Distribution of inositol 1,4,5-trisphosphate receptor mRNA in mouse tissues

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Received 30 April 1990

Northern blot analysis demonstrated that the concentration of inositol 1,4,5-trisphosphate (IP_3) receptor mRNA was greatest in cerebellar tissue. Moderate amounts of IP_3 mRNA were present in brain tissue without cerebellum and tissue of the thymus, heart, lung, liver, spleen, kidney, and uterus. Small amounts of IP_3 receptor mRNA were observed in skeletal muscle and testicular tissue. Regional distribution of IP_3 mRNA in various tissues was also examined by in situ hybridization. A considerable amount of IP_3 receptor mRNA was located in smooth muscle cells, such as those of the arteries, bronchioles, oviduct and uterus. In addition, secondary oocytes surrounded by Graafian follicles in the ovary were found to have large amounts of IP_3 receptor mRNA. The present studies suggest a functional importance of the IP_3 second-messenger system in these cell types.

Inositol 1,4,5-trisphosphate receptor; Second messenger; Cerebellum, Smooth muscle; Oocyte; Hybridization, in situ

1. INTRODUCTION

The phosphatidylinositide (PI)-dependent signal transduction pathway is now considered to play an important role in controlling the functions of a variety of cell types [1]. The physiological action of hormones, neurotransmitters and growth factors triggers receptor-activated PI hydrolysis by activating the conversion enzyme phospholipase C. This is the primary event of transmembrane signalling by which these extracellular signals are translated into two intracellular second messengers, inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). These second messengers are known to lead to cellular responses: IP_3 contributes to the release of Ca^{2+} into the cytoplasm from intracellular Ca^{2+} storage sites such as the endoplasmic reticulum (ER) while DAG activates protein kinase C.

The second messenger IP_3 must bind to a specific IP_3 receptor [2,3], before Ca^{2+} is released [4,5] from the intracellular Ca^{2+} storage sites. The primary structure of the IP_3 receptor protein was recently determined by cloning its cDNA from a cDNA library of the mouse cerebellum [6,7]. In order to understand its functional importance, we have now examined the expression and localization of IP_3 receptor mRNA in many tissues, using hybridization techniques. All tissues tested possessed the IP_3 receptor mRNA. Our results show that a considerable amount of IP_3 receptor mRNA is generally present in smooth muscle cells, which seems to relate to the fact that IP_3 can stimulate smooth-muscle contraction by inducing Ca^{2+} release from the sarcoplasmic reticulum (SR) [8,9]. We also observed large amounts of IP_3 receptor mRNA in secondary oocytes in which IP_3 may induce Ca^{2+} release, an event known to occur in *Xenopus* oocytes [10].

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; PI, phosphoinositide; DAG, 1,2-diacylglycerol; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum

2. MATERIALS AND METHODS

Sixty-day-old mice (ICR) were used.

Poly(A)$^+$ RNA preparation and Northern blot hybridization were performed as previously described [7].

For in situ hybridization, paraffin sections from various tissues (6 μm) were prepared and hybridized with ^{35}S-labeled probes essentially as previously reported [11]. After the hybridization reaction, the sections were dipped in NTB2 nuclear track emulsion (Kodak, USA) and autoradiography was performed [11]. The sections were lightly stained with 0.1% Cresyl violet (Merck, FRG) and examined by light microscopy.

A hybridization probe was prepared by random-primed labeling of the IP_3 receptor cDNA with Klenow enzyme (Takara Shuzo, Japan) and [α-^{35}S]dCTP (Amersham, UK) [7]. pUC118 vector DNA, linearized with restriction endonuclease *Eco*RI (Takara Shuzo) and ^{35}S-labeled, was used as a non-specific DNA probe.

3. RESULTS AND DISCUSSION

Our results show that the IP_3 receptor is extraordinarily abundant in the mouse cerebellum. Fig. 1

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00145793/90/$3.50

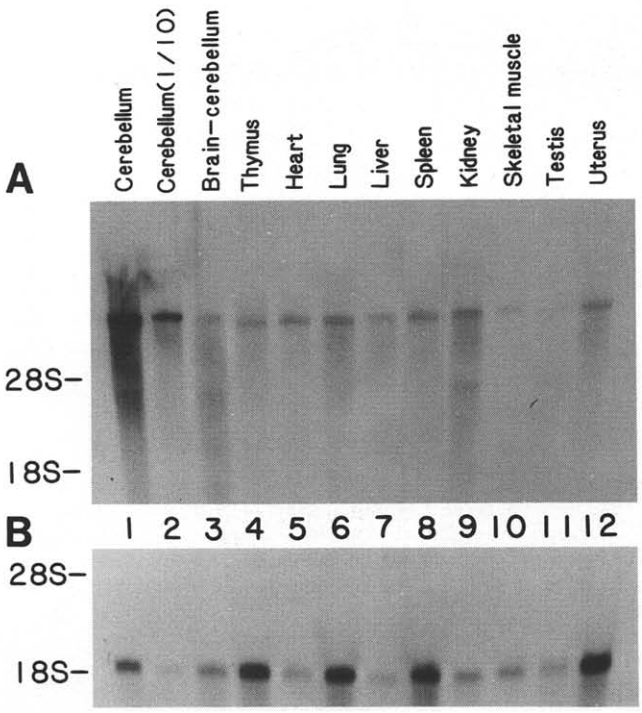

Fig 1. Northern blot hybridization analysis. (A) 5 μg of poly(A)$^+$ RNAs per lane except for cerebellum (1/10), 0.5 μg/lane, were hybridized with the IP_3 receptor cDNA probe (B) The same blot was probed with the β-actin cDNA as a control.

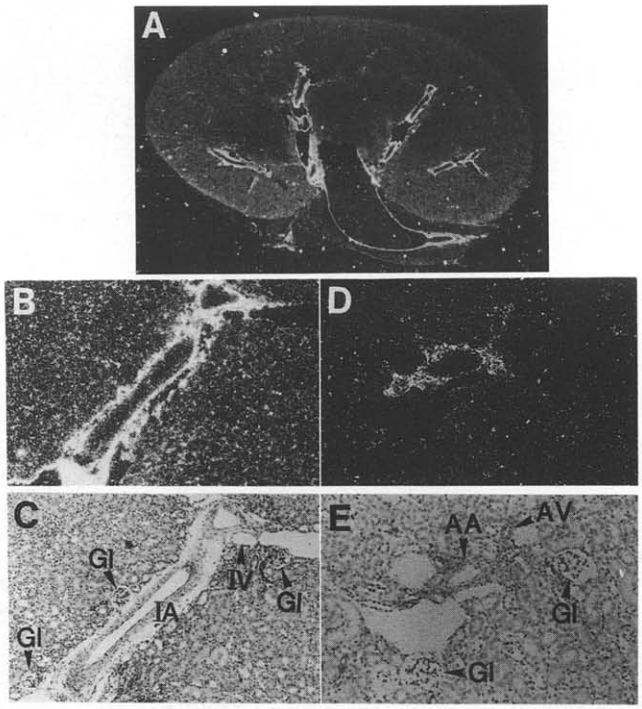

Fig. 2. Localization of the IP_3 receptor mRNA in the kidney sections. Dark-field (A, B and D) and bright-field (E) autoradiograms of mouse kidney sections hybridized with the ^{35}S-labeled IP_3 receptor cDNA. (C) Bright-field photo of the hematoxylin/eosin-stained section adjacent to the section (B). (D) and (E) are the same section. IA, interlobar artery; IV, interlobar vein; AA, arcuate artery; AV, arcuate vein; GI, glomerulus.

clearly shows that the IP_3 receptor mRNA is enriched in the cerebellum, having a concentration dozens of times greater than in any other brain area or in the peripheral tissues tested (lanes 1 and 2). We previously localized the quantitatively predominant sites of IP_3 receptor in cerebellar Purkinje neurons by immunohistochemical and in situ hybridization histochemical analyses [3,7]. The transcriptionally active expression of IP_3 receptor suggests the importance of PI turnover in this neuron. For example, it is suggested that synaptic transmission between Purkinje neurons and granule neurons may be mediated by a quisqualate-preferring glutamate receptor coupled with the PI turnover system [12,13]. There have been numerous recent reports on the existence of the PI turnover system in a variety of tissues. Our results indicate that all tissues tested thus far have the IP_3 receptor mRNA (Fig. 1), suggesting its importance in basic cellular function of all cell or tissue types, in addition to its cell-specific roles. Moderate amounts of IP_3 receptor mRNA were present in tissue of the brain without cerebellum and in tissue of the thymus, heart, lung, liver, spleen, kidney and uterus. However, we detected only a small amount of IP_3 receptor mRNA in skeletal muscle and testicular tissue.

Figs 2 and 3 show the results of in situ hybridization with tissue sections. In kidney sections (Fig. 2), arteries were visualized as strongly hybridizing sites while veins also produced a positive signal. Mesangium cells in glomeruli, in which the PI turnover system is known to occur [14,15], did not produce obvious hybridization signals. In lung sections, bronchioles were strongly labeled while arteries were also labeled (data not shown). These data indicate that vascular and bronchial smooth muscles possess large amounts of IP_3 receptor. This result may relate to recent findings that endothelin, a peptide released from endothelial cells [16] and renal and tracheal epithelial cells [17,18], acts as a potent vasoconstrictor and bronchoconstrictor, in a manner similar to that of angiotensin II, by stimulating the PI turnover system [19]. Strongly-labeled regions were also observed in the oviduct and uterus sections (Fig. 3), especially the smooth-muscle cell layers of both tissues, the tunica muscularis of the oviduct, and the myometrium of the uterus. Smooth-muscle contraction is generally thought to be activated by IP_3-induced Ca^{2+} released from the SR [8,9,20]. Our results show that smooth muscles generally possess quantitatively large amounts of IP_3 receptor which probably functions as one of the suppliers of Ca^{2+} which is required for the activation of smooth-muscle contraction. In contrast with smooth muscle, skeletal muscle had a weak expression of IP_3 receptor (Fig. 1). Recently, we reported the amino acid sequence

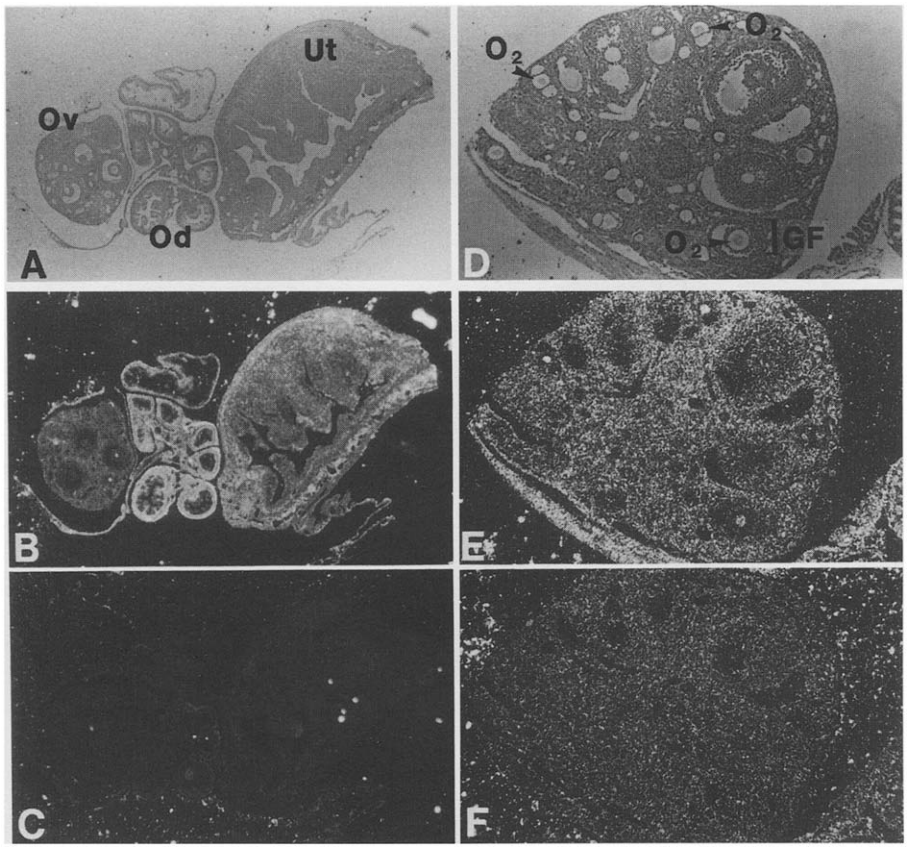

Fig. 3. Localization of the IP_3 receptor mRNA in the ovary, oviduct and uterus sections. Bright-field (A and D) and dark-field (B, C, E and F) autoradiograms. (A), (B), (D) and (E) sections were hybridized with the IP_3 receptor cDNA probe, and (C) and (F) with the pUC118 probe as a control. Ov, ovary; Od, oviduct; Ut, uterus; O_2, secondary oocyte; GF, Graafian follicle.

homology of the IP_3 receptor with the ryanodine receptor found in large quantities in skeletal muscle [7,21]. IP_3 and ryanodine receptors are involved in Ca^{2+} release from the ER and SR, respectively. Therefore, the ryanodine receptor is found predominantly in, and is responsible for, skeletal muscle contraction, whereas the IP_3 receptor may be primarily involved in a regulatory cellular response. Our data show that the testis has only a small amount of IP_3 receptor mRNA (Fig. 1). However, Nordquist et al. [22] reported that in testis the intensity of mRNA bands hybridizing to PCD6 clone, which is part of the IP_3 receptor cDNA, appeared to be almost the same as that in kidney tissue. With regard to this discrepancy, we believe that the testis preparation of Nordquist et al. [22] contained the ductus deferens (= vas deferens) which is composed of smooth muscle, while our preparation did not. In ovary sections (Fig. 3), large amounts of IP_3 receptor mRNA were observed in the secondary oocytes (O_2) within the Graafian follicles. IP_3-induced Ca^{2+} release is known to occur in *Xenopus* oocytes [10]. At this stage mature oocytes may prepare for the IP_3-induced Ca^{2+} release associated with fertilization which may be required for the egg-induced modification of the zona pellucida that results in the polyspermy block [23].

Acknowledgements: This work was supported by grants from the Science and Technology Agency of the Japanese Government and the Japanese Ministry of Education, Science and Culture.

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