JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1571–1576

Received January 25, 2006
Accepted April 3, 2006
DOI 10.1211/jpp.58.12.0002
ISSN 0022-3573

Ageing to arrhythmias: conundrums of connections in the ageing heart

Sandra A. Jones

Abstract

The proportion of the population that is elderly continues to increase, leading to an increasing need to address problems chiefly associated with old age. Progressive ageing of the heart is associated with an increasing incidence of arrhythmias and disorders of the normal origin of the heartbeat, the sinoatrial node. This intrinsic pacemaker of the heart has an increasing tendency with age to lose its dominant role in pacing the heart, and regulation of heart rate becomes erratic. This 'sick sinus syndrome' is associated with fainting, palpitations, shortness of breath and sudden death. Current treatment of this condition is by implantation of an artificial pacemaker, an intervention increasingly required with age. The current evidence suggests that the normal heartbeat fails due to changes in the expression of critical proteins that ensure the correct production and conduction of the cardiac action potential. Depletion of a protein directly responsible for providing electrical connections between the cells of the heart, connexin 43, appears to leave the normal cardiac pacemaker disconnected and unable to drive the heart. This process may be associated with age-dependent changes in stress-related signalling. Simple interventions such as exercise could impact on the processes hypothesized to be involved and may offer a means to preserve the stability of the electrical activity of the heart into old age without pharmacological manipulation.

Introduction: the high incidence of pacemaker dysfunction in the elderly

It has been predicted that by 2035 one-quarter of the Western population will be classed as elderly, i.e. ≥65 years old (Lakatta 2002). In normal healthy humans the intrinsic heart rate (the heart rate without any autonomic input) declines from 107 beats min^{-1} at 25 years to 90 beats min^{-1} at 50 years, then to 70 beats min^{-1} at 85 years (Jose & Collison 1970; Di Gennaro et al 1987; Opthof 2000; Brignole 2002). Similar observations have been reported in rodent models, such as the guinea-pig, where the intrinsic heart rate was shown to decline from 177 beats min^{-1} at 1 month to 152 beats min^{-1} in the senescent animal (Di Gennaro et al 1987; Alings & Bouman 1993; Jones et al 2004). This age-dependent change represents an intrinsic change in the pacemaker properties of the normal dominant pacemaker of the heart, the sinoatrial node (SAN). Accompanying this normal progressive change in the function of the sinoatrial node is an increasing incidence of sinus dysfunction. Intrinsic sinus dysfunction is at its highest incidence within the elderly population (Rubenstein et al 1972; Hartel & Talvensaari 1975; Rodriguez & Schocken 1990). Those suffering sinus dysfunction experience symptoms ranging from palpitations to dizziness, syncope with persistent fatigue and confusion. These symptoms arise because of a decreased ability to regulate heart rate to maintain adequate cardiac output and episodes of cardiac arrhythmia. Initial clinical diagnosis can be made by 12-lead electrocardiogram (ECG), sometimes in conjunction with exercise testing, confirmed by documenting sinus dysfunction electrocardiographically in association with symptoms. Clinically the dysfunction is observed as rhythm disturbances, pauses in the pacemaking of the SAN, exit block of the cardiac action potential from the SAN and arrhythmias. Without treatment, sinus dysfunction can result in sudden death (Psaty et al 1997; Ross & Kenny 2000). Current medical therapy is limited in efficacy and the majority of patients presenting with sinus dysfunction eventually require surgical implantation of a permanent artificial pacemaker. The elderly account for around 80% of all artificial pacemaker implants (Gregoratos 1999). Patient life expectancy can be increased by implantation

Institute of Membrane and Systems Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

Sandra A. Jones

Correspondence: Sandra A. Jones, Level 10, Worsley Building, Institute of Membrane and Systems Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT , UK. E-mail: s.a.jones@leeds.ac.uk

Acknowledgements: I would like to thank Dr Matthew Lancaster for his assistance in the preparation of this manuscript.

of an artificial pacemaker, drastically reducing the probability of sudden death. These patients, however, still have a substantial mortality rate due to their advanced age, and over 50% will die because of a non-cardiac related event (Flaker et al 2003). Why does the elderly heart become so susceptible to arrhythmias and the SAN fail? Is there something that could be done during the lifespan to prevent this apparent degeneration of the heart as it ages?

Propagation of the cardiac action potential at the sinoatrial node

Located in the wall of the right atrium, the SAN is the primary pacemaker of the heart, where the heartbeat is generated. The initiation of the cardiac action potential at the leading pacemaker site at the centre of the SAN and propagation across the remaining cardiac tissue is determined by the complex interaction of many ion channels. These ion channels give rise to the action potential, but also link the cells of the heart forming the pathways, allowing the rapid propagation of the action potential and co-ordinated activation of the heart muscle. Disruption of either the ion channels producing the cardiac action potential or those forming the electrical links between the cardiac cells could be responsible for the failure of the cardiac pacemaker with age and an increasing susceptibility to arrhythmias.

Whereas desmosomes are responsible for the intercellular physical adhesion of cardiac myocytes, electrical connections are provided by gap junctions that are composed of the cardiac connexin (Cx) proteins: Cx40, Cx43 and Cx45 (Honjo et al 2002). The Cx proteins have different spatial distributions, different voltage dependencies of conductance, single-channel conductance and permeability properties, which are capitalized on to ensure propagation of the action potential through the heart in the correct manner (Jongsma & Wilder 2000). Under normal physiological conditions, within the cardiac tissue of a healthy young individual, effective electrical coupling ensures the uniform propagation of the action potential across the myocardium, resulting in the whole heart behaving as a unified "functional syncytium". The conduction velocity of the action potential through the myocardium is largely dependent on the number of gap junctional proteins present at each intercalated disc and the constitution of these gap junctions (Saffitz et al 2000).

Remodelling of the gap junctions, by loss, gain or derangement of the connexin proteins, in the myocardium can significantly alter the function of the heart at both the cellular and whole organ level. Gap junctional remodelling can lead to changes in action potential duration, the effective refractory period of the tissue and conduction velocity. These changes can facilitate the generation of re-entrant wavelets, commonly observed in arrhythmias. Once initiated it has been observed that common atrial arrhythmias tend to progressively become persistent, resulting in the establishment of points of no return to normal cardiac rhythm. This persistence of atrial arrhythmias has been associated with structural changes and gap-junctional remodelling apparently induced by and associated with the arrhythmic activity (Spach 1994; Allessie et al 2001).

The prevalence of atrial arrhythmias increases with age and is highly prevalent in the elderly, currently affecting 10% of the population over 70 years of age (Kannel et al 1983). Clinical studies performed on different aged patients (ranging from 14 to 84 years of age) without a history of atrial arrhythmias determined a direct correlation between age and the vulnerability of the atria to arrhythmias, concluding that those at most risk were the elderly (Sakabe et al 2003) and suggesting that the mechanism responsible for inducing arrhythmias in the elderly is different to that in the young. Many now agree that the elderly demonstrate slower conduction of the cardiac action potential than their young counterparts and this is likely to be a major contributor towards atrial conduction abnormalities and the initiation of arrhythmias (Anyukhovsky et al 2004). This disruption of electrical coupling has been shown to increase with age at the cellular level due to remodelling of the gap junctions (Kostin et al 2002; Jones et al 2004).

We must, however, also consider the location at which gap junctional remodelling occurs: disturbances at the primary pacemaker, the SAN, and another area that can exhibit spontaneous pacemaking in the heart and serves as the bridge between atrial electrical activity and the ventricle, the atrioventricular node (AVN), can provide a potent substrate for the development of arrhythmias. Disruption of electrical propagation at any of these points will electrically disconnect areas of the heart from their normal source of electrical activation and potentially lead to erratic recruitment of the heart muscle and dispersion of the cardiac action potential. Such disruption will potentially present clinically as palpitations, syncope or sudden death.

Ageing disconnects the nodes of the heart

Cx43 is the principle cardiac protein responsible for rapid conduction of the action potential across the heart. As such, the conduction velocity of the action potential is highly dependent on the amount of Cx43 protein present as gap junctions per cardiac myocyte, as controlled by the balance in the rate of Cx protein synthesis vs degradation (Beardslee et al 1998). Interestingly, however, both the anatomically defined sinoatrial node and atrioventricular node have regions that lack Cx43 expression, both in terms of mRNA and protein regardless of species (SAN: Opthof 1988; Oosthoek 1990; Van Kempen et al 1996; AVN: Nikolski et al 2003).

These regions of limited electrical conductivity are thought to protect the nodes, preserving their ability to function as dominant pacemakers and sources of cardiac initiation. If there was normal conductivity between the surrounding cardiac mass and the nodes, the tissue would be unable to depolarize and initiate a propagating cardiac action potential whilst effectively electrically suppressed by a large surrounding mass of electrically connected tissue with a resting membrane potential of −80 mV. In the face of such electrical suppression, for the nodal tissue to instigate an action potential and raise surrounding tissue to threshold for a propagating action potential to occur would require very large current fluxes. With the relative electrical disconnection of the nodes the centre of the nodes is able to depolarize, producing an action potential that

then propagates outward progressively, depolarizing a larger and larger mass of tissue with improving electrical connection to the bulk cardiac tissue. Once a sufficiently large mass of tissue has been depolarized the action potential will propagate readily to the bulk of the heart without suppression.

The region lacking Cx43 protein within the SAN region has been shown to increase in size during progressive ageing of the guinea-pig. The newly born guinea-pig shows uniform expression of Cx43 protein across the nodal region; by 1 month, however, a region lacking Cx43 protein within the SAN develops (Jones et al 2004). From 1 month onwards the size of the region lacking Cx43 protein increases as ageing progresses until in the eldest animal, examined at 38 months, the area was found to have increased to its maximal size of ~48 mm^2, 14-fold larger than that observed in the 1-month-old animal (Jones et al 2004). The data demonstrate that the aged animal shows a substantial loss of Cx43 protein in comparison with its young counterpart within the sinoatrial node region. As a consequence, the conduction of the action potential slows. Contrasting the propagation velocity of action potential in the region that lacks Cx43 protein with Cx43-expressing regions, the action potential is conducted at a velocity five-fold slower in this area of the SAN compared with the surrounding tissue (Yamamoto et al 1998). A progressive reduction in action potential propagation velocity has now been shown in several species with increasing age (Alings & Bouman 1993; Jones et al 2004). Furthermore, studies of elderly patients known to suffer severe atrial conduction abnormalities have revealed reduced levels of Cx43 protein expression (Kostin et al 2002), similar to those seen in the elderly described by Jones et al (2004). However, the mechanism(s) responsible for the reduced Cx43 has not been determined.

The apparent age-dependent increase in electrical uncoupling is, however, not the only structural change that affects the functioning of the cardiac pacemaker. It has also been shown that the distance the action potential has to propagate from the centre of the SAN, where it is initiated, to reach the bulk of the atrial muscle progressively increases with age (Jones et al 2004). This change is apparently due to the continued growth of the sinoatrial node region during the lifespan. Combined with the reduction in action potential conduction velocity the changes in the sinoatrial node tissue lead to a significant increase in the time taken for conduction of the action potential from the initiation site to the surrounding tissue with age (Jones et al 2004). Taken to its logical conclusion these changes would be expected to lead to blockage of the propagation of the cardiac action potential from the node, rendering it unable to drive pacemaking of the heart. In addition, should the conduction time become extended sufficiently this provides the opportunity for other subsidiary pacemaking tissues, which normally have a slower intrinsic rate of spontaneous activity, to become the dominant pacemaker of the heart. Regulation of cardiac activity by the autonomic nervous system can become limited and abnormal when ectopic pacemakers (those outside the node) become dominant and the heart has an increased susceptibility to arrhythmias.

As with the SAN, an inverse correlation between the velocity of conduction of the action potential and increasing age has also been shown in the AVN (Gillette et al 1982). A detailed study still remains to be completed, but should the AVN show a similar change in connexin expression and associated apparent electrical disconnection, this could explain the increase in cases of conduction block and increased susceptibility to arrhythmias in the ventricle as well as the atria with age.

On a further structural issue there is debate over the effect of changes in the constitution of the cardiac tissue during ageing. Collagen and desmin have been shown to be expressed in a continuous manner across the whole SAN area at all ages (Opthof et al 1985; Alings & Bouman 1993; Jones et al 2004). Some studies, however, have shown increased fibrosis and fat infiltration in the aged heart (Opthof et al 1985; Song et al 1999). This tissue remodelling would be expected to disrupt normal conductance pathways and predispose to arrhythmias by breaking up the conducting wave of depolarization into wavelets. However, these factors do not appear to directly affect the pacemaker function of the SAN (Alings & Bouman 1993), and therefore cannot be considered to contribute to the observed age-dependent pacemaker dysfunction.

Disruption of the conduction pathway appears to be critical to most arrhythmias. It is interesting that even studies on genetic abnormalities have associations with fibrosis and conduction disorders (van Veen et al 2005). This raises the question of whether ion channel mutations are the crucial factor that increases the risk of arrhythmias regardless of age in patients with these genetic disorders, or are the arrhythmias are a consequential cardiac remodelling? Indeed, a heterozygous sodium channel knockout mouse model of the SCN5A gene (which encodes the main cardiac sodium channel) showed only a slight effect on action potential conduction at a young age. However, with increasing age the mouse model exhibited 'disarrangement of gap junctions', which resulted in slowed action potential conduction (van Veen et al 2005). Short or long QT syndrome, Brugada syndrome and other genetic abnormalities of the cardiac ion channels require careful consideration of the 'changing state' of the cardiac tissue where a developing arrhythmic potential may have a resemblance to the remodelling that occurs naturally as we age. The development of a fuller understanding of this 'ageing' cardiac background, on which these and other diseases are superimposed, is therefore required to fully understand the syndromes and how to master their treatment.

Potential mechanisms underlying age-dependent changes in SAN function

A bewildering array of different signalling cascades exist that impact on cardiac function and protein expression. With ageing it has been postulated that several of the effects observed can be likened to what might be expected in a tissue under chronic stress. One notable stress-related signalling system that may have a role in instigating the effects of ageing is the stress-activated mitogen-activated protein kinases (SAPK). These have previously been demonstrated to be activated in cardiac myocytes in response to a variety of cellular stresses (Bogoyevitch et al 1995).

SAPKs are a highly conserved family of signalling molecules of which C-Jun amino-terminal kinase (JNK) is a member (Johnson & Lapadat 2002). Biochemical evidence has shown that JNK in a non-stressed state has glutathione S-transferase bound to inhibit activation; however, under conditions of stress JNK is phosphorylated and hence activated (Adler et al 1999). Activated JNK has been shown to be involved in a series of pathways leading to modification of Cx43 and hence cardiac conduction properties (Figure 1). Activated JNK has the potential to phosphorylate the Cx43 protein at serine residues located within the C-terminus (Hertzberg et al 2000). This modification has confusingly been attributed to increasing or decreasing the conductance of intercellular links; however, the main data obtained under key physiological conditions seems to indicate that an increase in conductance is the most likely outcome of this modification.

In contrast to the apparent short-term increase in cellular communication evoked by activated JNK, in the longer term activation of JNK has been associated with the stress-induced loss of Cx43 protein and mRNA expression in cardiac myocytes. This means activation of JNK causes a long-term decline in intercellular conductance (Petrich et al 2002; Nao et al 2003). Further studies of specific activation of JNK within the myocardium in vivo used transgenic mice where directed over-expression of JNK was achieved with the Cre/LoxP-mediated gene-switch system. The mice showed a consequential 90% loss of Cx43 protein expression and a 60% loss of Cx43 mRNA expression, leading to the conclusion that activated JNK reduces Cx43 expression at both the transcriptional and post-transcriptional levels in cardiac myocytes (Petrich et al 2002, 2004). Furthermore, activated JNK reduced the number of gap junctions by 5- to 10-fold, functionally observed as slowed conduction of the action potential. The animals also showed a high apparent incidence of arrhythmias and sudden death (Petrich et al 2002). To verify that activated JNK was responsible for the observed down-regulation of Cx43, an inhibitor of JNK was found to attenuate the Cx43 down-regulation (Petrich et al 2002).

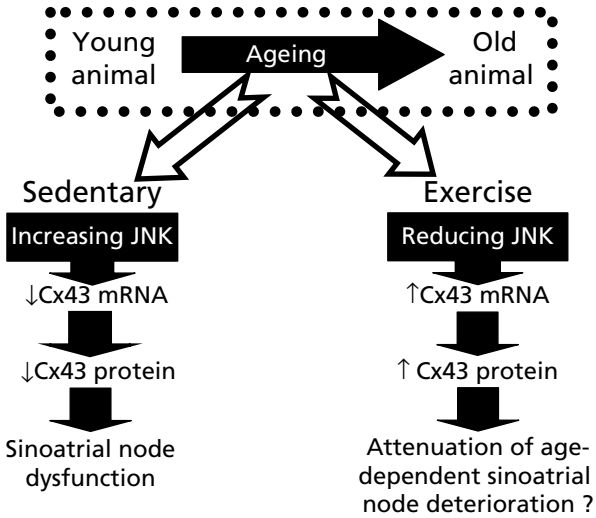

Figure 1 Activation of JNK and its hypothesized influence on Cx43 and SAN dysfunction. During natural ageing of the normal sedentary animal, activation of JNK increases. This in turn appears to lead to the observed reductions in Cx43 mRNA and Cx43 protein content within the sinoatrial node. The effects of this can be observed as slowed conduction of the action potential and dysfunction of the pacemaker in the elderly. Evidence suggests that the intervention of exercise can suppress JNK activation and ameliorate other age-related changes in gene-expression; hence, it is hypothesized, regular exercise may act to stabilize or restore the content of Cx43 mRNA and Cx43 protein, attenuating dysfunction of the sinoatrial node in the elderly population.

Although the direct relationship between JNK and Cx43 is unclear, both in-vitro and in-vivo studies have shown that activation of JNK reduces Cx43 protein expression by up to 90%. This model has clear parallels to the findings described above regarding changes in Cx43 with increasing age, but it remains to be clearly determined whether this stress-induced pathway is responsible for the changes occurring in the conducting system of the heart with age. Even if changes in JNK regulation prove not to be directly responsible for the age-dependent changes in Cx43 expression, this pathway still offers interesting opportunities for potentially modulating Cx43 expression to the benefit of the aged heart.

When considering the effect of stress-related factors on the aged heart it should be noted that the aged heart shows an apparently diminished capacity to respond to stress. For example, the aged heart exposed to an ischaemic insult is more vulnerable to damage compared with a young heart (Abete et al 1999).

First observed in 1962, the heat shock response has been recognized to possess a role in cellular protection (Ritossa 1962). Heat shock proteins (HSP), such as inducible HSP70, have been shown to provide cardiac protection against dysfunction and necrosis after events such as an ischaemic insult, reducing the occurrence of myocardial apoptosis (Suzuki et al 1997, 2000). An animal's capacity to express cardiac HSP70 diminishes with age, however, and the HSP70 response to ischaemia is blunted in the aged heart (Nitta et al 1994; Snoeckx et al 2001).

An implication of this work is that the aged heart is at greater risk should any cellular stress occur within the heart. There is no capacity to "tame" the insult as would occur within a young heart. As such, stresses that might have limited impact on a young heart will be more likely to lead to the stimulation of other stress-evoked pathways, such as the SAPK pathway, whose primary member is JNK. This is a working hypothesis on why the ageing heart may show the adaptations identified, but it, along with other stress-related mechanisms, needs further research to identify the specifics of these events and the potential to manipulate this process to the advantage of the elderly (Figure 1).

Improving the prospects for a steady cardiac rhythm in the elderly

Post-exercise gene expression analysis of elderly exercised animals compared to those that are sedentary has shown that adaptive physiological mechanisms induced by exercise can retard or even reverse some of the effects of ageing on gene expression in cardiac tissue (Bronikowski et al 2003; Iemitsu et al 2004). Regular exercise has been shown to prevent increases in JNK activation following stress

(Boluyt et al 2003). As such, regular exercise could potentially have a protective role to play in preventing remodelling of the cardiac conduction system as induced by JNK (Figure 1). If, as the evidence suggests, activation of JNK is a major factor responsible for down-regulation of Cx43 in the elderly, exercise could attenuate the JNK response and prevent the disconnection of the nodal tissue in the ageing heart.

Of further interest is whether the adoption of an appropriate exercise regime late in life, or potential pharmacological manipulation of the JNK pathway by perhaps an analogue of the activated-JNK inhibitor SP600125, could restore stable pacemaker function and protect against arrhythmias in the elderly. Appropriate regimes could reduce the need for artificial pacemakers and reduce the risk of sudden death, resulting in increasing longevity and quality of life.

References

Abete, P., Napoli, C., Santoro, G., Ferrara N., Tritto, I., Chiariello, M., Rengo, F., Ambrosio, G. (1999) Age-related decrease in cardiac tolerance to oxidative stress. *J. Mol. Cell. Cardiol*. **31**: 227–236

Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R., Davis, R. J., Ronai, Z. (1999) Regulation of JNK signalling by GSTp. *EMBO J*. **18**: 1321–1334

Alings, A. M. W., Bouman, L. N. (1993) Electrophysiology of the ageing rabbit and cat sinoatrial node – a comparative study. *Eur. Heart J*. **14**: 1278–1288

Allessie, M. A., Boyden, P. A., Camm, A. J., Kléber, A. G., Lab, M. J., Legato, M. J., Rosen, M. R., Schwartz, P. J., Spooner, P. M., Van Wagoner, D. R., Waldo, A. L. (2001) Pathophysiology and prevention of atrial fibrillation. *Circulation* **103**: 769–777

Anyukhovsky, E. P., Sosunov, E. A., Chandra, P., Rosen, T. S., Boyden, P. A., Danilo, P., Rosen, M. R. (2004) Age-associated changes in electrophysiologic remodelling: a potential contributor to initiation of atrial fibrillation. *Cardiovasc. Res*. **66**: 353–363

Beardslee, M. A., Laing, J. G., Beyer, E. C., Saffitz, J. E. (1998) Rapid turnover of connexin43 in the adult rat heart. *Circ. Res*. **83**: 629–635

Bogoyevitch, M. A., Ketterman, A. J., Sugden, P. H. (1995) Cellular stresses differentially activate c-Jun N-terminal protein kinases and extracellular signal-regulated protein kinases in cultured ventricular myocytes. *J. Biol. Chem*. **270**: 29710–29717

Boluyt, M. O., Loyd, A. M., Roth, M. H., Randall, M. J., Song, E. Y. (2003) Activation of JNK in rat heart by exercise: effect of training. *Am. J. Physiol. Heart Circ. Physiol*. **285**: H2639–2647

Brignole, M. (2002) Sick sinus syndrome. *Clinics in Geriatric Med*. **18**: 211–227

Bronikowski, A. M., Carter, P. A., Morgan, T. J., Garland, T. Jr., Ung, N., Pugh, T. D., Weindruch, R., Prolla, T. A. (2003) Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart. *Physiol. Genomics* **12**: 129–138

Di Gennaro, M., Bernabei, R., Sgadari, A., Carosella, L., Carbonin, P. U. (1987) Age-related differences in isolated rat sinus node function. *Basic Res. Cardiol*. **82**: 530–536

Flaker, G., Greenspon, A., Tardiff, B., Schron, E., Goldman, L., Hellkamp, A., Lee, K., Lamas, G. (2003) Death in patients with permanent pacemakers for sick sinus syndrome. *Am. Heart J*. **146**: 887–893

Gillette, P. C., Thapar, M. K., Zinner, A., McNamara, D. G. (1982) Effect of age and heart rate on the conduction of sinus node impulse to the ventricle. *Texas Heart Institute J*. **9**: 157–161

Gregoratos, G. (1999) Permanent pacemakers in older persons. *J. Am. Geriatr. Soc*. **47**: 1125–1135

Hartel, G., Talvensaari, T. (1975) Treatment of sinoatrial syndrome with permanent cardiac pacing in 90 patients. *Acta Med. Scand*. **198**: 341–347

Hertzberg, E. L., Saez, J. C., Corpina, R. A., Roy, C., Kessler, J. A. (2000) Use of antibodies in the analysis of connexin43 turnover and phosphorylation. *Methods* **20**: 129–139

Honjo, H., Boyett, M. R., Coppen, S. R., Takagishi, Y., Opthof, T., Severs, N. J., Kodama, I. (2002) Heterogeneous expression of connexins in rabbit sinoatrial node cells: correlation between connexin isotype and cell size. *Cardiovasc. Res*. **53**: 89–96

Iemitsu, M., Miyauchi, T., Maeda, S., Tanabe, T., Takanashi, M., Matsuda, M., Yamaguchi, I. (2004) Exercise training improves cardiac function-related gene levels through thyroid hormone receptor signaling in aged rats. *Am. J. Physiol. Heart Circ. Physiol*. **286**: H1696–1705

Johnson, G. L., Lapadat, R. (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK and p38 protein kinases. *Science* **298**: 1911–1912

Jones, S. A., Lancaster, M. K., Boyett, M. R. (2004) Ageing related changes of connexins and conduction within the sinoatrial node. *J. Physiol. (London)* **560**: 429–437

Jongsma, H. J., Wilder, R. (2000) Gap junctions in cardiovascular disease. *Circ. Res*. **86**: 1193–1197

Jose, A. D., Collison, D. (1970) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res*. **4**: 160–167

Kannel, W. B., Abbott, R. D., Savage, D. D., McNamara, P. M. (1983) Coronary heart disease and atrial fibrillation: The Framingham study. *Am. Heart J*. **106**: 389–396

Kostin, S., Klein, G., Szalay, Z., Hein, S., Bauer, E. P., Schaper, J. (2002) Structural correlate of atrial fibrillation in human patients. *Cardiovasc. Res*. **54**: 361–379

Lakatta, E. (2002) Cardiovascular ageing in health sets the stage for cardiovascular disease. *Heart, Lung and Circulation* **11**: 76–91

Nao, T., Ohkusa, T., Hisamatsu, Y., Inoue, N., Matsumoto, T., Yamada, J., Shimizu, A., Yoshiga, Y., Yamagata, T., Kobayashi, S., Yano, M., Hamano, K., Matsuzaki, M. (2003) Comparison of expression of connexin in right atrial myocardium in patients with chronic atrial fibrillation versus those in sinus rhythm. *Am. J Cardiol*. **91**: 678–683

Nikolski, V. P., Jones, S. A., Lancaster, M. K., Boyett, M. R., Efimov, I. R. (2003) Cx43 and dual-pathway electrophysiology of the atrioventricular node and atrioventricular nodal re-entry. *Circ. Res*. **92**: 469–475

Nitta, Y., Abe, K., Aoki, M., Ohno, I., Isoyama, S. (1994) Diminished heat shock protein 70 mRNA induction in ages rat hearts after ischemia. *Am. J. Physiol*. **267**: H1795–1803

Oosthoek, P. W., van Kempen, M. J. A., Wessels, A., Lamers, W. H., Moorman, A. F. M. (1990) Distribution of the cardiac gap junction protein, connexin43 in the neonatal and adult human heart. In: Marechal, G., Carraro, U. (eds) *Muscle and Motility: Proceedings of XIXth European Conference, Brussels*. Intercept, Andover, pp 85–90

Opthof, T. (1988) The mammalian sinoatrial node. *Cardiovasc. Drugs* **1**: 573–597

Opthof, T. (2000) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res*. **45**: 177–184

Opthof, T., de Jonge, B., Mackaay, A. J., Bleeker, W. K., Masson-Pevet, M., Jongsma, H. J., Bouman, L. N. (1985) Functional and morphological organization of the guinea pig sinoatrial node compared with the rabbit sinoatrial node. *J. Mol. Cell. Cardiol*. **17**: 549–564

Petrich, B. G., Gong, X., Lerner, D. L., Wang, X., Brown, J. H., Saffitz, J. E., Wang, Y. (2002) c-Jun N-terminal kinase activation mediates down-regulation of connexin43 in cardiomyocytes. *Circ. Res*. **91**: 640–647

Petrich, B. G., Eloff, B. C., Lerner, D. L., Kovacs, A., Saffitz, J. E., Rosenbaum, D. S., Wang, Y. (2004) Targeted activation of c-jun N-terminal kinase in vivo induces restrictive cardiomyopathy and conduction defects. *J. Biol. Chem*. **279**: 15330–15338

Psaty, B. M., Manolio, T. A., Kuller, L. H., Kronmal, R. A., Cushman, M., Fried, L. P., White, R., Furberg, C. D., Rautaharju, P. M. (1997) Incidence of and risk factors for atrial fibrillation in older adults. *Circulation* **96**: 2455–2461

Ritossa, F. M. (1962) A new puffing pattern induced by a temperature shock and DNP in Drosophila. *Experientia* **18**: 571–773

Rodriguez, R. D., Schocken, D. D. (1990) Update on sick sinus syndrome, a cardiac disorder of aging. *Geriatrics* **45**: 26–38

Ross, R. A., Kenny, R. A. (2000) Pacemaker syndrome in older people. *Age Ageing* **29**: 13–15

Rubenstein, J. J., Schulman, C. L., Yurchak, P. M., DeSanctis, R. W. (1972) Clinical spectrum of the sick sinus syndrome. *Circulation* **46**: 5–13

Saffitz, J. E., Laing, J. G., Yamada, K. A. (2000) Connexin expression and turnover: implications for cardiac excitability. *Circ. Res*. **86**: 723–728

Sakabe, K., Fukuda, N., Nada, T., Shinohara, H., Tamura, Y., Wakatsuki, T., Nishikado, A., Oki, T. (2003) Age-related changes in the electrophysiologic properties of the atrium in patients with no history of atrial fibrillation. *Jpn Heart J*. **44**: 385–393

Snoeckx, L. H., Cornelussen, R. N., Van Nieuwenhoven, F. A., Reneman, R. S., VanDer Vusse, G. J. (2001) Heat shock proteins and cardiovascular physiology. *Physiol. Rev*. **81**: 1461–1497

Song, Y., Yao, Q., Zhu, J., Luo, B., Liang, S. (1999) Age-related variation in the interstitial tissues of the cardiac conduction system; and autopsy study of 230 Han Chinese. *Forensic Sci. Int*. **104**: 133–142

Spach, M. S. (1994) Changes in the topology of gap junctions as an adaptive structural response of the myocardium. *Circulation* **90**: 1103–1106

Suzuki, K., Sawa, Y., Kaneda, Y., Ichikawa, H., Shirakura, R., Matsuda, H. (1997) In vivo gene transfection with heat shock protein 70 enhances myocardial tolerance to ischemia-reperfusion injury in rat. *J. Clin. Invest*. **99**: 1645–1650

Suzuki, K., Sawa, Y., Kagisaki, K., Taketani, S., Ichikawa, H., Kaneda, Y., Matsuda, H. (2000) Reduction in myocardial apoptosis associated with overexpression of heat shock protein 70. *Basic Res. Cardiol*. **95**: 397–403

Van Kempen, M. J., Vermeulen, J. L., Moorman, A. F., Gros, D., Paul, D. L., Lamers, W. H. (1996) Developmental changes of connexin40 and connexin43 mRNA distribution patterns in the rat heart. *Cardiovasc. Res*. **32**: 886–900

van Veen, T. A., Stein, M., Royer, A., Le Quang, K., Charpentier, F., Colledge, W. H., Huang, C. L., Wilders, R., Grace, A. A., Escande, D., de Bakker, J. M., van Rijen, H. V. (2005) Impaired impulse propagation in Scn5a-knockout mice: combined contribution of excitability, connexin expression, and tissue architecture in relation to aging. *Circulation* **112**: 1927–1935

Yamamoto, M., Honjo, H., Niwa, R., Kodama, I. (1998) Low-frequency extracellular potentials recorded from the sinoatrial node. *Cardiovasc. Res*. **39**: 360–372