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Cardiac Pacemaker (I_f) Current: Physiological and Pharmacological Properties

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ABSTRACT

Mammalian sinoatrial node (SAN) cells, the natural pacemaker cells of the heart, have an action potential characterized by the presence of a special phase, the slow diastolic (pacemaker) depolarization (phase 4), which drives pacemaker activity and has therefore attracted the interest of generations of cardiac physiologists. What is the basis of the pacemaker depolarization? Here the features of the "funny" (I_f) current of pacemaker cells and its involvement in the generation and autonomic regulation of heart rate are briefly addressed. I also address the involvement of I_f in the pharmacological control of cardiac chronotropism, and how defective "funny" channels can be responsible for inherited heart rhythm disturbances.

KEY WORDS: *cardiac pacemaker; sinus node; pacemaker current; ivabradine*

I_f GOVERNS THE GENERATION AND AUTONOMIC CONTROL OF HEART RATE

In the original report in the sinoatrial node (SAN) in 1979 (Brown et al., 1979), the "funny" I_f current was described as an inward current activated on hyperpolarization in the diastolic range of voltages (Figure 1). Its properties, as well as being relevant to the generation of the diastolic depolarization phase of the action potential, were shown to be apt to mediate the changes of diastolic depolarization slope, hence of the spontaneous rate, caused by ß-receptor stimulation by adrenaline.

Following its early description, much work was devoted to a thorough investigation of the properties of I_f in relation to kinetics, ionic nature and modulation by neuromediators (DiFrancesco, 1985; DiFrancesco, 1993). These studies confirmed that the pacemaker current is an essential mechanism in the generation of diastolic depolarization and thus in the intiation of the heartbeat, but also demonstrated the fundamental role of I_f in mediating the control of cardiac chronotropism by both the sympathetic and parasympathetic autonomic inputs (DiFrancesco et al., 1986; DiFrancesco et al., 1989).

Which is the mechanism by which I_f generates spontaneous activity and modulates cardiac rate? I_f is activated by hyperpolarization from a threshold near -40/-50 mV, as apparent in Figure 1a,b; its activation curve (i.e., the degree of current activation at steady-state, Figure 1c) shows that the current activation saturates at about -100/-100 mV, demonstrating that the I_f activation range overlaps that of the diastolic depolarization phase of the action potential (Figure 1a). As illustrated by plotting the fully-activated I/V relation in Figure 1c, I_f is inward at diastolic voltages, with a

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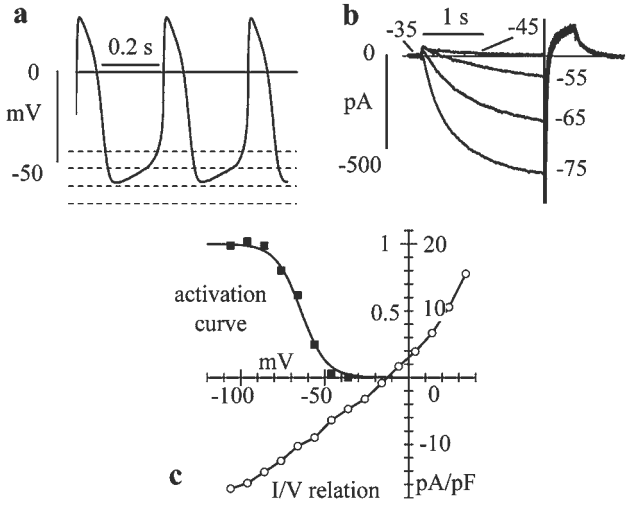

FIGURE 1. The "funny" I_f current. a, spontaneous activity recorded from isolated SAN myocytes reveals a slow diastolic "pacemaker" depolarization (phase 4 of the action potential) from about -60 to -40 mV. b, I_f current recorded from a SAN cell during hyperpolarizing steps from –35 mV to voltages in the range –45/–75 mV; the same voltage levels are drawn as broken lines in panel a to indicate that the range of If activation overlaps the diastolic depolarization. c, activation curve (left side of y-axis) and I/V relation (right side of the y-axis) of I_f. The activation curve represents the fraction of total current activated at each voltage; the I/V relation represents the voltage-dependence of the fully-activated current density, normalized to cell capacity.

reversal potential near -10/-20 mV, which reflects its mixed ionic Na^+/K^+ permeability (DiFrancesco, 1981a,b). Since the activation range of I_f overlaps that of diastolic depolarization (compare panels a and c in Figure 1), the current activates during repolarization of the action potential, and since I_f is inward at diastolic voltages, its activation is a suitable mechanism for the generation of the slowly developing pacemaker depolarization.

In addition to generating the diastolic depolarization phase of the action potential, hence rhythmic activity, I_f activation is the key process in the modulation of rate by autonomic transmitters. The mammalian pacemaker region (SAN) is densely innervated by the autonomic nervous system: sympathetic ß-adrenergic stimulation accelerates, and parasympathetic muscarinic stimulation slows cardiac rate. When isolated SAN cells are superfused with solutions containing low concentrations of autonomic agonists, changes of spontaneous rate are characterized by specific changes of the diastolic depolarization rate, without significant modifications of action potential duration and shape (Figure 2a). This indicates that the process responsible for the diastolic depolarization phase of the action potential (i.e. I_f activation) is the primary target of autonomic neurotransmitters.

Indeed, the original report of I_f in SAN cells (Brown et al., 1979) had already identified I_f as a functionally relevant target of adrenergic-induced positive chronotropic effect. Further experimentation then showed that ß-adrenergic stimulation increases I_f by displacing its current activation curve to more positive voltages, without modifying its conductance (DiFrancesco et al., 1986; Accili et al., 1997; Figure 2b). The depolarizing shift of I_f activation curve caused by ß-adrenergic stimulation is due to an increased level of intracellular cAMP, which acts as a second messenger in the modulation of funny channels (DiFrancesco & Tortora, 1991). This implies that ß-receptor stimulation accelerates rate by stimulating adenylate-cyclase activity and cAMP synthesis, which shifts the I_f activation curve to more positive voltages and thus increases the inward current during diastolic depolarization, ultimately leading to a faster depolarization rate.

Later studies showed that I_f is also strongly dependent upon parasympathetic stimulation, by a mechanism which is opposite to that elicited by β-adrenergic stimulation (DiFran-

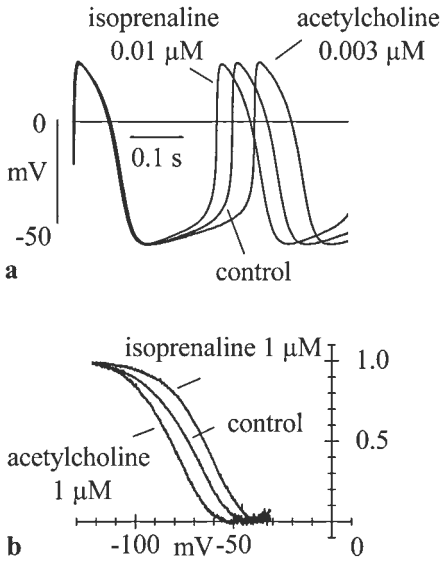

FIGURE 2. If mediates the modulation of cardiac rate by the autonomic nervous system. a, spontaneous activity recorded from a SAN myocyte; activity is accelerated by isoprenaline and slowed by acetylcholine, and rate changes do not involve changes of action potential shape or duration but are only associated to changes in the slope of diastolic depolarization. b, the I_f activation curve shifts to the right in the presence of isoprenaline and to the left in the presence of acetylcholine, thus increasing and decreasing, respectively, the current activated at each voltage; these effects are responsible for the changes of diastolic depolarization rate shown in panel a. [Modified with permission from DiFrancesco, 1993 (a) and Accili et al., 1997 (b)].

cesco & Tromba, 1988a). It has been known for a long time that vagal stimulation releases acetylcholine (Ach) and slows cardiac rate, and based on early experiments (Hutter & Trautwein, 1955) it was thought that the mechanism responsible for the slowing effect of ACh was an ACh-activated potassium current (Sakmann et al., 1983). This view was however challenged by the finding that ACh strongly inhibits I_f by shifting its activation curve to more negative voltages, an action opposite to that caused by catecholamines and due to a muscarinic-induced inhibition of adenylate-cyclase and cAMP reduction (DiFrancesco & Tromba, 1988a, DiFrancesco & Tromba, 1988b) (Figure 2a,b).

How could two different mechanisms, both effective to slow cardiac rate upon vagal stimulation, operate simultaneously? This puzzle was resolved by investigating the ranges of ACh concentration required to induce the two effects. We showed that while low doses of ACh (up to 0.01-0.03 μM) are able to inhibit I_f and slow the spontaneous frequency of SAN cells, much higher concentrations are required to activate the potassium conductance; indeed, the ACh dose required for half inhibition of I_f is some 20-fold lower than the dose half activating the K+ current (DiFrancesco et al., 1989). This finding introduced a novel concept in cardiac physiology, i.e. that the negative chronotropic effect of low-to-moderate vagal stimulatory tone is mediated by I_f inhibition, not by activation of a potassium current (DiFrancesco, 1993).

PHARMACOLOGICAL INHIBITION OF THE FUNNY CURRENT AND RATE-REDUCING AGENTS

The discovery of I_f and the detailed investigation of its properties were relevant not only in relation to the understanding of the basic principles underlying pacemaker generation and modulation, but also in that it provided the possibility to implement an approach to pharmacological control of heart rate based on the concept of I_f and its function. Since both experimental and mathematical modeling analysis (DiFrancesco & Noble, 1985) have clearly established a direct correlation between I_f and pacemaker activity, it is to be expected that I_f inhibition, such as the one that can be achieved by f-channel block, can lead to slowing of cardiac rate.

Several recently developed drugs able to specifically slow heart rate without substantial side-effects (the "heart rate-inhibiting" substances) have indeed been shown to act by selective blockade of f-channels. These agents may have a significant impact on specific cardiac therapies, particularly when slowing of heart rate is beneficial, such as chronic angina, ischemic heart disease and cardiac failure (DiFrancesco & Camm, 2004).

The slowing action of ivabradine (now in the market with the commercial name of Procoralan), and the ivabradine-induced inhibition of I_f, as recorded in single SAN myocytes, are shown in Figure 3a,b.

At the concentration used in Figure 3a (0.3 μM), the bradycardic action of the drug clearly involves a reduced steepness of the diastolic depolarization phase, without substantial alterations of either action potential shape or duration. This implies that the action of the drug reflects primarily a specific inhibition of the funny current. Also, the drug has a "physiological" type of action since it mimics the slowing induced by moderate cholinergic activity (compare with Figure 2a), although the mode by which ivabradine inhibits f-channels is complex and is quite different from a simple leftward shift of the activation curve (Bucchi et al, 2002). Block of the f-channels by ivabradine can be demonstrated by applying repetitive activating/deactivating voltage-clamp protocols during exposure to the drug (Figure 3b). The blocking action of ivabradine on f-channels has been investigated with some detail (Bois et al., 1996; Bucchi et al., 2002). The data show that ivabradine inhibits f-channels with a high degree of specificity relative to other channels expressed in the SAN and acts as an "open f-channel" blocker, implying that the molecule can reach the blocking site within the pore only when channels are open during hyperpolarization. At the same time, ivabradine blocks f-channels preferentially during depolarization, because it is a positively charged molecule. The open-channel block, requiring hyperpolarization, and the preferential block on depolarization are contradictory properties. However, this behaviour is the basis of the "use-dependent" action of ivabradine, i.e. the

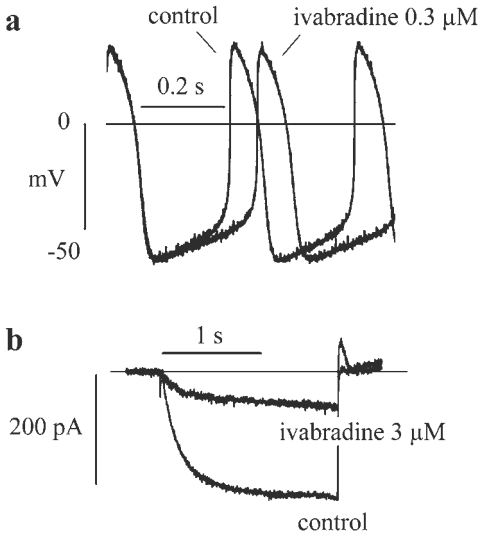

FIGURE 3. Ivabradine slows pacemaker rate and blocks f-channels. a, spontaneous activity recorded from a single SAN myocyte is slowed by ivabradine 0.3 μM, through a decreased rate of diastolic depolarization. b, ivabradine-induced block of If recorded during repetitive activating/deactivating steps (-100/+5 mV) applied every 6 s from a holding potential of -35 mV.

requirement of repetitive channel opening/ closing cycles for block to develop. This is a useful property because it implies that higher degrees of block develop at higher frequencies of opening/closing cycles; therefore, the rate-reducing action of the drug increases at higher (tachycardic) heart rates, when in fact it is more valuable.

Further analysis shows that the blocking action of ivabradine on f-channels differs qualitatively from that of other rate-reducing agents such as ZD7288 and zatebradine (UL-FS49) (DiFrancesco, 1994; Shin et al., 2001). Experimental evidence indeed indicates that the ivabradine block of I_f is not simply a voltage-dependent block such as that proposed for zatebradine, or a channel state-dependent block as the one proposed for ZD 7288. Rather, the ivabradine-induced block of I_f can be shown to depend on the direction of current flow, more than on voltage per se (Bucchi et al., 2002). A possible interpretation of these features is that ivabradine block involves a competition between drug molecules and permeating Na+ and/or K+ ions for a common binding site within the f-channel pore.

RHYTHM DISTURBANCES ASSOCIATED TO F-CHANNEL MODIFICATIONS

As well as physiologically (by the autonomic transmitters) and pharmacologically (i.e. by the rate-limiting agents), f-channel behavior can be altered genetically, by sequence mutations. Since HCN channels, the molecular constituents of native f-channels, were cloned, several structure-function studies have thoroughly demonstrated that the normal function of channels can be modified by alterations of the channel sequence, hence of its structure/gating relation (see Chen et al., 2002). This evidence, along with the established notion that f-channels are responsible for generation and modulation of cardiac rhythm, lead to an obvious question: can naturally defective f-channels cause rhythm disturbances?

This question has been recently answered by the finding of a large Italian family where a specific HCN4 mutation (S672R) in the cyclic-nucleotide-binding domain (at the C-terminus) leads to inherited sinus bradycardia (Milanesi et al., 2006). Of the four known HCN isoforms, HCN4 is the most expressed in SAN tissue. In the family investigated, each of the bradycardic individuals (rates between 43 and 60 bpm, mean of 52.2±1.4 bpm, n=15) carried the same HCN4 mutation, while all individuals with normal heart rates (range 64 to 81 bpm, mean of 73.2±1.6 bpm) had normal HCN4 sequences, indicating tight correlation between the HCN4 mutation and the bradycardic phenotype. When expressed heterologously in HEK293 cells, mutated channels had an activation curve which was shifted to more negative voltages relative to wild-type channels (by about 9 mV). When identical amounts of wild-type and mutated channel cDNA's were transfected in order to produce heteromeric channels such as those occurring in heterozygotes, the HCN4 activation curve was about 5 mV more negative than that of normal channels (Milanesi et al., 2006). The mutation thus mimicked the action of a low dose of ACh (between 10 and 30 nM) (see Fig. 2b), which can fully explain the bradycardic phenotype associated to it.

CONCLUSION

It is well established that the funny current plays an essential role in the generation of the diastolic depolarization of cardiac pacemaker cells and in the autonomic modulation of heart rate. Recent results show that the relevance of funny channels to pacemaker activity applies not only to physiological conditions, but also to pathological conditions, since defective channels have been shown to underlie an inherited rhythm disturbance such as sinus bradycardia (Milanesi et al., 2006).

Direct control of cardiac chronotropism can be achieved by exploiting the features of funny channels. For example, pharmacological control of rate can be achieved by the use of drugs such as the funny channel inhibitor ivabradine, which reduces in a controlled way the amount of I_f current during diastolic depolarization, and hence heart rate, by selective f-channel blockade (Bucchi et al., 2002).

The properties of funny channels can also be exploited to the opposite aim, i.e. to enhance the pacemaker function. Pacemaker activity, for example, can be transferred to quiescent cardiac cells by transfecting HCN channels and/or delivering stem cells expressing HCN channels (Rosen et al., 2004). "Biological pacemakers" based on the development of the above techniques and exploitation of the properties of funny channels may in due time become feasible alternatives to electronic pacemakers.

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