Expanded Materials and Methods

RNA extraction, poly(A)⁺ RNA Northern blot
Total RNA from frozen rat tissue samples was extracted using TRIzol Reagent (GIBCO BRL), and poly(A)⁺RNA was isolated. Rat ET-1, NGF, BNP, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA were obtained by RT-PCR from the heart and cloned into the pCRII plasmid. Inserts were labeled with [α-³²P]-dCTP by the random priming technique. Poly(A)⁺RNA (2.5 µg) was run on a 1% MOPS/formaldehyde-agarose gel, and northern blots were performed as described previously.¹

Transmission electron microscopy
The hearts were washed three times with PBS. The initial fixation was performed for 2 h in PBS containing 2.5% glutaraldehyde. The hearts were processed and embedded in epoxy resin. Ultrathin sections were double stained in uranyl acetate and lead citrate, and viewed under a JEM-1200EX transmission electron microscope, as described previously.²

Western blot analysis
Western blot analysis was performed as described previously.³ Samples were homogenized in lysis buffer [20 mM tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, with one complete mini® (Roche) tablet per 10 ml buffer]. The membrane was incubated with rabbit polyclonal antibody to TH (CHEMICON, AB152; 1:400) and mouse monoclonal antibody to PSA-NCAM (CHEMICON, MAB5324; 1:500) at 4°C for 24 and 48 h, respectively. The membrane was incubated with horseradish peroxidase-conjugated antibody to rabbit and mouse IgG (Amersham Pharmacia Biotech) for TH and PSA-NCAM, respectively, and the signals were visualized by chemoluminescence with SuperSignal® West Pico (PIERCE).

ELISA for NGF
An ELISA kit was purchased from Promega Corporation (Madison, Wisconsin, USA), and the assay was performed according to the manufacturer’s instructions.

References