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Epibatidine and pain

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In 1992 the chemical epibatidine (fig. 1), isolated from the skin of the Equadorian poison-arrow frog *Epipedobates tricolor*, was shown to be 200 times more potent than morphine in blocking heat nociception in the mouse. The study was carried out using the hotplate test, which measures the time taken for a mouse to show a behavioural response (jumping or paw licking) when stood on a heated plate maintained at 55°C.[63] Epibatidine had been described 10 years earlier by Daly and colleagues at the National Institutes of Health, Bethesda, USA, but had been isolated in such small amounts that it could not be characterized. However, so potent was the antinociceptive effect of the compound – less than 5 $\mu g\ kg^{-1}$ i.p. causes an antinociception response in the mouse – that the effect was used as a bioassay during the further isolation and purification of epibatidine. The antinociceptive effect of epibatidine was not blocked by the opioid antagonist naloxone, suggesting a therapeutic action different from that of morphine and a potentially novel therapeutic mechanism of providing pain relief. This was an exciting finding because it opened up the possibility of a new therapy, without the adverse effects of opiates, for the treatment of severe pain. However, epibatidine was extremely toxic, causing hypertension, respiratory paralysis and seizures, with death occurring at doses not much higher than those required for antinociception.[11 65] Extensive modification of the compound would be necessary to provide a useful therapeutic agent. Such was the interest in epibatidine that by 1993 synthetic material was available in larger amounts.[14 36] Synthesis of the optical isomers of epibatidine quickly followed,[28] potentially offering an important advantage in describing the pharmacology of the compound. Epibatidine is the exo-isomer of the two possible geometric isomers (fig. 1) and can exist as two enantiomers (+)- or R-epibatidine and (−)- or S-epibatidine. The natural compound is the (+) isomer,[72] although there is little difference in pharmacological activity between the (+) and (−) isomers.[6 20 72] The endo-isomer (fig. 1) is inactive.[27 58] These advances in synthesis of epibatidine's isomers made it possible to discover more about this exceptionally potent compound.

A news article in *Science* in 1993[12] stated that the "race is on to turn the potential (of epibatidine) into reality". In this review, I shall consider the progress that has been made towards realizing this goal, including the development of less toxic analogues, such as ABT-594, that retain a wide spectrum of antinociceptive actions.[9]

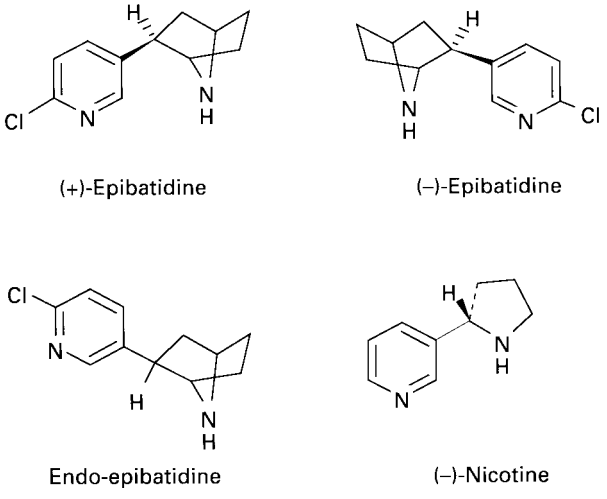

Figure 1 Structure of epibatidine isomers.

Epibatidine and analgesia

ANTINOCICEPTION

In spite of the high level of interest in this compound, the antinociceptive activity of epibatidine has been investigated only in rodents. The effect of epibatidine was originally observed in the hotplate assay, where at 2.5 $\mu g\ kg^{-1}$ i.p. the compound causes significant hotplate "analgesia".[63] This compares with a value of approximately 10 $mg\ kg^{-1}$ for morphine in the same test. Most studies on this compound have used a similar hotplate technique[6 10 58 65] (fig. 2) but a further heat nociceptive test, the tail-flick test, which measures latency to removal of the tail from a radiant light source, has also been used.[6 21 56] Other tests applied have been footshock, where the nociceptive stimulus is an electric current and the end point is vocalization,[11] and carrageenan-induced hyperalgesia in the rat hindpaw.[58] In all these experimental paradigms epibatidine is approximately equi-active and equi-efficacious with morphine (fig. 2), except in the footshock vocalization assay, where it was observed to be less efficacious. There have been no reports of the

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use of epibatidine as an antinociceptive agent in non-human primates or as an analgesic agent in humans, presumably because of its low margin of safety.

Mode of action

The original reports on epibatidine showed that the antinociceptive action of the compound was not reversed by naloxone, although in mice the drug elicited a Straub tail response; this response is customarily used as a indicator of morphine-like action, that is, activation of μ-opioid receptors. However, synthetic (+)- and (−)-epibatidine do not produce a Straub tail response,[11 58] suggesting that the observation with the natural material may be a toxic syndrome produced by high doses, or attributable to other substance(s) present in the frog skin extracts. Furthermore, a Straub tail response is induced in mice by several other drugs, including agonists acting at nicotinic and 5-hydroxytryptamine (5-HT) receptors. Thus, there is no pharmacological evidence for an opioid mechanism of action for epibatidine and in confirmation of this there are numerous reports that the compound does not bind to opioid receptors.[6 56 58 65] In fact, the compound shows no affinity for a variety of neuronal receptors including benzodiazepine, serotonergic, dopaminergic, adrenergic and $GABA_A$ receptors, and receptors for bradykinin, CCK, excitatory amino acids, CGRP and neurokinin.[6 56 58 65] Epibatidine does have low affinity for the muscarinic acetylcholine receptor but its binding profile suggests antagonist action at this site.[58]

On the other hand, the similarity of the structure of epibatidine (fig. 1) to that of nicotine was soon recognized and it was subsequently demonstrated that the compound has high affinity for nicotinic acetylcholine receptor (nAChR) binding sites labelled by [^{3}H]nicotine or its derivatives.[6 56 58 65] Nicotine has long been recognized to be active in tests for antinociception,[46] although its low activity and poor side-effect profile precluded any further development. The antinociceptive actions of nicotine are prevented by the nicotinic cholinergic receptor channel blocker mecamylamine, but not by the ganglion blocker hexamethonium, which does not pass into the central nervous system; this suggests a central mechanism of action.[1 60 67] In confirmation of this, the quaternary methiodide derivative of nicotine is inactive after s.c. administration but active after i.c.v. administration.[1] Direct injection of nicotine into the central nervous system enhances potency when compared with peripheral administration,[38 60] and antinociception correlates with nicotine concentration in mouse brain.[67]

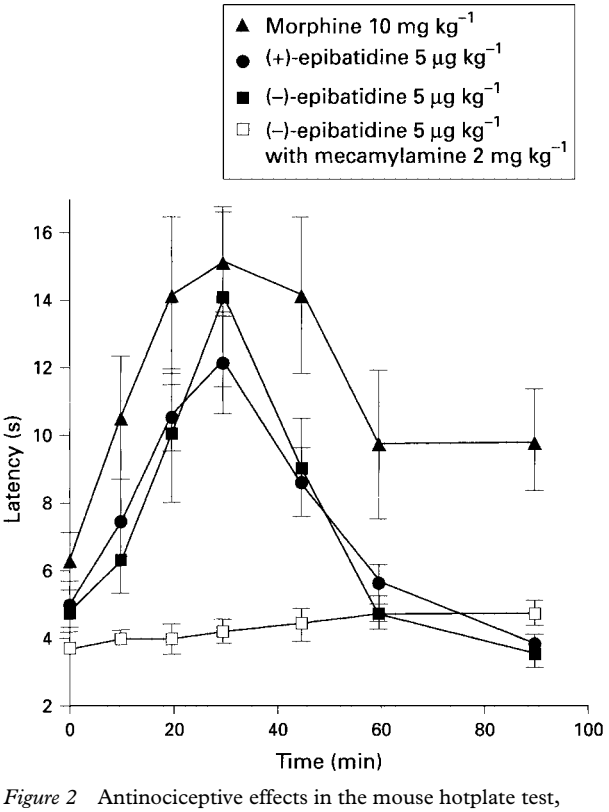

Figure 2 Antinociceptive effects in the mouse hotplate test, measured as time to appearance of the first behavioural response. All drugs were administered i.p. Figure drawn from data in reference 6.

The antinociceptive properties of epibatidine are remarkably similar to those of nicotine in most respects. Antinociception elicited by epibatidine is prevented by mecamylamine,[8 10 11 20 56–58 65] but not by hexamethonium.[20 56] There is one report that hexamethonium partially blocks the action of epibatidine in the tail-flick assay in the rat,[57] which indicates a possible peripheral component in addition to a central component; caution should be used in this interpretation, however, as quaternary compounds like hexamethonium may cross the blood–brain barrier in sufficient amounts to act centrally.[5 24]

Although epibatidine has qualitatively similar antinociceptive properties to those of nicotine, it does show some differences. Whereas nicotine exhibits tolerance to its antinociceptive action after acute or chronic administration, there is no substantial tolerance developed to epibatidine, at least in mice.[21] In addition, epibatidine is considerably more potent. In the various antinociceptive tests carried out in rodents, epibatidine is effective as an antinociceptive agent at approximately 5 μg kg^{-1}; nicotine is approximately 200–300 times less potent, given by various routes,[6 20 56–58] and morphine is approximately 1000 times less potent. The antinociceptive effect of both epibatidine and nicotine is rapid in onset (3–5 min) but whereas the action of nicotine is short-lived (10 min) the effect of epibatidine lasts up to 1 h.[10 56] Epibatidine readily enters the brain after tail-vein administration in the mouse, with high levels in the thalamus and superior colliculus. Maximum concentrations are attained at 30 min and epibatidine is still present at 4 h, showing slow clearance from the brain.[42] This does not appear to relate to the rapid onset and relatively short duration of action reported by several groups. Indeed, after direct central (i.c.v.) administration higher concentrations of epibatidine are required than when the compound is given peripherally (s.c.).[58] On the other hand epibatidine also shows an antinociceptive response after intrathecal administration at the same doses that are active after s.c. administration.[20 40] This may indicate a mechanism of action primarily at the spinal level. However, supraspinal activation of descending inhibitory pathways is a common mechanism for blocking pain transmission at the spinal level and so preventing impulses reaching higher centres. The

presence of a central nicotinic cholinergic pathway that modulates antinociception by activation of descending inhibitory pathways has been proposed. At the spinal level this seems to involve α-adrenergic and serotenergic as well as cholinergic systems.[37] The potential role of these transmitters is discussed later.

The above discussion confirms that the antinociceptive action of epibatidine is mediated by activation of nAChR. However, nicotinic agonists have a multitude of effects through interaction with central and peripheral (ganglionic and neuromuscular junction) nAChR. Unfortunately, from a drug therapeutic viewpoint, epibatidine shows a full spectrum of nicotinic effects, with changes seen in animal activity, body temperature and cardiovascular and respiratory systems at doses comparable with those affording antinociception. For example, in the hotplate test, significant antinociception in mice is associated with the same dose range of epibatidine as temperature and locomotor effects,[10 27 40 65] which are also thought to involve central nAChR.[40 45] This wide spectrum of actions attributable to epibatidine is a problem from two viewpoints. First, it makes the compound unacceptable as an analgesic agent for clinical use and second, as measures of antinociception invariably involve a behavioural end-point (that is, withdrawal of tail or foot) in response to a heat stimulus, then effects on several nicotinic systems could cause problems in interpretation of data from animal experiments. In particular, if motor activity is decreased then an increased latency to the behavioural response after the nociceptive stimulus will be seen and interpreted as an "analgesic" response. Motor defects could be contributing to, or even responsible for, the observed antinociception. Consequently, there is doubt as to whether real analgesia would be seen with these compounds in man.

It is therefore important to be able to separate any real antinociceptive action of epibatidine from motor and temperature effects in animal experimental models. Although effects on rodent activity are seen at antinociceptive doses it has been suggested that motor impairment may not be evident at these levels and therefore may not interfere with the behavioural measures.[63] In support of this, the reduction in motor performance has a slightly different time course from antinociception, which may indicate that locomotor changes are not entirely responsible for the antinociceptive response.[10] Furthermore, the antagonist mecamylamine blocks the effects of epibatidine on activity, temperature and antinociception when given before the epibatidine but when given after epibatidine blocks only the latter two effects and may enhance the nociceptive response.[10] This indicates a separation of antinociception from other, interfering, nicotinic effects of epibatidine, and perhaps a role for different nAChR in the various responses. Indeed, there are several lines of pharmacological evidence for differential receptor interactions at central nAChR. Some nicotinic agonists have antinociceptive properties in the mouse, whereas other have not been reported to show such activity.[23] In rats i.t. epibatidine affords a nociceptive response, which may be caused by the release of excitatory amino acids, at lower doses than its antinociceptive effect.[40] Unlike nicotine, epibatidine shows no evidence of anxiolytic activity, which again indicates different nAChR, although it is possible that toxic effects mask any anxiolytic activity.[65] However, the best pharmacological evidence for the activation of different subtypes of nAChR would come from studies with antagonists. There are two types of blockers of central nAChR: competitive, which act at the acetylcholine binding site and may be typified by dihydro-β-erythroidine; and non-competitive channel blockers such as mecamylamine. Mecamylamine has no selectivity but dihydro-β-erythroidine has some differential activity across central nAChR.[2 33] Both antagonists reduced the effect of epibatidine in the mouse hotplate test, though dihydro-β-erythroidine may be less effective[6 58] and does not appear to inhibit the antinociceptive response to i.t. epibatidine.[40]

All these differences support the concept that action at heterogeneous nicotinic receptor types within the central nervous system, either in terms of receptor recognition or efficacy of the different ligands, may mediate different actions and that targeting specific receptor types may provide leads to develop selective agents.

Epibatidine and nicotinic receptors

NICOTINIC RECEPTORS

Biochemical and molecular studies of nAChR indicate a high potential for subtype diversity within this class of receptors. Nicotinic receptors for acetylcholine are members of the ligand-gated ion channel superfamily. The nAChR is a protein complex composed of five peptide subunits that provide a ligand binding site and which make up an ion channel that allows the passage of Na^+ and Ca^{2+} ions, after agonist binding. Each of the five subunits is comprised of an extracellular, relatively hydrophilic *N*-terminal domain where acetylcholine binds, three hydrophobic transmembrane domains a larger intracellular loop and a fourth hydrophobic transmembrane domain.[47] Four different types of peptide subunit can make up the channel, namely α, β, γ and δ. The neuromuscular junction nAChR is composed of α, β, γ and δ subunits and is termed the α1β1γδ receptor. However, δ and γ subunits are not found in the central nervous system, where only α and β subunits have been described, although these have a somewhat different amino acid composition from the α and β subunits that are part of the neuromuscular junction receptor. Indeed, in brain tissue from several species, including human, genes coding for 11 different subunits have been described.[41 47] These 11 genes code for eight α-subunits (α2–9) and three β-subunits (β2–4). Consequently receptors could exhibit a great deal of molecular diversity, whether composed of a selection of these α and β subunits or even of α-subunits alone. The subunits that make up the receptor govern its functional properties and pharmacological properties. The binding site for acetylcholine is on an α-subunit, but it is the particular combination of subunits, and consequently of amino acid residues lining the channel, that controls the properties of the channel and of the receptor, such as ligand specificity, ion selectivity, open times and rate of desensitization.

In spite of the extensive molecular diversity theroetically made possible by combination of the

eight α-subunits and three β-subunits, only three different recognition sites have been described in brain homogenates using ligand-binding techniques.[44 61] These are sites with high affinity for (−)[^{3}H]nicotine (affinity 0.5–5 nM), sites with high affinity (0.5 nM) for α-bungarotoxin and sites with high affinity for neuronal bungarotoxin. At these latter two sites (−)-nicotine has affinity at the μM level. [^{3}H]epibatidine binding to homogenates of brain[35] and spinal cord[40] shows further heterogeneity with high-affinity (40–70 pM) and low-affinity (230–360 pM) sites. Neither of these sites appears to be the α-bungarotoxin binding sites. The high-affinity binding sites for [^{3}H]nicotine show a relationship with the distribution in brain of an nAChR consisting of the α4β2 subunit combination. In fact 90% of high affinity [^{3}H]nicotinic binding sites in rat brain can be precipitated by antibodies raised against α4 and β2 subunit peptides, suggesting that this is the predominant nAChR receptor in brain.[29 75] Similar studies indicate that the receptors defined as α-bungarotoxin binding sites may be composed only of α-7 subunits. These sites appear to be highly permeable to Ca^{2+} ions; they quickly activate and inactivate and may play an important role in synaptic transmission.[3] Another subunit combination that is of special relevance to studies of epibatidine is the α3-containing receptor combined with either β2 or β4, which is thought to be the ganglionic-type nicotinic receptor.[43]

As in many fields of neuroscience, the study of nAChR has benefited enormously from the ability to express individual receptor subunits in *in vitro* cell systems and in different combinations. Ligand binding to specified subunit combinations can then be studied in isolation, and the efficacy with which different ligands activate these different subunit combinations to alter cellular events can be measured. From these studies it has been shown that the number of possible receptors is restricted, because not all are able to make functional receptors. In fact just 11 different functional receptors have been identified by co-expression methods using cDNAs for different subunits. There are several recent comprehensive reviews on different nAChR.[13 17 41]

Epibatidine actions at nicotinic receptors

At central nAChR, epibatidine has much higher affinity than acetylcholine or nicotine (table 1). At the predominant α4β2 receptor[32 66] (+)- and (−)-epibatidine have very high affinity, around 20 times greater than that of (−)-nicotine. In addition, epibatidine is a potent agonist at the α4β2 receptor, being some 200 times more potent than (−)-nicotine, and also more efficacious[32] (table 2). Like nicotine, epibatidine has less affinity for the α7 receptor (300-fold less than its affinity for the α4β2 subtype), but this is still 100 times better than the affinity of nicotine for this receptor (table 1) and epibatidine is about 40 times more potent than nicotine as an agonist at this receptor[32] (table 2).

Epibatidine differs from acetylcholine and nicotine in possessing high affinity for peripheral nicotinic receptors, the α1β1δγ neuromuscular receptor and the α3 subunit containing receptors that constitute the ganglionic-type receptors (table 1). At both of these nAChR types, epibatidine is a potent agonist

Table 1 Affinity (*K*i) of epibatidine isomers for different nicotinic acetylcholine receptors (nAChR) *in vitro*

	*K*i (nM)			
	α4β2	α7	α3β2/4	α1β1δγ
(−)-Epibatidine	0.06	16	0.15	5.7
(+)-Epibatidine	0.05	22	0.23	2.5
(−)-Nicotine	1.0	2000	5	10 000

All receptors are human clones except torpedo α1β1δγ. Data from reference 66 except α3β2/4 data, which are from references 31 and 43. Values are from receptors in different expression systems, so comparisons can be only approximate.

Table 2 Agonist potency of epibatidine at different nicotinic acetylcholine receptors (nAChR) *in vitro*

	EC_{50} (nM)			
	α4β2	α7	α3β4	α1β1δγ
(±)-epibatidine	17	1100	19	1600*
(−)-nicotine	4000 (PA)	40 000	14 000	250 000

Data are from references 32, 55 and 31, and are for human receptors except α1β1δγ (torpedo). Values are from receptors in different expression systems and using different measures and so should be compared approximately. PA = partial agonist. *Data for (+)-epibatidine.

(table 2). At the ganglionic-type nAChR endogenously expressed in the human neuroblastoma cell line IMR-32,[43] epibatidine is >1000-fold more potent than (−)-nicotine and at α3β4 receptor injected into oocytes, epibatidine is approximately 1000 times more potent than nicotine or acetylcholine with an EC_{50} of around 20–130 nM.[31] These *in vitro* data agree with findings that show that epibatidine behaves as a potent agonist in isolated preparations of rat and guinea-pig autonomic ganglia[11] and is a potent ganglionic agonist *in vivo*, leading to nicotinic-like cardiovascular effects and respiratory stimulation in the anaesthetized rat that are reversed by the ganglion-blocker trimethaphan.[27] At high doses the compound produces a depolarizing blockade. In contrast, no effect was observed on neuromuscular transmission, suggesting the compound has higher potency at ganglionic as compared with neuromuscular nicotinic receptors. This agrees with findings *in vitro*, where the potency of epibatidine is at least 10 times higher at receptors containing α3 subunits than at those containing α1 (neuromuscular-type) subunits[31] (table 2). Even so, epibatidine has high affinity in the low nM range for the neuromuscular receptor type and has the highest affinity of all nicotinic agonists at the α1β1δγ neuromuscular receptor.

One perhaps surprising difference between nicotine and epibatidine is in the stereochemistry of the interaction with neuronal nAChR. In addition to labelling more sites in central nervous tissue, epibatidine binds and acts at the nAChR in a non-stereoselective fashion.The (−)- and (+)-isomers have similar affinity and similar potency in pharmacological assays and as antinociceptive agents.[6 20 58] In contrast (−)-nicotine binds 20 times more effectively to nAChR than the (+)-isomer. However, this appears

to be a property specific to *N*-methyl substituted compounds, like nicotine, because (−)- and (+)-nornicotine are not differentiated in binding to nAChR.[77]

Visualization of central nicotinic receptors with epibatidine

While nicotine and related compounds label only subsets of nicotinic receptors, epibatidine labels a much wider range of nAChR. This ability of [^{3}H]epibatidine to label nicotinic receptors has been used to map nicotinic receptor sites in brain.[53] The distribution of nicotinic binding sites in the central nervous system, labelled by [^{3}H]nicotine, shows sites in layers III and IV of the cerebral cortex, the thalamus, interpeduncular nucleus and superior colliculus, with lower levels in the hippocampus and hypothalamus and layers I and IV of the cerebral cortex.[16] This probably reflects the distribution of the predominant $\alpha4\beta2$ receptor. The binding of [^{3}H]epibatidine closely follows this pattern but in addition [^{3}H]epibatidine labels areas in the visual system (optic nerve, optic chiasm, optic tract, pretectal nuclei, superficial grey of the superior colliculus), and areas that are thought to be involved in the integration of olfactory and sensory signals (medial habenula, fasciculus retroflexus). It is possible that this represents binding to receptors containing the $\alpha3$ subunit.[53] In fact [^{125}I]epibatidine labels nicotinic receptors in superior cervical ganglia that do not express mRNA coding for $\alpha2$ or $\alpha4$ subtypes and are likely to contain the $\alpha3$ subunit. The distribution of sites labelled by neuronal bungarotoxin and α-bungarotoxin are very different, suggesting these are not labelled by [^{3}H]epibatidine.

Derivatives of epibatidine such as [^{125}I]IPH[22 52] and [^{18}F]FPH,[34] in which the C1 substituent is replaced by [^{125}I] or [^{18}F] respectively, match the distribution of [^{3}H]epibatidine. These and [^{11}C]-*N*-methylepibatidine[73] may be useful for *in vivo* imaging of brain nicotinic receptors. [^{3}H]epibatidine itself has been used to show selective losses of receptors in brains from patients with Alzheimer's disease, suggesting that the $\alpha4\beta2$ subtype might be the most vulnerable in this disease.[71]

Mechanism of antinociceptive action of epibatidine

NEUROTRANSMITTER RELEASE

Evidence has been provided to show that epibatidine acts as an antinociceptive agent through central nAChR, and that such receptors also mediate many other actions of nicotinic agonists. As discussed above, the antinociceptive properties may not be real because behavioural end-points are used in the animal tests, Nevertheless, it is important to understand what effects different central nAChR mediate, and how this could explain the observed antinociceptive response. In particular, activation of nicotinic receptors has been shown to cause the release of neurotransmitters. Epibatidine and other nicotinic agonists have been reported to release catecholamines by a Ca^{2+} dependent mechanism,[4 59] excitatory amino acids[40] and 5-HT.[74] As pathways involving these transmitters have been implicated in nociceptiye processing,[26] such effects may contribute to the antinociceptive actions of nicotinic agents.

Epibatidine produces a concentration-dependent increase in [^{3}H]dopamine release from rat striatal slices and [^{3}H]norepinephrine release from slices of hippocampus and thalamus.[59] The release profile is similar to that of (−)-nicotine with similar maximal effects, although epibatidine is 50–4000 times more potent, depending on the response measured. This difference is caused by the differential activity of nicotine across the different measures, because epibatidine is equally potent for each effect. The release of neurotransmitters in these experiments was blocked by mecamylamine and the release of norepinephrine, but not dopamine, was blocked by tubocurare. A similar profile is seen with nicotine-evoked release of norepinephrine.[76] Dihydro-β-erythroidine has the opposite profile. These antagonist studies indicate that different nAChR subtypes may be involved.

A role for adrenergic systems in the antinociceptive action of epibatidine has to be confirmed. However, antinociceptive responses to epibatidine and nicotine in the tail-flick test are significantly attenuated by pretreatment with phenoxybenzamine to remove adrenergic receptors and by *N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), a neurotoxic agent leading to a reduction in norepinephrine concentrations, some reduction in 5-HT concentrations but no reductions in dopamine concentrations.[57] This implicates noradrenergic systems in the antinociceptive actions of epibatidine and nicotine. In a similar tail-flick procedure yohimbine, a non-selective adrenergic antagonist, is reported not to block epibatidine-induced antinociception in mice or rats,[56] although others have shown an inhibition of nicotine-induced antinociception by yohimbine.[15 67] Thus the actual role of adrenergic receptors remains unclear.

The pharmacology of dopamine release from striatal slices suggests a role for nAChR different from norepinephrine release. However dopamine is unlikely to be involved in the antinociceptive response to epibatidine because nicotine-induced antinociception is not blocked by the dopamine DA1-receptor antagonist SCH23390 or the DA2-receptor antagonist sulpiride.[19] Rather, dopamine is important in other aspects of epibatidine's nicotinic pharmacology. *In vivo* epibatidine induces turning behaviour and an increase in locomotor activity, at least in some models, which are blocked by D1 and D2 antagonists.[59] The time course of these responses is relatively slow, occurring 15 min after injection and lasting for 60 min. Moreover, the doses of epibatidine needed are large compared with those that give an antinociceptive response. In addition, animals trained to discriminate nicotine recognize epibatidine as a full agonist 190 times more potent than nicotine itself. This nicotine cue probably involves dopamine release.

Opioids are well known to mediate their effects partly by inhibiting the release of nociceptive neurotransmitters, in particular substance P [see reference 62 for review] from primary sensory afferent neurons in the dorsal horn of the spinal cord. Nicotinic receptors are well represented in this area[39 78] and it is

conceivable that a similar mechanism could exist for these compounds. However, in peptidergic cells expressing ganglionic-type receptors, epibatidine enhances the release of substance P in a mecamylamine-sensitive manner.[55] This could be the mechanism behind the pain response seen after i.t. epibatidine,[40] and may conceivably be responsible for the antinociceptive action by depleting stores of nociceptive neurotransmitters. In addition, these receptors desensitize very rapidly and thus desensitization of neurons to noxious stimuli may be a potential antinociceptive mechanism.

ROLE OF CALCIUM

Although the role of Ca^{2+}-dependent neurotransmitter release in the antinociceptive action of epibatidine is not proven, there is direct evidence of a role for Ca^{2+} ions in the antinociceptive response to nicotinic agonists. Agents that raise intracellular calcium, namely the calcium channel agonist (±)BAY K 8644 (voltage-dependent channels), glyburide (ATP-gated K^+ channels), thapsigargin (intracellular stores) and A23187 (extracellular stores), when given intrathecally, all significantly potentiate antinociception afforded by nicotine given s.c. in the mouse tail-flick test, without causing an antinociceptive action themselves at the doses and times used.[18] Nicotine antinociception is also blocked by nifedepine, again indicating involvement of L-type Ca^{2+} channels, although the interaction is probably indirect.[18] Similarly the Ca^{2+} channel activator (−)BAY K 8644 enhances epibatidine antinociception, and its isomer (+)BAY K 8644, an L-type Ca^{2+} channel blocker, inhibits the response.[8] The antinociceptive actions of epibatidine are not blocked by the α-bungarotoxin-sensitive antagonist methyllycaconitine, suggesting that these receptors are not involved in the antinociceptive action of epibatidine.[57] As discussed earlier, these are likely to be α7-type receptors that are highly permeable to Ca^{2+} ions. Neuronal nAChR are known to be highly permeable to Ca^{2+} ions, although less than α7-type receptors.[50 68 69] Moreover, external Ca^{2+} potentiates agonist action at neuronal nAChR by enhancing ionic current amplitudes and agonist affinity, probably by binding to an allosteric site on the receptor.[30 51] In addition to neurotransmitter release these Ca^{2+} fluxes may also be involved in longer-term plasticity changes.[50 69]

The future for epibatidine and nicotinic analgesics

Because of the undesirable pharmacological properties of opioids and public debate about their use, inadequate doses of these drugs are often prescribed and given.[64] There is no doubt that there is considerable interest in nicotinic agonists as analgesic agents that would present a clinical profile very different from that of the morphine-like analgesics. The wide diversity of nAChR suggests that the many nicotinic actions are mediated by different receptors. An understanding of these receptors and the roles they play should allow specific agonists and antagonists to be designed. For example, the isoxazole analogue of epibatidine, named epiboxidine (fig.3), has approximately 10-fold lower affinity than epibatidine for [^{3}H]nicotinic binding in rat cortical membranes.[7] In the hotplate assay the compound is about 10-fold less potent than epibatidine and at a maximal antinociceptive dose is considerably less lethal. In contrast, at the ganglion-type receptor in PC12 cells, epiboxidine has similar potency to epibatidine. Thus binding to central sites recognized by [$^+$H]nicotine, which represents the predominant α4β2 type, appears to be related to antinociception. Therefore one way to obtain an improved side-effect profile is to reduce the potency of epibatidine for receptors other than the α4β2 subtype. Considerable strides towards this aim have been made. Two compounds, A-85380[66] and ABT-594[9] (fig. 3) have the same affinity as epibatidine for the α4β2 subtype but have, respectively, >3000 or $>100\,000$ lower affinity for the α7 and neuromuscular receptor subtypes. With A-85380, the functional activity seen at the ganglion-type receptor expressed in IMR cells is at a similar level to activity at the α4β2 receptor expressed in HEK cells,[66] although ABT-594 is reported to show reduced cardiovascular effects.[9] In rats, ABT-594 is approximately equi-efficacious with morphine in three different models of pain, in a mecamylamine-reversible manner. The compound is effective in a heat nociception assay, in blocking the second phase of the pain response after injection of formalin into the hind paw, a model for persistent pain, and in an allodynia model of neuropathic pain. Surprisingly, in view of the findings with epibatidine in peptidergic cells,[55] ABT-584 does inhibit capsaicin-induced release of substance P from spinal cord slices, and in dorsal horn neurons selectively inhibits activity in neurons activated by thermal and mechanical stimuli, but not by non-noxious stimuli. In addition the compound may activate descending pathways after injection into the brain stem.

Compounds like ABT-594 represent a major advance in the potential of nicotinic ligands to be developed as analgesic agents. It must be borne in mind that, although selectivity for central nAChR of the α4β2 type confirms that antinociception occurs *via* a centrally mediated mechanism, many of the other effects of nicotinic agonists are also centrally mediated. In addition, it is difficult to separate antinociceptive effects of nicotinic agonists from behavioural effects and antinociception with these compounds has been measured only in rodents. Therefore it is an open question whether analgesia without adverse nicotinic effects will be seen in humans with this class of drugs. In man, studies have suggested that tobacco smoking may invoke an analgesic effect,[25 54] although others show that smoking

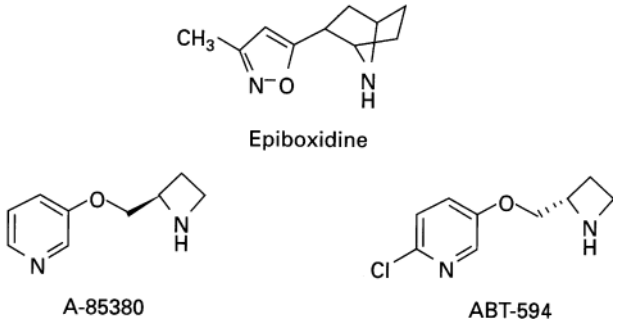

Figure 3 Epibatidine analogues.

has no analgesic effect[70] and may heighten sensitivity to pain.[48 49] ABT-594 will soon be undergoing safety trials in humans and may then undergo efficacy trials. The outcome of such studies with a highly efficacious (and, it is hoped, safe) nicotinic agonist will tell us whether such agents will be added to the armamentarium of the pain clinic. If they are, then the frog from Equador has done mankind a great service.

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References

1. Aceto MD, Awaya H, Martin BR, May EL. Antinociceptive action of nicotine and its methiodide derivatives in mice and rats. *British Journal of Pharmacology* 1983; **79**: 869–876.
2. Alkondon M, Albuqueque EX. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *Journal of Pharmacology and Experimental Therapeutics* 1993; **265**: 1455–1473.
3. Alkondon M, Reinhardt S, Lobron C, Hermsen B, Maelicke A, Albuquerque E. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. II. The rundown and inward rectification of agonist elicited whole cell currents and identification of receptor subunits by in situ hybridization. *Journal of Pharmacology and Experimental Therapeutics* 1994; **271**: 494–506.
4. Arqueros L, Naquira D, Zuinino E. Nicotine-induced release of catecholamines from rat hippocampus and striatum. *Biochemical Pharmacology* 1978; **27**: 2667–2674.
5. Asghar K, Roth LJ. Entry and distribution of hexamethonium in the central nervous system. *Biochemical Pharmacology* 1971; **20**: 2787–3795.
6. Badio B, Daly JW. Epibatidine, a potent analgetic and nicotinic *agonist Molecular Pharmacology* 1994; **45**: 563–569.
7. Badio B, Garraffo HM, Plummer CV, Padgett WL, Daly JW. Synthesis and nicotinic activity of epiboxidine: an isoxazole analogue of epibatidine. *European Journal of Pharmacology* 1997; **321**: 189–194.
8. Bannon AW, Gunther KL, Decker MW, Arneric SP. The influence of BAY K 8644 on (±)-epibatidine-induced analgesia. *Brain Research* 1995; **678**: 244–250.
9. Bannon AW, Decker MW, Holladay MW, Curzon P, Donnelly-Roberts D, Puttfarken PS, Bitner RS, Diaz A, Dickenson AH, Porsolt RD, Williams M, Arneric SP. Broad-spectrum non-opioid analgesic activity by selective modulation of neuronal nicotinic acetylcholine receptors, *Science* 1998; **279**: 77–81.
10. Bannon AW, Gunther KL, Decker MW. Is epibatidine really analgesic? Dissociation of the activity, temperature, and analgesic effects of (±)-epibatidine. *Pharmacology, Biochemistry andBehavior* 1995; **51**: 693–698.
11. Bonhaus DW, Bley KR, Broka CA, Fontana DJ, Leung E, Lewis R, Shiek A, Wong EHF. Characterization of the electrophysiological, biochemical and behavioral actions of epibatidine. *Journal of Pharmacology and Experimental Therapeutics* 1995; **272**: 1199–1203.
12. Bradley D. Frog venom cocktail yields a one-handed painkiller. *Science* 1993; **261**: 1117.
13. Brioni JD, Decker MW, Sullivan JP, Arneric SP. The pharmacology of (−)- nicotine and novel cholinergic channel modulators. *Advances in Pharmacology* 1997; **37**: 153–213.
14. Broka C. Total synthesis of epibatidine. *Tetrahedron Letters* 1993; **34**: 3251–3254.
15. Christensen MK, Smith DF. Antinociceptive effects of the stereoisomers of nicotine given intrathecally in spinal rats. *Journal of Neural Transmission* 1990; **80**: 189–194.
16. Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine and [^{125}I]bungarotoxin. *Journal of Neuroscience* 1985; **5**: 1307–1315.
17. Colquhoun LM, Patrick JW. Pharmacology of neuronal nicotinic acetylcholine receptor subtypes. *Advances in Pharmacology* 1997; **39**: 191–219.
18. Damaj MI, Welch SP, Martin BR. Involvement of calcium and L-type channels in nicotine-induced antinociception. *Journal of Pharmacology and Experimental Therapeutics* 1993; **266**: 1330–1338.
19. Damaj MI, Martin BR. Is the dopamine system involved in the central effects of nicotine in mice? *Psychopharmacology* 1993; **111**: 106–108.
20. Damaj MI, Creasy KR, Grove AD, Rosecrans JA, Martin BR. Pharmacological effects of epibatidine optical enantiomers. *Brain Research* 1994; **664**: 34–40.
21. Damaj MI, Martin BR. Tolerance to the antinociceptive effect of epibatidine after acute and chronic administration in mice. *European Journal of Pharmacology* 1996; **300**: 51–57.
22. Davila-Garcia MI, Musachio JL, Perry DC, Xiao Y, Horti A, London ED, Dannals RF, Kellar KJ. [^{125}I]IPH, an epibatidine analog, binds with high affinity to neuronal nicotinic cholinergic receptors. *Journal of Pharmacology and Experimental Therapeutics* 1997; **282**: 445–451.
23. Decker MW, Brioni JD, Bannon AW, Arneric SP. Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapies. *Life Sciences* 1995; **56**: 545–570.
24. Domino EF. Behavioral, electrophysiological, endocrine and skeletal muscle actions of nicotine and tobacco smoking. In: Redmond A, Izard C, eds. *Electrophysiological Effects of Nicotine*. Amsterdam: Elsevier/North Holland Biomedical Press, 1979; 133–146.
25. Fertig JB, Pomerleau OF, Sanders B. Nicotine-produced antinociception in minimally deprived smokers and ex-smokers. *Addictive Behaviors* 1986; **11**: 239–248.
26. Fields HL, Heinricher MM, Mason P. Neurotransmitters in nociceptive modulatory circuits. *Annual Review of Neuroscience* 1991; **14**: 219–245.
27. Fisher M, Hangfu D, Shen T, Guynet P. Epibatidine, an alkaloid from the poison frog *Epipedobates tricolor*, is a powerful ganglionic depolarising agent. *Journal of Pharmacology and Experimental Therapeutics* 1994; **270**: 702–707.
28. Fletcher SR, Baker R, Chambers MS, Hobbs SC, Mitchell PJ. The synthesis of (+) and (−)-epibatidine. *Journal of the Chemical Society Chemical Communications* 1993; **15**: 1216–1218.
29. Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of α4 and β2 subunits and is up-regulated by chronic nicotine treatment. *Molecular Pharmacology* 1992; **41**: 31–37.
30. Galzi JL, Bertrand S, Corringer PJ, Changeux JP, Bertrand D. Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor. *EMBO Journal* 1996; **15**: 5824–5832.
31. Gerzanich V, Peng X, Wang F, Wells G, Anand R, Fletcher S, Lindstrom J. Comparative pharmacology of epibatidine: a potent agonist for neuronal nicotinic acetylcholine receptors. *Molecular Pharmacology* 1995; **48**: 774–782.
32. Gopalakrishnan M, Monteggia LM, Anderson DJ, Arneric SP, Sullivan JP. Stable expression, pharmacologic properties and regulation of the human nicotinic acetylcholine α4β2 receptor. *Journal of Pharmacology and Experimental Therapeutics* 1996; **276**: 289–297.
33. Harvey SC, Maddox FN, Luetje CW. Multiple determinants of dihydro-β-erythroidine sensitivity on rat neuronal nicotinic receptor α-subunits. *Journal of Neurochemistry* 1996; **67**: 1953–1959.
34. Horti A, Scheffel U, Stathis M, Finley P, Ravert HT, London ED, Dannals RF. Fluorine-18-FPH for PET imaging of nicotinic acetylcholine receptors. *Journal of Nuclear Medicine* 1997; **38**: 1260–1265.
35. Houghtling RA, Davila-Garcia MI, Hurt SD, Kellar KJ. Characterization of [^{3}H]epibatidine binding to nicotinic cholinergic receptors in rat and human brain. *Molecular Pharmacology* 1995; **48**: 280–287.
36. Huang DF, Shen TY. A versatile total synthesis of epibatidine and analogs. *Tetrahedron Letters* 1993; **34**: 4477–4480.
37. Iwamoto ET, Marion L. Adrenergic, serotenergic and cholinergic components of nicotine anti-nociception in rats. *Journal of Pharmacology and Experimental Therapeutics* 1993; **265**: 777–789.
38. Kammerling SG, Wettstein JG, Sloan JW, Su TP, Martin WR. Interaction between nicotine and endogenous opioid mechanisms in the unanesthetized dog. *Pharmacology, Biochemistry and Behavior* 1982; **17**: 733–740.
39. Khan IM, Yaksh TL, Taylor P. Ligand specificity of nicotinic acetylcholine receptors in rat spinal cord: studies with nico-

tine and cytisine. *Journal of Pharmacology and Experimental Therapeutics* 1994; **270**: 159–166.
40. Khan IM, Yaksh TL, Taylor P. Epibatidine binding sites and activity in the spinal cord. *Brain Research* 1997; **753**: 269–282.
41. Lindstrom JM. Nicotinic acetylcholine receptors. In: North RA, ed. *Handbook of Receptors and Channels*. Boca Raton, Florida: CRC Press, 1994; 153–175.
42. London ED, Scheffel U, Kimes AS, Kellar KJ. In vivo labeling of nicotinic acetylcholine receptors in brain with [^{3}H]epibatidine. *European Journal of Pharmacology* 1995; **278**: R1-R2.
43. Lukas RJ. Expression of ganglia-type nicotinic acetylcholine receptors and nicotinic ligand binding sites by cells of the IMR-32 human neuroblastoma clonal cell line. *Journal of Pharmacology and Experimental Therapeutics* 1993; **265**: 294–302.
44. Marks MJ, Stitzel JA, Romm E, Wehner JM, Collins AC. Nicotinic binding sites in rat and mouse brain: Comparison of acetylcholine, nicotine and α-bungarotoxin. *Molecular Pharmacology* 1986; **30**: 427–436.
45. Martin TJ, Suchocki JS, May EL, Martin BR. Pharmacological evaluation, of the antagonism of nicotine's central effects by mecamylamine and pempidine. *Journal of Pharmacology and Experimental Therapeutics* 1990; **254**: 45–51.
46. Mattila MJ, Ahtee L, Saarnivaara L. The analgesic and sedative effects of nicotine in white mice, rabbits and golden hamsters. *Annales Medicinae Experimentalis et Biologiae Fenniae* 1968; **46**: 78–84.
47. McGhee DS, Role LW. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annual Review of Physiology* 1995; **57**: 521–546.
48. Milgrom-Friedman J, Penman R, Meares R. A preliminary study on pain perception and tobacco smoking. *Clinical and Experimental Pharmacology and Physiology* 1983; **10**: 161–169.
49. Miller L. Recent developments in the study of the effects of cigarette smoking on clinical pharmacokinetics and clinical pharmacodynamics. *Clinical Pharmacokinetics* 1989; **17**: 90–108.
50. Mulle C, Choquet D, Korn H, Changeux JP. Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. *Neuron* 1992; **8**: 135–143.
51. Mulle C, Lena C, Changeux JP. Potentiation of nicotinic receptor response by external calcium in rat central neurons. *Neuron* 1992; **8**: 937–945.
52. Musachio JL, Horti A, London ED, Dannals RF. Synthesis of a radioiodinated analog of epibatidine: (+)-exo-2-(2-iodo–5-pyridyl)-7-azabicyclo-[2.2.1]heptane for the in vitro and in vivo studies of nicotinic acetylcholine receptors. *Journal of Radiolabeled Compounds and Radiopharmaceuticals* 1997; **39**: 39–48.
53. Perry DC, Kellar KJ. [^{3}H]epibatidine labels nicotinic receptors in rat brain: an autoradiographic study. *Journal of Pharmacology and Experimental Therapeutics* 1995; **275**: 1030–1034.
54. Pomerleau O. Nicotine as a psychoactive drug: anxiety and pain reduction. *Psychopharmacology Bulletin* 1986; **22**: 865–869.
55. Puttfarken PS, Manelli AM, Arneric SP, Donnelly-Roberts DL. Evidence for nicotinic receptors potentially modulating nociceptive transmission at the level of the primary sensory neuron: studies with F11 cells. *Journal of Neurochemistry* 1997; **69**: 930–938.
56. Qian C, Li T, Shen TY, Libertine-Garahan L, Eckman J, Biftu T, Ip S. Epibatidine is a nicotinic analgesic. *European Journal of Pharmacology* 1993; **250**: R13–R14.
57. Rao TS, Correa LD, Reid RT, LLoyd GK. Evaluation of anti-nociceptive effects of neuronal nicotinic acetylcholine receptor (nAChR) ligands in the rat tail-flick assay. *Neuropharmacology* 1996; **35**: 393–405.
58. Rupniak NMJ, Patel S, Marwood R, Webb J, Traynor JR, Elliott J, Freedman SB, Fletcher SR, Hill RG. Antinociceptive and toxic effects of (+)-epibatidine oxalate attributable to nicotinic agonist activity. *British Journal of Pharmacology* 1994; **113**: 1487–1493.
59. Sacaan AI, Menzaghi F, Dunlop JL, Correa LD, Whelan KT, Lloyd GK. Epibatidine: a nicotinic acetylcholine receptor agonist releases monoaminergic neurotransmitters: in vitro and in vivo evidence in rats. *Journal of Pharmacology and Experimental Therapeutics* 1995; **276**: 509–515.
60. Sahley TL, Berntson GG. Antinociceptive effects of central and systemic administrations of nicotine in the rat. *Psychopharmacology* 1979; **65**: 279–283.
61. Schultz H, Loring R, Aizenman E, Zigmond R. Autographic localization of putative nicotinic receptors in the rat brain using [^{125}I]neuronal bungarotoxin. *Journal of Neuroscience* 1991; **11**: 287–297.
62. Smith TW, Traynor JR. Opiates and potent analgesics. In: Feldman SA, Paton W, Scurr C, eds. *Mechanisms of Drugs in Anaesthesia.* London: Edward Arnold, 1993; 399–412.
63. Spande TF, Garraffo HM, Edwards MW, Yeh HJC, Pannell L, Daly JW. Epibatidine: a novel (chloropyridyl)azabicycloheptane with potent analgesic activity from an Ecuadoran frog. *Journal of the American Chemical Society* 1992; **114**: 3475–3478.
64. Spence A. *Report of the working party on pain after surgery*. London: The Royal College of Surgeons of England and College of Anaesthetists, 1990.
65. Sullivan JP, Decker MW, Brioni JD, Donnelly-Roberts D, Anderson DJ, Bannon AW, Kang CH, Adams P, Piattoni-Kaplan M, Buckley MJ, Gopalakrishnan M, Williams M, Arneric SP. (±)-epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors. *Journal of Pharmacology and Experimental Therapeutics* 1994; **271**: 624–631.
66. Sullivan JP, Donnelly-Roberts D, Briggs CA, Anderson DJ, Gopalakrishnan M, Piattoni-Kaplan M, Campbell JE, McKenna DG, Molinari E, Hettinger A-M, Garvey DS, Wasicak JT, Holladay MW, Williams M, Arneric SP. A-85380 [3-(2(S)-azetidinylmethoxy)pyridine]: in vitro pharmacological properties of a novel, high affinity α4β2 nicotinic acetylcholine receptor ligand. *Neuropharmacology* 1996; **35**: 725–734.
67. Tripathi HL, Martin BR, Aceto MD. Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels. *Journal of Pharmacology and Experimental Therapeutics* 1982; **221**: 91–96.
68. Vernino S, Amador M, Luetje CW, Patrick J, Dani JA. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron* 1992; **8**: 127–134.
69. Vernino S, Rogers M, Radcliffe KA, Dani JA. Quantitative measurement of calcium flux through muscle and neuronal nicotinic acetylcholine receptors. *Journal of Neuroscience* 1994; **14**: 5514–5524.
70. Waller D, Schalling D, Levander S, Edman G. Smoking, pain tolerance, and physiological activation. *Psychopharmacology* 1983; **79**: 193–198.
71. Warpman U, Nordberg A. Epibatidine and ABT418 reveal selective losses of α4β2 nicotinic receptors in Alzheimer brains. *Neuroreport* 1995; **6**: 2419–2423.
72. Watt AP, Verrier HM, O'Connor D. Resolution of synthetic (+) and (−)-epibatidine by chiral high performance liquid chromatography and identification of the natural enantiomer. *Journal of Liquid Chromatagraphy* 1994; **17**: 1257–1264.
73. Westera G, Patt JT, Jankowski K. Epibatidine-derivatives: ligands for the neuronal nicotinic acetylcholine receptor. In: *Annual Report Paul Scherrer Institute.* Villigen, Switzerland: Paul Scherrer Institute, 1996. (http://www.psi.ch/www.irp_hn/clinic.html)
74. Westfall TC, Grant H, Perry H. Release of dopamine and 5-hydroxytryptamine from rat striatal slices following activation of nicotinic cholinergic receptors. *General Pharmacolology* 1983; **14**: 321–325.
75. Whiting P, Schoepfer R, Lindstrom J, Priestley T. Structural and pharmacological characterization of the major brain nicotinic acetylcholine receptor subtype stably expresses in mouse fibroblasts. *Molecular Pharmacology*, 1992; **40**, 463–472.
76. Wonnocott S, Irons J, Rapier C, Thorne B, Lunt GG. Presynaptic modulation of neurotransmitter release by nicotinic receptors. In: Nordberg A, Fuxe K, Holmstedt B, Sundwall A, eds. *Progress in Brain Research*. Amsterdam: Elsevier, 1990; 157–163.
77. Zhang X, Nordberg A. The competition of (−)-[^{3}H]nicotine binding by enantiomers of nicotine, nornicotine and anatoxin-A in membranes and solubilibised preparations of different brain regions of rat. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1993; **348**: 28–34.
78. Zoli M, LeNovere N, Hill JA, Changeux JP. Developmental regulation of nicotininc ACh receptor subunit mRNA's in the rat central and peripheral nervous systems. *Journal of Neuroscience* 1995; **15**: 1912–1939.