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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1897–1900
Bioorganic & Medicinal Chemistry Letters

Activation and desensitization of heteromeric neuronal nicotinic receptors: implications for non-synaptic transmission

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Received 20 August 2003; revised 20 August 2003; accepted 26 February 2004

Abstract—Consideration of the activation and desensitization properties of neuronal nicotinic acetylcholine receptors (nAChRs) predicts that there should be a range of concentrations over which low ambient levels of agonist can continuously open nAChR channels. These findings support the idea that postsynaptic nAChRs may participate in unconventional cellular signaling mediated by the release of acetylcholine from diffusely distributed non-synaptic cholinergic varicosities.

1. Introduction

In order to generate a fast nicotinic synaptic potential, nAChRs must be localized to a postsynaptic zone in close apposition to a presynaptic cholinergic terminal. Only this type of arrangement will permit the rapid and transient interaction of high concentrations of the neurotransmitter acetylcholine (ACh) with the relatively low affinity nAChRs, as typified at the neuromuscular junction.[1] However, despite the widespread innervation of the brain by central cholinergic systems,[2] there are just a handful of examples of fast nicotinic potentials in the CNS.[3–5] One likely explanation for the lack of synaptic responses is the low incidence (≈10%) of asymmetric synaptic contacts made by the abundant cholinergic varicosities.[6] Further it has been suggested that most cholinergic terminals appear to be randomly distributed with respect to neighboring tissue. These data alone suggest that postsynaptic nAChRs are not in a position to detect high levels of ACh, and may participate in an alternate form of chemical communication that results from the diffuse release of transmitter–volume transmission.[6]

2. Cholinergic-nicotinic nature of synaptic signaling in the habenulo-interpeduncular tract

If intercellular signaling does occur in a diffuse manner then a number of factors need to be taken into consideration, including how far ACh can travel, how fast and effective is enzymatic hydrolysis, and importantly the properties of the nAChRs. These aspects of transmission will be considered in relation to a model cholinergic–nicotinic system in the CNS—the habenulo-interpeduncular tract. The medial habenula (MHb) receives cholinergic input from the basal forebrain, and in turn the cholinergic neurons of the MHb project to the interpeduncular nucleus (IPN).[2] Cholinergic neurons, labeled with an antibody against the vesicular acetylcholine transporter (anti-VAChT; green), surrounded by putative cholinergic terminals, doubly labeled with anti-VAChT and an antibody against the presynaptic marker synaptophysin (yellow), can be clearly observed in the MHb (Fig. 1).

However, notwithstanding the presence of both postsynaptic nAChRs and cholinergic terminals, there is no evidence for fast nicotinic synaptic transmission in either the MHb[7] or the IPN.[8] Thus, one is left to conclude that nAChRs in these brain regions must participate in an unconventional form of signaling, of which the most likely is volume transmission.[6]

It has been further argued that, in densely innervated regions, such as the MHb and IPN, the release of ACh may override the effects of diffusion and enzymatic hydrolysis, therefore providing an ambient cholinergic tone.[6] Indeed it has been estimated that ACh may be continuously present in the CSF at a concentration ≈20 nM, and can reach levels nearing 1 μM in the presence of cholinesterase inhibitors.[9] Thus, it is important to evaluate how this low range of agonist concentrations interacts with nAChRs.

Keywords: Addiction; Acetylcholine.
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doi:10.1016/j.bmcl.2004.02.081

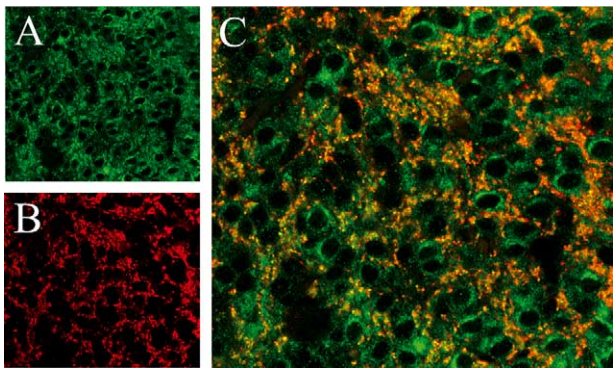

Figure 1. Cholinergic cell bodies and synaptic terminals in the MHb nucleus. Paraformaldehyde (2%) perfusion-fixed brain slices (50 μm) containing the MHb were prepared. Slices were incubated in both (A) anti-VAChT (1:500; Chemicon AB1588) with labeling revealed by a secondary Alexa Fluor 488 antibody (1:200; Molecular Probes) and (B) anti-synaptophysin (1:200; Chemicon MAB368) with labeling revealed by a secondary Alexa Fluor 594 antibody (1:200; Molecular Probes). Images were obtained using a confocal microscope system. The images in (A) and (B) have been adjusted for background staining by comparison with slices in which the primary antibody was not included. Images in (A) and (B) are shown merged and enlarged in (C).

3. Properties of nAChRs in the MHb

All known nAChRs can be both activated and desensitized by a variety of cholinergic agonists, including ACh,[10] although the concentration ranges and time courses for inducing these processes depend on the precise subunit composition.[11] In general though, substantial activation of neuronal nAChRs requires rapid pulses of high concentrations of agonist, whereas lower doses produce desensitization by promoting the formation of a high affinity desensitized state(s). In this respect the neuronal receptors closely resemble nAChRs at the neuromuscular junction.[12]

In the MHb, nAChRs are thought to be a relatively homogeneous population of heteromeric α3β4 subunit-containing receptors,[13,14] (but see ref. 15). In regard to the activation and desensitization of these channels by nicotine, a full description has already been made.[16] In brief, the EC_{50} for activation is ≈100 μM, whereas half-maximal pseudo steady-state desensitization occurs at ≈300 nM (Fig. 2A). Continuous exposure with concentrations above 300 nM will therefore force the majority of channels into the desensitized state(s), however, there comes a point where activation starts to occur before desensitization is complete: superimposition of the activation and desensitization curves reveals the concentration range over which this occurs (Fig. 2A; *shaded area*). Such 'window currents' allow receptors to maintain some level of activity even in the face of constant agonist application.[17] For MHb channels the 'window' is open over an agonist concentration range of ≈500 nM–50 μM with the peak occurring at 6 μM nicotine. Assuming that ACh behaves in a similar manner to nicotine,[13] then high nanomolar concentrations of ambient transmitter could persistently activate some channels. While, these concentrations of agonist seem quite high compared to reported low nanomolar concentrations of ACh in the CSF,[9] the local concentration of transmitter could be much higher depending on the amount of secreted acetylcholinesterase.[6] With respect to other neuronal nicotinic receptors, the very high affinity state of the α4β2 nAChR (e.g., ref. 18) would likely be more readily activated by these lower ACh concentrations than the α3β4 channels.

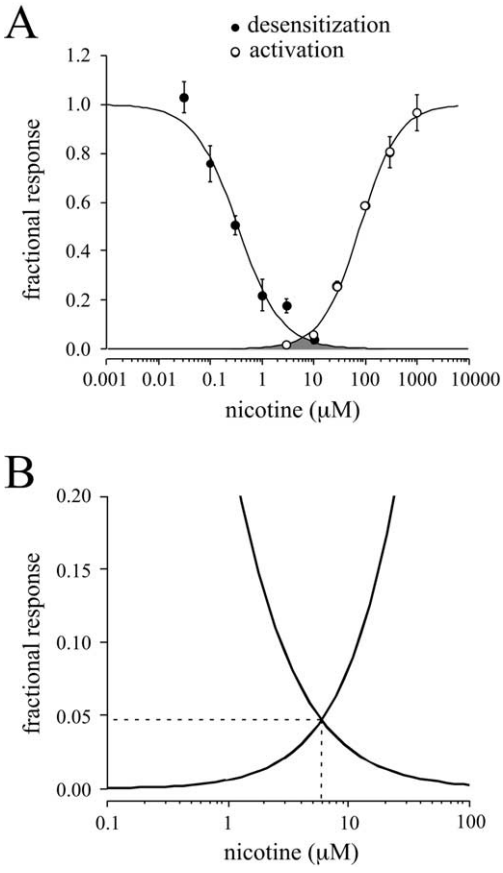

Figure 2. 'Window currents' mediated by nAChRs in the MHb. (A) Concentration–response curves for activation and desensitization of nAChRs by nicotine in whole cell patch clamp recordings. Activation was measured as the peak current resulting from the rapid application of agonist. Desensitization was measured from the change in peak amplitude of a test pulse of agonist following 10 min incubation in different concentrations of nicotine. The 'window current' is shown shaded. (B) Enlargement of the 'window current' portion of the dose–response curve showing that peak steady-state activation of ≈5% nAChRs occurs at 6 μM. Adapted with permission from ref 16.

At the peak of the 'window current' only about 5% channels will be activated (Fig. 2B), but this will be significant if there are a large number of nAChRs on the cell surface. Previous studies have estimated that there are ≈100 functional channels in a 10 μm^2 MHb cell membrane patch.[19] Assuming a uniform density of channels, based on a mean whole cell capacitance of 17 pF,[20] there will be ≈17,000 nAChRs in a MHb neuron. From the concentration–response curve, 6 μM nicotine could activate several hundred channels. Thus, substantial activation of nAChRs could occur in the presence of nanomolar concentrations of ambient transmitter. It should also be noted that the amplitude of the 'window current' may be markedly affected by modulation of steady-state desensitization, possibly via

Ca^{2+} and phosphorylation.[21] Such regulation would shift the desensitization curve to the right or left, providing more or less activation at a given concentration of ACh.

4. nAChRs as Ca^{2+} gates

Although nAChRs are thought primarily to serve as regulators of membrane voltage, they also cause the influx of Ca^{2+}.[22,23] In addition, they act as sensors of extracellular Ca^{2+}, because their activity, and as a consequence Ca^{2+} influx, is proportional to the local Ca^{2+} levels.[23,24] It may be, however, that this secondary action of nAChRs, namely Ca^{2+} influx, becomes of paramount importance if nAChRs are activated only by ambient levels of ACh. In this case, the net current influx and membrane depolarization may be small, but because under these conditions a proportion of channels are expected to be continuously active, nAChRs may provide an effective pathway for conveying information about the state of extracellular Ca^{2+}, by means of Ca^{2+} influx. Thus if nAChRs are a major contributor to intracellular Ca^{2+} homeostasis, then the concentration of intracellular Ca^{2+} will be proportional to its concentration in the extracellular space.

The high density of nAChRs on the small MHb cells (diameter ≈15 μm),[13] makes it likely that they will significantly contribute to the intracellular Ca^{2+} concentration, even at low levels of channel activation. Towards this end, it can be demonstrated that a low micromolar concentration of ACh (10 μM) can produce a significant elevation of intracellular Ca^{2+} (Fig. 3). Under non-voltage clamp physiological conditions, such a signal will be amplified by the Ca^{2+}-mediated potentiation of nAChRs,[23,24] and possibly through activation of voltage-dependent Ca^{2+} channels, and the release of Ca^{2+} from intracellular stores.[25–27]

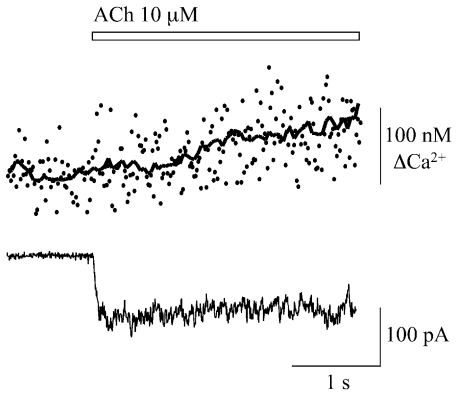

Figure 3. Elevation of intracellular Ca^{2+} in MHb neurons by low concentrations of transmitter. Under whole cell patch clamp, application of ACh (open bar) produces both an inward current (lower trace) and an increase in intracellular Ca^{2+} (upper trace). The solid line indicates a smooth fit through the data points. Intracellular Ca^{2+} concentrations were estimated using the ratiometric dye, indo-1, loaded through the patch pipette.

5. Conclusions

In summary, it is suggested that the biophysical properties of α3β4 nAChRs in the MHb may make these receptors suitable for detection of ambient levels of ACh. At present it is not known whether there is sufficient ACh release in this brain region to overcome breakdown by cholinesterases—a complication that may be related to the in vitro nature of the experiments performed so far. However, in the absence of any evidence for direct synaptic transmission, it is argued that the dense population of non-synaptic nAChRs in the MHb may be in a position to receive diffusely released transmitter.

The properties of nAChRs also provide a rationale in terms of nicotinic drug design. Compounds with agonist activity may be limited in their ability to stimulate nAChRs because of marked channel desensitization. Indeed it is plausible that some of the effects of therapeutic agents may arise through antagonism (rather than agonism) of nAChRs, due to a desensitization-induced block of the action of endogenous ACh. Drugs that do not interact as strongly with desensitized states of nAChRs could prove useful, not only in terms of their medicinal value, but also as research tools.

Acknowledgements

The author would like to thank, Xiaochuan Guo for providing the Ca^{2+} data, Arash Eslami for help with the immunohistochemistry, and Dr. P. Read Montague for helpful discussions. This work was supported by NIH grants NS31669 and DA11940.

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