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Polyamines in Human Brain: Regional Distribution and Influence of Aging

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Abstract: Although much evidence has implicated polyamines in brain development and function, little information is available on these substances in human brain. We examined the influence of regional distribution and aging on putrescine, spermidine, and spermine levels in autopsied human brain. In the adult brain, concentrations of spermidine were the highest, followed by spermine and putrescine. All three polyamines showed a distinct and uneven distribution profile among the 10 examined brain areas. Spermidine levels were especially high in white matter and thalamus (20 and 9.3 nmol/mg of protein, respectively), whereas spermine concentrations were highest in cerebellar cortex (3.4 nmol/mg of protein). High levels of putrescine were observed in cerebral cortices, putamen, and hippocampus (0.7–1.2 nmol/mg of protein), with lowest levels in cerebellum and thalamus (0.3–0.5 nmol/mg of protein). No statistically significant influence of aging (1 day to 103 years; n = 57) on either putrescine or spermine levels in occipital cortex was observed. In contrast, spermidine levels increased markedly from birth, reaching maximal levels at ~40 years of age (+228% increase in the mean 41-year-old group vs. 6-week-old group), which were maintained up to senescence. These observations in human brain thus differ from those reported in the rodent, in which levels of all three polyamines show a pronounced postnatal reduction. Our data support the notion that polyamines may have roles in both postnatal brain development and in mature brain function. **Key Words:** Polyamines—Spermine—Spermidine—Putrescine—Aging—Regional distribution.
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The major cellular polyamines, putrescine,[1] spermidine, and spermine are polyvalent cations that are ubiquitously distributed in mammalian cells (Pegg and McCann, 1982). Polyamines are involved in many diverse cellular and physiological processes including cellular growth and differentiation (Pegg and McCann, 1988), regulation of nucleic acid and protein synthesis (Slotkin and Bartolome, 1986), and stabilization of lipids. Polyamines have multiple functions within the CNS, including roles in brain development (Fozard et al., 1980; Bell and Slotkin, 1988), nerve growth and regeneration (Kauppila, 1992), response to brain injury and stress (Dienel and Cruz, 1984; Paschen et al., 1988), brain metabolism (Seiler and Bolkenius, 1985), regulation of ionic flux and neuronal ion channels (Iqbal and Koenig, 1985; Scott et al., 1993), and modulation of several neurotransmitter receptors in the brain (Koenig et al., 1989; Wasserkort et al., 1991; Williams et al., 1991). As might be expected from the regulatory nature of these compounds, polyamine synthesis is a tightly controlled process (see Fig. 1). Putrescine is formed from the enzymatic decarboxylation of ornithine by ornithine decarboxylase (ODC; EC 4.1.1.17). *S*-Adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.5) catalyzes the decarboxylation of *S*-adenosylmethionine to form decarboxylated *S*-adenosylmethionine, the donor of aminopropyl groups for spermidine and spermine synthesis. In addition, spermine may be metabolized back to spermidine, spermidine to putrescine, and putrescine to GABA through the polyamine interconversion pathway. The key rate-limiting enzyme of the interconversion pathway is spermidine/spermine *N*-acetyltransferase. All three of the abovementioned enzymes are highly regulated, inducible enzymes with a high turnover rate (Pegg and McCann, 1988).

Induction of the polyamine system may occur in response to a variety of stimuli, such as hormones, growth factors, tumor promoters, cerebral ischemia, mechanical and thermal brain injury, neurotoxin insult, neuronal deafferentation, and seizure activity (Pajunen et al., 1978; Dienel and Cruz, 1984; Crozat et al., 1992; Paschen, 1992).

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Abbreviations used: 1,6-DAH, 1,6-hexanediamine; FMOC, 9-fluorenylmethylchloroformate; ODC, ornithine decarboxylase (EC 4.1.1.17; L-ornithine carboxy-lyase); SAMDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50; *S*-adenosylmethionine carboxy-lyase); TCA, trichloroacetic acid.

[1]Although putrescine is a diamine, for the sake of simplicity, putrescine will be referred to as a polyamine.

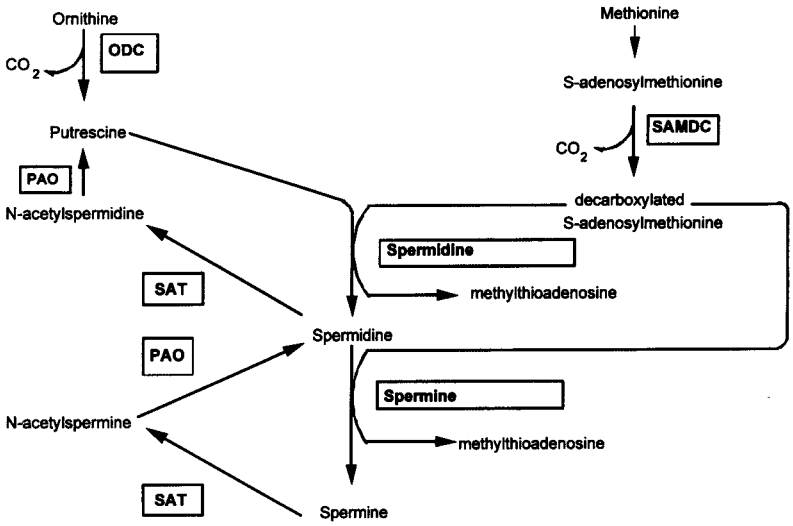

FIG. 1. Polyamine metabolism. SAT, spermidine/spermine *N*-acetyltransferase; PAO, polyamine oxidase.

Neurochemical studies of the influence of aging on brain polyamine levels in the rodent have shown that polyamine levels decline precipitously during postnatal development to low levels in mature brain (Janne et al., 1964; Suorsa et al., 1992). However, to our knowledge, only sparse information is available regarding polyamine levels in normal human brain (Perry et al., 1967; Shaw and Pateman, 1973; Sturman and Gaull, 1975, McAnulty et al., 1977; Chaudhuri et al., 1983). Previous studies have been limited either by the age range of the samples (fetal and infant brain, McAnulty et al., 1977; Chaudhuri et al., 1983), or by the number of cases (n = 2; Shaw and Pateman, 1973) or brain areas (whole brain, Perry et al., 1967; occipital lobe, Sturman and Gaull, 1974) examined. The present study describes the regional distribution of putrescine, spermidine, and spermine and the influence of aging (1 day to 103 years) on polyamine levels in neurologically and neuropsychiatrically normal human brain.

MATERIALS AND METHODS

Autopsied brain was obtained from 57 subjects who died without evidence of neurological or psychiatric disease or brain pathology (mean postmortem time, 12.3 ± 0.8 h, mean ± SEM). At time of autopsy, each brain was dissected sagittally into two equal halves. One half-brain was used for routine histopathological analyses, whereas the other half was frozen at −80°C for biochemical analysis.

Brain sample preparation, polyamine derivatization with 9-fluorenylmethyl chloroformate (FMOC), and reverse-phase HPLC analyses were conducted based on previous methods (Einarsson et al., 1983; Sabri et al., 1989), with modifications. Brain tissue was stored at −80°C before use, thawed on ice, and prepared by sonicating at a concentration of 400 mg of tissue/ml for 15 s in distilled H_2O on ice. An aliquot (20 μl) was removed for protein determinations (Coomassie Blue protein assay, Sigma). The samples were deproteinized by mixing an equal volume of sonicate and 10% (wt/vol) trichloroacetic acid (TCA) so that the final TCA concentration was 5% (vol/vol). The samples were vortexed for 10 s before centrifugation at 4°C at 12,000 rpm for 30 min in an Eppendorf microfuge. The supernatant was collected, the pH adjusted to neutral with NaOH and filtered (0.2 μm). The samples were derivatized by adding 0.1 ml of supernatant to a tube containing 1 ml of borate buffer (0.1 *M*, pH 9.6), 1 ml of acetone, and 10 μl of internal standard, 100 μg/ml solution of 1,6-hexanediamine (1,6-DAH). All tubes were vortexed before the addition of 100 μl FMOC in acetone (0.01 *M* made fresh). The tubes were vortexed for 30 s and the derivatization allowed to proceed for 10 min at room temperature. After this time, 2.0 ml of hexane/ethyl acetate (1:1, vol/vol) were added and the tubes vortexed for 30 s. The upper solvent layer containing the polyamine derivatives was removed and the extraction process repeated. The solvent was dried in a rotary vacuum extractor and the derivatized polyamines were reconstituted in 1 ml of HPLC-grade ethanol. The derivatives were filtered (0.2 μm) before HPLC analysis. The efficiency of the derivatization and extraction procedures was checked using radiolabeled standard, which indicated that >93% of label was extracted in the upper phase (data not shown).

A 10-μl sample was injected onto the HPLC column (5 μm, ODS 2). The equipment consisted of a binary solvent delivery system equipped with a gradient controller and integrator (Spectraphysics). A fluorometer (Perkin-Elmer LS30) was used to monitor the elution of polyamine derivatives from the column (excitation wavelength 254 nm, emission wavelength 316 nm). The derivatives were separated using 20% A (consisting of 3 ml glacial acetic acid, 1 ml triethylamine per L of distilled H_2O, pH 4.2)/80% B (methanol) for 7 min, 10% A/90% B from 7.2 to 22 min, then 20% A/80% B for 18 min, at a flow rate of 1.5 ml/min. Putrescine and polyamine peaks were identified by comparison of retention time with authentic standards.

Stock solutions of putrescine, spermidine, spermine, and 1,6-DAH were prepared at 100 μg/ml and used to construct standard curves (0–10 ng of putrescine, 0–50 ng of spermidine, 0–20 ng of spermine, and 10 ng 1,6-DAH injected). Polyamine levels in individual samples were calculated from the peak height ratios of derivatized polyamines and the internal standard 1,6-DAH.

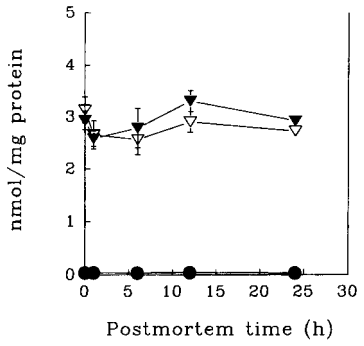

FIG. 2. Influence of postmortem time on putrescine (●), spermidine (▽), and spermine (▼) levels in whole rat brain homogenate (n = 5 brains/time point). The data shown are mean polyamine levels ± SEM.

Putrescine, spermidine, and spermine were purchased as chloride salts from Sigma, methanol and ethanol were HPLC grade, and all other chemicals were of the highest grade available.

RESULTS

The postmortem stability of polyamines under controlled conditions was assessed by measuring polyamine levels in brain of rodents (adult male Wistar rats) at various time intervals after decapitation (n = 5 per group). The decapitated heads were stored at 4°C for the specified postmortem time before brain removal and freezing at −80°C. As shown in Fig. 2, no significant changes in whole brain levels of putrescine, spermidine, or spermine were observed over a 24-h postmortem period (one-way ANOVA, $p > 0.05$). In a similar manner, in our autopsied human brain study, no statistically significant group differences (one-way ANOVA) or correlations (Pearson's product moment) for postmortem interval and polyamine levels were observed ($p > 0.05$) for the three polyamines in any of the 10 brain areas examined, with the exception of a significant positive correlation between spermidine levels and postmortem time in frontal cortex ($r = 0.93$, $p < 0.01$). Polyamine levels were also measured in biopsied temporal cortex obtained from three individuals (age, 43 ± 11 years) who had undergone temporal lobe resection for intractable epilepsy. The biopsied tissue was taken from nonepileptogenic regions as determined by electrocorticography with surface and temporary depth electrodes (Morrison et al., 1994). No significant changes in polyamine levels were observed in autopsied compared with biopsied human temporal cortex (putrescine levels, 1.1 ± 0.2 and 1.3 ± 0.2 nmol/mg of protein; spermidine levels, 5.0 ± 1.9 and 5.3 ± 1.6 nmol/mg of protein; spermine levels, 1.9 ± 0.7 and 2.3 ± 0.3 nmol/mg of protein, respectively). Our data demonstrate that brain polyamine levels are not increased as a consequence of death-associated hypoxia.

As shown in Fig. 3, a heterogeneous regional distribution of all three polyamines was observed in adult brain (mean age, 61 ± 6 years; one-way ANOVA, $p < 0.05$). Of the 10 regions examined, mean putrescine levels were high in temporal and occipital cortex (1.0 and 1.2 nmol/mg of protein, respectively) and low in cerebellar cortex and thalamus (0.3 and 0.5 nmol/mg of protein, respectively). Highest spermine levels were present in cerebellar, occipital, and temporal cortices (1.9–3.4 nmol/mg of protein) and lowest in hippocampus (1.1 nmol/mg of protein), whereas spermidine was most concentrated in white matter and thalamus (20 and 9.3 nmol/mg of protein) with relatively low levels in frontal, insular, and cerebellar cortices (3.7–4.7 nmol/mg of protein).

Figure 4 shows the influence of aging on mean polyamine levels in occipital cortex of 57 neurologically and neuropsychiatrically normal subjects, aged 1 day to 103 years, who were grouped into six age groups with mean ages of 6 weeks (n = 6; range, 21 h to 3.6 months; 6.3 ± 2 weeks, mean ± SEM), 1 year (n = 9; range, 8 months to 2 years 10 months; 1 year 5 months ± 2.4 months), 19 years (n = 7; range, 11–

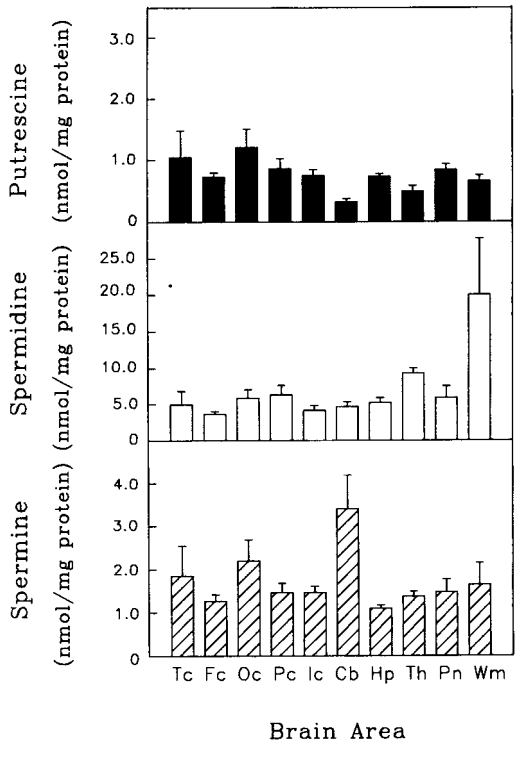

FIG. 3. Distribution of polyamine levels (nmol/mg of protein) in various regions of human brain. The data shown are mean determinations ± SEM obtained from seven brains per region, except white matter where n = 3. One-way ANOVA revealed significant differences in putrescine ($p < 0.05$), spermidine ($p < 0.00001$), and spermine levels ($p < 0.01$) in the different brain regions. Fc, frontal cortex; Tc, temporal cortex; Pc, parietal cortex; Oc, occipital cortex; Cb, cerebellar cortex; Ic, insular cortex; Pn, putamen; Th, medial–dorsal thalamus; Wm, white matter.

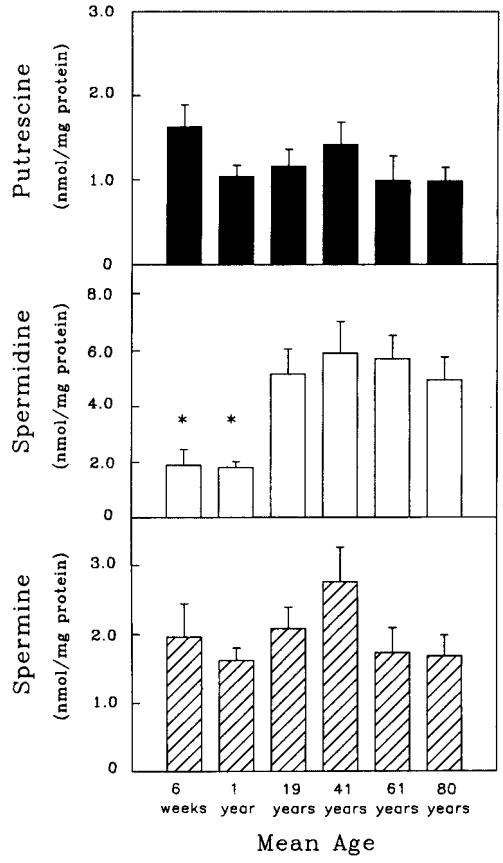

FIG. 4. Influence of aging on putrescine and polyamine levels in occipital cortex of neurologically normal human brain (n = 57). The data shown are mean determinations ± SEM. *Significant differences in spermidine levels of the younger age groups compared with the adult ($p < 0.05$, Student's two-tailed t test).

28 years; 18.6 ± 2.6 years), 41 years (n = 9; range, 30–48 years; 40.6 ± 2.2 years), 61 years (n = 12; range, 52–69 years; 61.4 ± 1.5 years); 80 years (n = 14; range, 70–103 years; 80.2 ± 2.6 years). The mean postmortem intervals of the age groups were not significantly different (one-way ANOVA, $p > 0.05$). The mean freezer storage times of the two youngest age groups (4 years) were about one-half that of the adults (8 years). However, there were no statistically significant correlations between polyamine levels and freezer storage time ($p > 0.05$).

A one-way ANOVA of the grouped data showed a statistically significant increase in spermidine levels with increasing age ($p < 0.002$), with mean spermidine levels being increased 228% in the adult brain (mean age, 41 years) compared with the youngest group (6 weeks) and reaching a plateau at ~40 years of age. Regression analyses revealed a highly significant correlation of spermidine concentration with age from birth to adulthood (1 day to 50 years; $r = 0.71$, $p < 0.01$; 1 day to 103 years; $r = 0.40$, $p < 0.01$). Although no significant influence of aging on putrescine levels was observed, the concentration of putrescine was ~60% higher in the youngest group (mean age, 6 weeks) compared with the oldest group (mean age, 80 years). Spermine levels were not influenced by age ($p > 0.05$ for both one-way ANOVA and Pearson's correlation).

DISCUSSION

To our knowledge, this is the first comprehensive study describing the distribution and effects of aging on putrescine and polyamine levels in neurologically normal human brain. In our investigation human brain polyamine levels were similar to those previously reported in brain of rodents and nonhuman primates and are the same order of magnitude as those reported by Sturman and Gaull (occipital cortex, 1974) and Shaw and Pateman (1973) in human brain (see Table 1). The levels of putrescine, spermidine, and spermine determined by Perry and coworkers (1967) in whole human brain (see Table 1) are ~20–40 times lower than values reported here, or by other authors. This discrepancy may be due to differences in the polyamine recovery efficiencies and sensitivities of the different methodologies used.

A heterogeneous and discrete distribution of all three polyamines was observed in autopsied human brain. Our finding that putrescine levels were highest in occipital and cerebral cortices, putamen, and hippocampus, and lowest in the cerebellar cortex and thalamus, can be compared with a previous animal (mongolian gerbil, Paschen et al., 1988) study in which putrescine levels were lower in cerebellar cortex than in hippocampus or parietal cortex. In adult human brain, spermidine levels were markedly higher in white matter and thalamus than in the other brain regions examined (see Fig. 3); however, unlike an earlier report (Shimizu et al., 1964), substantial concentrations of spermidine were present in gray matter. Our regional distribution data are consistent with the results of earlier studies on spermidine and spermine distribution in human brain, in which levels of spermine were found to be highest in the visual (occipital) and cerebellar cortices, and spermidine levels were greatest in richly myelinated areas (Shaw and Pateman, 1973; McAnulty et al., 1977). In human brain the activity of SAMDC, the key enzyme of spermidine and spermine biosynthesis, is high in occipital and other cortical regions and caudate but barely detectable in white matter (Morrison et al., 1993). The reason for the low SAMDC activity and relatively high spermidine and spermine levels in white matter is unknown but could be explained by specific uptake of spermidine and spermine into white matter by transport processes similar to those previously described in various cell types (Khan et al., 1990, 1993; Toninello et al., 1992).

ODC activity and putrescine levels in rodent (Anderson and Schanberg, 1972) and nonhuman primate (Sturman and Gaull, 1975) brain are highest in early

TABLE 1. *Polyamine levels (pmol/mg of protein) in mammalian brain*

Putrescine	Human	Rat	Gerbil	Monkey	Mouse
Fetal			—	—	—
Whole		2,500[c]			
Cortex	1,300[b]				
Occ. lobe	4,300[m]				
Cb. cortex	700[b]				
Infant		—	—		
Whole	300[i]				3,000[o]
Occ. lobe	(1,270)			900[n]	
Cb. cortex	6,000[g]				
Brainstem	10,250[g]				
Adult					
Whole	25[i]	(36)			200[o]
Occ. cortex	550[m] (1,210)			400[n]	
P. cortex	(860)		102[p]		
Cb. cortex	(320)	37[e]	83[p]		
Hp	(740)	100[a], 50[p]	105[p]		
Thalamus	(490)		98[p]		
Putamen	(850)	262[d]			
Striatum			128[p]		

Spermidine	Human	Rat	Monkey	Mouse	Rabbit	Dog	Sheep
Fetal			—	—	—	—	—
Whole	—	6,510[c]					
Cortex	1,270[b]						
Occ. lobe	3,600[m]						
Cb. cortex	1,260[b]						
Infant		4,000[h]			—	—	—
Whole	868[i]			10,000[o]			
Occ. lobe	(1,832)		2,700[n]				
Cb. cortex	1,300[g]						
Brainstem	3,000[g]						
Adult							
Whole	627[i]	2,700[h], 4,650[f] (3,130)		3,200[o]			
Occ. cortex	4,100[m] (5,860)		5,500[n]				
T. cortex	840[k] (4,963)						
F. cortex	1,180[k] (3,660)						
P. cortex	(6,330)	1,830[j]					
Cb. cortex	730[k] (4,680)	3,860[e], 4,060[j]			670[k]		1,100[k]
Hp	1,360[k] (5,220)	1,490[k], 1,800[a], 5,120[e]			820[k]		
Thalamus	2,420[k] (9,310)	1,020[k]			1,180[k]	130[k]	2,200[k]
Putamen	1,260[k] (5,900)						
W. matter	4,520[k] (20,000)						
Striatum		3,100[d], 2380[j]			2,590[k]	2,790[k]	4,010[k]
Cortical (gray)	1,767[f]	860[k]			510[k]	900[k]	980[k]

Spermine	Human	Rat	Monkey	Mouse	Rabbit	Dog	Sheep
Fetal			—	—	—	—	—
Whole	—	4,510[c]					
Cortex	1,300[b]						
Occ. lobe	3,310[m]						
Cb. cortex	440[b]						
Infant		—			—	—	—
Whole	402[i]			7,800[o]			
Occ. lobe	(1,760)		4,200[n]				
Cb. cortex	1,600[g]						
Brainstem	1,000[g]						
Adult							
Whole	84.7[i]	2,750[f] (2,930)		3,000[o]			
Occ. cortex	2,210[m] (2,200)		3,000[n]				
T. cortex	430[k] (1,850)						
P. cortex	(1,460)	1,930[j]					
Cb. cortex	950[k] (3,400)	2,310[j], 1,870[e]			860[k]		720[k]
Hp	430[k] (1,090)	1,060[k], 1,700[a], 3,000[e]			590[k]		
Thalamus	390[k] (1,370)	900[k]			820[k]	690[k]	750[k]
Putamen	290[k] (1,470)						
W. matter	760[k] (1,640)				1,370[k]	950[k]	110[k]
Striatum		1,690[d], 1,730[j]					
Cortical (gray)	747[i]	630[k]			520[k]	690[k]	750[k]

Our data are presented in parentheses for comparison. Occ., occipital; Cb., cerebellar; T., temporal; F., frontal; P., parietal; Hp, hippocampus; W. matter, white matter. The selected data were converted to the units shown assuming protein was 10% of tissue.

[a] Baudry and Najm, 1994; [b]Chaudhuri et al., 1983; [c]Desiderio et al., 1987; [d]Desiderio et al., 1988; [e]Ientile et al., 1988; [f]Janne et al., 1964; [g]McAnulty et al., 1977; [h]Pearce and Schanberg, 1969; [i]Perry et al., 1967; [j]Shaskan et al., 1973; [k]Shaw and Pateman, 1973; [l]Shimizu et al., 1964; [m]Sturman and Gaull, 1974; [n]Sturman and Gaull, 1975; [o]Suorsa et al., 1992; [p]Paschen et al., 1990.

life and decrease to low adult levels. In the human, Sturman and Gaull (1974) reported that putrescine levels in fetal brain are approximately eightfold higher than in mature brain. Although we did not observe any precipitous reduction in putrescine levels during postnatal development, putrescine concentration was, in fact, higher in the youngest (6 weeks) age group compared with adult brain. Our observation of markedly increased spermidine in human brain from birth to adulthood is consistent with a previous study performed in rhesus monkey (Sturman and Gaull, 1975) but differs from the profile seen in rodent brain (Shimizu et al., 1964; Shaskan et al., 1973; Suorsa et al., 1992) in which levels of all three polyamines decrease after birth. Because spermidine and spermine have stimulatory effects on glutamate receptor function (Ransom and Stec, 1988; Ransom and Deschenes, 1990; Williams et al., 1991), these polyamines may be involved in human postnatal developmental processes in which the polyamine-activated NMDA-preferring glutamate receptor is implicated, such as regulation of neuronal survival, synaptic reorganization, connectivity, and plasticity (for review, see McDonald and Johnston, 1990). The essential role polyamines have in brain development has been demonstrated in rodent models (Fozard et al., 1980; Slotkin et al., 1983; Bell and Slotkin, 1988) in which inhibition of polyamine biosynthesis resulted in fetal death or retarded brain development. Although the precise roles of polyamines on developing neurons are not fully understood, several studies have now associated polyamines with neurotrophic functions, such as enhancement of neuronal survival and control of axonogenesis, neurite elongation, and synaptogenesis (Slotkin and Bartolome, 1986; Abe et al., 1993; Chu et al., 1994).

Our observation that polyamine levels are maintained throughout adulthood suggests functions for these cations in mature brain other than those associated with rapid growth. These may include regulatory functions such as posttranscriptional modification of proteins (Folk et al., 1980), modulation of G-protein activity (Bueb et al., 1992), regulation of brain receptor function (Ransom and Stec, 1988; Koenig et al., 1989; Wasserkort et al., 1991; Williams et al., 1991), and control of synaptic and neuronal activity (Wedgewood and Wolstencroft, 1977; Iqbal and Koenig, 1985; Scott et al., 1993). In our study, the concentrations of spermidine and spermine determined in various regions of adult human brain are in the range 55–1,410 and 25–120 μM, respectively. Experimental studies indicate that both spermidine and spermine potentiate NMDA-receptor responses and modulate N-type Ca^{2+} channel function at concentrations of 0.2–200 μM (Pullan et al., 1990; Carter, 1994). This suggests potential roles in the human brain for polyamines in the phenomenon of long-term potentiation and the processes of learning and memory (Morris et al., 1986; Collingridge and Bliss, 1987; Mondadori et al., 1988). In this regard, spermine has recently been shown to facilitate the generation of long-term potentiation in the dentate gyrus of rodent brain in vivo (Chida et al., 1992).

In summary, we present here baseline information on putrescine, spermidine, and spermine levels in human brain. These data will be useful for future studies directed toward assessing the contribution of the polyamine system to human brain developmental and neurodegenerative disorders.

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REFERENCES

Abe K., Chida N., Nishiyama N., and Saito H. (1993) Spermine promotes the survival of primary cultured brain neurons. *Brain Res.* **605,** 322–326.

Anderson T. R. and Schanberg S. M. (1972) Ornithine decarboxylase in developing rat brain. *J. Neurochem.* **19,** 1471–1481.

Baudry M. and Najm J. (1994) Kainate-induced seizure activity stimulates the polyamine interconversion pathway in rat brain. *Neurosci. Lett.* **171,** 151–154.

Bell J. M. and Slotkin T. A. (1988) Co-ordination of cell development by the ornithine decarboxylase/polyamine pathway as an underlying mechanism in developmental neurotoxic events. *Prog. Brain Res.* **73,** 349–361.

Bueb J.-L., DaSilva A., Mousli M., and Landry Y. (1992) Natural polyamines stimulate G-proteins. *Biochem. J.* **282,** 545–550.

Carter C. (1994) Brain polyamines: intra- and intercellular messengers and neurotoxins? in *The Neuropharmacology of Polyamines* (Carter C., ed), pp. 255–295. Academic Press, New York.

Chaudhuri D., Choudhury I., and Mukherjea M. (1983) Ontogeny of polyamines in relation to nucleic acids in brain and spinal cord of the developing human fetus. *Dev. Brain Res.* **10,** 143–145.

Chida N., Saito H., and Abe K. (1992) Spermine facilitates the generation of long-term potentiation of evoked potential in the dentate gyrus of anesthetized rats. *Brain Res.* **593,** 57–62.

Chu P.-J., Saito H., and Abe K. (1994) Polyamines promote neurite elongation of cultured rat hippocampal neurons. *Neurosci. Res.* **19,** 155–160.

Collingridge G. L. and Bliss T. V. P. (1987) NMDA receptors—their role in long term potentiation. *Trends Neurosci.* **7,** 288–293.

Crozat A., Palvimo J. J., Julkunen M., and Janne O. A. (1992) Comparison of androgen regulation of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase gene expression in rodent kidney and accessory sex organs. *Endocrinology* **130,** 1131–1144.

Desiderio M. A., Sessa A., and Perin A. (1987) Polyamine and diamine oxidase activity in maternal, embryonal, and fetal tissues of rat after chronic ethanol consumption. *Biochem. Biophys. Res. Commun.* **142,** 843–848.

Desiderio M. A., Zini I., Davalli P., Zoli M., Corti A., Fuxe K., and Agnati L. F. (1988) Polyamines, ornithine decarboxylase, and diamine oxidase in the substantia nigra and striatum of the male rat after hemitransection. *J. Neurochem.* **51,** 25–31.

Dienel G. A. and Cruz N. F. (1984) Induction of brain ornithine decarboxylase during recovery from metabolic, mechanical, thermal, or chemical injury. *J. Neurochem.* **42,** 1053–1061.

Einarsson S., Josefsson B., and Lagerkvist S. (1983) Determination of amino acids with 9-fluorenylmethylchloroformate and reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **282,** 609–618.

Folk J. E., Park M. H., Chung S., Schrode J., Lester E. P., and Copper H. L. (1980) Polyamines as physiological substrates for transglutaminases. *J. Biol. Chem.* **255,** 3695–3700.

Fozard J. R., Port M. L., Prakash N. J., Grove J., Schechter P. J., Sjoerdsma A., and Koch-Weser J. (1980) L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. *Science* **208,** 505–508.

Ientile R., De Luca G., DiGiorgio R. M., and Macaione S. (1988) Glucocorticoid regulation of spermidine acetylation in the rat brain. *J. Neurochem.* **51,** 677–682.

Iqbal Z. and Koenig H. (1985) Polyamines appear to be second messengers in mediating Ca^{2+} fluxes and neurotransmitter release in potassium-depolarized synaptosomes. *Biochem. Biophys. Res. Commun.* **133,** 563–573.

Janne J., Raina A., and Siimes M. (1964) Spermidine and spermine in rat tissues at different ages. *Acta Physiol. Scand.* **62,** 352–358.

Kauppila T. (1992) Polyamines enhance recovery after sciatic nerve trauma in the rat. *Brain Res.* **575,** 299–303.

Khan N. A., Masson I., Quemner V., and Moulinoux J. P. (1990) Coupling of Na^+ with the spermidine transporter protein in NIH BALB/c 3T3 cells. *Cell. Mol. Biol.* **36,** 345–348.

Khan N. A., Sezan A., Quemener V., and Moulinoux J. P. (1993) Polyamine transport regulation by calcium and calmodulin: role of $Ca(2^+)$-ATPase. *J. Cell. Physiol.* **157,** 493–501.

Koenig H., Trout J. J., Goldstone A. D., Iqbal Z., Lu C. Y., and Siddiqui F. (1989) Polyamines as mediators of NMDA receptor excitotoxicity in chick retina: protection by α-difluoromethylornithine. *Neurology* **39** (Suppl. 1), 216.

McAnulty P. A., Yusuf H. K. M., Dickerson J. W. T., Hey E. N., and Waterlow J. C. (1977) Polyamines of the human brain during normal fetal and postnatal growth and during postnatal malnutrition. *J. Neurochem.* **28,** 1305–1310.

McDonald J. W. and Johnston M. V. (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev.* **15,** 41–70.

Mondadori C., Weiskrantz L., Buerk H., Petschke F., and Fagg G. E. (1989) NMDA receptor antagonists can enhance or impair learning performance in animals. *Exp. Brain Res.* **75,** 449–456.

Morris R. G. M., Anderson E., Lynch G. S., and Baudry M. (1986) Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature* **319,** 774–779.

Morrison L. D., Becker L., and Kish S. J. (1993) *S*-Adenosylmethionine decarboxylase in human brain. Regional distribution and influence of aging. *Dev. Brain Res.* **73,** 237–241.

Morrison L. D., Sherwin A. L., Carmant L., and Kish S. J. (1994) Activity of *S*-adenosylmethionine decarboxylase, a key regulatory enzyme in polyamine biosynthesis, is increased in epileptogenic human cortex. *Arch. Neurol.* **51,** 581–584.

Pajunen A. E. I., Hietala O. A., Virransalo E. L., and Piha R. S. (1978) Ornithine decarboxylase and adenosylmethionine decarboxylase in mouse brain—effect of electrical stimulation. *J. Neurochem.* **30,** 281–283.

Paschen W. (1992) Polyamine metabolism in reversible cerebral ischemia. *Cerebrovasc. Brain Metab. Rev.* **4,** 59–88.

Paschen W., Schmidt-Kastner R., Hallmayer J., and Djuricic B. (1988) Polyamines in cerebral ischemia. *Neurochem. Pathol.* **9,** 1–20.

Paschen W., Hallmayer J., Mies G., and Rohn G. (1990) Ornithine decarboxylase activity and putrescine levels in reversible cerebral ischemia of mongolian gerbils: effect of barbiturate. *J. Cereb. Blood Flow Metab.* **10,** 236–242.

Pearce L. A. and Schanberg S. A. (1969) Histamine and spermidine content in brain during development. *Science* **166,** 1301–1303.

Pegg A. E. and McCann P. P. (1982) Polyamine metabolism and function. *Am. J. Physiol.* **243,** 212–221.

Pegg A. E. and McCann P. P. (1988) Polyamine metabolism and function in mammalian cells and protozoans. *ISI Atlas of Science* (Biochemistry) 11–18.

Perry T. L., Hansen S., and MacDougall L. (1967) Amines of human whole brain. *J. Neurochem.* **14,** 775–782.

Pullan L. M., Keith R. A., La Monte D., Stumpo R. J., and Salama A. I. (1990) The polyamine spermine affects ω-conotoxin binding and function at N-type voltage sensitive calcium channels. *J. Auton. Pharmacol.* **10,** 213–219.

Ransom R. W. and Deschenes N. L. (1990) Polyamines regulate glycine interaction with the *N*-methyl-D-aspartate receptor. *Synapse* **5,** 294–298.

Ransom R. W. and Stec N. L. (1988) Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor–ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* **51,** 830–836.

Sabri M. I., Soiefer A. I., Kisby G. E., and Spencer P. S. (1989) Determination of polyamines by precolumn derivatization with 9-fluorenylmethyl chloroformate and reverse-phase high-performance liquid chromatography. *J. Neurosci. Methods* **29,** 27–31.

Scott R. H., Sutton K. G., and Dolphin A. C. (1993) Interactions of polyamines and neuronal ion channels. *Trends Neurosci.* **16,** 153–160.

Sebille A. and Bondoux-Jahan M. (1980) Motor function recovery after axotomy: enhancement by cyclophosphamide and spermine in rat. *Exp. Neurol.* **70,** 507–515.

Seiler N. and Bolkenius F. N. (1985) Polyamine reutilization and turnover in brain. *Neurochem. Res.* **10,** 529–544.

Shaskan E. G., Haraszti J. H., and Snyder S. H. (1973) Polyamines: developmental alterations in regional disposition and metabolism in rat brain. *J. Neurochem.* **20,** 1443–1452.

Shaw G. G. and Pateman A. J. (1973) The regional distribution of the polyamines spermidine and spermine in brain. *J. Neurochem.* **20,** 1225–1230.

Shimizu H., Kakimoto Y., and Sano I. (1964) The determination and distribution of polyamines in mammalian nervous system. *J. Pharmacol. Exp. Ther.* **143,** 199–204.

Slotkin T. A. and Bartolome J. (1986) Role of ornithine decarboxylase and the polyamines in nervous system development: a review. *Brain Res. Bull.* **17,** 307–320.

Slotkin T. A., Seidler F. J., Whitmore W. L., Weigel S. J., Slepetis R. J., Lerea L., Trepanier P. A., and Bartolome J. (1983) Critical periods for the role of ornithine decarboxylase and the polyamines in growth and development of the rat: effects of exposure to α-difluoromethylornithine during discrete prenatal or postnatal intervals. *Int. J. Dev. Neurosci.* **1,** 113–127.

Sturman J. A. and Gaull G. E. (1974) Polyamine biosynthesis in human fetal liver and brain. *Pediatr. Res.* **8,** 231–237.

Sturman J. A. and Gaull G. E. (1975) Polyamine metabolism in the brain and liver of the developing monkey. *J. Neurochem.* **25,** 267–272.

Suorsa A., Hietala O., and Pajunen A. (1992) Developmental expression of ornithine and *S*-adenosylmethionine decarboxylases in mouse brain. *Biochem. Biophys. Res. Commun.* **184,** 1114–1118.

Toninello A., Dalla Via L., Siliprandi D., and Garlid K. D. (1992) Evidence that spermine, spermidine, and putrescine are transported electrophoretically in mitochondria by a specific polyamine uniporter. *J. Biol. Chem.* **267,** 18393–18397.

Wasserkort R., Hoppe E., Reddington M., and Schubert P. (1991) Modulation of A1 adenosine receptor function in rat brain by the polyamine spermine. *Neurosci. Lett.* **124,** 183–186.

Wedgewood M. A. and Wolstencroft J. H. (1977) Effects of spermine and spermidine on single brain stem neurons. *Neuropharmacology* **16,** 445–453.

Williams K., Romano C., Dichter M. A., and Molinoff P. B. (1991) Modulation of the NMDA receptor by polyamines. *Life Sci.* **48,** 469–479.