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REVIEW

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Hypoxia and persistent sodium current

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Abstract During prolonged depolarization of excitable cells, some voltage-activated, tetrodotoxin-sensitive sodium channels are resistant to inactivation and can continue to open for long periods of time, generating a "persistent" sodium current (I_{NaP}). The amplitude of I_{NaP} is small [generally less than 1% of the peak amplitude of the transient sodium current (I_{NaT})], activates at potentials close to the resting membrane potential, and is more sensitive to Na channel blocking drugs than I_{NaT}. It is thought that persistent Na channels are generated by a change in gating of transient Na channels, possibly because of a change in phosphorylation or protein structure, e.g. loss of the inactivation gate. Drugs that block Na channels can prevent the increase in $[Ca^{2+}]_i$ in cardiac cells during hypoxia. Hypoxia increases the amplitude of I_{NaP}. Paradoxically, NO causes a similar increase in I_{NaP} and the effects of both can be inhibited by reducing agents such as dithiothreitol and reduced glutathione. It is proposed that an increased inflow of Na^+ during hypoxia increases $[Na^+]_i$, which in turn reverses the Na/Ca exchanger so that $[Ca^{2+}]_i$ rises. An increase in I_{NaP} and $[Ca^{2+}]_i$ could cause arrhythmias and irreversible cell damage.

Keywords Hypoxia · Sodium channels · Inactivation · Sodium current

Persistent sodium current

In the classical description of the ionic basis of action potentials, Hodgkin and Huxley (1952) described voltage-activated sodium currents that activate rapidly and then inactivate more slowly within tens of milliseconds. Some years later, tetrodotoxin (TTX)-sensitive plateau potentials were recorded in cerebellar Purkinje neurons (Llinas and Sugimori 1980) and TTX-sensitive inward rectification in neocortical neurons (Stafstrom et al. 1982). The results pointed to a TTX-sensitive, voltage-activated sodium current that did not inactivate rapidly in these neurons.

A couple of years later, the newly available single-electrode voltage clamp was used to record the persistent sodium current for the first time directly in mammalian neurons (French and Gage 1985). A similar "threshold sodium current" was recorded at about the same time in squid axon (Gilly and Armstrong 1984). A more comprehensive description of the persistent sodium current in neurons in the CA1 region of hippocampal slices appeared several years later (French et al. 1990). Because the current was so small, it was necessary to subtract current traces recorded before and after exposure of cells to high concentrations of TTX to be certain that currents recorded were indeed sodium currents and not due to drifts in leakage current with time. It was found that the maximum amplitude of the persistent sodium current was less than 1% of the maximum amplitude of the transient sodium current, that it activated at more negative potentials than the transient sodium current, and that it was resistant to inactivation even during depolarizations lasting many seconds. Although the current is small, its resistance to inactivation means that it can produce significant changes in $[Na^+]_i$ if it turns on for many seconds.

A similar persistent sodium current was also recorded in skeletal muscle (Gage et al. 1989) and cardiac muscle (Saint et al. 1992). Persistent sodium current is probably responsible for the fibrillations commonly seen in denervated skeletal muscle. Several heritable forms of myotonia and periodic paralysis are caused by impaired inactivation of sodium channels causing a persistent sodium current that may either initiate abnormal bursts of action potentials (myotonia) or cause paralysis by

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depolarizing fibers to a refractory, inexcitable state. Furthermore, enhanced persistent sodium currents underlie arrhythmias in one form of the long-QT syndrome (Lazzara 1996; Wattanasirichaigoon and Beggs 1998).

Because single channel currents occurring late during depolarization have characteristics similar to transient sodium channels activated in the same patch, it was suggested that the persistent sodium current was generated by a change in the gating mode of a transient sodium channel (Alzheimer et al. 1993). Subsequent recordings from recombinant α-subunit Na^+ channels support this view (e.g. Chen et al. 2000; Cummins et al. 1998; Fleischhauer et al. 1998; Raman and Bean 1997; Wang et al. 1996).

Effects of hypoxia

There is mounting evidence that an increase in persistent sodium current is an early and fundamental event in hypoxia. Drugs that selectively block this current might reduce damage to cells during ischaemia or hypoxia. The shortage of effective drugs that can be used to prevent this damage is largely due to our lack of information about the exact sequence of biochemical events linking insufficient oxygen supply to cell death (Lipton 1999).

Oxygen is required for the survival of cells because of its central role as the final acceptor of electrons in the mitochondrial respiratory chain, making the synthesis of ATP possible via oxidative phosphorylation. Even transient localized oxygen deficits, such as those that occur during head injuries or unstable coronary syndromes, can produce irreversible cell damage. The high lipid content and oxygen metabolism of the brain render it particularly vulnerable to oxidative damage. This may be a result of increased levels of free radicals or compromised defences (e.g. antioxidant levels) against the radicals in the brain during hypoxia. Normal cellular defences against hypoxia include a high intracellular content of reduced glutathione (GSH) and a low content of oxidized glutathione (GSSG). This keeps protein thiol groups in the reduced state. The tissue content of GSH is decreased whereas that of GSSG is increased during hypoxia and this creates a situation where SH groups may be modified.

Neuronal hypoxia

Neuronal death caused by decreased or interrupted O_2 delivery has been attributed to changes in intracellular pH, decreased ATP levels, free radical production, increases in $[Na^+]_i$ and/or $[Ca^{2+}]_i$, and to membrane depolarization (Lipton 1999). These changes are accompanied by activation of damaging proteases and phospholipases and release of free radicals. Neuronal cell death may be described as having three stages: (1) early intracellular ionic and chemical changes, (2) activation of damaging enzymes and (3) changes in cellular functions and structures, eventually leading to cell death. The delay before cell death occurs varies greatly (from minutes to hours or weeks), depending on the nature of the insult and the cell type. Dying cells release chemicals that endanger cells in the surrounding area (penumbra) where blood flow is not completely cut off. These surrounding cells are in a state of "shock" and either survive or die, depending on what happens in the minutes to hours that follow. A large number of the damaging changes occurring in the neurons are secondary to an increase in intracellular Na^+ levels (Lipton 1999).

Cardiac hypoxia

Arrhythmias

Although cardiac muscle appears to be more resistant to hypoxia than neurons, more than half the deaths following an ischaemic episode occur suddenly and have been attributed to arrhythmias. The cause of these arrhythmias is not well understood (Carmeliet 1999). It is known that electrophysiological changes occur rapidly following ischaemia without any evidence of irreversible membrane damage, indicating that the underlying mechanisms are likely to be due to transient biochemical and ionic alterations within or near the sarcolemma of the ischaemic myocyte. It has been suggested that abnormalities of action potentials (afterdepolarizations), which become prominent following cardiac hypoxia or ischaemia, may trigger the arrhythmias. Two kinds of afterdepolarizations have been described: an early afterdepolarization occurring during repolarization of an action potential and a delayed afterdepolarization occurring when repolarization is complete or nearly complete. The ionic mechanisms responsible for these afterdepolarizations are not well understood. It has been assumed that they are caused by a net increase in inward currents that are activated under hypoxic or ischaemic conditions. Computer models suggest that small increases in a persistent sodium current would cause significant lengthening of the action potential duration (Sakmann et al. 2000) that may cause lethal arrhythmias. Early afterdepolarizations are depressed by a reduction in external Na^+ concentration ($[Na^+]_o$) and by the specific Na^+ channel blocker TTX (Coraboeuf et al. 1980), suggesting that a TTX-sensitive Na^+ current is involved. Consistent with this idea, veratridine, which causes persistent activation of Na^+ channels, induces early afterdepolarizations which can be completely eliminated with TTX. Interestingly, the Na^+ channel blocker TTX abolishes early afterdepolarisations in myocytes obtained from heart failure patients (Maltsev et al. 1998). Delayed afterdepolarizations are Ca^{2+}-dependent events that are evoked by a variety of conditions that induce intracellular Ca^{2+} overload and are thought to play a role in reperfusion arrhythmias.

Cell damage

Cardiac ischaemia causes a rapid decline in contractile performance and, depending on factors like the severity and duration of the insult, eventually cell death (Allen et al. 1993). The amount of muscle damage that occurs is related to the level of increase in Ca^{2+} (Allen et al. 1993). A significant amount of this Ca^{2+} enters via reverse action of the Na^+/Ca^{2+} exchanger, triggered by raised levels of $[Na^+]_i$ (see model in Fig. 1 and Haigney et al. 1994). This model depends on an early increase in $[Na^+]_i$ that is primarily responsible for the increase in $[Ca^{2+}]_i$.

How does $[Na^+]_i$ rise during hypoxia?

It has been proposed that an abnormal Na^+ influx is one of the first steps in the hypoxic cascade leading to a rise in $[Ca^{2+}]_i$. This increase in $[Na^+]_i$ could have many causes, e.g. reduced Na^+-ATPase activity, Na^+ entry via the Na^+/H^+ exchanger or Na^+ entry through voltage-operated, TTX-sensitive Na^+ channels (Carmeliet 1999; Lipton 1999). At least in cardiac muscle, the first two mechanisms are not thought to play a major role early in ischaemia (up to 30 min) (Xiao and Allen 1999). Evidence for an increased influx of Na^+ through TTX-sensitive Na^+ channels and a consequent increase in $[Ca^{2+}]_i$, causing cell damage during hypoxia/ischaemia in cardiac muscle and neurons, includes the following observations (for a review, see Taylor and Narasimhan 1997):

1. An increase in $[Na^+]_i$ during hypoxia and ischaemia has been measured (e.g. with fluorescent probes such as SBF1) (Haigney et al. 1994; Xiao and Allen 1999).
2. The increase in $[Na^+]_i$ during hypoxia is caused by enhanced influx and not reduced efflux of Na^+ (Friedman and Haddad 1994b; Haigney et al. 1994; Nishida et al. 1993; Renlund et al. 1984).
3. An increase in $[Na^+]_i$ precedes the rise in $[Ca^{2+}]_i$ (Friedman and Haddad 1994a; Haigney et al. 1994). Reducing the $[Na^+]_o$ or low concentrations of Na^+ channel blockers (that do not inhibit action potentials) have been shown to block any rises in $[Ca^{2+}]_i$ (Fried et al. 1995; Friedman and Haddad 1993; Haigney et al. 1994).
4. Na^+ channel blockers block the rise in extracellular glutamate concentration, the decrease in ATP levels and cell damage during hypoxia (Fried et al. 1995; Haigney et al. 1994; Renlund et al. 1984). This probably explains why hypoxic glutamate release in the brain is not sensitive to Ca^{2+} channel blockers but can be blocked by TTX (Taylor and Meldrum 1995). Hypercontractures that occur in myocytes during ischaemia can be prevented with Na^+ channel blockers (Haigney et al. 1994; Nishida et al. 1993).
5. The transient Na^+ current would inactivate rapidly and could not underlie a sustained Na^+ influx during hypoxia. In fact, there is evidence that the transient Na^+ current of neurons is reduced during hypoxia (Cummins et al. 1993; O'Reilly et al. 1997).
6. Low concentrations of TTX (100 nM) block the increase in $[Na^+]_i$ during cardiac ischaemia (Xiao and Allen 1999). It has been shown that the persistent sodium current is blocked by lower concentrations of TTX (nM), lidocaine and quinidine than the transient sodium current (Hammarstrom and Gage 1998; Ju et al. 1994).

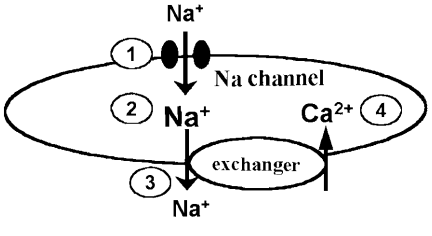

Fig. 1. An increase in Na^+ influx leads to an increase in the concentration of intracellular Ca^{2+}. (*1*) Hypoxia turns on persistent Na^+ channels. (*2*) The Na^+ concentration rises. (*3*) The Na^+/Ca^{2+} exchanger reverse. (*4*) $[Ca^{2+}]_i$ rises

We have shown in cardiac myocytes (Ju et al. 1996) and in hippocampal neurons (Hammarstrom and Gage 2000) that the persistent, TTX-sensitive Na^+ current increases during hypoxia. Since the persistent Na^+ current is thought to underlie cell damage and arrhythmias associated with hypoxia, this O_2 sensor may be a prime target for future anti-ischaemic and antiarrhythmic drugs.

Responses of other ion channels to a decrease in oxygen tension

Other ion channels also respond to hypoxia but, in contrast to persistent sodium channels, their activity tends to be decreased rather than increased (Lopez-Barneo et al. 2001). Regulation of ion channels by O_2 tension was first described in chemoreceptive type I cells of the carotid body, where K^+ channels were inhibited by hypoxia (Lopez-Barneo et al. 1988). Similar effects of hypoxia on a range of ion channels have now been reported (Peers 1997). In several studies, the mechanisms involved in O_2 sensing, such as direct redox modulation of the channel and/or sensing by membrane-bound, haem-containing structures, have been explored. It has been reported that plasma membranes contain flavin, cytochromes of *b* type, non-haem iron, coenzyme Q, α-tocopherol, thiol groups and maybe copper. One possibility for an oxygen sensor is the presence in the plasma membrane of NAD(P)H oxidase, a flavoprotein that catalyzes the production of H_2O_2 (Cross et al. 1990). Electron donors such as NADH normally transfer electrons to molecular O_2. Hypoxia may, however, prevent electron transfer through the electron transport systems (e.g. complex III of electron transport chain) (Cross et al. 1990). Consequently, electron donors may

accumulate in the chain, and this may affect the redox state of specific amino acid residues in the Na^+ channel protein. NAD(P)H oxidase is co-localized with a chemoreceptor K^+ channel and is thought to modulate activity of this channel (Youngson et al. 1993). Recent observations have suggested that NAD(P)H oxidase does not play a role in oxygen sensing in rabbit carotid body chemoreceptor cells (Obeso et al. 1999) or in pulmonary artery smooth muscle cells (Archer et al. 1999) [but the NAD(P)H oxidase blocker diphenyleneiodonium (DPI) should be used with caution to establish a relationship as it may inhibit K^+ and Ca^{2+} currents directly (Wyatt et al. 1994)]. Furthermore, mice can lack the gp91 phox subunit of the NAD(P)H oxidase and still preserve their O_2 sensing (Archer et al. 1999). This latter observation may still not rule out NAD(P)H oxidase as an O_2 sensor, but could mean that there are other gp91 phox analogues present. Alternatively, O_2 may be needed for forming coordination complexes between the channel protein and metal-containing proteins located near the channel subunits, and thereby regulate the conformational state of the molecule (Lopez-Barneo 1994). Hypoxia modulates another type of K^+ channel activity in excised patches and it was suggested that this effect is mediated by a membrane-bound and iron-containing protein [but not NAD(P)H-oxidase] associated with the channel (Jiang and Haddad 1994). This O_2 sensor was activated by iron chelators but was not blocked by the NAD(P)H oxidase blocker DPI. We believe that there is an auxiliary protein involved in O_2 sensing by the Na^+ channel, but at present we can only speculate about exactly how the Na^+ channel senses O_2 levels.

How does the persistent Na^+ channel sense oxygen tension?

We found that cardiac and neuronal persistent Na^+ channels respond to hypoxia in inside-out patches of membrane (Hammarstrom and Gage 2000; Khoury et al. 1999a). On average, the mean current increased about 20-fold during hypoxia. Although these patches of membrane had been removed from the influence of intracellular metabolic processes, they were still affected by hypoxia. There must be a direct effect of O_2 tension on the membrane that does not depend on changes in the cell metabolism. This discovery raises the unexpected possibility of identifying a novel, local (membrane-associated) link between hypoxia and activation of persistent Na^+ channels. The interruption of this process could reduce or prevent the intracellular accumulation of Na^+ and Ca^{2+} ions that damage cells during hypoxia. This oxygen sensor may provide a new target for anti-ischaemic drugs. The hypoxic rise in persistent Na^+ channel activity that we have recorded (Hammarstrom and Gage 2000; Ju et al. 1996) appeared to follow a significant decrease in the level of O_2 and channels were most likely responding to a PO_2 between ~45 and 0 mmHg. Na^+ channels may not be as responsive to small changes in O_2 levels as some K^+ channels which appear to respond to gradual changes of PO_2 from 120 to 70 mmHg (for review, see Lopez-Barneo 1996), but this needs to be tested.

Where and how do the oxygen sensor and Na^+ channel interact?

As mentioned above, several studies have examined the mechanisms involved in oxygen sensing but, until recently, the part of an ion channel that responds to oxygen tension had not been determined. Recently, Fearon and colleagues made use of three naturally occurring splice variants of the human isoform to identify a 39 amino acid sequence at the C-terminal end of the L-type Ca^{2+} channel that is essential for oxygen sensing (Fearon et al. 2000). This was the first identification of a structural region involved in oxygen sensing. There is no sequence homology between this region and the C-terminal of TTX-sensitive brain or cardiac Na^+ channels (unpublished observations). However, both the 39 amino acid sequence of the Ca^{2+} channel and the C-terminal end of the Na^+ channel contain several cysteines. These cysteine residues and potential disulfide bonds are possible candidates for redox modulation. Hypoxia could potentially cause the formation or disruption of intermolecular mixed disulfides, resulting in conformational change(s) in the channel protein, ultimately affecting channel gating. It is interesting and perhaps relevant that the $\beta 2$-subunit of the Na^+ channel is also thought to be linked via a disulfide bond to the α-subunit (Messner and Catterall 1986).

Effects of nitric oxide

Ischaemia has been reported to cause an increase in NO production in several different kinds of cells (Gess et al. 1997; Malyshev et al. 1999; Shibata et al. 1996), including cardiac myocytes (Kitakaze et al. 1995; Zweier et al. 1995). Excessive release of NO during hypoxia may well lead to abnormal electrical activity, as NO readily binds to and modulates the gating of a variety of ion channels (e.g. Bolotina et al. 1994; Lei et al. 1992). It has recently been reported that NO increases persistent Na^+ channel activity in excised patches from hippocampal neurons and cardiac myocytes (Hammarstrom and Gage 1999; Khoury et al. 1999b) and whole cells, and in outside-out patches from cardiac myocytes (Ahern et al. 2000). In fact, both exogenous and endogenous NO activate persistent Na^+ currents in posterior pituitary slices and ventricular myocytes (Ahern et al. 2000). It was concluded that NO acts by oxidizing the cardiac persistent Na^+ channel protein, or a closely associated regulatory protein, contained within the plasma membrane. A possible candidate for the latter is the haem

group of cytochromes which form the plasma membrane electron transport chain present in mammalian cells (Crane et al. 1991). In any case, our results show that persistent Na^+ channels in cardiac muscle and central neurons can be modulated and regulated by oxidants such as NO and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in a similar manner to hypoxia. It may be that the increased production of NO reported during hypoxia/ischaemia (Kitakaze et al. 1995; Zweier et al. 1995) contributes to the increase in persistent Na^+ current during hypoxia.

Effects of reducing agents

There are now several studies of the mechanisms involved in O_2 sensing; possible mechanisms include redox modulation and/or oxygen sensing by membrane-bound, iron-containing structures (Lopez-Barneo et al. 2001). The augmented hypoxic Na^+ channel activity that we have recorded in excised patches could be abolished by reducing agents such as reduced glutathione (GSH) and dithiothreitiol (DTT) (Hammarstrom et al. 2001; Khoury et al. 1999a). This suggests that, in both cardiac myocytes and hippocampal neurons, a redox reaction occurs at or near the Na^+ channel during hypoxia. In fact, hypoxia appears to act like an oxidizing agent. The fact that (1) it can be reversed by the reducing agents DTT and GSH (Hammarstrom et al. 2001; Khoury et al. 1999a) and (2) it is not easily reversible by washing out the reducing agents suggest that the increased Na^+ channel activity occurs as a result of a covalent modification or disulfide-bond formation at or near the channel protein. At present we can only speculate about exactly how the Na^+ channel senses O_2 levels. What we do know, however, is that in both rat cardiac myocytes and hippocampal neurons (Hammarstrom and Gage 2000) the O_2 sensor is attached to the plasma membrane and involves a redox reaction.

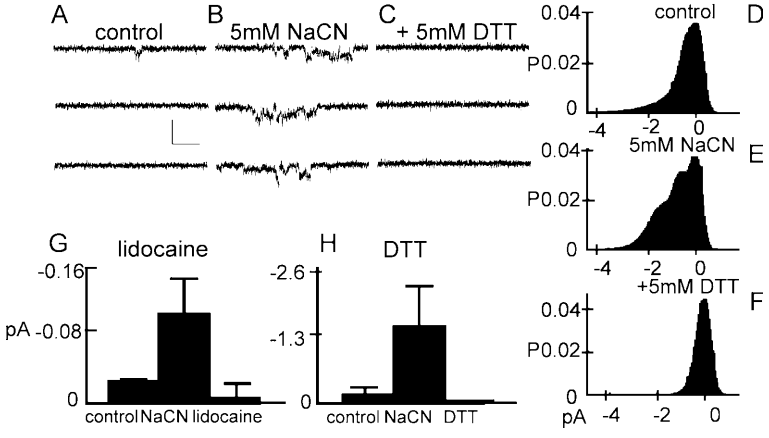

Fig. 2A–H. Effect of NaCN, DTT and lidocaine on channel activity in inside-out patches from a hippocampal neuron. Three traces recorded at –30 mV: **A** control; **B** during exposure to 5 mM NaCN; **C** after the addition of 5 mM DTT. *Scale bars*: 1 pA and 100 ms. *Broken lines* in **B** indicate closed current levels. **D–F**: all-points histograms. **G–H**: mean currents measured over 2–4 min during exposure to 5 mM NaCN, 100 μM lidocaine or 2–5 mM DTT. Vertical bars, +/–1 SEM

Effects of cyanide

We have previously shown that sodium cyanide increases persistent Na^+ channel activity recorded from intact ventricular myocytes (Hammarstrom and Gage 1998; Ju et al. 1996). We initially thought that this was due to the "classical" effect of NaCN in inhibiting reoxidation of cytochrome a_3 of the mitochondrial electron transport chain. However, we have subsequently recorded an increase in persistent Na^+ channel activity during exposure to NaCN in inside-out patches from hippocampal neurons (Fig. 2) (Hammarstrom and Gage 1999). Cyanide is thought to act as a reducing agent in some preparations (Karnik and Khorana 1990). More recently it has been shown that cyanide may modulate recombinant NR1/NR2B NMDA-receptor channel activity in a similar manner to the oxidizing agent DTNB (Arden et al. 1998). It was suggested that the latter effect was due to cyanolation, or the formation of thiocyanate adducts. We recently showed that the effect of NaCN on persistent Na^+ channels in inside-out hippocampal patches could be reversed by DTT as during hypoxia (Hammarstrom and Gage 1999). This would suggest that NaCN is either having a direct effect on the Na^+ channel, as it appears to have on NMDA-receptor channels (Arden et al. 1998), or may be interacting with some haem structure or an electron transport component present in, or anchored to, the plasma membrane that in turn influences Na^+ channels. The probability that mitochondria are attached to our inside-out patches has not been excluded and more experiments are needed to exclude this possibility. Experiments with inside-out patches from pancreatic β-cells showed that mitochondria are often present in the cytoplasmic plug attached to the tip of patch pipette, and it was suggested that mitochondria and K_{ATP} channels form functional microcompartments in pancreatic β-cells (Rustenbeck

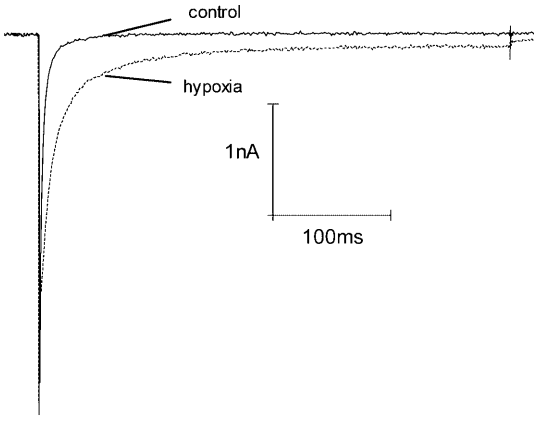

Fig. 3. Representative whole-cell recording from HEK293 cell expressing the human cardiac α-subunit of the Na^+ channel (hH1). Currents were TTX-subtracted and generated by voltage steps to –40 mV from a holding potential of –80 mV under control conditions (*solid line*) and after ~3–4 min of hypoxia (*broken line*) (a 300 ms prepulse to –130 mV preceded the depolarizing steps). In this cell, hypoxia caused a marked slowing of decay of I_{Na} and a very large increase in the amplitude of *I* NaP

et al. 1999). This interesting idea needs to be tested further in patches from other cells.

β1- and β2-subunits of the sodium channel

Voltage-gated Na^+ channels contain a principal α-subunit (of which there are several isoforms) and probably at least one of the auxiliary β-subunits, β1, β2 or β3. The β1-subunit when co-expressed with the rat brain type IIa α-subunit increases the peak Na^+ current and the rate of activation, causes a five-fold increase in the rate of inactivation and increases the number of channels detected by saxitoxin binding (Fozzard and Hanck 1996). The biophysical and physiological consequences of co-expressing the β1-subunit with the cardiac α-subunit are currently controversial and appear to vary in different expression systems. When the two cardiac subunits are co-expressed in HEK293 or HEK293t cells, there is a depolarizing shift in the channel kinetics when compared with channels formed by the α-subunit alone (Wright et al. 1999; Xiao et al. 2000). In contrast, when the two subunits are co-expressed in Chinese hamster oocytes, there is no, or only a modest, hyperpolarizing shift in inactivation (Bennett et al. 1993; Isom et al. 1992; Nuss et al. 1995). It is interesting that when the β1-subunit is co-expressed with the human heart α-subunit (hH1) the channels formed have a reduced affinity for lidocaine (Makielski et al. 1996) and the effects of saturated and monounsaturated fatty acids are abolished (Xiao et al. 2000). Interaction points between the two subunits are thought to include the S5-S6 loops (Makita et al. 1996; McCormick et al. 1998), the C-terminus of the α-subunit (Xiao et al. 1998) and the extracellular domain of the β-subunit (McCormick et al. 1998). Interactions between the β1-subunits and the α-subunit of Na^+ channels may be important for the hypoxic response and could contribute to the altered excitability of cardiac myocytes that has been observed.

Preliminary results

In pilot experiments, we have expressed the α-subunit of the human cardiac Na^+ channel (hH1) in mammalian HEK293 cell lines, and have recorded a very small persistent Na^+ current that increases during hypoxia (Fig. 3), behaviour similar to that seen in neurons and cardiac cells (Hammarstrom and Gage 1998; Ju et al. 1996). These results show that the hH1 subunit alone can give rise to persistent Na^+ currents that respond to hypoxia similar to native cardiac sodium channels. However, the smaller than normal size of the currents suggests that other factors, perhaps β-subunits or G-proteins, are also involved.

Discussion

At present, we do not know whether the persistent Na^+ current depends on changes in the major pore-forming α-subunit or the involvement of β-subunits (some mammalian cell lines can express endogenous subunits). Similarly, we do not know whether hypoxia affects one of the channel subunits (or its interactions) directly or whether it affects other regulatory proteins – "oxygen sensors". The association of β-subunits with the cytoplasmic side of certain voltage-gated channels may be reversible, allowing dissociation and re-association. Hence, regulation of the individual β-subunits and their interactions with the α-subunit may well contribute to altered excitability of cardiac or neuronal cells during both normoxia and hypoxia. In this context, it is of interest that the β1-subunit modifies the action of various drugs and that the β2-subunit appears to be covalently linked via disulfide bonds to the α-subunit.

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