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Tissue-specific expression of mRNAs encoding α- and β-catalytic subunits of protein phosphatase 2A

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The mRNAs encoding the catalytic subunits of the type 1 phosphatase and the α and β forms of phosphatase 2A (termed 2Aα and 2Aβ) were detected in all the porcine tissues examined (brain, heart, muscle, liver, kidney and ovaries). Total phosphatase 2A transcripts were about 10 times more abundant in brain and heart than in the other tissues while the distribution of phosphatase 1 mRNA showed significantly less variation. Phosphatase 2Aα mRNA was about 10 times more abundant than phosphatase 2Aβ mRNA in most tissues. Similarly, both the α and β forms of the mRNA of the catalytic subunit of the phosphatase 2A were present in all the rat tissues examined. In addition to the 2 kb mRNA generally expressed, rat tissues also express a 2.8 kb mRNA related to 2Aα and a 1.3 kb mRNA related to 2Aβ.

Protein phosphatase; mRNA expression

1. INTRODUCTION

Several protein phosphatases have been purified and classified on the basis of sensitivity to inhibitor proteins, dependence on metal ions and substrate specificity (reviewed in [1]). Both the type 1 and 2A phosphatases purified from various tissues appear to be composed of a catalytic subunit bound to regulatory proteins [1]. Phosphatase 1 and phosphatase 2A also dephosphorylate a common set of substrates although with different efficiencies [2]. Phosphatase 2A can be distinguished from phosphatase 1 by its lack of sensitivity to inhibitor-1 and inhibitor-2, two potent protein inhibitors of phosphatase 1 activity [2]. Interestingly, these two inhibitor proteins are themselves substrates of phosphatase 2A [3,4].

The catalytic subunits of both these phosphatases have been cloned by several groups [5–10]. There are at least two forms of the catalytic subunit of phosphatase 2A (termed α and β) which show 98% sequence identity at the protein level and are also highly conserved between species [5–10]. In addition, protein phosphatase 1 was found to show 49% sequence identity to phosphatase 2A at the protein level [9].

In order to gain some insight into the physiological significance of the two isoforms of phosphatase 2A catalytic subunit, we have investigated their mRNA expression in several porcine and rat tissues. In addition, since phosphatases 1 and 2A exhibit similar substrate specificities in vitro, we have also compared the tissue distribution of the mRNAs which code for these two enzymes.

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2. MATERIALS AND METHODS

2.1. *Northern blot analysis*

Total RNA from porcine and rat tissues was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction [11] followed by precipitation in 2.5 M LiCl. Poly(A^+) RNA from rat tissues was selected once on oligo(dT) cellulose [12]. Total RNA (20 μg) from each porcine tissue and 10 μg of poly(A^+) RNA from each rat tissue were fractionated on 1% formaldehyde-agarose gels and electrotransferred onto Zeta-probe (BioRad) membranes. Hybridization to specific probes

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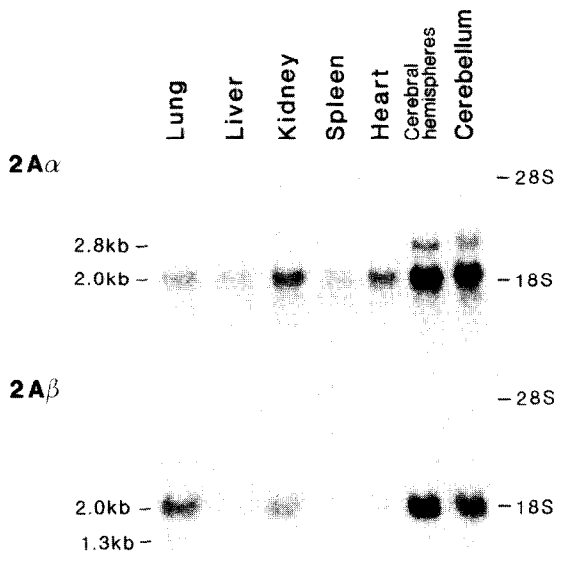

Fig.1. Phosphatase 2Aα- and 2Aβ-specific probes. The α-specific probe was prepared by isolating the *Dra*I fragment of the porcine or human 2Aα cDNA clone and the β-specific probe was prepared by isolating either the *Dra*I-*Eco*RI fragment of the porcine or the *Eco*RI-*Acc*I fragment of the human 2Aβ cDNA clones. Fragments αDD400 and βDE250 are located in the 3′-non-translated regions of the α and β cDNAs, respectively, which do not share any significant sequence homologies [5]. The filled boxes represent the coding region of the cDNA and the open boxes represent the 5′- and 3′-non-coding regions.

(see fig.1) was carried out in the presence of 50% formamide, 5 × SSC, 5 × Denhardts, 1% SDS, 0.05% sodium pyrophosphate and 250 μg/ml denatured DNA at 42°C. Following hybridization, the filters were washed as described in the figure legends.

2.2. *Preparation of phosphatase 1 and phosphatase 2Aα and β probes*

The synthetic probe used to quantitate type 1 phosphatase mRNA was based on the rabbit sequence reported by Berndt et al. [9]. Two 60-mer oligonucleotides, corresponding to nucleotides 314 to 374 (upper strand) and 358 to 448 (lower strand) were annealed and filled in using the Klenow fragment of DNA polymerase in the presence of [α-^{32}P]dATP. This generated a probe 105 bp long with a specific radioactivity of 2.3×10^9 cpm per μg DNA. Specific probes to the α and β forms of the type 2A phosphatase were made using the fragments indicated in fig.1 from either the porcine [5] or human [6] cDNAs and labeled by the random priming method [13]. The specific radioactivities of the porcine 2Aα, human 2Aα, porcine 2Aβ (βDE250) and human 2Aβ (βEA300) probes were 2.2×10^9, 2.0×10^9, 2.0×10^9 and 2.65×10^9 cpm per μg DNA, respectively.

3. RESULTS

3.1. *Northern analysis of phosphatase 2A mRNA expression in porcine tissues*

Fig.2 shows the relative levels of phosphatase 2Aα, 2Aβ and phosphatase 1 mRNAs expressed in porcine tissues. Both the 2Aα- and 2Aβ-specific probes hybridized to 2 kb mRNAs in all the tissues. Using the phosphatase 1 probe based on the rabbit cDNA sequence (see section 2), we detected a 1.6 kb mRNA in all the tissues, indicating a high level of sequence conservation between species and the usefulness of the probe in quantitating phosphatase 1 mRNAs in porcine tissues.

Table 1 shows that in most of the tissues examined the α form of the catalytic subunit of phosphatase 2A was about 8–12 times more abundant than the β form. The mRNAs encoding both isoforms of phosphatase 2A were found to be about 10 times more abundant in brain and heart than in the other tissues while the phosphatase 1 mRNA showed only about a 3-fold variation between tissues.

3.2. *Northern analysis of phosphatase 2A mRNA expression in rat tissues*

Fig.3 shows the relative abundance of phosphatase 2Aα mRNA in various rat tissues using the αDD400 human cDNA probe. The probe hybridized to a 2 kb mRNA as well as to a less abundant 2.8 kb mRNA. The level of the 2 kb 2Aα mRNA as well as that of the related 2.8 kb mRNA was found to be highest in brain tissues (both in cerebral hemispheres and cerebellum).

Poly(A^+) RNA from rat tissues was also hybridized to a human phosphatase 2Aβ probe (βEA300) isolated from the 5′-end of the cDNA (fig.3). Under the washing conditions used (see figure legend) no significant cross hybridization to the α mRNA was detected with this probe. The 2Aβ probe detected a 2 kb mRNA and to a much lesser extent also a 1.3 kb mRNA. The 2 kb 2Aβ mRNA, like the 2Aα mRNA, was found to be most abundant in brain tissues. The 3′-probe (βDE250) isolated from both the porcine and human phosphatase 2Aβ cDNAs did not hybridize to the rat RNA under the conditions described and only showed weak hybridization under lower stringency conditions (not shown). No attempt has

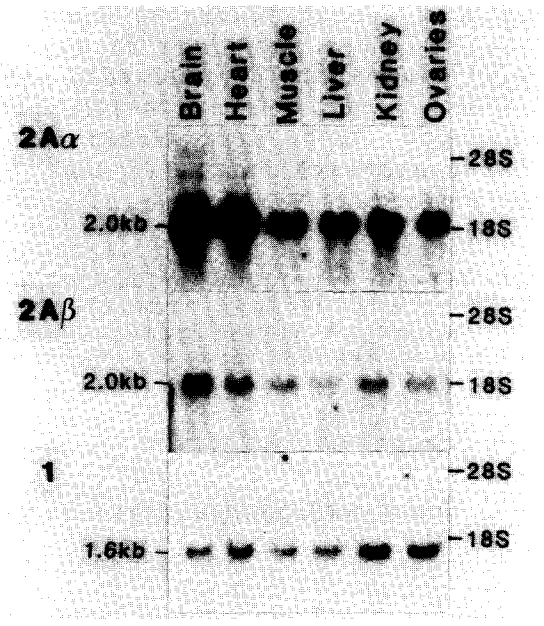

Fig.2. Northern blot analysis of porcine tissues using specific probes for phosphatase 2Aα, 2Aβ and phosphatase 1. Specific cDNA probes for phosphatase 2Aα and 2Aβ were the porcine αDD400 and βDE250 fragments (see fig.1). Following hybridization the filters were washed 3 times in 1 × SSC containing 1% SDS at 60°C for 30 min. Autoradiography was performed at −70°C with 2 intensifying screens for 4 days (2Aα and 2Aβ probes) and 7 days (phosphatase 1 probe). Approximate mRNA sizes and the 18 S and 28 S rRNA markers are shown.

Table 1

Relative levels of phosphatase catalytic subunit mRNAs in porcine tissues

Tissues	Relative mRNA abundance		
	2Aα	2Aβ	1
Brain	209 (5)	42 (1)	17
Heart	191 (8)	24 (1)	27
Muscle	25 (8)	3 (1)	13
Liver	22 (8)	3 (1)	14
Kidney	34 (11)	3 (1)	37
Ovaries	23 (12)	2 (1)	33

The data presented in fig.2 were quantitated by densitometric scanning of the autoradiograms using a Shimadzu scanner. Due to differences in the lengths of the α and β probes, the intensity of the β bands obtained after scanning was multiplied by a factor of 1.6. The numbers in parentheses are the ratios of 2Aα:2Aβ mRNAs. Direct comparison of the relative levels of phosphatase 1 and phosphatase 2A mRNAs was not possible

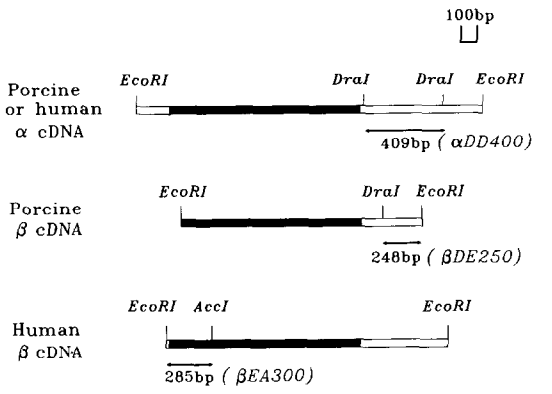

Fig.3. Northern blot analysis of rat tissues using specific probes for phosphatase 2Aα and 2Aβ. The specific human cDNA probes, αDD400 and βEA300 (see fig.1), were used to detect the 2Aα and 2Aβ mRNAs. Following hybridization the filter probed with the αDD400 fragment was washed 3 times in 1 × SSC containing 1% SDS at 60°C for 30 min and that probed with the βEA300 fragment was washed 4 times in 0.1 × SSC containing 1% SDS at 65°C for 30 min. Autoradiography was performed at −70°C with 2 intensifying screens for 3 days (2Aα probe) and 4 days (2Aβ probe). Approximate mRNA sizes and the 18 S and 28 S rRNA markers are shown.

been made in this study to quantitate the α:β ratio in the rat tissues due to the absence of homologous probes.

4. DISCUSSION

Both the α and β forms of phosphatase 2A were found to be expressed in all the porcine and rat tissues examined. A single mRNA species of 2 kb was found for each isoform in all the porcine tissues except brain, where some indication of higher molecular mass mRNA species was detected with the 2Aα probe. In rat tissues, however, a 2.8 kb mRNA related to the α form and a 1.3 kb mRNA related to the β form were also detected in addition to the predominant 2 kb form. These two additional mRNA species which were also not detected in several human cell lines (manuscript in preparation) are probably the same as the 2.8 kb and 1.3 kb forms observed in rabbit skeletal muscle [8]. Currently, there is no evidence to suggest whether these less abundant forms are the products of distinct genes or the result of alternative mRNA processing. In all the porcine tissues studied the α mRNA was found to be about 10 times more abundant than the β mRNA. This may reflect dif-

ferences in the relative transcription rates of the two genes, in the rates of mRNA turnover or in gene dosage. Recently, we have also examined the expression of the α and β forms of phosphatase 2A in 11 human cell lines (in preparation). In this analysis the ratio of α:β mRNA levels was found to vary from about 1:1 in A 1146 to 27:1 in MCF-7 cells. This large variation in α:β ratios may be due to differences in the physiological states of the cells or to cell-specific expression of the isoforms, suggesting that the levels of α and β mRNAs might be controlled independently.

In porcine tissues the phosphatase 2A mRNAs encoding both isoforms were found to be about 10-fold more abundant in brain and heart than in the other tissues studied. A high abundance of phosphatase 2A mRNA was also observed in rat brain but not in rat heart. Measurements of phosphatase 2A activity in rabbit tissues [3] indicated that the highest levels of phosphatase 2A activity were found in brain and heart. Western blot analysis of phosphatase 2A protein levels in bovine tissues [14] showed, however, that very high levels of the protein could be detected in the brain but heart tissue expressed the protein at the same level as the other tissues analyzed. Taken together, these data suggest that brain tissues from these species expressed high levels of phosphatase 2A mRNA compared to other tissues, while the level of phosphatase 2A mRNA in heart may be species-dependent. In contrast to the distribution of phosphatase 2A mRNA in different tissues, significantly less variation (2–3-fold) was observed in the levels of the phosphatase 1 mRNA. This could imply that a subset of phosphatase 2A substrates may be involved in tissue- or cell-specific functions.

The detection of mRNAs encoding both isoforms in several species including pig, rat, human (in preparation) and rabbit [8,10] suggests that the presence of different isoforms of the catalytic subunit of phosphatase 2A may be a general phenomenon. This observation implies that these different isoforms may play distinct physiological roles by having attained different substrate specificities through their differential association with regulatory subunits.

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REFERENCES

[1] Ballou, L.M. and Fischer, E.H. (1986) Enzymes (3rd Edn), pp.311–361.
[2] Ingebritsen, T.S., Stewart, A.A. and Cohen, P. (1983) Eur. J. Biochem. 132, 255–261.
[3] Ingebritsen, T.S., Stewart, A.A. and Cohen, P. (1983) Eur. J. Biochem. 132, 297–307.
[4] Tonks, N.K. and Cohen, P. (1984) Eur. J. Biochem. 145, 65–70.
[5] Stone, S.R., Hofsteenge, J. and Hemmings, B.A. (1987) Biochem. 26, 7215–7220.
[6] Stone, S.R., Hofsteenge, J. and Hemmings, B.A. (1987) Adv. Prot. Phosphatases 4, 375–389.
[7] Green, D.D., Yang, S. and Mumby, M.C. (1987) Proc. Natl. Acad. Sci. USA 84, 4880–4884.
[8] Da Cruz e Silva, O.B., Alemany, S., Campbell, D.G. and Cohen, P.T.W. (1987) FEBS Lett. 221, 415–422.
[9] Berndt, N., Campbell, D.G., Caudwell, F.B., Cohen, P., Da Cruz e Silva, E.F., Da Cruz e Silva, O.B. and Cohen, P.T.W. (1987) FEBS Lett. 223, 340–346.
[10] Da Cruz e Silva, O.B. and Cohen, P.T.W. (1987) FEBS Lett. 226, 176–178.
[11] Chomsczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[12] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[13] Feinberg, A.P. and Vogelstein, B. (1984) Anal. Biochem. 137, 266–267.
[14] Mumby, M.C., Green, D.D. and Russell, K.L. (1985) J. Biol. Chem. 260, 13763–13770.