Drug development for treatment of cardiac arrhythmias: targeting the gap junctions

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Reentrant excitation (12) is likely the most important mechanism for serious life-threatening arrhythmias. Traditionally, a major focus of antiarrhythmic drug development has been to explore the actions of drugs designed to block conduction in reentrant pathways(s). During the latter part of the 20th century, numerous new drugs were introduced clinically. Their major targets were and still are sarcolemmal ion channels (mainly Na\(^+\), Ca\(^{2+}\), and K\(^+\)), reducing current flow to prolong refractoriness and to slow or block conduction (23). The overall experience with the lack of uniform efficacy of these antiarrhythmic drugs suggests that there is a missing factor that is important for successful pharmacological therapy of reentrant arrhythmias. Although the role of intercellular communication through gap junctions has long been known to be an important factor governing conduction properties (24), it was not considered as a major target for antiarrhythmic drug development during this time. It was not until the important studies of Spach et al. (32, 33) beginning in the 1980s on anisotropic propagation that the significance of intercellular coupling as a cause of reentry rose to a level of prominence. Therefore, it became logical to test the concept that drugs acting on gap junctions might have antiarrhythmic actions (35). Our approach, and that of others working in this area at that time (early 1990s), was to elucidate the effects of blocking gap junctional conductance on reentrant excitation (3, 4, 22). Although there were no drugs available that had a specific blocking effect on cell coupling, some chemicals that decreased gap junctional conductance, such as heptanol (3, 4, 22, 34), were used as model drugs. We showed that a preferential slowing of conduction and eventual conduction block caused by heptanol stopped anisotropic reentry in a rabbit model (22). Another approach that we took was to increase intracellular calcium to block gap junctions and reentrant excitation with the L-type cardiac-specific calcium current “enhancer” Bay Y 5959 (5). These studies indicated that the block of gap junction function can be antiarrhythmic. In contrast, rotigaptide, a drug specifically developed to target gap junctions for the treatment of arrhythmias, enhances coupling (8, 11, 17). The studies described in the article published in this issue of the American Journal of Physiology-Heart and Circulatory Physiology by Kjølbye et al. (16) from the laboratory of Rosenbaum show how enhanced coupling may have an important antiarrhythmic effect.

Rotigaptide development can be traced to a group of peptides with antiarrhythmic properties [originally named the antiarrhythmic peptides (AAPs)] that were discovered in the early 1980s (1, 8, 17). Rotigaptide has been shown to improve conduction and decrease conduction block in experimental acute ischemia caused by coronary occlusion in the canine heart (36). In the current study by Kjølbye et al. (16) in this issue, it was shown that rotigaptide abolished ischemia-induced discordant alternans and prevented the occurrence of ventricular fibrillation that is associated with it (16).

The Rosenbaum laboratory (25) has been at the forefront of investigation on how T-wave alternans is related to sudden cardiac death; this group has provided strong evidence that it arises from alternation of repolarization of the transmembrane ventricular muscle action potential (25). Action potential duration in some regions of the heart may alternate in a long-short-long pattern, while other regions alternate in a short-long-short pattern (spatially discordant alternans). However, since the flow of current among cells during the repolarization phase of ventricular muscle action potentials tends to suppress large repolarization gradients, such alternans is promoted by a decrease in cell coupling (25). In the current study (16), discordant alternans was produced by rapid pacing in the presence of ischemia, a pathological event that reduces the coupling among myocardial cells (18). The link between discordant alternans and reentrant arrhythmias is that discordant alternans creates large gradients of refractoriness of sufficient magnitude to cause unidirectional conduction block and reentry. Rotigaptide, by enhancing cell coupling, attenuated discordant alternans, abolishing the gradients of refractoriness, thereby preventing the occurrence of fibrillation (16).

The mechanism of the antiarrhythmic effect of rotigaptide on gap junctions is unique; it is proposed to be prevention of dephosphorylation of connexin 43 (Cx43), the principle gap junction protein in ventricular myocardium (2, 16). Cx43 is known to be regulated by phosphorylation (19). In ventricular myocytes after ischemia, loss of the P2 band that is indicative of phosphorylated Cx43 protein in standard Western blots has been described previously (13). Although this has been termed a “dephosphorylation,” it is actually a change in the individual phosphorylated residues within the carboxy-terminal domain of Cx43 (19, 31). Two of the residues that lose their phosphates during an ischemic event are Ser\(^{297}\) and Ser\(^{368}\). These dephosphorylation events have been shown to be blocked by rotigaptide (2). Thus, the loss of the P0 band, or so-called dephosphorylated species, in the study by Kjølbye et al. (16) (termed a “hyperphosphorylation” of Cx43) caused by rotigaptide is likely a maintenance of the phosphorylation of these two key serine residues. This becomes important when trying to determine the exact molecular mechanism of the action of rotigaptide. Knowing which residues are phosphorylated can lead to an understanding of the underlying kinases involved and an understanding of the specificity of the result. This remains to be worked out. Drug action on kinases in other tissues might lead to unspecified adverse effects.
As a result of this study and the previous ones on rotigaptide cited above, it appears that the concept of increasing gap junction conductance as an antiarrhythmic intervention is an excellent one and rotigaptide studies may pave the way for the eventual development of an effective drug to prevent life-threatening cardiac arrhythmias. However, there are several issues that must be considered relating to this line of drug development. 1) The proposed mechanism of action of rotigaptide is prevention of dephosphorylation of Cx43 that accompanies acute metabolic stress such as that accompanying myocardial ischemia. However, the role of dephosphorylation of Cx43 in other pathological situations with diminished gap junction coupling not involving metabolic stress is unknown. Therefore, a drug that targets dephosphorylation might not be effective in these conditions (10, 26, 28, 29, 30). In addition, the role of phosphorylation state in controlling conductance of gap junctions formed by other connexins is unknown, for example, Cx40 a major connexin in the atria that may be involved in atrial fibrillation. 2) Gap junction remodeling accompanies some chronic diseases such as myopathies and chronic ischemic heart disease (10, 26, 27, 28, 29, 30). Remodeling includes a decrease in number of gap junction connections resulting from the interruption of communication between cells by fibrosis (32, 33) and downregulation of Cx43 formation or trafficking to the intercalated disk. Another feature of remodeling in some situations is lateralization of gap junctions where there is deposition of increased amounts of Cx on lateral membranes of myocytes (27). This deposition may represent movement of Cx43 out of the intercalated disk, decreasing gap junctions in the disk leading to uncoupling (9). For reasons that are not yet understood, lateralization can be associated with slow conduction and block and be a primary cause of reentry (27, 37). In the situation in which there is a significant decrease in the quantity of gap junctions and/or lateralization, increasing gap junction conductance might not be an effective means of improving conduction. Since there are many fewer connections in remodeled myocardium than in normal myocardium, blocking the few remaining gap junctions might be a more effective way of stopping reentry. Spear et al. (34) have shown that heptanol can cause conduction block in regions of nonuniform anisotropy in infarct border zones in concentrations that have little effect on conduction in normal ventricular muscle. 3) Even in acute ischemia where increasing gap junction conductance has been shown to stop reentrant tachyarrhythmias, the effects on eventual infarct size must be considered. It has been shown that the so-called “kiss of death” action of gap junctions causes cells coupled to damaged regions in many different kinds of tissues to die in clusters via the passage of apoptotic signals from the damaged region through Cx43 gap junction channels (6, 21). The reduction of intercellular coupling by decreasing gap junction conductance is a response of the myocardium to limit the damage caused by an insult such as a coronary occlusion (7). Studies looking at myocardial infarct size in a mouse model have shown that decreasing the levels of Cx43 via knockout of one allele in murine hearts (Cx43−/−) caused a decrease in infarct size in this model (14). It thus may be of importance to determine overall infarct size in models where rotigaptide is used as an antiarrhythmic agent after coronary artery occlusion since to limit infarct size the effective intervention may be to increase phosphorylation and gap junction conductance (20).

REFERENCES


