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EPIBATIDINE AND ANALOGS - NEW TRENDS IN THE DEVELOPMENT OF COGNITIVE ENHANCERS AND STRONG ANALGETICS

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Summary

*Epibatidine, the natural alkaloid isolated from the skin of the Ecuadorian rainforest poison-dart frog, *Epipedobates tricolor* and some of its synthetically prepared analogs are strong selective agonists of the nicotinic acetylcholine receptors. All of these substances have an analgesic effect and could be used in medical practice. This is very promising for medicine in the fight against pain as well as against dementia, because nicotinic agonists have been found to improve the performance of a variety of memory tasks in rodents and they are probably beneficial in Alzheimer's disease treatment.*

Key words: Toxin; Epibatidine; Nicotinic acetylcholine agonist; Alzheimer's disease; Pain.

Introduction

Natural aging of brain and particularly degenerative-induced changes in brain of patients with dementia is characterized by decreasing of its cognitive functions. Compounds that have cognitive enhancer activity are known as nootropic drugs or cognitive enhancers and there are now several drugs that can help prevent age associated memory loss. Cognitive enhancement therapy has progressed a long way in the past several years and cognitive enhancers have also been taken in the prevention and in the treatment of Alzheimer's disease (AD). The AD is the most widespread form of dementia. Because AD is characterized by cholinergic deficiency, important cognitive enhancers are drugs which ameliorate cholinergic transmission in brain. There are at present centrally active acetylcholinesterase inhibitors as for instance tacrin (Cognex), donepezil (Aricept) and rivastigmine (Exelon), which act as indirect cholinolytics. Other possible compounds are those with direct effect on cholinergic receptors, agonists of muscarinic and nicotinic

cholinergic receptors. In the last decade nicotinic receptors in the hippocampus, cerebral cortex and Meynert's nucleus basalis were characterized as very important for the cognitive function. These brain areas are mostly impaired in patients with AD. Because nicotinic agonists have been found to improve the performance of a variety of memory tasks of rodents, they are probably beneficial in AD treatment.

Epibatidine represents a new class of nicotinic agonists. It is isolated from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*. Therefore, the aim of this article are epibatidine and its synthetic analogs as potential cognitive enhancers. Epibatidine has also a very strong analgesic effect with a mechanism different from morphine, therefore this compound as well as some of its derivatives and analogs are very interesting as potential painkillers. From this point of view compounds derived from the structure of epibatidine with a strong analgesic affect are very interesting for acute and disaster medicine.

Nicotinic effects on cognitive performance in animals

Studies of nicotinic agonist and antagonist effects on cognitive performance have shown that nicotine and other nicotinic agonists can improve performance of cognitive tasks, while nicotinic antagonists can impair such performance (16). In behavioral pharmacology studies with nicotine or nicotinic agonists have been found to improve working memory function in the radial-arm maze (27). Acute nicotine has also been found to facilitate performance in passive avoidance (11, 47). Other selective nicotinic agonists have been also found to facilitate performance in many behavioral studies, which were recently summarized by Levin and Simon (28). From these studies the improvement of working memory by nicotinic agonists is clear, and reference memory is relatively unaffected. Other studies have shown that nicotinic agonists also facilitate performance in aged animals exhibiting deficits in spatial working memory (29) or in animals with poor passive avoidance performance due to a choline-deficient diet (37). Nicotine-induced memory improvements have been blocked by the nicotinic antagonist mecamylamine (2, 27).

Nicotinic effects on cognitive performance in human

In the last decade smoking has been found by several studies to reduce the incidence of AD (10, 26, 42) which stimulated the interest on nicotine as therapeutic drug (40). This was especially clearly seen in subpopulations with a genetically associated high risk for AD (42). Up to now nicotine injections have been found to improve attention and memory (24, 35). Nowadays nicotine and nicotinic agonists promise novel treatments for a variety of cognitive disorders including those associated with AD. Development of more specific nicotine-like drugs may separate the cognitive enhancing effects of nicotine from its cardiovascular and other adverse side effects. Very interesting and hopeful compound of this type is epibatidine, animal alkaloid from the skin of poison-dart frog *Epipedobates tricolor*.

Epibatidine

Epibatidine is alkaloid isolated from the skin of

the Ecuadorian rainforest poison-dart frog, *Epipedobates tricolor* (see color suppl. p. I, Fig. 1).

Chemically it is a derivative of 2-chloropyridine and 7-azabicyclo[2.2.1]heptane. The structural similarity with nicotine makes possible that epibatidine binds to nicotinic receptors. In many experiments, it has been demonstrated that both enantiomers of epibatidine are strong antagonists of nicotinic acetylcholine receptors (25). In pharmacological experiments in mice it was shown that epibatidine possessed 200 times more analgesic activity than morphine. The pharmaceutical use of epibatidine as an analgesic is not possible due to its extreme toxicity. However epibatidine serves as a useful leading compound in the search for derivatives, which possess comparable analgesic activity, but reduced toxicity (17). Little toxic derivatives of epibatidine with high affinity to nicotinic acetylcholine receptors are potential anti-AD drugs.

Epibatidine Analogs

The structure of epibatidine is unique among analgesics, in addition of being the only known representative of a new class of alkaloid. Although unique in these two areas, the activity of epibatidine can be accommodated within a well defined structure-activity model for general CNS activity. Synthesis of epibatidine analogues is currently being undertaken, in conjunction with pharmacological evaluation, to further develop the nicotinic acetylcholine structure-activity model.

The synthetic modification of epibatidine may be divided into three areas: a) Alkaloid ring modification, b) Substitution at the alkaloid nitrogen, and c) Heteroaromatic ring replacement (Fig. 2).

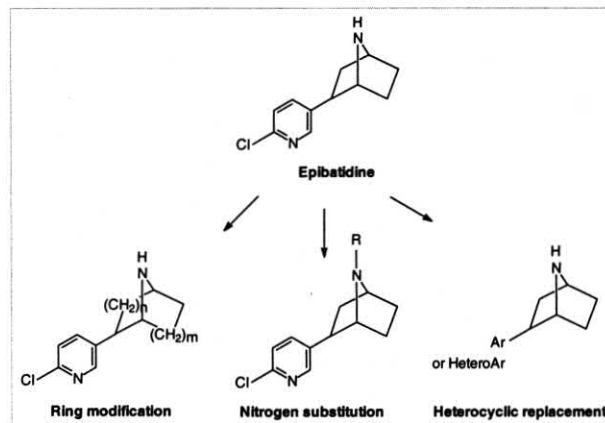


Fig. 2: The most frequent synthetic modification of epibatidine molecule.

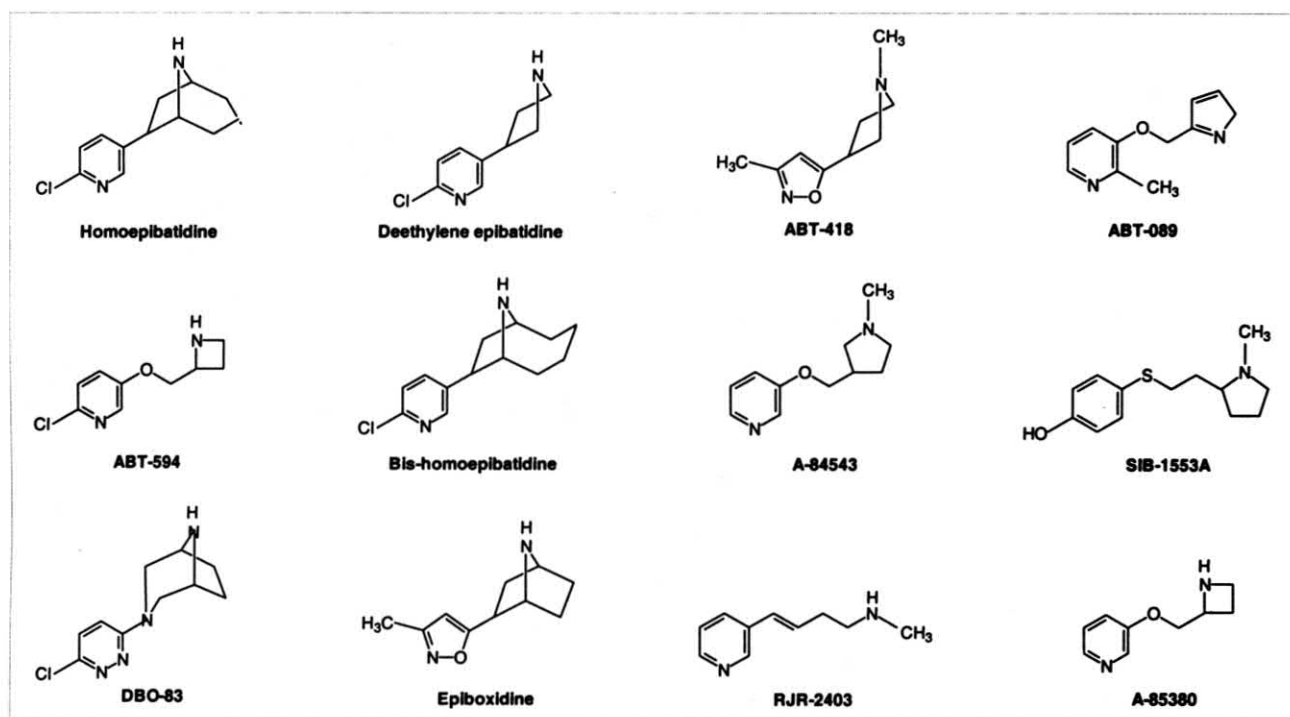


Fig. 3: Chemical structures of some nicotinic acetylcholine receptor agonists derived from the structure of epibatidine tested as yet in the preclinical experiments.

Structural formulas of the most important, so far prepared epibatidine derivatives, appearing as strong pharmacologically active compounds, are summarized in Fig. 3.

Interaction of Epibatidine and Analogs with Nicotinic Acetylcholine Receptor Subtypes

The neuronal nicotinic acetylcholine receptors belong to the family of ligand-gated ion channels which are widely distributed in the brain. Molecular studies have revealed the existence of several α (α_2 - α_9) and β (β_2 - β_4) subunits (36) forming various subtype combinations and different patterns of distribution of nicotinic acetylcholine receptors in brain (34). In the central nervous system β_2 - subunit appears to be the most widely expressed, but other subunit combinations were also found (43). The functional roles played by these subtypes are being intensively investigated. Experimental studies have shown that hippocampal neurons with nicotinic receptors $\alpha_4\beta_2$, $\alpha_3\beta_2$, and α_7 appear to be important for working memory functions. These receptors were found in high concentrations in hippocampus on interneurons and seem to regulate neurite outgrowth (19). Little is currently known about the functional role of α_7 type of nicotinic

receptors in the brain but it is evident that together with $\alpha_4\beta_2$ type are important for aspects of cognitive processes such as learning and memory and aging processes connected with neurodegenerative diseases such as Alzheimer's disease (45). Recently has been found that epibatidine binds with very high affinity to human α_3 , α_4 , and α_7 type subunits of nicotinic acetylcholine receptors (20) and that regional distribution is different from nicotine (32).

Epibatidine is at least 20 times more potent than nicotine at the $\alpha_4\beta_2$ type in rat, but has more than 150 times higher affinity than nicotine at the α_7 type in rat brain and more than 300 times in *Torpedo* electroplax (10). Compound, such as epibatidine, may be expected to demonstrate little separation between beneficial and toxic doses, but some derivatives may be more selective and may enjoy an improved therapeutic window.

Effects of Epibatidine and Analogs on Pain

Epibatidine shows potent activity, greater than 200 times more active than morphine, in eliciting a Straub-tail reaction and inducing hot-plate analgesia. The poor inhibition of [3 H]-dihydromorphine binding by epibatidine, together with its poor antagonism by the general opioid antagonist naloxone, indicates

that the activity of epibatidine is due to a non-opioid mechanism. The truly exciting discovery was that epibatidine's mechanism of action appeared to be non-opioid. Many potent pain-relieving drugs are opiates, morphine being a very familiar example.

Morphine is an effective and potent analgesic; however, the potential for addiction and the development of morphine tolerance are major drawbacks to its use. Several major pharmaceutical companies have focused their efforts on discovering better analgesics. When Badio and Daly (5) showed that epibatidine's effect was not blocked by naloxone, an opioid antagonist, this revelation produced much enthusiasm in the hope for a better drug.

If epibatidine did not exert its analgesic effect through opioid receptors, how then did it produce the pain relief? Shortly after the publication of the structure of epibatidine, several research groups, including Daly's, determined the answer by examining epibatidine's interaction with nicotinic acetylcholine receptors (10, 18, 33). These receptors are activated by nicotine - hence their name.

Epibatidine bind to and activate these receptors in extremely low concentrations ($K =$ about 55 pM). The finding that the analgesic effects of epibatidine are blocked by mecamylamine (a noncompetitive nicotinic antagonist), along with previous research illustrating potentially beneficial effects of nicotine (12), sparked a resurgence in the medicinal chemistry of nicotine and nicotinic analogs.

Structures of nicotine and epibatidine are very similar. Both contain a six-membered pyridine ring; both contain a basic nitrogen linked to the pyridine ring by one or two carbons; both basic nitrogens are part of a five-membered ring (in epibatidine, the five membered ring is a part of the azabicycloheptane structure). Dukat et al. (18) showed that energy-minimized molecular models of the compounds could be overlayed such that major structural features are in similar positions in space (see color suppl. p. I, Fig. 4).

This modeling experiment was the first to show in a three dimensional fashion that epibatidine and nicotine may interact with similar receptor features.

Many authors have suggested nicotine pharmacophores (7, 8, 38). With some simplification, all the models contain a hydrogen bond acceptor atom (e.g., pyridine N or carbonyl O) and a center of positive charge (e.g., protonated basic nitrogen), separated by a distance of approximately 4.8 Å.

This distance is often referred to as the „inter-nitrogen distance“ because most, although not all, nicotinic analogues contain a pyridine nitrogen and a more basic nitrogen. With the emergence of epibatidine as a high affinity nicotinic agonist, Glennon et al. (22) reevaluated the nicotinic pharmacophore and produced a model which indicated an optimal internitrogen distance of 5.1-5.5 Å. In 1996, a research group at Abbott Laboratories published their work in which they synthesized a series of pyridyl ether compounds which are nicotinic agonists, some as potent as epibatidine. Molecular modelling studies incorporating these new agents suggested that a inter nitrogen distance closer to 6.1 Å may be optimal for interaction at the nicotinic receptor (1). There is still much work to be done before a precise nicotine pharmacophore can be agreed upon by the medicinal chemists.

Synthetically prepared analogues of epibatidine have been tested for analgesic activity with different result. Homoepibatidine was comparable in analgesic response to epibatidine, deethylene epibatidine however was much less potent and caused analgesia at only high doses (46).

The most important epibatidine derivative was compound ABT-594 because it seemed to work against different types of pain and produced little side effects (39). Lastly published data indicate that ABT-594 is a centrally acting neuronal nicotinic acetylcholine receptor agonist with potent antinociceptive effect which does not have the unwanted side effects of morphine, such as respiratory depression and constipation (15). The presence of chlorine atom in a position 2 of pyridine moiety is very important, because the dechlorinated analog, compound A-85380, is less potent and has a less attractive safety profile (14, 41). In numerous pain assays ABT-594 proved a greatly enhanced improvement to that of morphine analgesic ability and was 30-70 times more potent. ABT-594 reduces the release of substance P and so inhibits the pain cascade and do not produces overt physical dependence with repeated administration and do not elicits withdrawal signs when administration is discontinued (4). Phase I clinical trials of ABT-594 look very promising, and the medication is now being tested in Europe on humans (23).

The diazabicyclic pyrazine derivative of epibatidine, DBO-83, is another very interesting high affinity nicotinic acetylcholine receptor agonist (21) as well as compound A-84543 (1).

Effect of Epibatidine and Analogs on Cognitive Performance

It is not possible to divide analgesic effects of these nicotinic acetylcholine receptor agonists from their cognitive activity and it is possible that some of them can probably be utilized as painkiller as well as Alzheimer's disease therapeutics. More compounds of epibatidine type are now in the phase of preclinical trial. It is very important that these compounds are selective agonists of $\alpha_4\beta_2$ and α_7 nicotinic acetylcholine receptor because these receptors might be the most vulnerable in AD and a possible targets for therapeutic strategies (44).

One epibatidine analogue is the compound ABT-418, where chlorpyridine ring is replaced by a methylisoxazol ring (3). ABT-418 represents a prototype of a new class of nicotinic agonists designed for the potential treatment of human dementias having a low profile of toxicity (13). Also other compound from this series, ABT-089, is very promising cognitive compound (30).

Another interesting analogue is epiboxidine, a hybrid between epibatidine and ABT-418, 20-fold less toxic than epibatidine (6). Other derivatives of this type are compounds SIB-1553A and RJR-2403 (9, 31).

Conclusions

Epibatidine's structure was found to be a new class of animal alkaloids possessing a 7-azabicyclo [2,2,1]heptane structure with a 2-chloro-5-pyridyl substituent. This unique molecule is structurally similar to nicotine and also has very similar pharmacological properties. The chance of epibatidine ever being used as a medicinal agent is quite low because of its toxicity, but new analogues and derivatives of this compound have been and are being synthesized and many of them have practically the same pharmacological effects without its toxicity. This is very promising for medicine in the fight against pain as well as against dementia, particularly Alzheimer's disease.

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Fig. 1: The Ecuadorian rainforest poison-dart frog *Epipedobates tricolor*, the natural source of epibatidine. The picture was kindly obtained from the Jennifer Patterson's Page of Pointlessness (<http://www.geocities.com/CollegePark/2290/frogs/index/html>) and for the print edited by Microsoft Photo Editor.

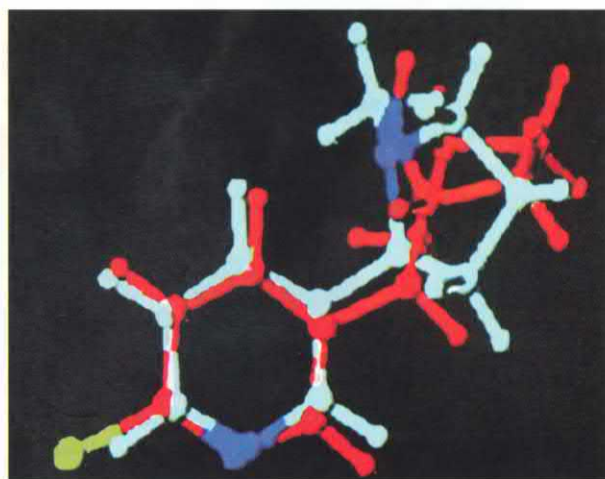


Fig. 4: The computerized superposition of nicotine (cyan) and epibatidine (red) molecules. Nitrogens are blue and chlorine is green. This picture was assumed with kindly allowance from the document of M. J. Dowd, graduate student of department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, USA (<http://www.phe.vcu.edu/feature/epi/index.html>).