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Increase in T-type calcium current in atrial myocytes from adult rats with growth hormone-secreting tumors

(GH_3 cells/insulin-like growth factor I/cardiac muscle)

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ABSTRACT **Growth hormone (GH) has pronounced effects on protein synthesis and cell growth in cardiac muscle from adult animals, although the mechanism of its action is not understood. Because Ca^{2+} has been implicated as a regulator of mitogenic processes in a number of tissues, we investigated whether GH affects the transmembrane movement of Ca^{2+} through voltage-activated channels of cardiac myocytes. Atrial and ventricular myocytes were isolated from adult rats with GH-secreting tumors and studied electrophysiologically by using patch-clamp techniques. Tumor-bearing rats re-enter an active growth phase and double their body weight over age-matched controls 8 weeks after introduction of the tumor. Atrial myocytes from tumor-bearing animals showed a 3-fold increase in the density of T-type Ca^{2+} current compared with cells from control animals, although the voltage dependency of activation and inactivation of T-type current was not altered. The increase in T-current density of atrial myocytes preceded by at least a week any measurable change in heart weight, body weight, or myocyte size. L-type Ca^{2+} currents in atrial and ventricular cells were not affected. The results suggest that a tumor-derived growth factor, most likely GH, can cause a specific enhancement of T-type Ca^{2+} current in atrial myocytes.**

Growth hormone (GH) has pronounced effects on somatic and visceral tissues in postnatal animals (1, 2). Skeletal and cardiac muscle are particularly sensitive to the growth-promoting actions of this hormone and respond to elevated serum GH levels with increased synthesis of muscle-specific proteins and increases in muscle mass even in adult animals (2–4). Because Ca has been implicated in the control of cell growth in a number of tissues (5) and given the general importance of Ca^{2+} in muscle function, we wondered whether GH would affect transmembrane Ca^{2+} movements in differentiated muscle cells. An influx of extracellular Ca^{2+} through voltage-sensitive ion channels is particularly important to the functional response of cardiac myocytes. Mammalian cardiac myocytes display two types of voltage-activated Ca^{2+} currents, referred to as T- and L-type current, which are distinguishable by the voltage range of their activation, the extent of their inactivation by depolarizing holding potentials, and their pharmacology (6, 7). In this study, we investigated whether Ca^{2+} currents are altered in mature cardiac myocytes once the cells reenter an active growth phase in rats with a GH-secreting tumor. We found a specific enhancement of T-type Ca^{2+} current in atrial myocytes that precedes any change in heart weight or myocyte size.

MATERIALS AND METHODS

Induction of GH-Secreting Tumors. GH_3 cells, a cell line originally isolated from a rat pituitary tumor, form a discrete, well vascularized tumor when implanted s.c. in female Wistar–Furth rats (3, 4, 8, 9). Tumors derived from GH_3 cells secrete GH indistinguishable from native rat GH, as determined by its biological and immunological activity (9, 10). Tumor-bearing rats undergo pronounced acromegaly with the largest percentage increase in muscle tissue occurring in the heart (3). GH_3 cells (CCL 82.1, American Type Culture Collection) were cultured in Ham's F-10 medium supplemented with 2.5% fetal bovine serum, 15% horse serum, 0.5% glutamine-pen/strep (Irvine Scientific) and Fungizone (0.75 μg/ml) at 37°C under 5% CO_2. About 10^6 cells were injected s.c. into the right flank of 3-mo-old female Wistar–Furth rats. Myocytes from treated rats and age-matched controls were studied 2, 3.5, 5.5, and 8 weeks after GH_3 cell inoculation.

Preparation of Myocytes. Hearts from rats were mounted in a Langendorf apparatus and retrogradely perfused with oxygenated and warmed solutions (35°C). They were initially perfused with a Ca^{2+}-free Tyrode's solution containing 135 mM NaCl, 5.4 mM KCl, 5 mM $MgCl_2$, 0.33 mM NaH_2PO_4, and 10 mM Hepes at pH 7.3 for 8, 10, and 12 min for rats weighing 200, 300, and 400 g, respectively. Enzymatic isolation of myocytes was achieved by a 15-min perfusion of an enzyme solution made by adding 30 μM Ca^{2+}, collagenase (type B, lot 119805) at 0.5 mg/ml and neutral protease at 0.03 mg/ml (both obtained from Boehringer Mannheim) to Ca^{2+}-free Tyrode's solution. Both atria and the free wall of right ventricle were separately minced in a recovery medium (11) containing 85 mM KCl, 30 mM K_2HPO_4, 5 mM $MgSO_4$, 1 mM K_2EGTA, 2 mM Na_2ATP, 5 mM pyruvic acid, 5 mM creatine, 20 mM taurine, and 20 mM glucose at pH 7.2. Dispersed cells were kept in this solution for 1 hr at room temperature and than transferred to recording solutions. Cells were randomly selected except that very small and very large myocytes were excluded to avoid problems associated with small currents or large capacitative transients, respectively.

Recording Solutions. The composition of the pipette and bath solutions was chosen to allow isolation of ion flow through Ca^{2+} channels by blocking other ionic currents. The bath solution contained 140 mM choline chloride, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, 10 mM Hepes, and 10–30 μM tetrodotoxin at pH 7.4 (titrated with CsOH). The pipette solution contained 140 mM CsCl, 10 mM Cs_2EGTA, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 3 mM Na_2ATP, 0.2 mM GTP, and 10 mM Hepes at pH 7.4 (titrated with CsOH). A Ca^{2+} channel antagonist, dihydropyridine (−)202–791, was supplied by A. Lindenmann and H. Stahelin of Sandoz, Basel.

Data Acquisition and Analysis. Whole-cell Ca^{2+} currents were recorded using patch-clamp techniques. Pipettes (0.5–1 MΩ) were fabricated from borosilicate glass capillaries and

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Abbreviations: GH, growth hormone; $I_{Ca,T}$, low-threshold T-type Ca^{2+} current; $I_{Ca,L}$, high-threshold L-type Ca^{2+} current.

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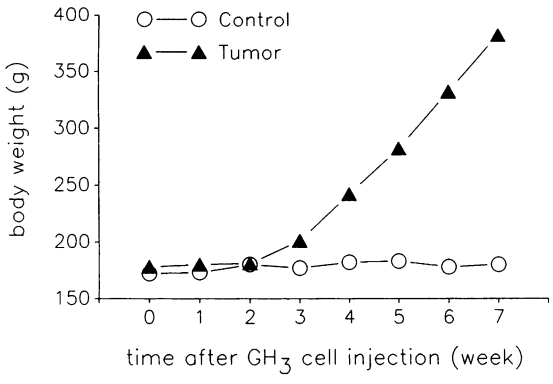

FIG. 1. Effect of GH-secreting tumors on body weight. ▲, GH_3 cell-injected rats; ○, untreated, age-matched control animals. For both groups $n = 4$, and SD is smaller than symbol size.

coated with Sylgard. Cell capacitance and series resistance were calculated from the capacitance transient produced by a pulse from −80 to −90 mV. Series resistance was usually within 1–5 MΩ and was compensated by >80%. Currents were elicited every 3.5–5 s, filtered at 1 KHz, and digitalized at 10 KHz. Current traces shown in *Results* are corrected for capacity and leakage currents.

Steady-state inactivation of Ca^{2+} current was measured as I/I_{max}, where I is the peak current elicited by a test pulse after a 1-s-long conditioning pulse to various voltages, and I_{max} is the maximal peak current in the absence of the conditioning pulse. The voltage dependency of activation was determined by measuring the peak conductance at various test potentials. Peak conductance was calculated from the peak current according to $g(V, \text{peak}) = I(V, \text{peak})/(V\text{-}E_{rev})$; E_{rev} is the apparent reversal potential obtained by extrapolating the linear part of the I/V curve to its intersection with the voltage axis (12). Data describing the steady-state inactivation (I/I_{max}) and activation (g/g_{max}) of Ca^{2+} current were fit by Boltzmann equation.

All experiments were performed at 20–22°C. No differences were found in the Ca^{2+} currents of the myocytes isolated from control rats aged 3–5 mo, and they were treated as a single group. Average values are expressed as means ± SD (n). Student's t test was used to determine statistical significance.

RESULTS

Growth in Rats Bearing GH-Secreting Tumors. The effect of GH-secreting tumors on the growth of rats is illustrated in Fig. 1 and Table 2. The body weight of rats injected with GH_3 cells begins to increase ≈3 weeks after inoculation and increases steadily thereafter. The body weight of controls changes little over the same period and averages 176 g. The onset of growth corresponds roughly to the time when a tumor can be first palpated at the inoculation site. After 8 weeks, tumor-bearing rats weigh 415 g, on average, or more

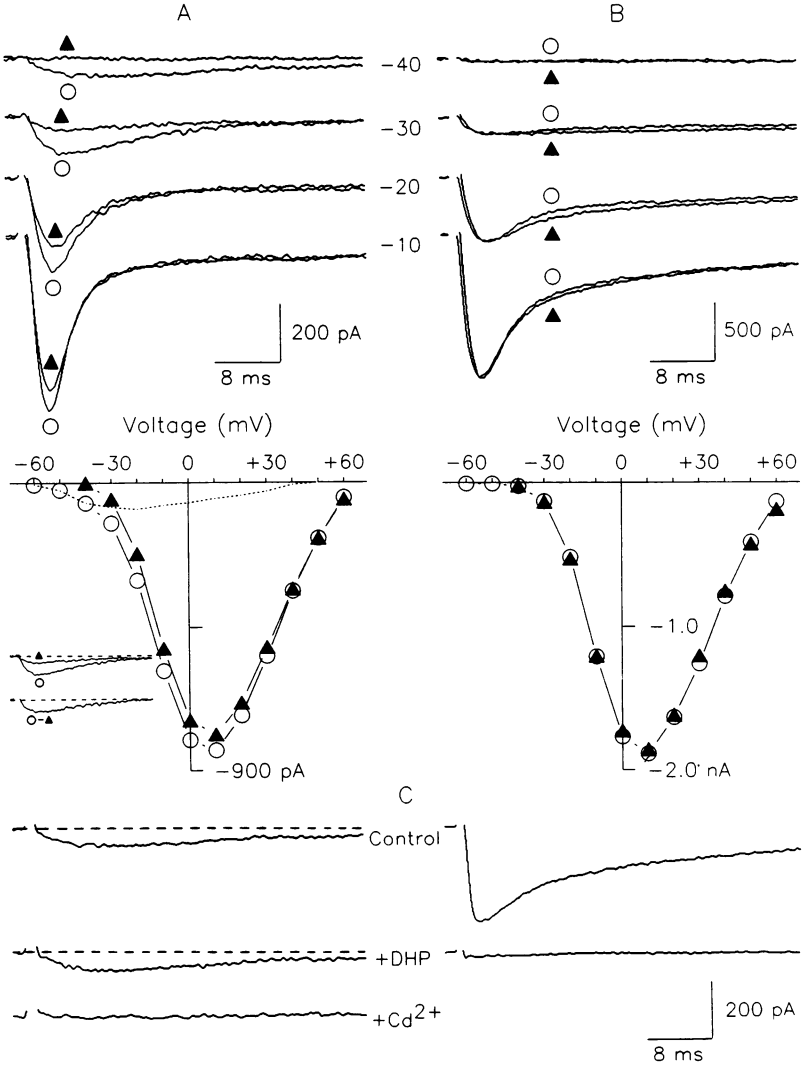

FIG. 2. Ca^{2+} currents recorded from atrial and ventricular myocytes isolated from control rats. (*A*) Atrial cell representative current records elicited at test potentials of −40 to −10 mV from holding potentials of either −50 mV (▲) or −90 mV (○). Peak I/V relationship is shown below the traces. For test potentials below +40 mV, peak current is larger for holding potentials of −90 mV compared with holding potentials of −50 mV, indicating the existence of both $I_{Ca,T}$ and $I_{Ca,L}$ (see text). (*Insert* by I/V curve) Example of subtraction technique used to isolate $I_{Ca,T}$ (○—▲) by using different holding potentials (test potential of −30 mV). Dotted line in I/V curve illustrates voltage dependency of $I_{Ca,T}$. Cell capacitance was 72 pF. (*B*) Ventricular cell representative traces from cell held at either −90 mV (○) or −50 mV (▲). Peak I/V relationship is shown below records. Holding potential does not affect peak current, indicating that only $I_{Ca,L}$ is present in ventricular myocytes. Cell capacitance was 138 pF. (*C*) Pharmacological separation of Ca^{2+} current types for the same atrial cell in *A*. Traces on left show $I_{Ca,T}$ (test pulse step from −90 to −40 mV). $I_{Ca,T}$ is unaffected by 1 μM (−)202–791 but is blocked by additional 0.5 mM Cd^{2+}. Traces on right show $I_{Ca,L}$ (test pulse step from −50 to 40 mV) being completely blocked by 1 μM (−)202–791.

than twice as much as age-matched controls. Previous studies have described similar effects of GH-secreting tumors on the body weight of Wistar–Furth rats (3, 4).

Ca^{2+} Currents in Atrial and Ventricular Myocytes of Control Rats. Ca^{2+} currents were elicited in atrial and ventricular myocytes isolated from control rats by depolarizing the membrane to various test potentials from holding potentials of either −90 or −50 mV.

In atrial cells (Fig. 2*A*) two distinct components of Ca^{2+} current are seen in the data traces. A low-threshold, T-type Ca^{2+} current ($I_{Ca,T}$) is activated by small test pulses; it is clearly seen in the records at a test pulse to −40 mV in cells held at −90 mV. T-type current is inactivated by depolarizing holding potentials so that no $I_{Ca,T}$ is recorded from cells held at −50 mV and depolarized to −40 mV. A high-threshold L-type Ca^{2+} current ($I_{Ca,L}$) is activated by larger test potentials (to −30 mV or above) and is not inactivated when the holding potential is increased to −50 mV. Because $I_{Ca,T}$ is inactivated at depolarizing holding potentials, currents recorded from cells held at −50 mV describe the behavior of $I_{Ca,L}$ uncontaminated by $I_{Ca,T}$. Currents recorded from cells held at −90 mV show the combined behavior of both $I_{Ca,T}$ and $I_{Ca,L}$. The peak current–voltage (I/V) relationship for Ca^{2+} currents from the same cell held at either −50 mV or −90 mV is shown at bottom of *A*. Subtracting the currents obtained from the same test potential but different holding potentials allows isolation of $I_{Ca,T}$, plotted on the I/V curve. In general, the amplitude of $I_{Ca,L}$ was much larger than that of $I_{Ca,T}$. The average peak $I_{Ca,T}$ density in control atrial cells was 0.41 ± 0.22 pA/pF (n = 29), and that for $I_{Ca,L}$ was 9.7 ± 3.1 pA/pF (n = 19).

Only $I_{Ca,L}$ was recorded from ventricular cells (Fig. 2*B*). Ca^{2+} current was activated only after large test pulses (to −30 mV or above). Depolarizing holding potentials did not affect magnitude of the Ca^{2+} current in ventricular cells, as seen by the identical peak I/V curves recorded at −50 mV and −90 mV. Small differences were seen in the kinetics of $I_{Ca,L}$ inactivation in ventricular cells at different holding potentials, as has been reported (13). Average, peak $I_{Ca,L}$ density in ventricular cells was 12.9 ± 4.1 pA/pF (n = 32).

$I_{Ca,L}$ and $I_{Ca,T}$ can also be distinguished pharmacologically (Fig. 2*C*). $I_{Ca,L}$ of both atrial and ventricular cells was sensitive to block by dihydropyridines, whereas atrial $I_{Ca,T}$ was insensitive to this class of Ca^{2+} channel antagonists. Both $I_{Ca,L}$ and $I_{Ca,T}$ were blocked by 0.5 mM Cd^{2+}, a nonspecific blocker of Ca^{2+} channels.

These results are consistent with numerous studies of Ca^{2+} currents in cardiac myocytes (14–16).

Selective Increase in the Density of T-Type Calcium Current in Atrial Cells from Rats Bearing GH-Secreting Tumors. Ca^{2+} currents were recorded in myocytes isolated from rats 8 weeks after GH_3 cell inoculation (Fig. 3). $I_{Ca,T}$ and $I_{Ca,L}$ were identified based on their voltage dependency, inactivation, and pharmacology, as described above for the cells from control animals.

Atrial cells from tumor-bearing rats were remarkable because of their unusually large $I_{Ca,T}$ (Fig. 3*A*). The large $I_{Ca,T}$ is readily seen in the peak I/V relationship for atrial cells as

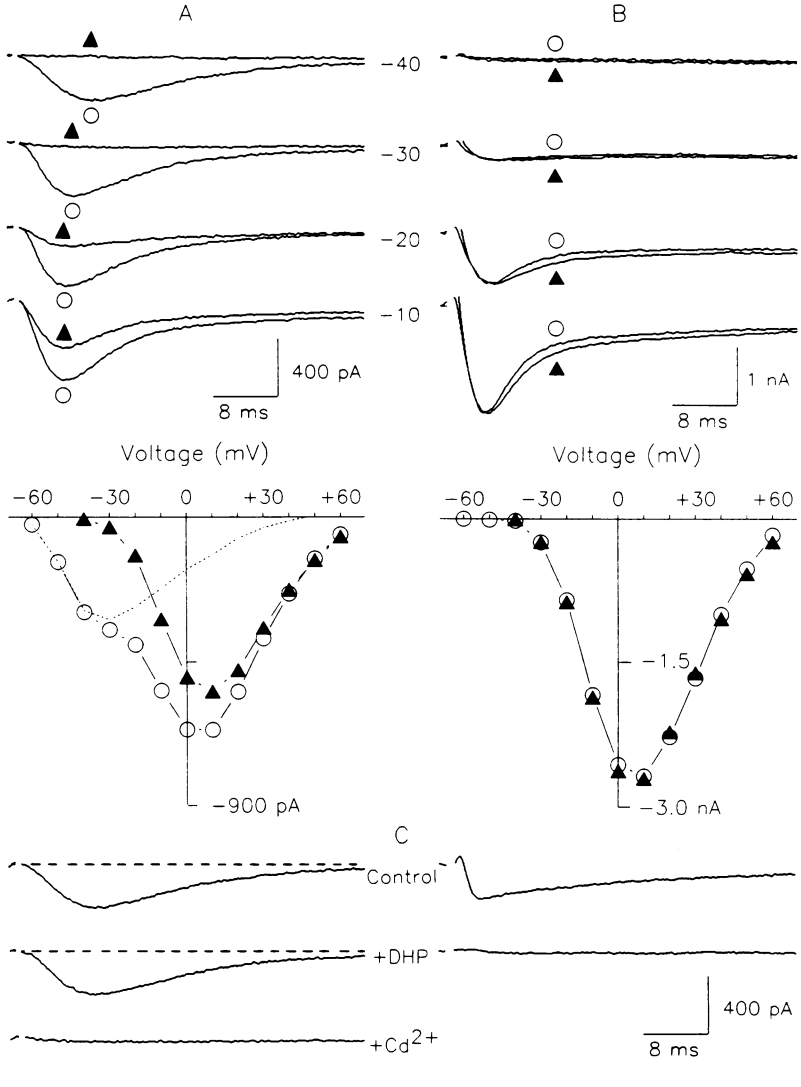

FIG. 3. Ca^{2+} currents from atrial and ventricular myocytes isolated from rats with GH-secreting tumors. (*A*) Atrial cell current traces recorded after changes of membrane potential, −40 mV to −10 mV from holding potentials of either −50(▲) or −90 mV (○). Peak I/V relationship is shown below data traces. Both $I_{Ca,T}$ and $I_{Ca,L}$ are present as seen by the difference in Ca^{2+} current amplitude at holding potentials of −50 and −90 mV. Note large $I_{Ca,T}$ elicited at low test potentials in this cell compared with controls (Fig. 2*A*). Dotted line on I/V curve represents peak I/V relationship for $I_{Ca,T}$. Cell capacitance was 105 pF. (*B*) Ca^{2+} currents from ventricular cells. Holding potentials were −50 (▲) and −90 mV (○). Only $I_{Ca,L}$ is present. Cell capacitance was 190 pF. (*C*) Pharmacological separation of Ca^{2+} current types for the same atrial cell in *A*. Data traces at left show $I_{Ca,T}$ (depolarizing from −90 to −40 mV) being unaffected by 1 μM (−)202–791 but blocked by 0.5 mM Cd^{2+}. $I_{Ca,L}$ at right (depolarizing from −50 to 40 mV) is blocked by 1 μM (−)202–791.

a large increase in the current at small test potentials for cells held at −90 mV (compare to Fig. 2A). The average, peak $I_{Ca,T}$ density in atrial cells from tumor-bearing rats was 1.24 ± 0.51 pA/pF (n = 23) or three times the average recorded from control cells. This difference is highly significant ($P < 0.001$).

To determine whether changes in the voltage dependency of the inactivation and activation of atrial $I_{Ca,T}$ accompanied the increase in current density, we compared the steady-state inactivation and activation of $I_{Ca,T}$ in control and tumor-bearing rats at various test potentials (Fig. 4A). The voltage dependency of the steady-state inactivation and activation of $I_{Ca,T}$ is unchanged in tumor-bearing rats (Table 1).

$I_{Ca,L}$ was unchanged in both ventricular and atrial myocytes from tumor-bearing rats (compare Figs. 2B and 3B; Fig. 4B). Average, peak $I_{Ca,L}$ density was 12.9 ± 4.1 (n = 32) and 12.2 ± 3.1 pA/pF (n = 28) in ventricular cells from control and tumor-bearing rats, respectively, and 9.7 ± 3.1 (n = 19) and 9.8 ± 2.8 pA/pF (n = 19) in atrial cells from control and tumor-bearing rats, respectively. We found no difference in the voltage dependency of steady-state inactivation or activation of $I_{Ca,L}$ from either cell type. Data are shown only for atrial cells (Fig. 4B and Table 1).

The Increase in Atrial T-Type Current Density Precedes the Increase in Heart Weight in the Tumor-Bearing Rats. To establish the temporal correlation between the increase in $I_{Ca,T}$ density in atrial cells and growth of the tumor-bearing rats, we measured body weight, cell capacitance, heart weight, and Ca^{2+} currents in control rats and in rats 2, 3.5, 5.5, and 8 weeks after GH_3 cell inoculation. Cell capacitance is an electronic measure of surface-membrane area and, thus, a sensitive, albeit indirect, measure of cell size. Significant changes, amounting to a 2-fold increase, in peak $I_{Ca,T}$ density are seen in atrial cells isolated from rats 2 weeks after

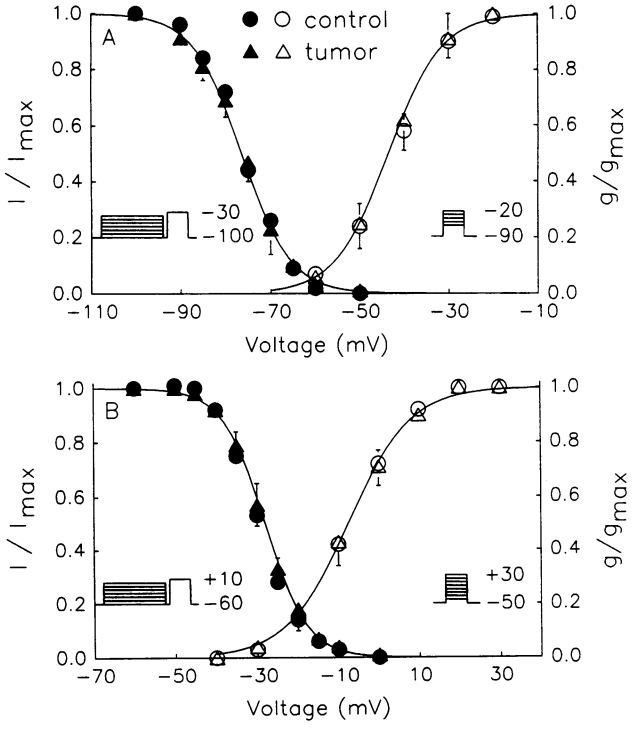

FIG. 4. Comparison of the voltage dependency of steady-state inactivation (●, ▲) and activation (○, △) of atrial $I_{Ca,T}$ and $I_{Ca,L}$ from control rats (○, ●, SD shown upward) and rats bearing GH-secreting tumors (△, ▲, SD shown downward). (A) $I_{Ca,T}$ (●, n = 1; ○, ▲, n = 3; △, n = 4). (B) $I_{Ca,L}$ (●, n = 6; ○, n = 4; ▲, △, n = 5). Data fit was by Boltzmann function; values for $V_{0.5}$ and k are shown in Table 1. Smooth curves are the fits for the tumor-bearing rats. (*Inserts*) Pulse protocols used to generate data.

Table 1. Parameters, $V_{0.5}$ and k, used to fit Boltzmann equation

	T current, mV				L current, mV			
	Inactivation		Activation		Inactivation		Activation	
	$V_{0.5}$	k	$V_{0.5}$	k	$V_{0.5}$	k	$V_{0.5}$	k
Control	−76.0	4.9	−42.5	−6.3	−29.0	4.8	−7.5	−7.2
Tumor	−76.4	5.4	−43.5	−6.0	−28.0	5.1	−7.5	−7.7

inoculation (Table 2). However, body weight, heart weight, and cell capacitance remain unchanged at this time. Body and heart weight increase above control values at 3.5 weeks, whereas average cell capacitance does not increase significantly until 5.5 weeks. The percentage increases in heart weight, cell capacitance, and peak $I_{Ca,T}$ density over control values are compared directly in Fig. 5. The earlier response of $I_{Ca,T}$ in tumor-bearing animals relative to changes in heart weight or cell capacitance is evident.

DISCUSSION

Growth in Tumor-Bearing Rats. Growth in rats with GH-secreting tumors can be best described as a generalized acromegaly in which somatic and visceral enlargement occur in proportion to tumor mass (3, 4, 8, 17). Animals with large tumors (7 weeks or so after inoculation) are reported to have significantly altered cardiovascular function including decreases in peripheral resistance and heart rate accompanied by increases in stroke volume and cardiac output (17). Such changes are presumably a response to the volume overload that accompanies the increase in somatic, visceral, and tumor mass. No pathological changes are present in ventricles from tumor-bearing rats (8, 18).

Is the Increase in T-Type Current Density Induced by GH? Because a significant increase in $I_{Ca,T}$ density in atrial myocytes occurs well before any change in body or heart weight (Fig. 5), it appears likely that the increase results from the influence of a circulating growth factor or factors released by the tumor rather than from the hemodynamic effects that accompany significant changes in body size. We feel the most logical candidate for this circulating agent is GH, which is secreted by GH_3 tumors in large amounts. Although the tumors also secrete prolactin (19), the induced cardiomegaly has been linked specifically to the increase in serum GH levels in tumor-bearing animals (3). The data of Yamashita *et al.* (20) show a doubling of GH levels as early as 3 weeks after inoculation with GH_3 cells, and increases are detectable at the second week. Ultimately, GH levels can increase >50-fold in tumor-bearing rats (4, 8, 17, 20). Many of the somatotropic effects of GH are mediated by insulin-like growth factor I (IGF-I), which was initially thought to be of hepatic origin (2). Although serum levels of IGF-I are known to increase 2- to 4-fold in rats 8 or 9 weeks after inoculation with GH_3 cells (4, 20), a study of the time course of the increase in serum concentration is not available. IGF-I may also function by means of a paracrine or autocrine mechanism. The concentration of IGF-I in rat heart is transiently increased 2–12 hr after a single injection of GH (21). Increased levels of IGF-I mRNA are found in the hearts of tumor-bearing rats (22). Thus the increase in $I_{Ca,T}$ density reported here may result from a direct effect of GH on atrial cells or a secondary effect of increased levels of IGF-I.

Is T-Type Current Linked to Growth in Atrial Cells? The physiological role of the $I_{Ca,T}$ in cardiac myocytes is not completely understood. Although found in many atrial myocytes (7, 14, 16), $I_{Ca,T}$ usually contributes only a small fraction to the total Ca^{2+} current in atrial cells. In ventricular myocytes, $I_{Ca,T}$ seems rare and has been reported most frequently in cells from guinea pig (15, 23). Interestingly, $I_{Ca,T}$ is prominent in embryonic cardiac tissue. Embryonic ven-

Table 2. Changes in body weight, heart weight, cell capacitance, and peak T-type current density after GH_3 cell inoculation

	Control	Time			
		2 wk	3.5 wk	5.5 wk	8 wk
BW, g	176 ± 7 (9)	177 ± 8 (5)	224 ± 4 (5)*	304 ± 12 (5)*	415 ± 17 (9)*
HW, mg	715 ± 45	725 ± 42	927 ± 28*	1256 ± 70*	1745 ± 105*
C_{cell}, pF	57 ± 15 (29)	57 ± 16 (34)	63 ± 14 (33)	87 ± 23 (30)*	94 ± 28 (23)*
I_T, pA/pF	0.41 ± 0.22	0.83 ± 0.19*	1.01 ± 0.39*	1.10 ± 0.35*	1.24 ± 0.51*

BW, body weight; HW, heart weight; C_{cell}, cell capacitance; I_T, peak T-type current density.
*Value significantly different from control values with $P < 0.001$.

tricular cells display robust T-type currents that are apparently lost as the tissue matures (24, 25). The transient expression of $I_{Ca,T}$ in developing cells is not unique to cardiac myocytes. Similar changes have been reported for other tissues including skeletal muscles (26, 27), smooth muscle (28), sensory neurons (25), and motoneurons (29), suggesting a possible association of $I_{Ca,T}$ with growth and development in some tissues (7, 25). Our finding that atrial cells in adult rats stimulated to reenter an active growth phase have increased $I_{Ca,T}$ density is consistent with this notion.

The GH-dependent effect we have described is specific for $I_{Ca,T}$. $I_{Ca,L}$ density in both atrial and ventricular myocytes was unaffected. Particularly striking was the fact that large changes in $I_{Ca,T}$ density occur well before a measurable change in heart weight or myocyte size. Although this observation is consistent with the idea that $I_{Ca,T}$ augmentation is correlated with events leading up to cell growth, we saw no evidence of induction of $I_{Ca,T}$ in ventricular cells, even though these cells also increase in size. The difference in response of atrial and ventricular cells to GH-secreting tumors may involve a fundamental difference in the capacity of the tissues to respond to growth factors. Cardiac myocytes are generally believed to have permanently lost their ability to proliferate in adult animals (30). However, Rumyantsev (31) has suggested that mammalian atrial cells have a much greater ability for DNA synthesis and nuclear proliferation than ventricular cells. Perhaps the presence of residual $I_{Ca,T}$ in atrial cells and its induction by GH-secreting tumors is a reflection of this difference. If so, it raises the possibility that $I_{Ca,T}$ may play a role in the regulation of growth processes in atrial myocytes.

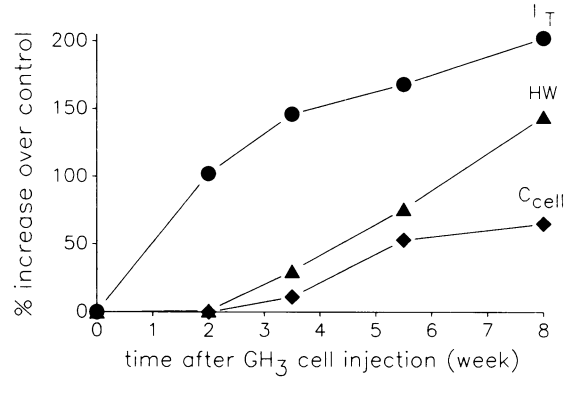

FIG. 5. Effect of GH-secreting tumors on the growth of heart (HW, ▲), increase of cell capacitance (C_{cell}, ◆), and peak $I_{Ca,T}$ density (I_T, ●) in atrial cells. Significant increases in $I_{Ca,T}$ density occur well before any measurable change in heart weight and cell capacitance.

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