Differential autocrine modulation of atrial and ventricular potassium currents and of oxidative stress in diabetic rats

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In contrast to numerous studies on diabetic ventricles, few studies have addressed atrial pathology. This is of importance, because the most common arrhythmia (9) is atrial fibrillation (AF). AF leads to changes in several atrial currents (49, 58). A transient K⁺ current is attenuated, although not in all conditions (9). K⁺ currents controlling atrial repolarization are mainly similar to those in ventricles (21, 55), although differences in channel expression levels lead to differences in repolarization patterns (28).

Diabetes (in humans and in rat models) activates a cardiac renin-angiotensin system (RAS) (10, 11). This elevates cardiac levels of ANG II, which acts by autocrine, paracrine, or intracrine mechanisms (2). ANG II, through numerous signaling pathways, plays a major role in cardioprotective and maladaptive mechanisms (7, 52). Chronically elevated ANG II attenuates K⁺ currents (57) and enhances oxidative stress (25), an important feature of diabetes (13, 14). The involvement of ANG II in K⁺ current attenuation was suggested both by direct measurements of ANG II elevation (10, 11, 44) and by the fact that inhibition of either the angiotensin-converting enzyme (ACE) or blockade of ANG II receptors leads to augmentation of attenuated K⁺ currents (35, 39) and associated channel proteins (42, 43). Furthermore, relief of ANG II-mediated oxidative stress also augments these currents (41, 56). The autocrine regulation of K⁺ currents and enhanced ANG II levels and oxidative stress in diabetes is sex dependent (40, 41, 43, 44). ANG II levels are elevated and K⁺ currents are attenuated to a significantly greater extent in males. This is presumably due to inhibition of the RAS by estrogen (12).

Very little is known about RAS activation and its potential modulation of K⁺ currents in atrial cells (A cells) under pathological conditions. A cells secrete a natriuretic peptide (ANP), which plays a key role in cardiovascular homeostasis (6, 19, 36, 53). Mice lacking ANP receptors develop hypertension and hypertrophy and exhibit ventricular arrhythmias and sudden death (18, 29). Cardiac-specific ANP receptor attenuation also compromises protection against adverse conditions such as pressure overload (30). Importantly, the protective role of ANP is linked to its ability to inhibit the RAS, thus countering detrimental effects of RAS activation (18, 30). Furthermore, the action of natriuretic peptides persists under diabetic conditions, when other protective mechanisms, such as the nitric oxide system, are compromised (53).

Based on earlier studies, we hypothesized that interaction of ANP and the RAS could result in different patterns of K⁺ current modulation in A cells and ventricular cells (V cells), as well as a potentially different degree of oxidative stress. Our

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objectives were 1) to establish whether atrial K+ currents are modified in diabetes, 2) to determine whether A cells exhibit RAS activation and elevated oxidative stress, 3) to investigate whether the RAS modulates atrial K+ currents, and 4) to investigate whether natriuretic peptides modulate K+ currents and oxidative stress under diabetic conditions and whether this relates to interaction with the RAS.

**METHODS**

This study conforms to the National Institutes of Health Guide for Care and Use of Laboratory Animals. All of the experiments were done according to guidelines established and approved by the Animal Care and Use Committee of the University of Calgary.

**Animals.** Age-matched control (untreated) and diabetic male Sprague-Dawley rats (250–300 g) were used. Type 1 diabetes was induced by intraperitoneal injection of streptozotocin (100 mg/kg) 8–14 days before experiments. In one subset of experiments, diabetic female rats were also used.

Cell isolation was achieved by enzymatic dispersion. Rats were anesthetized by CO2 inhalation and euthanized by cervical dislocation. Hearts were perfused, after aortic cannulation, with a solution (at 37°C, bubbled with 100% O2 containing (in mM) 113 NaCl, 4.7 KCl, 1.2 MgSO4, 0.6 KH2PO4, 0.6 NaHPO4, 12 NaHCO3, 12 KCl, 5.5 glucose, 10 HEPES, 30 taurine, and 10 2,3-butanedione monoxime (BDM). This was followed after 5–6 min by the same solution, also containing l-arginine (0.02–0.25 mg/ml, Roche), trypsin (0.14 mg/ml), and 12.5 μM CaCl2. After 7–8 min, the free wall of the right ventricle or both atria were cut into pieces. After shaking was completed, tissue pieces were filtered and cell suspensions were collected and stored at room temperature in a solution containing no enzymes, 20 mM taurine, 5 mg/ml albumin, and 0.1 mM CaCl2.

**Current recording.** Cells were placed on a stage of an inverted microscope and perfused (at 21–22°C) with a solution containing (in mM) 150 NaCl, 5.4 KCl, 1 CaCl2, 1 MgCl2, 5 HEPES, and 5 glucose (brought to pH 7.4 with NaOH). L-type calcium current was blocked by 0.3 mM CdCl2. Currents were recorded by whole cell voltage clamp, using 500–ms pulses to membrane potentials ranging from −110 to +50 mV. Digitized currents (2 kHz) were normalized to cell size by dividing by cell capacitance. Recording pipettes (2–3 MΩ resistance) contained (in mM) 120 potassium aspartate; 30 KCl, 4 KOH). Two currents were studied. The transient outward current (Ito), which determines early repolarization and the action potential plateau level, was measured (and is presented) both as the peak outward current and as the difference between peak current and the current at the end of 500-msec pulses.

The sustained outward current (Islow), measured at the end of 500-msec pulses, reflects a mixture of delayed rectifier currents (28) that determine late repolarization. Current densities were compared in the absence or presence of drugs. Results from different days were pooled. It should be noted that changes in current densities reflect changes in channel expression, which are measurable only after incubations of >5 h.

Superoxide production was detected by using dihydroethidium (DHE, Molecular Probes), a cell-permeable fluorescent dye that is oxidized by superoxide to ethidium bromide, a nucleic acid stain. Its fluorescent intensity indicates the relative level of superoxide production (14). Myocytes were suspended in a 800-μl solution containing (in mM) 120 NaCl, 5.4 KCl, 1.2 MgSO4, 1.2 Na H2PO4, 5.6 glucose, 20 NaHCO3, 10 BDM, and 5 taurine and 100 μM calcium and 0.2% fatty acid-free BSA. DHE (5 μM) was added to the cells, which were incubated in a light-protected incubator (30 min, 37°C). Suspensions were centrifuged (2 min, <1,000 g), and pellets were resuspended in 20–50 μl PBS. A small aliquot (10–15 μl) was fixed (with 90% glycerol) onto a slide.

DHE fluorescence was quantified by using an Olympus IX70 inverted epifluorescence microscope and a SPOT RT-cooled CCD camera. All images (1,600 × 1,200 pixels) were collected with the same camera settings. DHE fluorescence was analyzed with the use of two methods (41). First, a lower pixel intensity threshold was determined (using SPOT software) for images of individual nuclei, so that only light from the nucleus remained in the image. The mean fluorescence intensity of pixels above threshold for each nucleus was determined by using software designed for this purpose. In the second method, a boundary was drawn around each nucleus and the mean fluorescence intensity of pixels within the nuclear boundary (region of interest) was determined from an intensity histogram by using Photoshop software. Both methods of analysis gave similar results.

Cellular ANG II content was measured by ELISA, using a commercial kit (Peninsula). Standard curves were constructed, and optical densities of samples (in triplicates) were read from these curves (all values falling within the calibration curve). ANG II levels were normalized for protein content, measured in the same samples (using a bichinchoninic acid protein assay kit from Pierce). Values were similar to those reported by others (10).

**ANG II immunofluorescence.** ANG II is mainly localized in subcellular granules (10, 11). In our experiments, atria and ventricles were embedded in optimum cutting temperature compound (Electron Microscopy Sciences), frozen in 100% ETOH on dry ice for 30 min, and stored at −80°C. Frozen tissue was cut into 8-μm sections, using a cryostat (−20°C). Sections were mounted on coated slides (VWR) and stored at −80°C. For ANG II labeling, sections were air dried (room temperature) and fixed (1% formaldehyde). After being washed (3 times) with PBS, the tissue was permeabilized by using 1% Triton-X (10 min, room temperature). Nonspecific binding was blocked by using 2% BSA + 5% normal goat serum. A rabbit anti-ANG II antibody (Peninsula) was added (1:200 dilution in PBS + 2% BSA) and left overnight in a humid chamber. After tissues were washed in PBS, secondary antibody (anti-rabbit IgG conjugated to Alexa 488; Molecular Probes) was added (1:100 in PBS, 2% BSA). After 1 h in a humid chamber, the slides were washed in PBS. Finally, a mounting medium was added (Media, Inc.), and the slides were covered and stored at 4°C. As a control, ANG II (0.06 μg) was added to 1 μl of antibody. The preabsorption of ANG II antibody yielded unstained slides. These verified antibody specificity and were used to determine background fluorescence.

Quantification of ANG II was done as with DHE, using atrial and ventricular sections labeled with anti-ANG II. As noted above, background was determined from sections exposed to preabsorbed primary antibody, followed by secondary antibody. Multiple background images were collected for each preparation, and the background value used for the analysis was taken as the mean of the background light intensities determined from these images. All sections were labeled at the same time under the same conditions, and images were collected using the same camera settings. Background fluorescence was subtracted, and the number of pixels in each image with light intensities above background was determined. This value was used as one measure of ANG II levels. A second measure was obtained by computing the total above-background light intensity. This was done by summing the intensities of all of the pixels in the image that had values above background. Similar results were obtained from both methods of analysis. Several control and diabetic hearts were used, and a large number of images (>30) were collected and analyzed for each tissue type. As can be seen in one of the images shown in RESULTS (see Fig. 5A), some tissue sections did not occupy the entire field in an image. Inclusion of these images in the analysis could bias the results because there were fewer cells in the field. To avoid this possibility, the percentage of image area occupied by cardiac tissue was determined for each image and used to correct the fluorescence intensity values for that image.

To test the validity of the general method of analysis used to compare DHE fluorescence or anti-ANG II immunofluorescence
different cell or tissue samples, fluorescent microspheres were suspended in solution and different dilutions of the spheres were pipetted onto microscope slides. Images of the suspensions in randomly chosen fields on the slides were then collected, and the fluorescence in the images was analyzed by determining both the total number of pixels above background in the images and the total fluorescence intensity of all the pixels that were above background. Figure 1 summarizes the results of this analysis for six experiments. In five of the experiments, the microspheres were diluted in water and backgrounds that eliminated out-of-focus light were subtracted from the images. In the sixth experiment, the spheres were diluted in water and then added to suspensions of unlabeled isolated cells that provide a background of autofluorescence that was then subtracted from the images. Results from the two types of experiments are combined in Fig. 1. The results shown indicate that, although there is a slight deviation from linearity for the least concentrated samples, the method of analysis was able to predict relative changes in concentration quite well. The deviation of the results from the expected relative fluorescence at the higher dilutions is likely due to the greater effect of variance in the background light level on the measurements made when the number of beads in the fields was low.

**Drugs.** Quinapril-HCl was prepared as stock solution in 0.5% ethanol and diluted to the final concentration (1 μM for in vitro experiments and 6 mg/l for in vivo experiments). Final ethanol concentrations were <0.005%, and the pH of the solutions was 7.4.

**Statistics.** Results are given as means ± SE. Mean values were compared by using t-test or ANOVA, with the Student-Newman-Keuls multiple comparisons test. P values of <0.05 were considered significant.

## RESULTS

The first set of experiments compared peak and sustained outward currents in A cells from control and diabetic rats. In contrast to V cells (39), there was no significant current attenuation in diabetic A cells. Figure 2A shows current traces in atrial myocytes from control (left) and diabetic (right) rats (same protocol used in subsequent figures). STZ, streptozotocin. B: mean current densities as a function of membrane potential [transient (I_{trans}; left) and sustained (I_{sus}; right) outward current] in cells from control (○) and diabetic (●) rats. C: difference currents (I_{trans} – I_{sus}) are also not significantly different.

![Fig. 2. Outward K⁺ currents in atrial cells. A: current traces obtained in response to pulses given from −80 mV to potentials ranging from −10 to +50 mV in atrial cells (A cells) from a control (left) and a diabetic (right) rat (same protocol used in subsequent figures). STZ, streptozotocin. B: mean current densities as a function of membrane potential [transient (I_{trans}; left) and sustained (I_{sus}; right) outward current] in cells from control (○) and diabetic (●) rats. C: difference currents (I_{trans} – I_{sus}) are also not significantly different.](image-url)
quinapril (1 μM, >5 h). For comparison, Fig. 3B shows the effects of this protocol in V cells.

These results suggest that the RAS is less (or not) activated in diabetic A cells or that the effects of ANG II are suppressed.

Our laboratory (44) previously measured ANG II levels in V cells, using ELISA. However, this was not feasible with A cells, because enzymatic dispersion yields considerably fewer cells. We therefore used immunofluorescent ANG II labeling of ventricular and atrial sections (as in Ref. 11). Concordant with our earlier results and with Ref. 11, we confirmed that ANG II labeling is significantly increased (see METHODS) in diabetic V cells, compared with control (4 rats, >30 sections in each group). This is shown in Fig. 4, which also shows the lack of labeling when the ANG II antibody was preabsorbed with ANG II (Fig. 4C).

Based on the electrophysiological results with A cells (above), we hypothesized that no differences in ANG II would be found between control and diabetic A cells. The results with atrial sections confirmed this, although, surprisingly, ANG II labeling was high in both control and diabetic A cells, as shown in Fig. 5.

These results highlight a major difference between diabetic V and A cells in that diabetes elevates ANG II levels only in ventricles. If current attenuation depends on increased levels of ANG II, the absence of ANG II changes presumably underlies the lack of attenuation of \( \mathit{I}_\text{to} \) and \( \mathit{I}_\text{sus} \) magnitude in diabetic A cells. Furthermore, high ANG II levels in control A cells may...
underlie the smaller baseline currents, compared with V cells. In V cells, ACE inhibition presumably suppresses ANG II levels within 5 h, because K⁺ currents are augmented after incubation with quinapril for 5–9 h. It is possible that with high baseline ANG II levels in A cells, ACE inhibition might not reduce ANG II levels sufficiently to enable augmentation of K⁺ currents (Fig. 3).

We subsequently tested whether atrial-ventricular differences result from a suppression of ANG II effects in A cells, rather than from differences in ANG II levels per se.

ANG II induces oxidative stress, mainly through the activation of NADPH oxidase and the generation of superoxide ions (25). These can be measured by using fluorescent DHE. DHE interacts with superoxide, binding to nuclear DNA proportionately to superoxide levels (14). Superoxide ion levels were compared in A and V cells, as a measure of ANG II-induced oxidative stress. DHE fluorescence was significantly higher in V cells from diabetic (n = 3) compared with control (n = 3) rats. This confirms the presence of oxidative stress, a prominent feature of diabetes (13, 14). Figure 6, A–C, shows V cells from a control (Fig. 6A) and a diabetic (Fig. 6B) rat, as well as mean fluorescence intensity (Fig. 6C) in one paired comparison. We subsequently isolated V and A cells from diabetic rats and compared DHE fluorescence in cells from the same hearts. In five out of seven rats, A cells had significantly (P < 0.005) or very significantly (P < 0.0001) lower DHE fluorescence than V cells, indicating lower atrial oxidative stress. Figure 6, D–F, shows sample cells and the mean fluorescence intensity from one of these comparisons.

These experiments show that A cells are partially protected from the development of oxidative stress that occurs in V cells. This may underlie the absence of K⁺ current attenuation in these cells. The lack of sensitivity of atrial K⁺ currents to quinapril and a lower oxidative stress suggest that the RAS is either less activated in diabetic A cells and/or that its effector pathways are inhibited.

Earlier work suggested that the RAS can be inhibited by the ANP. We hypothesized that if the more abundant ANP in A cells (6, 19, 53) inhibits effects of the RAS, then the addition of exogenous ANP may reverse some of the effects of activated RAS in V cells. We therefore compared the effects of ANP on ventricular K⁺ currents in control and diabetic cells. Earlier reports suggested dose-dependent actions of ANP, with maximal effects at 1 μM (54). We used ANP at concentrations ranging from 100 nM to 1 μM.

In V cells from diabetic (male) rats, in which the RAS activity and ANG II levels are elevated (10, 44), 300 nM ANP (5–9 h) significantly augmented both Iₒ and Iₗₘₜₜ, as shown in Fig. 7. Iₒ, densities (at +50 mV) were 13.3 ± 1.1 (32 cells, 3 rats) and 18.4 ± 1.25 (33 cells, 3 rats) pA/pF (P < 0.004) in the absence or presence of ANP, respectively. The corresponding values for Iₗₘₜₜ were 4.1 ± 0.1 and 5.1 ± 0.2 pA/pF (P < 0.00001). An addition of 1 μM ANP produced similarly significant augmentation of both currents (5 rats, 51 untreated and 42 treated cells). In a small group of cells, 100 nM ANP significantly augmented Iₗₘₜₜ. Iₒ was also augmented but not significantly in the smaller sample of cells.

In V cells from control rats, in which the RAS is not activated, even the higher concentration of ANP was without effect. The mean densities (at +50 mV) of Iₒ in the absence and presence of ANP (1 μM, 5–9 h) were 24.7 ± 2.1 (21 cells, 3 rats) and 25.1 ± 1.0 (19 cells, 3 rats) pA/pF, respectively (P > 0.05). The corresponding values for Iₗₘₜₜ were 7.3 ± 0.3 and 7.5 ± 0.6 pA/pF (P > 0.05) (results not shown).

ANP also had no effects in V cells from diabetic females, in which ANG II levels are not augmented (44), indicating that...
RAS preactivation is required for ANP action. This result is also shown in Fig. 7.

In diabetic A cells, the RAS is apparently not activated above control (Fig. 5), and/or effects of ANG II may be blocked. Concordantly, ANP was without effect. Mean \( I_{\alpha} \) densities (at +50 mV) in the absence and presence of ANP (1 \( \mu \)M, 5–9 h) were 12.4 ± 1.3 (27 cells, 5 rats) and 13.3 ± 1.4 (24 cells, 5 rats) pA/pF, respectively (\( P > 0.05 \)). The corresponding values for \( I_{\text{sus}} \) were 5.9 ± 0.3 and 6.2 ± 0.4 pA/pF (\( P > 0.05 \)) (not shown).

ANP has been shown to protect against oxidative damage (31). We thus investigated whether ANP inhibits induction of oxidative stress by ANG II. Cells were prepared from three diabetic rats and divided into two groups. One was incubated in ANP (1 \( \mu \)M for 5h), and the other was left untreated. Subsequent labeling of cells from both groups showed that in all three pairs, ANP caused a significant attenuation in DHE fluorescence, indicating reduced levels of superoxide ions. One example is shown in Fig. 8.

Our results suggest that preactivation of the RAS is required for augmentation of \( I_{\alpha} \) and \( I_{\text{sus}} \) by ANP, which acts by inhibiting this system. ANP effects were tested under conditions in which the RAS is either not activated or is directly inhibited. First, male diabetic rats were given the ACE inhibitor quinapril in vivo for 3 wk (6 mg/l in the drinking water) before induction of diabetes. ANG II content in V cells from diabetic rats, without \( (n = 4) \) or after quinapril treatment \( (n = 4) \), was 47.1 ± 9.2 and 3.6 ± 0.5 pg/mg protein, respectively \( (P < 0.005) \), as shown in Fig. 9A.

In V cells from these quinapril-treated diabetic rats, \( I_{\alpha} \) and \( I_{\text{sus}} \) are still lower than in the control, despite the reduction in ANG II levels (see DISCUSSION). However, in these cells, ANP (1 \( \mu \)M) no longer augments \( I_{\alpha} \) and \( I_{\text{sus}} \), as shown in Fig. 9, B and C. Mean \( I_{\alpha} \) (at +50 mV) was 14.8 ± 1.0 (37 cells) and 15.2 ± 0.8 (39 cells) pA/pF in the absence or presence of ANP, respectively \( (P > 0.05) \). Corresponding \( I_{\text{sus}} \) values were 4.6 ± 0.2 and 4.9 ± 0.2 pA/pF \( (P > 0.05) \).

Finally, V cells from three diabetic male rats were exposed to ANP in the presence of excess (300 nM) ANG II. As shown in Fig. 10, the augmentation of \( I_{\alpha} \) and \( I_{\text{sus}} \) by ANP was significantly reduced, suggesting that excess ANG II counters the inhibitory effect of ANP on the RAS.

**DISCUSSION**

In summary, the present work provides several novel results. The major finding is that cardiac autocrine mechanisms, modulating \( K^+ \) currents and oxidative stress, are strikingly different in V and A cells. In contrast to the situation in V cells, atrial \( K^+ \) currents are not attenuated after the onset of Type 1 diabetes (Fig. 1). In addition, atrial \( K^+ \) currents are insensitive to the ACE inhibitor quinapril and to ANP, in contrast to V cells (Figs. 2 and 6). A further novel and important finding is that oxidative stress is lower in A cells than in V cells from the same diabetic hearts (Fig. 5). ANP may be a major contributor to the lower oxidative stress in A cells, as suggested by the reversal of augmented oxidative stress in diabetic V cells by ANP (Fig. 7). Finally, diabetic conditions augment ANG II in V, but not A, cells (Figs. 3 and 4).

**Interpretation.** Our hypothesis was that the activation of the RAS and ensuing ANG-mediated effects would be modified by ANP. Basal levels of ANP are higher in A cells (6) and presumably prevent activation of the RAS (Fig. 4) that occurs in V cells (Figs. 2 and 6). A further novel and important finding is that oxidative stress is lower in A cells than in V cells from the same diabetic hearts (Fig. 5). ANP may be a major contributor to the lower oxidative stress in A cells, as suggested by the reversal of augmented oxidative stress in diabetic V cells by ANP (Fig. 7). Finally, diabetic conditions augment ANG II in V, but not A, cells (Figs. 3 and 4).

**ANP and RAS preactivation.** The interaction with ANP may occur at the level of cGMP. cGMP, which is the major second messenger that is elevated by ANP (6, 19, 53), is increasingly recognized as a modulator of gene expression (19, 32) and could therefore be a positive modulator of some \( K^+ \) channels. ANG II inhibits...
cGMP signaling (17), which could be one of the mechanisms leading to current attenuation. The presence of ANP may counteract this action of ANG II, as well as ANG II-mediated oxidative stress.

Several lines of evidence support this. ANP augments K⁺ currents and suppresses oxidative stress in V cells from diabetic males (Figs. 6 and 7), but not if the RAS is inhibited before the onset of diabetes by quinapril (Fig. 8) or if the RAS is not activated, as in control males or in diabetic females (44). Furthermore, excess ANG II suppresses current augmentation by ANP (Fig. 10), suggesting a reciprocal inhibition between ANG II and ANP (17). This complexity is supported by reports showing inhibition of some ANP actions by ANG II (34).

Further work is required to elucidate the signaling pathways involved, to establish which channel isoforms are altered in V and A cells, and to determine whether there is a selective sensitivity to ANP of these isoforms.

Concordant with our study, other pathologies also exhibit similar atrial-ventricular differences. For example, in hyperthyroid dogs, ANG II receptor densities increase in ventricles but not in atria (37). In a rat model of cirrhosis, ventricular but not atrial K⁺ currents are attenuated, although mechanisms underlying this difference are unknown (51). Several cardiac pathologies lead to elevation in natriuretic peptides in both ventricles and atria (6). However, transcriptional control of peptide expression in atria and ventricles is different, because this expression is suppressed by ACE inhibition in ventricles but not atria (6). This suggests a different linkage between the RAS and ANP pathways in the two cell types. This is supported by our findings (Fig. 4) that diabetes leads to increased ANG II levels in V but not A cells.

In contrast to diabetic conditions, atrial K⁺ currents are modulated under other pathophysiological conditions, includ-
ing metabolic stress, heart failure, or AF (49). Oxidative stress during AF is associated with augmented superoxide production (8), as well as current attenuation (48, 49), although oxidative stress may occur after abnormal (mechanical) function, rather than being a causative agent. Interestingly, ventricular tachypacing and AF are also associated with augmented RAS activity (15), with elevated ANG II levels, presumably underlying oxidative stress (8). ACE inhibitors and ANG II receptor blockers are reportedly of benefit for AF (16, 45), although this may be limited to subsets of patients (16). In paced canine hearts, ANG II levels increase more in atria than in ventricles (15). This suggests that different pathologies activate different mechanisms and/or that significant species differences are present.

The complexity of regulatory mechanisms may also involve temporal changes in the involvement of the RAS. Shinagawa et al. (45) found short- but not long-term protective effects of ACE inhibition in AF. This may be linked to similar time-limited effects of ANP (47).

In diabetes, plasma ANP levels are elevated (22), but the expression of a closely related (brain natriuretic) peptide is chamber dependent, increasing only in atria (4). Interestingly, regulation of ANP actions may be defective in diabetes (27), although other reports indicate the persistence of protective action (53). Our results suggest that, at least in diabetes, high natriuretic peptide levels in A cells prevent further increase of ANG II and limit oxidative stress, presumably by inhibiting RAS activation. This results in unal-

Fig. 9. Effects of quinapril treatment. A: ANG II content (measured by ELISA) in diabetic male V cells in absence (open bar) or presence (hatched bar) of quinapril (6 mg/I for 3 wk) in drinking water before STZ injection (continued until cell isolation). After quinapril treatment, ANP no longer augments outward currents. B: current traces in absence (left) or presence (right) of ANP (1 μM, 9 h). C: mean I_to (left), I susp (middle), and I_diff (right) densities (at +50 mV) in absence or presence of ANP in cells from quinapril-treated rats.

Fig. 10. ANG II suppresses ANP effects. A: currents obtained in male diabetic V cells (3 rats) with no ANP (left, n = 18), after ANP (1 μM, 6.5 h, n = 15), and with ANP and 300 nM ANG II (n = 15), added 30 min before ANP (8 h of ANP). B: mean I_to (left), I susp (middle), and I_diff (right) densities (at +50 mV) show significant (*P < 0.05) augmentation of both currents by ANP, as well as significant suppression of this effect by ANG II.
tered atrial $K^+$ currents that are insensitive to ACE inhibition.

**Limitations.** ANP was not directly measured in this study. However, numerous reports suggest that ANP is found predominantly in A cells (6). Ventricular ANP can increase in some pathological conditions (6). Our results suggest that if this occurs in diabetic conditions, the magnitude is small and insufficient to prevent activation of the RAS and increases in ANP II (Fig. 3 and Refs. 10, 11, and 44). Furthermore, exogenous ANP augments attenuated ventricular (Fig. 6) but not atrial currents. The differences in oxidative stress (Fig. 5), largely ANP II dependent (13, 14, 25), also suggest lower ANP levels in V cells. Non-ANG II-related causes of oxidative stress (13) may explain why atrial and ventricular differences were not found in all cases.

Another limitation of this study lies in the fact that right and left atria were pooled. Several studies suggest differences between right and left atria, in terms of oxidative stress (8) or left atria were pooled. Several studies suggest differences largely ANG II dependent (13, 14, 25), also suggest lower ANP levels in V cells. Non-ANG II-related causes of oxidative stress (13) may explain why atrial and ventricular differences were not found in all cases.

Further analysis of the oxidative stress in the atria (19) also demonstrated that reparative processes involved in cardiac cellular homeostasis.

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