Minireview

Direct Up-regulating Effect of G_s on the Whole-cell L-Type Ca Current in Cardiac Cells

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In heart cells a number of membrane receptors are primarily coupled to Ca channels via signal transducing guanine nucleotide binding proteins (*G* proteins) which most often exert their action on the channel through intracellular signaling pathways (Rodbell 1980; Berridge and Irvine 1984; Hescheler et al. 1986; Trautwein et al. 1986; Birnbaumer et al. 1987; Isenberg et al. 1987; Kaczmarek 1987; Pelzer et al. 1990). One of them, the G_s protein, is known to stimulate the adenylate cyclase (AC)/cAMP/ protein kinase A (PKA) cascade in response to β-adrenergic receptor occupation. Recently, however, some evidence has been obtained for a direct action of G_s on single cardiac Ca channels (Yatani et al. 1987; Imoto et al. 1988). Herein, we briefly review our recent data on the effect of *G* protein activation on the macroscopic L-type Ca current (I_{Ca}) in guinea-pig ventricular myocytes, which are consistent with the idea of a direct action of G_s on cardiac Ca channels.

Experimental Approaches

All experimental data reported here were obtained on enzymatically isolated guinea-pig ventricular myocytes using the whole-cell patch-clamp technique (Hamill et al. 1981). Most often, the recording pipette was filled with MICS, a minimal intracellular solution that contained Cs^+ as the major cation and Mg^{2+} as a cofactor in *G* protein activation but did not contain ATP, cAMP or other supplements for channel phosphorylation. Free Ca^{2+} concentration in this solution was buffered at 10^{-8} mol/l. Even though MICS disfavours channel phosphorylation, in some experiments phosphorylation-pathway inhibitory (PI) agents were added, such as AMP-PNP, a non-hydrolysable ATP analogue

(Yount 1975; Whitehouse et al. 1983), R_p—cAMPs which prevents PKA activation by cAMP (Van Haastert 1984), and DNP, a blocker of endogenous ATP production (McDonald and MacLeod 1973). In the external solution Na and K were replaced by TMA to allow pulsing from relatively negative holding potentials (-100 to -60 mV) without occasioning transient Na current. A dual tight-seal pipette method (Shuba et al. 1990) was applied in many of the experiments. This allowed "control" dialysis of the myocyte via the first pipette to be followed by a rapid pressure-enhanced "test" dialysis via the second pipette which was usually filled with MICS supplied with *G* protein activators or inhibitors.

Effect of G protein activation on Ca current in conditions of low intracellular enzymatic activity

It is well known that dialysis of heart cells with non-supplemented solution results in a rapid rundown of whole-cell I_{Ca} (Irisawa and Kokubun 1983). This was usually observed during myocyte dialysis with MICS in a single pipette (P1) mode. However, after initial Ca-current rundown presure-assisted infusion of MICS supplied with 0.1 mol/l of GTP-γ-S (a non-hydrolysable GTP analogue which persistently activates *G* proteins) by means of a second pipette (P2) was followed by a 30 ± 15 % (mean, SD) increase of I_{Ca} within 2 min. The increase in Ca-current amplitude was accompanied by noticeable slowing of the decay phase of I_{Ca} and a significant increment of the stationary component of inward current. In addition, a hyperpolarizing shift of about 5 mV on the peak and half-peak voltages of the I-V relation occurred. None of these effects was detected during dialysis with MICS via P2. An effect similar to that of GTP-γ-S was obtained with another non-hydrolysable GTP analogue, GMP-P(NH)P (0.1 mol/l) which also can induce persistent activation of *G* proteins (Selinger et al. 1981; Breitweieser and Szabo 1985). However, the I_{Ca} increment in this case was smaller — 18 ± 10 % (mean, SD). In contrast GDP-β-S (0.1 mmol), which does not activate *G* proteins but blocks *G* protein activation by GTP analogues (Cockroft and Gomperts 1985), had no effect on Ca-current amplitude but abolished the stimulatory effect of subsequently applied GTP-γ-S.

Which G proteins are involved?

I_{Ca} in mammalian cardiomyocytes is modulated by three major enzymatic pathways, and each of them is coupled to specific membrane receptors by corresponding *G* proteins. i. G_s couples the AC/cAMP/PKA cascade to the

β-adrenergic receptor (Rodbell 1980; Birnbaumer et al. 1987). Stimulation of AC by G_s results in the phosphorylation of the Ca channel and the subsequent increase of channel activity (Kameyama et al. 1985; Trautwein et al. 1986). ii. G_i couples muscarinic and adenosine receptors to the same AC pathway. But in contrast to G_s, it inhibits AC activity, and this underlies the mechanism by which muscarinic agonists and adenosine inhibit the effect of β-adrenergic stimulation of I_{Ca} (Hescheler et al. 1986; Isenberg et al. 1987). ADP-ribosylation by pertussis toxin (PTX) inactivates G_i (Ui 1984; Brown and Birnbaumer 1988). iii. G_p couples several membrane receptors including muscarinic (Tajima et al. 1987; Pelzer et al. 1990) to phospholipase C system. Stimulation of this system results in generation of diacylglycerol (DAG) and inositol trisphosphate (IP_3) (Berridge and Irvine 1984). DAG activates Ca-dependent phospholipid-sensitive protein kinase C (PKC) which can increase Ca channel activity via phosphorylation (Lacerda et al. 1988), whereas IP_3 was shown to exert a direct stimulatory effect on Ca channel (Vilven and Coronado 1988). Aside from acting through the intracellular enzymatic pathways, G_s, G_i and G_p theoretically may have a direct action on Ca channel. Thus, the enhancement of I_{Ca} in guinea-pig ventricular myocytes by GTP analogues may be the net outcome of the activation of G_s, G_i and G_p.

The positive contribution of G_i can be ruled out since PTX treatment of the cells which leads to G_i inactivation did not change the magnitude of I_{Ca} enhancement by GTP analogues. PTX treatment, however, caused an increase of basal Ca-current amplitude (see also Hescheler et al. 1986) and some shortening of the latency to peak stimulation during cell dialysis with GTP-γ-S-containing solution in a single pipette mode (5 min compared to 9 min for untreated cells).

Two protocols were designed to mimic possible direct and indirect stimulatory effect of G_p on I_{Ca}. First, extrnal applications of 10 μmol/l acetylcholine to activate G_p via muscarinic receptors during the myocyte dialysis with MICS plus 1 mmol/l GTP (for facilitating receptor-stimulated G protein activation) failed to produce any change in control I_{Ca}. Second, external (as well as internal) applications of TPA (1 μmol/l), a direct activator of PKC, and of IP_3 (1 μmol/l) together with unfavourable conditions for PKC-dependent phosphorylation (low $[Ca^{2+}]_{in}$, ATP-free) also failed to produce any I_{Ca} stimulation (actually, they had a small inhibitory effect). Thus, in our experimental conditions direct or indirect up-regulating effects of G_p on Ca channels can be excluded.

The results summarized indicate that activation of G_s might be responsible for the enhancement of I_{Ca} by GTP analogues in our experimental conditions.

Does G_s exert a direct effect on the Ca channel?

Activation of G_s by GTP γ-S may have two consequences. First, G_s stimulates

the AC/cAMP/PKA cascade which results in the phosphorylation of the Ca channel and the augmentation of I_{Ca}. Second, G_s may have a direct up-regulating effect on the Ca channel. Even though dialysis with MICS certainly disfavours the effect of G_s via cAMP-dependent phosphorylation, we tried to suppress it further by using phosphorylation inhibitory (PI) agents (see *Experimental Approaches*). Cell dialysis with PI-MICS instead of MICS accelerated I_{Ca} rundown almost two times. Inclusion of GTP (1 mmol/l) into the PI-MICS did not change the time course of I_{Ca} rundown. To test for the availability of the AC/cAMP/PKA system during cell dialysis with PI-MICS + GTP we externally applied 1 μmol/l forskolin (FSK), an AC activator. After 3 to 5 min of cell dialysis FSK produced a nearly twofold increase of I_{Ca} amplitude, being completely ineffective after 8 to 9 min. This indicates that 8—9 min are sufficient to completely turn off the AC/cAMP/PKA cascade. In contrast, after 9 min of dialysis with PI-MICS β-adrenergic agonist isoproterenol (ISO) (0.1 μmol/l) which acts via G_s was still able to elicit a 30—35 % stimulation of I_{Ca}, and application of GTP-γ-S via a second pipette also produced a rapid enhancement (about 30 %) of I_{Ca}.

The most reasonable explanation for these results is that G_s exerts a direct action on Ca channels. However, the approximate constancy of the effect of non-hydrolysable GTP analogues in MICS and PI-MICS dialysed cells seems to be confusing. Indeed, one might expect some additional stimulation in MICS dialysed cells due to the action of G_s via the AC/cAMP/PKA cascade. A possible explanation for this contradiction was that significant AC stimulation requires receptor-facilitated activation of G_s, whereas the direct effect of G_s on the Ca channel can be achieved by activation of the same (or even different) G_s protein(s) by non-hydrolysable GTP analogues. This would be the case if G_s and AC are uncoupled in the resting state, and receptor occupation is necessary for both G_s activation and activated G_s/AC coupling.

A dual pathway for I_{Ca} stimulation was demonstrated in the experiments with FSK and ISO. ISO which acts via G_s produced only about 30 % increase of I_{Ca} after block of the AC/cAMP/PKA system (almost 3-fold increase in ATP-containing dialysate (Kameyama et al. 1985)). FSK, a direct AC activator, was completely ineffective in the same conditions. Moreover, the overall stimulation of I_{Ca} caused by forskolin in ATP-containing dialysates was usually 75 % of that caused by ISO. This difference as well as the 30 % of stimulation produced by ISO in presence of PI agents, which is similar to that produced by non-hydrolysable GTP analogues, can be attributed to the direct action of G_s on Ca channels.

Finally, the direct action of G_s on the Ca channel was demonstrated by the infusion into the cell of pre-activated G_s (kindly provided by L. Birnbaumer,

Houston, USA) in 6 nmol/l concentration. The augmentation of I_{Ca} was similar to that observed with non-hydrolysable GTP analogues.

Concluding remarks

There is strong evidence for a direct coupling of β-adrenergic receptors to Ca channels via G_s in cardiac cells. The contribution of the direct G_s action to the overall up-regulating effect of β-adrenergic stimulation, which also involves the indirect pathway via cAMP-dependent phosphorylation is relatively small and its physiological significance is not clear. According to Yatani and Brown (1989), a direct action of G_s on Ca channels is much faster than the action via the multi-step AC/cAMP/PKA cascade. The short latency of the direct mechanism will permit beat to beat changes in response to sympathetic nerve stimulation. Another possibility is that the direct interaction of the Ca-channel with activated G protein primes channels for further regulation by other agents. Finally, a direct action of G_s on the Ca channel would become important during cardiac dysfunction when heart cells have a diminished capacity for channel up-regulation via the AC/cAMP/PKA system.

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