Inositol 1,4,5-Trisphosphate Receptor in Heart: Evidence for its Concentration in Purkinje Myocytes of the Conduction System

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Abstract. Inositol 1,4,5-trisphosphate (IP$_3$) is one of the second messengers capable of releasing Ca$^{2+}$ from sarcoplasmic reticulum/ER subcompartments. The mRNA encoding the intracellular IP$_3$ receptor (Ca$^{2+}$ channel) has been detected in low amounts in the heart of various species by Northern blot analysis. The myocardium, however, is a heterogeneous tissue composed of working myocytes and conduction system cells, i.e., myocytes specialized for the beat generation and stimulus propagation. In the present study, the cellular distribution of the heart IP$_3$ receptor has been investigated. [3H]IP$_3$ binding experiments, Western blot analysis and immunofluorescence, with anti-peptide antibodies specific for the IP$_3$ receptor, indicated that the majority of Purkinje myocytes (the ventricular conduction system) express much higher IP$_3$ receptor levels than atrial and ventricular myocardium. Heterogeneous distribution of IP$_3$ receptor immunoreactivity was detected both at the cellular and subcellular levels. In situ hybridization to a riboprobe generated from the brain type 1 IP$_3$ receptor cDNA, showed increased accumulation of IP$_3$ receptor mRNA in the heart conduction system. Evidence for IP$_3$-sensitive Ca$^{2+}$ stores in Purkinje myocytes was obtained by double immunolabeling experiments for IP$_3$ receptor and cardiac calsequesstrin, the sarcoplasmic reticulum intralumenal calcium binding protein. The present findings provide a molecular basis for the hypothesis that Ca$^{2+}$ release from IP$_3$-sensitive Ca$^{2+}$ stores evoked by a$_1$-adrenergic stimulation is responsible for the increase in automaticity of Purkinje myocytes (del Balzo, U., M. R. Rosen, G. Malfatto, L. M. Kaplan, and S. F. Steinberg. 1990. Circ. Res. 67:1535-1551), and open new perspectives in the hormonal modulation of chronotropism, and generation of arrhythmias.

1. Abbreviations used in this paper: CS, calsequesstrin; IP$_3$, inositol 1,4,5-trisphosphate; PtInsP$_2$, phosphatidyl inositol 4,5-bisphosphate; SR, sarcoplasmic reticulum.

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The Journal of Cell Biology, Volume 121, Number 2, April 1993 345-353 345
left bundle branches and the peripheral network of ventricular conduction system myocytes, i.e., the Purkinje myocytes.

In this paper, we have investigated the cellular distribution of the IP$_3$ receptor in cardiac muscle by biochemical, immuno histochemical and in situ hybridization methods. We found that the majority of Purkinje myocytes express much higher IP$_3$ receptor levels than atrial and ventricular myocytes, and other portions of the conduction system. Heterogeneous distribution of IP$_3$ receptor immunoreactivity was detected both at the cellular and subcellular levels. The biological relevance of these observations is discussed in relation to phenomena such as automaticity, chronotropism and arrhythmias.

**Materials and Methods**

**Materials**

$[^3]$H]IP$_3$ and mouse anti-desmin mAbs were obtained from Amersham International (Buckinghamshire, UK), alkaline-phosphatase-conjugated anti-rabbit IgGs were obtained from Sigma Chemical Co. (St. Louis, MO) goat rhodamine-labeled anti-mouse IgGs from Cappel Laboratories (Malvern, PA) and swine fluorescein-labeled anti-rabbit IgGs from Dako Corporation (Carpinteria, CA).

**Tissue Sources**

Adult rats were killed by decapitation and their cerebella and hearts were quickly removed and stored at $-80^\circ$C until used. Bovine and horse hearts were obtained from a local slaughterhouse. Samples of different cardiac regions corresponding to both working myocardium and conduction system were dissected out. Analogous samples were excised from mongrel dog and chicken hearts. Whole hearts were dissected out from bovine fetuses collected at 3, 4, and 5 mo of gestational age. All samples were immediately frozen in liquid nitrogen and stored at $-80^\circ$C until used.

For immunohistochemical studies performed with anti calretinin (CS) antibodies, tissues were fixed with 4% paraformaldehyde in PBS for 2 h at 4°C, cryoprotected with 18% sucrose and frozen in liquid nitrogen.

cRNA Probes and Abs

A rat IP$_3$ cDNA (clone p12a; Mignery et al., 1990) containing a 2-kb EcoRI fragment of the 3' coding and noncoding regions of the type 1 IP$_3$ receptor mRNA, was used for in situ hybridization analysis. The 19-mer COOH-terminal peptide of the mouse/rat type 1 IP$_3$ receptor sequence (cf. Furuchi et al., 1989; Mignery et al., 1990) was synthesized, conjugated to keyhole limpet hemocyanin (protein carrier) by Multiple Peptide Systems (San Diego, CA), and used as immunogen in rabbits. Antibody specificity for the IP$_3$ receptor was ascertained by Western blot of cerebellar microsomal fractions (see Fig. 1 a).

The mouse polyclonal antibody specific for dog cardiac CS were obtained as described (Biral et al., 1992). The specificity of the antibody was assessed by Western blot (Biral et al., 1992).

Mouse mAbs specific for either the myosin heavy chain subunit (BA-DS) or the $\alpha$ myosin heavy chain (BA-G5) were obtained and characterized as described in Schiaffino et al. (1989) and Rudnicki et al. (1990), respectively.

**Isolation of Microsomal Fractions and Biochemical Assays**

Microsomes from rat cerebellum and different areas of bovine heart (atrium, ventricular wall, septum and false tendon) were prepared by differential centrifugation as described by Volpe et al. (1991). Protein concentration was assessed by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Microsomal fractions were analyzed by SDS PAGE (5-10% linear gradient), transferred to nitrocellulose and reacted with antibodies as described (Volpe et al., 1991).

$[^3]$H]IP$_3$ binding was carried out essentially as described (Alderson and Volpe, 1989) in a medium containing $0.1$ M KCl, $50$ mM Tris-Cl, pH 8.3, $1$ mM EDTA, $150$ $\mu$g of microsomal protein, $50$ nM $[^3]$H]IP$_3$, in the absence and presence of $5$ $\mu$M cold IP$_3$ for total and nonspecific binding, respectively. Protein and rinsings were as described (Volpe et al., 1991).

**Immunocytochemistry**

Indirect immunofluorescence was performed as previously described (Sartore et al., 1978; Gorza et al., 1986). Paraformaldehyde-fixed cryosections were quenched before incubation with antibodies in $0.2$ M Tris-glycine, pH 7.4, for $30$ min at room temperature. 5- to $10$-$\mu$m cryosections were incubated with adequate dilutions of anti-IP$_3$ receptor Abs for $20$ min at $37^\circ$C. After several washes with PBS, sections were incubated with fluorescein-conjugated anti-rabbit IgGs, rinsed again in PBS, mounted with glycerol and observed with a Zeiss Axioplan microscope equipped with epifluorescence optics.

For double-labeling immunofluorescence, anti-IP$_3$ receptor antibodies were mixed with mouse anti-CS or anti-myosin heavy chain Abs in the first incubation. Anti-mouse and anti-rabbit secondary antibodies, labeled with rhodamine or fluoresceine, respectively, were sequentially applied, after absorption with heterologous Ig in order to eliminate interspecies cross-reactivity (cf. Vitadello et al., 1990).

**In Situ Hybridization**

Antisense and sense cRNA probes were transcribed from the 2-kb pl2a cDNA clone (Mignery et al., 1990) after linearization with Hind III and BamHI, respectively, of the Bluescript plasmid and labeled with $[^3]$S as described (Ausoni et al., 1991; Gorza et al., 1993). Probes were digested to 50-100 nucleotides by mild alkaline hydrolysis. Cryosections were fixed with formaldehyde, digested with proteinase K (Gorza et al., 1993), and hybridized overnight at $52^\circ$C with $10$ nM of probe at a concentration ranging between $2.5$ and $20$ × $10^4$ cpm/$\mu$l, and washed at $65^\circ$C with $50$% formamide, $2 \times$ SSC and $0.1$ M DTT. Sections were dehydrated, dipped in Kodak NTB-2 autodioradiographic emulsion diluted 1:1 with water, and exposed for 7-21 d at $4^\circ$C. Slides were developed with Kodak D 19 for $3.5$ min, fixed and examined with a Zeiss Axioplan microscope equipped with dark field optics. Serial sections of the rat heart were processed for routine hematoxylin-eosin histology.

**Results**

**Biochemical Studies**

The bulk of the experiments were carried out on bovine heart because the false tendon could be identified, dissected out and used also for biochemical analysis. The false tendon contains, within a coat of collagen fibers, bundles of Purkinje myocytes, sympathetic nerves and their terminals, some blood vessels and fibroblasts (Sommer and Jennings, 1986).

Microsomal fractions were isolated from different areas of bovine heart, and probed with polyclonal anti-peptide antibodies specific for the IP$_3$ receptor in Western blot experiments. Fig. 1 shows that false tendon microsomes (lane b) contained an immunologically reactive polypeptide having the same electrophoretic mobility of the rat cerebellum IP$_3$ receptor (lane a; about 260,000 D), whereas microsomes isolated from the ventricle wall or the conduction-system-free septum were not reactive (lanes c and d, respectively). Specific $[^3]$H]IP$_3$ binding sites were quantitated in microsomes of atrium, ventricle, free septum and false tendon. Table I shows that false tendon microsomes contained $640$ fmol IP$_3$ bound/mg of protein, i.e., levels of IP$_3$ receptor about eightfold higher than any other heart region investigated. The apparent discrepancy between low level of $[^3]$H]IP$_3$ binding and lack of immunoreactivity reactivity in working myocardium microsomes (Fig. 1), could be due to the lower detection sensitivity of blotting techniques.

The results of both Western blot and $[^3]$H]IP$_3$, binding experiments per se do not warrant the conclusion that the IP$_3$
Figure 1. Western blotting of microsomal fractions obtained from different areas of bovine heart with anti-IP$_3$ receptor antibodies. SDS PAGE (5–10% linear gradient), electrophoretic transfer to nitrocellulose and immunoblot were carried out as described in Materials and Methods. Microsomes (100 µg of protein) derived from rat cerebellum (lane a), bovine heart false tendon (lane b, FT), ventricle wall (lane c, V) and free septum (lane d, S) were probed with anti-IP$_3$ receptor Abs. Bio-Rad (Bio-Rad Laboratories) molecular weight standards are indicated by arrows. IP$_3$R, IP$_3$ receptor.

Table I. [3H]IP$_3$ Binding to Microsomal Fractions Isolated from Bovine Atrium, Ventricle, Free Septum, False Tendon and Cerebellum

|                | fmol/mg of protein |
|----------------|--------------------|
| Atrium         | 85.8 ± 9.4 (4)     |
| Ventricle      | 88.0 ± 7.8 (5)     |
| Free septum    | 83.6 ± 13.8 (5)    |
| False tendon   | 640.4 ± 55.3 (5)   |
| Cerebellum     | 8860.3 ± 310.6 (3) |

Experiments were carried out as described in Materials and Methods. Data are given as mean ± SD for the number of different preparations shown in parenthesis. Scatchard plot analysis of [3H]IP$_3$ binding to cerebellum and false tendon microsomes yielded $K_a$ values of 12.3 and 14.1 nM, respectively, to a single class of binding sites.

The cellular compartmentalization of IP$_3$ receptor immunoreactivity in Purkinje myocytes of the bovine heart appears to occur very early during prenatal development. A population of myocytes of the ventricular septum of a 3-mo-old bovine fetus, corresponding to the right and left bundle branches, reacted strongly with anti-IP$_3$ receptor antibodies (RB in Fig. 3 a), at variance with the weak reactivity displayed by ventricular myocytes (V in Fig. 3 a). Higher magnification images of serial sections (Fig. 3 b and c) clearly show that labeling for the IP$_3$ receptor (b) was restricted to myocytes also reactive for anti-myosin antibodies (c). Comparable results were obtained in later stages of development (4- and 5-mo old fetuses): bundles of large myocytes were strongly reactive with anti-IP$_3$ receptor antibodies (Fig. 3 d). In serial section (e), Purkinje myocytes were unambiguously identified on the account of their reactivity with antibodies specific for α myosin heavy chain, a marker of Purkinje myocytes in developing bovine heart (Thornell et al., 1984). Other conduction system myocytes e.g., nodal myocytes, were as weakly labeled as working myocytes (not shown).

In Situ Hybridization

Analysis at the mRNA level was performed by in situ hybrid-$\lambda$
Immunofluorescence staining of bovine heart cryosections with anti-IP3 receptor (b, c, e-g), anti-myosin heavy chain (d) and anti-desmin (h) antibodies. The diagram in a outlines the composition and anatomical localization of the heart conduction system: sino-atrial node (SAN) myocytes, the pacemaker, atrio-ventricular nodal (AVN) myocytes, the atrio-ventricular His bundle, the right and left bundle branches and the peripheral network of ventricular conduction system myocytes (P), i.e., the Purkinje myocytes. (b) Bundles of the AVN myocytes; arrows and arrowheads point to strongly and weakly reactive myocytes, respectively. (c and d) Section through bundles of the right bundle branch, double labeled with either anti-IP3 receptor (c) or anti-myosin (d) antibodies: c, note weakly-labeled areas close to cell-to-cell junctions. (e) Peripheral Purkinje strands. (f) IP3 receptor-labeled bundles of the right bundle branch; arrows and arrowheads point to strongly and weakly reactive Purkinje myocytes, respectively. (g and h) Serial sections labeled with anti-IP3 receptor (g) and anti-desmin (h) antibodies: among Purkinje myocytes, only a few are strongly labeled by anti-IP3 receptor antibodies (arrow, g), the majority being weakly reactive (arrowhead, g). In h, arrow and arrowhead point to Purkinje myocytes identified on the account of their reactivity with anti-desmin antibodies. Abbreviation: V, ventricle. Bar, b-f, 60 μm; g and h, 160 μm.

Antisense cRNA hybridized strongly to rat cerebellar Purkinje neurons (Fig. 4a), as expected, given the high density of IP3 receptor in these cells (Worley et al., 1987; Furuichi et al., 1989; Satoh et al., 1990; Villa et al., 1991; Takei et al., 1992). Fig. 4b represents a hematoxylin-stained section serial to those of Fig. 4, c and d. Hybridization signals varied among rat cardiac myocytes: working ventricular myocytes were weakly reactive, whereas conduction system myocytes of either the atrioventricular node (AVN) and the His bundle (H), as shown in Fig. 4c, or subendocardial myocytes, probably corresponding to Purkinje bundles (P), as shown in Fig. 4d, hybridized strongly to IP3 cRNA. Hybridization to sense cRNA was negative (not shown). In the rat heart, strong hybridization to IP3 receptor mRNA was detected in smooth muscle myocytes of coronary vessels (inset in Fig. 4d; cf. Furuichi et al., 1990).

Only a very weak signal was observed in canine Purkinje
Figure 3. Immunofluorescence staining of bovine embryonic heart cryosections with anti-IP$_3$ receptor (a, b and d), anti-myosin heavy chain (c), and anti-α myosin heavy chain (e) antibodies. Serial sections of the ventricular septum of a 3-mo old fetus were labeled with anti-IP$_3$ receptor (b) and anti-myosin heavy chain (c) antibodies; labeling for IP$_3$ receptor is detectable in myosin-positive myocytes of the right bundle branch (arrows, b and c). Serial sections of the ventricular myocardium of a 5-mo-old fetus were labeled with anti-IP$_3$ receptor (d) and anti-α myosin heavy chain (e) antibodies: IP$_3$ receptor-positive bundles of large conduction myocytes were detected. Abbreviations: V, ventricle, RB, right bundle branch. Bar, a, 60 μm; b-e, 30 μm.

Gorza et al. IP$_3$ Receptor in Purkinje Myocytes

neurons with rat IP$_3$ receptor cRNA and no signal at all in both bovine and canine myocytes (not shown).

Subcellular Localization and Heterogeneous Distribution of the IP$_3$ Receptor and Calsequestrin

The cellular and subcellular distribution of both IP$_3$ receptor and CS, the SR intra-lumenal low-affinity, high-capacity Ca$^{2+}$ binding protein (MacLennan and Wong, 1971) was investigated by immunofluorescent labeling in bovine Purkinje myocytes.

Double labeling revealed uneven distribution of the two proteins in IP$_3$ receptor-positive Purkinje myocytes. The IP$_3$ receptor labeling was widespread throughout the cytoplasm, showing regional staining differences (Fig. 5, a and b), i.e., the staining was more intense in the central cytoplasmic region (see also Fig. 2 c) that is filled with intermediate filament proteins in bovine Purkinje myocytes (Thornell et al., 1985). On the other hand, the punctate staining of CS (cf. Jorgensen et al., 1984) was more evident in, but not limited to, subsarcolemmal areas (Fig. 5, c and d) where myofibrils are concentrated. In all panels, large arrows point to myocytes showing strong reactivity for the IP$_3$ receptor in central areas apparently devoid of CS; small arrows point to subsarcolemmal regions reactive for both the IP$_3$ receptor and CS.

Discussion

Heterogeneous Distribution of the Cardiac Type 1 IP$_3$ Receptor and Its Concentration in Purkinje Myocytes

In this paper we demonstrate, for the first time, that the type 1 IP$_3$ receptor is highly concentrated in the majority of Purkinje myocytes, the distal elements of the heart conduction system, and some fascicles of the atrio-ventricular node. We also show much lower amounts of IP$_3$ receptor in the working myocardium and other portions of the conduction system. The differential distribution of the IP$_3$ receptor between conduction system myocytes and working myocytes is also observed during embryonic development, i.e., at the earliest stage examined, conduction myocytes are found to express a higher level of IP$_3$ receptor.

There are, thus, two levels of heterogeneity whose functional relevance remains to be investigated: one among the different subpopulations of the heart conduction system and the working myocytes, the other between and within the Purkinje myocytes themselves. In the latter cases, the heterogeneity of Purkinje myocytes pertains both to the subcellular and cellular level, as judged by serial section analysis: there are, in fact, areas close to cell–cell junctions which are weakly stained (Fig. 2 c) as compared to central cytoplasmic areas; moreover, either individual myocytes within a bundle (Fig. 2 f) or entire bundles of conduction system myocytes (cf. Fig. 2, f-h) are weakly labeled as compared to either adjacent myocytes or bundles.

A rat type 2 IP$_3$ receptor isoform, the product of a distinct gene (Sudhof et al., 1991), has been recently described. Ligand binding studies on recombinant IP$_3$ receptor proteins, have shown that type 2 IP$_3$ receptor displays slightly higher affinity for IP$_3$ than type 1 IP$_3$ receptor. Analysis of the deduced amino acid sequence indicates divergence in the COOH-terminal 19-mer peptide, chosen to raise the antibodies specific for type 1 IP$_3$ receptor and used in this study. Low degree of homology is also observed in the 3'
Figure 4. In situ hybridization. Dark fields micrographs of IP3 receptor cRNA hybridized to rat cerebellum (a) and heart (b and c). Antisense probe hybridization was detected in the soma and dendrites of cerebellum Purkinje neurons (a), in coronary smooth muscle myocytes (inset, d), in subendocardial Purkinje myocytes (d, P, arrowhead), and in conduction myocytes of the AV node (AVN) and the His bundle (H; c); myocytes of the ventricular free wall (V) and septum (S) were weakly reactive (c and d). b, which represents a section serial to those of c and d, was stained with hematoxylin and eosin. Bar, a, c, d, and inset, 240 μm; b, 400 μm.

coding and noncoding regions of type 1 and 2 IP3 receptor mRNAs (Sudhof et al., 1991). Although we cannot rule out with certainty that other IP3 receptor isoforms are expressed (up to four distinct IP3 receptor cDNAs have been described in the mouse; Ross et al., 1992), the specificity of the antibodies and cRNA probes used along with our [3H]IP3 binding data, strongly indicate that the type 1 IP3 receptor represents a major isoform in Purkinje myocytes.

IP3-sensitive Ca2+ Stores Purkinje Myocytes

In Purkinje myocytes, the SR is composed of three continuous membrane domains, the free SR, corbular SR and junctional SR (Sommer and Jennings, 1986) whose relative development shows regional variations. The corbular and junctional SR share ultrastructural features and contain intralumenal granules largely made up of CS (Jorgensen and Campbell, 1984; Jorgensen et al., 1988). Junctional SR profiles are observed exclusively in the subsarcolemmal rim all around myofibrils. Free and corbular SR, as well as ER cisternae are also observed in myofilament-free areas of central cytoplasm (Sommer and Jennings, 1986).

The IP3 receptor is a ligand-gated Ca2+ channel (Maeda et al., 1991), found to be concentrated in the limiting membrane of smooth-surfaced ER subcompartments in both chicken and mammalian cerebellum Purkinje neurons (Satoh et al., 1990; Volpe et al., 1991; Takei et al., 1992). CS, an intralumenal low-affinity, high-capacity Ca2+ binding protein (MacLennan and Wong, 1971), is localized in striated muscle SR subcompartments, i.e., terminal cisternae (Jorgensen et al., 1984), and ER moderately dense-cored vacuoles in chicken cerebellum Purkinje neurons (Villa et al., 1991;
Volpe et al., 1991). The occurrence of both CS and IP\textsubscript{3} receptor on the same subcellular compartments is taken as evidence of functional IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} stores (Volpe et al., 1991; Takei et al., 1992; reviewed in Meldolesi et al., 1992).

In the present study, Purkinje myocytes are labeled by antibodies for both the IP\textsubscript{3} receptor and CS (Fig. 5), thus indicating the existence of IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} stores. The labeling pattern indicates that the IP\textsubscript{3} receptor is not restricted to subsarcolemmal SR but possibly present in corbular SR (or parts of it) and ER subcompartments, scattered throughout the cell (Sommer and Jennings, 1986), which might be in luminal continuity with junctional SR (cf. Volpe et al., 1992). The finding that central areas of a number of Purkinje myocytes are strongly reactive for the IP\textsubscript{3} receptor and poorly reactive for CS (Fig. 5), points to subcellular heterogeneity of Ca\textsuperscript{2+} stores in conduction system cells.

The relevance of the present observations and the detailed ultrastructural features of Ca\textsuperscript{2+} stores in Purkinje myocytes will be fully understood after completion of EM immunogold labeling experiments, which are in progress. Molecular heterogeneity of intracellular Ca\textsuperscript{2+} stores has been already demonstrated in the Purkinje neuron of the chicken, where different subcellular membrane compartments were found, by EM immunogold labeling, to be variably endowed with either CS or IP\textsubscript{3} receptor (Villa et al., 1991; Volpe et al., 1991; Takei et al., 1992).

**Functional Significance of the IP\textsubscript{3} Receptor in Purkinje Myocytes**

After plasma membrane depolarization, transient increases of [Ca\textsuperscript{2+}], appear to be caused by other entry of extracellular Ca\textsuperscript{2+} and release of Ca\textsuperscript{2+} from the SR (Wier, 1980; Fabiato, 1985) of Purkinje myocytes. However, the physiological role of Ca\textsuperscript{2+} release has remained elusive thus far. In Purkinje myocytes, IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} stores may be involved in the increase of automaticity after \(\alpha_1\)-adrenergic stimulation. del Balzo et al. (1990) have recently shown that WB 4101, a competitive inhibitor of \(\alpha_1\)-adrenergic receptor in canine Purkinje myocytes, antagonizes the \(\alpha_1\)-adrenergic
positive chronotropic response, and prevents norepinephrine-dependent inositol phosphate accumulation. del Balzo et al. (1990), thus, suggested that the products of PI(4,5)P₂ hydrolysis, either diacylglycerol, IP₃ or both, are "mechanistically involved in the α₁-adrenergic positive chronotropic response." Moreover, automatic arrhythmias induced by α₁-adrenergic stimulation in experimental models of "ischemic" and "reperfusion" canine Purkinje myocytes (Anyukhowsky and Rosen, 1991; Molina-Viamonte et al., 1991), were abolished by pretreatment with WB 4101. IP₃-induced transient elevation of [Ca²⁺]ᵢ may bring about decrease of membrane potential and, in turn, increased automaticity via activation of a non-specific cation conductance (Colquoun et al., 1981; Kass and Tsien, 1982; Molina-Viamonte et al., 1990). Thus, IP₃-induced Ca²⁺ release appears to be causally related to both increase in automaticity and generation of some arrhythmias. The demonstration of the high level of IP₃ receptor in Purkinje myocytes provides a molecular basis for this interpretation.

In Purkinje myocytes, the heterogeneous distribution of the IP₃ receptor might be related to that of phospholipase C-coupled pertussis toxin-insensitive G proteins, which appear to be functionally present only in 50% of Purkinje myocytes (see del Balzo et al., 1990). The transduction pathway activated by α₁A-adrenergic agonists and controlled by a pertussis-insensitive G protein appears to be developmentally regulated. At birth almost all Purkinje myocytes respond to α₁-adrenergic agonists with an increase in automaticity (see del Balzo et al., 1990, and references therein) whereas only 50% of adult Purkinje myocytes do so. The IP₃ receptor immunolabeling pattern observed in adult and embryonic Purkinje myocytes is formally consistent with these changes, insofar as Purkinje myocytes are far less heterogeneous as compared to adult ones. However, other mechanisms might be responsible for the negative chronotropism induced in about 50% of adult Purkinje bundles upon stimulation with α₁-adrenergic agonists. A pertussis toxin-sensitive G protein has been shown to be expressed after birth and to couple activation of α₁A-adrenergic receptor subtype to a Na⁺-K⁺ pump, whose operation would determine the decrease in automaticity (Zaza et al., 1990). Interestingly, specific α₂ and α₃ isoforms of the Na⁺-K⁺ pump are expressed in conduction system myocytes (Zahler et al., 1992). It remains to be ascertained whether both transduction pathways are functional in adult Purkinje myocytes or are segregated to distinct Purkinje myocytes subpopulations.

Sincere thanks are due to Dr. Thomas C. Sudhof (Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX) for the generous gift of the p22α cDNA clone.

Work supported by institutional funds from the Consiglio Nazionale delle Ricerche of Italy, grant GM-40088-03 from the National Institutes of Health, and funds from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy.

Received for publication 29 July 1992 and in revised form 15 January.

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