Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1859–1861

Neuronal nicotinic receptors and epilepsy, from genes to possible therapeutic compounds

Ronald C. Hogg and Daniel Bertrand*

Department of Physiology, Medical Faculty, 1 rue Michel Servet, Ch-1211 Geneva 4, Switzerland

Accepted 13 October 2003

In the search for new therapeutic compounds to fight neurological diseases it is now possible to establish strategies based on a combination of the latest genetic and physiological findings. This is illustrated by the identification of an association between a form of genetically transmissible epilepsy, the autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and a mutation in CHRNA4, the gene coding for the α4 subunit of the nicotinic acetylcholine receptor (nAChR).[1] Since this initial observation several studies have confirmed this association and have shown that ADNFLE is associated with either a mutation in CHRNA4 or CHRNB2, the gene that codes for the β2 subunit of the nAChR.[2–5]

Mutations in CHRNA4 and CHRNB2 in individuals suffering from ADNFLE give rise to single nucleotide polymorphisms, which cause either the exchange of a single amino acid or in one case the insertion of an extra amino acid in the receptor protein. Single amino acid mutations in the nAChR subunit can potentially modify several aspects of receptor behaviour and since all the mutations give rise to comparable clinical symptoms it is probable that the modification of function underlying the disease is consistent across all the ADNFLE mutant receptors. Central nAChRs receptors displaying a high affinity for nicotine result from the assembly of α4 and β2 subunits, it was therefore expected that mutation[2,3,6] in either of these subunits could result in dysfunction of the receptor complex.

Physiological studies carried out on mutant receptors formed from expression of the cDNAs corresponding to CHRNA4 and CHRNB2 in the *Xenopus* oocyte expression system has allowed us to examine the properties of human receptors that harbor the mutations found in the different families of ADNFLE sufferers. Heteromeric expression of the mutant subunit with its wild type α or β partner indicate that while every mutation identified so far causes multiple modifications of receptor function the only common trait shared by all the mutants identified so far is an increase in sensitivity to the agonist acetylcholine (ACh). In this work we examine in view of our most recent knowledge the characteristics of a molecule that would be designed to counterbalance the detrimental effects caused by these mutations.

The most obvious therapeutic strategy for the treatment of ADNFLE would be to reduce the enhanced responses of the mutant α4β2 nAChRs. Due to the ubiquitous nature of the nAChR in the CNS (see ref 7), widespread inhibition of nAChRs would be expected to have detrimental effects on CNS function. In humans, 11 genes have been identified coding for neuronal nAChR subunits and are classified as α2–α7, α9 and α10 and β2–β4. Studies have shown that functional receptors are formed from a pentameric assembly of subunits around a central ion-conducting pore. In most cases a single type of α and β subunit combine in an $(\alpha)_2(\beta)_3$ stoichiometry,[8] (see Fig. 1A) with the exception of β3 and putatively α5 and α6, which do not form functional receptors when expressed alone or with a single type of α or β subunit. The α7 and α9 subunits can form functional homomeric pentamers when expressed in mammalian or amphibian cells, however, α9 preferentially assemble with α10, but expression is limited to the inner ear and a few ganglia.[9–11]

The heteromeric nature of the receptor coupled with differential expression of subunits throughout the central and peripheral nervous system contributes a functional and pharmacological diversity between nAChR subtypes. These differences in pharmacological properties between nAChR subtypes present the possibility to

* Corresponding author. Tel.: +41-22-379-53-56; fax: +41-22-379-54-02; e-mail: daniel.bertrand@medecine.unige.ch

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2003.10.075

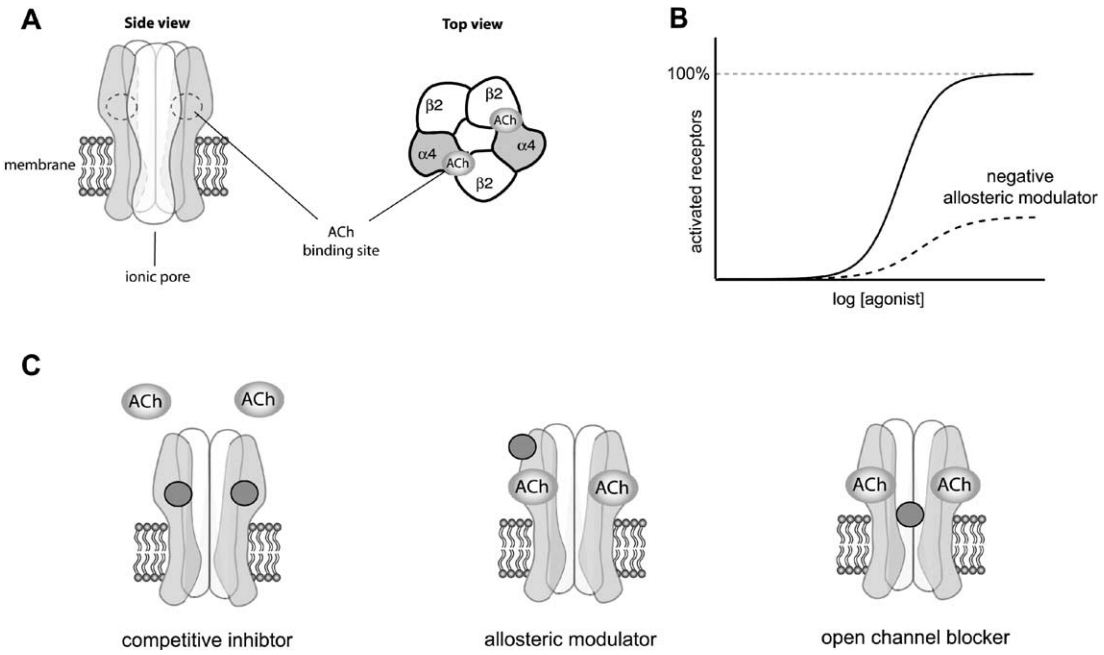

Figure 1. Ligands can modify nAChR function via different mechanisms. (A) schematic representation of an nAChR formed from the assembly of two α4 and three β2 subunits, showing the ionic pore and ACh binding site. (B) Negative allosteric effectors shift the equilibrium in favour of a closed state causing a loss of receptor function. This type of receptor inhibition may be useful in compensating for the increased activity of mutant receptors in ADNFLE. (C) Competitive antagonists compete with ACh for the ligand-binding site, receptor inhibition can be overcome by a higher concentration of agonist. Allosteric modulators bind to a site on the nAChR distinct from the ACh binding site and change the equilibrium constant between open and closed states of the receptor. Channel blockers, bind in the pore region of the channel, blocking conduction. Open channel blockers, such as carbemazepine have access to their binding site only when the channel is open and often show a use-dependent increase of block.

target ligands to specific areas of the nervous system, which raises hopes for the design of a molecule that could interact selectively with a defined receptor subtype. This may be possible due to the existence of numerous nAChR subunits, which combine to form receptors each with a unique pharmacology. It is therefore desirable to develop ligands, which not only interact with a specific receptor subtype, but also selectively inhibit mutant nAChRs displaying enhanced agonist sensitivity.

Functional studies on the nAChR have identified several classes of molecules, which modulate receptor activity via different mechanisms. Properties of nAChRs can be described by an allosteric model (see, refs 12 and 13) and the effects of different classes of ligands which interact with the receptor can be described according to which state of the receptor is stabilized. Functionally, nAChR channels can exist in two conformations that are either closed or open with the latter state stabilized by the ligand. Ligands stabilizing the open state are agonists (full or partial), while compounds that stabilize a closed state are antagonists. Two classes of antagonists exist, competitive antagonists, which prevent binding of the natural ligand ACh and non-competitive antagonists, which bind to a site on the receptor distinct from the agonist-binding site. Typical non-competitive antagonists are channel blockers, which inhibit ionic flux through the receptor by steric blockade of the ionic pore. Another class of compounds are ligands which modulate receptor function via an allosteric mechanism form. Positive allosteric effectors facilitate the transition from the resting to open states resulting in a larger response to a given agonist concentration, while negative allosteric effectors inhibiting the receptor by increasing the energy barrier for the transition from the closed to open state (Fig. 1B). Several steroid hormones, which readily cross the blood–brain barrier, cause inhibition of nAChR function via an allosteric mechanism. The use of a negative allosteric effector may be a promising strategy to reduce the enhanced responses at the mutant α4β2 receptors. Corticosterone, dexamethasone, progesterone and hydrocortisone have all been demonstrated to inhibit nAChRs via an allosteric mechanism.[14–20] However, none of these compounds are sufficiently selective between nAChR subtypes to be of use as selective inhibitors. The challenge remains to develop new ligands which display similar functional profiles but with greater subunit specificity and ideally a specificity for receptors displaying mutations. Numerous subtype specific ligands, such as the alkaloids nicotine, cytisine and epibatidine, the peptides α-bungarotoxin, α-cobratoxin and the α-conotoxins bind preferentially to different nAChR subtypes. The α-conotoxins continue to be an important source of nAChR subunit-specific ligands, with the recent report of an α-conotoxin selective for α6 subunits.[21] Molecular genetic techniques allow the scanning of the genome for conserved motifs of a particular conotoxin class, following the identification of these genes peptides can then be synthesized in sufficient quantities for functional testing. Subtle re-engineering of these peptides[22,23] and other molecules is also a promising source of new selective ligands, see refs 24 and 25 for recent reviews. Using these techniques the goal of selectively inhibiting only mutant receptors, which display enhanced agonist sensitivity, may be attainable.

An important property of the nAChR is desensitization of the response in the presence of a constant agonist concentration. Desensitization corresponds to the stabilization of one of the closed states of the receptor and can follow a rapid or slow timecourse. Fast desensitization immediately follows the peak response and has a timecourse of several milliseconds, whereas slow desensitization is a progressive reduction of the response, which can take from seconds to minutes and is observed in the prolonged presence of a low or subthreshold concentration of agonist. This property of the nAChR could potentially be exploited to reduce the current through these receptors.

An increased sensitivity to the anti-epileptic drug carbamazepine (CBZ), which has been shown to be a non-competitive inhibitor of nAChRs in in vitro experiments[26] (Fig. 1C), has been reported amongst some NFLE and ADNFLE sufferers. CBZ abolished seizures in approximately 20% of patients suffering from NFLE and reduced the intensity of the seizure by at least 50% in an additional 48% of cases.[27] In vitro experiments first showed that the α40-S248F and α4-L-776ins3 ADNFLE mutant receptors are approximately fourfold more sensitive to inhibition by CBZ than wild type α4β2 receptors.[2] Although presently, due to the low number of cases, no statistically significant linkage can be drawn between CBZ sensitivity and the severity of the patients' condition, it appears that ADNFLE mutations may show an altered sensitivity to certain inhibitors.[28] This confirms the possibility to selectively target receptors displaying a gain of function. The finding that a genetic component can determine the response to a drug provides a further means for the precise targeting of receptors. A pharmacogenomic approach to treatment will combine information from genetic screening with knowledge determined from in vitro experiments on the mechanisms of ligand action to predict the most suitable treatment strategy for each individual. The possibility to tailor a treatment to a particular genetic makeup may help to reduce undesirable side effects.

References and notes

1. Steinlein, O. K.; Mulley, J. C.; Propping, P.; Wallace, R. H.; Phillips, H. A.; Sutherland, G. R.; Scheffer, I. E.; Berkovic, S. F. *Nat. Genet.* **1995**, *11*, 201.
2. Picard, F.; Bertrand, S.; Steinlein, O. K.; Bertrand, D. *Epilepsia* **1999**, *40*, 1198.
3. Phillips, H. A.; Favre, I.; Kirkpatrick, M.; Zuberi, S. M.; Goudie, D.; Heron, S. E.; Scheffer, I. E.; Sutherland, G. R.; Berkovic, S. F.; Bertrand, D.; Mulley, J. C. *Am. J. Hum. Genet.* **2001**, *68*, 225.
4. De Fusco, M.; Becchetti, A.; Patrignani, A.; Annesi, G.; Gambardella, A.; Quattrone, A.; Ballabio, A.; Wanke, E.; Casari, G. *Nat. Genet.* **2000**, *26*, 275.
5. Favre, I.; Phillips, H. A.; Friedli, M.; Bertrand, S.; Antonarakis, S. E.; Goudie, D.; Roberts, R.; Scheffer, I. E.; Marini, C.; Berkovic, S. F. J. C. M.; Bertrand, D., submitted.
6. Rodrigues-Pinguet, N. O.; Jia, L.; Li, M.; Figl, A.; Klaassen, A.; Truong, A.; Lester, H. A.; Cohen, B. N. *J. Physiol.* **2003**, *18*, 18.
7. Hogg, R. C.; Raggenbass, M.; Bertrand, D. *Rev. Physiol. Biochem. Pharmacol.* **2003**, *147*, 1.
8. Anand, R.; Conroy, W. G.; Schoepfer, R.; Whiting, P.; Lindstrom, J. *J. Biol. Chem.* **1991**, *266*, 11192.
9. Lips, K. S.; Pfeil, U.; Kummer, W. *Neuroscience* **2002**, *115*, 1.
10. Morley, B. J.; Simmons, D. D. *Brain Res. Dev. Brain Res.* **2002**, *139*, 87.
11. Wang, N.; Orr-Urtreger, A.; Korczyn, A. D.; Morley, B. J.; Simmons, D. D.; Lips, K. S.; Pfeil, U.; Kummer, W.; Elgoyhen, A. B.; Vetter, D. E.; Katz, E.; Rothlin, C. V.; Heinemann, S. F.; Boulter, J. *Prog. Neurobiol.* **2002**, *68*, 341.
12. Edelstein, S. J.; Schaad, O.; Henry, E.; Bertrand, D.; Changeux, J. P. *Biol. Cybern.* **1996**, *75*, 361.
13. Changeux, J. P. *Trends Pharmacol. Sci.* **1990**, *11*, 485.
14. Ke, L.; Lukas, R. J. *J. Neurochem.* **1996**, *67*, 1100.
15. Paradiso, K.; Zhang, J.; Steinbach, J. H. *J. Neurosci.* **2001**, *21*, 6561.
16. Valera, S.; Ballivet, M.; Bertrand, D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9949.
17. Garbus, I.; Bouzat, C.; Barrantes, F. J. *Neuroreport.* **2001**, *12*, 227.
18. Bertrand, D.; Valera, S.; Bertrand, S.; Ballivet, M.; Rungger, D. *Neuroreport* **1991**, *2*, 277.
19. Bouzat, C.; Barrantes, F. J. *J. Biol. Chem.* **1996**, *271*, 25835.
20. Barrantes, F. J.; Antollini, S. S.; Bouzat, C. B.; Garbus, I.; Massol, R. H. *Kidney Int.* **2000**, *57*, 1382.
21. Dowell, C.; Olivera, B. M.; Garrett, J. E.; Staheli, S. T.; Watkins, M.; Kuryatov, A.; Yoshikami, D.; Lindstrom, J. M.; McIntosh, J. M. *J. Neurosci.* **2003**, *23*, 8445.
22. Hogg, R. C.; Miranda, L. P.; Craik, D. J.; Lewis, R. J.; Alewood, P. F.; Adams, D. J. *J. Biol. Chem.* **1999**, *274*, 36559.
23. Luo, S.; Nguyen, T. A.; Cartier, G. E.; Olivera, B. M.; Yoshikami, D.; McIntosh, J. M. *Biochemistry* **1999**, *38*, 14542.
24. Astles, P. C.; Baker, S. R.; Boot, J. R.; Broad, L. M.; Dell, C. P.; Keenan, M. *Curr. Drug Target CNS Neurol. Disord.* **2002**, *1*, 337.
25. Gotti, C.; Carbonnelle, E.; Moretti, M.; Zwart, R.; Clementi, F. *Behav. Brain Res.* **2000**, *113*, 183.
26. Ortells, M. O.; Barrantes, G. E. *Br. J. Pharmacol.* **2002**, *136*, 883.
27. Provini, F.; Plazzi, G.; Tinuper, P.; Vandi, S.; Lugaresi, E.; Montagna, P. *Brain* **1999**, *122*, 1017.
28. Leniger, T.; Kananura, C.; Hufnagel, A.; Bertrand, S.; Bertrand, D.; Steinlein, O. K. *Epilepsia* **2003**, *44*, 981.