Connecting the renin–angiotensin–aldosterone system with sudden death

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“Sudden death is nature’s way of telling you to slow down”! This quote has been attributed to Woody Allen, but I have not asked him personally and have not yet tested the hypothesis. After all, “who needs it?” In this issue of J Mol Med, Iravanian et al. explore mechanisms responsible for sudden death that are strongly influenced by the renin–angiotensin system [1]. The issue is germane since blockade of the renin–angiotensin–aldosterone system, either through inhibition of angiotensin-II (Ang II) generation or inhibition of mineralocorticoid receptor signaling, is said to decrease ventricular arrhythmias and sudden death [2]. Since we are amply equipped to block the renin–angiotensin–aldosterone system, we could have an opportunity to reduce sudden death, which afflicts about 25% of all patients with heart disease. Elucidating the responsible mechanisms is therefore of paramount importance.

The authors used mice with overexpression of angiotensin-converting enzyme (ACE) in the heart; the overexpression increases the amount of local Ang II [1]. The mice have increased propensity to develop ventricular tachycardia (VT) when stimulated and also have reduced expression of connexin 43 in the heart. The mice were treated with the ACE inhibitor captopril or the angiotensin receptor blocker (ARB) losartan. Both drugs reduced the propensity to VT and increased connexin 43 expression. The authors included whole cell patch clamping and were able to show a leftward shift of conductance and steady-state inactivation curves. They also verified gap junctional conductance.

The connexins are four-pass transmembrane proteins, six of which assemble to form a hemichannel or “connexon” (Fig. 1). When the connexons in the plasma membrane of two cells in contact are aligned, they form a continuous aqueous channel that connects the two cell interiors. A gap junction includes many of such connexon pairs in parallel. Furthermore, the connexons serve to hold the two cells a certain distance apart. The single units are termed “connexins” and are delineated by their molecular weights. Most cells express more than one type of connexin and the two connexin proteins can assemble into heteromeric configurations, namely the connexon. Moreover, different connexin proteins can assemble into a tetrameric connexon that has its own specific properties [2]. By virtue of its name, we can conclude that connexin 43 is sized around 43 kDa. Connexin 43 is otherwise known as gap junction alpha-1 protein and is encoded by the GJA1 gene [3, 4]. The encoded connexin 43 protein is the major protein of gap junctions in the heart. Connexins are thought to have a crucial role in the synchronized contraction of the heart and in embryonic development [5]. A related “intron-less” pseudogene has been mapped to chromosome 5. Mutations in this gene have been associated with oculodentodigital dysplasia and heart malformations. Thus, we could assume that the authors of the present report are on to something.

The group first observed a marked propensity of mice with cardiac ACE overexpression to die suddenly in an earlier study [6]. The mortality of transgenic animals was >75% at 100 days, while no control mice died. In a follow-up study, the authors observed that atrial connexin 40 and connexin 43 protein levels, as well as the ventricular cardiac sodium channel (Scn5a), were reduced in the hearts of mice with ACE overexpression [7]. They also showed that a connexin blocker, 1-heptanol (0.5 mM), produced an electrophysiological phenotype similar to that seen in ACE cardiac

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Fig. 1 Gap junctions are composed of hemichannels, or “connexons”. Connexons are constructed from six connexin molecules, identified in terms of their molecular weight (in kilodaltons). Gap junctions are essential for the depolarization of cardiac muscle, embryonic development, and the conducted responses in blood vessels. A gap junction forms a sort of molecular sieve. The connexons hold the interacting plasma membranes a fixed distance apart (gap). Regulation of these remarkable structures is the object of intense study

transgenic mice. Our laboratory made similar observations in a quite different model, namely the double transgenic rat (dTGR) harboring both human renin and angiotensinogen genes [8]. Untreated dTGR showed early increased perivascular and interstitial fibrosis, connective tissue growth factor expression, and monocyte infiltration compared with control rats. Furthermore, left ventricular mRNA expression of potassium channel subunit Kv4.3 and connexin 43 were significantly reduced in the dTGR. The expression of these proteins, however, could be restored with losartan treatment. VT could be induced in almost all dTGR in our study but only in 33% of losartan-treated dTGR. In another study involving dTGR, we observed that n-3 polyunsaturated acids also improve electrical remodeling in this model and restore connexin 43 proteins to their normal localization at the intercalated discs [9]. In an Ang II infusion mouse model, connexin 43 turned up again, as we observed that adoptive regulatory T cell transfer also promoted normalization of connexin 43 gap junction localization and reduced the propensity to arrhythmias [10]. Thus, there appears to be consensus. Ang II-induced mechanisms potentiate the proclivity to arrhythmias and disordered connexin 43 function. Effective treatments are available to ameliorate that state-of-affairs.

However, there is even more evidence from other sources. Stretch may be an important stimulus to connexin 43 remodeling. Hussain et al. [11] hypothesized that localized stretch *in vivo* causes connexin 43 remodeling, with associated changes in conduction, mediated by the renin–angiotensin–aldosterone system. They used an open chest canine model and stretched the right ventricle for 6 h. Connexin 43 protein was reduced by this maneuver and electrical properties were impaired; both were improved by ARB administration. Stein et al. [12] investigated old mice. Old age is known to be a risk for sudden death. In their studies, not only ARB but also mineralocorticoid receptor blockade was employed. Again connexin 43 turned up. The authors suggested that chronic renin–angiotensin–aldosterone inhibition limited aging-related interstitial fibrosis. They attributed the effect to fibrosis; however, connexin 43 expression correlated with arrhythmia potential.

Our discussion has centered around the target organ damage aspect of the renin–angiotensin–aldosterone system and connexins. However, the beginning of the renin story also warrants attention. Absence of connexin 40 leads to ectopic juxtaglomerular renin expression and abrogates recruitment of renin-expressing cells in the adult kidney but does not disturb renin expression during kidney development. These findings implied a role for connexins in renin release. To find an explanation for these observations, the Kurtz laboratory [12] analyzed the expression pattern of major vascular connexins in normal juxtaglomerular epithelioid cells, in recruited renin-expressing cells, and in fetal renin-expressing cells. They found that during renal development, the appearance of renin-producing cells paralleled the expression of connexin 40 and, to a lesser extent, connexin 45. In the adult kidney, juxtaglomerular epithelioid cells expressed several connexins, which localized to arteriolar smooth muscle cells. Recruitment of renin-producing cells in adult kidneys in response to long-term salt deprivation in mice correlated with the reappearance of connexin 40. In any event, connexins are essential in the recruitment of renin-producing cells in the kidney.

Ang II appears to be present intracellularly and can exert signaling events from within the cell and perhaps even within the nucleus. We found earlier that intracellular Ang II binds to intracellular Ang II receptors and elicits an increased Ca^{2+} signal in the Ang II-injected cells [13]. Thereafter, cells in the immediate neighborhood exhibit similar signals. Cell–cell contact was necessary for the Ang II-mediated effects. Our data suggested that intracellular Ang II could stimulate a cluster of cells from a single cell via the release of second messengers. However, the passage of the message was not elucidated. Could connexins have been responsible? In the paper, we speculated about the role of connexins including connexin 43. Most interestingly, heptanol prevented a surge in Ca^{2+} in adjacent cells. Accordingly, current evidence points to roles for connexins

in important regulatory mechanisms in the vasculature and the kidney [14]. Regulation of the renin–angiotensin–aldosterone system, tubuloglomerular feedback, and salt and water reabsorption are included in these activities. Connexins may help regulate blood pressure and may be involved in hypertension and diabetes. As the present report indicates, connexins may also be involved in risk for sudden cardiac death. One sobering clinical thought is that in man, the arrhythmia effects attributable to Ang II could be relatively small. ACE inhibitors, ARB, and mineralocorticoreceptor blockers are in widespread use. A major clinical decrease in sudden death has been claimed but not prospectively documented [15]. However, even small improvements are welcome. Should we ask Woody Allen if his renin–angiotensin–aldosterone system is adequately blocked?

Respectfully,

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