Adult Cardiac Sca-1-positive Cells Differentiate into Beating Cardiomyocytes

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Although somatic stem cells have been reported to exist in various adult organs, there have been few reports concerning stem cells in the heart. We here demonstrate that Sca-1-positive (Sca-1+) cells in adult hearts have some of the features of stem cells. Sca-1+ cells were isolated from adult murine hearts by a magnetic cell sorting system and cultured on gelatin-coated dishes. A fraction of Sca-1+ cells stuck to the culture dish and proliferated slowly. When treated with oxytocin, Sca-1+ cells expressed genes of cardiac transcription factors and contractile proteins and showed sarcomeric structure and spontaneous beating. Isoproterenol treatment increased the beating rate, which was accompanied by the intracellular Ca2+ transients. The cardiac Sca-1+ cells expressed oxytocin receptor mRNA, and the expression was up-regulated after oxytocin treatment. Some of the Sca-1+ cells expressed alkaline phosphatase after osteogenic induction and were stained with Oil-Red O after adipogenic induction. These results suggest that Sca-1+ cells in the adult murine heart have potential as stem cells and may contribute to the regeneration of injured hearts.

The heart has long been thought to adapt to increased work and loss of cardiomyocytes by the cellular hypertrophy of residual cardiomyocytes, but not by the proliferation of mature cardiomyocytes or the differentiation of undifferentiated cells. Recently, several reports have suggested that adult cardiomyocytes can proliferate under certain pathologic conditions and that there are cells expressing stem cell markers in the adult heart (1–4). It has been reported that Sca-1- and e-kit-positive (+) cells exist in the adult heart (5) and that adult murine hearts contain potential stem cells; side population (SP)1 cells (6, 7). However, it remains to be clarified whether these cells have the characteristics of stem cells such as abilities of self-renewal and differentiation into various types of cells including mature cardiomyocytes.

Sca-1 is a member of the Ly-6 family and has first reported as one of the cell surface markers of hematopoietic stem cells (8). Recently many reports have demonstrated that multipotent stem cells derived from bone marrow and skeletal muscle express Sca-1. Okumoto et al. (9) have reported that Sca-1+ cells from bone marrow differentiate into hepatocyte when treated with hepatic growth factor. Gujo et al. (10) have reported that adult mesenchymal stem cells from bone marrow abundantly express Sca-1 and differentiate into cardiomyocyte in vivo. Qu-Petersen et al. (11) have shown that skeletal muscle-derived stem cells, which highly express Sca-1, contribute to the regeneration of the skeletal muscle in a mouse model of Duchenne muscle dystrophy. They also demonstrated that the skeletal muscle-derived stem cells were able to differentiate into neural cells and endothelial cells. Asakura et al. (12) have reported that ~90% of SP cells in skeletal muscle express Sca-1. It has been reported that skeletal muscle-derived Sca-1+ and CD34+ cells restore dystrophin in mdx mice (13) and that CD34+ cells in the interstitial spaces of skeletal muscle, which highly express Sca-1, differentiate into adipocytes, endothelial, and myogenic cells (14). These findings suggest that Sca-1 might be important evidence for somatic stem cells.

Currently little is known about the humoral or growth factors that induce cardiomyogenic differentiation. It has been shown that ectopic application of bone morphological protein (BMP) 2 and 4 elicits cardiogenic responses in the chick in vivo system (15), and fibroblast growth factor (FGF) 2 and 4, combined with BMP-2 or BMP-4 can induce cardiogenesis in chick non-precordial mesoderm (16). The non-canonical Wnt/c-Jun-N-terminal kinase pathways have been reported to be essential for cardiac induction in frog and chick embryo systems (17, 18). These factors are prerequisites for early cardiac differentiation but are not sufficient for accomplishing differentiation into mature beating cardiomyocytes. Recently Paquin et al. (19) have reported that oxytocin induces differentiation of P19 embryonic carcinoma cells to beating cardiomyocytes. In support of the role of oxytocin in cardiac development, the oxytocin receptor is increased at the protein level in the murine heart from day 7 of gestation, when cardiac differentiation starts.
cardiomyocytes with oxytocin. Although the precise mechanism of the effect of oxytocin is not clear, oxytocin may play an important role in the differentiation into cardiac myocytes from primitive cells including adult somatic stem cells. Here, we first report that a novel population from Sca-1+ cells derived from the adult murine heart proliferates and differentiates into beating cardiomyocytes with oxytocin treatment.

EXPERIMENTAL PROCEDURES

Animals and Reagents—Wild mice (C57Bl/6) were purchased from Takasugi Experimental Animals Supply, Co, LTD, Japan. All protocols were approved by the Institutional Animal Care and Use Committee of Chiba University. Phycoerythrin (PE) -conjugated anti-Sca-1 (anti-Ly6A/E), anti-c-kit (anti-CD117) antibodies were purchased from eBio-science (San Diego, CA), PE-conjugated anti-CD34, anti-CD45, and biotin-conjugated anti-Sca-1 antibodies were purchased from BD Biosciences (San Jose, CA). rabbit polyclonal anti-connexin 43 (Zymed Laboratories, South San Francisco, CA). Fluorescent secondary antibodies were purchased from Jackson ImmunoResearch Laboratory (Bar Harbor, ME). Other reagents were obtained from Sigma-Aldrich (St. Louis, MO). Osteocalcin (29) was performed using 0.1% SDS, 2% donkey serum, 2% bovine serum albumin and 0.2% Nonidet P-40 for blocking. The samples were fractionated by 2% agarose gel electrophoresis.

RNA Extraction and Reverse Transcriptase-PCR—Total RNA was extracted from the adult murine heart, liver, and Sca-1+ cells by RNA bee reagent (TEL-TEST, Friendswood, TX). Reverse transcriptase (RT)-PCR of genes of cardiac transcription factors, including Csx/Nkx-2.5 (22), GATA4 (23), muscle enhancer factor-2C (MEF-2C) (24), and cardiac structural proteins, including α-actin (30)−actin (27), and oxytocin receptor (19), alkaline phosphatase (28), and osteocalcin (29) were performed using 0.1 μg/ml of streptomycin at 37 °C in humid air with 5% CO₂. Twenty-four hours after seeding, the cells were treated with 10 μM 5-azacytidine for the initial 72 h or 100 ng/ml oxytocin (WAKO, Japan). After treatment, the medium was changed every 3 days.

Characterization of Cardiac Muscle-derived Stem Cells for Flow Cytometric Analysis—Sca-1+ cells were isolated by the MACS system with biotin-conjugated anti-Sca-1 antibody and anti-biotin micro beads. Magnetic sorting was repeated twice, and the cells were incubated with PE-conjugated anti-CD45 antibody, PE-conjugated anti-CD34 antibody, and PE-conjugated anti-c-kit antibody, respectively for 10 min on ice and washed with PBS supplemented with 3% FBS. The percentages of CD45+, CD34+, and c-kit+ cells were analyzed by the EPICS ALTRA flow cytometer using EXPO32 software (Beckman Coulter, Miami, FL).

Immunocytochemistry—Cells were fixed with 4% paraformaldehyde for 15 min at room temperature. After preblocking with PBS containing 2% donkey serum, 2% bovine serum albumin and 0.2% Nonidet P-40 for

| Primer | Product size | Annealing temperature |
|--------|--------------|-----------------------|
| α-MHC  | 302 bp       | 65°C                  |
| β-MHC  | 205 bp       | 66°C                  |
| MLC-2a | 286 bp       | 54°C                  |
| MLC-2v | 499 bp       | 55°C                  |
| GATA4  | 216 bp       | 55°C                  |
| MEF-2C | 303 bp       | 61°C                  |
| ALP    | 450 bp       | 62°C                  |
| Osteocalcin | 92 bp     | 62°C                  |
| β-Actin| 583 bp       | 60°C                  |

(20). Although the precise mechanism of the effect of oxytocin is not clear, oxytocin may play an important role in the differentiation into cardiomyocytes from primitive cells including adult somatic stem cells. Here, we first report that a novel population from Sca-1+ cells derived from the adult murine heart proliferates and differentiates into beating cardiomyocytes with oxytocin treatment.
30 min, primary antibodies in PBS containing 2% donkey serum, 2% bovine serum albumin and 0.1% Nonidet P-40 were applied overnight in 4°C. Subsequently cells were washed three times in PBS, and then fluorescein isothiocyanate- or Cy5-conjugated secondary antibodies were applied to visualize expression of specific proteins. Nuclear staining was performed with TO-PRO-3 (Molecular Probes, Eugene, OR). Images of cells were taken by laser confocal microscopy (Radiance2000, Bio-Rad, Hercules, CA).

**Phase Contrast Live Imaging**—Live images were taken by a Zeiss inverted microscope (Carl Zeiss, Jena, Germany) equipped with phase-contrast objectives and an AxioCam camera. Live image of beating cells were obtained by a chilled CCD camera (Hamamatsu) using I-O DATA Videorecorder software.

**Measurement of Intracellular Ca²⁺ Concentration**—Intracellular Ca²⁺ concentration ([Ca²⁺]i) in beating cells derived from cardiac Sca-1+ cells was measured as previously described (31). The beating cells on gelatin-coated glass coverslips were incubated in HEPES (loading) solution containing 1 μM fluo 3-acetoxymethyl ester (fluo 3-AM; Molecular Probes) at 36°C in the dark for 30 min. The loading solution was prepared by diluting a 100 μM fluo 3 stock solution, which contained 0.45% Pluronic F127 (Molecular Probes), 10% dimethyl sulfoxide, and 90% FBS. HEPES solution consisted of (in mM) 126 NaCl, 4.4 KCl, 1.0 MgCl₂, 1.08 CaCl₂, 24 HEPES, 13 NaOH, 11 glucose, and 0.5 probenecid (pH 7.4). The coverslips were washed twice with dye-free HEPES solution and placed in a flow-through chamber on the microscope. Fluo 3-loaded beating cells were excited by 480-nm light and emitted fluorescence was recorded at 530 nm by a photomultiplier tube (AIM-10, InterMedical, Co, Japan) and digitized (PowerLab 2/20, AD-instruments, Castle Hill, Australia). Curve fits were performed with Origin 21 software (MicroCal Software, Northampton, MA). The intensity of the fluorescence at 530 nm increased with an increase in [Ca²⁺]i.

**Estimation of Pluripotency**—Osteogenic differentiation of Sca-1+ cells from the adult murine heart was induced in IMDM supplemented with 10% FBS, 50 μM ascorbic acid 2-phosphate, 0.1 μM dexamethasone, and 10 μM β-glycerophosphate.
Cell Surface Antigens of Sca-1+ Cells Derived from the Adult Murine Heart—Flow cytometric analysis revealed that Sca-1+ cells were enriched to over 90% when adult murine cardiac cells were sorted twice with the MACS system using PE-conjugated anti-Sca-1 antibody and anti-PE micro beads (Fig. 1A). The number of purified Sca-1+ cells was ~1 × 10^5 cells. Limana et al. (33) have estimated the number of cardiomyocytes in an adult murine heart as ~3 × 10^8. Therefore the percentage of cardiac Sca-1+ cells was ~0.3% of the total number of cardiomyocytes. Next we examined other cell surface antigens such as CD45, CD34, and c-kit in Sca-1+/H11001 cells. When Sca-1+ cells were cultured with vehicle (Fig. 3, lane A), two weeks after treatment with 5-azacytizine, a fraction of cells expressed sarcomeric myosin heavy chain (Fig. 4D), and tropomyosin (data not shown) were also expressed. Notably, staining of each contractile protein showed a fine striated pattern. Connexin 43 was expressed at the junction between two cardiac troponin T-expressing cells (Fig. 4E). These findings indicate that treatment with oxytocin induced differentiation of Sca-1+ cells, derived from the adult murine heart, into mature cardiomyocytes, which had well-organized structures and electrical junctions. After treatment with 5'-azacytizine, a fraction of cells expressed sarcomeric myosin heavy chain in fibrillar pattern (Fig. 4F), but not cardiac troponin T (data not shown). Next we sorted cardiac Sca-1+ cells on the basis of CD45 expression and cultured with oxytocin. Some of the Sca-1+/CD45+ cells expressed sarcomeric myosin after oxytocin treatment, but none of the Sca-1+/CD45+ cells expressed myosin (data not shown), suggesting that Sca-1+ cells that can differentiate into cardiomyocytes are in CD45− population.

Cardiac contraction is regulated by heat to beat change in [Ca^{2+}]. To ascertain that the spontaneous beating of differentiated cardiac Sca-1+ cells depends on intracellular level of Ca^{2+}, we analyzed [Ca^{2+}]^i transients of the beating cells. As shown in the upper panel of Fig. 5A, the spontaneous beating of differentiated Sca-1+ cells was accompanied with [Ca^{2+}]^i transients. After treatment with 10^{-5} M isoproterenol for 5 min, the frequency of [Ca^{2+}]^i transients was increased in comparison with control (Fig. 5A, upper panel versus lower panel). Next we examined the predominant subtype of β receptors, which mediates changes in beating rate. Differentiated cardiac Sca-1+ cells were treated with vehicle (PBS), propranolol, CGP20712A (β1-selective blocker), or ICI118551 (β2-selective blocker) for 30
Fig. 3. RT-PCR analysis of cardiac genes. Sca-1+ cells after treatment with oxytocin (lane OT) or 5’-azacytizine (lane A) expressed Csx/Nkx-2.5, GATA4, MEF-2C, α-MHC, β-MHC, MLC-2a, MLC-2v, and cardiac α-actin. Although Sca-1+ cells before treatment (lane P) expressed Csx/Nkx-2.5 and GATA4 slightly, none of cells after treatment with vehicle (lane V) or oxytocin antagonist combined with oxytocin (lane OT+OTA) expressed any cardiac transcription factors. Heart (lane H) and liver (lane L) were used as positive and negative controls, respectively.

Fig. 4. Immunocytochemical analysis of cardiac proteins. A–E, cardiac differentiation of Sca-1+ cells after oxytocin treatment. Cells were double-stained using anti-GATA4 antibody (A, green), anti-ANF antibody (B, green), and anti-cardiac troponin T antibody (A and B, blue). Cells were stained with anti-MLC-2v (C, green) and anti-sarcomeric myosin heavy chain antibodies (D, green), and nuclei were stained with TO-PRO-3 (C and D, blue). Cells were double-stained using anti-cardiac troponin T antibody (E, green) and anti-connexin 43 antibody (E, blue). F, differentiation of Sca-1+ cells after 5’-azacytizine treatment. Cells were stained with anti-sarcomeric myosin heavy chain (F, green) and TO-PRO-3 (F, blue). Bars, 50 μm.

In this report, we have first demonstrated that adult cardiac Sca-1+ cells can differentiate into beating cardiomyocytes in vitro by treatment with oxytocin. When treated with oxytocin, the Sca-1+ cells expressed cardiac genes including Csx/Nkx-2.5, GATA4, MEF-2C, α-MHC, β-MHC, MLC-2a, MLC-2v, and cardiac α-actin, and cardiac proteins including GATA4, cardiac troponin T, tropomyosin, MLC-2v, sarcomeric myosin heavy chain, ANF, and connexin 43. Furthermore, some of Sca-1+ cells showed well organized sarcomere and spontaneous beating. Although transient treatment with 5’-azacytizine also induced expression of cardiac genes in Sca-1+ cells, it did not induce expression of cardiac troponin T, assembly of sarcomere or spontaneous beating. These results suggest that treatment with 5’-azacytizine induces differentiation of Sca-1+ cells into cardiomyocytes incompletely and that oxytocin is a more potent inducer of cardiac differentiation than 5’-azacytizine.

P19 teratocarcinoma cells differentiate into beating cardiomyocytes after treatment with Me2SO and have been considered as a good model of in vitro cardiogenesis (34, 35). Several

DISCUSSION

In this report, we have first demonstrated that adult cardiac Sca-1+ cells can differentiate into beating cardiomyocytes in vitro by treatment with oxytocin. When treated with oxytocin, the Sca-1+ cells expressed cardiac genes including Csx/Nkx-2.5, GATA4, MEF-2C, α-MHC, β-MHC, MLC-2a, MLC-2v, and cardiac α-actin, and cardiac proteins including GATA4, cardiac troponin T, tropomyosin, MLC-2v, sarcomeric myosin heavy chain, ANF, and connexin 43. Furthermore, some of Sca-1+ cells showed well organized sarcomere and spontaneous beating. Although transient treatment with 5’-azacytizine also induced expression of cardiac genes in Sca-1+ cells, it did not induce expression of cardiac troponin T, assembly of sarcomere or spontaneous beating. These results suggest that treatment with 5’-azacytizine induces differentiation of Sca-1+ cells into cardiomyocytes incompletely and that oxytocin is a more potent inducer of cardiac differentiation than 5’-azacytizine.

P19 teratocarcinoma cells differentiate into beating cardiomyocytes after treatment with Me2SO and have been considered as a good model of in vitro cardiogenesis (34, 35). Several
essential transcription factors in cardiomyogenesis such as GATA4 (34), Csx/Nkx-2.5 (34), and MEF-2C (36) are up-regulated in P19 cells treated with Me2SO. Recently Paquin et al. (19) have reported that oxytocin induces P19 embryonic carcinoma cells to differentiate into cardiomyocytes. Treatment with oxytocin as well as with Me2SO induced colony formation of beating cardiomyocytes, expression of cardiac proteins, and oxytocin receptor proteins. In this study, cardiac Sca-1+ cells expressed low levels of oxytocin receptor mRNA that were positively regulated by oxytocin itself, and pretreatment with oxytocin antagonist inhibited oxytocin-induced oxytocin receptor up-regulation. Oxytocin receptors are coupled to Gq/11 class GTP-binding proteins and stimulate the generation of inositol trisphosphate and diacylglycerol, leading to Ca2+ release and activation of protein kinase C (37). Oxytocin stimulates cell proliferation through calcium (38, 39) and protein kinase C pathways (38). Cassoni et al. (40) have reported that oxytocin stimulates cell

![Graph](image)

**FIG. 5.** Physiological analysis of differentiated cardiac Sca-1+ cells. A, [Ca2+]i transients of beating cells derived from cardiac Sca-1+ cells before (upper panel) and after (lower panel) treatment with isoproterenol. B, the effects of subtype-specific β receptor blockers on isoproterenol-induced increase in beating rate of differentiated cardiac Sca-1+ cells. Preincubation with 10−6 M propranolol (nonselective β blocker) and 10−7 M CGP20712A (β1-selective blocker) reduced isoproterenol-induced increase in beating rate significantly but 10−7 M ICI118551 (β2-selective blocker) did not. *, p < 0.01 versus control; **, p < 0.05 versus isoproterenol only; n.s., not significant.

![RT-PCR analysis](image)

**FIG. 6.** RT-PCR analysis of oxytocin receptor expression in Sca-1+ cells. Oxytocin receptor mRNA was present at low levels in Sca-1+ cells before treatment (lane P). After oxytocin treatment, the oxytocin receptor mRNA expression was up-regulated (lane OT), but oxytocin antagonist inhibited oxytocin-induced oxytocin receptor up-regulation (lane OT+OTA).

![Bone and fat cell differentiation](image)

**FIG. 7.** Osteogenic and adipogenic differentiation potential of Sca-1+ cells derived from the adult murine heart. A, osteogenic differentiation of Sca-1+ cells was induced by treatment with ascorbic acid 2-phosphate, dexamethasone, and β-glycerophosphate for 3 weeks. Alkaline phosphatase staining (blue) was used for detection of osteocytes. B, RT-PCR experiment clearly revealed that osteogenic marker mRNAs such as alkaline phosphatase (ALP) and osteocalcin were induced in Sca-1+ cells by treatment with osteogenic inducers (lane P for pretreatment, lane V for vehicle treatment, lane O for osteogenic induction). Sca-1+ cells treated with oxytocin never expressed osteogenic marker mRNAs (lane OT for oxytocin treatment). β-actin was used as an internal control. C, adipogenic differentiation of Sca-1+ cells was induced by treatment with adipogenic mixture (MDI-I) for twelve days. Oil-Red O staining showed adipogenic differentiation of Sca-1+ cells. Hematoxylin was used for counterstaining of nuclei. Bars, 50 μm.
proliferation through oxytocin receptors that lead to an increase in intracellular Ca²⁺ and tyrosine phosphorylation. Tyrosine phosphorylation in oxytocin signaling has been reported to activate both p38 mitogen-activated protein kinase and extracellular signal-regulated kinase 2 (41, 42). The mechanism by which oxytocin stimulates tyrosine phosphorylation has not been elucidated, but may be mediated by Gβγ subunit dissociating from Gα subunit. Oxytocin inhibits the proliferation of human brain tumors (43), breast cancer cells (44), and adenocarcinoma of endometrium (45) via the cyclic adenosine monophosphate-protein kinase A pathway. Tahara et al. (46) have reported that the RhoA/Rho-kinase cascade is involved in oxytocin-induced rat uterine contraction. Among the considerable diversity of oxytocin-mediated signaling pathways, the specific pathway that activates cardiogenesis is currently unknown. Recently post-translational modification of cardiac transcription factors has been reported to be important for their transcriptional activities. Rho-like GTPases can phosphorylate GATA4 via activation of the p38 mitogen-activated protein kinase pathway, which enhances the potency of GATA4 (47). MEF2 is stimulated by calmodulin kinase activation in the heart (48). It remains to be determined which oxytocin signaling pathways are important for differentiation of cardiomyocytes.

It has been reported that c-kit+, Sca-1+, lineage-, and CD34-flow fraction of bone marrow cells contain hematopoietic stem cells, which contribute to long term multilineage reconstitution of the blood system in mice (49). Orič et al. (50) and Gojo et al. (10) have reported that c-kit+ bone marrow cells and c-kit+ bone marrow-derived mesenchymal cells transdifferentiate into cardiomyocytes in vitro, suggesting that c-kit is one of the cell surface markers of multipotent stem cells in bone marrow. The multipotential stem cells also reside in skeletal muscle, although the origin of the stem cells is still controversial (51). Skeletal muscle-derived stem cells reported by Qu-Petersen et al. (11) and Torrente et al. (13) highly express CD34 and Sca-1 but not c-kit and CD45 and differentiate into neural and endothelial cells. In our study, cardiac Sca-1+ cells expressed low levels of c-kit, suggesting that the features of stem cell markers on cardiac stem cells is distinct from bone marrow-derived stem cells and rather similar to skeletal muscle-devised stem cells.

Tamaki et al. (14) isolated CD34+ and CD45− cells from the interstitial space of skeletal muscle, which highly expressed Sca-1 but not other endothelial progenitor cell markers. The CD34+/CD45− cells differentiated into adipocytes, endothelial and myogenic cells and expressed Bcrp1/ABCG2 gene mRNA, which is an important determinant of the SP phenotype. Recently Polesskaya et al. (52) have reported that CD45+/Sca-1+ cells from injured skeletal muscle differentiate into myoblasts much more than CD34−/Sca-1 cells. Because the hematopoietic restricted expression of CD45 antigen, skeletal myogenic CD45+/Sca-1+ cells might be of hematopoietic origin. In our study, cardiac Sca-1+ cells expressed low levels of CD34 and ~40% of the cardiac Sca-1+ cells expressed CD45, one of hematopoietic cell markers. We sorted cardiac Sca-1+ cells on the basis of CD45 expression and cultured them with oxytocin. Some Sca-1+/CD45− cells expressed sarcomeric myosin after oxytocin treatment, but no Sca-1+/CD45+ cells expressed myosin (data not shown), suggesting that Sca-1+ cells that can differentiate into cardiomyocytes are in the CD45− population. Therefore, in terms of the expression of CD34 and CD45, the cardiac muscle stem cells are distinct from the previously reported skeletal muscle-derived stem cells.

Sca-1+ cells from the adult heart expressed GATA4 and Csx/Nkx-2.5, but not Oct-3/4 before treatment with oxytocin (data not shown), suggesting that the Sca-1+ cells are committed to cardiomyocytes to some degree. Makino et al. (24) have reported that mouse bone marrow-derived mesenchymal stem cells (CMG cells) differentiate into cardiomyocyte after 5′-aza-cytosine treatment. Although the cell surface antigens of CMG cells were not analyzed, the bone marrow-derived mesenchymal stem cells, which differentiated into cardiomyocytes after 5′-aza-cytosine treatment in vitro, expressed Sca-1, c-kit, and CD34 (10), suggesting that the cardiac Sca-1+ cells are different from bone marrow-derived mesenchymal stem cells. Cardiac Sca-1+ cells differentiated into osteocytes and adipocytes in appropriate conditions, suggesting that cardiac Sca-1+ cells have the intragerm layer multipotency. It remains to be determined whether the cardiac Sca-1+ cell population contains stem cells capable of differentiating to extra germ layer lineage.

The spontaneously beating differentiated cardiac Sca-1+ cells showed [Ca²⁺], transients and treatment with isoproterenol increased the frequency of [Ca²⁺], transients and beating rate. The similar response to isoproterenol has been reported in adult murine cardiomyocytes (53), embryonic stem cells-derived cardiomyocytes (54), and CMG cells (55). The β₁-selective blocker, CGP20712A, significantly reduced isoproterenol-induced increase in beating rate to the same extent as the non-selective β-blocker, propranolol, but the β₂-selective blocker, ICI118551, did not. These results suggest that the β₁ receptor is the predominant subtype that mediates the changes in beating rate of cardiomyocytes derived from Sca-1+ cells.

During the preparation of this article, two studies on cardiac stem cells were reported (56, 57). They have shown that c-kit+ or Sca-1+ cells derived from the adult murine heart express cardiac genes and proteins after the cardiogenic induction. We showed for the first time that there are potential adult cardiac stem cells that have an ability to proliferate and differentiate into various types of cells including beating cardiomyocytes in vitro. Although the role of cardiac stem cells is uncertain, our results suggest their possible role in cardiac repair. In addition, the understanding of precise molecular mechanisms of the differentiating process of cultured cardiac stem cells may provide us with new insights into cardiac development and regeneration.

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**REFERENCES**

1. Kajstura, J., Leri, A., Finato, N., Di Loreto, C., Beltrami, C. A., and Anversa, P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8801–8805
2. Leri, A., Barluchì, L., Limana, F., Deptała, A., Darzynkiewicz, Z., Hinte, T. H., Kajstura, J., Nadal-Ginard, B., and Anversa, P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8626–8631
3. Beltrami, A. P., Urbanek, K., Kajstura, J., Yan, S. M., Finato, N., Bussani, R., Nadal-Ginard, B., Silvestri, F., Leri, A., Beltrami, C. A., and Anversa, P. (2001) N. Eng. J. Med. 344, 1750–1757
4. Quaini, F., Urbanek, K., Beltrami, A. P., Finato, N., Beltrami, C. A., Nadal-Ginard, B., Kajstura, J., Leri, A., and Anversa, P. (2002) N. Eng. J. Med. 346, 5–15
5. Anversa, P., and Nadal-Ginard, B. (2002) Nature 415, 240–243
6. Asakura, A., and Rudnichi, M. A. (2002) Exp. Hematol. 30, 1339–1345
7. Hierihly, A. M., Seale, P., Lobo, C. G., Rudnichi, M. A., and Megeny, L. A. (2002) FASEB J. 16, 239–243
8. van der Rijn, M., Heimfeld, S., Spangrude, G. J., and Wiessman, I. L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4634–4638
9. Okumoto, K., Saito, T., Hatatori, R., Hs, J. J., Adachi, T., Takeda, T., Sugahara, K., Watanabe, H., Saito, K., Togashi, H., and Kawata, S. (2003) Biochem. Biophys. Res. Commun. 304, 691–695
10. Gojo, S., Gojo, N., Takeda, Y., Moro, T., Abe, H., Kyo, S., Hata, J., and Umezawa, A. (2003) Exp. Cell Res. 288, 51–59
11. Qu-Petersen, Z., Deasy, B., Jankowski, R., Ikezawa, M., Cummins, J., Puchta, R., Mytinger, J. C., Gates, C., Wernig, A., and Huard, J. (2002) J. Cell Biol. 157, 851–864
12. Asakura, A., Seale, P., Girgis-Gabardo, A., and Rudnichi, M. A. (2002) J. Cell Biol. 159, 123–134
13. Torreira, Y., Tremblay, J. P., Pinti, F., Belicchi, M., Rossì, B., Sironi, M., Fortunato, F., El Fahime, M., D’Angelo, M. G., Caron, N. J., Constantin, G., Paulin, D., Scarlato, G., and Bresolin, N. (2001) J. Cell Biol. 152, 335–348
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The MLC-2v panel was duplicated in the cardiac α-actin panel of the original Fig. 3. We have now replaced the cardiac α-actin panel with the correct panel. This correction does not change the interpretation of the results or the conclusions.