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Neurons Expressing Phosphodiesterase 10A mRNA in Rat Brain : *In situ* Hybridization

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Abstract

The mRNA expression of phosphodiesterase (PDE) 10A, a cGMP-binding cAMP PDE and cAMP-inhibited cGMP PDE, was examined in rat brain by *in situ* hybridization. The Northern blot analysis demonstrated expression of a single species of about 9,100-base long mRNA in various parts of rat brain. The most abundant expression of PDE10A mRNA was detected in the caudate-putamen, or striatum.

In situ hybridization demonstrated that neurons in rat brain expressed PDE10A mRNA. Morphology of PDE10A neurons was different from one brain region to another ; mostly from medium-sized to small granule cells. The morphologically same types of neurons were not always positive even in single nuclei or regions. A number of PDE10A neurons were located in the layer VI of the cerebral cortex and the caudate-putamen. Many or a moderate number of PDE10A neurons were observed in the external plexiform layer and the internal granule cell layer of the olfactory bulb, anterior olfactory nucleus, olfactory tubercle, piriform cortex, layer II and III of the cerebral cortex, dentate gyrus and fields CA 1 to CA3 of the hippocampus, amygdaloid nuclear complex, the granule cell layer of the cerebellum and the deep cerebellar nuclei. Solitary or scattered PDE10A neurons were found throughout the brain. The distinctive distribution of PDE10A neurons in rat brain suggests that this degradation enzyme plays indispensable role(s) in functions proper to given neuronal populations.

Key Words : intracellular signal transduction, phosphodiesterase, cGMP, cAMP, *in situ* hybridization

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Introduction

The second messengers such as cAMP and cGMP play pivotal roles in a variety of intracellular signaling pathways and in functions of various tissues.[1,6] Intracellular levels of cAMP and cGMP are regulated both through synthesis by adenylyl and guanylyl cyclases and hydrolysis by phosphodiesterases (PDEs). Activities of PDEs are directly involved in the steady-state levels and the rate of degradation of the cyclic nucleotides. Therefore, PDEs have been thought of as regulators of the cyclic nucleotide second messengers by modulating the amplitude and the duration of cyclic nucleotide signals.

PDEs form a superfamily of enzymes that catalyze the hydrolysis of cAMP and cGMP. On the basis of their biochemical properties, inhibitor profiles, and amino acid sequences, PDEs are subdivided into 11 major families. Each family contains several distinct genes, and members of a family also express alternative splice variants in different tissues. The alternative splicing appears to be highly tissue-specific, providing a mechanism for selective expression of different PDE isozymes in cells and tissues. It is assumed that different PDE isozymes participate in discrete signal transduction pathways and are implicated in different cell type-specific properties.

The molecular diversities of PDE isozymes are characterized with the biochemical and pharmacological properties, and correlated with their tissue-specific expression of the PDE genes.[1,6] Furthermore, cellular localization of diverse forms of PDE gene transcripts would help to understand roles of the different isozymes in the intracellular signal transduction pathways.[9,13,20~23,31,32] In nervous tissues, cellular and subcellular localizations of some PDEs suggest that the enzymes are closely implicated in neuronal functions such as synaptic transmission and sensory coding.[3,14,19,25] Some PDEs are elaborately regulated during the period of neuronal differentiation and synaptogenesis.[2,13,21,22] These cytological and developmental studies are additionally instructive and informative for further understanding cell- and developmental stage-specific roles of PDE isozymes.

PDE10A, or one of the two newest PDE genes, was recently cloned and characterized as cGMP-binding cAMP-PDE and cAMP-inhibited cGMP-PDE.[9,10,16,18,28] The cloning studies have reported that splice variants of the PDE10A transcripts are expressed in a tissue-specific manner, and also preliminarily the preferential localization of PDE10A mRNA expressing neurons are observed in the caudate-putamen and olfactory tubercle.[9] Since the other genes of the PDE families are also expressed in the same brain regions, the more data for comparison are inevitably necessary to determine cell-specific expression of distinct PDE isozymes and to understand the physiological reasons for the existence of multiple PDE isozymes in the brain. The present study has examined the cellular localization of PDE10A mRNA in adult rat brain using *in situ* hybridization, and shows the distinctive regional and cellular localizations of PDE10A mRNA in rat brain.

Materials and Methods

Animals used in the present study were Sprague-Dawley male rats at the age of postnatal 10 weeks (SPF/VAF Crj:CD[SD], 350±20g, Charles River Inc.). The rats were kept for more than 1 week in an artificial condition of 12-hour day/night cycle and 25℃. For *in vitro* RNA hybridization analysis, the animals were deeply anesthetized with pentobarbital sodium (Nembtal, 1 mL/kg body weight, i.p., Dinabott Inc.) before sacrificing. Brains were removed from skulls and dissected into 6 parts; olfactory bulb and tubercle, cerebral cortex, caudate-putamen, hippocampal formation, cerebellum, and brainstem. These brain regions and the other body tissues (lung, heart, liver, kidney and testis) were frozen in powdered dry ice, and kept at −80℃ until use.

For *in situ* hybridization, the anesthetized animals were transcardially perfused with 150 ml 0.9% saline, followed by 500 ml 4% paraformaldehyde in 0.1 M Sörensen's phosphate buffer (pH 7.2). Brains were removed from skulls, postfixed in the same fresh fixative for another 24 hours at 4℃,

and then rinsed in 20% sucrose in 0.1 M phosphate buffer (pH 7.2) for 24～48 hours at 4℃. The brains were frozen in powdered dry ice and cut at 15 μm in thickness in either a frontal or sagittal plane on a cryostat. Sections were thaw-mounted on gelatin-coated slides and stored at －80℃ until use. Microscopic analysis revealed that there were no appreciable differences in hybridization signals according to the duration of storage. The handling and treatment of the animals conformed to the guidelines of the Animal Study Committee of the Kagawa Prefectural College of Health Sciences.

Probe

The PDE10A probe was a 45-mer antisense oligonucleotide which corresponds to the sequence of the catalytic domain of rat PDE10A2 (GenBank: Acc. ID. AB027155, site 1969-2013) (Fig. 1). The specificity of the probe was analyzed with the aid of the computer-assisted homology search program (Blast Search [BLASTN]) at the internet site of the National Center for Biotechnology Information (NCBI). The PDE10A probe was designed to distinguish the target mRNA specifically among sequences of the PDE gene families, but the probe recognize all splice variants of the rat PDE10A gene (PDE10A2 ～ 6) (Fig. 1). Five splice variants of PDE10A transcripts in rat code the almost entire portion of the enzyme except for the N-terminal variations (Fig. 1). Another major form of splice variants in human (PDE10A1 splice variant) has not so far been identified in rat.

The PDE10A probe was labeled by incubating with terminal deoxynucleotidyl transferase (TdTase) and digoxigenin-linked dUTP/unlabeled dATP at 37℃ for 60 minutes (3'-end tailing kit, Roche Diagnostics Inc.). In the detection protocol of labeled probes were used the alkaline phosphatase-conjugated anti-digoxigenin antibody and nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP). The labeled probe showed the detection ability at as low as 10^{-17} mol/mL/dot on Nylon membrane (Immobilon-Ny, Millipore Inc.).

In vitro RNA Analysis

RNA extraction from tissues was carried out according to a protocol of Chomczynski and Sacchi. [5] In brief, tissues were homogenized in the buffer (pH 7.0) containing 4 M guanidinium thiocyanate. The homogenate was deproteinized with a mixture of acid phenol and chloroform.

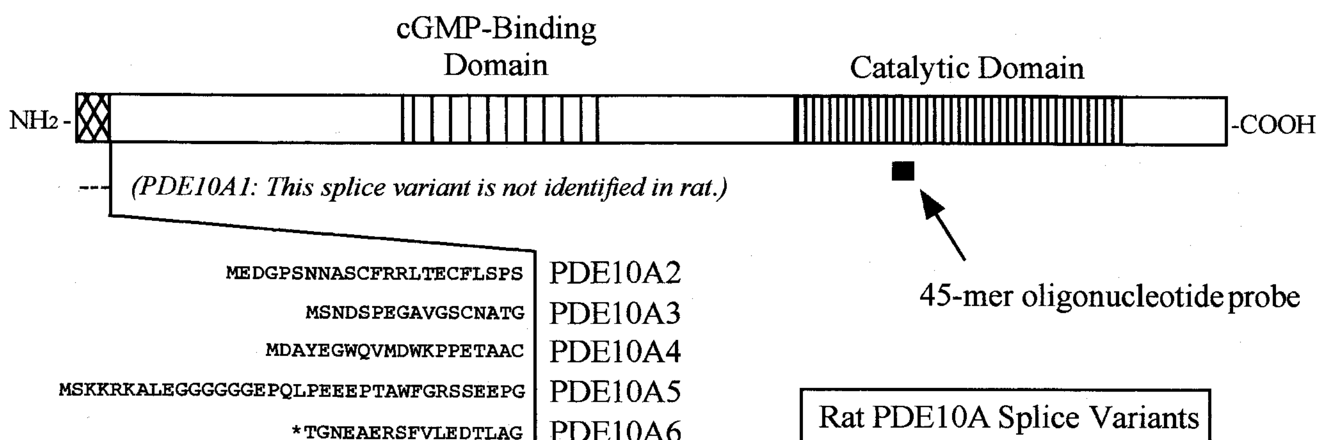

Fig. 1 Schematic representation of splice variants of rat PDE10A isozymes. The antisense PDE10A probe is a 45-mer oligonucleotide recognizing the mRNA sequences of the catalytic domain of the enzyme (solid bar). Since splice variant motifs are restricted to the N-terminal of the enzyme molecule, the antisense PDE10A probe recognizes all splice variants of the PDE10A gene.

Total RNA from the tissues was collected by ethanol precipitation. A portion of the total RNA was further purified to obtain poly(A)$^+$ RNA by passing through the oligo(dT)-cellulose column.

Both total RNA and poly(A)$^+$ RNA were used for the Northern blot analyses. Total RNA was prepared at 20 μg/lane and poly(A)$^+$ RNA at 6 μg/lane. Following electrophoresis in formalin-denatured agarose gel and capillary transfer to non-charged Nylon membrane, the membranes were baked at 80℃ and then exposed to 254 nm ultraviolet rays at 60×10^3 μJ/cm^2. Total and poly(A)$^+$ RNA samples were also used for dot blot analysis to survey the PDE10A mRNA expression in various tissues.

The membranes prepared were hybridized with the labeled PDE10A probe(1 pmol/mL) in hybridization solution(4 ×SSC[600 mM NaCl, 60 mM sodium citrate], 50% formamide, 0.12 M phosphate buffer[pH 7.2], 1% sarcosyl, 1× Denhardt's solution[0.02% bovine serum albumin F-V, 0.02% Ficoll, 0.02% polyvinylpyrrolidone K-90], 30 μg/mL carrier DNA, 200 μg/mL poly (A)RNA)for 18 ~ 21 hours at *T*m-20℃. Posthybridization wash was carried out in 1×SSC (150 mM NaCl and 15 mM sodium citrate)at *T*m-20℃ for 1 hour. Then the membranes were incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (dilution 1 : 1,000 ~ 1 : 4,000)for 1 ~ 2 hour at room temperature and the enzyme reaction was carried out with NBT/BCIP(1 : 50)at room temperature. For a chemiluminescence detection protocol, NBT/BCIP was substituted with CDP-*Star*(CDP-*Star* : chloro-substituted 1, 2-dioxetane chemiluminescent substrate, Roche Diagnostics Inc.). Following 5-minute luminescence reaction in CDP-*Star* solution, membranes were exposed to X-ray film like autoradiography.

The melting temperature(*T*m)value of the probe was calculated according to the following formulas :

(a) For Na$^+$concentrations above 0.4 M ;

$Tm = 81.5 + 0.41(\%\ GC) - 0.72(\%\ formamide)$

(b) For Na$^+$concentrations of 0.01 ~ 0.2 M ;

$Tm = 81.5 + 16.6(\log[Na^+]) + 0.41(\%\ GC) - 675/n$

(n= probe length)

In situ Hybridization

The procedures of *in situ* hybridization were essentially same to the protocol of the Northern blot analysis described above. In brief, prior to hybridization, brain sections were immersed in buffered 4% paraformaldehyde for 10 ~ 30 minutes, rinsed in 1×SSC for 15 minutes, and then dehydrated in ethanol. Hybridization were carried out with 1 pmol/mL labeled PDE10A probe in the hybridization buffer (see above) at *T*m -20℃ for 20 ~ 24 hours. Posthybridization stringency wash was carried out at either *T*m -5℃, *T*m-10℃, *T*m-15℃, or *T*m-20℃ for 1 hour. In the detection protocol for hybridization signals, brain sections were incubated with anti-digoxigenin antibody(1 : 1,000 ~ 2,000)at 25℃ for 4 hours to overnight, and then with NBT/BCIP(1 : 50)at 25℃ overnight.

Results

The expression of PDE10A mRNA was detected in different parts of rat brain by *in vitro* and *in situ* hybridizations. In total RNA analysis, the caudate-putamen(or striatum)alone showed signals in both the dot blot and Northern blot hybridizations(Fig. 2A ; Fig. 3A-c and B-c). Signals of the caudate-putamen in the Northern blot hybridization showed a single species of about 9,100-base long mRNA, based on 18s- and 28s-ribosomal RNA size-markers by gel electrophoresis(Fig. 3A-c and 3B-c).

Poly(A)$^+$ RNA selection proves to be effective for demonstrating the expression of a small amount of PDE10A mRNA in tissues(Fig. 2B). Testis was used as a positive control tissue, since it had been reported to be rich in PDE10A mRNA. It is worthy of note that the signal of poly(A)$^+$ RNA(1.16 μg)in the cerebral cortex was stronger than that(2.0 μg)in testis(Fig. 2B). The intensity of hybridization signals of poly(A)$^+$ RNA samples were increased in both the dot blot and Northern blot analyses, and different parts of rat brain exhibited positive signals (Fig. 2C ; Fig. 3C). In poly(A)$^+$ RNA analysis, hybridization signals revealed a single species of about 9,100-

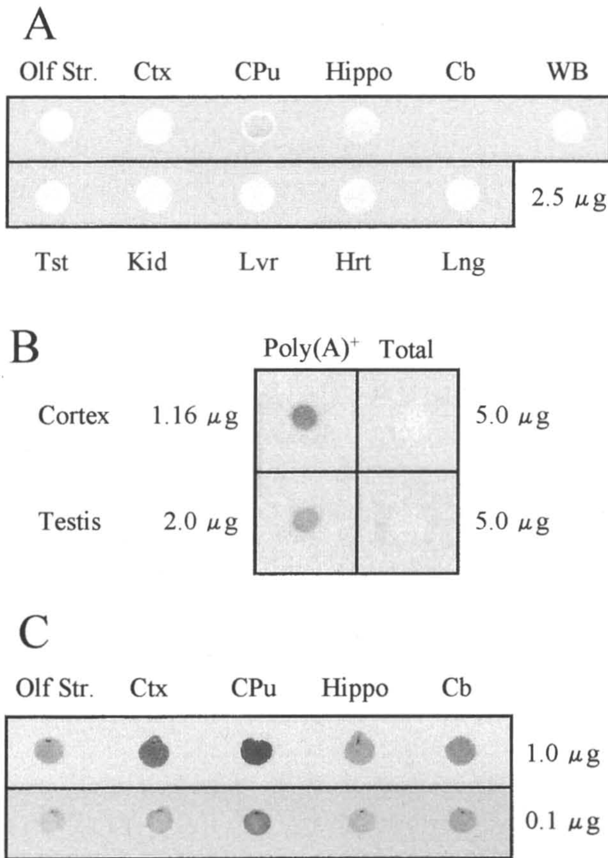

Fig. 2　Dot blot hybridization signals of PDE10A mRNA using total and poly(A)+ RNA samples. A：Total RNA analysis (2.5 μg/mL/dot) from different tissues. The caudate-putamen alone exhibited hybridization signal. The dot of the cerebellum is missing because of mis-spotting. B：Comparison of the detection efficiency between total and poly (A)+ RNA. Poly(A)+ RNA selection improved hybridization signals. C：Dot blot hybridization of poly(A)+ RNA from different brain regions. All regions examined showed hybridization signals (1.0 μg/mL/dot and 0.1 μg/mL/dot). Cb：cerebellum, CPu：caudate-putamen, Ctx：cerebral cortex, Hippo：hippocampus, Hrt：heart, Kid：kidney, Lng：lung, Lvr：liver, Olf Str.：olfactory structures, Tst：testis, WB：whole brain.

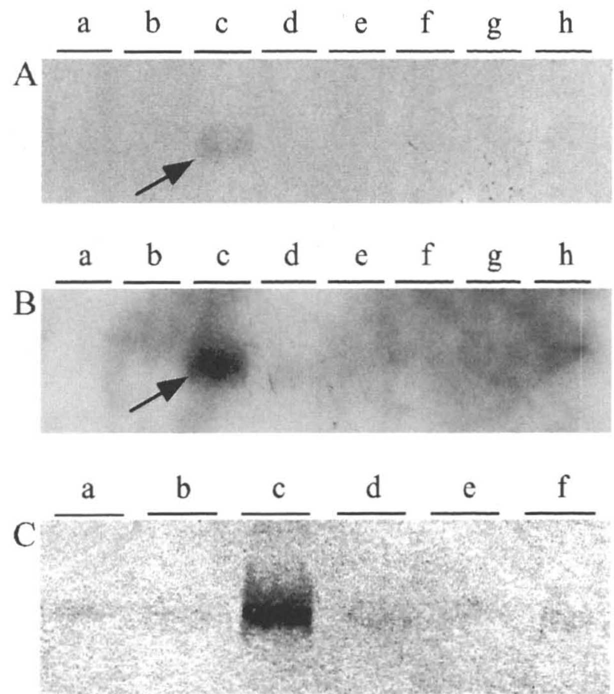

Fig. 3　Northern blot hybridization signals of total and poly(A)+ RNA samples from different brain regions. A and B：Hybridization signal (20 μg/lane total RNA, ca. 9, 100-base mRNA) processed with different detection protocols; NBT/BCIP (A) and CDP-Star (B) for alkaline phosphatase. a：central olfactory structures, b：cerebral cortex. c：caudate-putamen (or striatum), d：hippocampus, e：cerebellum, f：upper brain stem, g：lower brain stem, h：whole brain. In both cases the caudate-putamen alone exhibited hybridization signals of PDE10A mRNA. C：Hybridization signals (ca. 6.5 μg/lane poly(A)+ RNA, ca. 9, 100-base mRNA) processed for NBT/BCIP were detected in various brain regions with a markedly strong signal in the caudate-putamen. a：central olfactory structures, b：cerebral cortex. c：caudate-putamen (or striatum), d：hippocampus, e：cerebellum, f：whole brain.

base PDE10A mRNA (Fig. 3C), the molecular size of which was the same to that detected in total RNA analysis.

In situ Hybridization

On the basis of cell morphology and location in brain, cells expressing PDE10A mRNA were distinguished as neurons (Fig. 4). A positive neuron in the cerebral cortex showed a typical feature of neuron with a round perikarya stained purple-brown, a large pale nucleus, and a clear nucleolus (Fig. 4).

PDE10A neurons were primarily observed in forebrain structures and in the cerebellum. PDE10A neurons were numerous in the layer VI

of the cerebral cortex and the caudate-putamen (or striatum) (Fig. 5A and 5B). Many or a moderate number of positive neurons were found in various brain regions : the external plexiform layer and the internal granule cell layer of the olfactory bulb, anterior olfactory nucleus, olfactory tubercle, piriform cortex, layer II and III of the cerebral cortex, dentate gyrus (Fig. 5C) and fields CA1 to CA3 of Ammon's horn of the hippocampus, amygdaloid nuclear complex, the granule cell layer of the cerebellum (Fig. 5 D) and the deep cerebellar nuclei. Solitary or scattered PDE10A neurons were also seen in the periglomerular region of the olfactory bulb, accessory olfactory nucleus, tenia tecta, layer IV of the cerebral cortex, accumbens nucleus, globus pallidus, septum, thalamic nuclei, hypothalamus, habenular nucleus (medial and lateral),

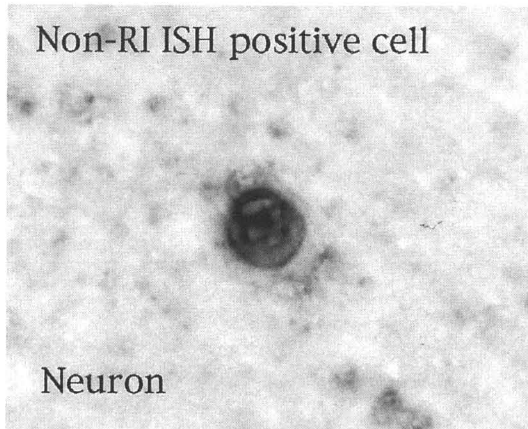

Fig. 4 A positive neuron showing *in situ* hybridization signal of PDE10A mRNA.

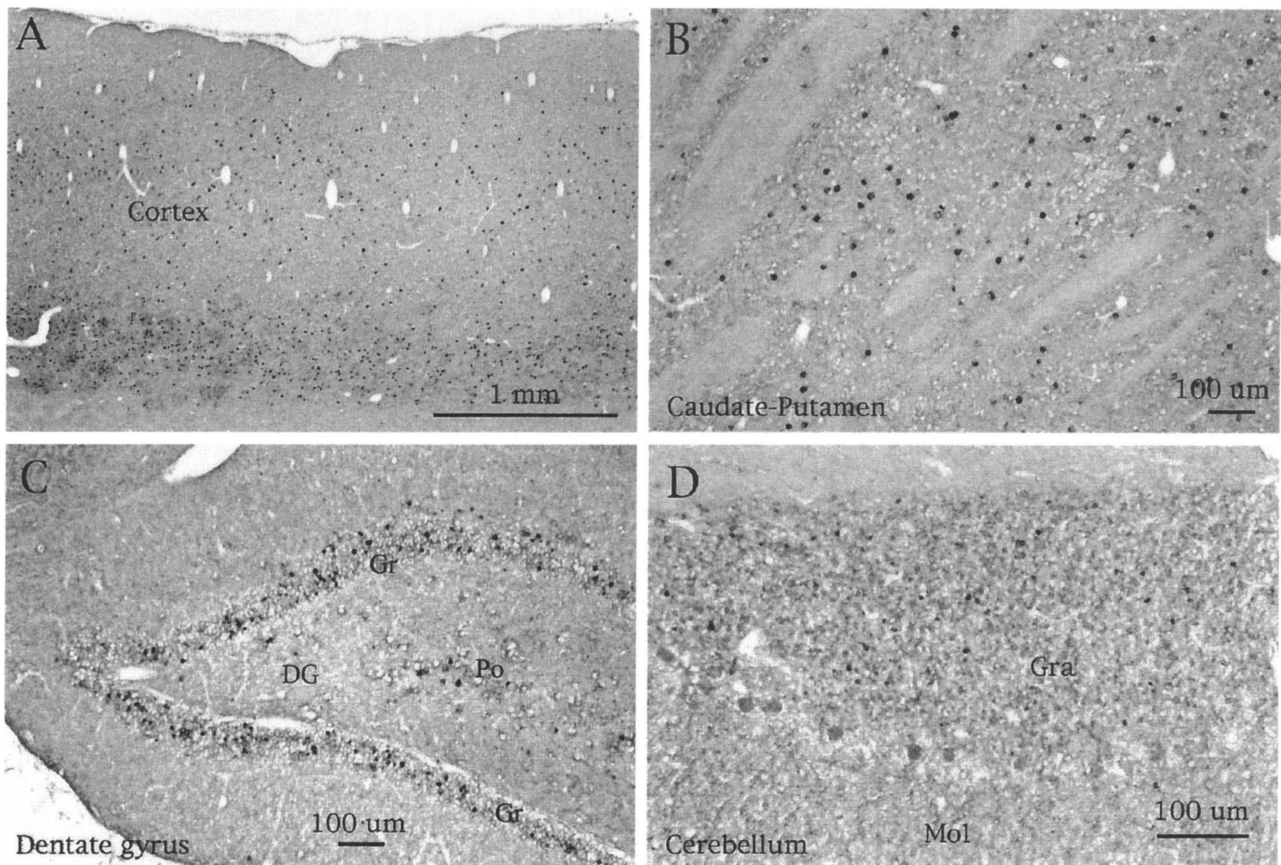

Fig. 5 *In situ* hybridization signals of neurons in various regions of rat brain (sagittal plane). A : Cerebral cortex. A number of positive neurons were observed in the layer VI, followed by the layer of II and III with the reduced number. B : Caudate-putamen (or striatum). Medium-sized positive neurons were distributed in the entire nucleus. C : Dentate gyrus (DG). Some neurons in the granular and polymorph layers (Gr and Po) of DG were positive. D : Cerebellar cortex. Many granule cells in the granule cell layer (Gra) of the cerebellum showed positive signals. Mol : molecular layer of the cerebellum.

Table 1 Location and distribution of PDE10A neurons in rat brain.

Brain region		Subdivision	Location	Number/unit area	Cell type	Distribution
Telencephalon	Olfactory structures	Olfactory bulb	Glomerular layer	a few	periglomerular cell	scattered
			External plexiform layer	moderate number	granule cell	scattered
			Internal granule cell layer	moderate number	granule cell	scattered
		Accessory olfactory bulb		a few	medium-sized	scattered
		Anerior olfactory nucleus		moderate number	medium-sized	scattered
		Olfactory tubercle	Pyramidal cell layer	moderate number	medium-sized	scattered
		Tenia tecta		a few	medium-sized	scattered
		Piriform cortex		moderate number	medium-sized	scattered
	Cerebral cortex	Layer II		many	medium-sized	
		Layer III		many	medium-sized	
		Layer IV		a few	medium-sized	scattered
		Layer VI		numerous	medium-sized	
	Hippocampal region	CA 1, 2, 3 & 4 of Ammon's horn	Pyramidal cell layer	moderate number	poyramidal neurons	scattered
		Dentate gyrus	Granule cell layer	moderate number	granule cells	scattered
	Basal ganglia and related structures	Caudate-putamen (Striatum)		numerous	medium-sized	
		Globus pallidus		very few	medium-sized, large	scattered
		Accumbens nucleus		a few	medium-sized	scattered
		Amygdaloid nuclear complex		moderate number	medium-sized	scattered
Diencephalon	Epithalamus	Epithalamus	Habenular nuclei (medial, lateral)	a few	medium-sized	gathered
	Thalamus	Lateral nuclear group	Lateroposterior nucleus (LP)	a few	medium-sized	scattered
			Laterodorsal nucleus (LD)	a few	medium-sized	scattered
		Ventral nuclear complex	ventrolateral nucleus (VL)	a few	medium-sized	scattered
			Ventroposterior medial nucleus (VPM)	a few	medium-sized	scattered
			Ventroposterior lateral nucleus (VPL)	a few	medium-sized	scattered
		Ventral posterior nuclear group	Medial geniculate body (MGB)	a few	medium-sized	scattered
			Lateral geniculate body (LGB)	a few	medium-sized	scattered
	Hypothalamus	Hypothalamus	Hypothalamic nuclei	a few	medium-sized	scattered
Mesencephalon		Sperior colliculus	Sperior colliculus	a few	medium-sized	scattered
		Inferior colliculus	Inferior colliculus	a few	medium-sized	scattered
Cerebellum		Cerebellar cortex	Molecular layer	a few	small	scattered
			Purkinje cell layer	very few	large but not Purkinje cell	scattered
			Granule cell layer	many	granule cells	
		Deep cerebellar nuclei	Deep cerebellar nuclei	many	large neurons	
Lower brain stem	Reticular formation			a few	medium-sized, large	scattered

superior and inferior colliculi, the molecular layer and the Purkinje cell layer of the cerebellum (but no Purkinje cell was positive), and the reticular formation of the brain stem (Table 1).

PDE10A neurons varied in size in different brain regions from medium-sized to small granule cells (Fig. 5B vs 5D). The morphologically same types of neurons were not always positive within single nuclei or regions (Fig. 5A-D).

Discussion

The present study reports that neurons expressing PDE10A mRNA are distinctively localized in various regions of rat brain, and that the molecular sizes of the gene transcripts are about 9,100 bases in length. Splice variants of rat PDE10A transcripts have been reported to comprise two major forms (PDE10A2 and PDE10A3) and three minor forms (PDE10A4~6) with N-terminal variants.[9] The PDE10A probe used, however, recognizes all splice variants of the transcripts, because the probe is generated against a nucleotide sequence common to all splice variants of rat PDE10A transcripts, or the catalytic domain of the enzymes (Fig. 1). Therefore, it is probable that hybridization signals in the present study reveal the total expression of PDE10A transcripts.

In vitro hybridization revealed the most abundant expression of PDE10A transcripts in the caudate-putamen, or striatum. The high level of mRNA expression of PDE10A in this region have been recently reported in rat[9] and human.[10] The strong Northern blot hybridization signals were consistent with a number of PDE10A neurons in the nucleus, as also shown in a previous report.[9] The other 5 brain regions (olfactory bulb and tubercle, cerebral cortex, hippocampus, cerebellum, and brainstem) exhibited feeble signals of PDE10A transcripts, when poly(A)$^+$ RNA was analyzed. However, some brain regions, such as the cerebral cortex, hippocampus and cerebellum, contain many PDE10A neurons despite the low intensity of *in vitro* hybridization signals. This disparity may be ascribed to the population density of PDE10A neurons in single nuclei or regions; for instance, PDE10A transcripts in the cerebral cortex may be diluted with those derived from a large number of different types of cortical neurons. The scattered distribution of PDE10A neurons in rat brain may support the low intensity of Northern blot hybridization signals in the 5 brain regions except the caudate-putamen. Fujishige et al.[9] have reported PDE10A mRNA expression restricted in the caudate-putamen in rat, whereas they have also reported PDE10A mRNA expression in various regions of human brain[10], as demonstrated in rat brain in the present study. The latter two studies reveal the wide-spread but uneven expression of PDE10A transcripts in human and rat brains.

The molecular sizes of PDE10A transcripts in the present study were about 9,100 bases in length, regardless of different sources of brain regions and possible contributions of different splice variants of the transcripts. This size of the transcripts coincides with that of one of the two forms of PDE10A transcripts (9,000 ~ 9,500 bases) in mouse,[28] rat[9] and human,[10, 18] but the present study has failed to detect another truncated form of 3,500 ~ 4,0000-base mRNA reported in brain, thyroid gland and testis.[9, 10, 28] Interestingly, both the truncated and long forms of PDE10A mRNA are detected in some tissues (for instance, testis and brain of human[10] and mouse[28]), but only the truncated form is detected in rat testis.[9] Unlike these reports, only the long form of PDE10A transcripts is detected in brain and other tissues of human[18] and in rat brain in the present study. There are some inconsistent results hard to interpret between different investigations, even if by the same investigators. It is also unknown and remains to be clarified why the two forms of the PDE10A transcripts are expressed in several tissues described above.[10, 28]

In addition to the size-differences of the transcripts, there have been reported splice variants of PDE10A family. In human, two splice variants (PDE10A1[10, 18] and PDE10A2[16, 18]) have been isolated and PDE10A2 variant appears to be a major

species in some human tissues.[16] The unique N-terminus of the PDE10A2 isozyme provides a putative phosphorylation site by cAMP-dependent protein kinase (PKA), but the PDE10A1 isozyme is not phosphorylated by PKA because of lacking the phosphorylation site of PKA. Human PDE10A1 isozyme contains putative phosphorylation sites by protein kinase C(PKC).[10] Similarly, in rat, five splice variants of PDE10A transcripts (PDE10A2～6) have been isolated with N-terminal variations.[9] Different N-terminal sequences of rat PDE10A splice variants may be cell type-specific in tissues and may be regulated by different types of kinases and associated proteins. Rat PDE10A isozymes appear to be phosphorylated by PKA.[9] The rat variant corresponding to the human PDE10A1 transcript has not so far been isolated. These studies provide some evidences that different PDE10A isozymes are regulated by different ways of activation and deactivation in the intracellular signaling cascades, although the detailed properties of individual isozymes need to be clarified.

The cellular localizations of PDE10A transcripts showed the regional overlapping with neurons expressing PDE1 (PDE1A, PDE1B and PDE1C)[20, 31, 32] and PDE2 transcripts.[23] In a broad way, PDE1A2 neurons in mouse brain were localized mainly in the cerebral cortex, pyramidal cells of the hippocampus, medial habenula and amygdaloid nuclear complex.[31] PDE1B1 neurons are observed in *the deeper layers of the cerebral cortex*, *caudate-putamen*, *nucleus accumbens*, *olfactory tubercle*, and *dentate gyrus* of the hippocampus, and with decreased signals in the olfactory bulb and the piriform cortex, although sparsely distributed PDE1B1 neurons were found throughout brain.[20, 30] Splice variants of PDE1C1～5 transcripts are expressed in *the granule cell layer of the cerebellum*, *some Purkinje cells*, *central amygdaloid nucleus*, *interpolar spinal trigeminal nucleus*, and *the glomerular* and *external plexiform layers of the olfactory bulb*, as well as *in parts of the caudate-putamen* and olfactory tubercle.[32] Moreover, PDE2 transcripts in rat brain are detected intensely in *the medial habenula*, *CA1 ～ CA3 fields of the hippocampus*, and *dentate gyrus*. Hybridization signals are also observed in olfactory and entorhinal cotices, subiculum, and amygdaloid nuclear complex, and diffusely in the basal ganglia, cerebellum and hypothalamus.[23] Thus, the regions described above for PDE1 and PDE2 neurons are mostly same to those of PDE10A neurons in rat brain. The regional overlapping, however, does not mean the co-localization of different PDE isozymes in single cells.

In addition to the distribution of these PDE neurons, of much interest are the biochemical properties of each PDE isozyme. Isozymes of PDE1, PDE2 and PDE10 catalyze the hydrolysis of both cAMP and cGMP as substrates. Of them, PDE1A, PDE1B and PDE1C isozymes are activated by calmodulin and Ca^{2+}, and PDE1A isozymes are inactivated by phosphorylation by PKA while PDE1B isozyme by Ca^{2+}/calmodulin-dependent protein kinase (Ca^{2+}/CaMK II). The comparable enzymatic properties of PDE10A isozymes have been described above. The cAMP and cGMP hydrolytic activity by PDE2A isozymes are stimulated by allosteric binding of cGMP under normal substrate condition. In addition, PDE3A and PDE3B isozymes have a relatively high affinity for cAMP as substrate, and also have a high affinity for cGMP but the *V*max (maximum velocity of enzyme reaction) is rather low (i.e., cGMP-inhibited PDE). Cellular localizations of PDE3A and PDE3B neurons show the distinctive patterns through the entire brains, developing and mature.[21, 22] Therefore, PDE3 isozymes are also implicated in the metabolism of cyclic nucleotide second messengers in certain neurons. Furthermore, In vitro studies of the other PDE families (PDE5[15] PDE7[12, 13, 24] PDE8[26] and PDE9[14, 27]) imply that they also participate in the intracellular signaling cascades in brain tissues. Isozymes of the latter 5 PDE families exhibit the distinctive biochemical properties, too.

Although PDE10A as well as PDE1B and PDE2A neurons are not exclusively localized within the caudate-putamen, a large amount of hybridization signals of PDE transcripts and a number of PDE neurons in the nucleus appear to make it significant to correlate striatal functions

with different PDE isozymes. In addition, neurons containing dopamine receptors (D1R,[8, 29, 30] D2R[30] and D3R[4]) overlap the regions of many PDE neurons in rat brain. The caudate-putamen is the major component of the basal ganglia, where the content of dopamine affects behavioral and motor functions. Dopamine action is mediated by D1R and D2R which are expressed in distinct populations of striatal neurons[17]; D1R stimulates the adenylyl cyclases and increases intracellular cAMP, while D2R inhibits the enzyme activity. Dopamine differentially regulates the two striatal output pathways including striatonigral (D1R) and striatopallidal (D2R) neurons in rat.[11] D1R striatal neurons express preprotachykinin-A gene transcripts (phenotype : substance-P and substance-K, and also preprodynorphin gene products) while D2R striatal neurons pre-proenkephalin gene transcripts (phenotype : Met-enkephalin). In the caudate-putamen, different types of PDE isozymes with distinctive biochemical properties are localized in medium-sized striatal neurons. Those PDE neurons may be assigned to either D1R or D2R neurons, but D3R neurons are also present in the caudate-putamen. It is not so easy to figure out the whole network of the intracellular signaling cascades in striatal neurons, triggered even by dopamine, followed by the increase of cAMP and/or cGMP. Therefore, it will be important to determine phenotypes of interest in striatal neurons, and to correlate them with the associated signaling molecules and with known phenotypes of striatal neurons, as well as in other brain neurons.

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