Since effects of co-administration of NS-9283 and ABT-594 on motor function could interfere with the animal's ability to respond in the antinociceptive assays, a series of CNS-related assays, including rotarod/edge performance, locomotor activity and body temperature measurement, were employed in the current investigation. These tests were used to determine if motor deficits or hypothermia may be generating a false positive in the pain behavior studies, and to assess whether co-administration of PAM was affecting CNS-related behavior within the analgesic dose range. We have demonstrated that antinociceptive dose range of co-administration of NS-9283 (3.5 μmol/kg) with ABT-594 (up to 100 nmol/kg) has no effect on rotarod or edge performance. Co-administration of NS-9283 3.5 μmol/kg also did not alter ABT-594-induced hypothermia, a potential confounding parameter for nAChR agonists. These data, in combination with the lack of emesis or cardiovascular impairment in the co-administration of the PAM with nAChR agonist at a dose even higher than that active against neuropathic pain [see Lee et al., accompanying article], confirm the improved therapeutic index by co-administration of PAM with $\alpha4\beta2$ nAChR agonist in rodent pain models. However, the present data cannot rule out the possibility that side-effects might be exacerbated/potentiated at higher doses of the agonist.

Behavioral sensitization in rodents can be induced by many drugs of abuse and may indicate liability for human drug dependence [24]. The current investigation demonstrated that repeated exposure to nicotine or ABT-594 induces locomotor behavioral sensitization, which is thought to result from plasticity in the mesolimbic dopamine system, consisting of dopamine projections from the ventral tegmental area of nucleus accumbens [25,26]. The present study also provides evidence that PAM does not affect the behavioral changes after repeated nicotine or ABT-594 exposure in naïve rats, indicating that a dose of the PAM that adequately potentiates nAChR agonist-mediated antinociception does not potentiate nicotine-mediated behavioral sensitization, further supporting the potential development of co-administration of a PAM combined with nAChR agonist as an analgesic.

In the present study, we demonstrated co-administration of PAM enhanced nAChR agonist (ABT-594 up to 100 nmol/kg)-induced analgesic potency in nociceptive/inflammatory pain without exacerbating adverse effects, confirming the dissociation of analgesic activity and adverse effects. Thus, the combination therapy may be an alternative approach to the development of nAChR agonists for the treatment of pain.

Conflict of interest

The authors are employees of Abbott Laboratories.

Acknowledgement

This work was supported by Abbott Laboratories.

References

[1] Decker MW, Rueter LE, Bitner RS. Nicotinic acetylcholine receptor agonists: a potential new class of analgesics. Curr Top Med Chem 2004;4:369–84.
[2] Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 2009;8:733–50.
[3] Bannon AW, Decker MW, Holladay MW, Curzon P, Donnelly-Roberts D, Puttfarcken PS, et al. Broad-spectrum, non-opioid analgesic activity by selective modulation of neuronal nicotinic acetylcholine receptors. Science 1998;279:77–81.
[4] Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature 1999;398:805–10.
[5] Bitner RS, Nikkel AL, Curzon P, Donnelly-Roberts DL, Puttfarcken PS, Namovic M, et al. Reduced nicotinic receptor-mediated antinociception following in vivo antisense knock-down in rat. Brain Res 2000;871:66–74.
[6] Bannon AW, Decker MW, Curzon P, Buckley MJ, Kim DJ, Radek RJ, et al. ABT-594 [(R)-5-(2-azetidinylmethoxy)-2-chloropyridine]: a novel, orally effective antinociceptive agent acting via neuronal nicotinic acetylcholine receptors: II. In vivo characterization. J Pharmacol Exp Ther 1998;285:787–94.

[7] Lynch 3rd JJ, Wade CL, Mikusa JP, Decker MW, Honore P. ABT-594 (a nicotinic acetylcholine agonist): anti-allodynia in a rat chemotherapy-induced pain model. Eur J Pharmacol 2005;509:43–8.
[8] Bitner RS, Nikkel AL, Curzon P, Arneric SP, Bannon AW, Decker MW. Role of the nucleus raphe magnus in antinociception produced by ABT-594: immediate early gene responses possibly linked to neuronal nicotinic acetylcholine receptors on serotonergic neurons. J Neurosci 1998;18:5426–32.
[9] Rueter LE, Meyer MD, Decker MW. Spinal mechanisms underlying A-85380-induced effects on acute thermal pain. Brain Res 2000;872:93–101.
[10] Rowbotham MC, Duan WR, Thomas J, Nothaft W, Backonja MM. A randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of ABT-594 in patients with diabetic peripheral neuropathic pain. Pain 2009; 146:245–52.
[11] Arneric SP, Holladay M, Williams M. Neuronal nicotinic receptors: a perspective on two decades of drug discovery research. Biochem Pharmacol 2007;74:1092–101.
[12] Changeux J, Edelstein SJ. Allosteric mechanisms in normal and pathological nicotinic acetylcholine receptors. Curr Opin Neurobiol 2001;11:369–77.
[13] Weltzin MM, Schulte MK. Pharmacological characterization of the allosteric modulator desformylflustrabromine and its interaction with alpha4beta2 neuronal nicotinic acetylcholine receptor orthosteric ligands. J Pharmacol Exp Ther 2010;334:917–26.
[14] Seo S, Henry JT, Lewis AH, Wang N, Levandoski MM. The positive allosteric modulator morantel binds at noncanonical subunit interfaces of neuronal nicotinic acetylcholine receptors. J Neurosci 2009;29:8734–42.
[15] Lloyd GK, Williams M. Neuronal nicotinic acetylcholine receptors as novel drug targets. J Pharmacol Exp Ther 2000;292:461–7.
[16] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77–88.
[17] Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain 1996;64:493–501.
[18] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.
[19] Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol 1980;20:441–62.
[20] Chandran P, Pai M, Blomme EA, Hsieh GC, Decker MW, Honore P. Pharmacological modulation of movement-evoked pain in a rat model of osteoarthritis. Eur J Pharmacol 2009;613:39–45.
[21] Chin CL, Fox GB, Hradil VP, Osinski MA, McGaraughty SP, Skoubis PD, et al. Pharmacological MRI in awake rats reveals neural activity in area postrema and nucleus tractus solitarius: relevance as a potential biomarker for detecting drug-induced emesis. Neuroimage 2006;33:1152–60.
[22] Chin CL, Tovcimak AE, Hradil VP, Seifert TR, Hollingsworth PR, Chandran P, et al. Differential effects of cannabinoid receptor agonists on regional brain activity using pharmacological MRI. Br J Pharmacol 2008;153:367–79.
[23] Decker MW, Bannon AW, Buckley MJ, Kim DJ, Holladay MW, Ryther KB, et al. Antinociceptive effects of the novel neuronal nicotinic acetylcholine receptor agonist, ABT-594, in mice. Eur J Pharmacol 1998;346:23–33.
[24] Berridge KC, Robinson TE. Parsing reward. Trends Neurosci 2003;26:507–13.
[25] Addy NA, Fornasiero EF, Stevens TR, Taylor JR, Picciotto MR. Role of calcineurin in nicotine-mediated locomotor sensitization. J Neurosci 2007;27:8571–80.
[26] Domino EF, Tsukada H. Nicotine sensitization of monkey striatal dopamine release. Eur J Pharmacol 2009;607:91–5.