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Review

The Anrep effect: 100 years later

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Cingolani HE, Pérez NG, Cingolani OH, Ennis IL. The Anrep effect: 100 years later. *Am J Physiol Heart Circ Physiol* 304: H175–H182, 2013. First published November 16, 2012; doi:10.1152/ajpheart.00508.2012.—Myocardial stretch elicits a rapid increase in developed force, which is mainly caused by an increase in myofilament calcium sensitivity (Frank-Starling mechanism). Over the ensuing 10–15 min, a second gradual increase in force takes place. This slow force response to stretch is known to be the result of an increase in the calcium transient amplitude and constitutes the in vitro equivalent of the Anrep effect described 100 years ago in the intact heart. In the present review, we will update and discuss what is known about the Anrep effect as the mechanical counterpart of autocrine/paracrine mechanisms involved in its genesis. The chain of events triggered by myocardial stretch comprises *1*) release of angiotensin II, *2*) release of endothelin, *3*) activation of the mineralocorticoid receptor, *4*) transactivation of the epidermal growth factor receptor, *5*) increased formation of mitochondria reactive oxygen species, *6*) activation of redox-sensitive kinases upstream myocardial Na^+/H^+ exchanger (NHE1), *7*) NHE1 activation, *8*) increase in intracellular Na^+ concentration, and *9*) increase in Ca^{2+} transient amplitude through the Na^+/Ca^{2+} exchanger. We will present the experimental evidence supporting each of the signaling steps leading to the Anrep effect and its blunting by silencing NHE1 expression with a specific small hairpin interference RNA injected into the ventricular wall.

angiotensin II; NHE1; slow force response; stretch; transactivation

THE HEART IS UNDER continuous nervous, hormonal, and electrophysiological influence. Despite this, the cardiac muscle has intrinsic mechanisms to adapt cardiac output to changes in hemodynamic conditions. An increase in left ventricular end-diastolic volume, caused by either increasing aortic resistance to ejection or venous return, immediately leads to a more powerful contraction. This is the well-known Frank-Starling mechanism that allows the heart to increase its output after a rise in preload or to maintain it despite a greater afterload. However, after this initial rise in contractility and over the 10 to 15 min following a sudden stretch, myocardial performance continues to increase. Gleb Von Anrep showed in 1912 that after clamping the ascending aorta in a dog (acutely decreasing outflow and increasing intraventricular pressures), its heart initially dilated (72). This was followed by a progressive decline in end-diastolic volume over the following minutes toward the initial volume. Anrep interpreted these findings as secondary to a positive inotropic effect secondary to the release of cathecolamines by the adrenal glands that were receiving low blood flow. In 1959, Rosenblueth et al. (63) ruled out the role of the adrenal glands by reporting the phenomenon in an isolated cardiac preparation. Sarnoff et al. (66) in 1960 coined the term "homeometric autoregulation" to define in isolated hearts the progressive decrease in left ventricular end-diastolic pressure that occurs after its initial increase induced by an afterload rise. Subsequently, in 1973, this phenomenon was reproduced in isolated ventricular myocardium strips (56), describing both rapid and slow increments in developed force after muscle is stretched. The rapid change in force is thought to be the basis for the Frank-Starling mechanism and is mainly caused by an increase in myofilament Ca^{2+} responsiveness. The slow force response (SFR) to the change in length, proposed to be the in vitro equivalent of the Anrep phenomenon, is due to a progressive increase in the Ca^{2+} transient amplitude, as demonstrated by Allen and Kurihara in 1982 (3) and later on confirmed by other authors (2, 37) (Fig. 1, *A* and *B*). Physiologically, the Anrep effect constitutes a powerful mechanism by which the heart adapts to an abrupt increase in afterload, occurring after the Frank-Starling law takes place.

In the present review we will discuss the key steps in the stretch-triggered signaling pathways underlying the SFR or "Anrep effect", as well as its pathophysiological relevance.

Stretch-induced angiotensin II type-1/endothelin type-A receptor activation. The first step in the autocrine/paracrine chain of events triggered by myocardial stretch seems to be the release of angiotensin II (ANG II) and activation of ANG II type-1 (AT_1) receptors. The release of preformed ANG II after stretch was first reported by Sadoshima et al. (65) in isolated neonatal rat cardiomyocytes. These authors found that after 10 min of cyclic stretch, rat neonatal myocytes release ANG II, which will act as an initial mediator of the stretch-induced signaling cardiac hypertrophic program. Others also showed

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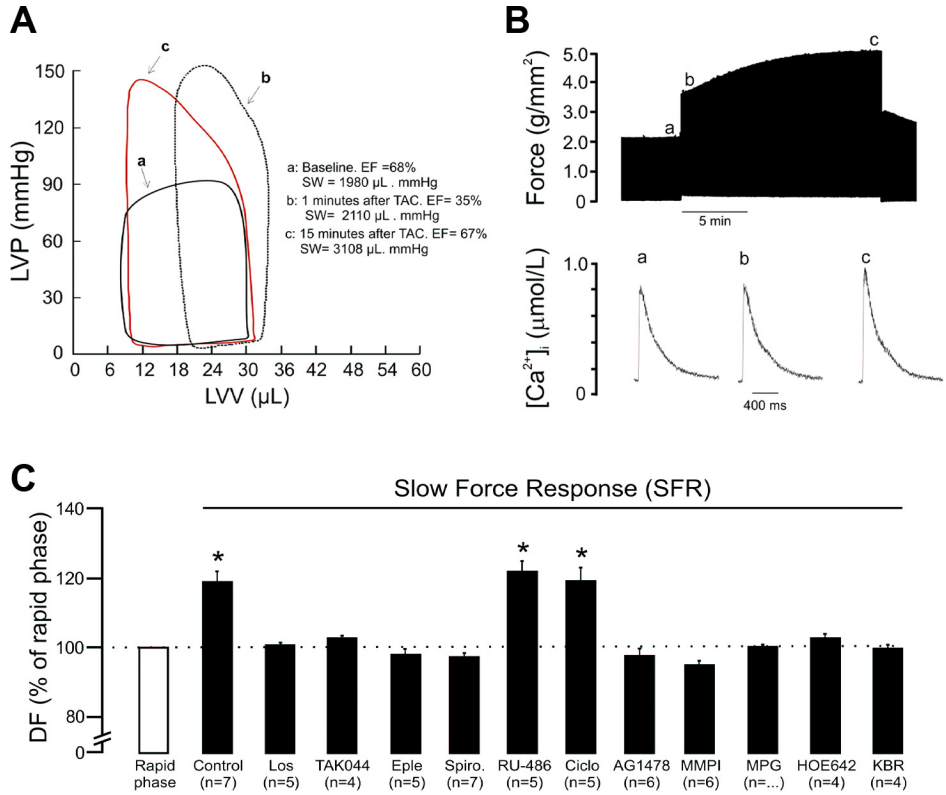

Fig. 1. The increase in contractility induced by the Anrep effect can be detected in the whole heart by pressure/volume loops (*A*). Note the changes from the *loop b* immediately after increasing aortic pressure to *loop c* 15 min after. LVP, left ventricular pressure; LVV, left ventricular volume; EF, ejection fraction; SW, stroke work; TAC, transverse aortic constriction. The Anrep effect is also observed in vitro in isolated papillary muscles by its equivalent slow force response (SFR) to stretch (*B*). $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration. In this case, we observed the increase in the calcium transient during the SFR (*B, bottom*). *C*: the SFR can be abolished by ANG II type-1 (AT_1) receptors blockade [losartan (Los)], endothelin (ET) receptors blockade (TAK044), mineralocorticoid receptor (MR) blockade [eplerenone (Eple); or spironolactone (Spiro)], epidermal growth factor receptor (EGFR) blockade (AG1478), metalloproteinase inhibition (MMPI), reactive oxygen species (ROS) prevention [*N*-(2-mercaptopropionyl)-glycine (MPG)], Na^+/H^+ exchanger (NHE1) inhibition (HOE642), and Na^+/Ca^{2+} exchanger (NCX) blockade [KBR7943 (KBR)]. The SFR was not inhibited by glucocorticoid receptors blockade (RU-486) or by inhibiting protein synthesis [cicloheximide (Ciclo)]. $*P < 0.05$ vs. rapid phase. Modified with permission from Cingolani and colleagues (12, 13, 16, 18, 70).

that blockade of AT_1 receptors inhibited the intracellular signaling cascade activated by stretch in cultured neonatal cardiomyocytes (41). Leri et al. (45) detected an increase in ANG II concentration in the culture medium while studying stretch-mediated apoptosis in adult cardiomyocytes. We were able to suppress the SFR in rat and cat ventricular myocardium by AT_1 receptor selective blockade (3, 57) (Fig. 1*C*). In addition, activation of NADPH oxidase (NOX) (one of the known effects of ANG II) with the consequential increase in reactive oxygen species (ROS) production, has been also detected after myocardial stretch (13).

In human heart, both atrial and ventricular myocardium exhibits stretch-dependent SFR (40). According to Pieske's group, the SFR does not depend on ANG II/endothelin (ET) release in the human ventricle but is depressed after myocardial Na^+/H^+ exchanger (NHE1) inhibition, one of the final mediators in the SFR. Instead, in human atrium, there seems to be an ANG II/ET-dependent, yet NHE1-independent, increase in force (40). These authors also propose an enhanced myofilament Ca^{2+} responsiveness through myosin light chain kinase-induced phosphorylation of myosin light chain 2 as a contributing factor to the SFR in both human atrium and ventricle (39, 40).

There is ample evidence demonstrating that several effects classically attributed to ANG II are actually caused by the release of ET from different cell types (5, 21, 24, 27, 34, 35, 51, 55, 59, 62). Ito et al. (35) reported that in order for ANG II to cause neonatal rat cardiomyocyte hypertrophy, an increased release/production of ET-1 was also necessary. These authors showed that the hypertrophic effect of ANG II can be prevented either by blocking ET type A (ET_A) receptors or using antisense oligonucleotides directed against prepro-ET mRNA. In addition, Liang and Gardner (46) studied stretch-induced brain natriuretic peptide production in cultured neonatal cardiac myocytes and found that expression of this gene was completely abolished not only by blocking AT_1 receptors with losartan but also by the ET_A receptor blocker BQ-123, implying a role for both ANG II and ET as autocrine/paracrine mediators. These authors also showed that ANG II and ET were serially arrayed, with ANG II working upstream ET-1, since the effect of ANG II on brain natriuretic peptide expression was blocked by BQ-123 (ET blocker), whereas that of ET was unaffected by losartan. This unidirectional ANG II-ET pathway was also reported by us studying stretch-mediated changes in intracellular pH (pH_i) in cat papillary muscle (14).

The SFR can be abolished by antagonizing ET receptors with the ET_A-ET_B nonselective blocker TAK044 (Fig. 1*C*) or with the ET_A-selective antagonist BQ-123. Interestingly, experiments performed by us in isolated cat ventricular myocytes demonstrated an increase in prepro-ET-1 mRNA in response to ANG II, a putative mediator of the SFR (17).

It is quite remarkable that when myocardial stretch is performed in the presence of cycloheximide (a protein synthesis inhibitor), the SFR is still present (12). The latter strongly suggests that the mechanisms leading to the Anrep effect are nongenomic, which seems not surprising given the short time frame in which the mechanical response occurs.

Activation of the mineralocorticoid receptors. Existing evidence linking ANG II/AT_1 and the mineralocorticoid receptors (MRs) (31, 43, 44) prompted us to explore MR activation as a critical step in the signaling cascade leading to the SFR. In fact, the SFR was suppressed by MR blockade with spironolactone or the more specific inhibitor eplerenone, but it was unaffected by either glucocorticoid receptors blockade of or protein syn-

thesis inhibition (Fig. 1*C*), supporting the idea that the MR activation seen after myocardial stretch in isolated rat papillary muscles is nongenomic and can be blunted by MR inhibition (12). The fact that stretching myocardium leads to MR activation is on line with the attractive hypothesis that stretch releases preformed ANG II from the myocyte, and this peptide—through AT_1 receptors activation—triggers the release of aldosterone (Ald), which in turn activates MRs in an autocrine/paracrine fashion. ANG II, the main stimulus for Ald release from the adrenal glands, has been reported to trigger the release/formation of Ald from the heart (67). However, this finding is controversial, since both hydroxylate 11-deoxycorticosterone (the required enzyme to synthesize Ald) and the steroidogenic acute regulatory protein (a crucial factor in the rate-limiting step for Ald biosynthesis) are found in very low amounts in myocytes (20, 30), and therefore a release of cardiac stored Ald was proposed as an alternative (30). Although the increase in Ald concentration constitutes the best recognized stimulus for MR activation, this receptor can be also activated in situations where Ald levels are normal or even low (28, 74, 76). On the other hand, the stretch-mediated myocardial activation of MRs does not necessarily imply the latter is a consequence of Ald release. In this regard, several other alternative Ald-independent mechanisms of MR activation should be considered: *1*) glucocorticoid-mediated MR activation, especially under conditions of enhanced ROS production (50); *2*) ligand-independent MR activation as the redox-sensitive Rac1-dependent activation (52); *3*) direct MR phosphorylation independent of its own ligand, as proposed by Kato et al. (36) for the estrogen receptor; and *4*) specific changes in MR conformation induced by strain, as proposed by Zou et al. (82) to explain AT_1 receptor activation by mechanical stretch. Obviously, the mechanism underlying activation of myocardial MRs by stretch deserves further investigation.

Transactivation of the epidermal growth factor receptor. The fact that ANG II failed to induce cardiac hypertrophy in transgenic mice overexpressing a mutant AT_1 receptor with consequent blunted epidermal growth factor receptor (EGFR) transactivation in the myocardium (79) leads us to hypothesize that if the mechanism leading to the SFR involved ANG II, it might as well involve transactivation of EGFR. As it can be appreciated in Fig. 1*C*, the SFR is blunted by different interventions that prevent EGFR transactivation, such as inhibiting matrix metalloproteinases, Src kinase, or specifically blocking EGFR (70).

Increased production of ROS from the mitochondria. When applied at low concentrations (1.0 nmol/l), ANG II increases sarcomere shortening in cat myocytes entirely through an autocrine cross talk with endogenous ET-1, and this effect is inhibited by interfering with mitochondrial function or by preventing oxidative stress (17). Since ANG II/AT_1 receptor activation initiates the signaling pathway leading to the SFR and ROS activates cardiac NHE1 (64), it was reasonable to expect that an increase in ROS production would be an important player in the stretch-triggered signaling cascade. This has been confirmed by finding roughly a 30% increase in ROS production accompanying the SFR and by blunting the SFR not only by scavenging ROS with *N*-(2-mercaptopropionyl)-glycine (Fig. 1*C*) but also after preventing mitochondrial ROS production. Furthermore, these ROS are originated in the mitochondria and seem to be NOX dependent (13). These results are aligned with previous reports describing the so-called "ROS-induced ROS-release" phenomenon in which NOX-dependent $O_2^{\cdot -}$ production triggers the opening of mitochondrial ATP-sensitive K^+ channels, inducing mitochondrial depolarization with consequent mitochondrial ROS production (9, 38, 81). Supporting the existence of a ROS-mediated NHE1 activation pathway, we have reported that stretch stimulates ERK1/2 and $p90^{RSK}$ redox-sensitive kinases, increasing their phosphorylation, an effect that is cancelled by AT_1 receptor blockade with losartan (19). In connection with this, it has been recently reported that stretching isolated myocytes induced a burst of NOX2-generated ROS in a process that seems to be mediated by microtubules (61). The latter could be involved in the "ROS-induced ROS release" mechanism, favoring the opening of mitochondrial ATP-sensitive K^+ channels and, therefore, the increase in mitochondrial ROS production that leads to activation of redox-sensitive kinases.

The chain of events initiated by myocardial stretch therefore begins with the release of ANG II followed by ET release, MR activation, and EGFR transactivation. These steps are upstream mitochondrial ROS release, the latter being responsible for kinase activation ($ERK_{1/2}$-$P90^{RSK}$) stimulating NHE1. An expected consequence, therefore, would be that Ald will activate NHE1 once EGFR transactivation takes place. This was indeed recently reported by De Giusti et al. (22). A critical point that we need to reiterate is the fact that these mechanisms responsible for the SFR are nongenomic, going from stretch-mediated releases of preformed ANG II/ET-1 to activation (phosphorylation) of kinases with the resultant posttranslational enhancement of NHE1 activity.

Increase in Ca^{2+} transient amplitude. A theoretical ionic model of ventricular myocyte used by Bluhm et al. (8) to analyze changes in sarcolemmal ion fluxes following step changes in cardiac muscle length predicted that a sudden increase in muscle length might induce changes in sarcolemmal Na^+ influx with a subsequent increase in intracellular Na^+ concentration ($[Na^+]_i$) and a concomitant increase in systolic Ca^{2+} entry through the Na^+/Ca^{2+} exchanger (NCX). How-

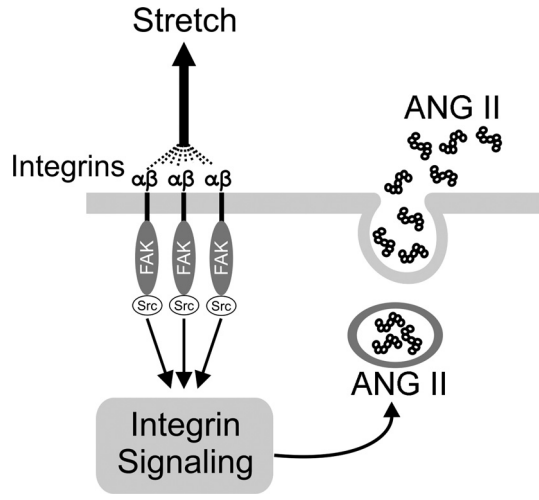

Fig. 2. Proposed model of stretch-activation in myocytes by Browe and Baumgartner (10) who proposed the activation of Cl^- current (I_{Cl} swell) by membrane deformation through a signaling pathway that involves focal adhesion kinases (FAK) and Src kinases activation. Modified with permission from Browe and Baumgartner (10).

ever, the mechanism by which the increase in $[Na^+]_i$ takes place was not proposed. Since NHE1 is an important Na^+ entry pathway in cardiomyocytes and it is activated by ANG II, ET-1, and Ald, it seems a suitable candidate for the stretch-mediated increase in Na^+ influx.

The first evidence suggesting myocardial stretch-mediated NHE1 activation emerged from experiments published in 1998 in which stretching cat papillary muscles maintained in bicarbonate-free medium (condition in which NHE1 is the only active pH_i regulatory mechanism) produced an intracellular alkalinization that was blunted by specific inhibition of the exchanger (14).

NHE1 regulates pH_i by exchanging intracellular H^+ for extracellular Na^+. Stimulation of the NHE1 could potentially increase myocardial force by two mechanisms: by alkalinizing the intracellular media and therefore augmenting myofilament Ca^{2+} sensitivity and/or by rising $[Na^+]_i$, which would favor an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) via the NCX. However, when experiments are performed under physiological conditions (using HCO_3^--containing buffers), little or no change in pH_i is detected (15). The lack of change in pH_i can be explained by the fact that many growth factors like ANG II simultaneously activate at least two different pH_i regulatory mechanisms: one alkalinizing the cell via NHE1 and, at the same time, another one acidifying it through the Na^+-independent Cl^-/HCO_3^- anion exchanger (23, 69). Whereas the latter is quite efficient maintaining pH_i, it cannot compensate for the increase in $[Na^+]_i$ triggered by the former. When the activation of the AE is prevented by a functional antibody, an increase in $[Na^+]_i$ takes place together with an increase in pH_i (15). Under this condition, myocardial stretch generates a greater SFR due not only to the increase in Ca^{2+} transient but also to the alkalization-dependent increase in myofilament Ca^{2+} responsiveness (26, 49, 54). Therefore, activation of NHE1 is detected in bicarbonate medium only by an increase in $[Na^+]_i$. This is of paramount relevance since under physiological conditions, stretch-mediated NHE1 activation will only augment $[Na^+]_i$ and not pH_i (3, 57). It is well known that an increase in $[Na^+]_i$ can lead to increases in $[Ca^{2+}]_i$ through the NCX as a result of decreased Ca^{2+} efflux (decreased forward mode) and/or increased Ca^{2+} entry (increased reverse mode). The increase in $[Na^+]_i$ induced by stretch or by exogenous ANG II or ET-1 (using doses of each compound that mimic the SFR) is prevented by blocking the NHE1(1, 3, 57, 59). The increase in myocardial $[Na^+]_i$ detected in our experiments was ~3–6 mmol/l. Increases of similar magnitude were detected by Baartscheer et al. (4) in the myocardium of rabbit failing hearts with enhanced NHE1 activity and by Luers et al. (47) after stretching rabbit myocardium. This increase in $[Na^+]_i$ shifts the reversal potential of NCX to a more negative voltage, allowing more time for NCX to operate in reverse mode during the action potential and promoting Ca^{2+} influx into the cell, the latter augmenting contractility. As reported by Bers et al. (6), cardiomyocytes have a limited capacity to buffer increases in $[Na^+]_i$, and NCX is more sensitive than the Na^+/K^+ ATPase pump to a change in $[Na^+]_i$ of this magnitude. As it can be appreciated in Fig. 1*C*, the SFR is abolished either by inhibiting NHE1 (HOE642) or the reverse mode of the NCX (KB-R7943). Although conceivably and experimentally proved, the above-described path to an increased Ca^{2+} transient is not uniformly accepted. Petroff et al. (60) working with isolated rat cardiomyocytes embedded in agarose and drawn into a polyethylene tubing proposed that stretching the tube activates the phosphatidylinositol 3-kinase pathway and phosphorylates endothelial nitric oxide synthase, with nitric oxide stimulating Ca^{2+} release from the sarcoplasmic reticulum and leading to the SFR. Unfortunately, these results could not be reproduced by other authors in papillary muscle or isolated myocytes (11). The potential role of the sarcoplasmic reticulum in the SFR has been also ruled out by other authors as well (7, 33, 37).

An alternative mechanism proposed by other investigators to explain the SFR involves the stretch-activated membrane channels (SACs). Stretch activates these nonselective cation channels, allowing Ca^{2+} and Na^+ entry. The latter would then permit $[Ca^{2+}]_i$ increase via NCX or even Ca^{2+} entry directly augmenting the Ca^{2+} transient amplitude driving the SFR. SACs were first described in skeletal muscle by patch clamping (32), but despite repeated attempts, they have never been patch clamped in ventricular myocardium (78), presumably because of their close colocalization with T tubules. A role for SACs in the SFR was suggested by Calaghan and White in 2004 (11), mainly as an addition to NHE1 activation in a 50–50% contribution manner. However, pharmacological strategies used to inhibit these channels were challenged based on the possible secondary actions of these compounds (80). Recently, Ward et al. (75) reported in mouse trabeculae that an opening of SACs appears to be the main contributor to the SFR after stretch. These authors showed that canonical transient receptor-operated channels (TRPCs) are sensitive to stretch in mouse myocardium. Specifically, TRPC1 and -6 isoforms appear to be the suitable stretch-activated nonselective cation channels mediating the SFR. They reported that streptomycin and gadolinium, compounds known to block SACs, blunted the SFR. These two compounds were reported to decrease the SFR in both rat papillary muscles and isolated myocytes (11). However, this effect was not detected in human ventricle (73). In addition to streptomycin and gadolinium, both of which seem to be nonspecific, Ward et al. (75) also inhibited the SFR using *Grammostola spatulata* mechanotoxin-4, a peptide isolated from a spider venom that seems to be more potent and specific blocking SACs. This compound blocks the SAC encoded by TRPC6 (68) and TRPC1 (48). At first glance, these results appear not to be in agreement with our proposal. However, we must consider the possibility that additional parallel or even in series pathways involving the aforementioned mechanisms could also exist. As an example, the TRPC6 may be necessary to trigger the release of ANG II after stretch or in any other step of the chain of events leading to the SFR.

Aligned with Ward's group, Kondratev et al. (42), working in mouse ventricular myocytes, reported that cariporide (NHE1 blocker) cannot prevent the rise in Na^+ after stretch but blunts the increase in Ca^{2+}, proposing that SACs, and not NHE1, are responsible for the increase in Na^+ after stretch. Interestingly, it has been recently proposed that TRPCs are necessary mediators of pathological cardiac hypertrophy in mice, in part through calcineurin-nuclear factor of activated T-cell signaling (77), a pathway that we reported to be sensitive to NHE1 inhibition in rat (25). The possibility that SACs are playing a role in series with the release of ANG II, or even a possible interaction between the extracellular matrix (ECM) and myocytes, could explain these discrepancies. In connection with the

latter, recent studies showed that mice lacking thrombospondin-4 (TSP-4), a matricellular protein thought to function as a matrix protein linker and whose expression is increased in heart failure (18), failed to show the Anrep effect or activate ERK1/2 (kinase upstream NHE1). Surprisingly, despite the Anrep phenomenon being absent in vivo from intact hearts as well as in vitro from papillary muscles from these knockout rodents not expressing TSP4, the SFR was detected in isolated single myocytes (without ECM and noncontractile cells) from this strain. These results lead to exiting alternative hypothesis. First, TSP4 (and possibly other matricellular proteins) may act as ECM-myocyte linkers, mechanically transducing force from myocyte to myocyte in between the ECM scaffold. Its absence allows slippage of myocytes with the consequent inadequate force transduction and diminished or absent Anrep effect. Second, TSP4 is normally present in the ECM (or cells others than myocytes), and its presence inactivate a yet-unknown Anrep-inhibiting factor; therefore, in the absence of the ECM (i.e.; isolated single myocytes), the Anrep is unaffected. The fact that in the mentioned study the addition of TSP4 to the $TSP4^{-/-}$ trabeculi preparation restored the Anrep effect suggests the latter concept, although further studies are necessary to elucidate this complex mechanism. Browe and Baumgarten (10) attached magnetic beads coated with antibodies to integrins and stretched myocytes by applying magnetic forces. They showed activation of Cl^- current (I_{Cl} swell). This Cl^- current

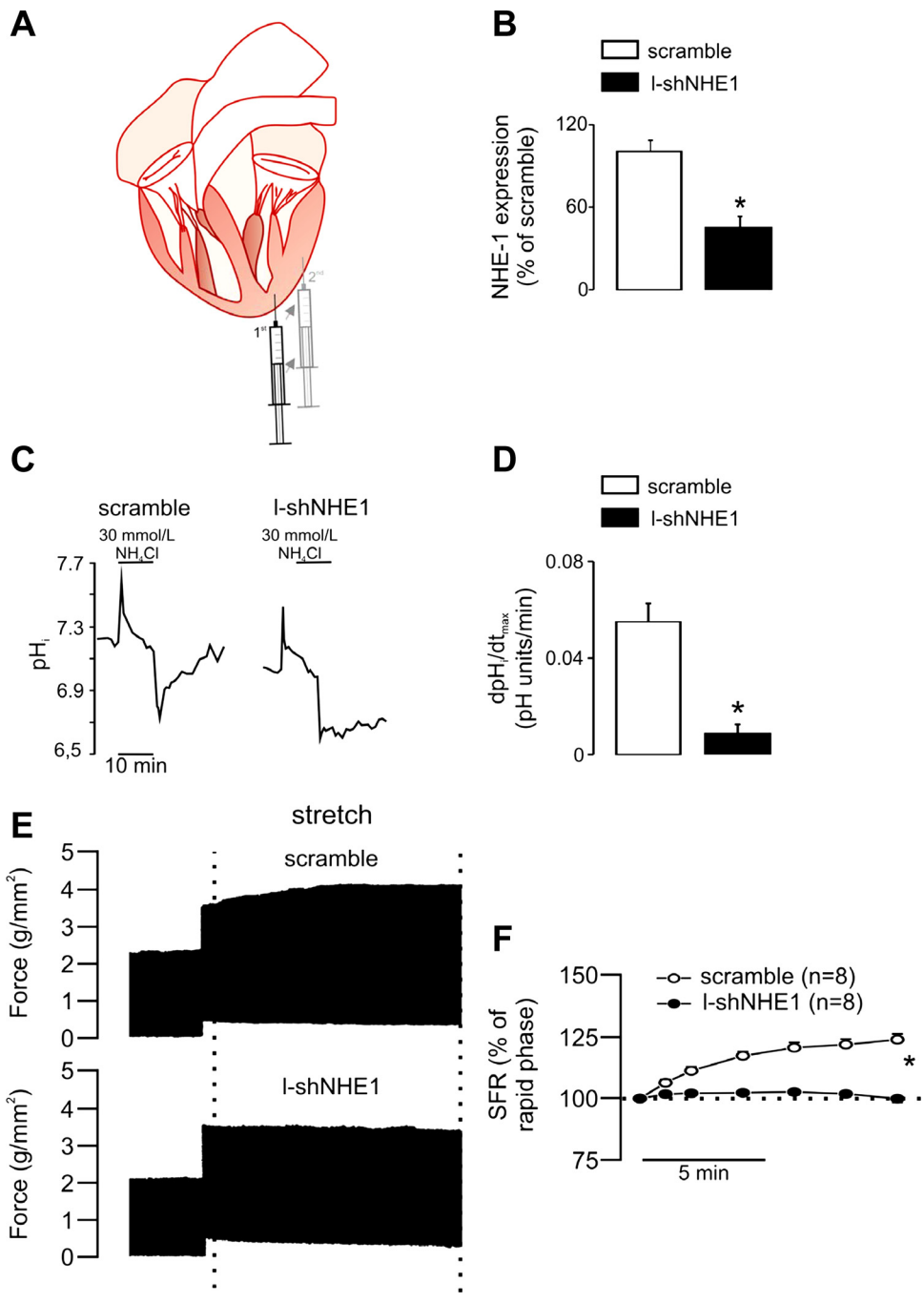

Fig. 3. *A*: schematic representation of the procedure followed to inject the lentivirus into the left ventricular wall. *B*: hearts injected with a small hairpin interference RNA (siRNA) against NHE1 (l-shNHE1) showed a significant reduction in the exchanger protein expression compared with the scramble-injected hearts. *C* and *D*: recovery from an acidic load promoted by an ammonium prepulse was dramatically impaired in papillary muscles from siRNA-injected hearts compared with scramble. pH_i, intracellular pH; dpH_i/dt_{max}, maximum change of pH_i over time. *E*: original force records of papillary muscles from a scramble (*top*)- and siRNA (*bottom*)-injected hearts subjected to a sudden increase in length. It can be appreciated the classical biphasic response to stretch in the scramble muscle (*top*) and the absence of SFR in the siRNA-injected one (*bottom*). *F*: averaged SFR expressed as percentage of the initial rapid phase from both groups of animals. Modified with permission from Perez et al. (58). $*P < 0.05$ vs. scramble.

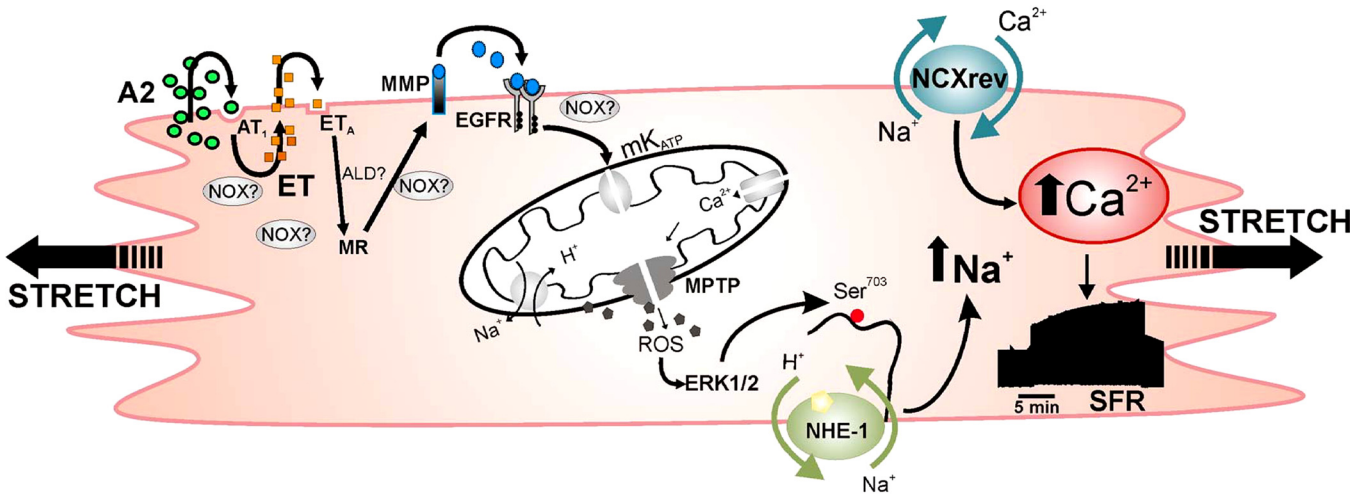

Fig. 4. Schematic representation of the proposed chain of events triggered by myocardial stretch. Stretch promoted release of preformed ANG II which through AT_1 receptor stimulation triggers the release/formation of ET. NADPH oxidase (NOX)-generated ROS may play a significant role in this or several other step of the signaling cascade. ET activates the ET type-A (ET_A) receptor, which activates MR and induces EGFR transactivation through a pathway that involves matrix metalloprotease (MMP) activation and possibly the ROS-sensitive Src kinase. EGFR activation triggers an intracellular signaling pathway that leads to mitochondrial ATP-sensitive potassium (mK_{ATP}) channel opening increasing ROS production and release through the mitochondrial permeability transition pore (MPTP). This causes ERK1/2 activation and NHE1 phosphorylation at Ser703 increasing NHE1 activity and intracellular Na^+ concentration that favors intracellular Ca^{2+} accumulation through the Na^+/Ca^{2+} exchanger (NCX). Ald, aldosterone. Modified with permission from Villa-Abrille et al. (70).

is known to be activated by membrane deformation (10). These authors proposed a stretch-activated signaling cascade similar to the one depicted in Fig. 2. A pathway that, according to our scheme, could be localized upstream the release of ANG II.

To complicate matters even more, the proposal stating that NHE1 is downstream ROS and redox-sensitive kinases, being therefore activated by mitochondrial ROS generation, was recently partially challenged by two studies from our own laboratory. In fact, we showed that either pharmacologically inhibiting NHE1 or silencing it with the administration of a specific small hairpin interference RNA (siRNA) targeted mitochondrial NHE1 and decreased ROS production (29, 71). Therefore, in addition to the idea that sarcolemmal NHE1 inhibition decreases $[Na^+]_i$, an additional role decreasing mitochondrial ROS formation seems to be also occurring and contributing to kinase activation.

We have recently shown that silencing NHE1 expression by a specific siRNA incorporated into a lentiviral vector and injected into the left ventricular wall cancels the SFR (58) (Fig. 3), providing evidence supporting NHE1 activation as a key mediator in the SFR.

In addition to the experimental evidence described in this section, a more recent mathematical model developed by Niederer and Smith (53) was used to revise the relative contribution of the different mechanisms mentioned above on the cause of the SFR to a step change in ventricular muscle length. Based on their simulations, the authors estimated that SACs play a significant role in producing the SFR, potentially in the presence of stretch-dependent NHE and Na^+-independent Cl^-/HCO_3^- anion exchanger activation. However, Kondratev et al. (42) proposed that the SFR was independent of NHE1 activation, although NHE1 inhibition prevented the stretch-induced cytosolic Ca^{2+} rise. Our finding that silencing the NHE1 by intramyocardial administration of a lentivirus coding for a specific siRNA against the NHE1 suppressed the SFR does not agree with the latter and provides evidence supporting the key role played by the NHE1 in the SFR development (58).

A quite interesting remark is the persistence of the stretch-induced NHE1 activation over time when we consider a time frame different from the 10–15 min after the onset of myocardial stretch. We have recently reported that 7 wk of transverse aortic constriction in mice resulted in cardiac hypertrophy with decreased cardiac performance along with increased activity of the redox-sensitive $p90^{RSK}$ kinase and NHE1 phosphorylation. AT_1 receptor blockade with losartan decreased $p90^{RSK}$ and NHE1 activation and cardiac hypertrophy development, maintaining contractility despite a higher workload (19). This finding underscores the long-term relevance of the mechanisms driving the Anrep effect, where NHE1 activation detected in vitro after a few minutes of stretch can persist in vivo after 7 wk of transverse aortic constriction.

Perspectives. There is enough evidence to suggest that the SFR effect takes place after a series of events, with the release of ANG II igniting this molecular cascade and ending with an increase in the Ca^{2+} transient through activation of the NCX. Interestingly, 50 years after Anrep reported his phenomenon, Sarnoff et al. (66) coined the word "autorregulación," a term transpiring that the mechanism resides in the myocardium itself. A century later, this is quite evident in light of the new cardiac autocrine/paracrine mechanisms described in the present review. Figure 4 depicts the proposed chain of events. However, the possibility that other pathways (acting in series or in parallel) in addition to those described herein could also be playing a role in this phenomenon cannot be denied. Our finding that NHE1 inhibition either pharmacologically or by silencing its expression cancels the SFR and decreases mitochondrial ROS formation opens new avenues of research in the field of cardiac mechanics, hypertrophy, and failure.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

H.E.C., N.G.P., O.H.C., and I.L.E. conception and design of research; H.E.C., N.G.P., O.H.C., and I.L.E. interpreted results of experiments; H.E.C., N.G.P., O.H.C., and I.L.E. edited and revised manuscript; H.E.C., N.G.P., O.H.C., and I.L.E. approved final version of manuscript; N.G.P. and O.H.C. performed experiments; N.G.P., O.H.C., and I.L.E. analyzed data; N.G.P., O.H.C., and I.L.E. prepared figures; I.L.E. drafted manuscript.

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