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I_f in non-pacemaker cells: Role and pharmacological implications

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Abstract

Pacemaker channels play a major role in the generation of sinoatrial rhythmic activity. However, their expression is not confined to specialized myocardial cells, such as primary and subsidiary pacemakers. Electrophysiological and molecular data collected over the last ten years have demonstrated that f-channels are also present in non-pacemaker cardiomyocytes, and become upregulated in cardiac hypertrophy and failure. Mislocalized expression and/or overexpression of f-channels are a consequence of electrophysiological remodeling and, from a clinical point of view, may represent an arrhythmogenic mechanism in heart failure, a condition associated with a high risk for sudden cardiac death. The potential arrhythmogenic role of I_f and the availability of selective f-channel blockers cause I_f to be a suitable therapeutic target in heart disease.

Keywords: Pacemaker current; Heart failure; I_f inhibitors

1. Introduction

Pacemaker ionic channels help to endow some types of specialized cells (either cardiomyocytes, neurons, or smooth muscle cells) with an intrinsic rhythmic activity. In the mammalian cardiac sinus node, the pacemaker current, termed I_f, plays a crucial physiological role in setting the heart rate and sensing its autonomic control (see [1] in this issue). The role of f-channels in non-pacemaker cardiac cells has long been disregarded. This flaw was based on the assumption that I_f expression is limited to the sinus node region and other parts of the conduction system, i.e., those cells possessing a well-defined diastolic depolarization phase. This conviction was also fueled by the lack of molecular assays, since genes coding for f-channels have been cloned only recently. Notwithstanding this view, first electrophysiological and then molecular data demonstrated that HCN channels are also present in non-pacemaker tissues. In our laboratory, the hypothesis of a "pacemaker" current in ventricular cells arose from the observation of a clear-cut *diastolic depolarization* phase during intracellular recordings from papillary muscles of hypertrophied rat hearts [2]. Two facts excluded the interpretation of this phenomenon as an experimental artifact: first, diastolic depolarization was absent in non-hypertrophied muscles challenged with similar experimental conditions. Second, and most important, this *diastolic depolarization* phase was prone to be steepened by β-adrenoceptor (βAR) stimulation, which exerted – in the hypertrophied ventricular tissue namely – a striking proarrhythmic action [2]. As reviewed in the previous article by DiFrancesco [1], the autonomic control of heart rate in pacemaker cells is largely mediated by the presence of the funny current I_f which is specifically modulated by intracellular cAMP levels. Thus, we postulated that the sensitivity to catecholamines and the presence of a diastolic depolarization were due to an *atypical* I_f occurrence in diseased ventricular tissue. This article will review the present knowledge concerning the presence and pharmacological modulation of I_f in common ventricular and atrial myocytes.

2. I_f in non-pacemaker cardiac cells

In ventricular myocytes, I_f is abundantly expressed during fetal and neonatal life [3–5]. Moreover, in murine beating myocytes derived from embryonic stem cells (ESC), I_f is detected early during differentiation [6]. At some stage of their in situ (or in vitro, for ESC-derived cardiomyocytes) electrophysiological maturation toward adult ventricular phenotype, these cells lose their capacity to generate spontaneous activity. Both in mouse and rat hearts, this is accompanied by a progressive decrease in I_f expression [3,4]. The current occurs in all myocytes from newborn rats, but is detected in one half and in one third of them at 2 or 4 weeks of age, respectively, and its amplitude is greatly reduced compared to cells from newborn rats [3] (Fig. 1).

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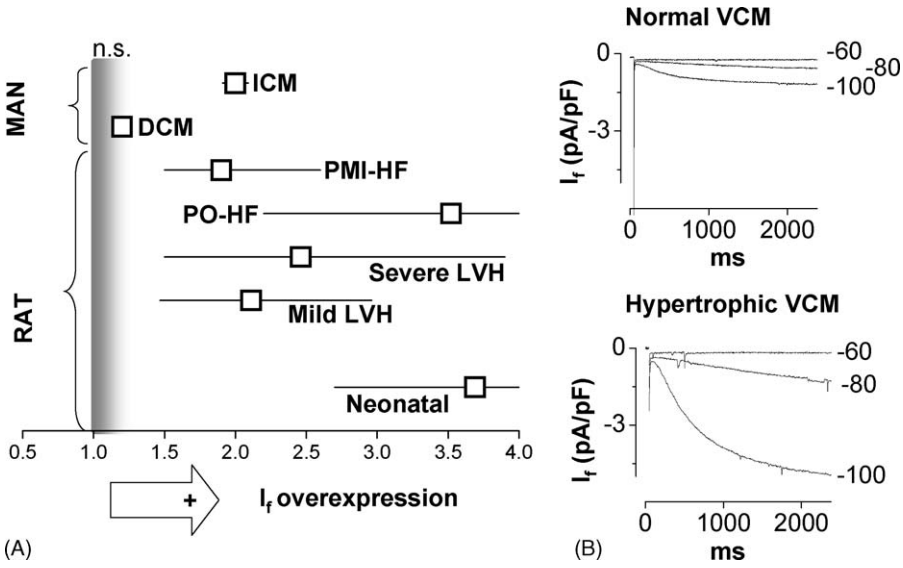

Fig. 1. Ventricular I_f expression is increased in cardiomyopathies. Each point represents the ratio between current density measured in VCMs from rat or human diseased hearts, and respective controls; bars are confidence intervals (95%). In newborn rats, values measured at 2 weeks are compared to those at 2 days after birth. Mild-LVH, severe-LVH: relative increase of I_f in rats with mild or severe left ventricular hypertrophy caused by aortic banding [9] or long-lasting pressure overload [8], respectively. PO-HF, PMI-HF: relative increase of I_f in rats with overt heart failure, resulting from uncompensated hypertrophy due to pressure-overload [8] or following a myocardial infarction due to coronary ligation [10], respectively. DCM, ICM: relative increase of I_f in patients undergoing cardiac transplantation for terminal dilated or ischemic cardiomyopathy, respectively [15]. For all conditions, the relative increase in I_f density was statistically significant versus controls, that is, normotensive rats, sham-operated rats, or undiseased donor hearts not transplanted for technical reasons, with the exception of DCM patients (n.s.: not significant).

Interestingly enough, ventricular myocytes can *re-express* I_f during adult life in particular circumstances, which – at variance with what is described for pacemaker tissues – relate to *pathophysiology* rather than to *physiology*. A striking upregulation of I_f expression has been observed in a variety of animal models of cardiac hypertrophy and failure [7–10]; in those circumstances I_f recovers its "fetal" role, so that a clear-cut diastolic depolarization can be detected in ventricular cardiomyocytes (VCMs) isolated from the diseased ventricles [7]. A similar behavior has been detected in all animal models of cardiac hypertrophy/failure tested so far, as well as in human failing hearts. To give an estimate of this phenomenon, we reported in Fig. 1 the relative increase observed in diseased VCMs when compared to control (undiseased) ones. The values were adjusted for cell size, taking into account that cardiomyocytes are "hypertrophic", that is, enlarged in myopathies: in fact, current amplitude is normalized compared to membrane capacitance, an index of cell size, and expressed as current density [11]. I_f was at least doubled in left VCMs from rats with mild or severe cardiac hypertrophy (LVH) caused by pressure overload (PO), and in rats with overt heart failure (HF) caused by high blood pressure or following myocardial infarction (PMI) (Fig. 1) where its overexpression reached values comparable with those observed in the neonatal stage. The degree of hypertrophy positively correlated with an increased I_f density [8], and changes in expression levels were most pronounced in those cardiac regions with highest overload [10,12], indicating that the processes leading to hypertrophy directly affected the level of channel expression. Typical traces of currents recorded in normal or hypertrophied cells are plotted on the left.

A further support to the pathophysiological role of I_f emerges from data obtained in failing human ventricles from transplanted patients [13,14]. A correlation with the severity of cardiac disease was obviously impossible in this setting, all patients being affected by terminal heart failure. Moreover, interpretation of the data is complicated by factors which are usual for this kind of studies (individual variability, long-standing therapies, paucity of human samples and difficulties in cell isolation, see also [11] for methods). However, interestingly enough, changes in I_f density were correlated with the etiology of the disease, I_f overexpression being greater in ischemic (ICM) than in idiopathic dilated (DCM) cardiomyopathy (Fig. 1) [15]. Also a different voltage-dependence was evident by comparing the I_f activation

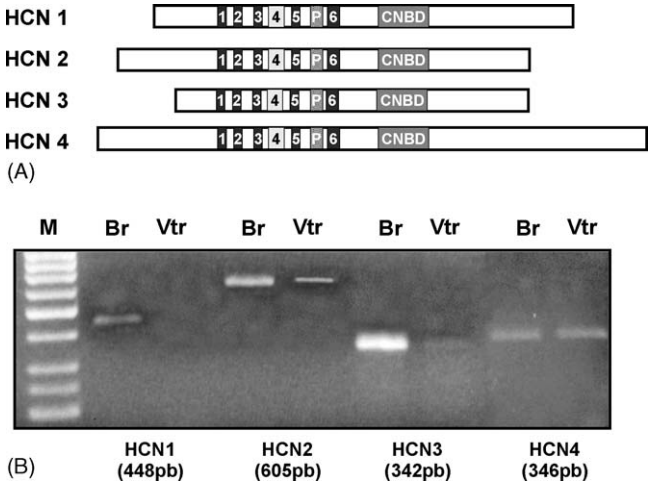

Fig. 2. Molecular determinants of I_f in non-pacemaker cells. (A) The four isoforms of the HCN family; (B) expression of mRNA encoding HCN isoforms in rat cardiac ventricles (Vtr) and brain (Br), by RT-PCR. PCR products of brain are presented as positive reference. Lane M is 100 basepair DNA ladder.