ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 457–466

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Role of neuronal nicotinic receptors in the transmission and processing of information in neurons of the central nervous system

Eliane Tribollet, Daniel Bertrand*, Mario Raggenbass

Department of Physiology, University Medical Center, 1, rue Michel-Servet, CH-1211 Geneva 4, Switzerland

Received 14 May 2001; received in revised form 27 September 2001; accepted 27 September 2001

Abstract

The properties of nicotinic acetylcholine receptors (nAChRs) were studied following exogenous expression in a host system or using whole-cell recordings in brain slices, autoradiography and immunohistochemistry. When expressed in HEK-293 cells, $\alpha4\beta2$ nAChRs displayed both a high and a low affinity component. The ratio of these two states was modified by chronic nicotine exposure, resulting in an enhanced sensitivity and a marked reduction in desensitization. Mutations in the gene coding for the $\alpha4$ subunit are responsible for a particular form of nocturnal epilepsy. When expressed in *Xenopus* oocytes, $\alpha4\beta2$ nAChRs containing these mutations displayed distinct alterations in agonist affinity, desensitization and calcium permeability. Magnocellular endocrine neurons in the supraoptic (SO) nucleus of the hypothalamus were found to express functional $\alpha7$-containing nAChRs, which could play a role in regulating neurohypophysial peptide secretion. Facial (VII), hypoglossal (XII) and vagal (X) motoneurons of young rats responded to ACh by a fast inward current. The nAChRs present in VII and XII nuclei were of the non-$\alpha7$-containing type, whereas those present in the X nucleus contained the $\alpha7$ subunit. In Bcl-2 transgenic mice, facial nerve axotomy caused nAChRs downregulation by interfering negatively with the expression of the $\alpha4$ subunit. Binding sites corresponding to $\alpha7$-containing nAChRs were also detected in spinal motor nuclei and axotomy provoked a reduction of the binding. Together, these data indicate that long-term exposure to nicotine can promote neuroadaptive changes in nAChRs and that genetic alterations of neuronal nAChRs can result in transmissible neurological diseases. They also suggest that these receptors probably play a role in the central regulation of autonomic functions, as well as in motor control.

Keywords: Acetylcholine; Axotomy; Brainstem; Hypothalamus; Motoneurons; Nicotinic antagonists; Reconstituted receptors; Whole-cell recordings

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are members of the superfamily of ligand-gated ion channels. Eleven mammalian genes coding for subunits of these receptors have now been identified (Elgoyhen et al., 2001; Galzi and Changeux, 1995). Functional neuronal nAChRs are widely distributed within the central nervous system. They can be located on axon terminals, where they play a well-documented role in modulating synaptic transmission, but they can also be found on cell bodies and/or dendrites, where they may mediate direct postsynaptic effects (Jones et al., 1999; MacDermott et al., 1999; McGehee and Role, 1995; Role and Berg, 1996; Wonnacott, 1997). Numerous functional studies have gradually highlighted our understanding of the role of these receptors in the central nervous system (Changeux et al., 1998; Dani et al., 2001; Lindstrom, 1997; Paterson and Nordberg, 2000). However, the precise contribution of these ligand-gated channels in brain function and/or dysfunction is still under investigation.

The present review summarizes recent studies done in our laboratory. In the first part, we will concentrate upon reconstituted nAChRs expressed in *Xenopus* oocytes or in transfected cell lines. In the second part, we will review electrophysiological and morphological experiments aimed at characterizing native nAChRs present in the endocrine hypothalamus and in brainstem motor nuclei, respectively. The data presented have already been published. In a final part, we present some preliminary, unpublished results on the axotomy-induced regulation of nicotinic binding sites in the brainstem and spinal cord.

* Corresponding author. Tel.: +41-22-702-53-56.
E-mail address: bertrand@cmu.unige.ch (D. Bertrand).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00700-6

2. Reconstitution of exogenous nAChRs in a host system

While recording of ligand-gated channel activity from dissociated neurons or in slices allows to examine the properties of native nAChRs, these studies are often limited for detailed characterization of the receptor properties. Main difficulties regarding these approaches are, on the one hand, the variability in receptor expression, and on the other hand, the limitation of proper drug delivery. It should be recalled that during synaptic transmission transmitter release is extremely fast and, for example, at the neuromuscular junction, it was proposed that ACh raise up to 1 mM in less than a millisecond.

To get a better understanding of a given receptor subtype in isolation, it is therefore necessary to control both the receptor composition and drug application. Conditions that best fulfill these two requirements are recording of receptors reconstituted by exogenous expression either in *Xenopus* oocytes or in transfected cell lines. The availability of a large series of cDNA coding for mouse, rat and human nAChRs further open the possibility to examine the functional similarities and differences of a given receptor subtype. These experiments therefore allow to best compare data obtained from animal experiments with possible hypotheses of nAChRs function in the human brain.

Striking differences of sensitivity and pattern of desensitization are observed between receptor obtained with different subunit combinations. The highly sensitive $\alpha4\beta2$ receptors, which are thought to be the most common in the brain, display a high sensitivity to ACh and a slow desensitization. At the other extreme are the low ACh sensitivity and highly desensitizing $\alpha7$ receptors (Couturier et al., 1990; Seguela et al., 1993). Because the ACh binding site is at the interface between the α (principal component) and the β (complementary component) subunits, a wide pattern of sensitivity and desensitization behavior can be expected when comparing different combinations. Indeed, $\alpha3\beta2$ receptors, a combination expressed in the CNS, display a low sensitivity and fast desensitization. Exchange of the β subunit in the $\alpha3\beta4$ combination, which is thought to be the major ganglionic receptor, results in a relatively low sensitivity to ACh but also in a slow desensitization. Construction of chimeric $\beta2$:$\beta4$ and $\beta4$:$\beta2$ subunits, made by exchanging the segment comprised between the N-terminal domain and the first transmembrane segment, revealed that the desensitization profile is determined by the N-terminal domain (Bohler et al., 2001).

A further complexity of the nAChRs was revealed by detailed analyses of the receptor dose–response relationship. Numerous determinations of $\alpha4\beta2$ ACh dose–response curve with less than 10 agonist concentrations all revealed that, while displaying a high sensitivity, these receptors are characterized by a curve with shallow slope, reflecting a low apparent cooperativity (Buisson et al., 1996). More accurate determination of $\alpha4\beta2$ nAChRs sensitivity revealed, however, a biphasic behavior of the concentration–response relationship. First reported for the rat $\alpha4\beta2$ reconstituted in *Xenopus* oocytes by Covernton and Connolly (2000), this biphasic sensitivity was more recently described and analyzed for the human receptor expressed in HEK-293 cells (Buisson and Bertrand, 2001). The major finding made in this work was that $\alpha4\beta2$ receptors display both a high and a low affinity component and that the ratio of these two states can be modified by chronic nicotine exposure. Thus, following nicotine exposure, the $\alpha4\beta2$ receptors display a higher ACh sensitivity than in control conditions. Concomitantly, chronic exposure to nicotine caused a marked reduction in desensitization. Differences with data obtained in previous studies (Fenster et al., 1999; Olale et al., 1997) may be attributable to the expression system. From the comparison with behavioral data that reveal, in certain conditions, a nicotine sensitization (Balfour et al., 2000), it may be argued that results obtained in cell lines correspond more closely to the in vivo receptor properties.

The identification in 1995 of an association between a missense mutation in the *CHRNA4* gene (coding for the $\alpha4$ subunit) and a particular form of nocturnal epilepsy (Phillips et al., 1995; Steinlein et al., 1995, 1997) shed a new light on the importance of nAChRs in the normal brain function. Reconstitution experiments made with this mutated $\alpha4$ subunit coexpressed with a control $\beta2$ subunit revealed the profound effects caused by this single amino acid substitution (Bertrand et al., 1998; Figl et al., 1998; Kuryatov et al., 1997; Picard et al., 1999; Weiland et al., 1996). This first observation was soon followed by the identification of another mutation in the *CHRNA4* gene in another family carrying the genetically transmissible phenotype of ADNFLE (Steinlein et al., 1997). This second mutation, which consists of a 3-bp insertion (776ins3), and, therefore, of an extra amino acid residue, also caused modifications of the receptor properties when tested in *Xenopus* oocytes. Since then, another mutation, C755T, was detected in the *CHRNA4* gene (Hirose et al., 1999). Several attempts were made in search of a common alteration that could explain the association between these genetic traits and the neurological phenotype. First reported, the S248F mutation causes a major reduction in the current amplitude and desensitization (Bertrand et al., 1998). In contrast, neither the 776ins3 mutation nor the C755T mutation causes a detectable reduction of the current amplitude or desensitization. In addition, while it was first shown that both the S248F and 776ins3 mutants display a lower calcium permeability than the control receptors (Kuryatov et al., 1997; Steinlein et al., 1997), we found no alteration of the calcium permeability in the C755T mutant. It should be pointed out, however, that all these results were obtained by injecting a mutated $\alpha4$ cDNA together with a control $\beta2$ cDNA. When considering expression in neurons, it should be recalled that patients carrying this mutations are heterozygous and that, therefore, unless imprinting is present, the two alleles should equally be expressed. To mimic this natural condition, new

experiments were designed in which both the affected and nonaffected α4 alleles were coinjected with a β2 subunit. Interestingly, in these experiments, the amplitude of ACh-evoked currents recorded in oocytes expressing the control and S248F mutation showed no differences with those of the control alone. Pronounced desensitization was, however, still observed and thereby confirmed the dominant nature of the mutations. Comparison of all the physiological data obtained in these experimental conditions suggests that a common trait of the three α4 mutations may be an increase in the receptor sensitivity to ACh (Moulard et al., 2001).

Because functional receptors must include the β2 subunit, it was predicted that mutations in the corresponding *CHRNB2* gene should also be associated with ADNFLE. The very recent identification of two unrelated families carrying a mutation in the *CHRNB2* gene indeed confirmed this prediction (De Fusco et al., 2000; Phillips et al., 2001). Characterization of the β2-V287M mutation revealed that, as for the α4 mutants, these receptors also display a higher sensitivity to ACh. While awaiting further experimental evidences, these data suggest that ADNFLE may be associated with an increase in the receptor sensitivity. Although it is tempting to conclude that ADNFLE results from an α4β2 gain of function, caution should still be taken. As previously demonstrated (Bertrand et al., 1998), increase in sensitivity can be associated with an increase in desensitization by prolonged exposure to agonists. Thus, it will be necessary to determine under physiological conditions if the receptors are more activatable or are more desensitized by the in situ level of neurotransmitter.

3. Native receptors

3.1. Endocrine hypothalamus

The supraoptic (SO) and paraventricular (Pa) nuclei of the hypothalamus contain the *magnocellular* endocrine neurons that synthesize vasopressin and oxytocin and project to the neural lobe of the hypophysis, where the peptides are released into the bloodstream in response to a variety of physiological stimuli. When injected into the SO, ACh stimulates the release of vasopressin, an effect that can at least, in part, be mimicked by nicotine (for review, see Renaud and Bourque, 1991). To investigate the neuronal mechanism of the action of ACh, we carried out whole-cell voltage clamp recordings in hypothalamic slices of young adult rats containing the SO (Zaninetti et al., 2000a). In the presence of atropine, an antagonist of muscarinic AChRs, ACh evoked a rapidly rising inward current, whose amplitude ranged between 30 and 500 pA. This ACh-evoked current was not affected by the blockers of glutamatergic synaptic transmission, 6-nitro-7-sulphamoylbenzo[*f*]quinoxaline-2,3-dione disodium (NBQX) and D-2-amino-5-phosphopentanoic acid (D-AP5), nor by the calcium channel blocker, Cd^{2+}. Its current–voltage relationship was linear at negative potentials and rectified inwardly at positive potentials (Fig. 1A). The ACh-evoked current was insensitive to dihydro-β-erythroidine (DHβE) at 1 μM (Fig. 1B), a

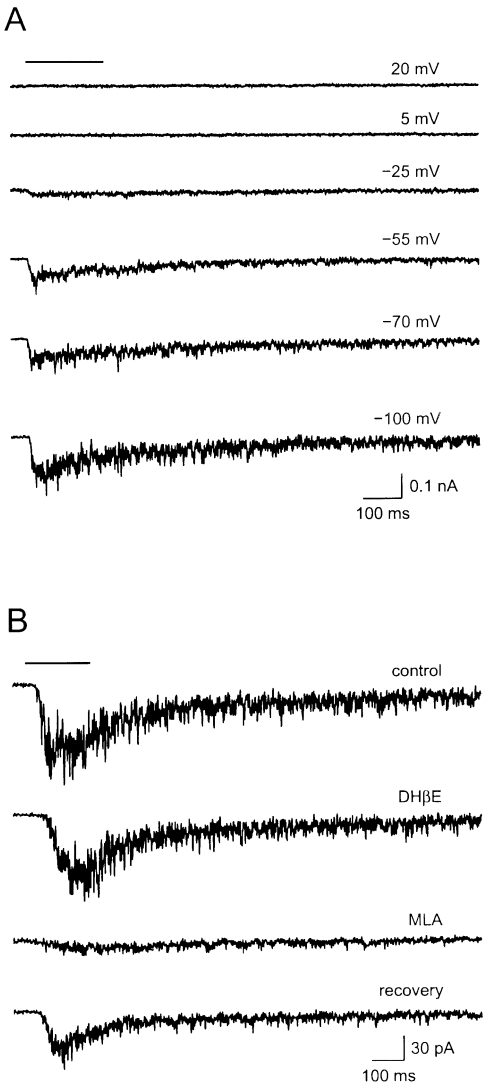

Fig. 1. Effect of ACh and nicotinic agonists on rat SO magnocellular neurons. (A) Voltage clamp records of the membrane current evoked by ACh (200 μM, 200 ms) in a SO neuron. Prior to ACh pressure ejection, the neuron was clamped at the potential indicated near each current trace. Note that the ACh-evoked current did not reverse at positive potentials, indicating that the ACh current–voltage relationship displayed inward rectification. (B) Voltage clamp records of the current evoked by ACh (200 μM, 200 ms) in a second SO neuron, in the normal perfusion solution (control), in the presence of 1 μM DHβE, in the presence of 1 nM MLA and following MLA washout (recovery). Note that the ACh-evoked response was insensitive to DHβE but was suppressed by MLA, suggesting that nAChRs in supraoptic neurons contain the α7 subunit. In this figure, as well as in Figs. 3 and 4, the horizontal bar above each top current trace indicates the time during which the ejection of the agonist lasted. *Methods:* Whole-cell recordings were performed in hypothalamic or brainstem slices, obtained from young adult (hypothalamus) or newborn (brainstem) animals. Neurons were visualized using IR DIC videomicroscopy. ACh was delivered by pressure ejection from a patch-type pipette positioned in the vicinity of the recorded neuron. All the recordings were done in the presence of the muscarinic antagonist, atropine (5 μM).

concentration sufficient to block currents mediated by $\alpha4\beta2$ nAChRs (Alkondon and Albuquerque, 1993; Buisson et al., 1996). It was reversibly abolished by methyllycaconitine (MLA) at 10 nM (Fig. 1B), a concentration at which currents mediated by $\alpha7$-containing nAChRs are fully antagonized (Palma et al., 1996). Similar results were obtained in acutely dissociated SO neurons. In this latter preparation, dose–response relationships yielded a half-saturating concentration (EC_{50}) of about 290 μM for ACh and about 110 μM for nicotine. We conclude that SO magnocellular endocrine neurons possess functional $\alpha7$-containing nAChRs. Immunohistochemical studies, performed using antibodies directed against vasopressin and oxytocin, indicated that responsiveness to nicotinic agonists was a property of vasopressin, as well as of oxytocin magnocellular endocrine neurons.

Consistent with the electrophysiological results, autoradiography revealed the presence in the SO of binding sites for [^{125}I]α-BgTx, a compound that binds specifically to $\alpha7$-containing nAChRs (Clarke et al., 1985; Couturier et al., 1990; Schoepfer et al., 1990), and binding sites for [^{3}H]epibatidine, a ligand selective for non-$\alpha7$-containing nAChRs (Houghtling et al., 1995; Perry and Kellar, 1995), were undetectable. Moreover, [^{125}I]α-BgTx binding could be efficiently displaced by the $\alpha7$-specific agonist (−)-2PABH (Fig. 2; Spang et al., 2000).

3.2. Brainstem motoneurons

In vertebrates, ACh-mediated transmission plays a crucial role in motor control. Muscle-type nAChRs mediate synaptic excitation of skeletal muscle by motoneurons and nAChRs are present on Renshaw cells, where they produce recurrent inhibition of spinal motoneurons (Curtis and Ryall, 1966a,b). Functional nAChRs, however, may also be present in motoneurons. To investigate this issue, whole-cell recordings were performed in motor nuclei in brainstem slices of young rats (Zaninetti et al., 1999). Facial (VII), hypoglossal (XII) and vagal (X) motoneurons responded to ACh by generating a fast inward current. Current–voltage relationships indicated that these currents decreased in amplitude when the membrane potential was shifted towards positive values and became undetectable above −10 mV (Fig. 3A). They did not reverse at positive potentials, indicating that the ACh current–voltage relationships typically displayed inward rectification (Fig. 4) (Bertrand et al., 1990; Forster and Bertrand, 1995).

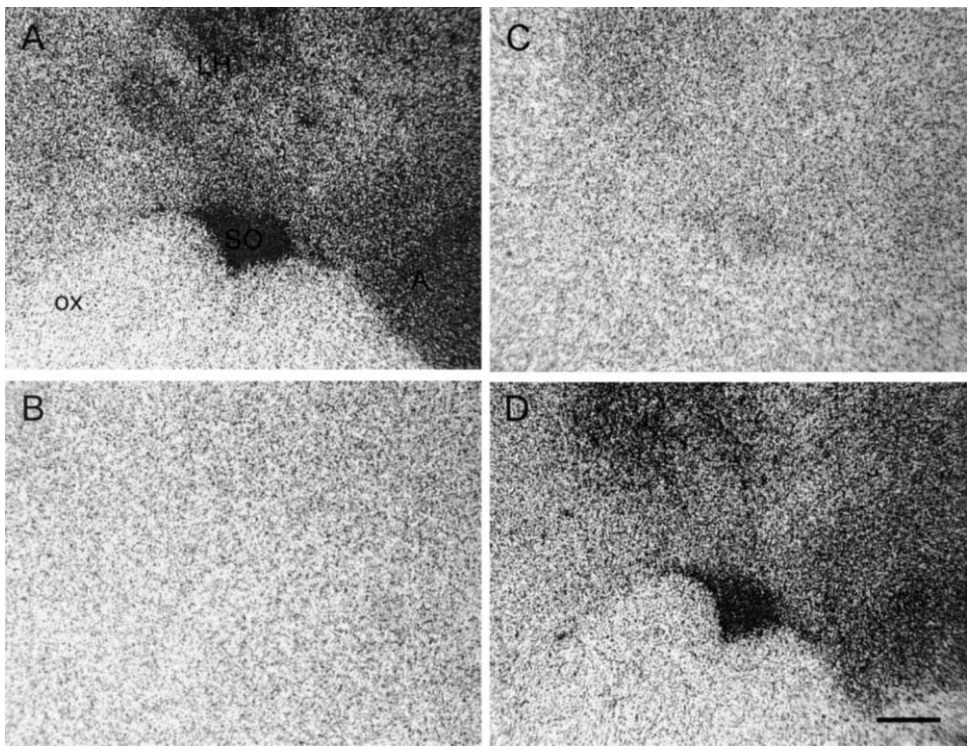

Fig. 2. Pharmacological properties of [^{125}I]αBgTx binding sites in the SO of the rat. Photomicrographs A–D illustrate autoradiograms obtained from four adjacent coronal sections 14 μm thick, cut through the hypothalamus of a 2-month-old male rat and incubated with 1 nM [^{125}I]αBgTx. The binding procedure was performed as previously described (Zaninetti et al., 2000a). (A) The section was incubated with [^{125}I]αBgTx alone. (B) Nicotine was present at 300 μM. (C) (−)-2PABH, a nicotinic agonist specific for $\alpha7$-containing nAChRs, was present at 10 μM. (D) (+)-2PABH, the inactive enantiomer, was present at 10 μM. Note the intense labeling of the SO and the moderate labeling in surrounding structures, in particular, the amygdala (A) and the lateral hypothalamic area (LH). This labeling is specific, since it can be displaced by both nicotine and (−)-2PABH but not by (+)-2PABH. Other abbreviation: ox, optic chiasma. Bar = 250 μm.

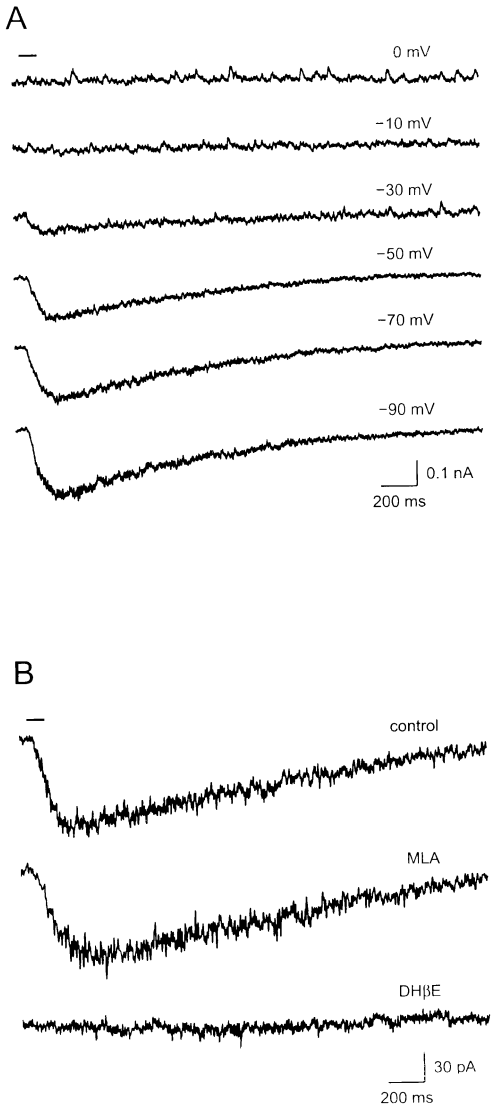

Fig. 3. Effect of ACh and nicotinic antagonists on Type VII motoneurons of the rat. (A) Voltage clamp records showing the current evoked by ACh (100 μM, 100 ms) while the motoneuron was kept at the potential indicated near each current trace. Note that the ACh-evoked current decreased in amplitude when the membrane potential was shifted towards positive values and became undetectable above −10 mV. (B) Voltage clamp records of the current evoked by ACh (100 μM, 100 ms) in a second motoneuron, in the normal perfusion solution (control), in the presence of 10 nM MLA and in the presence of 1 μM DHβE. Note that the ACh-evoked current was almost unaffected by MLA, but was suppressed by DHβE, suggesting that nAChRs present on Type VII motoneurons are not of the α7-containing type.

In Type VII and XII motoneurons, the ACh-evoked current was unaffected by the α7-specific competitive MLA, but was suppressed by DHβE. In addition, in Type VII motoneurons, the ACh effect was mimicked by epibatidine at 10 nM, a concentration at which this agonist is specific for heteromeric nAChRs (Gerzanich et al., 1995), and nicotine induced a slow inward current and desensitized the ACh-evoked current.

The pharmacological profile of the current evoked by ACh in X parasympathetic preganglionic motoneurons was more complex. In about half of the motoneurons, this current had a MLA-, as well as a DHβE-, sensitive component; in the remaining neurons, only the MLA-sensitive component was present (Fig. 4). This indicates that a majority of Type X motoneurons possess functional α7-containing nAChRs. An unexpected finding was that the current mediated by these receptors in Type X motoneurons had a time-to-peak of 40–160 ms, a value much longer than that reported for homomeric neuronal nAChRs (Gopalakrishnan et al., 1995) or for MLA-sensitive ACh-evoked currents in hippocampal neurons (Alkondon and Albuquerque, 1993). Recently, αBgTx-sensitive nicotinic responses also displaying slow time courses have been characterized in intracardiac ganglion neurons of the rat (Cuevas and Berg, 1998). It has been proposed that these responses may be due to nAChRs in which the α7 gene product is combined with another, as yet unidentified, subunit. Thus, in Type X motoneurons, as in intracardiac ganglion neurons, α7 may coassemble with another subunit that confers a slow time course to the responses.

In accordance with the electrophysiological data, autoradiography revealed that VII, X and XII nuclei of young rats contained binding sites for [^{3}H]epibatidine. Binding sites for [^{125}I]α-BgTx were present in the X nucleus but were almost undetectable in the VII and XII nuclei.

3.3. Motoneuron axotomy

Motoneuron axotomy is currently exploited as a model system for studying functional and morphological changes

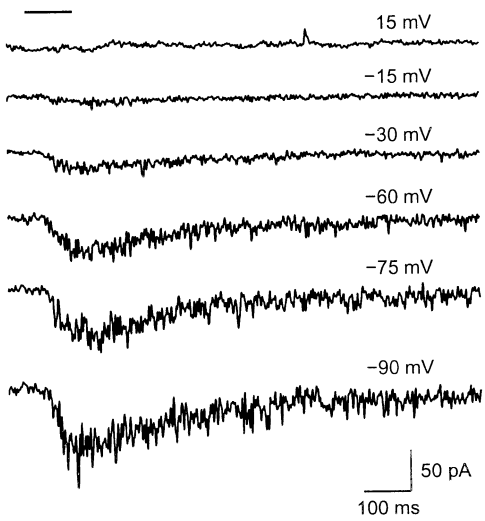

Fig. 4. Effect of ACh on a X parasympathetic preganglionic motoneuron of the rat. Voltage clamp records showing the current elicited by ACh (100 μM, 100 ms) in a X neuron held at the potentials indicated near the current traces. Recordings were performed in the presence of 0.5 μM DHβE. The ACh-evoked current was thus exclusively due to the activation of α7-containing nAChRs. Note the inward rectification in the ACh current–voltage relationship.

caused in motoneuron cell bodies by peripheral axon injury. As summarized above, Type VII motoneurons of newborn rats express functional nAChRs of the non-α7 type (Zaninetti et al., 1999). The effect of neonatal unilateral VII nerve transection on these receptors was determined using electrophysiological and immunohistochemical techniques (Zaninetti et al., 2000b). In rodents, motoneurons undergo apoptotic cell death if axon lesion occurs shortly after birth, whereas motoneurons survive and regenerate their axons if axotomy takes place in adulthood. To avoid cell death of axotomized motoneurons, our study was performed in newborn mice overexpressing the human *bcl-2* transgene. In these animals, indeed, the human *bcl-2* transgene can prevent the axotomy-induced apoptotic motoneuron death (Dubois-Dauphin et al., 1994). Moreover, the surviving motoneurons retain at least part of their electrophysiological and pharmacological properties (Alberi et al., 1996). We found that intact motoneurons responded to ACh by generating a rapidly rising inward current, which was insensitive to MLA but was suppressed by DHβE. This indicates that mouse Type VII motoneurons — like rat Type VII motoneurons — possess nicotinic receptors that are probably devoid of the α7 subunit. In striking contrast, axotomized motoneurons displayed little or no sensitivity to ACh. Axotomy did not affect the sensitivity of Type VII motoneurons to the glutamate receptor agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), nor to the glutamate receptor agonist *N*-methyl-D-aspartic acid (NMDA) or to the neuropeptide vasopressin, which excites Type VII motoneurons by generating a sodium-dependent inward current (Alberi et al., 1996). Immunohistochemical studies revealed that the α4 nicotinic receptor subunit was present in intact motoneurons but was undetectable in axotomized motoneurons. By contrast, the β2 subunit was comparable in intact and axotomized motoneurons. Thus, mouse VII nicotinic receptors are probably of the α4β2 type and axotomy reduces the expression of the α4 subunit.

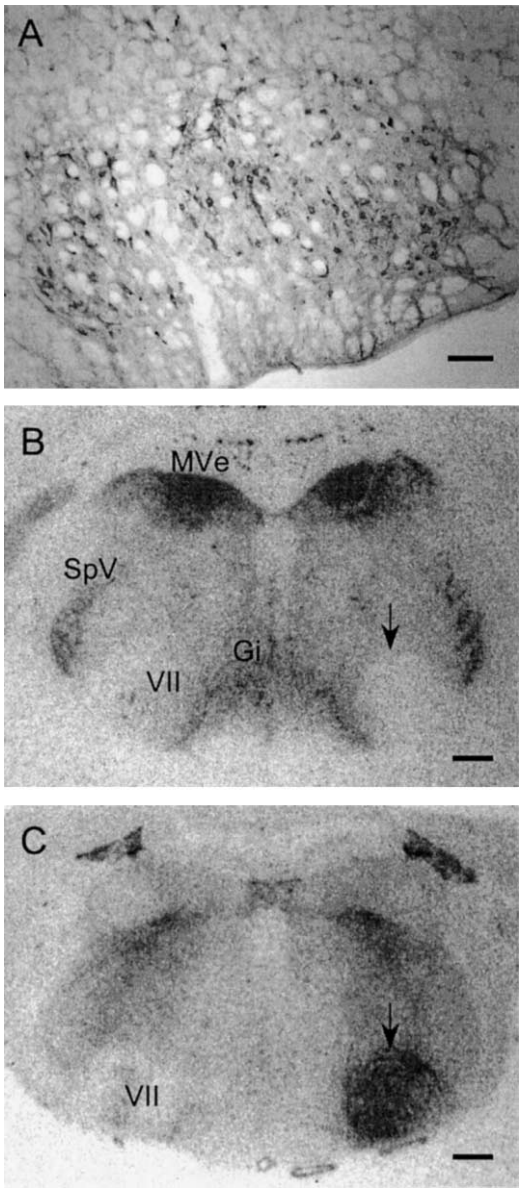

Fig. 5. Effect of axotomy on α7-containing nAChRs in the rat VII nucleus. (A) Immunostaining of the α7 subunit in the VII nucleus of an adult, intact animal. Note that all motoneurons are strongly immunoreactive for this subunit. (B) Autoradiogram showing the distribution of [^{125}I]αBgTx binding sites in a brainstem section obtained from an adult rat killed 14 days after axotomy of the right VII nerve. Note that in the VII nucleus, the density of [^{125}I]αBgTx binding is low both on the control and on the operated sides, although the staining intensity is slightly decreased on the lesionned side (arrow). (C) Autoradiogram obtained from a section adjacent to section B, showing binding of [^{125}I]VPA, a vasopressin antagonist selective for the V_{1a} vasopressin receptor subtype, which is strongly upregulated by axotomy (arrow), in contrast to [^{125}I]αBgTx binding. Gi, gigantocellularis reticular nucleus; MVe, medial vestibular nucleus; SpV, spinal V nucleus. Bars = 100 μm (A) and 1 mm (B and C). *Methods:* For immunocytochemistry, the rat was perfused through the left ventricle with 200 ml of 2% paraformaldehyde dissolved in 0.1M phosphate buffer (PBS; pH 7.4), the brain was dissected out, postfixed overnight in the same solution, immersed for 2 days in a 30% sucrose solution in PBS and cut in 50-μm-thick sections in a cryostat. Free-floating sections were preincubated for 1 h in 5% normal horse serum and incubated overnight at 4 °C with the monoclonal antibody mAb307 (kindly provided to us by Dr. J. Lindstrom; Dominguez del Toro et al., 1994) diluted 1/100 in 1% Triton X-100 in PBS. Immunostaining was performed using a biotinylated horse antimouse antibody and an avidin–biotin detection system (Vectastain Elite, ABC kit; Vector Laboratories, Burlingame, USA). [^{125}I]αBgTx binding sites were revealed using the procedure described in the legend of Fig. 2. V_{1a} vasopressin receptor labeling was performed as previously described (Tribollet et al., 1994), using [^{125}I]VPA at the concentration of 20–30 nM. Labeling of the axotomized facial nucleus was specific since it disappeared in the presence of 1 μM nonradioactive vasopressin (not shown).

4. Regulation of nicotinic binding sites by axotomy

An immunohistochemical study, done using three different antibodies raised against the α7 subunit — and which bind to the cytoplasmic loop of the subunit — suggested that α7-containing nAChRs are present in all brainstem motor

nuclei, as well as in the ventral horn of the spinal cord of adult rats (Dominguez del Toro et al., 1994). This is apparently in contrast with the electrophysiological and autoradiographical data obtained by us and by others, indicating that the nAChRs present in VII and XII nuclei are devoid of the α7 subunit (Clarke et al., 1985; Zaninetti et al., 1999). In an attempt to resolve this discrepancy, we repeated the immunohistochemical study in the VII nucleus using one of the monoclonal antibodies cited above. We found that this nucleus was indeed densely labeled, both in adult (Fig. 5A) and young rats (not shown). In parallel, we examined nAChRs distribution, using [^{125}I]αBgTx as ligand, in a rat in which one of the VII nerves had been sectioned 14 days prior to the labeling. We found that the density of [^{125}I]αBgTx binding sites was low both in the intact VII nucleus and in the lesioned VII nucleus (Fig. 5B). That axotomy had been effective was ascertained by the fact that in the lesioned nucleus, binding of [^{125}I]VPA, a vasopressin antagonist selective for the V_{1a} vasopressin receptor subtype, was upregulated (Tribollet et al., 1994). One explanation of the discrepancy between data yielded by immunohistochemical and binding studies may be that α7 subunits produced in the soma of Type VII motoneurons do not form functional α7-nAChRs receptors. Alternatively, it can be proposed that these cells express the α7-dup protein (Gault et al., 1998). Because this protein results from the expression of the duplicate exons 5–10, which encodes for the transmembrane and intracellular domains of α7, it is probably labeled by antibodies binding intracellular epitopes. However, the α7-dup protein is supposed to contain the DCBA cassettes in the extracellular domain and should, therefore, be devoid of agonist binding site. Thus, the presence of the α7-dup protein in Type VII motoneurons may explain the discrepancy between immunohistochemical and autoradiographic and electrophysiological studies.

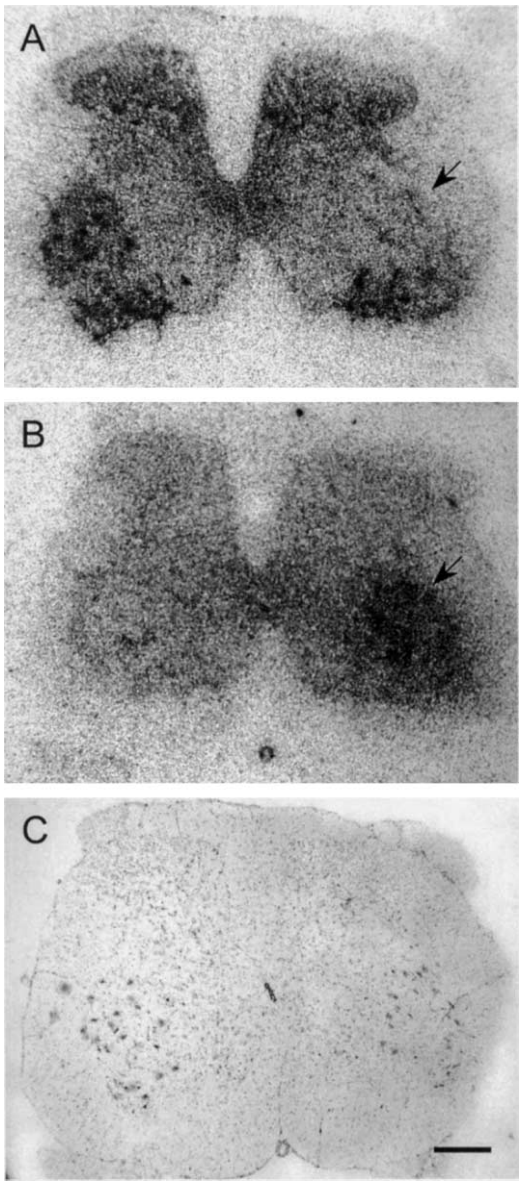

Fig. 6. Effect of axotomy on α7-nAChRs in lumbar spinal motor nuclei of the rat. (A) [^{125}I]αBgTx binding in the lumbar spinal cord of an adult male rat, 14 days following section of the right sciatic nerve at midthigh. (B) [^{125}I]VPA binding in a section adjacent to A. (C) Section used to generate autoradiogram A, stained with cresyl violet, showing the localization of motoneurones in the ventral horn. Note in A the dense specific binding of [^{125}I]αBgTx in motor nuclei on the intact, left side, and the marked reduction induced by axotomy on the operated side (arrow). In contrast, B shows that [^{125}I]VPA binding is low on the control side and is upregulated by axotomy (arrow). Bar = 500 μm. *Methods:* [^{125}I]αBgTx and [^{125}I]VPA binding were performed as described in the legends of Figs. 2 and 5, respectively.

In the spinal cord, immunolabeling and binding data were in good agreement. Indeed, using the same anti-α7 subunit antibodies, we and others (Dominguez del Toro et al., 1994) found strong labeling of motoneurons, and dense [^{125}I]αBgTx binding was observed in the ventral horn at all spinal levels, both in adult and young rats (Fig. 6A). Moreover, unilateral section of the sciatic nerve induced a marked decrease of [^{125}I]αBgTx binding in the lesioned spinal nuclei (Fig. 6A), whereas it induced upregulation of [^{125}I]VPA binding sites (Fig. 6B). This indicates that the ventral horn of the spinal cord contains bona fide α7 nAChRs. Whether [^{125}I]αBgTx binding sites present in spinal motor nuclei represent functional α7-containing nAChRs remains to be investigated.

5. Conclusion

Our studies on reconstituted nAChRs may be summarized as following: (i) Chronic exposure to nicotine can alter both the sensitivity and the desensitization properties of α4β2 receptors. These neuroadaptive changes may alter the normal functions of the central nicotinic cholinergic system and could play a role in nicotine addiction (Dani et al., 2001). (ii) Some mutations in the gene coding for the α4 subunit are associated with a particular form of nocturnal epilepsy. Reconstituted α4β2 nAChRs containing these

mutations appear to display distinct alterations in agonist affinity, desensitization and calcium permeability.

Our data on native nicotinic receptors suggest that by acting on nAChRs located in the SO, cholinergic systems of the brain may exert a selective control upon the hormonal activity of the posterior lobe of the pituitary gland. The SO, however, appears to be devoid, or almost devoid, of choline acetyltransferase-immunoreactive axons or axon terminals (Theodosis and Mason, 1988). This casts some doubts upon the existence of axo-somatic nicotinic synapses within the SO. Nevertheless, a functional role for nAChRs present on SO magnocellular neurons can still be suggested. (i) ChAT-immunoreactive terminals appear to make contacts with unlabeled dendrites immediately dorsolateral to the SO. Although the precise origin of these dendrites has not been determined, some of these contacts may be established with dendrites of magnocellular neurons reaching the perinuclear region dorsal to the nucleus. (ii) nAChRs in the SO may be activated by ACh diffusing from synaptic clefts or by ACh released from axonal nonsynaptic varicosities (Vizi and Lendvai, 1999).

We found that distinct classes of brainstem motoneurons possess functional nAChRs. Cholinergic terminals, as detected by vesicular acetylcholine transporter (VAChT) immunohistochemistry, are present in brainstem motor nuclei and in the ventral horn of the spinal cord (Gilmor et al., 1996; Hellstrom et al., 1999; Roghani et al., 1998). Thus, motoneurons may be the target of cholinergic innervation. By activating nAChRs, endogenous ACh could exert a regulatory role in somatic and visceral motor functions. An intriguing possibility is that somatic motoneurons — like Type VII or Type XII motoneurons — could influence the activity of neighboring motoneurons via recurrent axon collaterals and postsynaptic nAChRs, thus facilitating motor unit recruitment. Even more intriguing, the presence of nAChRs on brainstem motoneurons suggests that these neurons may establish autaptic contacts between their axon and their own soma or dendrites.

Some motoneuron pathologies, like amyotrophic lateral sclerosis, may at least, in part, arise from damage to motoneuron axons, leading to deficient axonal transport, reduced conduction velocity and progressive denervation of muscle (Cleveland, 1999; Flood et al., 1999). Several lines of evidence indicate that nAChRs could play a role in preventing neurodegeneration (Carlson et al., 1998; Kaneko et al., 1997; Kihara et al., 1998; Messi et al., 1997). Our finding that motoneuron axotomy downregulates nicotinic receptors expressed in these cells suggests that, in newborn animals, peripheral nerve injury may promote motoneuron degeneration by reducing motoneuron sensitivity to ACh. Alternatively, these two processes may be independent consequences of peripheral axotomy. The fact that following axotomy, the sensitivity to ACh was lost, whereas the sensitivity to AMPA, NMDA and vasopressin was retained, may reflect a differential effect of axotomy on the metabolic stability and/or the mechanism of membrane anchoring of different classes of receptors.

To conclude, the data described in the present work indicate that long-term exposure to nicotine can promote neuroadaptive changes in nAChRs and that genetic alterations of neuronal nAChRs can result in inheritance of neurological diseases. In addition, they suggest that these receptors can play a role in the central regulation of autonomic functions, as well as in motor control.

Acknowledgments

This work was supported in part by the Swiss National Science Foundation (grants to E.T., M.R. and D.B.). We thank Drs. Spang and Westera for providing the (−)-2PABH compound and Dr. J. Lindstrom for the monoclonal antibody mAb307.

References

Alberi S, Raggenbass M, de Bilbao F, Dubois-Dauphin M. Axotomized neonatal motoneurons overexpressing the bcl2 proto-oncogene retain functional electrophysiological properties. Proc Natl Acad Sci USA 1996;93:3978–83.

Alkondon M, Albuquerque EX. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons: I. Pharmacological and functional evidence for distinct structural subtypes. J Pharmacol Exp Ther 1993; 265:1455–73.

Balfour DJ, Wright AE, Benwell ME, Birrell CE. The putative role of extrasynaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 2000;113:73–83.

Bertrand D, Ballivet M, Rungger D. Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes. Proc Natl Acad Sci USA 1990;87:1993–7.

Bertrand S, Weiland S, Berkovic SF, Steinlein OK, Bertrand D. Properties of neuronal nicotinic acetylcholine receptor mutants from humans suffering from autosomal dominant nocturnal frontal lobe epilepsy. Br J Pharmacol 1998;125:751–60.

Bohler S, Gay S, Bertrand S, Corringer PJ, Edelstein SJ, Changeux JP, Bertrand D. Desensitization of neuronal nicotinic acetylcholine receptors conferred by N-terminal segments of the beta2 subunit. Biochemistry 2001;40:2066–74.

Buisson B, Bertrand D. Chronic exposure to nicotine upregulates the human (alpha)4((beta)2 nicotinic acetylcholine receptor function. J Neurosci 2001;21:819–29.

Buisson B, Gopalakrishnan M, Arneric SP, Sullivan JP, Bertrand D. Human alpha4beta2 neuronal nicotinic acetylcholine receptor in HEK 293 cells: a patch-clamp study. J Neurosci 1996;16:7880–91.

Carlson NG, Bacchi A, Rogers SW, Gahring LC. Nicotine blocks TNF-alpha-mediated neuroprotection to NMDA by an alpha-bungarotoxin-sensitive pathway. J Neurobiol 1998;35:29–36.

Changeux JP, Bertrand D, Corringer PJ, Dehaene S, Edelstein S, Lena C, Le Novere N, Marubio L, Picciotto M, Zoli M. Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. Brain Res Brain Res Rev 1998;26:198–216.

Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [3H]acetylcholine, [3H] nicotine, and [125I]-alpha-bungarotoxin. J Neurosci 1985;5:1307–15.

Cleveland DW. From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. Neuron 1999;24:515–20.

Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T, Ballivet M. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. Neuron 1990;5: 847–56.

Covernton PJ, Connolly JG. Multiple components in the agonist concentration–response relationships of neuronal nicotinic acetylcholine receptors. J Neurosci Methods 2000;96:63–70.

Cuevas J, Berg DK. Mammalian nicotinic receptors with alpha7 subunits that slowly desensitize and rapidly recover from alpha-bungarotoxin blockade. J Neurosci 1998;18:10335–44.

Curtis DR, Ryall RW. The acetylcholine receptors on Renshaw cells. Exp Brain Res 1966a;2:66–80.

Curtis DR, Ryall RW. The synaptic excitation of Renshaw cells. Exp Brain Res 1966b;2:81–96.

Dani JA, Ji D, Zhou FM. Synaptic plasticity and nicotine addiction. Neuron 2001;31:349–52.

De Fusco M, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, Ballabio A, Wanke E, Casari G. The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet 2000; 26:275–6.

Dominguez del Toro E, Juiz JM, Peng X, Lindstrom J, Criado M. Immunocytochemical localization of the alpha 7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. J Comp Neurol 1994;349:325–42.

Dubois-Dauphin M, Frankowski H, Tsujimoto Y, Huarte J, Martinou JC. Neonatal motoneurons overexpressing the bcl-2 protooncogene in transgenic mice are protected from axotomy-induced cell death. Proc Natl Acad Sci USA 1994;91:3309–13.

Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. Alpha 10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. Proc Natl Acad Sci USA 2001;98:3501–6.

Fenster CP, Whitworth TL, Sheffield EB, Quick MW, Lester RA. Upregulation of surface alpha4beta2 nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. J Neurosci 1999; 19:4804–14.

Figl A, Viseshakul N, Shafaee N, Forsayeth J, Cohen BN. Two mutations linked to nocturnal frontal lobe epilepsy cause use-dependent potentiation of the nicotinic ACh response. J Physiol 1998;513:655–70.

Flood DG, Reaume AG, Gruner JA, Hoffman EK, Hirsch JD, Lin YG, Dorfman KS, Scott RW. Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions. Am J Pathol 1999;155:663–72.

Forster I, Bertrand D. Inward rectification of neuronal nicotinic acetylcholine receptors investigated by using the homomeric alpha 7 receptor. Proc R Soc London, Ser B: Biol Sci 1995;260:139–48.

Galzi JL, Changeux JP. Neuronal nicotinic receptors: molecular organization and regulations. Neuropharmacology 1995;34:563–82.

Gault J, Robinson M, Berger R, Drebing C, Logel J, Hopkins J, Moore T, Jacobs S, Meriwether J, Choi MJ, Kim EJ, Walton K, Buiting K, Davis A, Breese C, Freedman R, Leonard S. Genomic organization and partial duplication of the human alpha7 neuronal nicotinic acetylcholine receptor gene (CHRNA7). Genomics 1998;52:173–85.

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. Mol Pharmacol 1995;48:774–82.

Gilmor ML, Nash NR, Roghani A, Edwards RH, Yi H, Hersch SM, Levey AI. Expression of the putative vesicular acetylcholine transporter in rat brain and localization in cholinergic synaptic vesicles. J Neurosci 1996;16:2179–90.

Gopalakrishnan M, Buisson B, Touma E, Giordano T, Campbell JE, Hu IC, Donnelly-Roberts D, Arneric SP, Bertrand D, Sullivan JP. Stable expression and pharmacological properties of the human alpha 7 nicotinic acetylcholine receptor. Eur J Pharmacol 1995;290:237–46.

Hellstrom J, Arvidsson U, Elde R, Cullheim S, Meister B. Differential expression of nerve terminal protein isoforms in VAChT-containing varicosities of the spinal cord ventral horn. J Comp Neurol 1999; 411:578–90.

Hirose S, Iwata H, Akiyoshi H, Kobayashi K, Ito M, Wada K, Kaneko S, Mitsudome A. A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. Neurology 1999;53:1749–53.

Houghtling RA, Davila-Garcia MI, Kellar KJ. Characterization of (+/–)(–)[3H]epibatidine binding to nicotinic cholinergic receptors in rat and human brain. Mol Pharmacol 1995;48:280–7.

Jones S, Sudweeks S, Yakel JL. Nicotinic receptors in the brain: correlating physiology with function. Trends Neurosci 1999;22:555–61.

Kaneko S, Maeda T, Kume T, Kochiyama H, Akaike A, Shimohama S, Kimura J. Nicotine protects cultured cortical neurons against glutamate-induced cytotoxicity via alpha7-neuronal receptors and neuronal CNS receptors. Brain Res 1997;765:135–40.

Kihara T, Shimohama S, Urushitani M, Sawada H, Kimura J, Kume T, Maeda T, Akaike A. Stimulation of alpha4beta2 nicotinic acetylcholine receptors inhibits beta-amyloid toxicity. Brain Res 1998;792:331–4.

Kuryatov A, Gerzanich V, Nelson M, Olale F, Lindstrom J. Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca2+ permeability, conductance, and gating of human alpha4beta2 nicotinic acetylcholine receptors. J Neurosci 1997;17:9035–47.

Lindstrom J. Nicotinic acetylcholine receptors in health and disease. Mol Neurobiol 1997;15:193–222.

MacDermott AB, Role LW, Siegelbaum SA. Presynaptic ionotropic receptors and the control of transmitter release. Annu Rev Neurosci 1999; 22:443–85.

McGehee DS, Role LW. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu Rev Physiol 1995; 57:521–46.

Messi ML, Renganathan M, Grigorenko E, Delbono O. Activation of alpha7 nicotinic acetylcholine receptor promotes survival of spinal cord motoneurons. FEBS Lett 1997;411:32–8.

Moulard B, Picard F, leHellard S, Agulhon C, Weiland S, Favre I, Bertrand S, Malafosse A, Bertrand D. Ion channel variation causes apilepsie. Brain Res Brain Res Rev (in press).

Olale F, Gerzanich V, Kuryatov A, Wang F, Lindstrom J. Chronic nicotine exposure differentially affects the function of human alpha3, alpha4, and alpha7 neuronal nicotinic receptor subtypes. J Pharmacol Exp Ther 1997;283:675–83.

Palma E, Bertrand S, Binzoni T, Bertrand D. Neuronal nicotinic alpha 7 receptor expressed in *Xenopus* oocytes presents five putative binding sites for methyllycaconitine. J Physiol 1996;491:151–61.

Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. Prog Neurobiol 2000;61:75–111.

Perry DC, Kellar KJ. [3H]Epibatidine labels nicotinic receptors in rat brain: an autoradiographic study. J Pharmacol Exp Ther 1995;275:1030–4.

Phillips HA, Scheffer IE, Berkovic SF, Hollway GE, Sutherland GR, Mulley JC. Localization of a gene for autosomal dominant nocturnal frontal lobe epilepsy to chromosome 20q 13.2. Nat Genet 1995;10:117–8.

Phillips HA, Favre I, Kirkpatrick M, Zuberi SM, Goudie D, Heron SE, Scheffer IE, Sutherland GR, Berkovic SF, Bertrand D, Mulley JC. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. Am J Hum Genet 2001;68:225–31.

Picard F, Bertrand S, Steinlein OK, Bertrand D. Mutated nicotinic receptors responsible for autosomal dominant nocturnal frontal lobe epilepsy are more sensitive to carbamazepine. Epilepsia 1999;40:1198–209.

Renaud LP, Bourque CW. Neurophysiology and neuropharmacology of hypothalamic magnocellular neurons secreting vasopressin and oxytocin. Prog Neurobiol 1991;36:131–69.

Roghani A, Shirzadi A, Butcher LL, Edwards RH. Distribution of the vesicular transporter for acetylcholine in the rat central nervous system. Neuroscience 1998;82:1195–212.

Role LW, Berg DK. Nicotinic receptors in the development and modulation of CNS synapses. Neuron 1996;16:1077–85.

Schoepfer R, Conroy WG, Whiting P, Gore M, Lindstrom J. Brain alpha-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this

branch of the ligand-gated ion channel gene superfamily. Neuron 1990;5:35–48.

Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J Neurosci 1993;13:596–604.

Spang JE, Bertrand S, Westera G, Patt JT, Schubiger PA, Bertrand D. Chemical modification of epibatidine causes a switch from agonist to antagonist and modifies its selectivity for neuronal nicotinic acetylcholine receptors. Chem Biol 2000;7:545–55.

Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 1995; 11:201–3.

Steinlein OK, Magnusson A, Stoodt J, Bertrand S, Weiland S, Berkovic SF, Nakken KO, Propping P, Bertrand D. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. Hum Mol Genet 1997;6:943–7.

Theodosis DT, Mason WT. Choline acetyltransferase immunocytochemical staining of the rat supraoptic nucleus and its surroundings. A light- and electron-microscopic study. Cell Tissue Res 1988;254:119–24.

Tribollet E, Arsenijevic Y, Marguerat A, Barberis C, Dreifuss JJ. Axotomy induces the expression of vasopressin receptors in cranial and spinal motor nuclei in the adult rat. Proc Natl Acad Sci USA 1994; 91:9636–40.

Vizi ES, Lendvai B. Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. Brain Res Brain Res Rev 1999;30:219–35.

Weiland S, Witzemann V, Villarroel A, Propping P, Steinlein O. An amino acid exchange in the second transmembrane segment of a neuronal nicotinic receptor causes partial epilepsy by altering its desensitization kinetics. FEBS Lett 1996;398:91–6.

Wonnacott S. Presynaptic nicotinic ACh receptors. Trends Neurosci 1997;20:92–8.

Zaninetti M, Tribollet E, Bertrand D, Raggenbass M. Presence of functional neuronal nicotinic acetylcholine receptors in brainstem motoneurons of the rat. Eur J Neurosci 1999;11:2737–48.

Zaninetti M, Blanchet C, Tribollet E, Bertrand D, Raggenbass M. Magnocellular neurons of the rat supraoptic nucleus are endowed with functional nicotinic acetylcholine receptors. Neuroscience 2000a;95: 319–23.

Zaninetti M, Dubois-Dauphin M, Lindstrom J, Raggenbass M. Nicotinic acetylcholine receptors in neonatal motoneurons are regulated by axotomy: an electrophysiological and immunohistochemical study in human Bcl-2 transgenic mice. Neuroscience 2000b;100:589–97.