(*22*) to produce the $G_{LTP}$-dependent changes responsible for LTP.

Intracerebroventricular injections of PT block LTP in mossy fiber–CA3 but not stratum radiatum—CA1 synapses (*23*). We were also unable to counteract LTP in the CA1 region by ventricular injection of the toxin (*24*). The CA3 region of the hippocampus in rats is strategically located close to the lateral ventricle, whereas the CA1 area is not. Therefore, PT in the ventricle would have better access to the CA3 region. However, intracerebral injections of the toxin directly above the hippocampus, as were used in this study, localize it in the vicinity of CA1 neurons. Taken together, our study and that of Ito *et al.* (*23*) suggest a G protein is involved in LTP in two separate synaptic systems in the hippocampus. Several differences have been found between LTP in the CA1 and CA3 areas. (i) LTP induction is associative in the CA1 area but not in mossy fiber–CA3 synapses (*25*). (ii) NMDA receptor blockade inhibits LTP induction in the CA1 region but not in the mossy fiber–CA3 system (*25, 26*). (iii) Intracellular injections of $Ca^{2+}$ chelators block LTP in the CA1 region but not in the mossy fiber–CA3 system (*20, 27*). A role for G proteins in these two apparently different pathways provides a common link in the LTP process. Furthermore, PKC activation by phorbol ester application can produce a potentiation of responses in both the stratum radiatum–CA1 and mossy fiber–CA3 synaptic systems. There is a good correlation between the distributions of phorbol ester binding sites in the brain and $G_o$ (*28*), a G protein that is inactivated by PT and can regulate PLC activity (*7*). Perhaps $G_o$ and $G_{LTP}$ are similar or identical proteins.


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# Autonomic Regulation of a Chloride Current in Heart


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**In isolated heart cells, β-adrenergic receptor stimulation induced a background current that was suppressed by simultaneous muscarinic receptor stimulation. Direct activation of adenylate cyclase with forskolin also elicited this current, suggesting regulation by adenosine 3′,5′-monophosphate (cAMP). This current could be recorded when sodium, calcium, and potassium currents were eliminated by channel antagonists or by ion substitution. Alteration of the chloride equilibrium potential produced changes in the reversal potential expected for a chloride current. Activation of this chloride current modulated action potential duration and altered the resting membrane potential in a chloride gradient–dependent manner.**


IN HEART, STIMULATION OF β-ADRENergic receptors activates adenylate cyclase, resulting in the production of cAMP and subsequent protein kinase activation. This pathway is involved in the regulation of several ionic currents. Furthermore, the response to β-adrenergic stimulation of at least two of these, the $Ca^{2+}$ current ($I_{Ca}$) (*1*) and the delayed rectifier $K^+$ current ($I_K$) (*2–4*), can be antagonized by acetylcholine (ACh) acting through muscarinic receptors (*4–5*). The transient outward current ($I_{to}$) is also enhanced by β-adrenergic stimulation (*6*). The $I_{to}$, which rapidly activates during membrane depolarization and helps repolarize the membrane potential (*7*), was initially suggested to be a $Cl^-$ conductance (*8*) but it is now believed to be carried predominantly by $K^+$ ions (*9*). Nevertheless, single $Cl^-$ channels with a unitary conductance of 55 pS can be observed in planer lipid bilayers


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into which cardiac sarcolemmal proteins have been incorporated (*10*). However, the function of these channels is unknown. We have demonstrated a whole-cell $Cl^-$ current in cardiac myocytes that is regulated by β-adrenergic and muscarinic receptors. When activated, this current can modulate the duration of the cardiac action potential and might explain, in part, inconsistent reports (*11*) of β-adrenergic–induced alterations of the resting membrane potential.

When isolated guinea pig ventricular cells (*12*) were exposed to isoproterenol (ISO) ($10^{-6}M$) under voltage-clamp conditions (*13*), there was an increase in the background current that was most evident at potentials positive to −40 mV (Fig. 1, control). At this range there is little outward current under control conditions because of rectification of the background $K^+$ conductance ($I_{K1}$). In guinea pig ventricle there is little, if any, $I_{to}$, which is common in other cardiac preparations (*6–9*). Therefore, when $Na^+$ current was inactivated with a depolarized holding potential and $I_{Ca}$ was blocked with nisoldipine [$10^{-6}M$, a concentration 100-fold greater than the median effective dose ($ED_{50}$) for $I_{Ca}$ inhibition] (*13a*), on depolarization the ISO-induced current appeared as an instantaneous current jump (Fig. 1, ISO) before activation of $I_K$. This response was observed in 89% of all cells tested ($n = 86$). Subsequent exposure to ISO plus ACh ($10^{-5}M$) resulted in the attenuation of the ISO-induced current (Fig. 1, ISO + ACh). The response to ISO could be blocked by propranolol ($10^{-5}M$) and the response to ACh could be blocked by atropine ($10^{-6}M$), indicating that regulation occurs via β-adrenergic and muscarinic receptors. The net ISO-induced current time appeared to be independent and exhibited a reversal potential of −46 ± 11 mV (SD, $n = 6$) with slight rectification in the outward direction (Fig. 2A). Direct activation of adenylate cyclase with forskolin ($10^{-6}M$) elicited a time-independent current with the same voltage dependence and reversal potential (Fig. 2B). This suggests that production of cAMP is involved in the regulation of this current.

That ISO-induced current could be elicited from nisoldipine-treated cells with a depolarized holding potential suggests that this current is not conducted through $Ca^{2+}$ or $Na^+$ channels. The ISO-induced current also persisted in the presence of $Cs^+$ and $Ba^{2+}$, indicating that it is not likely to be a $K^+$ current, and in the presence of oubain ($10^{-4}M$), suggesting that the electrogenic $Na^+$,$K^+$ pump is not involved. Furthermore, the reversal potential of the current was not affected by removing extracellular $Na^+$ or $K^+$, and intracellular $Ca^{2+}$ was buffered with 5 m*M* EGTA. Therefore, it is unlikely that the ISO-induced current is conducted through a nonselective cation channel.

Since the reversal potential was not affected by changing cation concentrations, we tested whether the current might be carried by an anion (for example, $Cl^-$). When the $Cl^-$ equilibrium potential was altered by changing the intracellular $Cl^-$ concentration, the reversal potential for this current shifted accordingly (Fig. 3). In addition, the voltage dependence of the current was linear when concentrations of $Cl^-$ were equal inside and outside of the cell; rectification became more pronounced as internal $Cl^-$ was reduced. These results indicate that the ISO-induced current is predominantly conducted by $Cl^-$; we therefore refer to it as $I_{Cl}$. This conclusion is further supported by our finding that the current at all potentials was reduced by 38.2 ± 9.9% ($n = 2$) in the presence of $10^{-4}M$ 9-anthracenecarboxylic acid (9-AC). Although there are no known specific, high-affinity blockers of $Cl^-$ currents (*14*), 9-AC reduces the $Cl^-$ conductance in skeletal muscle and epithelial preparations (*14, 15*). Unlike the $Cl^-$ conductance of some epithelial (*14*) and smooth muscle preparations (*16, 17*), $I_{Cl}$ in heart does not appear to require intracellular $Ca^{2+}$, because in all of our experiments the cells were dialyzed with 5 m*M* EGTA. However, this does not rule out the possibility that internal $Ca^{2+}$ may be involved in regulating $I_{Cl}$.

The intracellular $Cl^-$ concentration in Purkinje fibers and ventricular tissue is between 10 and 24 m*M* (*18*), which would mean that the $Cl^-$ equilibrium potential ($E_{Cl}$) is between −48 and −71 mV (external $Cl^-$, 151 m*M*). Therefore, during depolarization, a $Cl^-$ conductance would act as a repolarizing force. To test the physiological importance of $I_{Cl}$, we recorded action potentials in cells dialyzed with an internal solution containing 22 m*M* $Cl^-$ ($E_{Cl}$, −50 mV) before and after exposure to ISO and ACh (a representative experiment is illustrated in Fig. 4). Before recording action potentials (Fig. 4A), we monitored membrane current with 5-s depolarizing voltage-clamp steps (Fig. 4B). In the presence of ISO, $I_{Cl}$ was evident as a parallel shift of the time-dependent current $I_K$, in the outward direction. There was no apparent change in $I_K$ magnitude, as expected under these conditions (22°C) (*3*), and $I_{Ca}$ was blocked with nisoldipine. Associated with the appearance of $I_{Cl}$ was a pronounced decrease in action potential duration. Addition of ACh ($10^{-5}M$) reversed the ISO-induced changes in membrane current ($I_{Cl}$) and action potential duration, and the response to ACh was reversed on washout. These results suggest that $I_{Cl}$ may be significant in vivo. This conclusion is supported by the fact that $I_{Cl}$

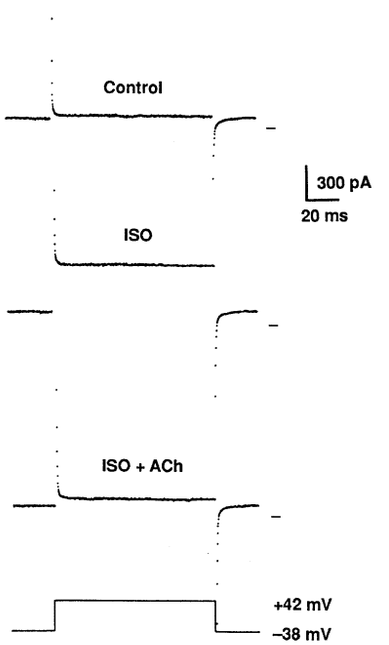


**Fig. 1.** Effect of ISO ($10^{-6}M$) and ISO plus ACh ($10^{-5}M$) on membrane currents. Changes in background conductance were monitored by applying 100-ms depolarizing test pulses to +42 mV from a holding potential of −38 mV. Solutions in (*13*). Zero current level is indicated to the right of the traces.

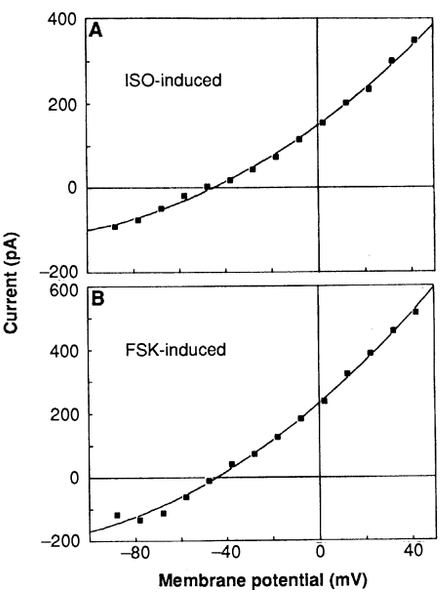


**Fig. 2.** (**A**) Current-voltage relation of ISO-induced currents. Difference currents were obtained by digital subtraction of currents recorded before from those recorded after exposure to ISO ($10^{-6}M$). The current was determined as the average of 100 consecutive data points taken 50 ms into the test pulse (traces digitized at a frequency of 3.33 kHz). (**B**) Current-voltage relation of forskolin (FSK) ($10^{-6}M$)-induced currents. Solutions in (*13*). Currents and measurements were obtained as in (A).

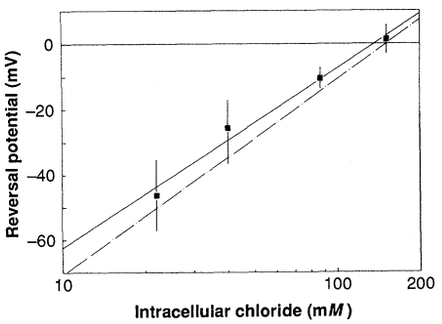


**Fig. 3.** Relation between intracellular $Cl^-$ concentration and reversal potential of the ISO-induced current. Each point represents the average of six separate experiments (± SD). The solid line is a linear regression fit to the data points (slope, 56 ± 3 mV). The broken line is the relation predicted for a $Cl^-$ electrode by the Nernst equation (slope, 59 mV). The $Cl^-$ equilibrium potential was altered by increasing the intracellular $Cl^-$ concentration (replacing glutamate) (*13*).

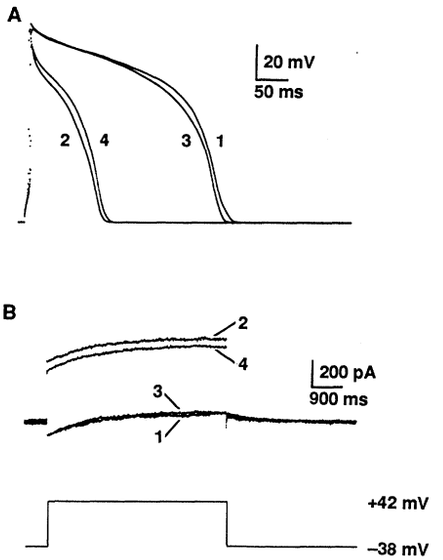


**Fig. 4.** Action potentials and membrane currents obtained from a cell exposed to ISO with and without ACh. (**A**) Membrane potential was measured by switching to current-clamp, and action potentials were evoked by an intracellular injection of current (2 ms; 440 pA). The resting membrane potential was −78 mV. Each action potential represents the average of seven digitized traces. Action potential duration (measured at 90% repolarization): (trace 1) control, 315 ms; (trace 2) ISO, 116 ms; (trace 3) ISO + ACh, 303 ms; and (trace 4) ISO and washout ACh, 143 ms. The short duration of the control action potential at room temperature (*12*) is due to the absence of $Ca^{2+}$ current. (**B**) Membrane currents recorded from the cell used to obtain data in (A). After each intervention and before action potentials were recorded, changes in membrane current were monitored. Currents were elicited by 5-s voltage-clamp steps to +42 mV from a holding potential of −38 mV. Numbered traces correspond to legend in (A). Qualitatively similar results were obtained in five experiments.

could be evoked by ISO concentrations as low as $10^{-11}M$. Concentrations of ISO greater than $10^{-8}M$ elicited a maximal $I_{Cl}$ that was maintained as long as the agonist was present (up to 20 min). However, ISO below $10^{-9}M$ caused an increase in the background conductance that peaked quickly, then subsided to a submaximal level in the continued presence of agonist.

The physiological significance of $I_{Cl}$ is further emphasized by the fact that we have observed this current in guinea pig atrial and rabbit ventricular cells as well. Because changes in background current are often difficult to observe in the presence of $I_{Ca}$ or $I_{to}$, $I_{Cl}$ may have gone unnoticed in the past. In addition, some of the noninactivating current that is increased by β-adrenergic stimulation and has been attributed to $I_{to}$ (*6*) may represent $I_{Cl}$. Identification of $I_{Cl}$ may also depend on the experimental conditions. In guinea pig ventricular myocytes, ISO has been reported to cause a depolarization of the resting membrane potential (*19*). In these experiments, cells may have been loaded with $Cl^-$ intracellularly, resulting in a depolarizing shift of $E_{Cl}$. This is consistent with the results of experiments in which we dialyzed cells with 152 m*M* $Cl^-$. Under these conditions exposure to ISO resulted in a depolarization of the resting membrane potential by 53.3 ± 3.2 mV ($n$ = 3). When cells were dialyzed with 22 m*M* $Cl^-$, $E_{Cl}$ was closer to the resting membrane potential and $I_{Cl}$ exhibited rectification. Under these conditions $I_{Cl}$ would contribute relatively little to the resting membrane conductance, which is dominated by $I_{K1}$. In these experiments, exposure to ISO caused a small (<2 mV) but consistent depolarization of the resting membrane potential ($n$ = 5). In some cardiac preparations additional mechanisms involving other membrane conductances (*11*) or changes in the activity of the electrogenic $Na^+,K^+$ pump (*20*) may also contribute to β-adrenergic modulation of the resting potential. The identification of single $Cl^-$ channels that are responsible for the whole-cell current described here will allow a more direct comparison to $Cl^-$ currents in noncardiac preparations as well as allow a detailed investigation of intracellular regulatory pathways.

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