Elsevier Editorial System(tm) for Journal of Chemical Neuroanatomy
Manuscript Draft

Manuscript Number: CHENEU-D-09-00072R1

Title: The human area postrema and other nuclei related with the emetic reflex express cAMP phosphodiesterases 4B and 4D

Article Type: Research Article

Keywords: PDE4B; PDE4D; solitary tract nucleus; dorsal vagal motor nucleus; in situ hybridization histochemistry.

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Abstract: Phosphodiesterase 4 (PDE4) inhibitors, i.e. rolipram, are being extensively investigated as therapeutic agents in several diseases. Emesis is one of the most common side effects of PDE4 inhibitors. Given the fact that the area postrema is considered the chemoreceptor trigger zone for vomiting, the present study investigates the regional distribution and cellular localization of the four gene transcripts of the PDE4 subfamily (PDE4A, PDE4B, PDE4C and PDE4D) in human brainstem. In situ hybridization histochemistry was used to locate the mRNA distribution of the four PDE4 subfamilies in the area postrema and related nuclei of human postmortem brainstem. We have found that in the brainstem PDE4B and PDE4D mRNA expression is abundant, and distributed not only in neuronal cells, but also in glial cells, and on blood vessels. The hybridization signals for PDE4B and PDE4D mRNAs in the area postrema were stronger than those in any other nuclei in the brainstem. They were also found in vomiting-related nuclei such as the nucleus of the solitary tract and the dorsal vagal motor nucleus. These findings suggest that cAMP signaling modification in the area postrema could mediate the emetic effects of PDE4 inhibitors in human brainstem.

Corrected version for *J Chem Neuroanat*

March 2010

The human area postrema and other nuclei related to the emetic reflex express cAMP phosphodiesterases 4B and 4D

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ABSTRACT

Phosphodiesterase 4 (PDE4) inhibitors, i.e. rolipram, are being extensively investigated as therapeutic agents in several diseases. Emesis is one of the most common side effects of PDE4 inhibitors. Given the fact that the area postrema is considered the chemoreceptor trigger zone for vomiting, the present study investigates the regional distribution and cellular localization of the four gene transcripts of the PDE4 subfamily (PDE4A, PDE4B, PDE4C and PDE4D) in human brainstem. *In situ* hybridization histochemistry was used to locate the mRNA distribution of the four PDE4 subfamilies in the area postrema and related nuclei of human postmortem brainstem. We have found that in the brainstem PDE4B and PDE4D mRNA expression is abundant and distributed not only in neuronal cells, but also in glial cells, and on blood vessels. The hybridization signals for PDE4B and PDE4D mRNAs in the area postrema were stronger than those in any other nuclei in the brainstem. They were also found in vomiting-related nuclei such as the nucleus of the solitary tract and the dorsal vagal motor nucleus. These findings suggest that cAMP signaling modification in the area postrema could mediate the emetic effects of PDE4 inhibitors in human brainstem.

Keywords. PDE4B; PDE4D; nucleus of the solitary tract; dorsal vagal motor nucleus; in situ hybridization histochemistry.

1. Introduction

cAMP plays a significant role as second messenger in signal-transduction pathways controlling multiple cellular processes in the brain. Cellular cAMP levels are regulated by the activities of two types of enzymes, adenylyl cyclase and cyclic nucleotide phosphodiesterase (PDE) (Bender and Beavo, 2006). There are eleven families of PDE that hydrolyze the cyclic nucleotides, either cAMP or cGMP, or both (Houslay and Milligan, 1997; Conti and Jin, 1999; Soderling and Beavo, 2000; Beavo and Brunton, 2002). The PDE4 family is composed of four subfamilies (PDE4A, PDE4B, PDE4C and PDE4D) encoded by different gene loci, and each of them has been shown to produce several (around 20) mRNAs by alternative splicing (Houslay et al., 1998; Houslay, 2001). They are characterized by a low K_m, Ca^{2+}-insensitivity, specificity for cAMP as a substrate and sensitivity to the specific inhibitor rolipram (Bolger, 1994; Beavo et al., 1994; Beavo, 1995). These subfamilies and their splice variants are widely expressed in many tissues including the brain (Bolger et al., 1994; Engels et al., 1995; Cherry and Davis, 1995; Iwahashi et al., 1996; Takahashi et al., 1999; 1999; Pérez-Torres et al., 2000; Lamontagne et al., 2001; McPhee et al., 2001; Miró et al., 2002; Cherry and Pho, 2002; D'Sa et al., 2005; Reyes-Irisarri et al., 2008), with the exception of PDE4C which is not expressed in rodent brain (Bolger et al., 1994; Engels et al., 1995; Suda et al., 1998; Pérez-Torres et al., 2000).

Rolipram is a prototypic PDE4 inhibitor (Wachtel, 1982) that binds to two sites, one is the low-affinity rolipram binding site (LARBS) and the other is the high-affinity rolipram binding site (HARBS) (Schneider et al., 1986; Jacobitz et al., 1996); both

sites are described as two distinct binding affinity states rather than separate sites (Souness and Rao, 1997). Some inhibitors bind with high affinity to both HARBS and LARBS (e.g., piclamilast). It has been shown that in truncated PDE4A mutants the inhibitor binding to both the HARBS and the LARBS is to the catalytic site (Jacobitz et al., 1996). Binding to the HARBS depends on the presence of the N-terminal region of the protein whereas it is not needed for the LARBS. It has been suggested that the HARBS and the LARBS mediate different effects of PDE4 inhibitors (Harris et al., 1989; Barnette et al., 1995; Duplantier et al., 1996; 1996; Souness et al., 1996). Ever since rolipram was developed clinically as anti-depressant (Wachtel, 1983; Wachtel and Schneider, 1986; Scott et al., 1991) and studied for Parkinson's disease reaching Phase II trials (Parkes et al., 1984), the side effects of nausea, vomiting, diarrhoea and headaches, have remain a problem, limiting clinical development (Hebenstreit et al., 1989).

PDE4 inhibitors have been extensively investigated as therapeutic agents in a variety of diseases such as asthma, chronic obstructive pulmonary disease, depression, schizophrenia and as cognitive enhancers (Torphy and Undem, 1991; Giembycz, 2000; O'Donnell and Zhang, 2004; Ghavami et al., 2006). The mechanism by which PDE4 inhibition results in increased vomiting and nausea in species such as dog and human (Bertolino et al., 1988; Hebenstreit et al., 1989; Heaslip and Evans, 1995) is not fully understood, but probably includes both central and peripheral sites of actions. Emesis induced by PDE4 inhibitors in peripheral sites was initially described with rolipram (Puurunen et al., 1978). This effect is presumably related to the ability of PDE4 inhibitors to increase parietal cell

cAMP content, enhancing acid gastric secretion, an effect which correlates with the affinity for HARBS on PDE4 (Barnette et al., 1998). Other studies on the potentiation of apomorphine-induced emesis in dogs by RO20-1724 (Carpenter et al., 1988) suggest that nausea and vomiting are likely to be produced, at least in part, via the direct stimulation emetic centers in the brain. Consistent with these studies the administration of rolipram or quinoline compound PMPQ in rats, elevated the Fos-like immunoreactivity in brain regions potentially relevant to the emetic effects of PDE4 inhibition (Bureau et al., 2006). The significance of emetic central sites has been additionally validated experimentally by the use of YM976, a PDE4 inhibitor with low emetogenic potential likely due to its poor brain penetration and low affinity for HARBS (Aoki et al., 2001). Some second generation rolipram-like compounds, such as cilomilast and roflumilast, that target in contrast the LARBS, are clinically advanced. Cilomilast has completed Phase III chronic obstructive pulmonary disease (COPD) clinical trials and Roflumilast is under development for asthma and COPD (Lipworth, 2005). The therapeutic window for anti-inflammatory action and their known side effects is probably not wide enough for cilomilast and may limit the use of roflumilast in asthma. Other “new” PDE4 inhibitors under development which appear to have an improved therapeutic window and to lack significant emetic action are tetomilast, oglemilast, apremilast, ONO 6126, IPL-455903 and IPL-512602 (Giembycz, 2008; Spina, 2008), though the molecular basis have not been disclosed.

The area postrema (AP), considered the chemoreceptor trigger zone for vomiting (Borinson and Wang, 1953; Carpenter et al., 1988) is located on the dorsal surface

of the medulla oblongata, at the caudal end of the fourth ventricle. It is a circumventricular organ serving as an interface between the brain parenchyma and the cerebrospinal fluid (CSF)-containing ventricles. The AP lacks a specific blood-brain diffusion barrier to large polar molecules and is thus anatomically positioned to detect emetic toxins in the blood as well as in the CSF. The AP along with the nucleus of the solitary tract (STN) and the dorsal vagal motor nucleus (DVMN) make up the so-called dorsal vagal complex, which is the major termination site of vagal afferent nerve fibers. Lesions of the AP prevent vomiting in response to most, but not all, emetogenic drugs.

The presence of mRNAs coding for PDE4A, PDE4B and PDE4D in the area postrema has been shown in rats (Takahashi et al., 1999; Pérez-Torres et al., 2000) and has suggested that cAMP signaling modification in this area could mediate the emetic effects of PDE4 inhibitors. As rodents do not possess an emetic reflex it is important to know the distribution of PDE4 subfamily members in human brainstem. Here, we describe experiments aimed at the analysis of both regional and cellular expression of the mRNA coding for PDE4A, PDE4B, PDE4C and PDE4D in nuclei related to the emetic reflex in human brainstem.

Material and Methods

Specimens

The human brain samples were provided by the Department of Human Anatomy and Physiology of the University of Padova. Approval of the local Ethics

Committies of the University of Padova was obtained. This study was performed on brainstems sampled during autopsy from 8 control adult subjects (6 males, 2 females; age range: 31-71 years; mean age: 53 years; mean postmortem delay 37h). Autopsies were performed within 48 h of death. Brainstems were cut after fixation in 10% formalin for 7 days and paraffin-embedded. Preliminary histological examination was performed on 5 µm thick transverse sections of the medulla oblongata, stained with haematoxylin-eosin, cresyl violet, Klüver-Barrera and luxol fast blue. In all cases, examination revealed the absence of acute, chronic, localized or diffuse brain pathology. In each case, 10 tissue sections, 14 µm thick, were cut on a microtome at the level of the area postrema and then mounted onto Histogrip-coated slides (Zymed, Carlsbad,CA, USA).

In situ hybridization histochemistry procedure

Oligonucleotide probes for PDE4A, PDE4B, PDE4C and PDE4D complementary to regions of each mRNA that share little similarity among the phosphodiesterase family have been described and used previously (Pérez-Torres et al., 2000). The oligonucleotides used were complementary to the following bases of the human cDNAs: 2323–2374 of PDE4A (GenBank acc. no. L20965); 2410-2455 of PDE4B (GenBank acc. no. L20971), 2333-2378 PDE4C (GenBank acc. no. U66347) and 1916-1960 of PDE4D (GenBank acc. no. U50159). They were custom-synthesized by Amersham Pharmacia Biotech (Little Chalfont, UK).

The oligonucleotides were 3'-end-labeled with terminal deoxynucleotidyl-transferase and [^{33}P]α-dATP (3000 Ci/mmol, New England Nuclear, Boston, USA). Labeled probes were purified by QIAquick Nucleotide Removal Kit (QIAGEN, Hilden, Germany).

The protocol for *in situ* hybridization histochemistry was based on previously described procedures with minor modifications (Tomiyama et al., 1997). Tissue sections were deparaffinized and fixed for 20 min at 4ºC in 4% paraformaldehyde in phosphate-buffered saline (1x PBS: 8 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 136 mM NaCl, 2.6 mM KCl), washed for 5 min in 3x PBS at room temperature, twice for 5 min each in 1x PBS, and incubated for 2 min at 21ºC in a solution of proteinase K (Calbiochem, San Diego, CA, USA) at a final concentration of 100 µg/ml in 50 mM Tris-HCl pH 7.5, 5 mM EDTA, washed twice in 1 X PBS, 5 min each and dehydrated through a graded series of ethanol and air dried.

For hybridization, labeled oligonucleotides were diluted to a final concentration of approximately $2x10^7$ cpm/mL in a solution containing 50% formamide, 4x SSC, 1x Denhardt's solution, 1% sarkosyl, 10% dextran sulfate, 20 mM phosphate buffer pH 7.0, 250 µg/mL yeast tRNA, 500 µg/mL salmon sperm DNA. Tissues were covered with 100 µL of hybridization solution, overlaid with Nescofilm (Bando Chemical Ind, Kobe, Japan) coverslips, and incubated overnight in humid boxes at 42ºC. Sections were then washed 4 times (45 min each) in 600 mM NaCl, 20 mM Tris-HCl pH 7.5, 1 mM EDTA at 60ºC. The sections were then briefly dipped in 70% and 100% ethanol, air-dried and dipped into Ilford K5 nuclear emulsion (Ilford, Mobberly, Chesire, UK) diluted 1:1 with distilled water. They were

exposed in the dark at 4°C for 6 weeks, and finally developed in Kodak D19 (Kodak, Rochester, NY, USA) for 5 min, and fixed in Ilford Hypam fixer (Ilford).

Several routine controls were carried out to determine the specificity of the hybridization signals, as we have previously described (Pérez-Torres et al., 2000). For each of the mRNA under study, several oligonucleotide probes complementary to different regions of the same mRNA were used independently as hybridization probes in consecutive sections showed identical patterns of hybridization. For a given oligonucleotide probe, addition in the hybridization solution of an excess of the same unlabeled oligonucleotide resulted in the complete abolition of the specific hybridization signal. The remaining autoradiographic signal was considered background. If the unlabeled oligonucleotide included in the hybridization was a different oligonucleotide, then the hybridization signal was not affected. The thermal stability of the hybrids was examined by washing at increasing temperatures: a sharp decrease in the hybridization signal was observed at a temperature consistent with the T_m of the hybrids (data not shown).

Microscopy and image processing

Tissue sections were examined in bright- and dark-field in a Wild 420 macroscope (Leica, Heerbrugg, Germany) and in a Nikon Eclipse E1000 microscope (Nikon, Tokyo, Japan) equipped with bright- and dark-field condensers for transmitted light and with epi-illumination. Microphotography was performed digitally using a DXM 1200 Digital still camera (Nikon, Tokyo, Japan) and

analySIS® 3.1 software (Soft Imaging System GmbH, Münster, Germany). Images were captured separately as TIFF files. All images were slightly enhanced in Photoshop 7.0 (Adobe Software, Mountain View, CA, USA) with the levels and bright/contrast tools. All images were treated equally to make backgrounds appear similar.

The area postrema and the vomiting-related nuclei localization were verified by the examination of cresyl violet-stained sections. PDE4A, PDE4B and PDE4D mRNA-containing cells were considered positive when accumulations of silver grains over the stained cellular profiles were at least three-fold higher than the average background for each one of the radioactively labeled oligonucleotides used. *In situ* hybridization histochemistry provides reliable information concerning the relative abundance of a given mRNA in different regions. However, caution must be taken in comparing the relative hybridization signals produced by different probes that detect different mRNA species. In addition to the actual abundance of the different mRNAs, the intensity of the hybridization signals observed can be affected by other factors, such as differences in the hybridization efficiency of the various probes or differences in the specific activities of the probes. Therefore, the results presented here have to be interpreted with this caveat in mind when comparing the abundance of the mRNAs coding for the different PDE4 enzymes.

Results

Distribution of mRNAs coding for PDE4 subfamilies

Table 1 summarizes the results obtained. In the area postrema (AP), very low hybridization signal for PDE4A, low for PDE4B, and strong for PDE4D were seen (Fig. 1). In the STN and the DMVN, no hybridization signal for PDE4A could be observed, whereas it was low for PDE4B and moderate for PDE4D (Figs. 1, 2). Hybridization levels for PDE4B and PDE4D were stronger in the AP than in the STN or DMVN. PDE4C mRNA expression was totally absent in the tissue sections analyzed in this study, which is consistent with our previous work in human and rat brain (Pérez-Torres et al., 2000) (not shown). We could not observe effects of age, gender and postmortem delay in the mRNAs analyzed as we have previously described (Pérez-Torres et al., 2000; 2003).

The distribution in other nuclei of the medulla oblongata was similar to the results that we have previously reported. In brief, PDE4B and PDE4D presented a diffuse hybridization signal in the reticular (ventral, medial and dorsal) nuclei. Somatosensory nuclei, such as cuneate, gracile and spinal trigeminal showed low to moderate hybridization signals for PDE4B and PDE4D. The hypoglossal nucleus weakly expressed PDE4B and PDE4D mRNA. In addition, low levels of PDE4B mRNA was observed in the inferior olivary complex, in both the principal and medial nuclei. Moderate hybridization signal for PDE4D was seen in the principal nucleus and low levels in the medial nucleus. Both PDE4B and PDE4D mRNAs were detected in the different nuclei of the solitary tract. Low to moderate levels

were visualized in both paracommissural and commissural solitary nuclei and lower levels in the intermediate solitary nucleus. PDE4B and PDE4D were found expressed at low levels in the cells of the locus coeruleus in the pons. Very low hybridization signals for PDE4A mRNA were seen in the lateral reticular nucleus, whereas no hybridization signal for PDE4A mRNA could be observed in the other nuclei in the medulla oblongata.

Cellular localization of mRNAs coding for PDE4 subfamilies

Very low hybridization signal for PDE4A was detected in general in the analyzed cell nuclei (Figs. 1A, 1D, 1G, 2A, 2D, 2G). Moderate numbers of silver grains for PDE4B were observed on blood vessels (Fig. 1H) and on neuronal and glial cells (Fig. 2B, 2E, 2H). Strong hybridization signal for PDE4D was also seen on blood vessels (Fig. 1I) and on neuronal and glial cells (Fig. 2C, 2F, 2I).

Discussion

By using oligonucleotide probes that selectively recognize the transcripts of the four PDE4 subfamilies forms of the PDE4 family (PDE4A, PDE4B, PDE4C and PDE4D), we have examined their regional distribution and cellular localization in human brainstem. Our results show that PDE4D mRNA expression was in general stronger than PDE4B. Additionally, PDE4B and PDE4D mRNA expression was abundant and distributed in neuronal cells and in glial cells, especially in areas

close to blood vessels. An important finding is that the signals for PDE4B and PDE4D mRNAs in the AP were stronger than those in any other nuclei in the brainstem. To our knowledge, this is the first detailed report of the regional distribution and cellular localization of the PDE4 subfamilies in human area postrema and other nuclei related to emesis.

We observed positive hybridization signals for PDE4B and PDE4D in several nuclei in human brainstem, especially in the AP. The AP has been implicated as a chemoreceptor trigger zone for vomiting (emesis) (Miller and Leslie, 1994). Emesis is one of the most common side effects of PDE4 inhibitors administration. The mechanism by which PDE4 inhibition results in increased vomiting and nausea in species such as dog and human (Bertolino et al., 1988; Hebenstreit et al., 1989; Heaslip and Evans, 1995) is not fully understood, but probably includes both central and peripheral sites of actions. Treatment of rats with the potent and selective PDE4 inhibitors 6-(4-pyridylmethyl)-8-(3-nitrophenyl) quinoline (PMNPQ) or rolipram elevated Fos-like immunoreactivity (an activity marker for a variety of neuronal populations in the brain) in brain regions associated with emesis such as the AP and STN (Bureau et al., 2006). Our finding suggests that cAMP signaling modification in this area could mediate the emetic effects of PDE4 inhibitors in human brainstem.

In the present study, hybridization signal for PDE4B and PDE4D mRNAs was stronger in the AP than in any other nuclei in human brainstem and those signals were expressed preferentially in association with blood vessels. Morphologically, the AP shares many features with the subfornical organ and the

vascular organ of the lamina terminalis, including rich vascularity (numerous blood vessels), modified ependyma, and a network of neuroglia (Weisinger et al., 1990). Porzionato and coworkers (Porzionato et al., 2005) evaluated the regional differences in microvessel density of the human medullary tegmentum and reported that DMVN and AP showed higher microvessel density with respect to STN, st and hypoglossal nucleus (XII). These findings indicate that the expression of PDE4B and PDE4D mRNAs in the AP might be regulated by chemical substances originating from blood vessels.

The anatomical distribution of PDE4B and PDE4D mRNAs presented in this study partially agrees with Lamontagne and coworkers (Lamontagne et al., 2001) who detected expression of PDE4D mRNA and immunoreactivity in the medulla and nodose ganglion of squirrel monkey and Cherry and Davis (1999) who reported the presence PDE4D immunoreactivity for mouse area postrema. However, and in contrast with our results, Lamontagne and coworkers were unable to find PDE4B expression in monkey mainly due, according to them, to the lack of selective antibodies for this subfamily and to inconclusive results obtained with their *in situ* hybridization experiments (Lamontagne et al., 2001).

The results in the present work are in agreement and expand to the human brain, our observations in the rat area postrema and medulla oblongata where we reported the expression of PDE4B and PDE4D mRNA (Pérez-Torres et al., 2000; Miró et al., 2002). Further evidence that supports the role of PDE4D in the emetic response is the increased reversion of alpha-2-mediated-anaesthesia, a behavioural surrogate of emesis in non vomiting species found with pro-emetic

PDE4 inhibitors (Robichaud et al., 2002a), that has also been demonstrated using PDE4D deficient mice (Robichaud et al., 2002b). In contrast, in the same study, PDE4B deficient mice exhibited the same sleep duration as their wild-type littermates under xylocaine/ketamine induced anaesthesia (Robichaud et al., 2002b). In our study, the AP contained higher levels of PDE4D mRNA than those of PDE4B mRNA in human brainstem (Table 1) when compared to their own expression in other brainstem nuclei, which is similar to what it has been previously described in the AP of rat brainstem (Takahashi et al., 1999; Pérez-Torres et al., 2000). The AP is a region which is known to mediate vomiting and nausea as emesis (Borinson and Wang, 1953; Carpenter et al., 1988). Based on their results, Miller and Ruggiero (1994) have proposed that there is not an unique group of neurons that might function as a “vomiting center”, but rather that those neurons involved in coordinating emesis could extend out from the AP and STN to an arc in the lateral tegmental field implicated in somato-autonomic integration. The substantial presence of PDE4D mRNA in some of these brainstem nuclei in this study points to its possible participation in emesis and other functions.

PDE4 inhibitors such as roflumilast and cilomilast have been proposed for the treatment of chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease, where they have shown modest but significant improvement in clinically relevant trials (Rabe et al., 2005; Lipworth, 2005). Rolipram and other PDE4 inhibitors produce memory-enhancing effects in some models and have anti-depressant-like activity in both preclinical and clinical models (Barad et al., 1998; Vitolo et al., 2002; Itoh et al., 2003; Gong et al., 2004; Ghavami et al., 2006).

Recent studies have postulated a role for PDE4 in schizophrenia (Kanes et al., 2007; Siuciak et al., 2007; Millar et al., 2007; Fatemi et al., 2008a), autism (Braun et al., 2007; Halene and Siegel, 2008) and bipolar disorder (Fatemi et al., 2008b).

Full exploitation of their therapeutic potential has been hampered though by the dose limitation imposed by the appearance of unwanted secondary effects, of which nausea has been consistently considered as one of the most prominent (Compton et al., 2001; Chung, 2006). It is not yet proven that PDE4D inhibition is the cause of emesis since there examples of PDE4 inhibitors that have anti-inflammatory activity with either no-emetogenic (Gale et al., 2002) or low emetogenic (Aoki et al., 2001) activity.

Conclusions

Our studies have demonstrated that PDE4B and PDE4D mRNAs are present in human brainstem, especially in the area postrema in association with blood vessels. This evidence, together with the expression of PDE4D in the nodose ganglia and the functional results on KO mice suggests that PDE4D may be associated with distinct functions of the area postrema such as emesis in the human. Nonetheless, the functional role of PDE4B in human brainstem, and especially in the AP, remains to be elucidated.

Acknowledgements:

This work was supported by grants from Ministerio de Educación y Ciencia and Fondo Europeo de Desarrollo Regional (SAF2006-10243). F.M. was on sabbatical leave from Hirosaki University School of Medicine. S.P.-T. was recipient of a predoctoral fellowship from CIRIT (Generalitat de Catalunya).

Conflict of Interest statement

"The authors declare that there are no conflicts of interest."

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Figure legends

Fig. 1. Distribution of mRNAs coding for PDE4 subfamilies in the human medulla oblongata and cellular localization in the area postrema (AP). A-I are bright-field photomicrographs from emulsion-dipped sections showing the presence of PDE4A (A, D, G), PDE4B (B, E, H) and PDE4D (C, F, I) mRNAs. D-I are higher magnifications of A-C from AP. Note absence of the labeling of PDE4A (D, G). Low densities of the labeling of PDE4B can be observed in AP (E) and located on and around blood vessels (H). High levels of hybridization for PDE4D are seen on and around blood vessels of AP (F, I). Sections (G-I) are stained with cresyl violet. Black arrows point to blood vessels. Black arrow heads indicate PDE4-hybridizing cells. White arrow heads are PDE4 non-hybridizing cells. DMVN, dorsal motor vagal nucleus; STN, nucleus of the solitary tract; st, solitary tract. Bar in A = 500 µm (applies to A - C). Bar in D = 100 µm (applies to D - F). Bar in G = 40 µm (applies to G - I).

Fig. 2. Cellular localization of mRNAs coding for PDE4 subfamilies in the dorsal motor vagal nucleus (DMVN) (A-C), nucleus of the solitary tract (STN) (D-F) and inferior olivary nucleus (ION) (G-I). Autoradiographic images are presented as bright-field photomicrographs from emulsion-dipped tissue sections in which autoradiographic grains are seen as black dots. All sections are stained with cresyl violet. Most cells exhibit few silver grains for PDE4A mRNA in DVMN (A), STN (D) and ION (G). Moderate labeling for PDE4B mRNA can be seen on neuronal and

glial cells in the three nuclei (B, E, H). High levels of hybridization for PDE4D mRNA are observed on neuronal and glial cells in the three nuclei (C, F, I). Arrows point to PDE4 mRNA expressing neurons. Arrow heads point to glial cells expressing PDE4 mRNAs. Bar in G = 40 μm (applies to A-I).

Table(s)

Table 1. Distribution of PDE4 mRNAs in the medullary and pontine nuclei

Region	PDE4A	PDE4B	PDE4D
Area postrema	-/+	+/++	+++
Nucleus of the solitary tract			
Paracommissural solitary n	-	+/++	+/++
Commissural solitary n	-	+/++	+/++
Intermediate solitary n	-	+	+
Dorsal motor vagal nucleus	-	+	++
Spinal trigeminal nucleus (Sp5)	-	+	++
Inferior olivary nucleus			
Principal n	-	+	++
Medial n	-	+	+
Hypoglossal nucleus	-	+	+
Lateral reticular nucleus	-/+	+	++
Cuneate nucleus	-	+	++
Gracile nucleus	-	+	+
Reticular formation nuclei	-	+	+
Locus coeruleus	-	+	+

The levels of intensity are indicated from '+' for low intensity up to '+++' for the highest intensity. '-', not detected.

Figure(s)

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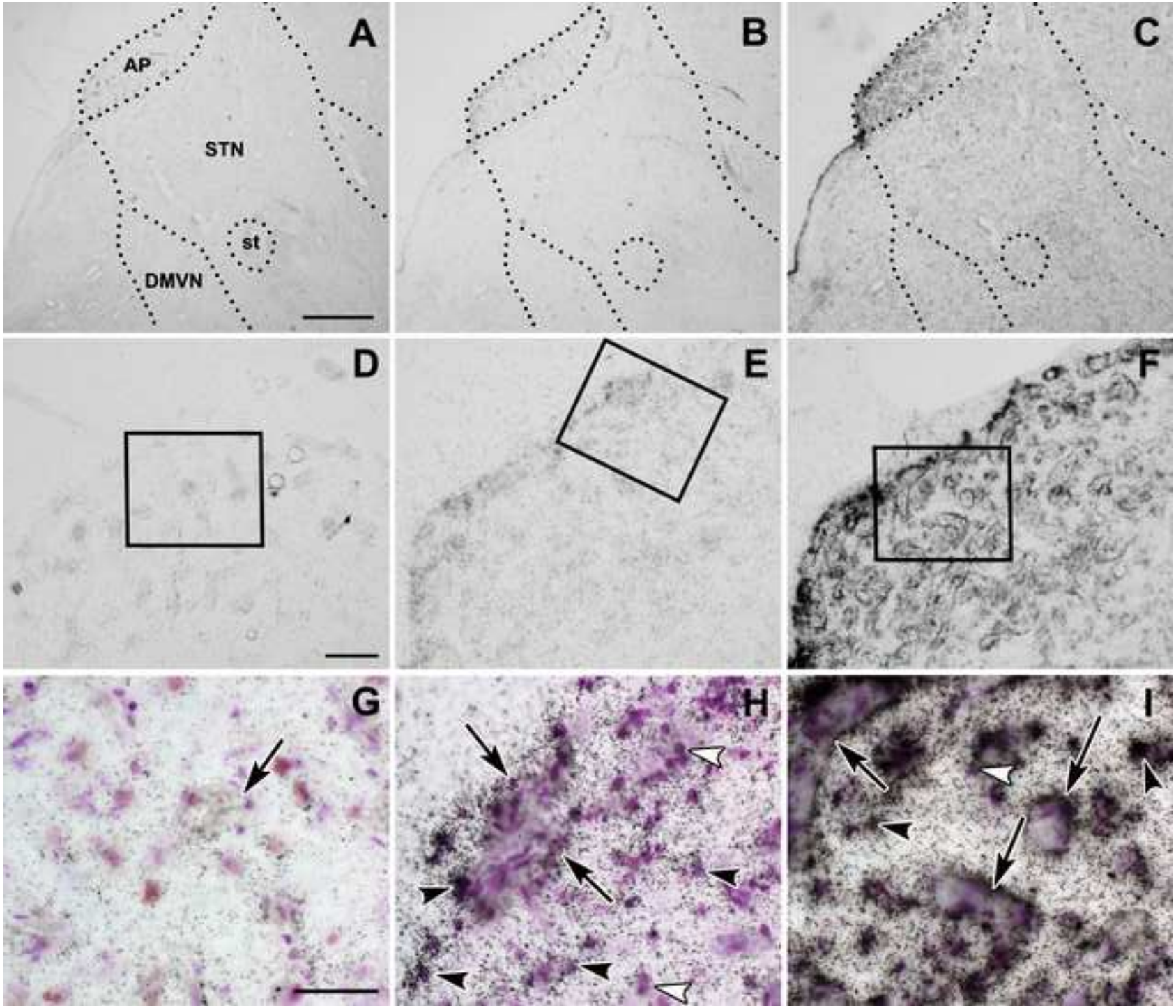

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