which transformation and increased proliferation are associated with a fully differentiated phenotype. In fact, the phenotype of *ras*-transformed LCL we have described is highly reminiscent of multiple myeloma, since in this malignancy there is overproduction of terminally differentiated plasma cells derived from more immature B cell precursors (*18*). We have recently determined that the more immature B cell malignancies such as lymphoma and chronic lymphocytic leukemia do not contain activated *ras* genes, whereas multiple myeloma is associated with N-*ras* activation in approximately 30% of the cases studied (*19*). These observations suggest that N-*ras* activation may be a late event during the development of at least some myeloma cases, leading to the acquisition of both a fully transformed and terminally differentiated phenotype.

In general, our observations underscore the notion that oncogenes may have distinct biological effects in different tissues and that the careful dissection of these effects will contribute to understanding both the function of proto-oncogenes and the role of their respective activated counterparts in the pathogenesis of different types of malignancies. The transformation assay based on EBV-infected human lymphoblasts represents a model system for understanding the mechanisms through which known oncogenes contribute to B cell lymphomagenesis, as well as for identifying novel tissue-specific oncogenes.

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Modulation of Calcium Channels in Cardiac and Neuronal Cells by an Endogenous Peptide

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Calcium channels mediate the generation of action potentials, pacemaking, excitation-contraction coupling, and secretion and signal integration in muscle, secretory, and neuronal cells. The physiological regulation of the L-type calcium channel is thought to be mediated primarily by guanine nucleotide–binding proteins (G proteins). A low molecular weight endogenous peptide has been isolated and purified from rat brain. This peptide regulates up and down the cardiac and neuronal calcium channels, respectively. In cardiac myocytes, the peptide-induced enhancement of the L-type calcium current had a slow onset (half-time ≈75 seconds), occurred via a G protein–independent mechanism, and could not be inhibited by α_1-adrenergic, β-adrenergic, or angiotensin II blockers. In neuronal cells, on the other hand, the negative effect had a rapid onset (half-time <500 milliseconds) and was observed on both T-type and L-type calcium channels.

CALCIUM CHANNELS ARE WIDELY distributed in excitable cells and mediate many biological processes. Cardiac L-type Ca^{2+} channels are primarily up-regulated by adenosine 3′,5′-monophosphate (cAMP)–dependent phosphorylation (*1–3*). Neuronal Ca^{2+} channels are inhibited by a variety of adrenergic and peptidergic receptors (*4–9*). L-type channels have been identified in vitro by their specific binding site to dihydropyridines (*10*). Because these stereospecific sites can be up- or down-regulated experimentally (*11, 12*), they may be regulated by putative endogenous ligands. A low molecular weight material isolated from the rat brain inhibits the nitrendipine-binding sites and decreases veratridine-induced ^{45}Ca uptake in rat brain synaptosomes (*13, 14*). We now show that this endogenous compound is an acidic peptide with a molecular size of about 1 kD, which interacts with Ca^{2+} channels, enhancing their activity in isolated cardiac myocytes and suppressing activity in the central neurons.

The nitrendipine-displacing material was extracted from rat brain homogenates prepared with trichloroacetic acid at 95°C. The supernatant was retained on silica Seppak cartridge (Waters, Milford, Massachusetts) (*13, 14*), and final purification was obtained by high-performance liquid chromatography (HPLC) with a Partisil-10 silica column (Whatman, Clifton, New Jersey) and a linear gradient from 5 to 45% methanol in methylene chloride for 40 min. The nitrendipine-displacing activity eluted in one single peak from a Partisil-10 column with a retention time (RT) of about 23 min. When this eluate was reinjected and eluted with the same gradient conditions, one major peak (RT, 22.90 min) and several smaller peaks (RT, 21.38 and 25.22 min) were obtained. The active material eluting in the peak with an RT of 22.90 min was resolved into two peaks by ^{252}Cf plasma desorption mass spectrometry (*15*). The major peak had the mass number of 948, whereas that of the smaller one was 1022 (Fig. 1).

The purified material inhibited in a concentration-dependent manner [^{3}H]nitrendi-

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Table 1. Agents that failed to modify or mimic the enhancing effect of the endogenous peptide on cardiac Ca^{2+} channels.

Agent	Concentration
Propanolol	5 μ*M*
WB-4101	2 μ*M*
cAMP	≤200 μ*M*
GTP-γ-S	200 μ*M*
GDP-β-S	2 m*M*
[Sar^1, Ile^8]AII	10 μ*M*
GABA	10 μ*M*
L-Glutamate	100 μ*M*
Glycine	100 μ*M*

pine binding in hippocampal and heart membranes (*13, 14*), although solutions that were three times more concentrated than those used for hippocampal membranes were needed to obtain a 50% decrease of [^{3}H]nitrendipine binding in heart membranes. In contrast, the nitrendipine-displacing material failed to alter the specific binding of radioligands that are markers for dopamine, serotonin, or glutamate receptors in brain membranes.

To determine whether the active material was a peptide, we exposed it to pronase, proteinase K, protease V8, or acid hydrolysis. The nitrendipine-displacing material was resistant to digestion by pronase and proteinase K. The incubation of the material with protease V8 (1 mg of protein per milliliter) suppressed its nitrendipine-displacing activity. The [^{3}H]nitrendipine-displacing activity was also lost after acid hydrolysis (5.7*N* HCl for 30 min at 100°C). Protease V8 cleaves peptide bonds specifically at the carboxyl-terminal site of either Asp or Glu residues (*16*); we determined the amino acid composition of the hydrolysate. This composition was 55% Asp, 25% Glu, 5% Gly, 5% Thr, and two unidentified peaks. Our attempt to determine the amino acid sequence of this material was unsuccessful, most likely due to blockade of free amino-end terminals by carbohydrates or pyrrolidine rings.

Ca^{2+} current (I_{Ca}) was measured in guinea pig ventricular myocytes, mouse neuroblastoma, GH3 cells, and cortical neurons with the whole-cell patch-clamp technique (*17*). Neurons were obtained from primary cell cultures, and cardiac cells were freshly isolated (*18*). We isolated I_{Ca} from other current components by using tetrodotoxin (TTX) in the external solution to block Na^+ current and by dialyzing the cell with Cs^+-containing solution to minimize the contribution of outward K^+ currents. The external solution generally contained 140 m*M* NaCl, 5.4 m*M* KCl, 1 m*M* $MgCl_2$, 2 to 5 m*M* $CaCl_2$, 0.001 m*M* TTX, 10 m*M* glucose, and 10 m*M* Hepes-NaOH, *p*H 7.4, at room temperature (21° to 23°C). The internal dialyzing solution contained 100 m*M* CsCl, 20 m*M* tetraethylammonium chloride, 5 m*M* magnesium adenosine triphosphate, ≤0.2 m*M* cAMP, 10 m*M* EGTA or 5 m*M* bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), and 10 m*M* Hepes-CsOH, *p*H 7.2. Solutions were quick-exchanged with a system of multibarreled pipettes, making it possible to change the solution surrounding the cell in less than 100 ms (*19*).

Before final purification by HPLC, the nitrendipine-displacing material had a biphasic effect on I_{Ca} of guinea pig ventricular myocytes. During application of the material, I_{Ca} first decreased and then increased toward a level that was only slightly larger than the control value. On washing, however, I_{Ca} rapidly increased by about 100% before decreasing again to the control level. This experiment suggested that the extracted material contained more than one type of agent, which produced inhibitory and enhancing effects on I_{Ca}.

In contrast, after further purification by HPLC, the active material produced only a monophasic enhancing effect on I_{Ca}. The effect of 1 unit of the HPLC-purified peptide on I_{Ca} is shown in Fig. 2, A and B. One unit of peptide decreased [^{3}H]nitrendipine binding by 50% in hippocampal membrane preparations. On the basis of the amino acid composition of the hydrolysate and the apparent molecular weight of the peptide, 1 unit corresponds to 1.0 to 2.5 n*M*. When the peptide was added, I_{Ca} increased from 0.86 to 2.1 nA in a monophasic way with a half-time of about 75 s (Fig. 2A). The current-voltage relation for I_{Ca} from the same experiment showed a threshold potential at about −40 mV, a maximum at +20 mV, and an apparent reversal potential at about +60 mV. The purified peptide enhanced the amplitude of I_{Ca} at all voltages examined, left the threshold and the reversal potential unaltered, but shifted the maximum by 5 to 10 mV in the hyperpolarizing direction (Fig. 2B). Comparison of the time course of inactivation in the presence and absence of endogenous peptide by appropriately scaling the peak of I_{Ca} showed that inactivation was slower in the presence of the peptide. This observation suggests that the endogenous peptide increases the mean open time of the channel. The results obtained in different (n = 19) preparations are summarized in Fig. 2C. The maximum peptide-induced increase in I_{Ca} was a sigmoid function of the endogenous peptide concentration with an apparent affinity constant of 1.2 units and a Hill coefficient of 1.

Potentiating effects of the peptide were also observed in rat and frog ventricular myocytes. The presence of the β-adrenergic blocker propanolol (5 μ*M*) or the α_1-adrenergic blocker WB-4101 (2 μ*M*) did not alter the enhancing effect of the peptide. The enhancing effect of the peptide on I_{Ca} was also independent of the presence of cAMP (≤200 μ*M*) or the stable guanosine 5′-triphosphate (GTP) analog, guanosine 5′*O*-(3-thiotriphosphate) (GTP-γ-S) (200 μ*M*), in the dialyzing solution. Addition of 2 m*M* of the stable guanosine diphosphate (GDP) analog, guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S), in the dialyzing solution, which suppressed the effect of exogenously added adrenaline (10 μ*M*) on I_{Ca}, also did not inhibit the enhancing effect of the peptide. These observations rule out the involvement of G proteins directly or indirectly in the enhancing action of the peptide on I_{Ca}. Acid hydrolysis of the peptide completely suppressed its enhancing effect on cardiac myocytes.

Because the hydrolyzed material contained a large fraction of glutamate and glycine, we examined possible effects of these amino acids on the I_{Ca} of myocytes. L-Glutamate and glycine at concentrations of 100 μ*M* failed to have any effect on I_{Ca} of guinea pig ventricular myocytes. Also, γ-aminobutyric acid (GABA) (10 μ*M*) failed to mimic the enhancing effect of the peptide on I_{Ca}. Because the molecular weight of the endogenous peptide was similar to that of

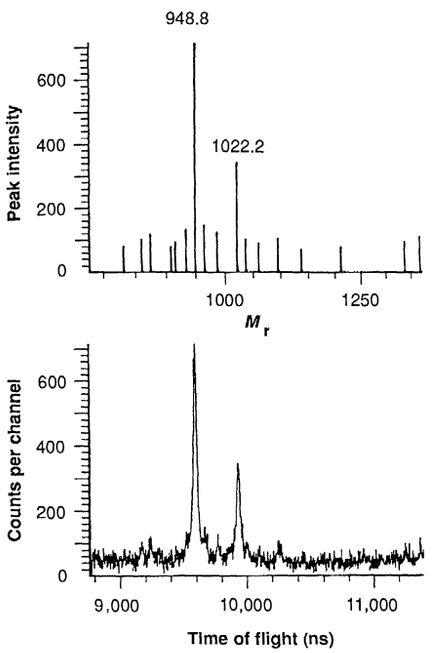

Fig. 1. Determination of the molecular mass of the purified peptide. The nitrendipine-displacing activity that eluted from a Partisil-10 column with a retention time of 22.90 min was resolved by ^{252}Cf plasma desorption mass spectrometry (*15*) into two peaks. (**Upper**) The major peak had mass number 948, and the smaller one had mass number 1022. (**Lower**) Raw data from which the mass was determined.

angiotensin II, we examined whether this material was either contaminated by angiotensin II, or acted by a similar mechanism. In rat ventricular myocytes, which were treated with high concentrations (10 μM) of angiotensin II inhibitor [Sar^1, Ile^8]AII, the endogenous peptide still produced its strong enhancing effect on I_{Ca}. This was consistent with the lack of any [^{3}H]nitrendipine-displacing activity of angiotensin II, [Val^5]AII, in hippocampal membranes. The amino acid composition of the endogenous peptide was significantly different than that of angiotensin II, specifically arginine and phenylalanine could not be detected in the hydrolyzed material.

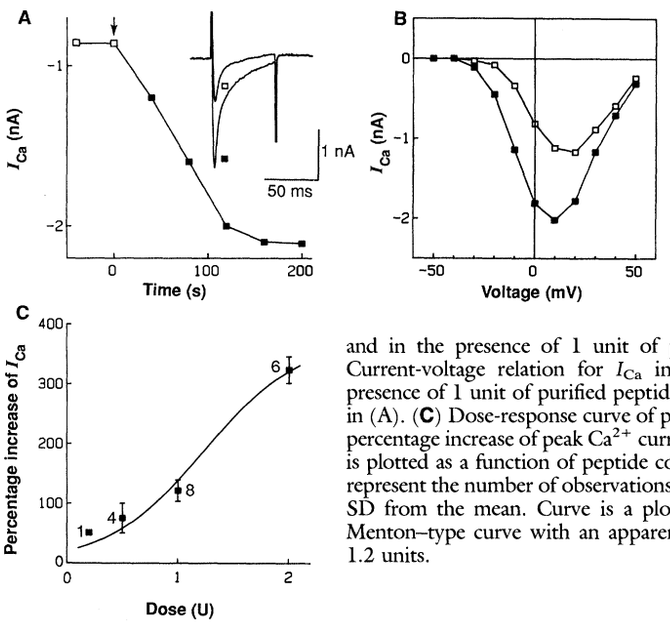

Fig. 2. Effect of purified peptide on I_{Ca} of guinea pig ventricular myocyte. (**A**) I_{Ca} was activated by depolarization from −50 mV to +10 mV, and peak I_{Ca} is plotted as a function of time. At arrow, 1 unit of purified peptide was applied to the cell, resulting in a monophasic increase in I_{Ca}. The inset shows I_{Ca} recorded in control (□) and in the presence of 1 unit of purified peptide (■). (**B**) Current-voltage relation for I_{Ca} in control (□) and in the presence of 1 unit of purified peptide (■). Same experiment as in (A). (**C**) Dose-response curve of purified peptide on I_{Ca}. The percentage increase of peak Ca^{2+} current induced by the peptide is plotted as a function of peptide concentration. The numbers represent the number of observations, and the error bars show 1 SD from the mean. Curve is a plot of theoretical Michaelis-Menton–type curve with an apparent dissociation constant of 1.2 units.

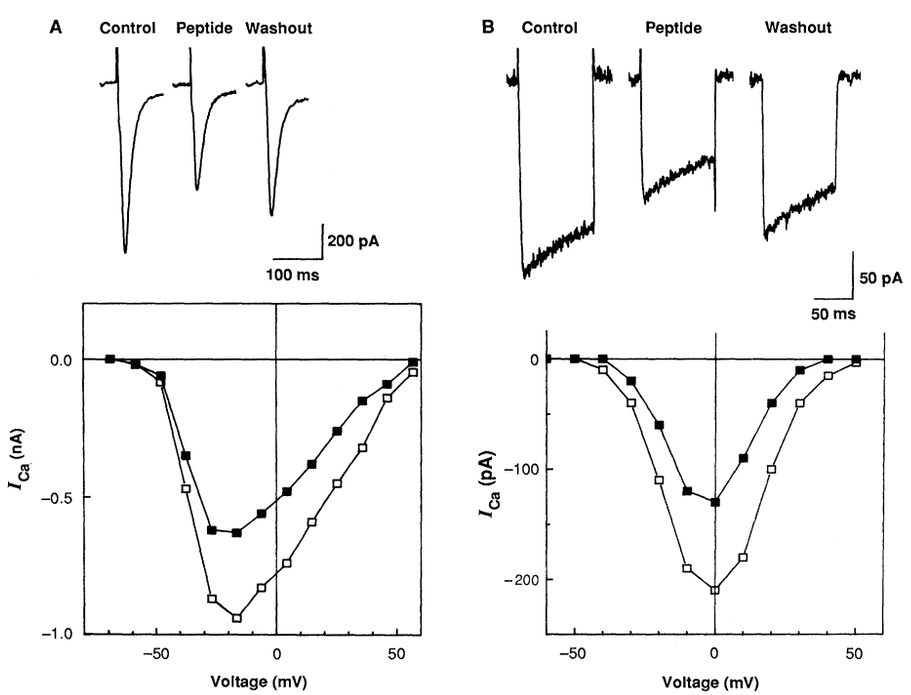

Fig. 3. Effect of purified peptide on T-type and L-type components of Ca^{2+} current in neurons. Cells were held at −80 mV and depolarized to potentials between −80 and +60 mV. (**A**) Effect of 1 unit of purified peptide on T-type Ca^{2+} current in a neuroblastoma cell (Nle-115). Upper panel shows peptide-induced block of I_{Ca} activated by depolarization to −20 mV. Lower panel shows peak I_{Ca} as a function of voltage in control (□) and in the presence of the peptide (■). (**B**) Effect of 1 unit of purified peptide on L-type Ca^{2+} current in a GH3 cell. Upper traces show peptide-induced block of I_{Ca} activated by depolarization to 0 mV. Lower panel plots peak I_{Ca} against voltage in control (□) and in the presence of the peptide (■). Traces were leak-subtracted by subtracting the current response to equal voltage steps in the presence of 5 mM Ni^{2+}. In the external solution, $CaCl_2$ (5 mM) was replaced with equal amounts of $BaCl_2$.

Table 1 summarizes the effect of the various possible agents that were used to determine the mechanism by which the endogenous peptide may exert its effect on cardiac Ca^{2+} channels. The endogenous peptide strongly enhances the cardiac Ca^{2+} channel through a novel and yet unknown mechanism. Because the onset of action of the peptide and its washout were slow as compared to the effect of other agents that enhance I_{Ca} (for example, adrenaline or Bay K 8644), we suspect that an intermediate reaction, most likely on the myoplasmic side of the membrane, mediates the enhancement of I_{Ca}.

We also examined the effect of the purified peptide on the Ca^{2+} channel current in three different types of neurons. The peptide consistently and rapidly suppressed the Ca^{2+} current in each type of the cells examined. Unlike its agonistic effect on cardiac myocytes, the antagonistic effect of the peptide occurred rapidly (within a fraction of a second). The effect of the peptide on the T-type Ca^{2+} channel current is shown for a neuroblastoma cell (Fig. 3A). Application of 1 unit of the peptide resulted in a 30 to 40% inhibition of I_{Ca} (n = 5). The effect was partially reversible on washout. Complete reversibility was obtained when the peptide was applied for shorter times (<1 min). A qualitatively similar inhibition of the L-type Ca^{2+} channel current was also observed in GH3 cells (n = 4) (Fig. 3B). Similar suppressive effects were also obtained in cortical neurons (n = 8). Thus, irrespective of neuronal cell type, the endogenous peptide blocked both the T- and L-type Ca^{2+} channels. The acid-hydrolyzed form of the peptide produced no measurable effects in neuroblastoma cells. However, in cortical neurons, rapid addition of acid-hydrolyzed material activated a transient Cl^- current lasting about 1 to 2 s. It is possible that this effect occurs secondary to the presence of constituent amino acids (for example, glutamate and glycine) in the hydrolyzed material. Consistent with this idea, 100 μM glycine activated a Cl^- current quite similar to that induced by the hydrolyzed material.

Our data thus show that the endogenous peptide modulates the Ca^{2+} channels of neuronal and cardiac cells in a different manner. Such specificity suggests subtle differences in the regulatory or selectivity sites of Ca^{2+} channels in heart and brain tissues. Although the effect of the peptide on cardiac myocytes and neurons is somewhat similar to the effect of catecholamines in these tissues [that is, enhancement of I_{Ca} in heart (*1*–

3) and its block in dorsal root ganglion neurons (6, 7)], the inability to suppress the enhancing effect of the peptide in cardiac cells dialyzed with GDP-β-S or in the presence of α_1- or β-blockers suggests that its effect is not mediated through a G protein–dependent pathway. We do not yet know whether a similar molecular mechanism mediates both the agonistic and antagonistic effects of the peptide on cardiac and neuronal Ca^{2+} channels. Our results, nevertheless, suggest the existence of endogenous peptide in the brain that may serve as a physiological regulator of Ca^{2+} channels by a novel mechanism.

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Specific Block of Calcium Channel Expression by a Fragment of Dihydropyridine Receptor cDNA

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Although the structure of rabbit skeletal muscle dihydropyridine (DHP) receptor, deduced from cDNA sequence, indicates that this protein is the channel-forming subunit of voltage-dependent calcium channel (VDCC), no functional proof for this prediction has been presented. Two DNA oligonucleotides complementary to DHP-receptor RNA sequences coding for putative membrane-spanning regions of the DHP receptor specifically suppress the expression of the DHP-sensitive VDCC from rabbit and rat heart in *Xenopus* oocytes. However, these oligonucleotides do not suppress the expression of the DHP-insensitive VDCC and of voltage-dependent sodium and potassium channels. Thus, the gene for DHP receptor of rabbit skeletal muscle is closely related, or identical to, a gene expressed in heart that encodes a component of the DHP-sensitive VDCC. The DHP-sensitive and DHP-insensitive VDCCs are distinct molecular entities.

SEVERAL TYPES OF VDCCs EXIST; THE most thoroughly studied channel is sensitive to DHPs (L-type) (1). The DHP receptor (DHPR) of skeletal muscle is one of the four or five subunits that presumably constitute the channel (2). Skeletal muscle DHPR may be the voltage sensor of excitation-contraction coupling (3), or be associated with functional VDCCs (4), or may serve both functions (5). The primary structure of the rabbit skeletal muscle DHPR, deduced from cDNA that encodes this protein, is as expected for an ion channel (5). However, the functional expression of the cDNA clones has not yet been demonstrated. To examine whether DHPR is indeed an important component of the channel, we used hybrid arrest of mRNA expression in *Xenopus* oocytes by complementary oligonucleotides (6). With skeletal muscle RNA, we could not achieve reproducible expression of the DHP-sensitive VDCC in the oocytes (7); therefore, we used heart RNA for this purpose.

Frogs were maintained and dissected and the follicles removed from the oocytes, as described (8, 9). RNA was isolated from hearts of 7-day-old rats or 10-day-old rabbits by a LiCl-urea method (10). Total RNA was used in this study; it induced Ca^{2+} channel currents similar to those induced by polyadenylated RNA (9). Injected oocytes were incubated for 3 to 5 days at 22°C in sterile ND96 solution (9) (96 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 5 m*M* Hepes-NaOH; *p*H, 7.5) supplemented with antibiotics and sodium pyruvate before the recording of the currents. Currents through VDCCs were recorded with the two-electrode voltage-clamp technique (9, 11) in a solution containing 40 m*M* Ba^{2+}, 2 m*M* K^+, 50 m*M* Na^+, and 5 m*M* Hepes-NaOH (*p*H, 7.5), the anion being either acetate or methanesulfonate.

Rat and rabbit heart RNA cause the appearance of two distinct VDCC currents in *Xenopus* oocytes (9) (Fig. 1): a transient current (I_{tr}) and a "slow" current (L-type; I_{sl}). I_{sl} was inhibited by DHP blockers (9) and potentiated by the DHP agonist BAY K 8644 (12) (Fig. 1C). I_{tr} was DHP-insensitive and inactivated at more negative voltages than I_{sl} (9) (Fig. 1B). After a complete block of Ba^{2+} currents by 0.5 m*M* Cd^{2+} or by the oligonucleotide treatment, depolarizations to potentials beyond −20 mV evoked only a slow, noninactivating outward current (I_{out}), which rarely exceeded 5 nA at 0 mV at the end of the depolarizing pulse.

The currents were quantified as shown in Fig. 1. I_{sl} was slightly underestimated because of its mild inactivation and the development of I_{out}. Presence or absence of I_{sl} was verified by testing the effect of BAY K 8644 on the currents (Table 1). The amplitude of I_{tr} was estimated by two independent procedures. The leak-subtraction procedure (Fig. 1A) overestimates I_{tr} because the total current at the end of the depolarizing pulse contains an outward current component (I_{out}). The "I_{tr}-inactivation" procedure (Fig. 1B) offsets the contribution of the leak current, I_{sl} and I_{out}, but I_{tr} is underestimated by 25 to 30% because its inactivation is incomplete at −20 mV (9).

Although the transient, BAY K 8644–insensitive Ba^{2+} current in native (not injected with RNA) oocytes (9, 10, 13) (Fig. 1C, part a) resembles the I_{tr} in heart RNA–injected oocytes, I_{tr} is always at least two (usually three to five) times larger than the native current (Table 1) (7). The possibility that the native VDCC is overexpressed in heart RNA–injected cells is unlikely for several reasons (14). The primary reason is that the expression of endogenous messages is reduced by exogenous RNA (15). Thus, we postulate that a major part of I_{tr} is directed by heart RNA.

The method for selective inhibition of RNA expression in *Xenopus* oocytes is based on work (16) that demonstrates that DNA

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