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Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease

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Abstract

The basic symptoms of Alzheimer's dementia, i.e., a loss in cognitive function, are due to impaired nicotinic cholinergic neurotransmission. To compensate for this impairment by drug treatment, blockers of the acetylcholine-degrading enzyme acetylcholinesterase are applied, even though this approach obviously is prone to many side-effects, including those of muscarinic nature. We have recently described a novel class of nicotinic acetylcholine receptor ligands which, similar to the action of benzodiazepines on $GABA_A$ receptors, allosterically potentiate submaximal nicotinic responses. The sensitizing effect is a consequence of facilitated channel opening in the presence of allosterically potentiating ligand (APL). Representative members of this class of ligands are the plant alkaloids physostigmine, galanthamine, and codeine. Because APLs could enhance nicotinic neurotransmission under conditions of reduced secretion and/or increased degradation of acetylcholine or reduced acetylcholine-sensitivity of nicotinic acetylcholine receptors, they could have a preventive and corrective action on impaired but still functioning nicotinic neurotransmission.

Keywords: Nicotinic acetylcholine receptor; Allosteric modulation; Alzheimer's disease; Galanthamine; Cholinergic deficit

1. Introduction

A large body of evidence, including autoradiographic and histochemical studies of autopsy brain tissue (Nordberg and Winblad, 1986; Whitehouse et al., 1986; Schröder et al., 1991; Perry et al., 1995), and brain imaging studies of patients (Nordberg et al., 1995), identifies the selective loss of nicotinic acetylcholine receptors as the biochemical parameter most closely associated with the severeness of the disease. In the upper cortical layers of the frontal cortex and in the temporal cortex, the loss of nicotinic acetylcholine receptors appears to concern predominantly an $\alpha 4$ subunit-bearing subtype rather than the $\alpha 7$ nicotinic acetylcholine receptor, as is suggested by histochemical studies (Martin-Ruiz et al., 1999; Wevers et al., 1999) and radioligand binding (Potter et al., 1999).

Of the many nicotinic acetylcholine receptor subtypes that are expressed in the mammalian brain, the $\alpha 4\beta 2$ and the $\alpha 7$ subtype are the most prominent ones. They are both found in postsynaptic as well as in presynaptic and perisynaptic locations (Albuquerque et al., 1996a; Alkondon et al., 1999b). The $\alpha 7$ nicotinic receptor displays functional properties quite different from those of the $\alpha 4\beta 2$ nicotinic receptor, among which are a much higher Ca^{2+} permeability, very fast desensitization and different pharmacology, including activation by choline and blockade by α-bungarotoxin (Castro and Albuquerque, 1995; Albuquerque et al., 1996b; Alkondon et al., 1997; Alkondon et al., 1999b). Due to its sensitivity to choline, the $\alpha 7$ nicotinic receptor can be chemically excited even after the natural transmitter has been enzymatically cleaved. $\alpha 7$ Nicotinic acetylcholine receptor therefore can respond not only to synaptic events of acetylcholine release but also to volume changes in acetylcholine/choline concentration. (Rapid desensitization of $\alpha 7$ nicotinic acetylcholine receptor and an appropriate refractory period may be prerequisites for the latter response mode.) Due to its Ca^{2+} perme-

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ability, α7 nicotinic receptor activation can produce metabotropic responses in the excited cell, including Ca^{2+}-controlled transmitter release and stimulation of gene transcription and protein biosynthesis. Very recently, the first electrophysiological studies of human cerebral cortical interneurons have been reported (Alkondon et al., 1999a). These studies established that both α4β2 and α7 nicotinic acetylcholine receptors are located on the somatodendritic regions of human interneurons, and as demonstrated by their ability to modulate GABA release, could be involved in inhibitory and disinhibitory mechanisms in the human cortex. The inhibitory action could enhance the signal-to-noise ratio of neuronal circuitry, whereas the disinhibitory action could lead to synaptic strengthening which is an essential element of the learning paradigm long-term potentiation (LTP) (Alkondon et al., 1999a).

Three major strategies have so far been applied to balance nicotinic cholinergic deficits, stimulation of acetylcholine synthesis, inhibition of acetylcholine degradation, and administration of nicotinic receptor agonists. Practically no therapeutic effects have been achieved by the administration of acetylcholine precursors (Feldman and Gracon, 1996). Administration of choline esterase inhibitors presently is the most commonly applied therapeutic approach. These inhibitors have proven albeit limited therapeutic value (Nordberg and Svensson, 1998), and most of them do not prevent progression of the disease to any significant extend (Rogers et al., 1998; Flicker, 1999). A number of nicotinic receptor agonists are presently in preclinical and clinical testings (Bjugstad et al., 1996; Menzaghi et al., 1997; Francis et al., 1999), even though they are difficult to dose, as higher levels may cause desensitization rather than increased activation of nicotinic receptors (Maelicke and Albuquerque, 1996). Other unsolved problems are drug transport to the targeted nicotinic receptor(s) in the brain and target selectivity (receptor subtype).

A novel approach to drug treatment in Alzheimer's disease is the application of allosteric modulators of nicotinic receptors (Maelicke and Albuquerque, 1996; Maelicke et al., 1995). Allosteric modulators are compounds that interact with the receptor via binding sites that are distinct from those for acetylcholine and nicotinic receptor agonists and antagonists. Consequently, modulators are not directly involved in the neurotransmission process they affect, and hence, usually do not induce compensatory processes, as agonists and antagonists may do (e.g., receptor desensitization, down-regulation of expression). Because Alzheimer's disease is associated with a deficit in nicotinic neurotransmission, allosteric modulators are needed to up-modulate (potentiate) the channel activity of nicotinic receptors in response to acetylcholine. Such properties are displayed by a novel class of nicotinic receptor ligands, named ''allosterically potentiating ligands'' (APLs) (Maelicke and Albuquerque, 1996; Schrattenholz et al., 1996).

Allosteric modulation of receptor activity is a quite common mechanism in neurotransmission. Arguably, the most prominent example is the benzodiazepines which positively modulate (potentiate) the activity of the $GABA_A$ receptor by facilitating opening of the receptor-integral Cl^- channel (increase in the probability of channel opening at given concentrations of GABA). This effect is the underlying principle of the anxiolytic action of benzodiazepines (McDonald and Twyman, 1992).

2. Results

In Fig. 1A, a representative example of allosteric potentiation of nicotinic responses is shown. Using 3-day old PC 12 cells of bipolar morphology, the response to 100 μM acetylcholine, in the absence of 1-methyl-galanthamine (me-Gal, first trace), was nearly doubled in peak amplitude when acetylcholine was applied together with 0.4 μM *N*-methyl-galanthamine (second trace). At the same concentration, *N*-methyl-galanthamine alone did not induce a significant whole-cell current (third trace). The level of response induced by the combined application of 100 μM acetylcholine and 0.4 μM *N*-methyl-galanthamine (second trace) was matched in amplitude, but not in the kinetics of inactivation, by the response to 1000 μM acetylcholine (fourth trace).

In Fig. 1B, the effect of *N*-methyl-galanthamine on the dose–response relationship for acetylcholine is displayed. The APL shifts the dose–response curve to lower concentrations of acetylcholine without changing the level of maximal response. This finding suggests that in the presence of *N*-methyl-galanthamine, the affinity of binding of

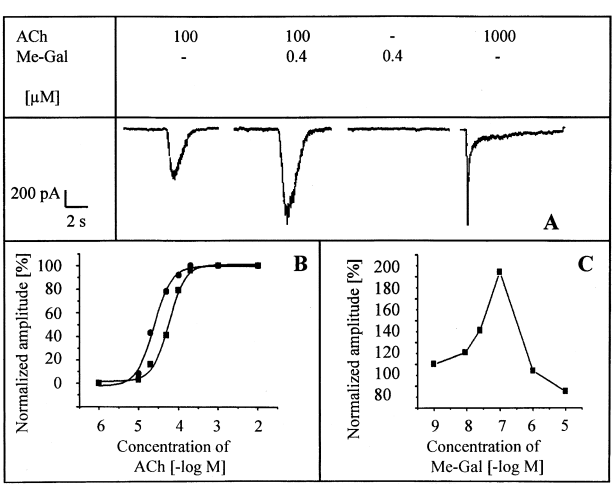

Fig. 1. Potentiation by 1-methyl-galanthamine of acetylcholine-elicited whole-cell responses of cultured PC12 cells. (A) Response recorded from a single PC12 cell of bipolar morphology from a 3-day old culture. (B) Effect of *N*-methyl-galanthamine on the dose–response relationship for acetylcholine. Note that potentiation is observed only at sub-maximal responses. (C) Change of the peak amplitude of response to acetylcholine (100 μM) versus the concentration of *N*-methyl-galanthamine applied.

acetylcholine to nicotinic acetylcholine receptor is increased, or the level of channel activation corresponding to given occupancies of the receptor is increased, or both.

The potentiating effect of *N*-methyl-galanthamine is observed only in a rather narrow window of APL concentration. As shown in Fig. 1C, potentiation of acetylcholine-induced response was limited to *N*-methyl-galanthamine concentrations below 1 μM. At higher concentration of *N*-methyl-galanthamine, direct channel blockade is becoming increasingly significant; it counterbalances and eventually reverses the potentiating effect.

In addition to PC12 cells, we have observed positive modulation by APL of nicotinic responses with many other cell types, including rat hippocampal neurons (Pereira et al., 1993a,b), SHSY5Y human neuroblastoma cells (Schrattenholz et al., unpublished), mouse fibroblast and HEK-293 human embryonic kidney cell lines stably expressing the rat $\alpha4\beta2$ nicotinic acetylcholine receptor (Pereira et al., 1994; Maelicke et al., unpublished) and the human $\alpha4\beta2$ nicotinic acetylcholine receptor (Samochoski et al., unpublished), respectively, and most recently, human brain tissue (Alkondon et al., 1999a). At the present stage of analysis, we cannot conclusively state whether only selected, or most, or all nicotinic acetylcholine receptor subtypes are subjected to positive allosteric modulation by APL.

Representative nicotinic APL are the plant alkaloids physostigmine (Phy), galanthamine (Gal), and codeine (Cod), and the neurotransmitter serotonin (5-HT). Most APL are rather lipophilic compounds, and they contain a tertiary nitrogen that is cationic at neutral pH, and which is located at a fixed distance from a phenolic hydroxyl group (Maelicke et al., 1995). These structural properties are similar to those of phenanthrene-type opioids and endorphines with narcotic activity. They are also found in non-narcotic drugs, such as certain dopaminergic agonists and antagonists, and some centrally acting cholinergic drugs.

3. Discussion

The key feature of Alzheimer's disease is a loss in cognitive function which includes loss of (short-term) memory and learning ability, impaired attention associated with relentlessness, disturbances of language, and emotional instability. All these functional deficits are the result of impaired neurotransmission in the central nervous system and probably involve several transmitter systems. Interestingly, the biochemical parameter best correlated with the severeness of Alzheimer's disease is a substantial loss in nicotinic receptors in the brain regions known to be essential for the behavioral tasks that are impaired in Alzheimer's disease. The question therefore arises, whether and how nicotinic receptors could affect other neurotransmission systems.

One mechanism of interaction of nicotinic cholinergic neurotransmission with other neurotransmission systems has recently been identified. It is the modulatory control of transmitter release by pre-synaptic nicotinic receptors, both the $\alpha4\beta2$ subtype and the Ca^{2+}-conducting $\alpha7$ nicotinic acetylcholine receptor subtype (Albuquerque et al., 1996b; Alkondon et al., 1999b). Thus, in the case of Alzheimer's disease, reduced expression of presynaptic nicotinic acetylcholine receptor could limit or even abolish the modulatory control of glutamate release, which in turn could lead to reduced capability in learning and memory. Moreover, because $\alpha7$ nicotinic acetylcholine receptor can be activated by choline (Alkondon et al., 1997, 1999b) and not only by acetylcholine as most other nicotinic receptors, choline may act as a ''retrograde messenger'' in the learning paradigm LTP, which is governed by glutamatergic neurotransmission. Similarly, a loss of presynaptic nicotinic acetylcholine receptor could also reduce the modulatory control of serotonergic neurotransmission, leading to the mood changes known to be associated with Alzheimer's disease. The recent suggestion of an ambient level of acetylcholine in the central nervous system (Descarries, 1998) is further evidence for acetylcholine/choline-controlled modulatory mechanisms. It is noteworthy in this regard that the $\alpha7$ nicotinic acetylcholine receptor has been linked to several additional psychiatric diseases, including schizophrenia (Leonard et al., 1996, Freedman et al., 1997) and Tourette's syndrome (Hsu et al., 1996). An additional mechanism of modulatory control by nicotinic receptors is provided by the considerable Ca^{2+} permeability of some subtypes which links nicotinic neurotransmission to intracellular signaling controlled by Ca^{2+}.

To focus on nicotinic acetylcholine receptor-modulated transmitter release, let us consider a synapse in which (i) only acetylcholine is acting, and (ii) two transmitters (ACh and another one, e.g., glutamate) are acting coincidentally. In the first case, the activation of presynaptic nicotinic receptors could induce, by way of entry of Ca^{2+}, the release of (additional) acetylcholine, which consequently would result in an (increased) postsynaptic response to acetylcholine. Such a mechanism would function as a modulatory feedback loop for acetylcholine, in that the more acetylcholine is released, the more acetylcholine or choline will bind not only to postsynaptic but also to presynaptic nicotinic receptors, with the latter further enhancing presynaptic release. Intense usage of nicotinic cholinergic synapses, within the time frame of insignificant diffusion and uptake processes, will hence lead to an up-regulation of synaptic response, such as is typical for the learning paradigm LTP.

In the second case, acetylcholine and/or choline are merely used as signaling molecules that report back to the presynaptic, ending the state of activity of the synapse. Thus, the more intense the synaptic usage, the more acetylcholine/choline is co-released with e.g., glutamate, and

the higher will be their synaptic concentration. At increased concentration due to more intense synaptic usage, more presynaptic nicotinic acetylcholine receptor will be activated, more transmitter (both acetylcholine and glutamate) will be released, and the stronger will be the postsynaptic response. Again, a positive feedback loop will result which is consistent with the requirements for a Hebb synapse. Also the second mechanism is a suitable basis for a learning paradigm.

Under conditions of Alzheimer's disease, and other nicotinic acetylcholine receptor-associated neurological diseases, the above natural mechanism of cognition acquisition may be impaired due to neurodegenerative loss of nicotinic acetylcholine receptor or loss of other functional properties. Should nicotinic neurotransmission be reduced but still functioning, nicotinic APLs could be employed as drugs to balance, at least in part, the existing nicotinic cholinergic deficit. By making the remaining nicotinic acetylcholine receptors more sensitive to acetylcholine/choline, a stronger response is produced than could be achieved in the absence of APLs (Fig. 1). There would be hardly any risk of overstimulation or desensitization of presynaptic nicotinic acetylcholine receptors by APL because (i) APLs do not produce themselves significant responses and (ii) APLs only potentiate submaximal acetylcholine-induced responses (Fig. 1).

Positive allosteric modulation by APL may be of particular importance in central nervous system synapses, in which the major neurotransmitter is not acetylcholine. As has been shown previously, presynaptic nicotinic acetylcholine receptors, in addition to modulating the release of acetylcholine, can also modulate the release of glutamate, serotonin, and GABA (Alkondon et al., 1997, 1999b; Levin 1998). Thus, APLs may help to improve learning and memory (due to enhanced glutamatergic neurotransmission), reduce emotional disturbances such as anxiety and depression (due to enhanced serotonergic neurotransmission), and provide behavioral stability, e.g., remove aggression and sexual disinhibition (due to enhanced GABAergic neurotransmission).

Recent findings from several laboratories suggest that chronic low-level stimulation of nicotinic receptors may up-regulate their expression (Rowell et al., 1987; Peng et al., 1997) and may slow neurodegeneration (Court and Perry, 1994). Moreover, there is initial evidence that chronic low-level stimulation of nicotinic receptors protects against β-amyloid toxicity by increased release of the terminally truncated secreted form of β-amyloid precursor protein. (Kihara et al., 1998). Similarly, the same treatment has been reported to protect from glutamate-induced neurotoxicity (Akaike et al., 1994). All these effects may be produced by the quasi metabotropic properties of nicotinic acetylcholine receptor subtypes that have a high Ca^{2+} permeability, e.g., the α7 nicotinic receptor.

Fig. 2 summarizes possible strategies of drug treatment in Alzheimer's disease based on the assumption that the reduced number of nicotinic receptors is the basic deficit that is linked to the major symptoms of the disease. Clearly, the most specific strategy, which therefore should be the least prone to unwanted side effects, is to selectively sensitize particular nicotinic acetylcholine receptor subtypes. The presently most common approach, i.e., inhibition of acetylcholinesterase, certainly is more likely to produce side effects, including those due to muscarinic overstimulation.

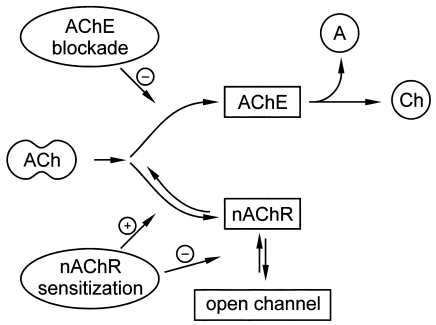

Fig. 2. Compensation by drugs of a nicotinic cholinergic deficit. After pre-synaptic release, acetylcholine interacts with the hydrolysing enzyme acetylcholinesterase (AChE) in the synaptic cleft, and with postsynaptic (and presynaptic) nicotinic receptors. Because association of acetylcholine to both macromolecules proceeds with very high, almost identical rate (Jürss et al., 1979), relative distribution is determined by the expressions levels and accessibility of acetylcholinesterase and nicotinic acetylcholine receptor. Eventually all acetylcholine molecules will be degraded to choline and acetate, and the breakdown products will be removed by diffusion and re-uptake. In this sense, interaction of acetylcholine with nicotinic acetylcholine receptor is a delay loop (Maelicke, 1984). Drugs can affect nicotinic neurotransmission in three ways: (i) by inhibition of acetylcholinesterase, thereby temporarily raising the synaptic level of acetylcholine and hence the probability of nicotinic acetylcholine receptor activation; (ii) by sensitisation of nicotinic acetylcholine receptor (e.g., by allosterically potentiating ligand); and (iii) by increasing the stability of the open-channel state. Most drugs presently employed in Alzheimer's disease act as anticholinesterases, whereas galanthamine (and physostigmine) also act as nicotinic acetylcholine receptor-sensitising ligands (APL).

Drugs like nicotinic APL that do not directly participate in the neurotransmission process they affect, are advantageous, as compared to agonists, in that they do not promote receptor desensitization and/or down-regulation of receptor expression. Because the maximal achievable level of receptor activation is not changed in the presence of APLs (they only enhance submaximal activation, see Fig. 1), they provide as a means of gentle balancing an existing nicotinic cholinergic deficit.

An agonist action of the established anticholinesterase physostigmine was first described by Katz and Miledi (1977) and Shaw et al. (1985). After we have shown that this agonist action actually is an allosteric potentiation of an intrinsic acetylcholine response (Schrattenholz et al., 1996), several other laboratories have reported similar findings (e.g., Zwart and Vijverberg, 1997; Sabey et al., 1999).

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