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Distribution of the α2, α3, and α5 nicotinic acetylcholine receptor subunits in the chick brain

A.S. Torrão[1], J.M. Lindstrom[2] and L.R.G. Britto[1]

[1]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil
[2]Department of Neuroscience, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Nicotinic acetylcholine receptors (nAChRs) are ionotropic receptors comprised of α and ß subunits. These receptors are widely distributed in the central nervous system, and previous studies have revealed specific patterns of localization for some nAChR subunits in the vertebrate brain. In the present study we used immunohistochemical methods and monoclonal antibodies to localize the α2, α3, and α5 nAChR subunits in the chick mesencephalon and diencephalon. We observed a differential distribution of these three subunits in the chick brain, and showed that the somata and neuropil of many central structures contain the α5 nAChR subunit. The α2 and α3 subunits, on the other hand, exhibited a more restricted distribution than α5 and other subunits previously studied, namely α7, α8 and ß2. The patterns of distribution of the different nAChR subunits suggest that neurons in many brain structures may contain several subtypes of nAChRs and that in a few regions one particular subtype may determine the cholinergic nicotinic responses.

Key words
- Cholinergic system
- Neurotransmitters
- Receptors
- Receptor subunits

Correspondence
A.S. Torrão
Departamento de Fisiologia
e Biofísica, ICB, USP
Av. Prof. Lineu Prestes, 1524
05508-900 São Paulo, SP
Brasil
Fax: 55 (011) 818-7426
E-mail: lrbritto@usp.br

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Neuronal nicotinic acetylcholine receptors (nAChRs) are composed of subunits that presumptively assemble in a pentameric structure around an ion channel. So far, eight ligand-binding (or α, α2-α9) subunits and three non-α or structural (or ß, ß2-ß4) subunits of the nAChRs have been characterized (1-6). Several nAChR subunits have been localized to the vertebrate brain by means of *in situ* hybridization to detect the mRNAs coding for those subunits, and by immunohistochemistry to detect the corresponding proteins (7-16). Since antibodies to detect the α2, α3, and α5 nAChR subunits are now available in our laboratory (3), we sought in the present study to evaluate the distribution of these three subunits in the chick mesencephalon and diencephalon. The results are compared with previous data on the localization of the α7, α8, and ß2 subunits in the same brain regions (7).

The immunohistochemical methods used here have been described in detail elsewhere (7). Rat monoclonal antibodies were employed that recognize the α2 (mAb321), α3 (mAb315), and α5 (mAb210) subunits (3, 17,18). Ten 1-2-week-old chicks (*Gallus gallus*) obtained from a local hatchery were

used in these experiments. The animals were maintained with food and water *ad libitum* on a 14:10 h light-dark cycle. The animals were deeply anesthetized with ketamine (5 mg/100 g body weight, *im*) and xylazine (1 mg/100 g body weight, *im*) and perfused through the heart with phosphate-buffered saline and 2% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. After 3-5 h of postfixation, the brains were transferred to a 30% sucrose solution in PB to ensure cryoprotection. Coronal sections (30 µm) of the frozen brains were cut with a sliding microtome and incubated free-floating with the primary antibodies diluted 1:500 to 1:1,000 in PB containing 0.3% Triton X-100 for 14-18 h at 4°C. After three washes (15 min each) in PB, the sections were incubated with a biotinylated rabbit anti-rat serum (Vector Labs., Burlingame, CA) diluted 1:200 in PB for 1 h at room temperature. The sections were washed in PB, and then incubated sequentially with the avidin-biotin-peroxidase complex (ABC Elite; Vector Labs.), 0.05% 3-3'-diaminobenzidine, and a 0.01% solution of hydrogen peroxide in PB. The reaction was intensified with 0.05% osmium tetroxide in water, and the material was mounted on gelatin- and chromoalumen-coated slides, dehydrated, cleared, and coverslipped with Permount (Fisher, Pittsburgh, PA). The main control for specificity of immunostaining was the omission of the primary antibodies from the procedure. In addition, in several experiments the mAbs were replaced with normal rat serum. Specific staining was abolished under either of these conditions. The identification of the different structures of the chick brain was based on a stereotaxic atlas (19). The number of labeled somata was scaled from 0 to 5, which represented 0% to 100% immunostained perikarya, in 20% steps. The intensity of neuropil staining was subjectively estimated, and scaled from 0 (absent) to 4 (very intense).

The mapping of the distribution of α2, α3, and α5 nAChR subunits revealed that these subunits present a differential localization in the chick brain. Immunoreactivity for the α5 subunit was observed in many more diencephalic and mesencephalic structures than immunoreactivity for the α2 and α3 subunits (Tables 1 and 2). In general, the staining for α2, α3, and α5 subunits was qualitatively rated as weak to moderate, with intense staining observed only in a few structures. It is important to stress that we observed a strong, specific staining in blood vessels with the antibody against the α3 nAChR subunit.

Somata staining for the α2 subunit appeared highly marked (5 on the scale of labeled somata) only in the nucleus reticularis superior, and moderate (scale = 3) only in the nucleus spiriformis lateralis. Neuropil immunoreactivity for the α2 subunit was intense (scale = 3) only in the nucleus interpeduncularis, moderate (scale = 2) in the nucleus reticularis superior, griseum tecti, and the nucleus spiriformis lateralis, and weak (scale = 1) in several other structures. Many structures were not labeled at all with the antibody against the α2 nAChR subunit.

Somata staining for the α3 nAChR subunit was found in several regions, but the positive perikarya were usually present in small numbers (scale = 1-2), except for the nucleus pontinus lateralis and the nucleus pretectalis, which exhibited moderate (scale = 3) to high (scale = 4) numbers of labeled cells, respectively. Likewise, neuropil immunoreactivity for the α3 subunit was generally weak (scale = 1), but was observed more often in mesencephalic than in diencephalic structures.

Somata staining for the α5 nAChR subunit appeared in very high numbers (scale = 5) in the nucleus spiriformis lateralis, high numbers (scale = 4) in the nucleus infundibuli hypothalami, and small (scale = 1-2) to moderate (scale = 3) numbers of labeled cells were found in several other areas. Neuropil staining for theα5 subunit was intense (scale

Table 1 - Distribution of α2, α3, and α5 in the chick diencephalon.

Number of labeled somata

0		absent
1	+	1-20% of all cells
2	++	21-40% of all cells
3	+++	41-60% of all cells
4	++++	61-80% of all cells
5	+++++	81-100% of all cells

Intensity of neuropil staining

0		absent
1	+	light
2	++	moderate
3	+++	intense
4	++++	very intense

Structures	α2		α3		α5	
	Somata	Neuropil	Somata	Neuropil	Somata	Neuropil
Intergeniculate leaflet			+	+	+	+
Nucleus anteromedialis hypothalami					+	+
Nucleus decussationis supraopticae		+	+		+	+
Nucleus dorsointermedius posterior						
Nucleus dorsolateralis anterior		+			+	+
Nucleus dorsolateralis posterior						
Nucleus dorsomedialis anterior						
Nucleus dorsomedialis hypothalami			+		+	+
Nucleus dorsomedialis posterior						
Nucleus geniculatus lateralis ventralis		+				+
Nucleus habenularis lateralis					+++	++
Nucleus habenularis medialis					+	
Nucleus inferioris hypothalami					+	+
Nucleus infundibuli hypothalami			+		+++++	++
Nucleus intercalatus thalami					+	+
Nucleus lateralis anterior			+			
Nucleus ovoidalis		+				+
Nucleus paramedianus internus			+	+	+	+
Nucleus paraventricularis		+	+	+	+	+
Nucleus periventricularis					++	++
Nucleus reticularis superior	+++++	++	++	+	+	+
Nucleus rotundus		+				+
Nucleus subrotundus			+	+	+	+
Nucleus superficialis parvocellularis						
Nucleus triangularis						
Nucleus ventrolateralis thalami					++	+
Nucleus ventromedialis hypothalami					++	+
Organum paraventricularis						+
Regio lateralis hypothalami			+		+	+
Stratum cellulare externum			+	+	+	
Suprachiasmatic nucleus					+	

= 3) in the nucleus spiriformis lateralis and the nucleus interpeduncularis, and low (scale = 1) to moderate (scale = 2) in many other areas.

This study showed that the nAChR subunits investigated here are widely distributed in the chick brain, especially in mesencephalic structures. The α3 subunit appeared to be also present in blood vessels, but the functional significance of this finding is unknown at present and remains to be further characterized. There is no information regarding the distribution of α2 and α3 in other species, but the present results on the distribution of the α5 nAChR subunit agree in general with data from an *in situ* hybridization study in the rat brain (16). Overall, the α5 subunit appears to be found in larger

Table 2 - Distribution of α2, α3, and α5 in the chick mesencephalon.

Scale for the number of labeled somata and intensity of neuropil staining as in Table 1.

Structures	α2		α3		α5	
	Somata	Neuropil	Somata	Neuropil	Somata	Neuropil
Area pretectalis			+	+	+	+
Area ventralis tegmenti (Tsai)	++	+	+	+	+++	++
Formatio reticularis, pars lateralis			+	+	+	+
Formatio reticularis, pars medialis			+	+	+	+
Griseum tecti		++	+	+	++	++
Locus coeruleus			+	+	+	+
Nucleus externus			+	+	+	++
Nucleus intercollicularis			+	+	+	
Nucleus interpeduncularis	+	+++	+	++	++	+++
Nucleus interstitialis						
Nucleus interstitio-pretecto-subpretect			+	+	++	+
Nucleus isthmi, pars magnocellularis			+		++++	+
Nucleus isthmi, pars parvocellularis						++
Nucleus isthmo-opticus						+
Nucleus lentiformis mesencephali			++	+	++	+
Nucleus mesencephalicus lateralis			+	+	+	+
Nucleus mesencephalicus profundus			+	+	+	+
Nucleus of Darkschewitsch			+	+	+	+
Nucleus of Edinger-Westphal			+		+	+
Nucleus of the basal optic root		+	+	+	++	++
Nucleus papillioformis			+	+		
Nucleus pontinus lateralis			+++	+	++	+
Nucleus pretectalis		+	++++	+	+	+
Nucleus pretectalis diffusus			+	+	+	+
Nucleus pretectalis medialis					+	+
Nucleus principalis precommissuralis			++	+	+	+
Nucleus ruber			+	+		
Nucleus semilunaris		+			+	+
Nucleus spiriformis lateralis	+++	++			+++++	+++
Nucleus spiriformis medialis			+	+		
Nucleus subpretectalis			+	+	++	+
Nucleus tegmenti pedunculo-pontinus			+	+	+	+
Nucleus trochlearis					+	+
Oculomotor complex					+	+
Raphe complex					+	+
Stratum album centrale of tectum						+
Stratum griseum centrale of tectum			+		+	++
Stratum griseum et fibrosum superficiale		+	+		+	++
Stratum griseum periventriculare						
Stratum opticum of the tectum						
Substantia grisea centralis		+	+	+	++	+

amounts and in more structures than α2 and α3. As seen in a previous study concerning the distribution of the α7, α8, and ß2 subunits (7), the α2, α3, and α5 subunits also have a differential localization in the nervous system, with a partial degree of colocalization. The subunits studied here were absent in several structures that were found to contain the α7, α8, and ß2 subunits, such as the nucleus ovoidalis, nucleus semilunaris, and the isthmic nuclei. In contrast, some structures that contain the latter subunits also appeared to contain the α2, α3, or α5 subunit, such as the nucleus spiriformis

lateralis, nucleus interpeduncularis, and the nucleus reticularis superior.

The present results indicate that nAChRs are diffusely distributed in the chick mesencephalon and diencephalon, with varying degrees of co-localization. The diversity of nAChRs and the co-localization of some subunits in particular structures of the chick brain, taken together with data from other studies (20), suggest the occurrence of a myriad of pharmacological and physiological cellular responses to acetylcholine.

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