Mevinolin, an inhibitor of cholesterol biosynthesis, drastically depresses Ca^{2+} channel activity and uncouples excitation from contraction in cardiac cells in culture

(cardiac cells/cholesterol/mevinolin/ Ca^{2+} channel)

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ABSTRACT Mevinolin (MK803), a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (K_i , 30×10^{-9} M), depressed *de novo* synthesis of cholesterol in 11-day chicken embryonic cardiac cells cultured in lipoprotein-deficient serum (LPDS). Cardiac cells exposed to different concentrations of mevinolin for 1–3 days presented different electrophysiological and mechanical properties: (i) The resting membrane potential, the rate of increase, and the shape of the action potential and contractile properties were changed at concentrations as low as $0.1 \mu\text{M}$ mevinolin. (ii) At a concentration of $1 \mu\text{M}$ mevinolin, the cardiac cells became quiescent and electrical stimulation induced action potentials of short duration without contraction. Isoproterenol and Bay K8644 were unable to restore excitability and contraction. Although the number of receptors for the tritiated Ca^{2+} channel blocker nitrendipine was the same in control and in mevinolin-treated cells, voltage-clamp data on isolated cardiac cells and $^{45}\text{Ca}^{2+}$ flux experiments on monolayers showed that most of the slow Ca^{2+} channel activity was lost in mevinolin-treated cells. These results suggest that the disappearance of Ca^{2+} channel activity is most probably at the origin of the loss of cardiac contractility.

All mammalian cells require free cholesterol both for biogenesis and for the function of their plasma membrane. This cholesterol is acquired from the exogenous source constituted by the low density lipoprotein fraction of lipoproteins (1, 2). It also comes from *de novo* synthesis within the cell (3). Previous work with chicken cardiac cells in culture has shown that cells cultivated in low density lipoprotein-free medium had a changed level of cholesterol in their plasma membrane, which was accompanied by changes in electrical properties (4).

This work analyzes the effect of mevinolin on excitability and contraction of cardiac cells in culture. Mevinolin (MK803) is a natural product of fungal origin that acts as an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (5–7). This enzyme catalyzes the conversion of hydroxymethylglutamate to mevalonate, which is the first and rate-limiting step in the cholesterol biosynthesis pathway (8). Mevinolin lowers serum cholesterol in dog (5, 9), in the normal human (10), and in hypercholesterolemic patients (11, 12). This molecule has been suggested as being a potentially useful drug in the treatment of hypercholesterolemia (5).

MATERIALS AND METHODS

Heart Cell Cultures. Ventricular cells from 11-day-old chicken embryos were dissociated by trypsinization as described (13). Cells were either cultured in the form of monolayers or as isolated cells. The standard medium used was supplemented with 5% lipoprotein-deficient serum (LPDS). Mevinolin was added every 12 hr for 1–3 days at concentrations ranging between 10 nM and $10 \mu\text{M}$, 2 days after plating.

Concentrations of mevinolin between 10 nM and $0.1 \mu\text{M}$ produced no apparent change in the cell morphology even after 3 days of treatment. For higher concentrations, between $1 \mu\text{M}$ and $10 \mu\text{M}$, mevinolin caused morphological changes of cardiac cells. Cells had a tendency to retract from neighboring and to round up. Cell death often occurred after 4 days when treated with $10 \mu\text{M}$ mevinolin.

Preparation of LPDS. LPDS was prepared as described (4) using fetal calf serum (Seromed, Munich, F.R.G.), adjusted to a density of 1.21 by addition of solid KBr, and centrifuged twice to remove all lipoproteins.

Cholesterol Determination. The amount of cholesterol in cultured embryonic cardiac cells was measured after extraction of lipids in chloroform/methanol (2:1, vol/vol), according to Bligh and Dyer (14). Total cholesterol was determined according to Ott *et al.* (15).

Incorporation of [^{14}C]Acetate into Lipids. Cardiac cells grown in the presence of different concentrations of mevinolin (2 days) were pulse-labeled overnight with [^{14}C]acetate (2 μCi per 5 ml of medium; 1 Ci = 37 GBq). Cardiac cells were digested in 0.8 ml of 0.1 M NaOH and 1 ml of water. Protein determination was done by the method of Hartree (16). Lipids were extracted and separated by thin-layer chromatography on Kiesel gel 60 plates (Merck) using either a mixture of benzene/ethylacetate/formic acid (80:20:1) or hexane/ether/formic acid (80:20:1) as developing solvents. After revelation in iodine vapor, areas corresponding to major lipid classes were scraped from the plate and radioactivity incorporated was determined by using a Packard model B2450.

Measurement of HMG-CoA Reductase Activity. Measurements of HMG-CoA reductase activity were carried out as described (17). Cardiac cells were exposed to mevinolin for 2 days before measurement.

ATP Determination. ATP content in cardiac cells cultured either in the presence or in the absence of mevinolin was determined with a commercial enzymatic assay (Sigma).

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Abbreviations: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LPDS, lipoprotein-deficient serum; TTX, tetrodotoxin; [^3H]en-TTX, [^3H]ethylenediamine TTX; V_{max} , maximum upstroke velocity; AP, action potential.

Electrophysiological and Contraction Measurements. Electrical recordings from 11-day chicken embryonic cardiac cells cultured in the form of monolayers were carried out as described (4). Glass microelectrodes had a resistance between 20 and 40 M Ω . Contraction was recorded simultaneously with the membrane voltage recording and analyzed as described (18).

Isolated chicken embryonic cardiac cells were voltage-clamped by the whole-cell variant of the patch-clamp technique (19). The external solution contained 140 mM tetraethylammonium chloride, 2.5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, 10 mM Hepes titrated to pH 7.4 with Tris. The pipette solution contained 140 mM CsCl, 5 mM EGTA, 10 mM Hepes titrated to pH 7.2 with CsOH. Patch pipettes (2–6 M Ω) were connected to the head stage (10 G Ω input resistance) of the recording apparatus (RK 300, Bio-Logic, Grenoble, France). The signal was filtered (2 kHz) using a Krohn-Hite filter (type 3340), stored and analyzed with a minicomputer system (model 6220; Plessey Systems, Irvine, CA). Linear leak currents were subtracted by linear extrapolation from leak currents obtained for small hyperpolarizing pulses. All experiments were performed at 20°C $\pm$ 2°C and with a holding potential V_H = –65 mV.

Binding Assays. Biochemical titrations of the Na⁺ channel were carried out as described (4) using homogenates prepared from chicken heart cells in culture and tritiated ethylenediamine tetrodotoxin ([³H]en-TTX) (20). Biochemical titrations of the Ca²⁺ channel were carried out using tritiated nitrendipine as described (21).

²²Na⁺ and ⁴⁵Ca²⁺ Flux Experiments. Rates of ²²Na⁺ uptake by cardiac cells in culture were determined as described (22). The preincubation medium was supplemented with 0.5 mM ouabain to inhibit the Na⁺, K⁺-ATPase and with veratridine (0.2 mM) and sea anemone toxin II (10 μ M) to activate the Na⁺ channel.

Determinations of nitrendipine-sensitive and insensitive rates of ⁴⁵Ca²⁺ uptake were made in the presence or absence of the Ca²⁺ channel agonist Bay K8644 as described (23).

RESULTS

Chicken embryonic heart cells have been cultured in LPDS to suppress the exogenous source of cholesterol and in the presence or in the absence of mevinolin, a potent inhibitor of *de novo* synthesis of cholesterol.

Mevinolin Inhibits HMG-CoA Reductase Activity and Decreases Levels of Cholesterol in Cardiac Cells in Culture. Mevinolin inhibited the HMG-CoA reductase activity of cardiac cells with a K_i value of 30×10^{-9} M (not shown). Cardiac cells cultured for 3 days in LPDS in the presence of mevinolin had a higher level of HMG-CoA reductase (mevinolin inhibition of the enzyme derepresses its synthesis), as observed in other cell types (6). At concentrations of mevinolin >0.1 μ M the level of HMG-CoA reductase activity was increased by a factor of 2–2.4 (from 0.25 nmol per mg of protein per min to 0.5–0.6 nmol per mg of protein per min).

The effect of mevinolin on [¹⁴C]acetate distribution among the major lipid classes—phospholipids, triglycerides, monoglycerides, cholesterol, and cholesterol esters—has been analyzed by pulse labeling cardiac cells for 12 hr with [¹⁴C]acetate. Fractions of [¹⁴C]acetate incorporated into lipids in control cells were 0.4 for monoglycerides, 0.29 for triglycerides, 0.18 for phospholipids, 0.10 for cholesterol, 0.02 for cholesterol esters, and 0.01 for other lipids. Mevinolin at 0.1 μ M reduced [¹⁴C]acetate incorporation into triglycerides by 20%, while incorporation into monoglycerides and phospholipids was slightly increased (15%). Incorporation of [¹⁴C]acetate into cholesterol and cholesterol esters was reduced by 90% and 80%, respectively. Mevinolin added twice a day for 2 days to cardiac cells cultured in LPDS

produced a decrease in the total cholesterol content of the cells as indicated in Table 1.

Mevinolin had no effect on the internal level of ATP at concentrations ranging from 0.1 nM to 1 μ M. This level remained constant at a value of 27 ± 2 nmol of ATP per mg of protein after 2 days of treatment.

Electrical and Contractile Activities of Chicken Embryonic Cardiac Cells in the Presence and in the Absence of Mevinolin. Fig. 1 shows that control cardiac cells grown in LPDS in the absence of mevinolin had a maximal diastolic potential of –75 mV and a maximum upstroke velocity ($\dot{V}_{max}$) of the action potential (AP) of 120 V/sec (Fig. 1A). Cells treated with 0.1 μ M mevinolin were less polarized (-45 ± 5 mV) than control cells and their contractility was slightly depressed (Fig. 1B). After a 2-day treatment with 1 μ M mevinolin, spontaneous electrical activity and beating were suppressed. Electrically evoked APs could be generated after prepolarization (Fig. 1C), but they did not produce contractions. Maximal diastolic potentials, $\dot{V}_{max}$ values, and cholesterol levels at different mevinolin concentrations are summarized in Table 1.

Sensitivities to TTX and isoproterenol of control and mevinolin-treated cardiac cells are presented in Fig. 2A. TTX (0.1 μ M) suppressed the AP and blocked spontaneous beating in control cells (Fig. 2A, traces a and b). Under these conditions, isoproterenol (1 μ M) restored both excitability and contraction (Fig. 2A, trace c). Addition of 10 nM nitrendipine drastically depressed the slow AP duration and blocked contraction as previously observed (21). In mevinolin-treated cardiac cells, TTX suppressed the evoked AP (Fig. 2A, traces d and e) and addition of isoproterenol after TTX blockade of Na⁺ channels (Fig. 2A, trace f) was unable to restore excitability and contraction.

Fig. 2B shows that Bay K8644 produced marked effects on both duration of the AP and contraction when added to control cardiac cells (Fig. 2B, traces a and b). Conversely, the electrically induced AP in mevinolin-treated cells (Fig. 2B, trace c) remained unchanged in the presence of 10 nM Bay K8644. Under these conditions, Bay K8644 did not restore contraction (Fig. 2B, trace d).

The voltage dependence of the Ca²⁺ current of isolated cardiac cells was similar to that found in other types of cardiac cells (24–28). The threshold was observed at about –40 mV and the maximum inward current was between 0 and +10 mV (Fig. 3A). No significant decrease of Ca²⁺ current was observed with these cells for periods up to 30 min.

Mammalian cardiac cells have been shown to contain two types of Ca²⁺ channels (L type and T type) with different voltage and time dependences (26, 27). Analysis of the current–voltage relationship for the Ca²⁺ current (not shown) indicates that only the slow Ca²⁺ channel (L type) is present in the embryonic avian cardiac cells.

Table 1. Effects of mevinolin on 11-day chicken embryonic cardiac cholesterol content electrical and mechanical activities

Mevinolin, M	Total cholesterol, μ g per mg of protein	$\dot{V}_{max}$, V/sec	Maximal diastolic potential, mV	Contraction spontaneous or induced
None	27 ± 3	120 ± 10	-70 ± 5	Normal
10^{-7}	20 ± 2	70 ± 20	-45 ± 5	Depressed
10^{-6}	17 ± 3	—	$-40 \pm 5^*$	Suppressed
3×10^{-6}	9 ± 2	—	$-35 \pm 5^*$	Suppressed

Cells were cultured for 3 days in LPDS and exposed to mevinolin for 2 days. $\dot{V}_{max}$ and maximal diastolic potential values are for spontaneously beating cells. All values are means $\pm$ SEM of 5–10 measurements from different series of culture.

*Membrane potential values are given for quiescent cells.

FIG. 1. Electrical and mechanical activities of cardiac cells from 11-day chicken embryos cultured as monolayers in the presence of LPDS (A–C) and in the presence of mevinolin (B and C). (A) Control AP. (B) After treatment with 0.1 μM mevinolin for 2 days. (C) After treatment with 1 μM mevinolin for 2 days. Lower traces give the amplitude of the contraction.

The pharmacological properties of the slow Ca^{2+} channel were similar to those of cardiac Ca^{2+} channels from different animal origins (23–28). Peak Ca^{2+} current amplitude increased after external application of isoproterenol (1 μM) (Fig. 3B) and of Bay K8644 (10 nM) (Fig. 3C). Fig. 3A shows that the Ca^{2+} current was strongly depressed in mevinolin-

FIG. 2. (A) Electrical and mechanical activities and sensitivity to TTX and isoproterenol of cardiac cells cultured in the form of a monolayer in the absence of mevinolin (traces a–c) and in the presence of 1 μM mevinolin (traces d–f). Curves: a, control AP; b, addition of 0.1 μM TTX; c, addition of 1 μM isoproterenol; d, AP after 2 days of treatment with 1 μM mevinolin; e, addition of 0.1 μM TTX; f, addition of 1 μM isoproterenol. (B) Electrical and mechanical activities and sensitivity to Bay K8644 of nontreated cardiac cells and in cells treated with 1 μM mevinolin. Traces: a, control AP from nontreated cells and contraction; b, addition of 10 nM Bay K8644; c, AP after treatment with 1 μM mevinolin; d, addition of 10 nM Bay K8644. Lower traces give the amplitude of the contraction.

FIG. 3. Ca^{2+} currents in normal and mevinolin-treated cardiac cells. Voltage-clamp experiments were performed on isolated cardiac cells cultured for 3 days with or without 1 μM mevinolin. (A) Superimposed Ca^{2+} current traces associated with depolarizing voltage steps of 24 (trace 1), 48 (trace 2), 70 (trace 3), and 85 (trace 4) mV from a holding potential of -65 mV. (Left) Control cell. (Right) Mevinolin-treated cell. (B and C) Ca^{2+} currents associated with a step depolarization to -1 mV from a holding potential of -65 mV. Control and 10 min after addition of 1 μM isoproterenol (B) or 10 nM Bay K8644 (C). HP, holding potential; TP, test pulse.

treated cardiac cells. However, Ca^{2+} channel blockers had the same blocking potencies on the decreased Ca^{2+} current as on nontreated cardiac cells (not shown). Conversely, mevinolin-treated cells had Ca^{2+} channels that were much less sensitive to isoproterenol (Fig. 3B) or Bay K8644 (Fig. 3C) than control cells. Both drugs produced a mean increase of Ca^{2+} current of only $\approx 15\%$, as compared to 50% in control cells.

Biochemical Investigations of the Properties of Voltage-Dependent Na^+ and Ca^{2+} Channels in Control and Mevinolin-Treated Cardiac Cells. Veratridine and sea anemone toxin II are specific for the gating system of the Na^+ channel (29). These two toxins are synergistic in their action and increase the rate of $^{22}\text{Na}^+$ entry through voltage-sensitive Na^+ channels of cardiac cells. Fig. 4 shows that the $^{22}\text{Na}^+$ uptake component that can be activated by the mixture of veratridine plus toxin II and inhibited by 0.1 μM TTX with a half-maximum effect at 4 nM is identical in both mevinolin-treated and nontreated cells (Fig. 4 *Inset A*).

Maximum binding capacities (B_{max}) and dissociation constants (K_d) for the [^3H]en-TTX-receptor complex were 38 ± 5 fmol per mg of protein and 1.3 ± 0.3 nM for control and 40 ± 4 fmol per mg of protein and 1.2 ± 0.2 nM for mevinolin-treated cells (Fig. 4 *Inset B*). Binding characteristics of [^3H]en-TTX remained constant between 3 and 5 days of culture (not shown).

Kinetics of $^{45}\text{Ca}^{2+}$ uptake by nontreated cardiac cells that had been incubated in a low K^+ medium (5 mM KCl) and in a high K^+ medium (40 mM KCl) in the presence and/or in the absence of 10 nM Bay K8644 and 1 μM nitrendipine are shown in Fig. 5 (*Inset A*). When cardiac cells were in a low

FIG. 4. Activation of TTX-sensitive $^{22}\text{Na}^+$ uptake by sea anemone toxin II and veratridine (1 min at 37°C) in chicken embryonic heart cells cultured in the presence and in the absence of mevinolin ($1\mu\text{M}$) for 2 days. Each bar gives the mean value $\pm$ SEM of three experiments. (Insets) Scatchard representation of the specific binding of $[^3\text{H}]\text{TTX}$ on chicken cardiac cells cultured in the presence (●) or in the absence (▲) of mevinolin. Dose-response curves for TTX were done on the initial rate of $^{22}\text{Na}^+$ uptake in chicken embryonic heart cells cultured in the presence (●) and in the absence of mevinolin (▲) after activation with the mixture of veratridine (Vera) and sea anemone toxin II (As_{II}).

K^+ medium, their membrane potential was at -75 mV (Table 1). Under these conditions, $^{45}\text{Ca}^{2+}$ uptake was not affected by nitrendipine (Fig. 5 Inset A). Addition of 10 nM Bay K8644 increased (2-fold) the initial rate of $^{45}\text{Ca}^{2+}$ uptake in nontreated cells (Fig. 5). This component of $^{45}\text{Ca}^{2+}$ uptake was blocked by nitrendipine. When cells were incubated in a high K^+ medium, they were depolarized to $-30 \pm 5\text{ mV}$ (not shown). Depolarization increased the initial rate of $^{45}\text{Ca}^{2+}$

uptake (3.5-fold) in nontreated cells (Fig. 5 Inset A). This increased uptake was inhibited by $1\mu\text{M}$ nitrendipine. When cardiac cells were treated by $1\mu\text{M}$ mevinolin, the rate of $^{45}\text{Ca}^{2+}$ uptake was neither affected by addition of Bay K8644 or nitrendipine (Fig. 5) in polarized conditions (5 mM K^+), nor by depolarization (40 mM K^+). Fig. 5 shows that isoproterenol ($10\mu\text{M}$) increased the component of $^{45}\text{Ca}^{2+}$ uptake induced by depolarization in control cells and that this increase was inhibited by $1\mu\text{M}$ nitrendipine. Conversely, in mevinolin-treated cells isoproterenol did not increase $^{45}\text{Ca}^{2+}$ uptake observed in depolarized conditions (Fig. 5). B_{max} and K_d values of the $[^3\text{H}]\text{nitrendipine}$ -receptor complex were $120 \pm 10\text{ fmol per mg of protein}$ and $0.5 \pm 0.1\text{ nM}$ for control and $100 \pm 10\text{ fmol per mg of protein}$ and $0.4 \pm 0.1\text{ nM}$ for mevinolin-treated cardiac cells (Fig. 5 Inset B).

DISCUSSION

Uptake, storage, and synthesis of cholesterol by cells is regulated by low and high density lipoproteins (1, 2). Previous work from this laboratory has shown that cardiac cells from 11-day chicken hearts had different electrophysiological properties when they were cultured in medium supplemented with fetal calf serum or with LPDS (4). These observations and those of others (30), who had treated heart myocytes with a mixture of high-density lipoproteins and sphingomyelin to deplete the cells of cholesterol, suggested that the cholesterol level in the plasma membrane was crucially involved in the control of excitability of cardiac cells.

The approach taken in this paper has been to analyze, using chicken cardiac cells cultivated in LPDS, the effects of mevinolin, a potent inhibitor of cholesterol biosynthesis, which is a potential new drug for the treatment of hypercholesterolemia (5–8).

Mevinolin blocked the activity of HMG-CoA reductase with a K_i of $30 \times 10^{-9}\text{ M}$. At concentrations $>0.1\mu\text{M}$, it decreased the incorporation of $[^{14}\text{C}]\text{acetate}$ into cholesterol and cholesterol esters. It also significantly decreased total cholesterol

FIG. 5. Time course of $^{45}\text{Ca}^{2+}$ uptake in chicken embryonic heart cells incubated in low (5 mM) or high (40 mM) K^+ solutions. Effects of isoproterenol and nitrendipine on initial rate of $^{45}\text{Ca}^{2+}$ uptake under depolarized (40 mM K^+) conditions, and of Bay K8644 and nitrendipine under polarized (5 mM K^+) conditions in cardiac cells cultured in the absence or presence of $1\mu\text{M}$ mevinolin. Uptake of $^{45}\text{Ca}^{2+}$ was measured for 20 sec at 37°C . Each bar gives the mean value $\pm$ SEM of five experiments. (Inset A) Cardiac cells from chicken embryos cultured in the absence of mevinolin. Uptakes of $^{45}\text{Ca}^{2+}$ were measured for increasing periods of time in a medium containing either 5 mM (□, ■, △, ▲) or 40 mM (○, ●, ○, ●) K^+ in the presence (□, ■) or absence (○, ●, △, ▲) of 10 nM Bay K8644 and in the presence (■, ●, ▲) or absence (□, ○, △) of $1\mu\text{M}$ nitrendipine. (Inset B) Scatchard representation of the specific binding of $[^3\text{H}]\text{nitrendipine}$ on cardiac cells cultured in the presence (●) or absence (▲) of mevinolin ($1\mu\text{M}$).

content in cardiac cells. At 0.1 μM mevinolin, the total cholesterol content was 26% lower than in nontreated cells.

Mevinolin concentration of 0.1 μM produced very significant effects on the maximal diastolic potential, the V_{max} of the AP, and the contractility of heart cells (Table 1). Changes in electrophysiological properties were not linked to variations of internal ATP concentration. Effects of mevinolin pretreatment on the resting potential may be due to an alteration of a K^+ conductance and/or of a change in the catalytic properties of the Na^+, K^+ -ATPase.

The most striking effect of mevinolin treatment (Figs. 1 and 2) is the tendency to uncouple excitation from contraction. Mammalian cardiac cells have both slow and fast Ca^{2+} channels (26, 27). Fast Ca^{2+} channels do not seem to be expressed in the embryonic chicken heart cell. Slow channels are known to be essential in excitation-contraction coupling in cardiac cells (31-33). Therefore, particular attention has been paid to these channels. The activity of the voltage-dependent slow Ca^{2+} channel in cardiac cells can be increased in two ways: (i) by isoproterenol treatment, which acts via cyclic AMP and phosphorylation of the channel protein (32-34); (ii) by treatment with the Ca^{2+} channel agonist Bay K8644, which activates $^{45}\text{Ca}^{2+}$ uptake and increases the peak Ca^{2+} current (24-28). Both treatments clearly increased Ca^{2+} channel activity in control chicken heart cells (Fig. 2). Isoproterenol produced a slow Ca^{2+} AP in cells that had been treated with TTX to suppress the fast Na^+ component of the AP. Under these conditions, isoproterenol triggered strong contractions. Bay K8644 prolonged the plateau phase of the AP and had cardiotoxic effects (Fig. 2B). None of these treatments had any effect on electrical and contractile properties of cells treated with mevinolin. Voltage-clamp data on isolated cells have confirmed these results by showing (i) that most, if not all, of the slow Ca^{2+} channel activity was lost in mevinolin-treated cells and (ii) that isoproterenol and Bay K8644 failed to produce a recovery of a normal Ca^{2+} channel activity in mevinolin-treated cells. The first important observation coming from biochemical results was that the number of receptors for the Ca^{2+} channel antagonist nifedipine was the same in control and in mevinolin-treated cells. The second observation came from Ca^{2+} flux experiments, which have clearly shown that activation of Ca^{2+} channels that was induced either by high K^+ , by isoproterenol, or by Bay K8644 in control cardiac cells was completely abolished in mevinolin-treated cells. These results clearly indicated that the Ca^{2+} -channel protein was present in the same amount before and after treatment with mevinolin but that it had become nonfunctional. The situation was different for the Na^+ channel: (i) the Na^+ -channel protein measured by the number of [^3H]en-TTX receptor sites was in the same amount in normal and mevinolin-treated cells, and (ii) the chemical activation of Na^+ channels by toxins was unchanged after mevinolin treatment.

All these results suggest that slow Ca^{2+} channel activity in chicken cardiac cells is particularly sensitive to cholesterol content in the plasma membrane. Disappearance of Ca^{2+} channel activity is most probably at the origin of the loss of cardiac contractility.

The effects of mevinolin on heart-cell excitability and contractility in the whole animal or in humans are difficult to evaluate from this work. Animals treated by mevinolin will of course have a partial inhibition of endogenous cholesterol synthesis in all tissues, but they will also be in a situation in which the low density lipoprotein fractions of lipoproteins will still provide exogenous cholesterol to cardiac cells. Therefore, the effects of mevinolin in animals or in humans will certainly be much less drastic than those observed here

with cardiac cells cultured in low density lipoprotein-free medium and in higher concentrations of the drug.

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