Stretch-induced Hypertrophic Growth of Cardiocytes and Processing of Brain-type Natriuretic Peptide Are Controlled by Proprotein-processing Endoprotease Furin*

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Yoshie Sawada‡‡, Masayuki Suda‡, Hironori Yokoyama‡, Tsugiyasu Kanda‡, Tetsuo Sakamaki‡, Shigeyasu Tanaka, Ryozo Nagaishi, Shuzo Abe** and Toshiyuki Takeuchi‡‡

From the Departments of ‡‡Molecular Medicine and ||Cell Biology, the Institute for Molecular and Cellular Regulation, the ‡Second Department of Internal Medicine, ||Department of Laboratory Medicine, Gunma University School of Medicine, Maebashi 371, and the **Union Co., Takasaki 370, Japan

When hypertrophic growth is induced in neonatal rat cardiocytes by stretching, the cardiocytes express high levels of brain-type natriuretic peptide (BNP) and the proprotein-processing enzyme furin. A BNP precursor, γBNP, possesses a furin-cleavable Arg-X-X-Arg motif, which is cleaved when γBNP is processed to form BNP-45. The Arg-X-X-Arg motif is found in many precursors of growth factors and growth-related proteins. To determine if furin converts γBNP to BNP-45 as well as other unidentified growth-promoting protein precursors to their active form that may induce hypertrophic growth in cardiocytes, we used two protease inhibitor systems, synthetic peptidyl chloromethyl ketones (CMK) (dec-Arg-Val-Lys-Arg-CMK and dec-Phe-Ala-Lys-Arg-CMK; where dec is decanoyl) and vaccinia vector-integrated native and variant α1-antitrypsins. The furin-specific inhibitors, dec-Arg-Val-Lys-Arg-CMK and variant α1-antitrypsin with the inhibitory determinant Arg-X-X-Arg, suppressed the stretch-induced hypertrophic growth of cardiocytes as well as the processing of γBNP to BNP-45. The other serine protease inhibitors and variant α1-antitrypsin against elastase, or thrombin, however, neither suppressed the hypertrophic growth nor prevented the processing of γBNP to BNP-45. Thus, we suggest that furin catalyzes the conversion of γBNP to BNP-45 as well as growth-promoting proproteins to their active form, which might induce hypertrophic growth in cardiocytes.

When cardiocytes undergo the stretch force, they adapt by developing cellular hypertrophy. Because cardiocytes are terminally differentiated and cannot proliferate by cell division, they exhibit hypertrophic growth, an increase in cell size and protein content. The hypertrophic growth of cardiocytes is attained through a series of multiple gene expressions (1–4) and phosphorylation reactions (4, 5) as follows: expression of immediate early genes such as c-fos, c-jun, and Egr-1; consequent protein kinase cascade of phosphorylation reactions and expression of peptide growth factors such as fibroblast growth factor and transforming growth factor-β (TGF-β)1; cardiac regulatory peptides such as atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP); the contractile proteins myosin light chain-2 and fetal-type β-myosin heavy chain; and probably extracellular matrix-degrading metalloproteinases in a remodeling step (6). Some of these proteins are produced as precursors, including TGF-β (7, 8), ANP (9, 10), BNP (10), and metalloproteinases including membrane-type matrix metalloproteinase (11) and stromelysin-3 (12). The precursors are cleaved to the active forms by endoproteases. Because TGF-β, BNP, membrane-type matrix metalloproteinase, and stromelysin-3 contain a cleavage site for the proprotein-processing endoprotease furin (7, 10–12), we examined whether furin might be another candidate gene expressed during the hypertrophic growth of cardiocytes.

Furin belongs to the yeast Kex2 endoprotease family, to which the neuroendocrine cell-specific endoproteases PC2 and PC3 also belong (13, 14). Furin is localized on the trans-Golgi networks of virtually all cell types including neuroendocrine cells (15). Cleavage by furin is specific for a unique amino acid sequence, -Arg-1-X-X-Arg-2 \( \downarrow \) X-3 (16). An additional basic amino acid at the -2 or -6 position facilitates more efficient cleavage (17). This furin-specific Arg-X-X-Arg (RXXR) motif is found in many growth-promoting peptides or proteins as listed above. In addition to this cleavage reaction, furin is known to exhibit another function, recycling between the trans-Golgi network and plasma membrane (15, 18). With this recycling, furin expression may increase protein flow through a constitutive pathway, which results in an augmented transport of growth-promoting peptides or proteins outside the cells.

One of the furin-cleavable peptides, BNP, regulates blood pressure, diuresis, natriuresis, and dilation of vascular smooth muscles (19, 20). In congestive heart failure and hypertrophic cardiomyopathy, BNP is known to be produced extensively from both the atrium and ventricle. Another important cardiac regulator, ANP, also increases these pathological conditions in the atrium and, in severe cases, in the ventricle where ANP is not normally expressed (21). Because the rise of BNP is more marked than that of ANP in congestive heart failure and hypertrophic cardiomyopathy, BNP is thought to be a more val-

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‡‡ To whom correspondence should be addressed: Dept. of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Showa-machi, Maebashi 371, Japan. Tel.: 011-81-272-20-8855; Fax: 011-81-272-20-8896; E-mail: tstake@news.gunma-u.ac.jp

1 The abbreviations used are: TGF-β, transforming growth factor-β; BNP, brain-type natriuretic peptide; irBNP, immunoreactive BNP; dec, decanoyl; CMK, chloromethyl ketones; ANP, atrial natriuretic peptide; RIA, radioimmunoassay; PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography; MCA, methylcelamamidie; boc, t-butyloxycarbonyl; pyr, pyroglutamyl; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; VV, vaccinia virus; PTHrP, parathyroid hormone-related protein; TLCK, tetrachloro-3-(4-tosylamido)-7-amino-2-heptanone; m.o.i., multiplicity of infection.
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**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The primary culture of neonatal ventricular cardiocytes was prepared according to the method previously described (28, 29), with a minor modification. Briefly, a ventricle was removed from 1- to 3-day-old Wistar rats, rinsed with phosphate-buffered saline (PBS), minced and dispersed with 0.08% trypsin in PBS for 10 min at 37 °C, and pipetted approximately 30 times. The suspension was lightly centrifuged, and the supernatant was removed. Three to four further digestions with the trypsin solution were performed, and the supernatants from each digestion were combined with Dulbecco’s modified Eagle’s medium (DMEM/Ham’s F12 medium (1:1, v/v, Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml amidocillin. The solutions were then centrifuged at 1300 rpm for 3 min. The pellet was gently resuspended in DMEM/F12/FBS, and the cells were plated onto a plastic dish (100 mm, Falcon, Oxnard, CA) and incubated at 37 °C for 90 min. During the incubation, most non-cardiocytes attached to the floor of the dish, while cardiocytes remained suspended. The non-attached cells were then seeded into six-well plates with a silicone membrane bottom (FLEX I culture plates; Flexcell Corp., McKeensport, PA) in 2 ml of DMEM/F12/FBS. The cells were incubated for 48 h at 37 °C under a humidified atmosphere of 95% air, 5% CO2. When the cells exhibited synchronous contractions, the cells were then utilized for stretch experiments.

**Mechanical Stretch of Cardiocytes**—The silicone membrane bottoms of the FLEX I culture plates were attached to suction tubes. The attachment was sealed tightly with plastic glue (Tayo Electric Ink., Tokyo, Japan) for vacuum suction. Membrane bottoms were distended using suction at 120 and 180 mmHg, with a minor modification. Briefly, a ventricle was removed from 1- to 3-day-old Wistar rats, rinsed with phosphate-buffered saline (PBS), minced and dispersed with 0.08% trypsin in PBS for 10 min at 37 °C, and pipetted approximately 30 times. The suspension was lightly centrifuged, and the supernatant was removed. Three to four further digestions with the trypsin solution were performed, and the supernatants from each digestion were combined with Dulbecco’s modified Eagle’s medium (DMEM/Ham’s F12 medium (1:1, v/v, Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml amidocillin. The solutions were then centrifuged at 1300 rpm for 3 min. The pellet was gently resuspended in DMEM/F12/FBS, and the cells were plated onto a plastic dish (100 mm, Falcon, Oxnard, CA) and incubated at 37 °C for 90 min. During the incubation, most non-cardiocytes attached to the floor of the dish, while cardiocytes remained suspended. The non-attached cells were then seeded into six-well plates with a silicone membrane bottom (FLEX I culture plates; Flexcell Corp., McKeensport, PA) in 2 ml of DMEM/F12/FBS. The cells were incubated for 48 h at 37 °C under a humidified atmosphere of 95% air, 5% CO2. When the cells exhibited synchronous contractions, the cells were then utilized for stretch experiments.

**Assessment of Cell Growth**—The hypertrophic growth of cardiocyte was assessed with the enlargement of cell size by a cell sorter, the protein/DNA ratio of cardiocytes was obtained from the values of protein and DNA per dish (5 × 105 cells). The total DNA content in homogenized cells was determined using a fluorescent dye 4',6-diamidino-2-phenylindole (31). 4',6-Diamidino-2-phenylindole was dissolved at 100 ng/ml in the buffer 100 mM NaCl, 10 mM EDTA, and 10 mM Tris, pH 7.0. Salmon sperm DNA was used as a standard. Fluorescence was measured by excitation at 360 nm and emission at 450 nm. Protein concentration was measured using the Bradford method (Bio-Rad) using bovine serum albumin as a standard.

**Radioimmunoassay for BNP**—Radioimmunoassay (RIA) was carried out using an RIA kit for rat BNP-45 (BIA 9085, Peninsula Laboratories, Belmont, CA), which utilizes an antibody to rat BNP-45 generated in rabbits in response to a synthetic rat BNP-45 (32, 33). The antibody was added to test samples and incubated for 16–24 h at 4 °C.125I-Labeled BNP (between 1 and 100 μCi/ml) was added and incubated again at 4 °C for 16–24 h. The second antibody (goat anti-rabbit immunoglobulin G serum mixed with normal goat serum) was added to the above mixture and then incubated at room temperature for 90 min. BIA buffer supplied with the kit was added, and the samples were vortexed and centrifuged for 20 min at 3000 rpm. The supernatant was removed, and the radioactivity in the pellets was counted in a gamma counter.

**BNP Extraction and HPLC Analysis**—BNP was extracted from cardiocytes or culture medium. For the BNP extraction from cardiocytes, the freeze-dried sample was homogenized in 5 ml of ice-cold 5% acetic acid (containing 0.3 mg/ml phenylmethylsulfonyl fluoride) using a ground-glass homogenizer, as described previously (32). The supernatants, obtained after centrifugation at 100,000 × g for 30 min, were loaded onto a Sep-Pak C18 cartridge (Millipore, Milford, MA). For the extraction from the culture medium the medium was lightly centrifuged to remove contaminating cells or cell debris, then lyophilized, and reconstituted to 4 ml by adding 4% acetic acid. The acido solution was passed through a Sep-Pak C18 cartridge.

After washing the cartridge with 0.5 ml acetic acid, adsorbed peptides were eluted with 60% acetonitrile containing 0.1% trifluoroacetic acid. Each eluate was evaporated under vacuum prior to lyophilization. Lyophilized samples were dissolved in 0.1% trifluoroacetic acid and applied to a reverse-phase HPLC analyzer (Hitachi L-6200). HPLC analysis was carried out using a nucleosil 10C18 column (4.6 × 150 mm, Chemco Pak, Osaka, Japan). The column was equilibrated with 0.1% trifluoroacetic acid and eluted with a linear gradient of 0–60% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. Aliquots of all fractions were subjected to RIA for BNP-45. The endogenous converting activity of BNP to BNP-45 was assumed as being measured using the yBPN and BNP-45 fractions in cardiocyte lysates and those released into the culture medium from the cardiocytes. The ratio between the two fractions was determined by separating the immunoreactive (ir) BNP on HPLC (34).

**Northern Blot Analysis**—Total RNA was isolated from cardiocytes and electrophoresed on a 1% agarose gel as described previously (28).
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35. RNA electrophoretic bands were blotted onto a nylon membrane (Hybond-N, Amersham Japan, Tokyo, Japan). Hybridization was performed in a solution consisting of 5 × SSPE (20 × SSPE, 3.6 mM NaCl, 200 mM NaHPO4, 20 mM EDTA, pH 7.4), 10 × Denhardt’s reagent (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 50% formamide, 1.4% sodium dodecyl sulfate (SDS), and 0.1 mg/ml salmon sperm DNA with either a 32P-labeled mouse furin cDNA fragment (924 base pairs; 36), or the 628-base pair rat BNP cDNA fragment (35). After washing, the membrane was exposed to x-ray film (Kodak, XAR 5).

Western Blot Analysis—For immunodetection of furin, the cells were cultured on coverslides. The nuclei for preparation of cell lysates in lysis buffer (70 mM Tris-HCl, pH 6.8, 11.2% glycerol, 3% SDS, 0.01% bromphenol blue, 5% 2-mercaptoethanol). Cell lysates were electrophoresed on a 7.5% polyacrylamide gel under the reducing condition. Separated proteins were blotted onto a nitrocellulose membrane and probed with rabbit anti-furin antiserum (ST-73) at 1:5000 dilution. Furin blots were detected using an enhanced chemiluminescence detection system (Amerham, Buckinghamshire, UK).

Immunocytochemical Studies—Immunostaining of furin was performed as described previously (34, 35). Briefly, the cells were cultured on the silicon membrane plate. After half of the membrane bottoms were stretched, non-stretched and stretched cells were fixed with 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 h at room temperature. The specimens were incubated for 2 h with rabbit anti-furin serum (ST-73) and then incubated with indocarbocyanine-conjugated affinity-purified donkey anti-rabbit immunoglobulin G (Jackson Immuno Research Lab, West Grove, PA), diluted with the blocking buffer (1% bovine serum albumin, 0.2% skim milk, 0.5% Triton X-100, 0.1% sodium azide), and incubated for 2 h, followed by mounting. The specimens were examined with an Olympus BX50 microscope with an incident illuminator.

Enzyme Assay—Processing enzyme activities in cardiocytes was assessed by using the three enzyme substrates pyroglutamyl-arginyl-furin serum (ST-73) and then incubated with indodicarbocyanine-conjugated affinity-purified donkey anti-rabbit immunoglobulin G (Jackson Immuno Research Lab, West Grove, PA), diluted with the blocking buffer (1% bovine serum albumin, 0.2% skim milk, 0.5% Triton X-100, 0.1% sodium azide), and incubated for 2 h, followed by mounting. The specimens were examined with an Olympus BX50 microscope with an incident illuminator.

Effect of Protease Inhibitors on Furin-type Enzyme—The potency of other processing enzyme inhibitors decanoyl-arginyl-valyl-lysyl-arginyl-methylcoumarinamide (pyr-Arg-Thr-Lys-Arg-MCA), boc-Ala-Gly-Pro-Arg-MCA, and boc-Gly-Lys-Arg-MCA are designed to assess furin, rat ANP precursor processing enzyme, and paired basic residue cleavage enzyme, respectively. The enzyme activity was measured by the production of liberated fluorescent aminomethylcoumarin (AMC) from the carboxyl terminus of a synthetic peptide substrate (34, 38). The enzyme source was prepared by cultured cardiocytes in PBS containing 1 mM CaCl2 and 2% octylglucoside, as described previously by Garten et al. (39). To 0.20 ml of cell lysate 20 nmol of a synthetic substrate was added to make a total volume of 0.25 ml consisting of 50 mM HEPES, pH 7.3, 1 mM CaCl2, and 2% octylglucoside. The enzyme reaction was performed at 37 °C for 1 h and then 2.0 ml of 5 mM EDTA was added to stop the reaction. The liberated AMC was fluorometrically measured by excitation at 380 nm and emission at 460 nm.

Effect of Protease Inhibitors on Furin-type Enzyme—The potency of other processing enzyme inhibitors decanoyl-arginyl-valyl-lysyl-arginyl-chloromethyl ketone (dec-Arg-Val-Lys-Arg-CMK) and decanoyl-phenylalanine-lysyl-arginyl-chloromethyl ketone (dec-Phe-Ala-Lys-Arg-CMK) (a kind gift of Dr. W. Garten, Marburg) was assessed by adding these inhibitors (up to 50 μM) to the above described enzyme assay of cardiocyte lysates (39, 40). The inhibitor dec-Arg-Val-Lys-Arg-CMK inhibits furin; and dec-Phe-Ala-Lys-Arg-CMK inhibits paired basic residue cleavage enzyme, respectively.

Another inhibition system of furin cleavage is the vaccinia virus (VV) infection (924 base pairs; 36), or the 628-base pair rat BNP cDNA fragment (35). RNA electrophoretic bands were blotted onto a nylon membrane (Hybond-N, Amersham Japan, Tokyo, Japan). Hybridization was performed in a solution consisting of 5 × SSPE (20 × SSPE, 3.6 mM NaCl, 200 mM NaHPO4, 20 mM EDTA, pH 7.4), 10 × Denhardt’s reagent (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 50% formamide, 1.4% sodium dodecyl sulfate (SDS), and 0.1 mg/ml salmon sperm DNA with either a 32P-labeled mouse furin cDNA fragment (924 base pairs; 36), or the 628-base pair rat BNP cDNA fragment (35). After washing, the membrane was exposed to x-ray film (Kodak, XAR 5).

Effect of Protease Inhibitors on the Hypertrophic Growth of Cardiocytes and the Processing of BNP—To examine the effect of the peptidyl-CMK on the stretched-induced hypertrophic growth of cardiocytes, the cells were incubated with the inhibitor for 4 h at a concentration of 50 μM. To examine the effect of α1-antitrypsin on hypertrophic growth, the cells were incubated with wild strain WR, VV-α1-PDX, VV-PIT, or VV-α1-PDX for 1 h (m.o.i. = 5, 20, or 50). The viral solution was washed out with PBS-M; DME/F-12/FBS was added; the cells were then treated for another 4 h; and the cells were harvested and applied to the cell sorter for analysis.

To examine the inhibitory effect of the peptidyl-CMKs on the conversion of y-BNP to BNP-45, cardiocytes were cultured for 8 h in the presence or absence of each peptidyl-CMK at a final concentration of 25 or 50 μM. Peptidyl-CMK was added 2 times at a 4-h interval because the half-life of the inhibitor is thought to be 4–8 h (39, 40). The effects of the trypsin inhibitors on the conversion of y-BNP to BNP-45 were also examined. The cells were cultured for 4 h in the presence or absence of 1-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone (TLCK) and fungal serine protease inhibitor leupeptin. Each inhibitor was added to the DME/F-12 culture medium at a final concentration of 100 μM. When the cells had been cultured for 4 h the culture medium was collected for HPLC analysis. The effect of α1-antitrypsins on the BNP processing was performed as described above. After stretching the medium was collected for HPLC analysis.

RESULTS

Hypertrophic Growth of Cardiocytes—We evaluated the stretch-induced hypertrophic growth of cardiocytes by four different experiments. The first method, measurement of cell size by a cell sorter has been utilized for evaluating hypertrophy of vascular smooth muscle cells and renal epithelial cells (28, 29, 43, 44). Cardiocytes were plated almost to confluency on a silicon membrane plate. The cells exhibited synchronous contractions at approximately 100–120 beats/min. Stretching of cardiocytes has been used for their hypertrophic growth (5, 24, 25, 27). The cells were stretched 10–15% longer than their original size for 48 h by pulling the membrane bottom with suction. The cells, with or without stretching, were harvested and applied to the cell sorter analysis to obtain a histogram of their size. When removed from the plate, spindle-shaped cardiocytes on a silicon membrane became rounded by microscopic observation (Fig. 1A). Approximately 4 × 106 cells were analyzed to obtain each histogram. The cell size is reflected to forward light scatter on the x axis of a histogram as described previously (28, 29). The histogram of stretched and non-stretched cells exhibited a bell-shaped distribution and that of stretched cells shifted to the right from that of non-stretched cells (Fig. 1B). The forward light scatter before stretching was 131.1 ± 26.8, whereas that after stretching was 141.6 ± 26.3. The difference was significant (p < 0.001) between the stretched and non-stretched cells as assessed by the two sample t test.

Second, hypertrophic growth of cardiocytes was measured by the incorporation of [14C]Phe into cellular protein (24, 25). The incorporation of [14C]Phe was approximately 2-fold in stretched cells than in non-stretched cells after only 2 h of stretching (Fig. 2A, p < 0.01). This increase continued even 8 h after the start of stretching. Third, we compared the rate of protein synthesis between stretched and non-stretched cardiocytes by using the equilibrium and pulse labeling method (26, 27). The rate was calculated by Equation 1 under “Experimental Procedures” from the ratio of the pulse labeling with [3H]Phe and equilibrium labeling with [14C]Phe in cellular protein and free radioisotope-labeled Phe in the culture medium (Table 1). The protein synthetic rate Ks of stretched cardiocytes (0.0305 ± 0.0009) was clearly larger than that of non-stretched cardiocytes (0.0266 ± 0.0020). Total cellular protein was increased approximately 20% more than that in non-stretched cardiocytes during 4-day stretching (Table 1). Fourth, the protein/DNA ratio of cardiocytes was evaluated by 4-h stretching. The
ratio for stretched cardiocytes was approximately 20% greater than that of non-stretched cardiocytes (Fig. 2B). Thus, stretching significantly increased the cell size and protein synthesis in cultured cardiocytes as described previously (24, 25, 27).

**Stretch-induced Expression of BNP**—Total RNA was isolated from cardiocytes after stretching for 48 h and then applied to Northern blot analysis. Fig. 3A shows that BNP mRNA was slightly visible in non-stretched cells, whereas the mRNA became more intense in stretched cardiocytes. In contrast, β-actin mRNA stayed in a similar level between stretched and non-stretched cardiocytes. Consistent with the BNP mRNA expression, the stretching induced approximately a 2-fold increase in the release of immunoreactive BNP (irBNP) into the culture medium (Fig. 3A). The rat BNP-45 corresponds to the position 51–90 amino acid region of the 90-residue rat ANP precursor (37). The molecular form of BNP was not markedly affected by stretching. In the culture medium of both stretched and non-stretched cardiocytes only a processed form of BNP-45 was observed. In the culture medium of stretched cardiocytes, the proportion of BNP-45 to irBNP in the cell extracts from stretched and non-stretched cardiocytes only a processed form of BNP-45 was observed. In the culture medium of both stretched and non-stretched cardiocytes (Fig. 3B). The rat BNP-45 corresponds to the position 51–90 amino acid region of the 90-residue γBNP (37). The molecular form of BNP was not markedly affected by stretching. In the culture medium of both stretched and non-stretched cardiocytes only a processed form of BNP-45 was observed. In the cell extracts from stretched and non-stretched cardiocytes consisted of two molecular forms, a major form of a rat ANP precursor processing enzyme; boc-Gly-Lys-Arg-MCA, which is a substrate for paired basic residue cleavage enzyme; pyr-Arg-Thr-Lys-Arg-MCA, which is designed to serve as a substrate for paired basic residue cleavage enzyme; pyr-Arg-Thr-Lys-Arg-MCA, which is designed to serve as a substrate for paired basic residue cleavage enzyme. When the proteolytic activity observed on the ANP precursor-processing enzyme substrate boc-Ala-Gly-Pro-Arg-MCA was set to equal 100%, the activity for the dibasic residue cleavage enzyme substrate was approximately 95%, while the activity for furin substrate was approximately 430% (Fig. 5A). Thus, cardiocyte lysates contained high cleavage activity to a furin-type substrate. However, we could not attribute this cleavage activity directly to the furin by the following two reasons. First, the cleavage activity of the furin substrate may be overestimated because the substrate pyr-Arg-Thr-Lys-Arg-MCA is cleaved not only by furin but also by monobasic- or dibasic-residue cleavage enzymes.

**Effect of stretching on fractional rates (K_s) of protein synthesis**

Total protein was measured using 5 × 10^5 cells. K_s values are the average of three experiments.

| Cardiocyte   | Total protein per culture | K_s \(h^{-1}\) |  
|--------------|---------------------------|----------------|
| Non-stretched| 417                       | 0.0266 ± 0.0002|
| Stretched    | 500                       | 0.0305 ± 0.0009|

**Protease Activities in Cardiocytes**—We assayed the proteolytic activities of the cardiocyte lysates by using three enzyme substrates: boc-Ala-Gly-Pro-Arg-MCA, which is a substrate for rat ANP precursor processing enzyme; boc-Gly-Lys-Arg-MCA, which is a substrate for paired basic residue cleavage enzyme; and pyr-Arg-Thr-Lys-Arg-MCA, which is designed to serve as a substrate for furin. When the proteolytic activity observed on the ANP precursor-processing enzyme substrate boc-Ala-Gly-Pro-Arg-MCA was set to equal 100%, the activity for the dibasic residue cleavage enzyme substrate was approximately 95%, while the activity for furin substrate was approximately 430% (Fig. 5A). Thus, cardiocyte lysates contained high cleavage activity to a furin-type substrate. However, we could not attribute this cleavage activity directly to the furin by the following two reasons. First, the cleavage activity of the furin substrate may be overestimated because the substrate pyr-Arg-Thr-Lys-Arg-MCA is cleaved not only by furin but also by monobasic-or dibasic-residue cleavage enzymes.
dibasic-site cleavage enzymes. Second, cardiocyte extracts contain at least another furin type yeast Kex2 endoprotease PACE4, although its substrate specificity is narrowly limited compared with that of furin (34, 45, 46). Cardiocytes contain more than one form of furin-type enzymes so that we need to purify this activity for its accurate characterization.

Since yeast Kex2 family endoproteases including furin and PACE4 require calcium for their activity, we examined the calcium dependence of this activity in non-stretched cardiocytes. The activity was maximal at 1 mM Ca\(^{2+}\) and then decreased with the increment of Ca\(^{2+}\) concentrations up to 10 mM (data not shown). Then the calcium chelator EDTA was added to the enzyme reaction. The activity was inhibited 70% in the presence of 5 mM EDTA (Fig. 5B). The calcium-dependent activity was more elevated than calcium-independent activity in stretched cardiocytes. This finding also suggested that the furin-type activity is elevated during hypertrophic growth of cardiocytes.

When the furin-type endoprotease inhibitor dec-Arg-Val-Lys-Arg-CMK was added to the enzyme reaction mixture, the peptidyl-CMK inhibited the liberation of AMC from the substrate pyr-Arg-Thr-Lys-Arg-MCA. An inhibitor concentration of 25 \(\mu\)M inhibited proteolysis of the furin substrate by approximately 73%, and a concentration of 50 \(\mu\)M produced approximately 87% inhibition (Table II). In contrast, the dibasic site cleavage enzyme inhibitor dec-Phe-Ala-Lys-Arg-CMK inhibited proteolysis of the furin substrate by approximately 28% at 25 \(\mu\)M and 44% at 50 \(\mu\)M. Thus, cardiocyte lysates contain high proteolytic activity of furin-type enzyme.

The Effect of the Peptidyl-CMK and \(\alpha\,\alpha\)-Antitrypsins on the Hypertrophic Growth of Cardiocytes—Because furin was highly expressed with the hypertrophic growth of cardiocytes, we won-
tion was performed at pH 7 in the presence of 1 mM CaCl₂. For measuring Ca²⁺-dependence EDTA was added to the reaction at a final concentration of 5 mM. The Ca²⁺-insensitive activity (filled part) was not remarkably affected by stretching. In contrast, Ca²⁺-sensitive activity increased approximately twice in stretched cardiocytes (hatched bar) as compared to non-stretched cardiocytes (open bar). The activity was assayed in the reaction containing 1 mM CaCl₂ with a concentration of 5 mM.

- For furin substrate pyr-Arg-Thr-Lys-Arg-MCA (hatched bar) were plotted. Cardiocyte lysate contained relatively high proteolytic activity to the furin-type enzyme substrate pyr-Arg-Thr-Lys-Arg-MCA. The activity was assayed in the reaction containing 1 mM CaCl₂ without 5 mM dec-Arg-Val-Lys-Arg-CMK and natural α₁-antitrypsin.

The reaction was performed at pH 7, in the presence of 1 mM CaCl₂. For measuring Ca²⁺-dependence EDTA was added to the reaction at a final concentration of 5 mM. The Ca²⁺-insensitive activity (filled part) was not remarkably affected by stretching. In contrast, Ca²⁺-sensitive activity increased approximately twice in stretched cardiocytes (hatched bar) as compared to non-stretched cardiocytes (open bar). The activity was assayed in the reaction containing 1 mM CaCl₂ with a concentration of 5 mM.

**TABLE II**

Effects of peptidyl CMK inhibitors on the furin-type activity to cleave pyr-Arg-Thr-Lys-Arg-MCA

| Inhibitor                  | Concentration | Residual activity |
|----------------------------|---------------|-------------------|
| Control                    | 100           | %                 |
| dec-Arg-Val-Lys-Arg-CMK    | 25            | 27                |
|                            | 50            | 13                |
| dec-Phe-Ala-Lys-Arg-CMK    | 25            | 72                |
|                            | 50            | 56                |

- For furin substrate pyr-Arg-Thr-Lys-Arg-MCA (hatched bar) were plotted. Cardiocyte lysate contained relatively high proteolytic activity to the furin-type enzyme substrate pyr-Arg-Thr-Lys-Arg-MCA. The activity was assayed in the reaction containing 1 mM CaCl₂ without 5 mM dec-Arg-Val-Lys-Arg-CMK and natural α₁-antitrypsin.

The reaction was performed at pH 7, in the presence of 1 mM CaCl₂. For measuring Ca²⁺-dependence EDTA was added to the reaction at a final concentration of 5 mM. The Ca²⁺-insensitive activity (filled part) was not remarkably affected by stretching. In contrast, Ca²⁺-sensitive activity increased approximately twice in stretched cardiocytes (hatched bar) as compared to non-stretched cardiocytes (open bar). The activity was assayed in the reaction containing 1 mM CaCl₂ with a concentration of 5 mM.

**FIG. 5.** **Proteolytic activities in cardiocyte lysate.** A, proteolytic activity of cardiocyte lysate to the three synthetic substrates: boc-Ala-Gly-Pro-Arg-MCA, boc-Gly-Lys-Arg-MCA, and pyr-Arg-Thr-Lys-Arg-MCA. The activity was assayed in the reaction containing 1 mM CaCl₂ at pH 7.0. Proteolytic activity for the ANP processing enzyme substrate boc-Ala-Gly-Pro-Arg-MCA was set to 100% (open bar). Relative activities for the dibasic site cleavage enzyme substrate boc-Gly-Lys-Arg-MCA (hatched bar) and for the furin substrate pyr-Arg-Thr-Lys-Arg-MCA (filled bar) were plotted. Cardiocyte lysate contained relatively high proteolytic activity to the furin-type enzyme substrate pyr-Arg-Thr-Lys-Arg-MCA.

B, proteolytic activity in the lysate of stretched (S, hatched bar including filled part) and non-stretched (N, open bar including filled part) cardiocytes to pyr-Arg-Thr-Lys-Arg-MCA. The reaction was performed at pH 7 in the presence of 1 mM CaCl₂. For measuring Ca²⁺-dependence EDTA was added to the reaction at a final concentration of 5 mM. The Ca²⁺-insensitive activity (filled part) was not remarkably affected by stretching. In contrast, Ca²⁺-sensitive activity increased approximately twice in stretched cardiocytes (hatched bar) as compared to non-stretched cardiocytes (open bar without filled part).

**FIG. 6.** **Effect of the furin-type enzyme inhibitor dec-Arg-Val-Lys-Arg-CMK on the hypertrophic growth of cardiocytes.** Cardiocytes were cultured with or without stretching in the presence or absence of 50 μM dec-Arg-Val-Lys-Arg-CMK for 4 h and then subjected to cell sorter analysis. The presence of inhibitor is marked by + and the absence of inhibitor by −. A, non-stretched cardiocytes in the absence of dec-Arg-Val-Lys-Arg-CMK (cell size by forward light scatter, 143.6 ± 26.8). B, non-stretched cardiocytes in the presence of 50 μM dec-Arg-Val-Lys-Arg-CMK (142.8 ± 25.3). C, cardiocytes stretched in the absence of the inhibitor (149.8 ± 25.2). D, cardiocytes stretched in the presence of 50 μM inhibitor (143.3 ± 26.9). Furin-specific inhibitor dec-Arg-Val-Lys-Arg-CMK was effective to suppress the hypertrophic growth of cardiocytes by stretching (p < 0.001, the histogram in D versus that in C).

When cardiocytes were stretched in the presence or absence of the inhibitor, the average forward light scatter was 143.3 ± 26.9 with the inhibitor and 149.8 ± 25.2 without the inhibitor (Fig. 6, C and D). We evaluated the difference in cell size against the non-stretched cells cultured without the inhibitor (Fig. 6A) using the two sample t test. There was no difference in the size of the cells that were not stretched (p > 0.05, Fig. 6B) nor were the stretched cells that were cultured with the inhibitor (p > 0.05, Fig. 6D). There was a significant difference, however, between the stretched cells cultured with and without the inhibitor (Fig. 6, C and D, p < 0.001). Thus, even under the stretch force, the inhibitor prevented the hypertrophic growth of cardiocytes (Fig. 6C).

Unfortunately we used up another peptidyl-CMK, dec-Phe-Ala-Lys-Arg-CMK, for the BNP processing experiment and could not examine the effect of the inhibitor against dibasic residue cleavage enzyme.

We then examined the effect of the peptidyl-CMKs on protein synthesis of stretched cardiocytes by two methods. First, we measured the incorporation of [¹⁴C]Phe in cellular protein in a similar manner to Fig. 2A. In the presence of 50 μM peptidyl-CMKs, especially furin-specific dec-Arg-Val-Lys-Arg-CMK, the increase of [¹⁴C]Phe incorporation was suppressed over 50% at both 2- and 8-h points (Fig. 7A). Second, the ratio of total protein/DNA in stretched cardiocytes was also suppressed to...
and causes cell lysis 48–72 h following infection (47, 48). Thus, cellular mRNAs for the effective translation of viral mRNAs. Vaccinia virus infection results in the rapid degradation of S A N observed at 2- and 8-h points after the start of stretching. CMK or dec-Arg-Val-Lys-Arg-CMK. The incorporation of [14C]Phe was synthesis. Thus, the inhibitory effect of furin-type enzyme inhibitor on hypertrophic growth of cardiocytes was demonstrated by cell sorter histogram as well as by protein synthesis.

To confirm further the suppressive effect of furin-type endopeptidase inhibitors on the hypertrophic growth, we used another type of inhibitor, natural α1-antitrypsin and its variants. Vaccinia virus infection results in the rapid degradation of cellular mRNAs for the effective translation of viral mRNAs and causes cell lysis 48–72 h following infection (47, 48). Thus, we observed the effect of α1-antitrypsin only 4 h after the infection. An expression experiment at 4 h after vaccinia infection is earlier than usual, compared with regular vaccinia gene expression experiments that are usually performed at 12–24 h after infection (48). The cardiocytes, while being stretched, were exposed to wild strain WR or each of vaccinia recombinant virus. When the WR strain, VV:α1-PIT exhibited a single peak of BNP-45 as determined by HPLC separation (Fig. 9), indicating that each inhibitor was not sufficient to suppress the conversion to BNP-45. In contrast, infection of VV:α1-PDX stayed smaller after stretching than that of cells expressing the WR strain, α1-NAT, and α1-PIT with the same statistical analysis used for Figs. 1B and 6. Thus, two different types of inhibitors against a furin-type enzyme neutralized the effect of stretching on the hypertrophic growth of cultured cardiocytes.

The Effect of Trypsin Inhibitors, Peptidyl-CMKs, and α1-Antitrypsins on the Processing of BNP—We observed co-elevation of BNP-45 and furin in the stretched cardiocytes (Figs. 3 and 4), but these data do not directly present evidence that furin catalyzes the conversion of γBNP to BNP-45. To obtain direct evidence, we used several endopeptidase inhibitors, trypsin inhibitors including leupeptin and TLCK, peptidyl CMKs, and vaccinia vector-integrated α1-antitrypsins. Leupeptin and TLCK inhibited less than 10% of the proteolytic activity to pyr-Arg-Thr-Lys-Arg-MCA in cardiocyte lysates (data not shown). These trypsin inhibitors were added to the cardiocyte culture to examine their effect on the processing of γBNP to BNP-45. The culture medium of cardiocytes without the inhibitor exhibited a single peak of BNP-45 as determined by HPLC (Fig. 3C). The addition of the inhibitors to the cardiocyte culture produced a tiny fraction of uncleaved γBNP as assessed with HPLC separation (Fig. 9), indicating that each inhibitor was not sufficient to suppress the conversion to BNP-45. In contrast, the addition of dec-Arg-Val-Lys-Arg-CMK (25 μM) produced some uncleaved γBNP, and a higher concentration (50 μM) produced much less conversion of γBNP to BNP-45 (Fig. 10). But a dibasic site cleavage enzyme inhibitor (dec-Phe-Ala-Lys-Arg-CMK) did not affect the conversion of γBNP, even at 50 μM.

We then examined the processing of BNP in cardiocytes by expressing three types of α1-antitrypsins in cultured cardiocytes. When the WR strain, VV:α1-NAT, and VV:α1-PIT were infected, cardiocytes secreted only a processed form of BNP-45 using cell sorter analysis (Fig. 8). Infection of VV:α1-NAT or VV:α1-PIT did not affect the stretch-induced enlargement of cardiac muscle compared with the control cell size infected with the WR strain (WR strain, 133.4 ± 69.4; VV:α1-NAT, 132.2 ± 72.0; VV:α1-PIT, 132.4 ± 70.4, by forward light scatter). In contrast, infection of VV:α1-PDX induced the suppression of the stretching-induced cell size enlargement (126.6 ± 66.6 by forward light scatter, p < 0.001 against the cells infected with the WR strain). The histogram of forward light scatter, however, was not bell-shaped but was distributed to the right with a peak to the left asymptomatically similar to a log normal distribution curve. Thus, the standard deviation became much larger than that shown in Fig. 6. We examined several different virus titers for infecting cardiocytes (m.o.i. = 5, 20, 50), but a histogram consistently revealed an asymmetrical distribution with any virus titer. The deformation of cell shape may be derived by the degradation of cytoskeleton, such as actin and tubulin mRNAs (49). Although the histogram of cell size was affected by vaccinia viral infection, the average size of cardiocytes expressing α1-PDX stayed smaller after stretching than that of cells expressing the WR strain, α1-NAT, and α1-PIT with the same statistical analysis used for Figs. 1B and 6. Thus, two different types of inhibitors against a furin-type enzyme neutralized the effect of stretching on the hypertrophic growth of cultured cardiocytes.
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diate early genes express several embryonal stage-specific genes including myosin light chain-2, β-myosin heavy chain, ANP, and mitogen-activated protein kinases, which are

without the precursor form γBNP. In contrast, expression of α1-PDX in the cardiocytes resulted in the appearance of γBNP (Fig. 11). The proportion of γBNP to BNP-45 was greater in α1-PDX-expressing cardiocytes than in those cultured in the presence of dec-Arg-Val-Lys-Arg-CMK. Thus, the two types of furin-specific inhibitors, peptidyl-CMK as well as the variant α1-antitrypsin, prevented the conversion of γBNP to BNP-45, while other serin protease inhibitors did not.

**DISCUSSION**

The present results indicate that 1) both BNP and furin are highly expressed in cardiocytes hypertrophied by stretching; 2) furin-specific inhibitors dec-Arg-Val-Lys-Arg-CMK and variant α1-antitrypsin α1-PDX suppressed the stretch-induced hypertrophic growth of cardiocytes, as well as the conversion of the BNP precursor form (γBNP) to the processed form (BNP-45).

Cardiocytes are known to exhibit hypertrophic growth in response to stretch force and pressure overload (1, 4, 24, 25, 27). We demonstrated stretch-dependent hypertrophic growth of cardiocytes using two methods, cell sorter analysis and protein synthesis (Figs. 1 and 2, Table I). The mechanical stress and some of the peptide growth factors are known to induce the expression of the immediate early genes c-fos, c-jun, c-myc, and egr-1 in cardiocytes (1–4, 24, 50). Consequently, these immediate early genes express several embryonal stage-specific genes including myosin light chain-2, β-myosin heavy chain, ANP, and mitogen-activated protein kinases, which are

Another natriuretic peptide BNP mRNA is elevated as rapidly as the immediate early genes but much faster than ANP mRNA does during the hypertrophic growth of cardiocytes (51). BNP as well as ANP serve to reduce extracellular body fluid to compensate for the pressure overload to the myocardium by inducing natriuresis, diuresis, and vasodilation (20). We confirmed the elevation of BNP mRNA and irBNP in stretched cardiocytes (Fig. 3).

Along with BNP, furin was also increased with hypertrophic growth of cardiocytes (Fig. 4). We assayed basic residue cleavage enzymes using the three substrates. Each fluorescent MCA-coupled peptide was used as a substrate for either the rat ANP precursor-processing enzyme, the paired basic residue cleavage enzyme, or furin (34, 38). The cardiocytes contained a high level of proteolytic activity that cleaves the furin-type substrate, and this activity was enhanced by stretching (Fig. 5). The furin-type proteolytic activity was extensively inhibited by two furin-specific inhibitors, dec-Arg-Val-Lys-Arg-CMK, and vaccinia virus-integrated variant α1-antitrypsin with Arg-X-X-Arg determinant, α1-PDX. These furin-specific inhibitors not only prevented the conversion of γBNP to BNP-45 (Figs. 10 and 11) but also suppressed the hypertrophic growth of cardiocytes (Figs. 6–8). Furin is a membrane protein localized in the trans-Golgi networks and recycles between the trans-Golgi networks and plasma membranes (15, 18), whereas α1-antitrypsin is a secretory protein (52). α1-PDX may inhibit furin activity at the trans-Golgi networks or during traffic to the plasma membrane. In addition, since furin is known to be secreted after truncation from the membrane-bound domain (53), we cannot exclude the possibility that α1-PDX inhibits furin activity outside the cells. Further study is required to pinpoint a site where α1-PDX inhibits furin activity.

Furin exerts an essential role in cell proliferation and de-differentiation by cleaving the RXXR motif on growth-related precursor peptides or proteins (13, 36). The RXXR motif is found in several cardiovascular regulatory peptide precursors including the amino terminus of BNP and CNP (10), and both amino and carboxyl termini of adrenomedullin (54), big endothelin (55), and parathyroid hormone-related protein (PTHrP) (56). This motif is also found in a variety of many other precursors for growth-promoting peptides or proteins (57). Cardiocytes produce regulatory peptides with the RXXR motif in-

![Fig. 8. Effect of α1-antitrypsin and its variants on the hypertrophic growth of cardiocytes.](image)

Cardiocytes were stretched for 4 h after infecting with the wild vaccinia strain WR, VV:α1-NAT, VV:α1-PIT, and VV:α1-PDX (m.o.i., 20) for 1 h. Cell size by forward light scatter: WR strain, 133.4 ± 69.6; VV:α1-NAT, 132.2 ± 72.0; VV:α1-PIT, 132.4 ± 70.4; VV:α1-PDX, 126.6 ± 66.6. α1-PDX was most effective to suppress the hypertrophic growth of cardiocytes by stretching (p < 0.001 against the histogram by the WR). Note that the distribution pattern of cell size shifted asymmetrically to the left with much higher standard deviation compared with the histogram in Figs. 1B and 6.

![Fig. 9. Effect of protease inhibitors leupeptin and TLCK on the processing of the γBNP to BNP-45 in cultured cardiocytes.](image)

Cardiocytes were cultured for 4 h in the presence of 100 μM each inhibitor. irBNP in the culture medium was separated using a reverse phase HPLC. A trace of a precursor form γBNP was detected in the culture with leupeptin and TLCK.
including BNP (19), TGF-β (58), endothelin (59), and PTHrP (60). Each peptide precursor undergoes a variety of distinct proteolytic reactions. BNP precursor γBNP consists of 108 amino acids in humans, 105 in pigs, and 95 in rats. γBNP is cleaved to a distinct species-specific size at the RXXR motif; 32 amino acid BNP in humans, 26 and 32 amino acids BNPs in pigs, and 45 amino acid BNP in rats (10). The processing of γBNP to BNP-45 is predicted to be carried out by furin (19). We previously demonstrated that BNP is co-elevated with furin but not with PACE4 in the same region of rat atrial and ventricular tissue after myocardial infarction (34). In the present study, the processing of BNP by furin was clearly proved by using two types of furin inhibitors (Figs. 9–11). TGF-β is activated through at least two proteolytic steps, cleavage of the precursor by furin, and removal of latent TGF-β binding protein from a 25-kDa TGF-β dimer probably by plasmin (7, 8). TGF-β does not appear to induce hypertrophic growth of cardiac cells but rather maintains the contractile function of cardiocytes (58). Recently endothelin was found to be produced from cardiocytes (59). Endothelin requires two processing reactions, proteolysis of the precursor to big endothelin by furin, followed by the cleavage of big endothelin by endothelin-converting enzyme-1 (61). The conversion of big endothelin to mature endothelin by endothelin-converting enzyme-1 is efficient (62). The first cleavage step of endothelin precursor to big endothelin by furin may be more rate-limiting because furin expression in cardiocytes is regulated by pressure load and stretching in cardiocytes (34). PTHrP is also known to be produced from cardiocytes and processed to mature form by furin (56, 60). PTHrP was shown to antagonize the creatine kinase-inducing action of parathyroid hormone in cardiocytes (63). It is not known whether PTHrP exhibits hypertrophic growth in cardiocytes, although PTHrP has growth-promoting activity in some studies (64).

In addition, cardiocytes produce ANP and angiotensinogen that require another type of processing reaction (20, 65). ANP appears to be cleaved by protease that is probably membrane-associated (66, 67). Formation of angiotensin II requires three proteolytic steps for its activation, cleavage of angiotensin I from angiotensinogen by renin, proteolytic activation of prorenin to renin by renin-converting enzyme, and conversion of decapeptide angiotensin I to octapeptide angiotensin II by angiotensin I-converting enzyme (65). In patients with chronic heart failure, angiotensin I-converting enzyme message was elevated 3-fold compared with normal individuals (68). But, this enzyme was not induced in stretched cardiocytes (69), in contrast to the increase of furin-type protease in these cells. Another type of angiotensin I-converting enzyme, chymase, also exerts its action in failing hearts (70), although chymase is localized in mast cells contained in the heart (71). With these examples, cardiocytes contain many kinds of processing proteases. Among these processing enzymes we suggest that furin might have an essential role in the control of the hypertrophic growth of cardiocytes by generating bioactive peptides or pro-

**FIG. 10.** Effect of the two protease inhibitors dec-Arg-Val-Lys-Arg-CMK and dec-Phe-Ala-Lys-Arg-CMK on the processing of the γBNP to BNP-45 in cultured cardiocytes. The inhibitor dec-Phe-Ala-Lys-Arg-CMK (for the dibasic site cleavage enzyme) was added to the culture medium at a final concentration of 50 μM and dec-Arg-Val-Lys-Arg-CMK (which inhibits furin) at concentrations of 25 and 50 μM. Each inhibitor was added two times at 4-h intervals to the culture medium. The last 4-h culture medium was used for HPLC analysis. A, no inhibitors; B, dec-Phe-Ala-Lys-Arg-CMK, 50 μM; C, dec-Arg-Val-Lys-Arg-CMK, 25 μM; D, dec-Arg-Val-Lys-Arg-CMK, 50 μM.

**FIG. 11.** Effect of α1-antitrypsin and its variants on the processing of the γBNP to BNP-45 in cultured cardiocytes. Cardiocytes were cultured for 4 h after infecting with the wild vaccinia strain WR, VV:α1-NAT, VV:α1-PIT, and VV:α1-PDX (m.o.i., 20) for 1 h. irBNP in the culture medium was separated using a reverse phase HPLC. A, no virus; B, wild vaccinia strain WR; C, VV:α1-NAT; D, VV:α1-PIT; E, VV:α1-PDX. Note that only α1-PDX was effective to inhibit the conversion of the γBNP to BNP-45 (E).
teins, including a number of growth factors and cardiovascular regulatory peptides.

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REFERENCES

1. Morgan, H. E., and Baker, K. M. (1991) Circulation 83, 13–25
2. Parker, T. G., and Schneider, M. D. (1991) Annu. Rev. Physiol. 53, 179–200
3. Chien, K. R., Kowtun, K. U., Zhu, H., and Chien, S. (1991) FASEB J. 5, 3037–3046
4. Komuro, I., and Yazaki, Y. (1993) Annu. Rev. Physiol. 55, 55–75
5. Yamashita, T., Komuro, I., Kudoh, S., Zou, Y., Shiomiya, I., Minuzo, T., Takano, H., Hirai, Y., Ueda, K., Kato, K., Kadawaki, T., Nagai, R., and Yazaki, Y. (1995) J. Clin. Invest. 96, 438–446
6. Cleutjens, J. P. M., Kandala, J. C., Guarda, E., Guntaka, R. V., and Weber, K. T. (1995) J. Mol. Cell. Cardiol. 27, 1281–1292
7. Doi, M., LaRuspa, M. H., Bianchette, G., Gentry, L. E., and Leduc, R. (1995) J. Biol. Chem. 270, 16168–16164
8. Taipale, J., Miyazono, K., Heldin, C.-H., and Keski-Oja, J. (1994) J. Cell Biol. 124, 171–181
9. Rosenweig, A., and Seidman, C. E. (1991) Annu. Rev. Biochem. 60, 229–255
10. Keller, K. J., and Goeddel, D. V. (1992) Circulation 86, 1018–1088
11. Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1993) Nature 370, 61–65
12. Pei, D., and Weis, S. J. (1995) Nature 375, 244–247
13. Smeekens, S. P. A. (1993) BioTechnology 11, 182–186
14. Halban, P. A., and Irminger, J.-C. (1994) Biochem. J. 299, 1–18
15. Molloy, S. S., Thomas, L., VanStrake, J. K., Stenberg, P. E., and Thomas, G. (1994) EMBO J. 13, 18–33
16. Molloy, S. S., Bresnahan, P. A., Leppia, S. H., Klmpel, K. R., and Thomas, G. (1992) J. Biol. Chem. 267, 16396–16402
17. Liu, Y.-C., Kawagishi, M., Mikayama, T., Inagaki, Y., Takeuchi, T., and Ohashi, H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8957–8961
18. Bosshart, H., Humphrey, J., Deignan, D., Davidson, J., Drazba, J., Yuan, L., Ouyang, Y., Peters, P. J., and Bonifacino, J. S. (1994) J. Cell Biol. 126, 1157–1172
19. Steinhelper, M. E. R. (1993) Circ. Res. 72, 984–992
20. Espiner, E. A. (1994) J. Intern. Med. 235, 527–541
21. Sato, Y., NakaK, R., Araki, H., Nakamura, K., Okumura, K., Obata, K., Takemura, G., Fujiwara, H., Sugawara, A., Yamada, T., Itoh, H., Mukoyama, M., Hosoda, K., Kawai, C., Ban, T., Yasue, H., and Imura, H. (1989) J. Clin. Invest. 83, 298–305
22. Mukoyama, M., NakaK, K., Hosoda, K., Suga, S., Saito, Y., Ogawa, Y., Shirakami, G., Jugousaki, M., Obata, K., Yasue, H., Kambayashi, Y., Inouye, K., and Imura, H. (1991) J. Clin. Invest. 87, 1402–1412
23. Hama, N., Itoh, H., Shirakami, G., Nakagawa, O., Suga, S., Ogawa, Y., Masuda, I., Nakasukis, K., Yoshimasa, T., Hashimoto, Y., Yamaguchi, M., Hori, R., Yasue, H., and Nakao, K. (1995) Circulation 92, 1558–1564
24. Komuro, I., Kudoh, S., Zou, Y., Shiomiya, I., Hiroi, Y., Mizuno, T., Maemura, K., Kurihara, H., Aikawa, R., Takano, H., and Yazaki, Y. (1996) J. Biol. Chem. 271, 3221–3228
25. Nishino, K., Yoshimasa, T., and Nakao, K. (1995) J. Clin. Invest. 96, 1280–1287
26. Permutt, D. H., and Pierce, J. A. (1989) Am. J. Physiol. 257, L147–L162
27. Vey, M., Schafer, W., Berghofer, S., Klenk, H.-D., and Garten, W. (1994) J. Cell Biol. 127, 1829–1842
28. Kitamura, K., Sakata, J., Kawaguchi, K., Kojima, M., Matsuo, H., and Eto, T. (1993) Biochem. Biophys. Res. Commun. 194, 720–725
29. Yanagisawa, M., Kurihara, H., Kinomura, S., Tohda, K., Matsuoka, M., Ohashi, H., Yamada, M., and Takeuchi, T. (1994) Biochemistry 32, 59–64
30. Aikawa, R., Takano, H., and Yazaki, Y. (1993) Cardiovasc. Res. 27, 25–31
31. Baynash, A. G., Hosoda, K., Giaid, A., Richardson, J. A., Emoto, N., Hammer, F. M., and Husain, A. (1993) Circ. Res. 73, 473–485
32. Schluter, K.-D., Wingender, E., Treff, W., and Sperling, R. (1994) J. Biol. Chem. 269, 10731–10737
33. Masfelder, T., Helwig, J.-J., and Stewart, A. F. (1996) Endocrinology 137, 3151–3153
34. Baker, K. M., Booz, G. W., and Dostal, D. E. (1992) Annu. Rev. Physiol. 54, 227–241
35. Poo, M. K., and Hjorth, M. (1984) J. Clin. Invest. 73, 965–972
36. Nichols, A. T., and Pologe, H. (1993) J. Cell Biol. 124, 1107–1118
37. Beaubien, G., Schafer, M. H., Weisflog, D. W., and Sporn, M. B. (1993) J. Biol. Chem. 268, 747–754
38. Kato, M., Sakata, Y., and Yazaki, Y. (1994) J. Biol. Chem. 269, 6755–6760
39. Vayssier, M., and Carpentier, R. (1994) J. Biol. Chem. 269, 4643–4649
40. Vayssier, M., and Carpentier, R. (1994) J. Biol. Chem. 269, 4650–4657
41. Vayssier, M., and Carpentier, R. (1994) J. Biol. Chem. 269, 4658–4663
Stretch-induced Hypertrophic Growth of Cardiocytes and Processing of Brain-type Natriuretic Peptide Are Controlled by Proprotein-processing Endoprotease Furin

Yoshie Sawada, Masayuki Suda, Hironori Yokoyama, Tsugiyasu Kanda, Tetsuo Sakamaki, Shigeyasu Tanaka, Ryozo Nagai, Shuzo Abe and Toshiyuki Takeuchi

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