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COMMENTARY

Therapeutic Potential of Neuronal Nicotinic Acetylcholine Receptor Agonists as Novel Analgesics

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ABSTRACT. Pharmacological treatments for pain have come largely from two classes of compounds—the opioids and the nonsteroidal anti-inflammatory drugs (NSAIDs). Because of deficiencies associated with these two classes of compounds, exploration of novel approaches to pain relief has intensified of late. Nicotine, a neuronal nicotinic acetylcholine receptor (nAChR) agonist, has long been known to have antinociceptive effects in both experimental animals and humans. The relatively modest antinociceptive effects and the toxicities associated with nicotine preclude its development as an analgesic agent. However, recent discoveries in the nAChR field have stimulated interest in nAChR-targeted compounds as potential analgesic agents. Epibatidine, a potent nAChR agonist, was found to have full efficacy relative to opioids in preclinical pain models. Although epibatidine is toxic, these observations demonstrated that modest efficacy is not a general limitation of nAChR agonists. Moreover, exploration of the molecular biology of nAChRs revealed evidence of receptor diversity, suggesting that nAChR subtype-selective agents less toxic than nicotine might be discovered; and early medicinal chemistry efforts already have resulted in compounds with improved safety profiles. For example, ABT-594 is a nAChR agonist with the antinociceptive efficacy of epibatidine, but with an improved safety profile. This commentary reviews recent findings with nAChR-targeted compounds, explores potential mechanisms responsible for nAChR-mediated antinociception, and raises issues that must be addressed in developing compounds of this class as analgesics. BIOCHEM PHARMACOL **58**;6:917–923, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. nicotinic acetylcholine receptor; epibatidine; nicotine; ABT-594; analgesia; pain

Pain represents a major unmet medical need. In the U.S. alone, more than 100 million people will suffer from moderate to severe pain during the course of a year; and pain is the number one reason for visits to physicians. By alerting people to tissue damage and potentially dangerous underlying conditions and motivating them to seek treatment, pain serves an adaptive function. Unrelieved pain, however, can be an impediment to normal function. Moreover, abnormal pain perception caused by damage to the nervous system can produce chronic neuropathic pain that persists even in the absence of noxious stimulation.

Current pharmacological therapies for pain come primarily from two classes of compounds—NSAIDs† and opioids [1, 2]. Both classes of compounds have long histories of clinical use and major deficiencies, and much of the drug discovery effort in the area of pain has focused on incremental improvements within these two classes of compounds. NSAIDs have primary utility in mild to moderate pain, particularly pain associated with inflammation. Intensive drug discovery efforts have been undertaken to address the gastric irritation produced by NSAIDs, a major limitation of the compound class. An important recent breakthrough in this area is the development of agents that target the inducible form of cyclooxygenase (COX-2) and may provide relief from inflammation and pain without producing gastric irritation. Opioids are beset with an array of adverse side-effects, including nausea and vomiting, constipation, sedation, and respiratory depression. In addition, tolerance to the analgesic effects and physical dependence can develop with repeated administration of opioids. Fear of dependence liability and scheduling issues probably account for the under-utilization of opioids in chronic pain. Moreover, there is also evidence that some neuropathic pain conditions are resistant to opioid therapy [3]. Important adjuncts to opioids and NSAIDs, particularly in the treatment of neuropathic pain, include a number of anticonvulsant and antidepressant medications [3–5]. Over the last 5–10 years, however, there has been a fundamental shift in the understanding of the mechanisms that contribute to pain perception; and newer drug discovery strategies have focused on attenuation of specific biochemical and physiological processes that augment pain signaling [6]. Among the novel approaches under development are neu-

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† *Abbreviations*: NSAIDs, nonsteroidal anti-inflammatory drugs; nAChR, nicotinic acetylcholine receptor; NRM, nucleus raphe magnus; and PPTgN, pedunculopontine tegmental nucleus.

rokinin-1 receptor antagonists, sodium channel antagonists, *N*-methyl-D-aspartate receptor antagonists, muscarinic cholinergic agonists, and compounds interacting with purinergic neurotransmission.

In addition, interest in nAChR agonists as potential analgesics has emerged. Nicotine, itself, has long been known to have antinociceptive properties [7]. In animal studies, however, high doses of nicotine are required to produce antinociception, and the antinociceptive effect is relatively modest and of short duration [8–10]. The stimulus for the recent resurgence in interest in nicotinically mediated analgesia was John Daly's discovery of epibatidine, a compound he originally isolated from the skin of an Ecuadorian tree frog, *Epipedobates tricolor* [11]. Epibatidine produces profound antinociceptive effects in rodents, with efficacy comparable to that of morphine and potency as much as 200 times greater than morphine. The antinociceptive effects of epibatidine can be prevented by the noncompetitive nAChR antagonist mecamylamine, but not by opioid antagonists; and *in vitro* functional studies have demonstrated that epibatidine is a potent nAChR agonist [11–13]. Thus, Daly discovered a potent and highly efficacious antinociceptive compound with a nAChR-mediated mechanism of action. This discovery, combined with important advances in the molecular biology of neuronal nAChRs and medicinal chemistry efforts targeting nAChR-based treatments for central nervous system disorders, sets the stage for intensified exploration of the analgesic potential of nAChR agonists.

Although epibatidine has antinociceptive properties in rodent pain models, the compound also produces profound hypothermia and ataxia, effects that also are observed at antinociceptive doses of nicotine [12]. These effects present an interpretive problem. Since antinociception in animal models typically is measured by assessing motor responses to painful stimuli, impaired motor performance could interfere with accurate assessment of antinociception. In addition, since many rodent models use heat as a noxious stimulus, hypothermia might alter test results by increasing the time required to raise the skin temperature. Thus, reduced responsivity to painful stimuli produced by epibatidine in these models might not truly represent decreased pain perception but could be an epiphenomenon related to ataxia and hypothermia.

Although the issue has not been resolved completely, there is evidence that hypothermia and ataxia do not account adequately for the antinociceptive effects of epibatidine [14], with evidence coming from experiments demonstrating dissociation of these effects. For example, mecamylamine administered prior to epibatidine prevents the antinociceptive, hypothermic, and ataxic effects of epibatidine in mice; but if mecamylamine is given after epibatidine, it can attenuate the hypothermic and ataxic effects of the compound without affecting antinociception. Similarly, attenuation of the hypothermic and ataxic effects of epibatidine can be observed with repeated administration under a regimen that does not affect antinociception. Thus, it appears that it is possible to maintain antinociception in animals whose hypothermic and ataxic responses to epibatidine have been attenuated. There are also electrophysiological findings consistent with an antinociceptive action of epibatidine. In anesthetized rats, the compound reduces event-related potentials in the brain that are generated by footshock, an effect that is also observed with morphine and one that would not be influenced by effects of epibatidine on motor performance [13].

A second issue related to the adverse side-effects of epibatidine is the relatively small separation between toxic and antinociceptive doses. As already noted, hypothermia and ataxia are present at antinociceptive doses in rodents. More importantly, epibatidine produces seizures at doses only slightly higher than those required for antinociception, and the separation between antinociception and death in mice is relatively small [12]. In addition, repeated dosing appears to potentiate this toxicity [13]. In an experiment in mice, twice daily administration of 0.1 μmol/kg of epibatidine, a dose that does not produce seizures or death when given acutely, killed more than 60% of the animals by day 5 of treatment. Epibatidine also has marked effects on the cardiovascular system in dogs.

Epibatidine, therefore, is a toxic compound not suitable for clinical development. The toxicity of epibatidine also raises questions regarding the potential of nAChR-targeted compounds as analgesics, the overriding issue being whether nAChR-mediated antinociception and toxicity are somehow inextricably linked. The diversity of nAChRs elucidated over the last decade suggests that the diverse effects of nicotinic agents may be mediated through distinct nAChR subtypes, but this has not been established.

Neuronal nAChRs, like their neuromuscular nAChR counterparts, are composed of five subunits surrounding a cation channel (see Refs. 15 and 16 for reviews). Subunits found in neuronal receptors, however, differ from those found in neuromuscular receptors. At present, eight α subunits (α2–α9) and three β subunits (β2–β4) have been identified in vertebrates. Not all combinations of subunits form functional receptors. For example, α2, α3, α4, and α6 subunits require the presence of β2 or β4 subunits, and β subunits require the presence of α subunits. The precise subunit combinations of native nAChRs have not been determined, and few pharmacological tools are currently available to identify nAChR subtypes. Ligands are available, however, that distinguish between the two most abundant subtypes in brain. In rat brain, cytisine is thought to bind primarily to a nAChR subtype containing α4 and β2 subunits [17], whereas α-bungarotoxin has high affinity for a subtype believed to be a homomer made up of five α7 subunits [18]. α-Bungarotoxin also has high affinity for neuromuscular-type nAChRs, which are not present in brain.

Although ligand binding is not entirely predictive of agonist potency, an approximation of compound selectivity can be obtained by competition binding studies with cytisine and α-bungarotoxin in brain tissue and α-bunga-

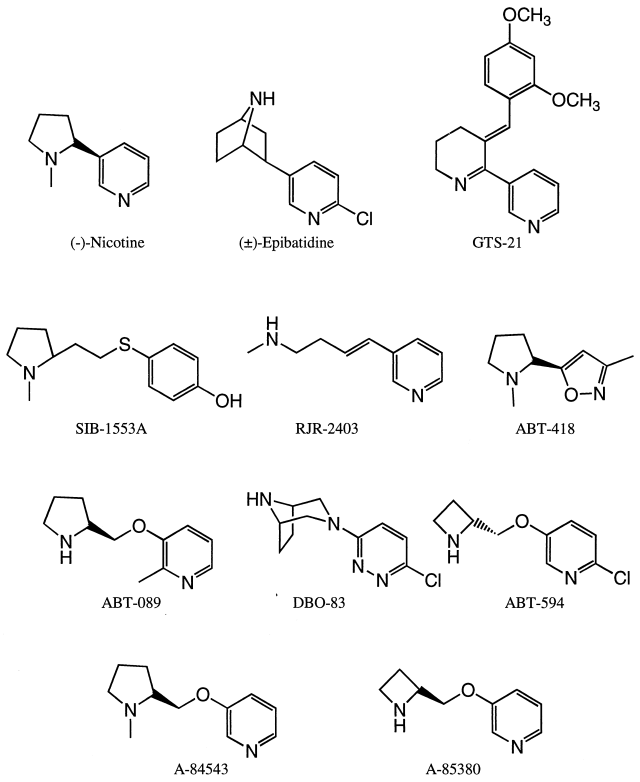

FIG. 1. Structures of some nAChR-targeted compounds.

rotoxin in *Torpedo* electroplax, a rich source of neuromuscular-type nAChRs. When these studies are conducted with epibatidine, it becomes clear that the compound has relatively high affinity for all three subtypes [11, 12, 19]. Epibatidine is at least 20 times more potent than (-)-nicotine at the α4β2-containing subtype in rat, but has more than 150 times higher affinity than (-)-nicotine at the α7-containing nAChRs in rat brain. Moreover, in *Torpedo* electroplax, epibatidine has more than 300 times the affinity of (-)-nicotine. Thus, epibatidine is a potent ligand at all of these nAChR subtypes. It is entirely possible, then, that the poor separation between doses of epibatidine producing toxic and beneficial effects is related to its apparent lack of selectivity.

Compounds, such as epibatidine, that are potent agonists at all nAChR subtypes may be expected to demonstrate little separation between beneficial and toxic doses. More selective compounds, however, may enjoy an improved therapeutic window. Precedence for the ability to separate beneficial and toxic effects of nAChR-targeted compounds has been established preclinically by several newly designed compounds intended as symptomatic treatments for Alzheimer's disease, including GTS-21, SIB-1553A, RJR-2403, ABT-418, and ABT-089 [20–28].

Additional compounds exhibiting nAChR-mediated antinociceptive properties have been described recently (see Fig. 1) [29]. A series of diazobicyclooctanes have been characterized, of which one analog (DBO-83) exhibited morphine-like potency and efficacy in preclinical pain models. These antinociceptive effects were blocked by mecamylamine but not naloxone, supporting a nAChR-mediated mechanism of action. The effects of DBO-83 on motor coordination and spontaneous motor activity differ significantly from epibatidine, suggesting that antinociception can be maintained with attenuated side-effect liabilities [30].

A second series of compounds, exemplified by ABT-594, that exhibit epibatidine-like potency in preclinical pain models with decreased side-effect liabilities have been described recently [31]. However, antinociceptive activity was not observed throughout this series, and it was poorly correlated with receptor affinity to the principal neuronal nAChR subtype (α4β2). For example, A-84543 exhibits subnanomolar affinity for the α4β2 binding site and is a full agonist at this site, but is inactive in rodent models of antinociception [32]. Two related compounds, A-85380 and ABT-594, exhibit nearly identical *in vitro* profiles in radioligand binding and functional assays [19, 33], yet ABT-594 exhibits broad-spectrum potent antinociceptive activity [34], whereas A-85380 is less potent and has a less attractive safety profile [35].

The optimized compound from a large series of pyridyl ethers, ABT-594, displays antinociceptive activity across a variety of rodent models of acute, persistent, and neuropathic pain [31, 34, 36–38]. This compound has affinity similar to that of epibatidine for the cytisine binding site in rat brain (K_i values = 0.037 and 0.043 nM for ABT-594 and (±)-epibatidine, respectively) [19]. However, ABT-594 has 60 times lower affinity for the α7 receptor in rat brain and 3000 times lower affinity for the neuromuscular-type receptor in *Torpedo* electroplax than epibatidine [19]. Thus, at least on the basis of binding affinity for these nAChR subtypes, ABT-594 shows enhanced selectivity relative to epibatidine. In *in vivo* testing, this enhanced selectivity appears to translate into an improved safety index. In mice, for example, ABT-594 has more than 5-fold greater separation between antinociceptive and lethal doses than does epibatidine [38]. However, whether this improved preclinical safety index means that the compound will be efficacious at nontoxic doses in humans has yet to be determined.

Whereas it is more selective than epibatidine, ABT-594 cannot be regarded as a "subtype-specific" compound. For example, the compound has similar potency and efficacy in cell lines expressing α3-containing and α4-containing nAChRs (EC_{50} = 340 and 140 nM in IMR-32 and K177 cells, respectively) [19]. Thus, the role of specific nAChR subtypes in producing antinociceptive or adverse effects is still uncertain, and the development of second-generation compounds will require extensive investigation of the mechanisms of nAChR-mediated antinociception.

Neuronal nAChRs are found throughout the pain pathway, so antinociceptive effects could, theoretically, be produced by actions at multiple sites. ABT-594, for example, can attenuate capsaicin-induced calcitonin gene-related peptide (CGRP) release *in vitro* from both the peripheral and central projections of primary afferents [19,

39]. The relevance of this observation to the antinociception produced by ABT-594 is unknown; but this effect may contribute to the demonstrated ability of ABT-594 to decrease the response of neurons in the dorsal spinal horn to noxious stimulation of the paw [34]. Notably, this latter effect can be produced by systemic injection or by direct injection into the spinal cord or the paw. Moreover, no effect on neuronal responses to innocuous stimuli is observed, suggesting that ABT-594 does not indiscriminately attenuate sensory processing.

Although actions in the periphery have been observed with ABT-594, the antinociceptive effects of the compound in animal models can be greatly reduced or even prevented by intracerebroventricular administration of the nAChR antagonist chlorisondamine [36]. Thus, it would appear that central nAChRs may be particularly important in producing the effects of this compound. This would be consistent with the ability to produce antinociception by direct injection of nAChR agonists into specific sites within the central nervous system [34, 40, 41].

Neurotransmitter release appears to play a critical role in producing the antinociceptive effects of nAChR activation. It is well established that nicotine can increase release of a number of neurotransmitters in the brain, including norepinephrine, dopamine, γ-aminobutyric acid, glutamate, serotonin, and acetylcholine [42–45]. Although the effects of nicotine on neurotransmitter release in the spinal cord have not been well studied, there is evidence that the antinociceptive effects of nicotine involve spinal release of several of these neurotransmitters. For example, direct injection of α_2-adrenergic, muscarinic cholinergic, or serotonergic antagonists into the spinal cord attenuates the antinociceptive effects of systemic nicotine or intracranial *N*-methylcarbachol [46, 47]. The effect of nicotine is not blocked completely by injection of a single antagonist, however: injection of combinations of antagonists is required for complete blockade of the effect. This latter observation suggests that nicotine activates multiple descending inhibitory pathways to produce antinociception.

Direct effects of nAChR agonists in the spinal cord may play a role in nAChR-mediated antinociception, but activation of descending inhibition originating in brainstem nuclei that project to the spinal cord is probably at least as important. Iwamoto [41] found several brain sites where nicotine could produce antinociceptive effects in the hot-plate and tail-flick tests. Of these, the NRM and the PPTgN were particularly sensitive. Cholinergic cells in the PPTgN project to the NRM, which is an important source of descending inhibitory input to the dorsal horn of the spinal cord. Activation of this projection, either by stimulation of the cell bodies in the PPTgN or the terminals in the NRM, plays an important role in the antinociceptive effects of the site injections of nicotine [41].

In addition to nicotine, a number of nAChR agonists, including *N*-methylcarbachol, epibatidine, ABT-594, and A-85380, produce antinociception when injected into the NRM [34, 35, 47, 48]. Moreover, systemic administration of ABT-594 activates neurons in the NRM, as assessed by c-fos expression [49]. The serotonin neurons in the NRM appear to mediate at least some of the antinociceptive effects of nAChR-targeted compounds. The α4-containing nAChRs are expressed by serotonergic neurons in this brain region [49]; and, as already noted, the antinociceptive effects of *N*-methylcarbachol injected into the NRM can be attenuated by spinal injection of serotonin antagonists [47]. In addition, lesions of descending serotonergic pathways produced by intra-NRM injection of 5,7-dihydroxytryptamine (5,7-DHT) significantly attenuate, but do not completely block, the antinociceptive effects of systemically administered ABT-594 [40]. The failure to block completely the effects of systemic ABT-594 with 5,7-DHT injected into the NRM suggests that other mechanisms are involved. Consistent with this latter interpretation is the finding that ABT-594 also activates the locus coeruleus, a source of noradrenergically mediated descending inhibition.

Activation of nAChRs in the NRM is antinociceptive in tests of acute thermal pain, such as the hot-plate and tail-flick assays; and the NRM, therefore, may play an important role in the antinociceptive effects of nAChR agonists. Activation of the NRM, however, does not appear to be required for nAChR-mediated antinociception, as the importance of the NRM varies as a function of the compound used. For example, inactivation of the NRM by a direct injection of the local anesthetic lidocaine completely prevents the antinociceptive effects of systemic administration of epibatidine or nicotine, but does not alter the effects of systemic administration of ABT-594 or A-85380 [35, 40]. One interpretation of these data is that the NRM mediates all of the antinociceptive effects of nicotine and epibatidine, but this seems unlikely. Nicotine can produce antinociception when injected into other brain regions [41], and both nicotine and epibatidine can produce antinociception when injected directly into the spinal cord [50, 51].

A more likely explanation of the differences in the impact of NRM inactivation on the antinociceptive effects of systemic nicotine or epibatidine and those of systemic ABT-594 or A-85380 may be related to differences in the balance of excitatory and inhibitory effects of these compounds on pain sensation. Neuronal nAChR agonists, like opioid agonists, can have both analgesic and hyperalgesic effects, depending on the specific site of injection in the central nervous system [52]. Since the summation of these effects probably determines the overall effect of systemic administration of these compounds, inactivation of an important "analgesic center," such as the NRM, could shift the balance enough to allow analgesic and hyperalgesic influences to cancel each other out. With epibatidine and nicotine, perhaps this is the case; with ABT-594 and A-85380, it is possible that removal of the NRM from the equation is not sufficient to shift the balance because the compounds have diminished activity at "hyperalgesic centers." If hyperalgesic and analgesic effects are mediated by

actions at different subtypes and/or release of different neurotransmitters, it is possible that more selective compounds could be developed with improved efficacy. In this regard, it is notable that intrathecal administration of epibatidine has been reported to produce behavioral signs of both antinociception and irritation, effects that have different time courses and different sensitivities to nAChR antagonists [51].

If it can be established that nAChR-targeted compounds can be sufficiently efficacious to warrant development as analgesics, several other issues must be addressed if the analgesic potential of this class of compounds is to be exploited fully. The sites and mechanisms of action need to be better understood. This is true not only for the antinociceptive effects of the compounds, but also for the adverse effects.

In particular, it will be important to determine if activation of specific nAChR subtypes is responsible for the antinociceptive effects. This task is made all the more difficult by our inability to assign a precise subunit composition to native nAChRs and our uncertainties regarding the validity of expressing subunit combinations in artificial systems, such as frog oocytes. Furthermore, the dearth of subtype-selective compounds makes it difficult to address this problem using traditional pharmacological approaches. Instead, this work likely will rely on alternative approaches, such as the use of subunit-selective antisense and knockout animals. Even then, the possibility exists that differences between rodent and human receptors may undermine the utility of preclinical models. Moreover, the mechanistic studies conducted to date have largely focused on a single class of pain—acute thermal pain. It is entirely possible that the mechanisms, and thus the nAChR subtypes, involved in the antinociceptive effects of nAChR agonists may vary as a function of the type of pain measured.

Another challenge for development of nAChR-targeted analgesics is the negative view of nicotine itself that stems from its presumed role in maintaining smoking behavior. Two issues are relevant here—the attitude of the public regarding the acceptability of compounds related to nicotine and the possibility that these compounds will be addictive.

Nicotine is widely viewed as a toxic substance. Although nicotine is toxic, the toxicity of the compound perhaps is exaggerated by the tacit assumption that the toxicities of smoking and those of nicotine are equivalent. Nicotine, of course, is only one of many compounds present in cigarette smoke, and the idea that nicotine is responsible for all of the negative consequences of smoking seems naive, although its critical role in maintaining this destructive behavior cannot be denied. The mechanisms of nicotine addition are not known, and the assumption that all nAChR-targeted compounds would prove to be addictive is unwarranted. There is some evidence that the mode of administration is important in establishing addiction. Part of the reason that smoking is addictive is probably related to the relatively rapid delivery of nicotine achieved by this method of administration [53]. In addition, the behavior of smoking itself may play a role in maintaining a "psychological" addiction. Indeed, it is not clear that slow, passive administration of nicotine, such as via the transdermal patch, has the same addictive potential as delivery of nicotine by smoking [53]. Furthermore, the psychopharmacology of nicotine differs from that of many other addictive substances. Nicotine does not produce euphoria or disrupt perceptual processing or judgment. Thus, nicotine is not generally regarded as a "recreational" drug or an intoxicant. Driving under the influence of nicotine, for example, is not illegal. These differences may explain some of the difficulties encountered when evaluating nicotine using preclinical models developed using other addictive substances. Preclinical tests such as place-preference yield inconsistent results [54, 55], and it is more difficult to train rats to self-administer nicotine than to self-administer drugs such as heroin or cocaine [56]. This makes preclinical evaluation of nAChR-targeted agents more difficult.

Based on the available data, development of nAChR-targeted compounds as analgesics represents an emerging area of research in pain control. There are some human experimental data confirming that nicotine can produce modest analgesic effects [57, 58], effects that are consistent with the modest effects produced by nicotine in preclinical models. Whereas the human data probably would not support the development of nicotine itself as an analgesic, these data provide at least some level of validation of the approach and of the preclinical data. Compounds with greater preclinical efficacy and potency, such as epibatidine and ABT-594, have not been evaluated in humans, so we do not yet know if the more impressive preclinical data with these compounds will translate into improved clinical efficacy. Moreover, it is unlikely that clinical studies will be conducted with epibatidine, given its toxicity; and although ABT-594 appears to be significantly less toxic than epibatidine in preclinical studies, it is not known whether or not this compound will be safe and effective in humans. Clearly, clinical data with nAChR-targeted compounds other than nicotine will be important to establish the potential of this approach to the development of novel analgesics.

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