effects appear to be derived from the fact that LTCC antagonists have a higher affinity for open or inactivated states of LTCCs. The antihypertensive and antianginal effects take advantage of the fact that the resting potential of smooth muscle is more depolarized than working cardiac myocytes. Thus, LTCC block and the associated vasodilation can be achieved without significant negative inotropy. The use-dependent nature of certain Ca^{2+} channel antagonists (such as verapamil) makes them particularly effective in the treatment of atrial tachyarrhythmia. These topics are covered in detail in Chapter 98.

■ REGULATION OF L-TYPE Ca^{2+} CHANNELS BY PROTEIN KINASES AND PHOSPHATASES

Phosphorylation of the LTCC complex by protein kinases and dephosphorylation by protein phosphatases are physiologically and pathologically relevant processes in cardiac myocytes. The primary physiologic regulation of LTCCs occurs via the β-adrenergic signaling pathways through activation of PKA. Phosphorylation of the LTCC complex via this pathway causes an increase in Ca^{2+} influx, SR Ca^{2+} loading, and SR Ca^{2+} release and underlies the associated increase in cardiac contractility. Abnormalities of this signaling cascade are well described in cardiac diseases that lead to congestive heart failure. Many clinically useful drugs, particularly β-adrenergic antagonists, are likely to impart a portion of their beneficial effects by influencing the phosphorylation state of the LTCC complex. Here we will briefly review the regulation of the LTCC complex via well-described signaling cascades with the understanding that drugs that activate or block these pathways will impart at least a portion of their cardiac effects via their influence on the cardiac LTCCs. It should be noted in this regard that calmodulin-dependent kinase II (CaMKII) is an important regulator of the LTCC. However, these effects have been reviewed in Chapter 2 and will not be discussed further here.

Effects of β-Adrenergic (cyclic Adenosine Monophosphate/Protein Kinase A) Signaling Pathways on L-Type Ca^{2+} Channels

Activation of the sympathetic nervous system is a major mechanism for controlling the rate and contractility of the normal heart. Catecholamines released from sympathetic nerves bind to β-adrenergic receptors on the cell surface, and this leads to phosphorylation of LTCC complex via activation of PKA (Fig. 16–4). Tsien[34] initially proposed that the stimulatory effect of cyclic adenosine monophosphate (cAMP) in heart cells was caused by PKA-mediated

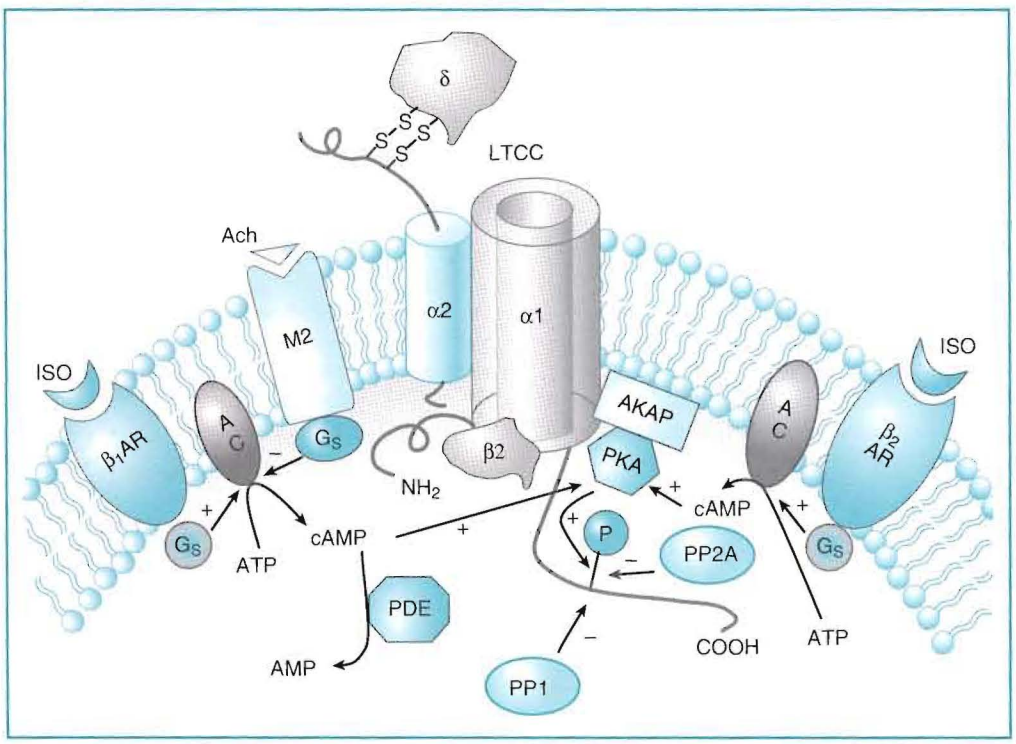

FIGURE 16-4 ■ Scheme of the cAMP/PKA pathway for LTCC regulation with β-adrenergic system as an example. When an agonist (e.g., isoproterenol [ISO]) binds to β-adrenergic receptors, β_1- and β_2-adrenergic receptor (β_1-AR and β_2-AR), the associated stimulatory G proteins (Gs) are activated and inhibitory Gβγ subunits are dissociated from Gαs. Activated Gαs then diffuses to activate adenyl cyclase (AC) that is attached to cell membrane. Active AC catalyzes the production of cyclic adenosine monophosphate (cAMP) and therefore local cAMP concentration increases dramatically. An elevated cAMP concentration activates protein kinase A (PKA) that is believed to be anchored close to LTCC by A kinase–anchoring proteins (AKAPs) and subsequently PKA phosphorylates LTCC. This diagram shows PKA-dependent phosphorylation of the α_1 subunit at the C-terminal tail (COOH). There are also PKA sites on β_2 but they are omitted for simplicity. Phosphorylated LTCC is dephosphorylated by protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1). The cAMP is cleaved by phosphodiesterases (PDE). Activation of the M2 cholinergic receptor may inhibit AC activity via G_i proteins. Ach, acetylcholine.

phosphorylation of LTCCs. These ideas were subsequently confirmed in many other laboratories. PKA-dependent phosphorylation of LTCCs causes a several-fold increase in $I_{Ca\text{-}L}$ and also shifts the voltage dependence of both activation and inactivation to more negative membrane potentials (Fig. 16–5, NF). Because PKA-dependent phosphorylation prolongs single-channel open time, the whole cell $I_{Ca\text{-}L}$ should decay more slowly. However, as discussed earlier, this effect is offset by an increase in $I_{Ca\text{-}L}$ that promotes Ca^{2+}-dependent inactivation. Single-channel experiments have shown that PKA-dependent LTCC phosphorylation increases channel availability and P_o and induces mode 2 gating without a change in single-channel conductance.[23] These effects can be blocked by PKA inhibitors such as H-89 and RpP -cAMP as well as by polypeptides such as PKA-I.

Two major subtypes of β adrenoceptors, β_1 and β_2, are thought to be present in cardiac myocytes, and activation of both receptors has been reported to phosphorylate LTCCs. There is very strong evidence supporting a role for β_1 receptor signaling to LTCCs. However, the functional relevance of the β_2 pathway is controversial and largely unresolved. Some studies[35] have found that zinterol, a β_2 adrenoceptor agonist, increases $I_{Ca\text{-}L}$. However, Nagykaldi et al[36] recently showed that the effects of zinterol on $I_{Ca\text{-}L}$ were blocked by β_1 antagonist CGP 20712A but not by β_2 antagonists. They concluded that the effect of zinterol occurred via a β_1 adrenoceptor because of its relatively low specificity. Other neurotransmitters and hormones such as histamine, glucagons, parathyroid hormone, and serotonin can also modulate $I_{Ca\text{-}L}$ in cardiomyocytes via the cAMP/PKA signal pathway. These effects are very small in comparison to those via β-adrenergic signaling.

Ser-1928 on α_{1c} subunit and Ser-478 and/or Ser-479 on the β_{2a} subunit are thought to be the PKA sites on the LTCC complex that are phosphorylated to cause an increase in $I_{Ca\text{-}L}$.[37] However, this hypothesis has not been well established with direct measurements. In addition, the role of the C-terminus of α_{1c}, at which Ser-1928 is located, is controversial because this portion of the protein can be cleaved by proteases or truncated by alternative splicing. Interestingly, it has been shown that the cleaved cytoplasmic C-terminus of the LTCC remains tethered to the membrane-imbedded α_1 subunit.[38] The relative roles of α_1 and β_{2a} phosphorylation in regulation of LTCCs also remain largely unestablished. Naguro and others[39] found that Ser-1901 in the so-called rat brain type II α_{1c} (corresponding to Ser-1928 in cardiac α_{1c}) is responsible for the increase in P_o upon phosphorylation by PKA, and phosphorylation of other PKA sites mediated the leftward shift of voltage-dependent activation. These are important issues for cardiac Ca^{2+} channels because they could lead to the development of calcium channel–specific drugs that specifically regulate LTCC phosphorylation. Another important issue is that PKA phosphorylation of LTCCs may require A kinase–anchoring proteins (AKAPs) to

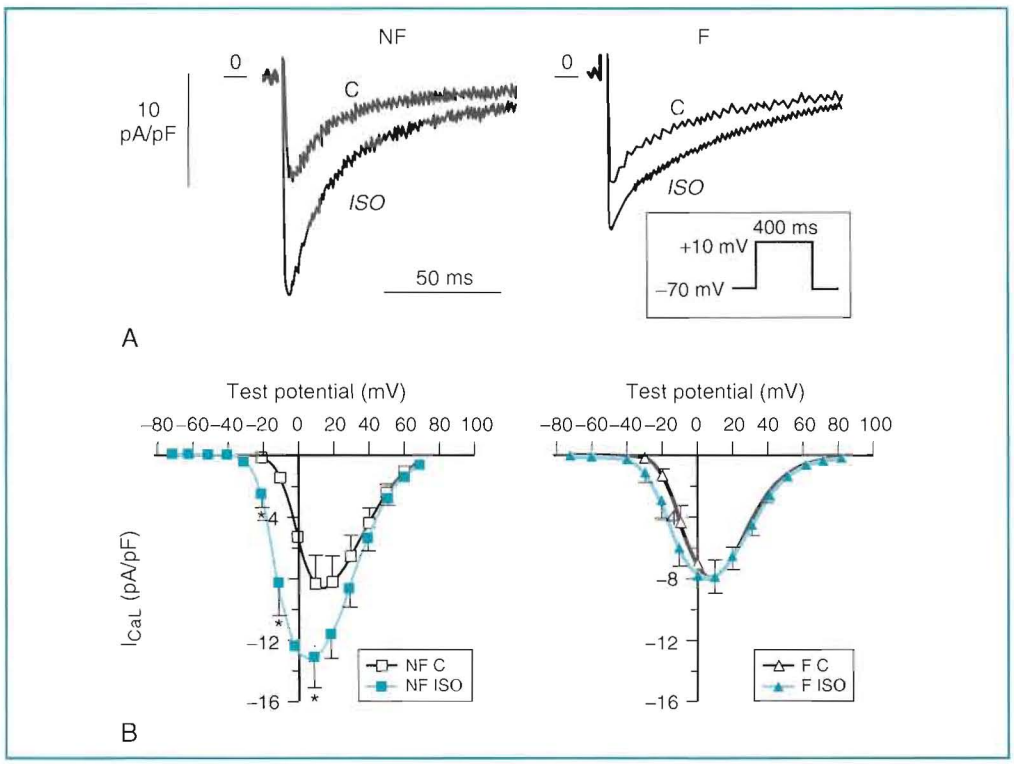

FIGURE 16-5 ■ Effects of 1 μM isoproterenol (ISO) on $I_{Ca\text{-}L}$ in nonfailing (NF, $n = 12$, $N = 6$) and failing (F, $n = 9$, $N = 4$) human ventricular myocytes. *A*, Sample current traces before and after bath application of 1 μM ISO. ISO increases $I_{Ca\text{-}L}$ significantly in NF but had little effect in F (124 ± 12%) myocytes versus either NF (190 ± 15%). *B*, Effect of ISO on $I_{Ca\text{-}L}$-voltage relationship in NF and F myocytes. ISO significantly increased maximal $I_{Ca\text{-}L}$ in NF myocytes but had minimal effect in F myocytes ($I_{Ca\text{-}L,ISO}/I_{Ca\text{-}L,ctr}$) in NF vs. F: 1.57 vs. 1.08). (From Chen X, Piacentino V 3rd, Furukawa S, et al: L-type Ca^{2+} channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. Circ Res 91:517–524, 2002.

anchor PKA in proximity to LTCCs. One study showed that PKA-dependent phosphorylation of α_{1c} required AKAP, whereas the phosphorylation of the β_{2a} subunit by PKA did not.[38] The role of these molecules in the phosphorylation defects in diseased myocytes is an important topic that needs to be studied. The signaling pathways that interact to regulate the phosphorylation state of the LTCC and thereby determine its activation state and the size of $I_{Ca\text{-}L}$ are shown diagrammatically in Figure 16–4 with β-adrenergic signaling pathway as an example.

In addition to the components mentioned above, phosphodiesterase (PDE), which cleaves cAMP into AMP and protein phosphatases (PP1 and PP2) that dephosphorylate LTCCs, also influence the phosphorylation state of the LTCC. A study in failing human myocytes suggested that the phosphorylation state of the LTCC is increased and that this results from a low activity of phosphatases that normally dephosphorylate the LTCC. Other studies in normal myocytes showed that phosphatase activity is an important determinant of cardiac LTCC properties.[40]

Regulation of L-Type Ca^{2+} Channels by Protein Kinase C in Cardiac Myocytes

Unlike the effect of PKA, the effect of PKC on $I_{Ca\text{-}L}$ is controversial. Some studies revealed stimulatory effects of PKC, whereas others showed inhibitory effects or biphasic responses.[37] *In vitro*, both the α_1 and β_2 subunits of LTCCs are good substrates for PKC-mediated phosphorylation with a stoichiometry of 2 to 3 moles of phosphate per mole of α_1 subunit and 1 to 2 moles of phosphate per mole of β_{2a} subunit.[37] The PKC sites are possibly located at the N-terminus (The-27 and The-31), and phosphorylation of these sites by PKC has been shown to cause $I_{Ca\text{-}L}$ inhibition.[41] Other studies suggested that PKC sites at regions other than N-terminus inhibit LTCC activity in the dephosphorylated state[42] and that phosphorylation of these sites relieves this inhibition and increases the P_o of LTCCs.

PKC has been proposed to mediate the electrophysiologic and contractile effects of many hormones and neurotransmitters that include α-adrenergic agonists, intracellular adenosine triphosphate (ATP), angiotensin II, glucocorticoids, arginine-vasopressin, and endothelin. Angiotensin II under perforated patch conditions (no cell dialysis) enhances $I_{Ca\text{-}L}$ possibly via PKC phosphorylation[43] but has little effect when $I_{Ca\text{-}L}$ is recorded with ruptured patch techniques that involved cellular dialysis. In normal rabbit ventricular myocytes, endothelin-1 had a biphasic effect on $I_{Ca\text{-}L}$, first inhibiting $I_{Ca\text{-}L}$ and then increasing it. Furthermore, endothelin-1 strongly attenuates the β-adrenergic stimulation of $I_{Ca\text{-}L}$.[44] The role of PKC as a modulator of the LTCC is an important unresolved issue that needs further study. What is clear is that the quantitative effect of PKC on LTCCs is significantly smaller than that of PKA.

Regulation of L-Type Ca^{2+} Channels by Protein Kinase G in Cardiac Myocytes

The role of protein kinase G (PKG) in regulating LTCCs is even more controversial and is beyond the scope of this chapter. The cyclic guanosine monophosphate (cGMP)/PKG pathway could influence the LTCC through at least three mechanisms[37]: (1) direct phosphorylation by PKG; (2): PKG-induced activation of phosphatases; and (3) cGMP-dependent activation or inhibition of PDEs that control cAMP. In brief, most studies have shown a direct inhibitory effect of PKG on $I_{Ca\text{-}L}$ with a stimulatory effect of cGMP being related to processes other than PKG phosphorylation, e.g., cGMP-dependent inhibition of PDEIII.

Abnormalities of L-Type Ca^{2+} Channel Regulation in Diseased Hearts

Blunted adrenergic responsiveness is a hallmark of heart failure and is responsible for the low exercise tolerance of patients with heart failure. The responsiveness of $I_{Ca\text{-}L}$ to β-adrenergic stimulation is diminished in heart failure[2] (see Fig. 16–5) and contributes to the depressed contractile reserve of the failing heart. The mechanisms responsible for these blunted effects are not firmly established with likely roles for reduced receptor numbers, decreased adenyl cyclase activity (leading to decreased cAMP production), increased coupling of G_i to β-adrenergic receptors, increased expression of β-adrenoceptor kinase (that desensitizes β-adrenergic responses), increased PDE activity, abnormalities in AKAP abundance and localization, increased activity of signaling pathways (such as the cGMP/PKG pathway) that antagonize the cAMP/PKA pathway,[45] and abnormalities of the LTCC phosphorylation state caused by reduced phosphatase activity.[46] These processes are all worthy of future investigation. Interestingly, currently effective heart failure medications such as β-adrenergic receptor blockers may impart part of their beneficial effects by altering the phosphorylation state of PKA target protein such as the LTCC.[2]

■ PHARMACOLOGY OF T-TYPE Ca^{2+} CHANNEL IN THE HEART

There are no highly specific T-type calcium channel antagonists available today. Mibefradil (also named Ro 40-5967) is the most specific antagonist available at present, but it still has potent effects on the LTCC. Structurally mibefradil is a derivative of tetralol and is unrelated to the three categories of classical LTCC antagonists. It suppresses T-type calcium current and shifts the steady-state inactivation to more negative voltages.[47] Recent studies have shown that mibefradil induces peripheral vasodilation and heart rate reduction, but no decrease in cardiac contractility in patients with heart failure. In addition, mibefradil inhibits neurohormonal releases of aldosterone from adrenal medulla and cortex and of noradrenaline from the sympathetic nerves.[48] Clinical trials have shown that mibefradil is an effective antianginal, antihypertensive, and anti-ischemic agent. However, trials in patients with congestive heart failure have been disappointing.[49] Unexpected metabolic drug interactions complicate the putative beneficial effects of mibefradil and have resulted in its withdrawal from the market. New specific T-type calcium channel antagonists without such drug interactions might be clinically useful and would help in basic research because they would allow the functional role of this channel to be more clearly defined.