Autocrine/Paracrine Determinants of Strain-activated Brain Natriuretic Peptide Gene Expression in Cultured Cardiac Myocytes*

(Received for publication, January 7, 1998, and in revised form, March 18, 1998)

Faquan Liang and David G. Gardner‡

From the Metabolic Research Unit and Department of Medicine, University of California, San Francisco, California 94143

The application of mechanical strain leads to activation of human brain natriuretic peptide gene promoter activity, a marker of hypertrophy, in cultured neonatal rat ventricular myocytes. We have used a combination of transient transfection analysis and reverse transcriptase-polymerase chain reaction to examine the role of locally produced factors in contributing to this activation. Conditioned media from strained, but not static, cultures led to a dose-dependent increase in human brain natriuretic peptide gene promoter activity. This increase was completely blocked by losartan or BQ-123, implying a role for angiotensin and endothelin as autocrine/paracrine mediators of the response to strain. Inclusion of the same antagonists in the cultures themselves led to only partial inhibition (∼60%), whereas inclusion of exogenous endothelin or angiotensin II resulted in amplification of the strain response. Angiotensin II and endothelin appear to be arrayed in series in the regulatory circuitry; the angiotensin response was blocked by BQ-123, whereas the endothelin response was unaffected by losartan. Mechanical strain was also shown to stimulate expression of the endogenous angiotensinogen, angiotensin-converting enzyme, and endothelin genes in this system. Collectively, these data indicate that locally generated angiotensin II and endothelin, acting in series, play an important autocrine/paracrine role in mediating strain-dependent activation of cardiac-specific gene expression.

Application of mechanical strain, or passive stretch, to myocardi cells in culture results in a series of phenotypic changes that closely resemble those that occur with myocyte hypertrophy in vivo. This includes activation of the immediate early gene family (e.g. c-fos, c-jun, c-myc, and egr-1), increased expression of the fetal gene program (e.g. atrial natriuretic peptide, α-skeletal actin, and β-myosin heavy chain), and increased protein synthesis (1, 2).

A number of recent studies have suggested that activation of autocrine/paracrine regulatory mechanisms in the myocardium may play an important, if not dominant, role in determining the myocyte response to hemodynamic load. Specific local regulators invoked as participating in this process include angiotensin II (AII) (3–6), endothelin (ET) (7–9), transforming growth factor β (TGF-β) (10, 11), fibroblast growth factor (11, 12), myotrophin (13), and cardiomyelin (14, 15), among others. Sadoshima et al. (3) suggested that locally produced angiotensin, stored in and secreted from the cardiac myocyte in vitro, plays a dominant role in effecting the response to mechanical strain. Losartan, an angiotensin type 1 (AT1) receptor antagonist, blocked strain-dependent increases in c-fos, α-skeletal actin and atrial natriuretic factor gene expression in their system. Yamazaki et al. (4, 5) confirmed that AII plays a significant role in determining the response to strain. In their hands, the induction of MAP kinase, MAP kinase kinase, Raf-1 kinase, or protein synthesis by strain was suppressed, although only by 50–70%, following blockade of the AT1 receptor (4, 5). This subtotal inhibition implies variable dependence of different responses on locally generated AII. In an independent study, the same group found that the potent vasoconstrictor endothelin (ET) also participates as an intermediate in the MAP kinase response to strain (7). This effect was found to be additive with that produced by AII suggesting that the two operate in parallel as autocrine/paracrine effectors of hypertrophy.

The cell culture studies have found corroboration in several whole animal models. Angiotensinogen, ACE, and renin gene expression have been identified in the heart (16, 17) and shown, in selected models, to increase with application of mechanical strain in vitro (3, 18) or hemodynamic overload in vivo (19–23), perturbations which are associated with the development of myocyte hypertrophy. This association appears to be mechanistic in nature. Subpressor AII infusions have been shown to promote ventricular hypertrophy in rats (24), whereas ACE inhibition has been shown to block the development of hypertrophy in a banded rat aorta model (19, 25, 26) and in the spontaneously hypertensive rat (27). Typically, the anti-hypertrophic effect of the ACE inhibitor is out of proportion to its afterload reducing properties (26). Furthermore, a number of anti-hypertensives that operate independently of the renin-angiotensin system (RAS) lack anti-hypertrophic activity despite comparable efficacy in lowering blood pressure (versus ACE inhibition) (25, 27). Of note, the endothelin antagonist BQ-123 has also been shown to prevent hypertrophy in the banded rat aorta model (28). Collectively, the existing data seem to imply an important role for AII, ET, and perhaps other locally produced autocrine/paracrine factors as signaling intermediates linking mechanical strain/hemodynamic overload to the phenotypic changes associated with hypertrophy. However, there is considerable variability in the magnitude of the contribution(s) of these putative autocrine/paracrine intermediates to different strain-dependent responses (3–5). Furthermore, there is little consensus regarding the functional relationship between these various factors and hypertrophy.
relationships that exist among these different factors during the development of hypertrophy. Finally, there is a paucity of data linking mechanical strain and/or its autocrine/paracrine effectors to cardiac-specific gene expression in the myocyte.

Recently, we found that application of mechanical strain to cultured neonatal cardiac myocytes in vitro resulted in significant activation of brain natriuretic peptide (BNP) gene promoter activity (29). BNP is a cardiac hormone that is normally produced at relatively low levels in the adult myocardium but increases dramatically with the development of cardiac hypertrophy (30, 31). In the present work we have used BNP promoter activation as a cardiac-specific marker of the response to strain to investigate the role of locally produced autocrine/paracrine mediators in this response.

**EXPERIMENTAL PROCEDURES**

**Materials—**Angiotensin II (AII), endothelin-1 (ET-1), BQ-123, and IRL-1038 were purchased from Peninsula Laboratories, Inc. (Belmont, CA). Losartan and PD123319 were provided by Merck Sharp and Dohme and Parke-Davis, respectively. Captoril was obtained from Hoffmann La-Roche. Anti-TGF-β antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-endothelin antisera and angiotensin I were obtained from Sigma. [32P]ATP was purchased from NEN Life Science Products. All oligonucleotides were synthesized by CuaChenc Inc. (Dulles, VA). The RT-PCR kit (AdvantageTM RT-for-PCR) was purchased from CLONTECH (Palo Alto, CA).

**Cell Culture and Stretch—**Ventricular myocytes were prepared from 1-day-old neonatal rat hearts by alternate cycles of 0.05% trypsin digestion and mechanical disruption as described previously (32). Cells (1 × 10^6) were cultured on collagen-coated Flex plates (Flexcell, Inc.; McKeep, PA) in Dulbecco’s modified Eagle’s-H21 medium containing 10% enriched calf serum (Gemini Bioproducts, Calabasas, CA), 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Medium was changed 24 h prior to initiation of the experiment. Cells were plated at a density of 10^6 cells/ml on the Flexcell Strain apparatus at a level of distension sufficient to promote ~20–25% increase in surface area at the point of maximal distension on the culture surface (33).

**Plasmids—**The construction of ~1 595 hBNPLUC (34) has been described previously. The ~1 329 rat endothelin-1 promoter-luciferase reporter construct (pLux-pre1) (35) was provided by M. Paul. The rat angiotensinogen expression vector (pRSV-AOG) (36) was provided by C. F. Deschepper.

**Transfection and Luciferase Assay—**Freshly prepared ventricular myocytes were transiently transfected with the indicated reporters and expression vectors (Gene Pulser, Bio-Rad) at 280 mV and 250 microfarads. Individual cultures were normalized for transfected DNA with pUC18. After transfection, cells were plated in 6-well collagen-coated Flex plates at a density of 1 × 10^5 cells per well in Dulbecco’s modified Eagle’s-H21 medium containing 10% enriched calf serum. Medium was changed 24 h after plating, and cyclical strain was applied. Cells were harvested and lysed in 60 μl of cell culture lysis reagent (Promega, WI). Protein concentration of each cell extract was measured using Coomassie protein reagent (Pierce). Cell lysates were processed (30 μg of protein per sample) and assayed for luciferase as described previously (29). To ensure reproducibility, experiments were repeated 3–5 times, using at least three different plasmid DNA preparations.

**RNA Isolation and RT-PCR—**Total RNA was isolated from the cultured ventricular myocytes by using the RNeasy mini kit from Qiagen (Valencia, CA). 1 μg of total RNA was used to synthesize and amplify cDNA using AdvageneRT-for-PCR, a conventional RT-PCR kit (CLONTECH). Angiotensinogen (AOG) cDNA was amplified to generate an 810-bp fragment by PCR with the following two primers: 5’-GACGGGTATACATCACCCTTTTCCCTCTC-3’ (sense, exon 2; positions 738–762 in the cDNA) and 5’-CTGCCACAGAATCTCATGAGGCCGAGCTCAG-3’ (antisense, exon 3; positions 882–853 in the cDNA). A 317-bp rat angiotensin-converting enzyme (ACE) cDNA fragment was synthesized by using 5’-CCCTGTTAACCCAGGATGGTTCAGAG-3’ (sense, exon 2; positions 279–303 in the cDNA) and 5’-GCCAGGCTTCCCCAGGCAAACAGCAC-3’ (antisense, exon 4; positions 595–571 in the cDNA). A 545-bp rat ET cDNA was synthesized using 5’-CTGGTGGCTCTCTGTCCTCTGTTGATGG-3’ (sense, positions 338–362 in the cDNA) and 5’-AGATCTCCAGCCACTGAGACACG-3’ (antisense, positions 883–819 in the cDNA). A 1000-bp rat renin cDNA was generated by using 5’-TGGATCGCTTGGTACCCCATC-3’ (sense, exon 3; positions 473–495 in the cDNA) and 5’-AGGAAGGCACCATTTGCTGGC-3’ (antisense, exon 9; positions 1479–1457 in the cDNA). A 366-bp rat GAPDH cDNA fragment was co-amplified along with the AOG, ACE, or ET cDNA fragments using 5’-TCCTGATACCATTTGG- CATCTGAGG-3’ (sense, positions 515–538 in the cDNA) and 5’-GTTCTGTGGAACTCAGACGAGACG-3’ (antisense, positions 891–868 in the cDNA).

**Southern Blot Analysis—**PCR products were electrophoresed on 2% agarose gel, denatured with 1.5 M NaCl and 0.5 M NaOH, then transferred to a nitrocellulose filter, and hybridized, respectively, with the following end-labeled oligonucleotides: AOG, 5’-GGGCACTTACAAGCTGTCGAAGACGAC-3’ (positions 372–402); ACE, 5’-GCTAAGAACACATCCAGCATCAGCAGACGAGACG-3’ (positions 486–505), renin, 5’-GCCAGCTTTCCTACTTCGAGCTGAGGACGAGG-3’ (positions 981–991), and GAPDH, 5’-CAGGCTGACGGAAGCAGCTGACGAGG-3’ (positions 689–719). Autoradiography was performed with an intensifying screen at room temperature for 1–12 h. Autoradiographic signals were quantified using the NIH Image program. Normalized data are presented as the ratio of AOG, ACE, or ET to GAPDH signal.

**Statistical Analysis—**Data were evaluated by one-way analysis of variance with Newman-Keuls test for significance.

**RESULTS**

To determine whether an autocrine/paracrine factor, capable of stimulating BNP gene promoter activity, is produced in myocyte cultures exposed to mechanical strain, we collected media from cultures subjected to cyclical distortion (1 Hz) or from cells cultured in a static environment for 24 h. These media were plated on a second group of ventricular myocytes (cultured in a static environment) that had previously been transfected with the ~1 595 hBNP luciferase reporter. As shown in Fig. 1, at full strength the strain-conditioned medium provided a level of BNP promoter activation which was 75–80% higher than that seen with medium conditioned by static cultures. Progressive dilution with fresh, unconditioned medium reduced the magnitude of the effect seen with the strain-conditioned medium but had little effect on that from the static cultures.

Next, we explored the roles of AII and ET as potential contributors to this autocrine/paracrine activity. As shown in Fig. 2A, both the AT-1 antagonist losartan and the ETa blocker BQ-123 completely blocked the ability of strain-conditioned media to activate the BNP promoter in transfected ventricular myocytes. PD123319, an AT-2 blocker, and IRL-1038, an ETa antagonist, were devoid of activity. Of note, none of these agents affected activity seen with conditioned media from the
static cultures. These studies indicate that both AII and ET can completely, or near completely, account for the autocrine/paracrine activity in the strain-conditioned media. This is further supported by the study presented in Fig. 2B that shows that antibody directed against the ET peptide effected a dose-dependent reduction in the strain-conditioned media-dependent stimulation of BNP promoter activity. Non-immune serum was devoid of activity.

When the inhibitors were included in the cultures during application of mechanical strain, somewhat different results were obtained. As shown in Fig. 3, PD123319 and IRL-1308 were still without activity in this paradigm. On the other hand, losartan, BQ-123, and the angiotensin-converting enzyme inhibitor captopril each reduced strain-dependent BNP promoter activity, but the inhibition, unlike that seen with the culture media, was partial (~60%) in each case. The combination of losartan and BQ-123 was no more effective that either agent used alone, implying that AII and ET employ a final common pathway to stimulate BNP promoter activity.

Given the findings with the AT-1 and ET-A antagonists, we next asked whether exogenous AII and ET would prove capable of directly stimulating hBNP promoter activity in these cultures. Both AII (Fig. 4A) and ET (Fig. 4B) effected a dose-dependent stimulation of this promoter. These effects were prevented by co-treatment with losartan and BQ-123, respectively (see Fig. 6, below), but not by PD123319 or IRL-1038 (data not shown). Of note, in each case the activation was further amplified if the peptide agonists were applied coincident with the strain stimulus (Fig. 4C). Once again the combination of AII and ET was no better than ET alone in effecting this response, supporting the hypothesis that they operate over similar circuitry in promoting activation of the BNP gene promoter.

We next explored the role of endogenous AII generation in contributing to basal and strain-dependent induction of the BNP promoter. Angiotensin I, the immediate precursor of AII, raised basal BNP promoter activity modestly and significantly amplified the response to strain (Fig. 5A). The latter effect was partially reversed by inclusion of either losartan or captopril in the cultures. Similarly, co-transfection of a rat angiotensinogen expression vector together with the BNP luciferase reporter resulted in a modest increase in basal activity and amplification of the strain response (Fig. 5B). Again, this increase was partially blocked by co-incubation with losartan or captopril indicating, as with AI above, an AII-dependent process. Collectively, the data suggest that these cultures have the capacity to both process angiotensinogen to AI (i.e. renin-like activity) and to convert AI to AII (i.e. converting enzyme activity) and that endogenous generation of AII is associated with activation of BNP gene transcription.
Next, we investigated the inter-relationship of AII and ET in promoting the autocrine/paracrine induction observed in Fig. 1. Since antagonists of either peptide completely blocked the stimulatory properties of strain-conditioned media and re-myocytes 1 h prior to application of the strain stimulus. The data are presented as means ± S.D. from four separate experiments. *, p < 0.01 versus the static control, and #, p < 0.05 versus its static counterpart.
versed the strain-dependent effect in cultured cells to a roughly equivalent extent, we postulated that the two were operating in series. To explore this possibility, we examined the effects of the AT-1 and ET_{4} antagonists on hBNP promoter induction by each of these two peptides. As shown in Fig. 6, neither antagonist had a significant effect on basal promoter activity. Losartan, as expected, completely blocked induction by AII. Noteworthy, however, BQ-123 also blocked the AII effect. As expected, BQ-123 blocked the ET induction; however, losartan was ineffective in this regard. This confirms our hypothesis that AII and ET operate in series. It appears that locally produced AII increases ET production which, in turn, leads to activation of the hBNP promoter and increased BNP gene expression.

AII has also been shown to increase production of TGF-β in cardiac myocytes (37, 38), and TGF-β is known to be a potent activator of those genetic programs typically associated with hypertrophy (10, 11). Therefore, we asked whether locally generated TGF-β might provide an additional level of autocrine/paracrine control. As shown in Fig. 7, addition of anti-TGF-β antibody resulted in a decrease in the activity of the transfected hBNP gene promoter in both the static and strained cultures; however, the magnitude of the strain effect (i.e. fold induction) was preserved. The specificity of the anti-TGF-β antibody effect was confirmed by the ability of purified TGF-β to partially reverse the inhibition. These findings imply that TGF-β is important for maintenance of basal promoter activity but does not contribute directly to generation of the response to mechanical strain.

We next asked whether mechanical strain would lead to increased expression of the individual components of RAS or endothelin in this system as a prelude to activation of hBNP promoter activity, and if so, whether the temporal pattern of induction would fit the model outlined above. We used RT-PCR to provide a semi-quantitative assessment of angiotensinogen, ACE, renin, and ET gene expression as a function of time following application of the strain stimulus. As shown in Fig. 8A, strain led to a time-dependent increment in angiotensinogen gene expression which was first apparent after 6 h of culture in the dynamic environment. The effect peaked at ~3-fold above basal after 24 h and then fell by 48 h. Rat renin gene expression could not be detected in these cultures using conditions that easily identified the renin gene transcript in prepa-

The present study demonstrates an important role for AII and ET as autocrine/paracrine contributants in the BNP gene transcriptional response to mechanical strain. Interestingly, virtually all of the soluble mediator-dependent contributions to the strain response could be linked to the presence of AII or ET in the culture medium. However, this contribution represented, at best, only 50–60% of the total response, implying the existence of an intrinsic stimulatory mechanism leading to BNP gene promoter activation independent of autocrine/paracrine activity. Additional support for this derives from the fact that the strain continued to demonstrate an independent stimulatory effect even in the presence of maximally active concentrations of AII and ET (see Fig. 4C).

Like a number of earlier studies, our findings support the
presence of an intrinsic renin-angiotensin system (16, 17) in the heart. Both angiotensinogen and ACE were found to be expressed in these myocyte cultures. Furthermore, expression of each was increased (within 6 h for angiotensinogen and within 12 h for ACE) following application of mechanical strain. Exogenous AI and overexpressed angiotensinogen amplified the response to strain, in each instance in an AII-dependent fashion. This together with the AII antagonist studies alluded to above support a key role for the cardiac RAS in the autocrine/paracrine amplification of the strain response. We did not, however, find evidence for expression of endogenous rat renin in these cultures, despite detection of the renin transcript in total RNA from rat kidney. This stands in contrast to other reports of renin expression in the heart (21–23), and may reflect differences in the sensitivity of the assays employed for detection. A true absence of renin gene expression here does not seem likely.

**Fig. 8.** Effect of strain on AOG (A), ACE (B), and ET-1 (C) mRNA levels in neonatal rat ventricular myocytes. Cells were subjected to cyclical strain for varying times, and total RNA was isolated. 1 μg of total RNA was converted to cDNA by reverse transcription and amplified by PCR with sense and antisense primers as described under “Experimental Procedures.” PCR products were electrophoresed on 2% agarose gel, transferred to a nitrocellulose filter, and hybridized with the end-labeled 32P-oligonucleotides. Autoradiographs were quantified using NIH Image. AOG, ACE, and ET-1 cDNA levels were normalized to GAPDH. C1, sample run solely with oligonucleotides encoding AOG, ACE, or ET-1, respectively. C2, sample run solely with oligonucleotides encoding GAPDH. Pooled data from three independent experiments are presented as means ± S.D., *, p < 0.01 versus static control, and #, p < 0.05 versus static control.
not negate the proposed involvement of the RAS in amplifying the response to strain. Relevant angiotensinogen processing activity could derive from circulating plasma renin in vivo (39) or, in the case of the in vitro model used here, from residual renin-like activity present in the serum used in culturing the myocytes. Alternatively, renin-like activity (independent of true renin) could be produced endogenously in the myocytes or non-myocytes themselves.

The antagonist studies support a role for the AT1 and ET<sub>A</sub> receptors in signaling the responses to AII and ET, respectively, and specifically exclude a role for AT2 and ET<sub>B</sub>. The fact that the effects of strain-conditioned media were completely reversed by AT1 blockade, ACE inhibition, or ET<sub>A</sub> blockade implies that AII and ET operate through a common final pathway in signaling the strain response. Our data support this and further indicate that the two are arrayed in series with AII leading to enhanced ET production which, in turn, leads to increased BNP gene transcription. This is somewhat at odds with the conclusions drawn by Yamazaki et al. (7) who found that AII- and ET-dependent increments in MAP kinase activity were additive in their myocyte cultures. The discrepancy is not readily explained but suggests that different regulatory circuitry may be involved in controlling expression of individual components of the hypertrophic phenotype. Alternatively, the discrepancy could reflect intrinsic differences between the single stretch system employed by Yamazaki et al. (7) and our cyclical strain system. Our studies do, however, concur with those of Ito et al. (8) who reported that ET functions as a direct intermediate in signaling AII-dependent increments in [3H]leucine incorporation into myocyte protein, those of Harada et al. (9) who found that AII-dependent increments in ANP or BNP secretion in myocyte/fibroblast co-cultures were suppressed by the ET antagonist BQ123, and those of Clavell et al. (40) who found that chronic ACE inhibition abolished increases in plasma ET levels during chronic canine thoracic inferior vena cava constriction.

The role of ET as an autocrine/paracrine mediator of the strain response has been controversial. Sadoshima et al. (3) found no increase in media ET levels despite a robust increase in AII levels. Yamazaki et al. (7), on the other hand, reported a marked stimulation of both ET secretion and gene expression in myocyte cultures subjected to mechanical strain. In our own studies ET gene expression was clearly activated by mechanical strain. The induction was first demonstrated after 24 h of continuous strain. Temporally, this is compatible with the proposed dependence upon AII in that angiotensinogen and ACE gene expression were increased a full 12–18 h earlier. This dependence was further supported by the finding that the strain-dependent induction of the ET gene promoter was completely reversible with either losartan or captopril, a finding which, notably, was not seen with the hBNP gene promoter, implying that strain-dependent activation of the ET promoter is heavily dependent upon the autocrine/paracrine effects of AII released into the culture media.

Our studies do not exclude a role for additional mediators distal to ET in the signaling cascade. AII has, in fact, been shown to stimulate production of TGF-β (37, 38), a well-documented hypertrophic agonist in the myocardium (11), and TGF-β has been linked to stimulation of ET secretion from the non-myocyte elements present in the myocardium (9). However, although specific antisera directed against TGF-β re-

![Fig. 9. Effect of strain on rat ET promoter activity in neonatal rat ventricular myocytes. A, 20 μg of −1329 rat ET luciferase was transfected into ventricular myocytes. After 24 h of culture, cells were subjected to mechanical strain for 48 h in the presence of 1 μM losartan or 10 μM captopril. Each drug was administered to the myocytes 1 h prior to application of the strain stimulus. B, cells were transfected with 20 μg of −1329 rat ET promoter-luciferase. After 24 h of culture, cells were treated with varying concentrations of angiotensin II for 48 h. C, cells were transfected with 20 μg of −1329 rat ET promoter-luciferase. After 24 h of culture, cells were treated with varying concentrations of endothelin-1 for 48 h. The data are expressed as means ± S.D. from four separate experiments. *, p < 0.01 versus static control, and #, p < 0.05 versus static control.](image-url)
duced basal hBNP promoter activity, they did not abrogate the response to strain. This implies that TGF-β plays an important role in supporting basal myocyte activity, a role advocated by others (41), but argues against a significant role for this particular growth factor as an autocrine/paracrine mediator of the strain response.

The cellular compartment responsible for synthesizing or processing the precursors for AII or ET cannot be established in the model employed here since low level contamination of the topographical relationship which they maintain with one another in the heart must be regarded as unresolved. Others, however, have documented both angiotensinogen and endothelin production within the cardiac myocytes themselves (8, 40, 43), so, at present, the role of angiotensin II and, secondarily, endothelin) released into the results from a combination of direct effects on the cardiac myocytes and, therefore, represent logical targets for pharmacological intervention where control of myocyte hypertrophy and progression to failure (43) is desired.

Acknowledgments—We are grateful to Karl Nakamura for assistance with preparation of the cells and to M. Paul and C. F. Descheppe for providing plasmids.

REFERENCES

1. Komuro, I., Kato, Y., Kaida, T., Shibazaki, Y., Kuraibayashi, M., Hoh, E., Takaku, F., and Yazaki, Y. (1991) J. Biol. Chem. 266, 1265–1268
2. Sadoshima, J., Jahn, L., Takahashi, T., Kuklik, T. J., and Izumo, S. (1992) J. Biol. Chem. 267, 10551–10560
3. Sadoshima, J., Xu, Y., Slayter, H. S., and Izumo, S. (1993) Cell 75, 977–984
4. Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., and Yazaki, Y. (1995) Circ. Res. 77, 258–265
5. Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., and Yazaki, Y. (1995) J. Clin. Invest. 96, 438–446
6. Woos, G. W., and Baker, K. M. (1996) Hypertension 28, 635–640
7. Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Hiroi, Y., Mizuno, T., Maemura, K., Kurihara, H., Aikawa, R., Takano, H., and Yazaki, Y. (1996) J. Biol. Chem. 271, 3221–3228
8. Ito, H., Hiroi, Y., Adachi, S., Tanaka, M., Tsujino, M., Kozue, A., Nomagami, A., Marumo, F., and Hiroi, M. (1993) J. Clin. Invest. 92, 398–403
9. Harada, M., Ito, H., Nakagawa, O., Ogawa, Y., Miyamoto, Y., Kowahara, K., Owaga, E., Igaki, T., Yamashita, J., Masuda, I., Yoshimasa, T., Tanaka, I., Saito, Y., and Nakao, K. (1997) Circulation 96, 3737–3744
10. Takahashi, N., Calderone, A., Izzi, N., Jun, M., Maki, T. M., Marsh, J. D., and Colucci, W. S. (1994) J. Clin. Invest. 94, 1470–1476
11. Parker, T. G., Packer, S. E., and Schneider, M. D. (1990) J. Clin. Invest. 85, 507–514
12. Weiner, H. L., and Swain, J. L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2683–2687
13. Mukherjee, D. P., McTernan, C. F., and Sen, S. (1993) Hypertension 21, 142–148
14. Pennica, D., King, K. L., Shaw, K. J., Luiz, E., Rullamas, J., Lesh, D., Park, J., and Marumo, F. (1993) J. Biol. Chem. 268, 1142–1146
15. Wollert, K. C., Taga, T., Saito, M., Marumo, F., Kishimoto, T., Guglielmi, C. C., Verna, A. B., Heath, J. K., Pennica, D., Wood, W. I., and Chien, K. R. (1996) J. Biol. Chem. 271, 9535–9545
16. Raman, V. K., Lee, Y. A., and Lindpaintner, K. (1995) Am. J. Cardiol. 76, 183–21D
17. Baker, K. M., Booz, G. W., and O’Hara, P. M. (1992) Am. J. Hypertens. 5, 275–280
18. Suyu, K. G., Chen, J. J., Shih, N. L., Chang, H., Wang, D. L., Lien, W. P., and Liew, C. C. (1995) Biochem. Biophys. Res. Commun. 211, 241–248
19. Baker, K. M., Brenal, I., and Izumo, S. (1990) Am. J. Physiol. 259, H234–H332
20. Sadoshima, J., Jahn, L., Takahashi, T., Kulik, T. J., and Izumo, S. (1992) J. Biol. Chem. 267, 10551–10560
21. Sadoshima, J., Xu, Y., Slayter, H. S., and Izumo, S. (1993) Cell 75, 977–984
22. Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., and Yazaki, Y. (1995) Circ. Res. 77, 258–265
23. Bruckshlegel, G., Holmer, S. R., Jandeleit, K., Grimm, D., Muders, F., Kroemer, E. P., Rietig, G. A. J., and Schunkert, H. (1995) Hypertension 25, 250–259
24. Linz, W., Scholzkens, B. A., and Ganten, D. (1989) Clin. Exp. Hypertens. 11, 1235–1350
25. Pfeffer, J. M., Pfeffer, M. A., Mirsky, I., and Braunwald, E. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3310–3314
26. Ito, H., Hiroe, M., Hirota, Y., Fujisaki, H., Adachi, S., Akimoto, H., Ohta, Y., and Marumo, F. (1994) Circulation 89, 2198–2203
27. Liang, F., Wu, J., Garami, M., and Gardner, D. G. (1997) J. Biol. Chem. 272, 28950–28956
28. Nakagawa, O., Ogawa, Y., Itoh, H., Suga, S., Komatsu, Y., Kishimoto, I., Nishino, K., Yoshimasa, T., and Nakao, K. (1995) J. Clin. Invest. 96, 1290–1297
29. Hasegawa, K., Fujiiwa, H., Oka, M., Miyamae, M., Fujiiwa, T., Suga, S., Mukoyama, M., Nakao, K., Imura, H., and Yasumasa, S. (1993) Circulation 88, 372–380
30. Kovacic-Milivojevic, B., and Gardner, D. G. (1992) Mol. Cell. Biol. 12, 292–301
31. Hayes, S. J., and Marumo, F. (1994) J. Biol. Chem. 273, 142–148
32. von Lutterotti, N., Catanzaro, D. F., Sealey, J. E., and Laragh, J. H. (1994) Hypertension 27, 753–758
33. Paul, M., Zinzin, M., Bocker, W., and Dyer, M. (1996) Hypertension 27, 683–687
34. Descheppe, C. F., and Beedel, T. L. (1996) Hypertension 16, 147–153
35. Lee, A. A., Dillmann, W. H., Mullenhuber, F. H. H. (1994) J. Clin. Exp. Hypertens. 16, 147–153
36. Akimoto, H., Marumo, F., and Hiroe, M. (1995) Science 266, 1280–1287
37. Lee, A. A., Dillmann, W. H., McCulloch, A. D., and Villareal, F. J. (1995) J. Biol. Chem. 270, 587–592
38. Von Lutterotti, N., Catanzaro, D. F., Sealey, J. E., and Laragh, J. H. (1994) Hypertension 27, 753–758
39. Clay, W. L., and Marsden, T. M. (1990) J. Clin. Invest. 86, 2056–2062
40. Roberts, A. B., Roche, N. S., Winken, K. S., and Borm, P. C. (1992) J. Clin. Invest. 90, 2056–2062
41. Fujisaki, H., Ito, H., Hirota, Y., Tanaka, M., Hata, M., Lin, M., Adachi, S., Akimoto, H., Marumo, F., and Hiroe, M. (1995) J. Clin. Invest. 96, 1059–1065
42. Saki, S., Miyayachi, T., Kobayashi, M., Yamaguchi, I., Goto, K., and Sugishita, Y. (1996) Science 384, 353–355