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Muscarinic Cholinergic Receptors in Human Infant Forebrain: [^{3}H]Quinuclidinyl Benzilate Binding in Homogenates and Quantitative Autoradiography in Sections

MICHAEL V. JOHNSTON[1,2,3], FAYE S. SILVERSTEIN[1], FREDERICK O. REINDEL[1], JOHN B. PENNEY, Jr.[2] and ANNE B. YOUNG[2]

Departments of [1]Pediatrics and [2]Neurology, University of Michigan Medical School and [3]The Center for Human Growth and Development, Ann Arbor, MI 48104 (U.S.A.)

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The ontogeny of muscarinic receptors in human brain was studied by comparing [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding in postmortem tissue from infants 1 week to 3 months of age with binding in adult specimens. Saturation analysis with [^{3}H]QNB and displacement studies with muscarinic antagonists and agonists in tissue homogenates demonstrated that binding sites in the infants' forebrain regions were present in adult or higher than adult concentrations (B_{max}). Binding affinity (K_d) and pharmacological characteristics were nearly identical at the two ages. Quantitative receptor autoradiography demonstrated more [^{3}H]QNB binding in the gray matter of infants than adults and revealed a marked difference between the two ages in the laminar distribution of binding sites in neocortex. In contrast to the adult pattern with higher binding in superficial layers 1–3 than in layers 4–6, the distribution in the immature cortex was inverted. These results suggest that muscarinic receptors in infants resemble closely those in mature brain. However, the topography of receptors in the immature neocortex is distinct and they are redistributed in a gradient from inside outward during postnatal development.

INTRODUCTION

Acetylcholine neurotransmitter pathways are involved in a variety of brain functions, including control of movement, maintenance of consciousness, learning and memory. Developmental studies of rodent brain and more limited data from human postmortem tissue indicate that maturation of biochemical markers for cholinergic pathways lags behind other systems such as catecholamine neurons[4,11,18]. In neocortex from rats and humans, the activity of the acetylcholine synthetic enzyme, choline acetyltransferase (ChAT), rises slowly to adult levels over an extended period postnatally[9]. This time course corresponds to the gradual postnatal acquisition of several motor and cognitive behaviors thought to be mediated by cholinergic neurotransmission[5,18]. These events are also accompanied by reorganization of cholinergic neurocircuitry in developing brain. For example, in humans, acetylcholinesterase containing islands are prominent in the infant corpus striatum but adults have a more homogeneous pattern[15]. Neonatal rat somatosensory cortex contains dense fibers staining for acetylcholinesterase in layer IV which disappear by 3 weeks of age[22]. Muscarinic receptors are also relatively concentrated in layer IV of 1 week old rat pups and receptors in layer I become apparent at later times to form the adult pattern[23]. Therefore, maturation of brain cholinergic innervation, both in terms of addition of nerve fibers and receptors and their reorganization into adult circuits, appears to be an important feature of postnatal brain development.

To examine the development of cholinergic neurocircuitry in human brain, we studied the binding characteristics of the muscarinic antagonist [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) in postmortem tissue from infants less than 3 months of age and compared them to brain from adults. The distribution and relative density of muscarinic binding sites was determined using quantitative in vitro autoradiography on

Correspondence: M. V. Johnston, Neuroscience Laboratory Building, 1103 East Huron, Ann Arbor, MI 48104, U.S.A.

frozen brain sections. These results were compared to analyses of [^{3}H]QNB binding to homogenates of tissue from the same brain specimens sampled in areas adjacent to the frozen sections. The results demonstrate that muscarinic receptor binding affinities and pharmacologic characteristics are similar at the extremes of age. However, the density of [^{3}H]QNB binding in infants generally exceeds that in adults. Furthermore, the neocortical laminar receptor distribution found in the perinatal period is markedly different from the adult pattern, suggesting that cholinergic neurocircuitry is reorganized during postnatal development.

MATERIALS AND METHODS

Postmortem tissue was obtained from the Pathology Department after removal at autopsy. The 5 infants ranged in age from 1 week to 3 months, and they died suddenly from congenital heart disease in 4 cases (aortic arch anomalies in 3 and vetricular septal defect in a fourth) and pulmonary insufficiency in one. All had short periods of hypoxemia prior to death but none had clinical evidence of significant brain hypoxia-ischemia and routine neuropathological examinations were unremarkable. None of the infants had apparent chromosomal anomalies. The 5 adults ranged in age from 55 to 74 years (mean = 56 years, S.D. = 18). All died suddenly, 4 of outpatient cardiac arrests and one during coronary bypass surgery. None had neurological diagnoses and routine neuropathological exams were normal. Brains were removed, cut into coronal slices approximately 2 cm thick, quick frozen on a glass plate on dry ice and stored frozen at −70 °C within 3–24 h after death.

Saturation analysis of [^{3}H]QNB binding as well as comparison of regional differences in maximal ligand binding and pharmacologic displacement studies were performed in homogenates of tissue adjacent to blocks used for autoradiography. Pieces of frozen gray matter were dissected so as to exclude major areas of white matter and homogenized 1:200 (wt/vol) by sonication in 50 mM Tris buffer, pH 7.7, at 4 °C. The homogenate was centrifuged at 48,000 *g* for 10 min, the supernatant was discarded and the pellet was resuspended in the same volume of buffer. Aliquots containing 1 mg of tissue were added to tubes, brought to a final volume of 4 ml of buffer and incubated for 1 h at 37 °C along with various concentrations (0.02–1 nM) of [^{3}H]QNB (29 Ci/mmol, New England Nuclear). Specific binding was determined in duplicate or triplicate as the difference between radioactivity bound in the presence or absence of 2 mM oxotremorine or 10^{-4} atropine, which gave identical results. For drug displacement studies, the competing muscarinic agonist or antagonist drugs (purchased from Sigma, St. Louis, MO) were added to the incubation mixture at the same time as the [^{3}H]QNB (0.3 nM) at a range of concentrations shown in the results. The incubation was terminated quickly by filtration of the suspension through GF/B (Whatman) glass fiber filters under vacuum. The filters were washed 4 times with 4 ml aliquots of the ice cold buffer, dried and placed in vials with ACS (Amersham) liquid scintillation fluid and trapped radioactivity was counted by liquid scintillation spectrometry. Protein was determined using the BioRad assay[3].

For data analysis, maximal binding capacity (B_{max}) and receptor affinity (K_d) were calculated using least squares regression analysis of Eadie–Hofstee plots of saturation isotherms. Displacement data for agonist or antagonists were analyzed using a computer program to perform least squares fit of the data to a one or two site model[29]. Inhibitory concentrations that displaced 50% of the maximal binding (IC_{50}s) were determined from log–logit plots.

The autoradiographic procedures were done by methods developed previously[31–34]. The entire blocks of tissue were placed in a Lipshaw 1800N cryostat to equilibrate at −20 °C and 30 μm frozen coronal sections of half or whole brain were cut and thaw mounted on gelatin-coated 3.25 × 4 in. lantern slides. For one of the infant specimens, tissue for examination was limited to smaller blocks of frontal, temporal and parietal cortex cut in the coronal plane. The glass mounted tissue sections were then brought to room temperature and immersed in 3 sequential 5-min washes with Dulbecco's phosphate buffered saline, pH 7.4. Then they were placed in a bath containing a saturating concentration of [^{3}H]QNB (1.4 nM) in the same buffers at 25 °C for 3 h. After incubation, the mounted sections were placed in two ice cold (4 °C) 5 min washes of buffer, then blown dry with a cooled stream of air. The sections and standards containing known amounts of radioactivity were

placed in contact with the emulsion of tritium sensitive film (Ultrofilm-3H, LKB) in an X-ray cassette and placed at 4 °C for 7–10 days exposure. The autoradiograms were developed for 5 min in D-19 developer (Kodak) at 20 °C. Binding of tritiated muscarinic antagonist in regions 0.5 × 0.5 mm was determined using a computerized spot densitometer to compare the film density to the standards[31]. Non-specific binding (less than 5% of total for infants and adults) was subtracted from each image and this was measured as binding in the presence of 10^{-4} M atropine.

After the films were developed, the sections were fixed over paraformaldehyde vapors at 60 °C for 4 h and then stained with cresyl violet. The developed film was then reoriented over the section. Section and film were examined under a dissecting microscope to determine the exact cortical areas from which measurements had been made.

RESULTS

Receptor binding in brain homogenates

To compare the maximal binding capacity (B_{max}) and affinity constants (K_d) in infants and adults, saturation analysis was performed on multiple regions of a single neonate and adult in the same assay. Additional brains were analyzed at a single saturating ligand concentration to measure B_{max} (Table I). Fig. 1 shows Eadie–Hofstee plots of a representative saturation binding study comparing adult and infant frontal cortex. Both plots are linear ($r^2 = -0.95$) and the

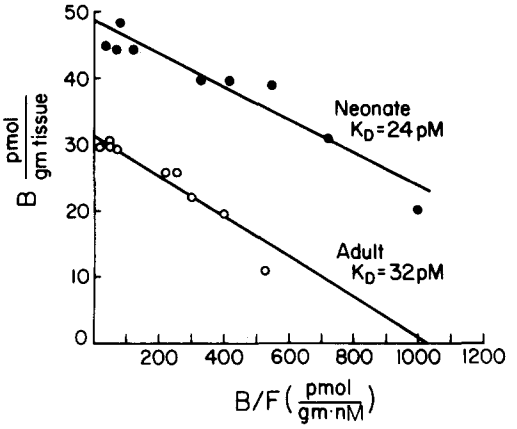

Fig. 1. Eadie–Hofstee plot of a representative [^{3}H]QNB saturation experiment comparing parietal cortex from a neonatal and adult human brain, performed as described in Methods.

B_{max} in infant brain exceeded that of the adult by 50% (47.5 pmol/g vs 32 pmol/g) while the K_d is somewhat lower in the infant than the adult (24 pM vs 32 pM).

Comparing the 3 infants and adults directly, the mean values for infants exceeded adult values in the 4 cortical regions by 6–43%, though this difference was not significant using Student's *t*-test. B_{max}s in all cortical regions at the same age were similar while binding in caudate nucleus in one subject was twofold higher than cortex. The equilibrium affinity constants were quite similar at the two ages and in parietal and temporal cortex the K_ds differed by less than 13%.

The pharmacological characteristics of [^{3}H]QNB

TABLE I

Regional [^{3}H]QNB binding characteristics in homogenates of human cerebral cortex

Values are means ± S.E.M.; n = number of brains. Some assays were carried out using 9 concentrations of [^{3}H]QNB and 1 mg of tissue as described in Methods to determine B_{max} and K_d from Eadie-Hofstee plots of saturation analyses. The K_d measurements in neonatal and adult parietal cortex are based on 6 and 2 separate saturation analyses, respectively. In others, a saturating (0.3 nM) concentration of [^{3}H]QNB was used to determine B_{max} alone. Tissue from adult and infant brains was generally assayed together on the same day. B_{max} values are expressed per gram of tissue (wet weight); mean values per gram of protein for frontal cortex are 716 pmol/g protein for infants and 413 pmol/g protein for adult cortex. The higher K_d value for caudate was derived from an experiment with 2 mg of tissue; the apparent K_d for [^{3}H]QNB has been shown to relate directly to the receptor concentration in the assay. Analogous gyri from brains at both ages were assayed.

Region	*B_{max} (pmol·g^{-1})*					*K_d (pM)*			
	(n)	*Infant*	*(n)*	*Adult*	*%*	*(n)*	*Infant*	*(n)*	*Adult*
Frontal lobe	(3)	43 ± 4	(3)	30 ± 6	−30	(1)	23	(1)	32
Parietal lobe	(3)	36 ± 6	(3)	25 ± 5	−31	(3)	23 ± 4	(3)	26 ± 6
Temporal lobe	(3)	37 ± 4	(2)	35 ± 8	−5	(1)	24	(1)	25
Occipital lobe	(1)	57	(2)	45 ± 3	−21	(0)	–	(0)	–
Caudate	(1)	101	(0)	–		(1)	69	(0)	–

TABLE II

Concentrations of agonists and antagonists that displaced 50% of [³H]QNB bound to homogenates of human cerebral cortex

Values were derived from log-logit plots of data from experiments in which various concentrations of displacers were incubated together with 1 mg of cerebral cortex and 0.3 nM [³H]QNB as described in Methods. The results for adult cortex closely resemble our data from rat cortex (carbamylcholine 1×10^{-4}; oxotremorine 1.5×10^{-6}; atropine 2.0×10^{-9}) and values from human adult cerebral cortex previously reported[4,10,12,37].

Displacer	*Infants IC_{50} (M)*	*Adults IC_{50} (M)*
Atropine	2.5×10^{-9}	3.2×10^{-9}
Scopolamine	3.6×10^{-10}	9.1×10^{-10}
Pilocarpine	1.7×10^{-5}	2.7×10^{-5}
Oxotremorine	3.0×10^{-6}	1.1×10^{-6}
Carbamylcholine	1.1×10^{-4}	2.1×10^{-4}

binding at the two ages were studied by displacement experiments with the antagonists, scopolamine and atropine, with a mixed agonist, pilocarpine and with the muscarinic agonists oxotremorine and carbachol (Table II, Fig. 2). The agonist and antagonist displacement curves in cortex from infants and adults were quite similar. Displacement curves for atropine (Fig. 2) and scopolamine (Table II) were nearly perfectly sigmoidal, consistent with binding to a single population of receptors. The curve for pilocarpine (Fig. 2) did not appear perfectly sigmoidal but the computer derived Hill coefficients (1.0 for adult and 0.93 for the infant) were close to unity and the computer program indicated that the data fit best to a one site model. In contrast, the Hill coefficients for displacement of [³H]QNB binding by oxotremorine and carbachol were considerably less than unity and the plots deviated markedly from sigmoidal shape. The non-linear curve fitting program fit the data to a two site model (Fig. 2). For carbachol, the Hill coefficient for the displacement curve was 0.42 ± 0.1 for adults and 0.54 ± 0.03 for the infant. Carbachol displaced 46% of [³H]QNB from a high affinity (K_i = 1.4×10^{-6} M) site and 54% from a site with an apparent affinity constant of 4×10^{-4} in adult brain. In the infant the distribution was nearly identical (Fig. 4) oxotremorine displaced 67% of the [³H]QNB from a 'low' affinity site in adult cortex ($K_i = 4 \times 10^{-6}$ M) and 33% from a site with an apparent affinity of 2×10^{-8} M. The data from infants resembled closely that from adults.

Quantitative [³H]QNB autoradiography

Since the binding data from homogenates indicated that [³H]QNB binds to similar receptor site populations in infants and adults, we analyzed the distribution and quantity of these sites in more detail using quantitative autoradiography. Because of the very high affinity (approximately 25 pM) of the [³H]QNB site, relatively large incubation volumes would be needed to perform saturation analysis under proper conditions (i.e. with a low (< 10%) per-

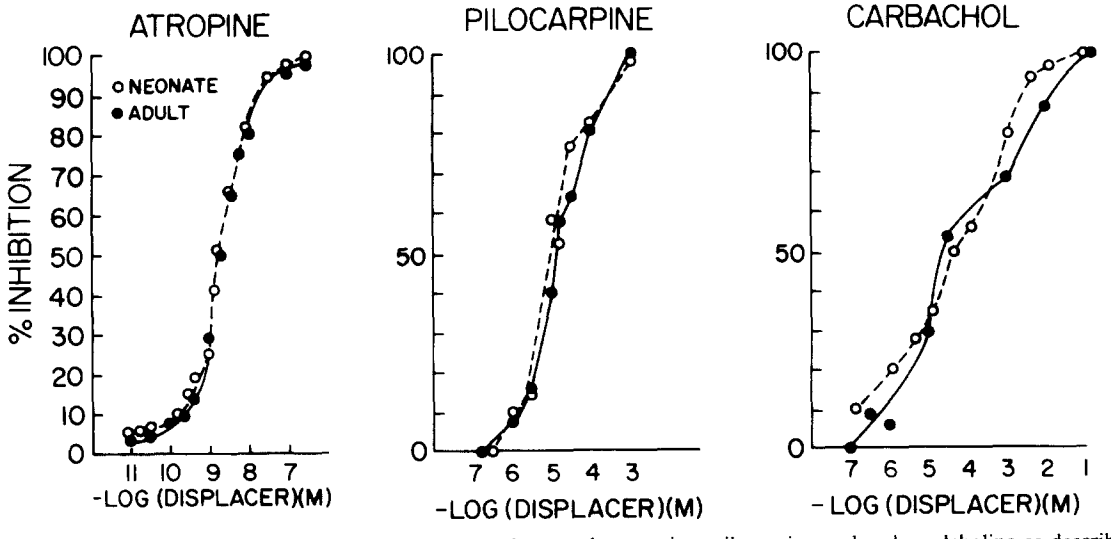

Fig. 2. Displacement of [³H]QNB binding to human cerebral cortex by atropine, pilocarpine and carbamylcholine as described in Methods and Table II. In contrast to displacement with antagonists, the data for carbamylcholine fit best to a two site model as described in Results.

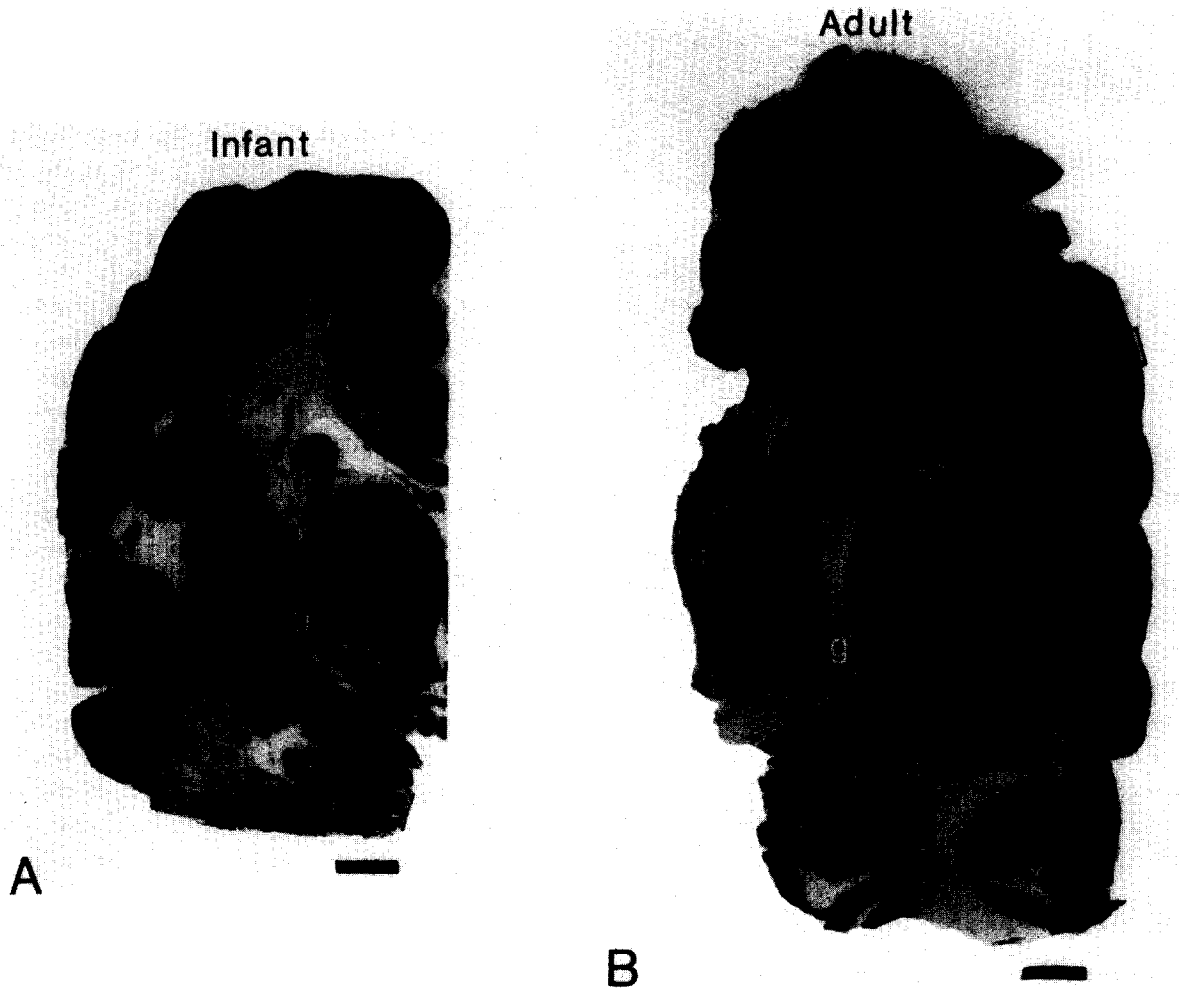

Fig. 3. [^{3}H]QNB binding to coronal sections of human cerebral hemispheres. A: 3-month infant. B: 60-year-old male. Binding is highest in the caudate nucleus (c) and putamen (p) of both adult and infant. High binding is also present in outer cortical layers of the adult, layer 4 of the infant, dorsal medial (d), anterior (a) (adult), and ventral lateral (v) (infant), thalamic nuclei, subthalamic nucleus (s) (infant) and stratum oriens of hippocampus (hp). Differences in the prominence of the thalamic nuclei in these specimens are due to differences in the level of sectioning and no major age related changes were apparent. Details of cortical binding from the areas in boxes are shown in Fig. 4. Abbreviations: i, insula; g, globus pallidum. Films were exposed to [^{3}H]QNB containing sections for 10 days at 4 °C. Scale bar = 1 cm. Part B is reproduced from ref. 33.

centage of total [^{3}H]QNB bound). Because of this consideration and the limited tissue available for analysis, binding was determined in the presence of a single saturating concentration of [^{3}H]QNB. Five infant and adult brains were available for analysis and a representative comparison is shown in Fig. 3. The major feature obvious from gross inspection is that the relatively dense binding in superficial layers of most regions of adult neocortex is much less apparent in the infants. However, one cortical region which does resemble the adult more closely is insular cortex. Dense binding is also present in the caudate and putamen and in the infant, it appears more concentrated than in the adult. The general features seen in the infant brain shown in Fig. 3 were duplicated in all the specimens we examined. There was no correlation between the mode of death and any alterations in the pattern of binding. Preliminary studies of one infant with pulmonary hypoplasia indicated that binding was lower in neonatal cortex but the autoradiographic pattern was the same as the other neonates[20].

Densitometric measurements of [^{3}H]QNB bound to several regions of the human brain are shown in

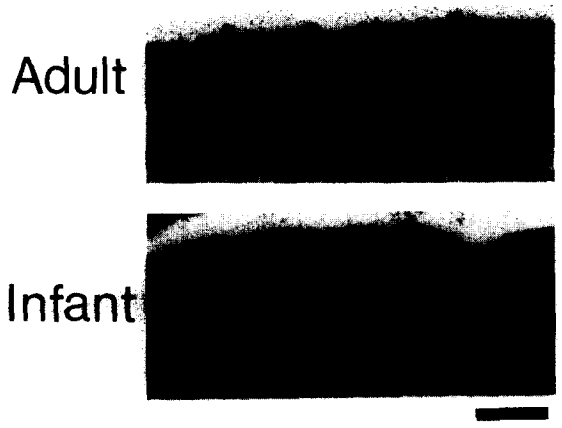

Fig. 4. Details of cortical binding from the boxed areas in Fig. 3. Arrows point to layer 2. Scale bar = 1 mm.

Table III. As in the homogenate studies, there were fewer muscarinic binding sites in adult than in infant but greater differences between the two ages were measured on autoradiograms than in homogenates. The autoradiograms demonstrated [^{3}H]QNB binding in regions of the adult brains that was 49–85% lower than analogous regions in infants and the group regional differences were almost all statistically significant. Overall, the absolute B_{max}s calculated from the autoradiographic data were 3–5 times those calculated from the homogenate binding.

The densitometric data also confirmed that the normal adult laminar distribution pattern (higher in superficial layers than in deeper layers) was reversed in the neonates. The ratio of binding in layer 1/layer 4 was 0.92, 0.87 and 0.83 in frontal, temporal and insular cortex respectively compared with ratios greater than 1 in adults. Fig. 4 demonstrates this inversion in an enlarged photograph of portions of neocortex shown in Fig. 3. Both inspection and quantitative densitometry are in agreement in demonstrating that the adult pattern develops postnatally from an infantile 'inside layer greater than outside' pattern. Furthermore, this reorganization occurs in association with an overall developmental decrease in muscarinic receptor density.

DISCUSSION

The results of [^{3}H]QNB binding experiments in tissue homogenates suggest that muscarinic receptors in cerebral cortex from human adults and young infants share very similar characteristics. Our results for receptor density and binding affinity are quite

TABLE III

Regional distribution of [^{3}H]QNB binding determined from autoradiograms of human brain

Values are mean ± S.E.M. of B_{max}s from the number of brains in parentheses. The B_{max} from each region was based on the means of 8 densitometer readings and converted to quantities of [^{3}H]QNB using standards as described in Methods. 'Layer 1' is the most superficial 1 mm of cortex and 'layer 4' is a 1 mm area including layer 4 based on comparison with Nissl staining of autoradiogram sections. The autoradiograms were prepared at a saturating concentration (1.4 nM) of [^{3}H]QNB. *P*-value denotes results of comparison using Student's *t*-test.

	(n)	*Infants*	*Specific binding at saturation (pmol/g tissue)*			
			(n)	*Adults*	*Δ%*	*P <*
Frontal cortex, layer 1	(4)	212 ± 44	(4)	99 ± 10	−53	0.05
Frontal cortex, layer 4	(4)	230 ± 48	(4)	68 ± 5	−70	0.02
L_1/L_4 ratio		0.92		1.45		
Temporal cortex, layer 1	(5)	215 ± 120	(5)	109 ± 44	−49	0.1
Temporal cortex, layer 4	(5)	245 ± 120	(5)	98 ± 45	−60	0.05
L_1/L_4 ratio		0.87		1.11		
Insular cortex, layer 1	(3)	220 ± 50	(4)	95 ± 15	−57	0.05
Insular cortex, layer 4	(3)	263 ± 60	(4)	87 ± 17	−67	0.05
L_1/L_4 ratio		0.83		1.09		
Caudate n.	(2)	331 ± 93	(3)	128 ± 39	−61	0.1
Lateral G.P.	(2)	82 ± 35	(4)	14 ± 2	−83	0.05
Medial G.P.	(2)	89 ± 51	(4)	16 ± 3	−82	0.1
N. basalis	(1)	188	(4)	28 ± 5	−85	

close to those reported previously for adult human and infant brain[4,10,12,37]. Brooksbank et al.[4] reported that the adult values for B_{max} for [^{3}H]QNB binding was 18% higher than for infants in the perinatal period (40.3 ± 7, n = 5) while our results found adult cortex to be 5–30% lower than infants. However, neither difference was statistically significant. The stability of muscarinic receptors in postmortem adult brain frozen after storage at 4 °C for up to 53 h has been established[37]. It is possible that the values for neonatal brain underestimate binding in fresh tissue. In neonatal rodents, freezing reduced apparent [^{3}H]QNB sites by 33% compared to fresh tissue but the same treatment reduced binding in adult tissue by only 11%[25]. The possible effect of perinatal events on muscarinic receptors is not clear from the studies.

In contrast to the linear Eadie–Hofstee plots found in [^{3}H]QNB saturation binding experiments, muscarinic agonists appear to bind in a non-linear fashion to several populations of receptors[1,2,16,39]. Displacement of [^{3}H]QNB with muscarinic agonists typically are not sigmoidal and Hill coefficients plotted from such experiments are significantly lower than unity. Antagonist and agonist competition experiments with homogenates of human brain confirmed these observations and demonstrated similar receptor characteristics at the extremes of age. In contrast to sigmoidal displacement of [^{3}H]QNB by atropine and scopolamine, carbachol displaced the antagonist ligand over several orders of magnitude with Hill coefficients in the adult of 0.42 and the infant of 0.54. The non-linear curve-fitting computer program fit data from both infants and adults to a two-site model in which half the sites are low affinity with an apparent K_i of approximately 10^{-4} M and half are high affinity with an affinity constant of 10^{-6} M. These results are similar to those for adult rat neocortex[27] (Silverstein and Johnston, unpublished results). In another study of adult human brain, carbachol displacement revealed apparent sites with affinities of $3–42 \times 10^{-6}$ M and $7–13 \times 10^{-4}$ M with 75% lower affinity sites[37]. In contrast to newborn rodents, in which there is postnatal acquisition of the population of high affinity muscarinic sites[25], very young human infants appear to have developed high affinity sites at birth.

Quantitative receptor autoradiography using emulsion and tritium-sensitive film has been described for [^{3}H]QNB and other neurotransmitter ligands and provides a higher level of resolution than is possible with homogenate binding studies[13,21,23,26,30,34,35,38,39]. Direct comparison of quantitative autoradiographic data and homogenate binding demonstrated consistent, significant absolute differences between the two methods. The B_{max} and apparent K_d are generally considerably higher when derived from autoradiograms than from homogenates[13,31]. This disparity may be due in part to the ability of autoradiography to resolve small regions of high receptor density which are averaged with low density areas (e.g. white matter) in homogenates. B_{max}s from the autoradiograms from the human tissue were 2–4 × higher than results from homogenate data and the differences between the adults and infants were greater and statistically significant in the autoradiograms. The autoradiographic data are in agreement with the trend suggested by the homogenate results indicating that infants have a higher muscarinic receptor density than adults in both cortical and subcortical regions. The higher receptor site density in infants may have several possible explanations. Muscarinic receptors may be generated in higher than adult densities in anticipation of the expansion of axo-dendritic connections that occurs in childhood[6,7]. It is possible also that the higher density is correlated with the relatively high synaptic density found in the infant's cerebral cortex and some pruning occurs later on[14,17]. It is noteworthy that we have also found higher than adult concentrations of binding sites for [^{3}H]muscimol, a GABAergic agonist and [^{3}H]flunitrazepam in these same specimens (Johnston, Penney and Young, unpublished observations). An 'overshoot' also occurs in the concentrations of the GABA synthetic enzyme L-glutamate decarboxylase in human neonatal brain and in [^{3}H]-GABA uptake in developing rats[8,11]. However, our human data for muscarinic receptors is distinctly different from the pattern reported for rats, which shows a steady rise in postnatal life[9,25,36].

The most striking developmental difference in muscarinic receptors between ages is seen in the autoradiograms of the cerebral cortex. In adults, muscarinic receptor binding is relatively dense in superficial layers I–III, and acetylcholinesterase positive fibers are also relatively dense there[19,22]. In contrast, layer IV of infant rodents and humans contains an early,

dense innervation with acetylcholinesterase fibers[23,24]. This region also contains a relatively high concentration of synapses in the immature cortex[28]. Our results indicate that layers IV–VI in the human infant are denser than more superficial layers. The inversion of the adult pattern of muscarinic binding in the infants is quantifiable from the autoradiograms using the ratio of binding in layer 1:layer 4 which is less than unity in infant cortical areas and greater than 1 in adults. Prominent muscarinic receptor binding in layer 4 with reduced superficial receptors has been observed in infant rat somatosensory cortex[23]. The human neonatal cortex seems to acquire muscarinic receptors from inside outward, in a fashion resembling the pattern for maturation of axo-dendritic connections[7]. The detailed localization of these receptors, their relationship to synaptogenesis and functional importance in infancy remain to be established. However, reorganization of the intracortical muscarinic receptor pattern appears to be an important feature of postnatal human development.

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REFERENCES

1 Birdsall, M. J. M., Burgen, A. S. V. and Hulme, E. C., The binding of agonists to brain muscarinic receptors, *Molec. Pharmacol.*, 14 (1978) 723–736.
2 Birdsall, M. J. M. and Hulme, E. C., Biochemical studies on muscarinic acetylcholine receptors, *J. Neurochem.*, 27 (1976) 7–16.
3 Bradford, M., A rapid, sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, *Ann. Biochem.*, 72 (1976) 248–253.
4 Brooksbank, B. W. L., Martinez, M., Atkinson, D. J. and Balazs, R., Biochemical development of the human brain: I. Some parameters of the cholinergic system, *Develop. Neurosci.*, 1 (1978) 267–284.
5 Burt, D. K., Hungerford, S. M., Crowner, M. L. and Baem, L. A., Postnatal development of a cholinergic influence in neuroleptic induced catalepsy, *Pharmacol. Biochem. Behav.*, 16 (1982) 533–554.
6 Chi, J. G., Dooling, E. C. and Gilles, F. H., Gyral development of the human brain, *Ann. Neurol.*, 1 (1977) 86–93.
7 Conel, J. L., *The Postnatal Development of the Human Cerebral Cortex*, Harvard University Press, Cambridge, (1939–1967), V 1–8.
8 Coyle, J. T. and Enna, S. J., Neurochemical aspects of ontogenesis of GABAergic neurons in the rat brain, *Brain Res.*, 111 (1976) 119–133.
9 Coyle, J. T. and Yamamura, H. I., Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain, *Brain Res.*, 118 (1976) 429–440.
10 Davies, P. and Verth, A. H., Regional distribution of muscarinic acetylcholine receptor in normal and Alzheimer's-type dementia brains, *Brain Res.*, 138 (1978) 385–392.
11 Diebler, M. F., Farkas-Bargeton, E. and Wehrle, R., Developmental changes of enzymes associated with energy metabolism and synthesis of some neurotransmitters in discrete areas of human neocortex, *J. Neurochem.*, 32 (1979) 429–435.
12 Enna, S. J., Bennett, J. P., Bylund, D. B., Creese, I., Burt, D. R., Charness, M. E., Yamamura, H. I., Simantrov, R. and Snyder, S. H., Neurotransmitter receptors binding: regional distribution in human brain, *J. Neurochem.*, 28 (1977) 233–236.
13 Gilbert, R. F. T., Hanley, M. R. and Iverson, L. L., [^{3}H]Quinuclidinyl benzilate binding to muscarinic receptors in rat brain: comparison of results from intact brain slices and homogenates, *Brit. J. Pharmacol.*, 65 (1979) 451–456.
14 Goldman-Rakic, P. S. and Rakic, P., Development and modifiability of the cerebral cortex, *Neurosciences Research Program Bulletin*, 1982, 611 pages.
15 Graybiel, A. and Ragsdale, C., Clumping of acetylcholinesterase activity in the developing striatum of the human fetus and young infant, *Proc. nat. Acad. Sci. U.S.A.*, 77 (1980) 1214–1218.
16 Hulme, E. C., Birdsall, N. J. M., Burgen, A. S. V. and Mehta, P., The binding of antagonists to brain muscarinic receptors, *Molec. Pharmacol.*, 14 (1978) 737–750.
17 Huttenlocher, P., Synaptic density in human frontal cortex — developmental changes and effects of aging, *Brain Res.*, 163 (1979) 195–205.
18 Johnston, M. V. and Coyle, J. T., Development of central neurotransmitter systems. In K. Elliott and J. Whelan (Eds.), *The Fetus and Independent Life*, Pitman, 1981, pp. 251–270.
19 Johnston, M. V., Young, A. C. and Coyle, J. T., Laminar distribution of cholinergic markers in neocortex: effects of lesions, *J. Neurosci. Res.*, 6 (1981) 597–607.
20 Johnston, M. V., Penney, J. B., Reindel, F. O. and Young, A. B., Characteristics of muscarinic acetylcholine receptors in neonatal and adult human brain, *Neurology*, 32 (1982) A66.
21 Kobayashi, R. M., Palkovits, M., Hruska, R. E., Rothschild, R. and Yamamura, H. I., Regional distribution of muscarinic cholinergic receptors in rat brain, *Brain Res.*, 15

(1978) 13–23.
22 Kristt, D. A., Development of neocortical circuitry: histochemical localization of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex, *J. comp. Neurol.*, 186 (1979) 1–16.
23 Kristt, D. A. and Kasper, E. K., High density of cholinergic muscarinic receptors accompanies high intensity acetylcholinesterase-staining layer IV of infant rat somatosensory cortex, *Develop. Brain Res.*, 8 (1983) 373–376.
24 Krmpotic-Nemanic, J., Kostovic, I., Kelovic, Z. and Nemanic, D., Development of acetylcholinesterase (ACHE) staining in human fetal auditory cortex, *Acta otolaryngol.*, 89 (1980) 388–392.
25 Kuhar, M. J., Birdsall, N. J. M., Burgen, A. S. V. and Hulme, E. C., Ontogeny of muscarinic receptors in rat brain, *Brain Res.*, 184 (1980) 375–383.
26 Kuhar, M. and Yamamura, H., Localization of cholinergic muscarinic receptors in rat brain by light microscopic radioautography, *Brain Res.*, 110 (1976) 222–249.
27 McKinney, M. and Coyle, J. T., Regulation of neocortical muscarinic receptors; effects of drug treatment and lesions, *J. Neurosci.*, 2 (1982) 97–105.
28 Molliver, M. E., Kostovic, I. and Van der Loos, H., The development of synapses in cerebral cortex of the human fetus, *Brain Res.*, 50 (1973) 403–407.
29 Munson, P. J. and Rodbard, D., Ligand: a versatile computerized approach for characterization of ligand-binding systems, *Analyt. Biochem.*, 107 (1980) 220–239.
30 Nonaka, R. and Maroji, T., Quantitative autoradiography of muscarinic receptors in rat brain, *Brain Res.*, 296 (1984) 295–303.
31 Pan, H. S., Frey, K. A., Young, A. B. and Penney, J. B., Changes in [^{3}H]-muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography, *J. Neurosci.*, 3 (1983) 1189–1198.
32 Penney, J. B., Frey, K. A. and Young, A. B., Quantitative autoradiography of neurotransmitter receptors, *Europ. J. Pharmacol.*, 72 (1981) 421–422.
33 Penney, J. B., Pan, H. S., Young, A. B., Frey, K. A. and Dauth, G. W., Quantitative autoradiography of [^{3}H]-muscimol binding in rat brain, *Science*, 214 (1981) 1036–1038.
34 Penney, J. B. and Young, A. B., Quantitative autoradiography of neurotransmitter receptors in Huntington's disease, *Neurology*, 32 (1982) 1391–1395.
35 Rotter, A., Birdsall, M. J. M., Burgen, A. S. V., Field, P. M., Hulme, E. C. and Raisman, G., Muscarinic receptors in the central nervous system of the rat. I. Technique for autoradiographic localization of the binding of [^{3}H]propylbenzilylcholine mustard and its distribution in the forebrain, *Brain Res. Rev.*, 1 (1979) 141–165.
36 Rotter, A., Field, P. M. and Raisman, G., Muscarinic receptors in the central nervous system of the rat. III. Postnatal development of binding of [^{3}H]propylbenzilylcholine mustard, *Brain Res. Rev.*, 1 (1979) 185–205.
37 Unden, A., Meyerson, B., Winblad, B., Sachs, C. and Bartfai, T., Postmortem changes in binding to the muscarinic receptor from human cerebral cortex, *J. Neurochem.*, 41 (1983) 102–106.
38 Wamsley, J. K., Palacios, J. M., Young, W. S. III and Kuhar, M. J., Autoradiographic determination of neurotransmitter receptor distribution in the cerebral and cerebellar cortices, *J. Histochem. Cytochem.*, 29 (1981) 125–135.
39 Wamsley, J. K., Zarbin, M. A., Nigel, J. M., Birdsall, N. and Kuhar, M. J., Muscarinic cholinergic receptors: autoradiographic localization of high and low affinity agonist binding sites, *Brain Res.*, 200 (1980) 1–12.