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Class I and IV Antiarrhythmic Drugs and Cytosolic Calcium Regulate mRNA Encoding the Sodium Channel α Subunit in Rat Cardiac Muscle

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SUMMARY

Previous studies have shown that chronic *in vivo* treatment with the antiarrhythmic drug mexiletine produces an increase in sodium channel number. We examined whether chronic mexiletine treatment would similarly regulate the level of mRNA encoding the cardiac sodium channel. RNA isolated from cardiac tissue was probed with a 2.5-kilobase cRNA transcribed with T7 RNA polymerase from the clone Na 8.4, which encodes nucleotides 3361–5868 of the α subunit of the R_{IIA} sodium channel subtype. Chronic mexiletine treatment produced a 3-fold increase in the level of mRNA encoding sodium channel α subunits. Previous studies of cultured skeletal muscle cells had suggested that chronic sodium channel blockade may mediate an increase in sodium channel mRNA by changes in cytosolic Ca^{2+} concentration. To address this issue, we assessed whether verapamil would also produce up-regulation of the level of mRNA encoding the sodium channel and whether the calcium ionophore A23187 would produce the opposite effect on mRNA level. Verapamil treatment increased sodium channel mRNA level up to 3-fold, whereas *in vitro* A23187 treatment decreased the mRNA level 5-fold. The combination of verapamil and mexiletine produced no further increase in the mRNA level, compared with that seen with the single agents, suggesting a convergent second messenger pathway for the actions of these two drugs. These data show that the level of mRNA encoding sodium channels is substantially increased during antiarrhythmic drug treatment and suggest that change in cytosolic Ca^{2+} concentration is the second messenger involved in the regulation of levels of mRNA encoding the α subunit of the cardiac sodium channel.

Class I antiarrhythmic drugs prevent arrhythmias by inhibiting cardiac sodium channels in a complex voltage- and frequency-dependent manner. Chronic *in vivo* treatment with the class I antiarrhythmic drug mexiletine produced an increase in cardiac sodium channel number, as measured by high affinity binding of batrachotoxin benzoate (1). Sodium channel number, as measured by high affinity binding of saxitoxin, was also increased in skeletal muscle cells in culture during chronic treatment with bupivacaine, another sodium channel blocker (2, 3). These results show that reduction of the electrical activity of cardiac and skeletal muscle cells causes a compensatory increase in sodium channel number and imply that the normal level of electrical activity of these cells is sufficient to cause a chronic decrease in sodium channel number.

The decrease in sodium channel number in skeletal muscle cells caused by the normal level of electrical activity may be mediated by calcium entering the cell during each action potential, because chronic elevation of cytosolic calcium by treatment of myocytes with the calcium ionophore A23187 or the calcium-releasing agent ryanodine substantially reduces sodium channel number (2, 3). The changes in sodium channel number in skeletal muscle cells in culture are correlated with comparable changes in the level of mRNA encoding sodium channel α subunits (4), indicating that regulation of transcription of mRNA or its processing and stability is primarily responsible for the regulation of sodium channel number. Levels of mRNA encoding other skeletal muscle proteins, such as the acetylcholine receptor, are regulated by depolarization and by changes in intracellular calcium concentration (5, 6). In this report, we describe the effects of the sodium channel blocker mexiletine and the calcium channel blocker verapamil on the levels of mRNA encoding sodium channel α subunits in rat ventricle and examine the role of calcium in this process in cardiac myocytes in cell culture.

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Materials and Methods

***In vivo* drug treatment protocols.** All experiments were done in a paired fashion. Pairs of weight-matched adult Sprague-Dawley rats were randomly assigned to receive either subcutaneous placebo, mexi-

ABBREVIATIONS: SSC, standard saline citrate; SDS, sodium dodecyl sulfate; kb, kilobase(s).

letine, verapamil, or the combination of verapamil and mexiletine at matched doses.

Two doses of mexiletine (50 and 75 mg/kg/day) were administered subcutaneously in 0.25 ml of saline at 9:00 a.m. daily (1). The dose of mexiletine used in this study is 6–20-fold less than the LD_{50} dose range (480–1080 mg/kg/day) (1) and is in the same dosage range that produced an increase in cardiac sodium channel receptor number (1). We reported previously that serum concentrations of mexiletine assessed 2 and 24 hr after subcutaneous administration of 50 mg/kg were below the lower detectable limit of our high performance liquid chromatography assay (0.1 μg/ml). Previous studies have documented that mexiletine blocks not only the sodium current but also the slow inward calcium current (7). Three doses of verapamil (3, 8, and 16 mg/kg/day) were administered subcutaneously in 0.25 ml of saline adjusted to pH 5.0. The doses of verapamil used in this study were designed to achieve concentrations of verapamil in the therapeutic range (8–10). After 3 days of *in vivo* treatment, pairs of placebo-, mexiletine-, and verapamil-treated rats were anesthetized with 30 mg/kg pentobarbital and their hearts were immediately removed for mRNA isolation. To assess the time dependence of changes in mRNA levels, pairs of rats were randomly assigned to treatment with placebo or verapamil (3 mg/kg/day) and they were killed and their hearts were removed after 0, 1, 2, or 3 days of treatment. In some of these animals, the entire forebrain was rapidly removed and RNA was prepared.

***In vitro* drug treatment protocols.** All experiments were done in a paired fashion. Neonatal rat cardiac cells in culture (10) for 24 hr (3×10^6 cells/plate) were then treated with verapamil (500 μg/liter), the calcium ionophore A23817 (1 μM), or placebo. Cells were maintained in culture for an additional 3 days with these treatments. Thereafter, the culture medium was removed and total cellular RNA was prepared.

Neonatal ventricular myocytes in culture. Cardiac myocytes were isolated from 1-day-old neonatal Sprague-Dawley rats by collagenase dispersion, yielding 3×10^6 cells/plate (11). The aortic root and atria were physically removed before dispersion. Myocytes were maintained in a medium containing 90% Dulbecco's modified Eagles medium, 10% newborn calf serum, 10 mg/ml streptomycin, and 10 mg/ml penicillin G (4).

Isolation of RNA. RNA was isolated using the method of Glisin *et al.* (12). The cells in culture were scraped in a medium containing 4 M guanidinium thiocyanate and homogenized. Similarly, whole hearts were homogenized in this 4 M guanidinium thiocyanate solution. This homogenate was layered over a 4-ml cushion of 6 M CsCl in an SW 41 tube and was centrifuged at 26,500 rpm for 20 hr. After centrifugation, the solution was removed and the tube and RNA pellet were air-dried briefly. After drying, the pellet was redissolved in water, 0.1 volume of 5 M ammonium acetate was added, and the RNA was precipitated by the addition of 2 volumes of absolute ethanol (4).

Gel electrophoresis and RNA blotting. RNA (10 μg) prepared as described above was electrophoresed in gels containing 2.2. M formaldehyde. After electrophoresis was complete, the gel was soaked in 50 mM NaOH, 1.5 M NaCl, for 0.5 hr and then soaked in 1 M Tris (pH 6.8), 1.5 M NaCl, for 0.5 hr (4). The RNA was transferred to nitrocellulose, which was baked in vacuum to fix the RNA to the filter (12, 13). Completeness of the transfer was verified by inspection of the ethidium bromide-stained gels.

Probe labeling. The plasmid Na 8.4, containing an insert spanning nucleotides 3361–5868 of the α subunit of the R_{IIA} rat brain sodium channel (14), was linearized using *Sal*I. T7 RNA polymerase transcription reactions were performed using standard protocols, with ^{32}P-labeled CTP included (15). After synthesis, the cRNA probe was separated from unincorporated nucleotides by Sephadex spun-column chromatography. A similar cRNA probe was synthesized using a random primer technique from a linearized plasmid containing a cDNA encoding elongation factor 1α, a constitutively expressed ribosomal protein.

Hybridization. The nitrocellulose was prehybridized in 50 mM Tris·HCl (pH 7.5), 6× SSC (0.877% NaCl, 0.44% sodium citrate, pH 7.0) 10× Denhardt's, 50% formamide, 0.5% SDS, with denatured salmon sperm DNA added to 0.5 mg/ml. Prehybridization was performed at 40° for 4 hr. Radiolabeled RNA probe was then added to a concentration of 5×10^5 cpm/ml of hybridization solution. Hybridization was performed at 65° for 12 hr. After hybridization, the nitrocellulose was washed four times at room temperature in 2× SSC, 0.5% SDS. The filters were then washed twice, for 30 min each time, at 65° in 0.2× SSC, 0.5% SDS. Radioactive signals were quantitated using an LKB laser densitometer (4). The densitometer was set to scan and integrate the intensity of a rectangular area corresponding to the autoradiographic bands. Background intensity was determined by scanning a region of identical shape and area above and below the 8.5-kb mRNA. These two background intensity determinations were averaged and subtracted from the intensity of the autoradiographic band at 8.5 kb.

Electrocardiographic interval measurements. Six adult rats were sedated with diazepam (17 μg/g) to avoid the use of general anesthetics such as pentobarbital or halothane, because these agents are sodium channel blockers (16–18). Surface electrocardiographic leads I and a VF were measured simultaneously and recorded on a Nicolet digital oscilloscope. After base-line measurements were obtained the same dose of mexiletine (75 mg/kg) was given, and surface QRS duration was measured 30 min after treatment.

Results

Effects of mexiletine treatment on the level of mRNA encoding the α subunit of the sodium channel. Adult rats were treated with mexiletine or placebo, and cardiac mRNA was isolated and probed with a 2.5-kb cRNA transcribed with T7 RNA polymerase from the clone Na 8.4, which encodes nucleotides 3361–5868 of the α subunit of the R_{IIA} sodium channel subtype from rat brain. Previous studies have reported substantial homology among sodium channels in rat skeletal muscle, brain, and heart in these homologous domains. The conditions of hybridization and wash do not distinguish among known isoforms of the sodium channel (4).

Fig. 1 shows a representative Northern blot hybridization of rat heart mRNA encoding the cardiac sodium channel, in animals treated with placebo or mexiletine (at doses of 50 mg/kg/day) for 24, 48, or 72 hr. Mexiletine (50 mg/kg/day) produced a significant (3-fold) increase in mRNA levels encoding the cardiac sodium channel ($p < 0.01$) but had no effect on mRNA encoding brain sodium channels. The mRNA levels were not further increased at doses of mexiletine of 75 mg/kg/day (Fig. 1). In order to examine the specificity of the effect on sodium channel mRNA, nitrocellulose blots were simultaneously probed with cRNA transcribed from the cDNA clone encoding the constitutively expressed ribosomal protein elongation factor 1α. Data were expressed as the ratio of densitometric integrals of the 8.5-kb band for the α subunit of the sodium channel to that of elongation factor 1α. Mean data are shown as a percentage of control. Mexiletine (50 mg/kg/day) substantially increased the level of mRNA encoding sodium channel α subunits, whereas the level of mRNA encoding elongation factor 1α was unchanged. These results show that overall changes in mRNA level or variability in gel loading cannot account for the increases in sodium channel mRNA levels seen during chronic *in vivo* mexiletine therapy.

In order to verify that mexiletine inhibits cardiac sodium channels *in vivo* in the dose range used, we examined its effects on the rate of rise of the ventricular action potential using electrocardiographic interval measurements. Mexiletine (75 mg/kg) significantly prolonged surface QRS from a mean of 62 ± 8 msec at base line to 70 ± 9 msec at 30 min of mexiletine treatment ($p < 0.05$), consistent with a reduction in the rate of

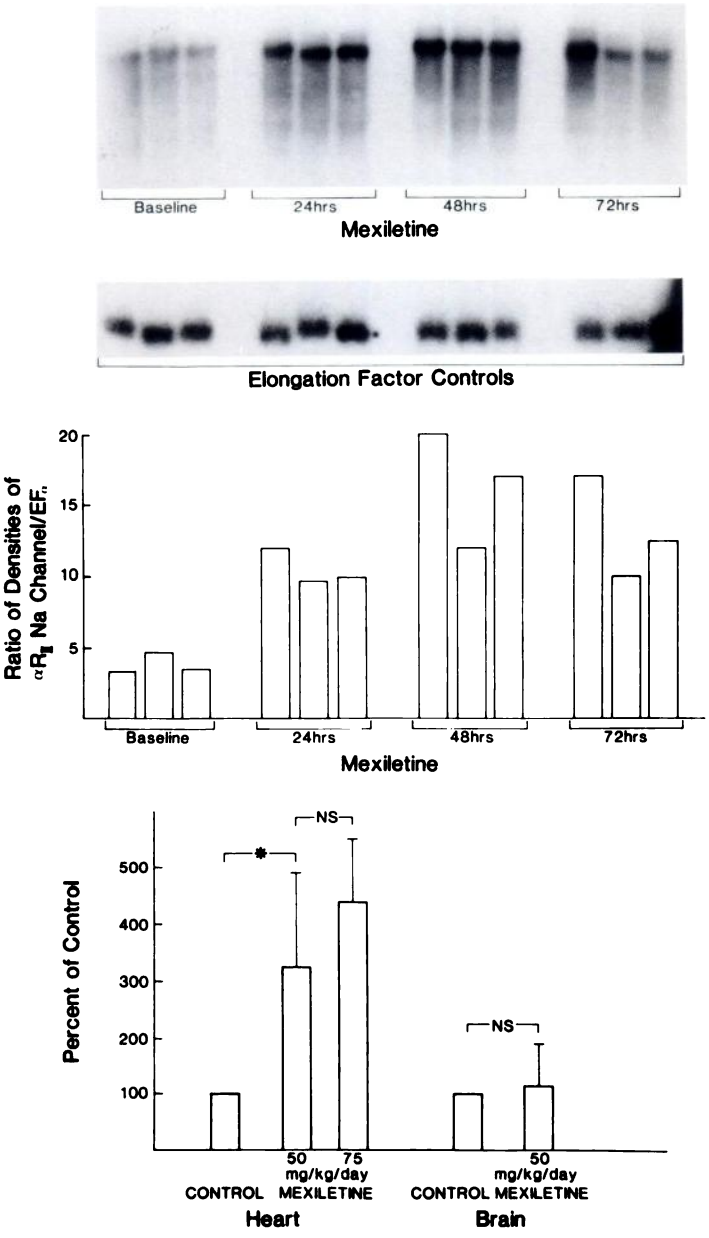

Fig. 1. Northern blot hybridization of rat heart mRNA encoding cardiac sodium channel in animals treated with placebo and mexiletine. *Upper, first three lanes*, mRNA from animals treated with placebo; *next three lanes*, mRNA from animals treated with mexiletine (50 mg/kg/day) for 24 hr; *next three lanes*, mRNA from animals treated with mexiletine (50 mg/kg/day) for 48 hr; *last three lanes*, mRNA from animals treated for 72 hr. *Middle*, ratio of the densitometric integral of the α subunit of the sodium channel to that of elongation factor 1α. *Lower*, mean data from all results are shown expressed as a percentage of control. Mexiletine increases mRNA encoding the α subunit of the sodium channel in heart but not in brain.

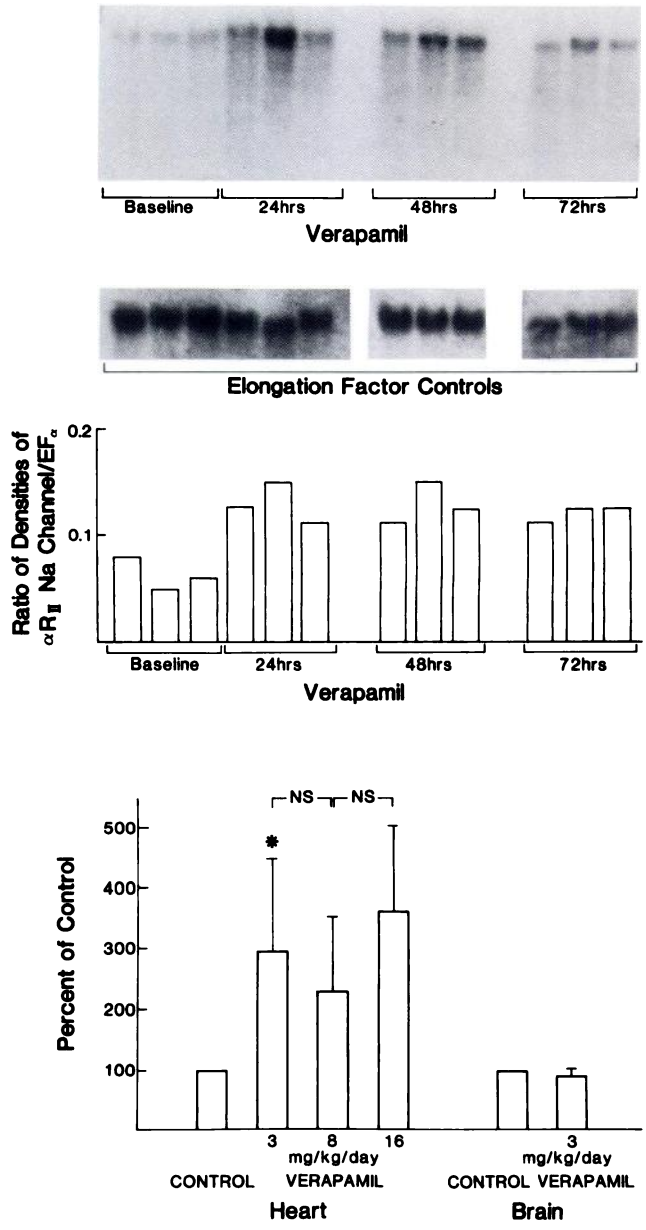

Fig. 2. Northern blot hybridization of rat heart mRNA encoding cardiac sodium channel in animals treated with placebo and verapamil at a dose of 3 mg/kg/day. *Upper, first three lanes*, mRNA from animals treated with placebo; *next three lanes*, mRNA from animals treated with verapamil (3 mg/kg/day) for 24 hr; *next three lanes*, mRNA from animals treated with verapamil (3 mg/kg/day) for 48 hr; *last three lanes*, mRNA from animals treated for 72 hr. Middle, ratio of the densitometric integral of the α subunit of the sodium channel to that of elongation factor 1α. *Lower*, ratio of the densitometric integral of the α subunit sodium channel band to that of elongation factor 1α. No increase in mRNA encoding the sodium channel was observed in brain during verapamil treatment. Mean data are expressed as a percentage of control. Verapamil significantly increases mRNA encoding the α subunit of the sodium channel.

rise of the action potential due to inhibition of sodium channels. The results indicate that modest changes in the rate of rise of the cardiac action potential are sufficient to up-regulate sodium channel mRNA levels.

Effects of verapamil treatment on the level of mRNA encoding the α subunit of the sodium channel. If the effect of normal cardiac electrical activity to reduce sodium channel mRNA level is mediated by calcium entry during the action potential, treatment with a calcium channel blocker like the class IV antiarrhythmic drug verapamil should mimic the effect of mexiletine. Fig. 2 shows representative Northern blot hybridizations of heart mRNA and a summary of results from animals treated with placebo or verapamil (3 mg/kg/day) over 72 hr and at doses of 3, 8, or 16 mg/kg/day. Verapamil produced a significant increase in levels of mRNA encoding the cardiac sodium channel, in a dose-dependent fashion, with no change in mRNA for elongation factor 1α. Fig. 2 (*upper*) illustrates the time course of increase in sodium channel mRNA level during verapamil treatment. The levels of mRNA encoding the α subunit of the sodium channel were substantially increased

by 24 hr of treatment and increased little thereafter. Densitometric assessment of levels of mRNA during treatment with placebo and verapamil (3 mg/kg/day) indicated a 3-fold increase in sodium channel mRNA in heart with no increase in brain, as observed for mexiletine.

Effects of combination treatment on the level of mRNA encoding the α subunit of the sodium channel. When mexiletine (50 mg/kg/day) and verapamil (3 mg/kg/day) were combined, the level of mRNA encoding the cardiac sodium channel was not additively increased (Fig. 3). Indeed, densitometric analysis suggests that the combination treatment produced no further increase or a decrease in mRNA levels, compared with treatment with one of the drugs.

Effects of drug treatment on sodium channel mRNA in cardiac myocytes in cell culture. To examine whether changes in cytosolic calcium concentrations modulate sodium channel mRNA levels, neonatal myocytes were treated for 3 days with placebo, verapamil, or A23187. Treatment with verapamil produced only a small increase in the level of mRNA encoding sodium channel α subunits, which was not statistically significant (Fig. 4). This may indicate that the asynchronous action potentials and contractions of cardiac myocytes in culture are insufficient to cause a substantial chronic reduction in sodium channel mRNA level that can be reversed by verapamil treatment. Nevertheless, the cell culture system allows chronic elevation of cytosolic calcium by treatment with a calcium ionophore. Treatment with A23187 produced a substantial decrease in the sodium channel mRNA level (Fig. 4), without an effect on the level of mRNA for elongation factor 1α (data not shown). Densitometric assessment of mRNA levels in autoradiograms during treatment with placebo and A23187 indicated at least a 5-fold reduction due to treatment with the calcium ionophore. These results are consistent with previous studies suggesting that calcium mediates the regulation of sodium channel mRNA by electrical activity in skeletal muscle cells (2–4).

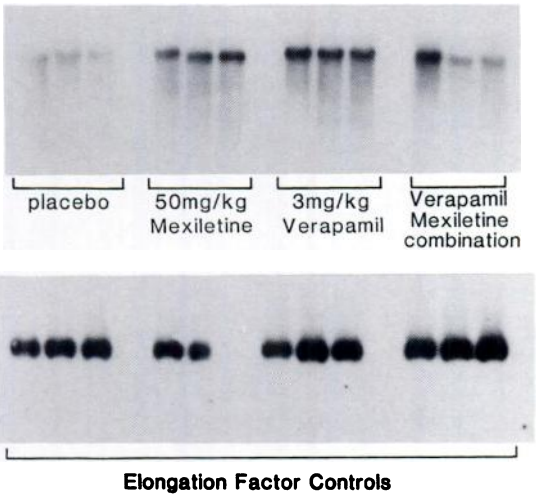

Fig. 3. Northern blot hybridization of rat heart mRNA encoding cardiac sodium channel in animals treated with mexiletine (50 mg/kg/day), verapamil (3 mg/kg/day), or their combination for 72 hr. *Middle*, simultaneous probes with cRNA encoding elongation factor 1α. The elongation factor in *lane 6* does not appear, likely due to a technical problem (bubble in gel). *Lower*, ratio of the integral of the α subunit sodium channel band to that of elongation factor 1α. The mean data from all results are expressed as a percentage of control.

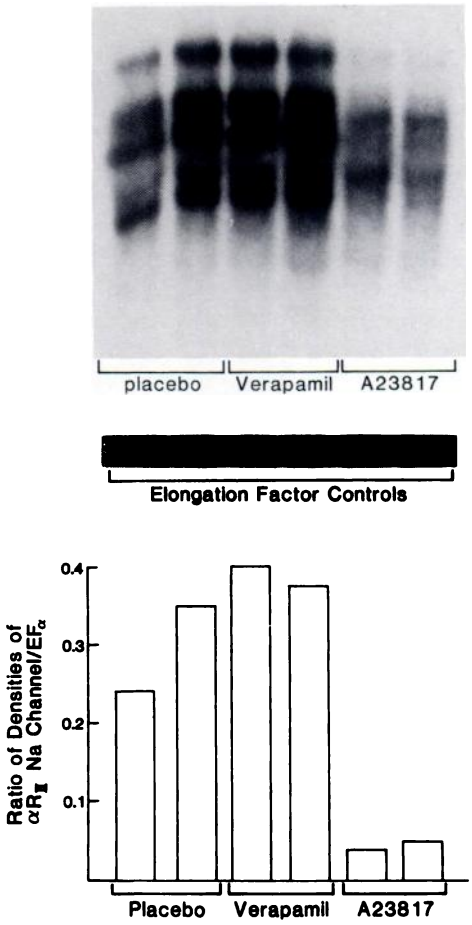

Fig. 4. Northern blot hybridization of rat heart mRNA encoding cardiac sodium channel in cultured neonatal myocytes during treatment with placebo, verapamil (500 μg/liter), or A23817 (1 μM). *Lower*, ratio of densitometric integral of the α subunit of the sodium channel band to that of elongation factor 1α. Treatment with A23817 substantially decreases sodium channel density.

Discussion

Effects of antiarrhythmic drugs on sodium channel mRNA. Our results provide the first evidence that sodium channel mRNA levels can be modulated by electrical activity and antiarrhythmic drugs *in vivo* in the heart. Chronic *in vivo* mexiletine treatment produced significant increases in levels of mRNA encoding the α subunit of the sodium channel in the rat heart. These data are in keeping with previous studies that report an increase in sodium channel number during the same chronic mexiletine treatment (1). Chronic *in vivo* treatment with the calcium channel blocker verapamil also produced a

significant increase in levels of mRNA encoding the α subunit of the sodium channel. This increase in sodium channel mRNA appeared within 24 hr of the onset of verapamil therapy. To assess whether mexiletine and verapamil treatment acted through a common second messenger system, we combined these two agents. If the increases in mRNA levels caused by the two drugs were mediated by independent second messenger pathways the combination of verapamil and mexiletine would be expected to produce an additive increase in mRNA, whereas if a common second messenger system was involved additivity might not be observed. We found that the combination of saturating doses of verapamil and mexiletine produced a similar or a smaller increment in levels of mRNA encoding the cardiac sodium channel, compared with that seen with treatment with a single drug. These data are consistent with a convergent second messenger system mediating the regulation in sodium channel mRNA levels seen with mexiletine and verapamil.

Treatment with sodium channel blockers such as bupivacaine has been reported to increase sodium channel receptor number in skeletal muscle cells and to produce a concomitant increase in levels of mRNA encoding the α subunit of the sodium channel (2–4). Calcium ionophores and calcium-releasing agents reduce the levels of sodium channels and the mRNA encoding them, consistent with a primary role for calcium as a second messenger regulating sodium channel expression in response to changes in electrical activity (2–4). These data suggest a common regulation system in skeletal muscle and cardiac muscle for levels of mRNA encoding the α subunit of the sodium channel. The findings that the calcium channel blocker verapamil and the calcium ionophore A23187 produced opposite effects on levels of mRNA encoding the cardiac sodium channel suggest that the cytosolic calcium concentration is involved in the regulation of mRNA levels in cardiac cells as well as in skeletal muscle cells.

Regulatory pathway. Based on the results of the present and past studies, we propose a pathway for regulation of the level of mRNA encoding the α subunit of the sodium channel wherein changes in cytosolic calcium concentration modulate the rate of transcription of α subunit mRNA or its processing and stability. Measurements of transcription rates in nuclear run-off assays are required to assess mRNA biosynthesis directly. The low level of transcription of sodium channel genes observed in brain (19), which has a considerably higher density of sodium channels than the heart, suggests that such direct measurements of sodium channel gene transcription would not be feasible in the heart. Transcription of other genes is modulated by changes in cytosolic calcium. For example, verapamil and the sodium channel blocker tetrodotoxin increased whereas A23817 decreased nicotinic acetylcholine receptor number in skeletal muscle cells, as measured by high affinity binding of α-bungarotoxin (5, 6). These changes in cell surface acetylcholine receptor density were paralleled by increases in levels of mRNA encoding the acetylcholine receptor. Therefore, the results of the present study parallel those observed when the regulation of another important cell membrane receptor, the acetylcholine receptor, is assessed.

Clinical relevance. Some patients who respond to acute intravenous administration of a class I antiarrhythmic drug develop resistance to that same drug at nearly identical concentrations when the class I drug is given chronically (20). One possible mechanism for this drug resistance is that chronic sodium channel blocker treatment produces an increase in the sodium channel number that decreases the pharmacodynamic response to these class I drugs. A testable prediction from the current study is that pretreatment of animals with the class IV antiarrhythmic drug verapamil will increase sodium channel number and result in a lesser response to acute intravenous class I drug therapy. The increased number of sodium channels caused by chronic treatment with these drugs may itself cause arrhythmias as a secondary consequence of therapy. Such arrhythmias would be particularly likely on withdrawal of treatment.

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References

1. Taouis, M., R. S. Sheldon, and H. J. Duff. Up-regulation of the rat cardiac sodium channel by *in vivo* treatment with class IA antiarrhythmic drug. *J. Clin. Invest.* **88**:375–378 (1991).
2. Sherman, S. J., and W. A. Catterall. Electrical activity and cytosolic calcium regulate levels of tetrodotoxin sensitive sodium channel in rat muscle cells. *Proc. Natl. Acad. Sci. USA* **81**:262–266 (1984).
3. Sherman, S. J., J. Chrivia, and W. A. Catterall. Cyclic adenosine 3′,5′-monophosphate and cytosolic calcium exert opposite effects on biosynthesis of tetrodotoxin-sensitive sodium channels in rat muscle cells. *J. Neurosci.* **5**:1570–1576 (1985).
4. Offord, J., and W. A. Catterall. Electrical activity, cAMP and cytosolic calcium regulate mRNA encoding sodium channel α subunits in rat muscle cells. *Neuron* **2**:1447–1452 (1989).
5. Klarsfeld, A., R. Laufer, B. Fontaine, A. Deviller-Theiry, and J. P. Changeux. Regulation of muscle AChR *alpha* subunit gene expression by electrical activity: involvement of protein kinase C and Ca^{2+}. *Neuron* **2**:1229–1236 (1989).
6. Shainberg, A., M. Freud-Silverberg, and H. Brik. Changes in the levels of acetylcholine receptors mediated by calcium concentration in sarcoplasmic reticulum. *Prog. Clin. Biol. Res.* **253**:303–314 (1987).
7. Ono, K., T. Kiyosue, and M. Arita. Comparison of the inhibitory effects of mexiletine and lidocaine on the calcium current of single ventricular cells. *Life Sci.* **39**:1465–1470 (1986).
8. MacLeod, B. A., M. Moult, K. M. Saint, and M. J. Walker. The antiarrhythmia efficacy of intravenous anipamil against occlusion and reperfusion arrhythmias. *Br. J. Pharmacol.* **98**:1165–1172 (1989).
9. Yeager, J. C., and M. E. Whitehurst. Verapamil prevents isoproterenol-induced cardiac failure in the rat. *Life Sci.* **30**:299–306 (1982).
10. Weinhouse, E., J. Kaplanski, and G. Genchih. Plasma and tissue levels of digoxin in the rat following pretreatment with verapamil. *Res. Commun. Chem. Pathol. Pharmacol.* **47**:469–472 (1985).
11. Kilborn, M. J., and D. Fedida. A study of the developmental changes in outward currents of rat ventricular myocytes. *J. Physiol. (Lond.)* **430**:37–42 (1990).
12. Glisin, V., R. Crkvenjakov, and C. Byus. Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry* **13**:2633–2637 (1973).
13. Thomas, P. J. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77**:5201–5205 (1980).
14. Auld, V. J., A. L. Goldin, D. S. Krafte, J. Marshall, J. M. Dunn, W. A. Catterall, H. A. Lester, and R. J. Dunn. A rat brain Na^+ channel α subunit with novel gating properties. *Neuron* **1**:449–461 (1988).
15. Melton, D. A., P. A. Krieg, M. R. Rebagliati, and T. Maniatis. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes for plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**:7035–7056 (1984).
16. Davis, L. D., and J. V. Tempte. Effects of cyclopropane and of hypoxia on transmembrane potentials of atrial ventricular and Purkinje fibers. *Circ. Res.* **18**:692–703 (1966).
17. Laforet, E. G., E. T. Angelakos, and A. H. Hegnauer. Ventricular-excitability in the unanesthetized dog and its modification by pentobarbital anesthesia. *J. Physiol. (Lond.)* **189**:596–598 (1957).
18. Hunt, G. B., and D. L. Ross. Comparison of effects of three anesthetic agents on induction of ventricular tachycardia in a canine model. *Circulation* **78**:221–226 (1988).
19. Scheinman, R. I., V. J. Auld, A. L. Goldin, N. Davidson, R. J. Dunn, and W. A. Catterall. Developmental regulation of sodium channel expression in the rat forebrain. *J. Biol. Chem.* **264**:10660–10666 (1989).
20. Duff, H. J., D. G. Wyse, D. Manyari, and L. B. Mitchell. Intravenous quinidine: relationships amongst concentration, tachyarrhythmia suppression and electrophysiologic actions in patient with inducible sustained ventricular tachycardia. *Am. J. Cardiol.* **55**:92–97 (1985).

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