

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circ. Res. 2004;94:32-38; originally published online Feb 12, 2004;

DOI: 10.1161/01.RES.0000121566.01778.06

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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Functional Basis of Sinus Bradycardia in Congenital Heart Block

Keli Hu, Yongxia Qu, Yuankun Yue, Mohamed Boutjdir

Abstract—Congenital heart block (CHB) is a conduction abnormality characterized by complete atrioventricular (AV) block. CHB affects fetuses and/or newborn of mothers with autoantibodies reactive with ribonucleoproteins 48-kDa SSB/La, 52-kDa SSA/Ro, and 60-kDa SSA/Ro. We recently established animal models of CHB and reported, for the first time, significant sinus bradycardia preceding AV block. This unexpected observation implies that the spectrum of conduction abnormalities extends beyond the AV node to also affect the SA node. To test this hypothesis, we investigated the functional basis of this sinus bradycardia by characterizing the effects of antibodies from mothers with CHB children (positive IgG) on ionic currents that are known to significantly contribute to spontaneous pacing in SA node cells. We recorded L- ($I_{Ca,L}$) and T- ($I_{Ca,T}$) type Ca^{2+} , delayed rectifier K^+ (I_K), hyperpolarization-activated (I_f) currents, and action potentials (APs) from young rabbit SA node cells. We demonstrated that positive IgG significantly inhibited both $I_{Ca,T}$ and $I_{Ca,L}$ and induced sinus bradycardia but did not affect I_f and I_K . Normal IgG from mothers with healthy children did not affect all the currents studied and APs. These results establish that IgG from mothers with CHB children causes substantial inhibition of $I_{Ca,T}$ and $I_{Ca,L}$, two important pacemaker currents in rabbit SA node cells and point to both $I_{Ca,T}$ and $I_{Ca,L}$ as major players in the ionic mechanism by which maternal antibodies induce sinus bradycardia in CHB. These novel findings have important clinical significance and suggest that sinus bradycardia may be a potential marker in the detection and prevention of CHB. The full text of this article is available online at <http://circres.ahajournals.org>. (*Circ Res.* 2004;94:e32-e38.)

Key Words: L-type calcium current ■ T-type calcium current ■ antibodies ■ congenital heart block

Congenital heart block (CHB), detected at or before birth in a structurally normal heart, is strongly associated with autoantibodies reactive with the intracellular soluble ribonucleoproteins 48-kDa SSB/La, 52-kDa SSA/Ro, and 60-kDa SSA/Ro.¹ CHB is presumed to be due to the transplacental passage of these IgG autoantibodies from the mother into the fetal circulation.² In addition to various degree of atrioventricular (AV) block, other neonatal abnormalities affecting the skin, liver, and blood elements are also associated with anti-SSA/Ro and -SSB/La antibodies in the maternal and fetal circulation and are grouped under the heading of neonatal lupus syndromes.³ To date, complete AV block is irreversible, although varying degrees of block have been noted, and second degree block has on rare occasion reverted to normal sinus rhythm.⁴

We have recently reported that perfusion of Langendorff perfused rabbit hearts with IgG from mothers with CHB children (positive IgG) caused sinus bradycardia preceding AV block using surface ECG and optical action potentials.⁵ A significant and unexpected sinus bradycardia was also observed in the animal models of CHB developed by either passive transfer of positive IgG into pregnant mice⁶ or by

active immunization of female mice⁷ or rabbits⁸ with SSA/Ro antigen. This high incidence of sinus bradycardia both in vitro and in vivo suggests the possible involvement of the sinoatrial (SA) node. Indeed, Brucato et al⁹ found sinus bradycardia in infants born to mothers seropositive to SSA/Ro antibodies. Based on these animal and clinical data, we hypothesized that positive IgG may affect SA node ion currents underlying the pacemaker, thus providing a functional explanation for this sinus bradycardia.

Pacemaker activity in SA node cells is known to be due to a complex interplay of various ionic currents.¹⁰ Among these currents, L-type Ca^{2+} current, $I_{Ca,L}$, plays a significant role in both the diastolic depolarization and upstroke phase. Delayed rectifier K^+ current, I_K , is also important in diastolic depolarization. Hyperpolarization-activated inward current, I_f , and T-type Ca^{2+} current, $I_{Ca,T}$, are operative in early and late phases of the diastolic depolarization, respectively. In addition, changes in the time-independent currents can also affect the electrical activity in SA node.¹⁰ In the present study, we focused on the major time-dependent pacemaker currents to understand the negative chronotropic mechanism of autoantibodies from mothers with CHB children.

Original received December 15, 2003; resubmission received January 28, 2004; accepted February 2, 2004.

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DOI: 10.1161/01.RES.0000121566.01778.06

Materials and Methods

Cell Isolation

All experiments were performed in accordance with animal studies subcommittee regulations at VA New York Harbor Healthcare System. The isolation of single SA node cells was performed by the chopping method¹¹ with a slight modification. New Zealand young rabbits weighing 0.9 to 1.5 kg were anesthetized with intravenous injection of pentobarbital sodium (40 mg/kg). The heart was rapidly excised and immersed in a normal Tyrode's solution containing the following (in mmol/L): 140 NaCl, 5.4 KCl, 1.0 MgCl₂, 1.8 CaCl₂, 0.33 NaH₂PO₄, 10 glucose, and 5 HEPES (pH 7.4). The SA node region was excised from the heart and strips about 0.5 to 1 mm wide were cut perpendicularly to the crista terminalis border. The strips were first incubated in an oxygenated Ca²⁺-free Tyrode's solution for 15 minutes, then in the Ca²⁺-free Tyrode's solution containing elastase (0.2 mg/mL, Boehringer Mannheim) and collagenase (85 U/mL, Worthington) for 45 to 60 minutes at 37°C. The cells were then dispersed by gentle trituration and were stored at 4°C in a KB solution containing the following (in mmol/L): K glutamate 70, KCl 30, KH₂PO₄ 10, MgCl₂ 1, taurine 20, glucose 10, and HEPES 10. The cells used in this study were visually identified as long spindle-shaped cells and showed faint striation and prominent centrally located nuclei.

Solutions

At the beginning of each experiment, all cells were first superfused with normal Tyrode's solution and then switched to the appropriate solution for each current to be studied. Unless otherwise indicated, the standard pipette solution contained (in mmol/L): 140 KCl, 0.5 MgCl₂, 10 HEPES, 10 EGTA, 0.1 Na₂GTP and 5 Na₂ATP (pH 7.2).

For both L-type ($I_{Ca,L}$) and T-type ($I_{Ca,T}$) Ca²⁺ currents recording, K⁺ currents were blocked with intracellular and extracellular Cs²⁺ and 4-aminopyridine (4-AP).⁷ $I_{Ca,T}$ was recorded in a Na-free and Tris Tyrode's solution with 20 μmol/L tetrodotoxin plus 1 μmol/L nifedipine. The composition of external solutions for $I_{Ca,L}$ contained (in mmol/L): NaCl 132, CsCl 5.4, CaCl₂ 1.8, MgCl₂ 1.8, NaH₂PO₄ 0.6, HEPES 10, dextrose 5 (pH 7.4), and for $I_{Ca,T}$, contained (in mmol/L): 140 Tris-Cl, 5.4 KCl, 1.0 MgCl₂, 2.5 CaCl₂, 10 glucose, 5 HEPES (pH 7.4). The internal solution for both $I_{Ca,L}$ and $I_{Ca,T}$ recordings was the same and contained (in mmol/L): CsCl 139.8, K₂EGTA 10, MgCl₂ 2, CaCl₂ 0.062, Na₂-creatine phosphate 5, HEPES 10, Na₂ATP 3.1, Na₂GTP 0.42 (pH 7.2). The normal Tyrode's solution was used for I_K in the presence of 10 μmol/L nifedipine and 5 mmol/L 4-AP, and for I_f in the presence of 1 mmol/L BaCl₂. The internal solution for I_K contained (in mmol/L): 150 KOH, 30 HCl, 10 NaCl, 2 CaCl₂, 5 EGTA, 5 MgCl₂, 0.1 Na₂-GTP, and 5 HEPES (pH 7.2 with aspartic acid). The standard pipette solution as mentioned above was used as the internal solution for I_f .

IgG Purification

Purification of IgG has been performed as previously described.^{5,7,8} Briefly, immunoglobulin fractions containing IgG were purified from serum by protein A-Sepharose columns and confirmed to be pure by electrophoresis. IgG were obtained from three mothers whose children have CHB. These IgGs are referred to as positive IgG and contain antibodies against 48-kDa SSB/La, 52-kDa SSA/Ro, and 60-kDa SSA/Ro, as tested by ELISA and immunoblot. Negative IgG (normal IgG) was purified from sera of three healthy mothers with healthy children, and tested negative for anti-SSA/Ro and anti-SSB/La antibodies by ELISA and immunoblot.

Electrophysiological Recordings

Current Recordings

Membrane currents were recorded with amphotericin-perforated patch clamp techniques.¹² Amphotericin B (6 mg) was dissolved in 100 μL dimethyl sulfoxide, from which 10 μL was added to a 3-mL pipette solution. The perforated patches were usually established within 10 minutes. The amphotericin-perforated patch recordings

were used to reduce dilution of intracellular components, a possible cause of rundown of some membrane currents.

Data were sampled with an A/D converter (Digital 1200, Axon Instruments) and stored on the hard disk of a computer for subsequent analysis. A programmable horizontal puller (Model P-87, Sutter Instrument Company) was used to pull the electrodes. Borosilicate glass electrode (outer diameter, 1.5 mm) with resistances of 2 to 5 MΩ when filled were connected to a patch-clamp amplifier (Dagan 3900A, Dagan Corporation). Junction potentials were zeroed before the pipette touched the cell and always compensated. Pipette series resistance was compensated to minimize the duration of the capacitive transient on 10-mV depolarization from -80 mV.

$I_{Ca,L}$ was activated by depolarization pulses from a holding potential of -40 mV. For some cells, a double pulse protocol consisting of a 150-ms prepulse from a holding potential of -80 to -40 mV followed by 300-ms depolarization to 10 mV was used to inactivate the fast Na⁺ current and $I_{Ca,T}$ and minimize the rundown of $I_{Ca,L}$. $I_{Ca,T}$ was elicited by depolarizing pulses from a holding potential of -80 mV. A 1000-ms depolarization pulse from a holding potential of -40 mV to the test potentials was used to record I_K . I_f was elicited on hyperpolarizations from a holding potential of -40 to -110 mV.

Action Potential Recordings

Action potentials (APs) were recorded during stable spontaneous electrical activity (current clamp conditions) using the same set-up for current recordings described above. Cells were superfused with Tyrode's solution containing the following (in mmol/L): NaCl 140.0, KCl 5.4, NaH₂PO₄ 0.33, CaCl₂ 1.8, MgCl₂ 0.5, glucose 5.5, and HEPES 5.0; pH was adjusted to 7.4 with NaOH. The pipette solution contained the following (in mmol/L): aspartic acid 100.0, KCl 30.0, MgCl₂ 0.5, ATP- Na₂ 5.0, GTP-Na₂ 0.1, EGTA 11.0, and HEPES 10.0; CaCl₂ 5.0 (pH was adjusted to 7.2 with KOH). Experiments were performed at 35±0.5°C.

Data Analysis

All results are presented as mean±SEM. Current density expressed as pA/pF was determined by dividing current amplitude with cell capacitance. Statistical significance was determined by a Student's *t* test for paired data. A value of $P \leq 0.05$ was considered significant.

Results

Effects of Positive IgG on $I_{Ca,L}$ and $I_{Ca,T}$

To determine the relative role of ionic currents affected by maternal antibodies from mothers with CHB children in single SA node cells, first, the effect of positive IgG (10, 50, 100, and 200 μg/mL) on peak $I_{Ca,L}$ was examined. The dose-response curve of positive IgG on $I_{Ca,L}$ yielded an IC₅₀ of 59.4 μg/mL (Figure 1A). Figure 1B shows the time course of a representative current recording elicited by 300 ms depolarizing pulses to 10 mV from a holding potential of -40 mV (activating $I_{Ca,L}$ preferentially over $I_{Ca,T}$) in a K⁺ free, Cs⁺-containing solution in the presence of 5 mmol/L 4-AP before, during application of, and after washout of positive IgG. Application of positive IgG (100 μg/mL) reduced the peak of $I_{Ca,L}$ from 191 to 112 pA (41.4%). The inhibition was partly reversed on washout of IgG (160.0 pA, 83.7% recovery). In the contrary, negative IgG (100 μg/mL) did not significantly alter the time course of $I_{Ca,L}$. Figure 1C shows current density-voltage (*I-V*) relations of $I_{Ca,L}$ during control and positive IgG (100 μg/mL). Positive IgG, but not negative IgG, significantly reduced $I_{Ca,L}$ at voltages between 0 and +30 mV (Figure 1C; 46.2%±5.6% at 10 mV, n=5; $P < 0.01$) without a significant shift in the steady state activation curve

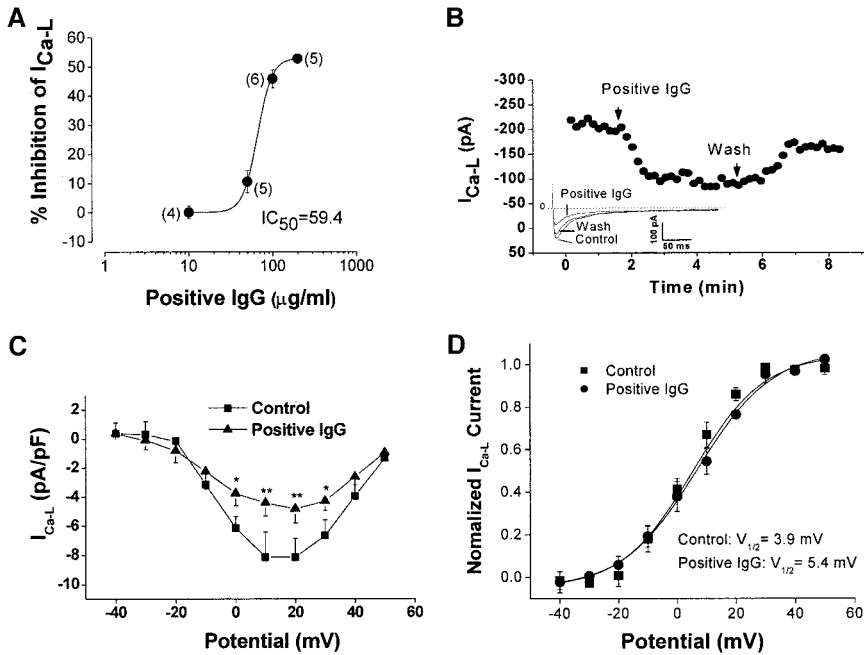


Figure 1. Effect of positive IgG on I_{CaL} . **A**, Dose-response relation for the effect of positive IgG on I_{CaL} at 10 mV from a holding potential of -40 mV. Curve was fit by non-linear regression to a sigmoidal function of the following form: $\text{effect} = 1/[1 + (K_d/[C])^n]$, where C is concentration, K_d (IC_{50}) is the concentration for half-maximal effect, and n is a constant. Each point represents mean \pm SEM data for 4, 5, or 6 cells at each positive IgG concentration, as indicated. **B**, Time course of peak I_{CaL} at 10 mV from single SA node cells before, during application of positive IgG, and after washout of positive IgG (100 $\mu\text{g}/\text{mL}$). Original traces for control, positive IgG, and washout are shown in the inset. **C**, Averaged current density-voltage relations of peak I_{CaL} in response to various depolarizing pulses from a holding potential of -40 mV before (control) and during application of positive IgG (100 $\mu\text{g}/\text{mL}$). * $P < 0.05$; ** $P < 0.01$. **D**, Voltage-dependent activation properties of I_{CaL} before (control, $n = 5$) and after positive IgG ($n = 5$). Normalized values were fitted to Boltzmann equation ($I/I_{\text{max}} = 1/[1 + \exp((V_m - V_{1/2})/k)]$) to obtain the midpotential ($V_{1/2}$) and slope factor (k). $V_{1/2} = 3.9 \pm 0.8$ mV, $k = 8.9 \pm 0.7$ for control, and $V_{1/2} = 5.4 \pm 0.9$ mV, $k = 12.1 \pm 0.9$ for positive IgG.

(Figure 1D; $V_{1/2} = 3.9 \pm 0.8$ mV in control versus $V_{1/2} = 5.4 \pm 0.9$ mV in positive IgG group, $n = 5$; $P = \text{NS}$).

Next, the effect of positive IgG on I_{CaT} was investigated. Figure 2A shows that the dose-response curve of positive IgG on I_{CaT} yielded an IC_{50} of 56.4 $\mu\text{g}/\text{mL}$. The time course of I_{CaT} before and during application of positive IgG (100 $\mu\text{g}/\text{mL}$) from representative cells is shown in Figure 2B. The inset of Figure 2B shows a current recording in response to a 200-ms depolarization test pulse to -40 mV (activating I_{CaT} preferentially over I_{CaL}) from a holding potential of -80 mV recorded in a Na-free and Tris Tyrode's solution in the presence of nifedipine (1 $\mu\text{mol}/\text{L}$) and tetrodotoxin (20 $\mu\text{mol}/\text{L}$). On depolarization, a peak inward current was elicited, mainly I_{CaT} . Similar to I_{CaL} , I_{CaT} was significantly reduced at 100 $\mu\text{g}/\text{mL}$ positive IgG (109 to 75 pA, 31.2%). I_{CaT} elicited at -40 mV from a holding potential of -80 mV was totally abolished by 40 $\mu\text{mol}/\text{L}$ Ni^{2+} ($n = 3$, data not shown). Negative IgG (100 $\mu\text{g}/\text{mL}$) did not affect I_{CaT} at all voltages tested. Figure 2C summarizes the effect of positive IgG on I - V relations of I_{CaT} . The average inhibition of I_{CaT} by positive IgG was $31.4 \pm 5.2\%$ at -40 mV and $44.1\% \pm 6.1\%$ at -20 mV ($n = 5$, $P < 0.01$).

Effects of Positive IgG on I_K and I_f

In the experiment shown in Figure 3, the effect of positive IgG on I_K was examined in normal Tyrode's solution with 10 $\mu\text{mol}/\text{L}$ nifedipine and 5 mmol/L 4-AP. Figure 3A shows the original superimposed traces recorded at 0 mV from a holding potential of -40 mV under control condition, and after superfusion with positive IgG. The current amplitudes measured near the end of depolarizing pulses and tail currents on repolarization to -40 mV were not affected by 100 $\mu\text{g}/\text{mL}$ positive IgG. Even a relatively high concentration of positive IgG (500 $\mu\text{g}/\text{mL}$) did not have significant effect on I_K (data not shown). Figure 3B shows I - V relations (current

measured at the end of pulse) in response to 1-second pulses to various potentials from -40 mV under control conditions, E-4031-sensitive current (subtracting the current after E-4031 from control) and after positive IgG. In the control, depolarization of the membrane to the test potentials between -40 to 60 mV activated a time-dependent outward current, I_K . In the presence of 5 $\mu\text{mol}/\text{L}$ E-4031, I_K was almost completely blocked and no obvious time-dependent outward current was noted. E-4031-sensitive current density-voltage relations show inward rectification indicating that the rapid component of I_K is predominant in our single SA node cells. Positive IgG had no effect on I_K . Similar findings were obtained in five other cells (step current at 0 mV: 6.0 ± 0.5 pA/pF for control versus 6.2 ± 0.6 pA/pF for positive IgG group, $n = 6$; $P = \text{NS}$).

The effect of positive IgG on hyperpolarization-activated current, I_f , is shown in Figure 4. I_f was elicited on hyperpolarization from a holding potential of -40 mV to the test potentials between -40 to -110 mV every 5 seconds in the normal Tyrode's solution with the presence of 1 mmol/L BaCl_2 . Application of positive IgG (100 $\mu\text{g}/\text{mL}$) did not significantly change the amplitude of I_f (Figure 4A). I - V relations for I_f is shown in Figure 4B (10.4 ± 1.1 pA/pF for control versus 10.0 ± 1.2 pA/pF for positive IgG, $n = 5$, $P = \text{NS}$). Similar to I_K , 500 $\mu\text{g}/\text{mL}$ IgG did not significantly affect the amplitude of I_f (data not shown).

Effects of Positive IgG on SA Node Action Potential (AP) Rate

The effects of positive IgG (100 $\mu\text{g}/\text{mL}$) were tested in spontaneously beating (162.5 ± 11.6 bpm, $n = 5$) SA node myocytes. A typical recording is shown in Figure 5. Figure 5A shows control APs at a firing normal sinus rhythm of 155 bpm in Tyrode's solution. After 1-minute superfusion of the SA node myocytes with positive IgG, there was sinus

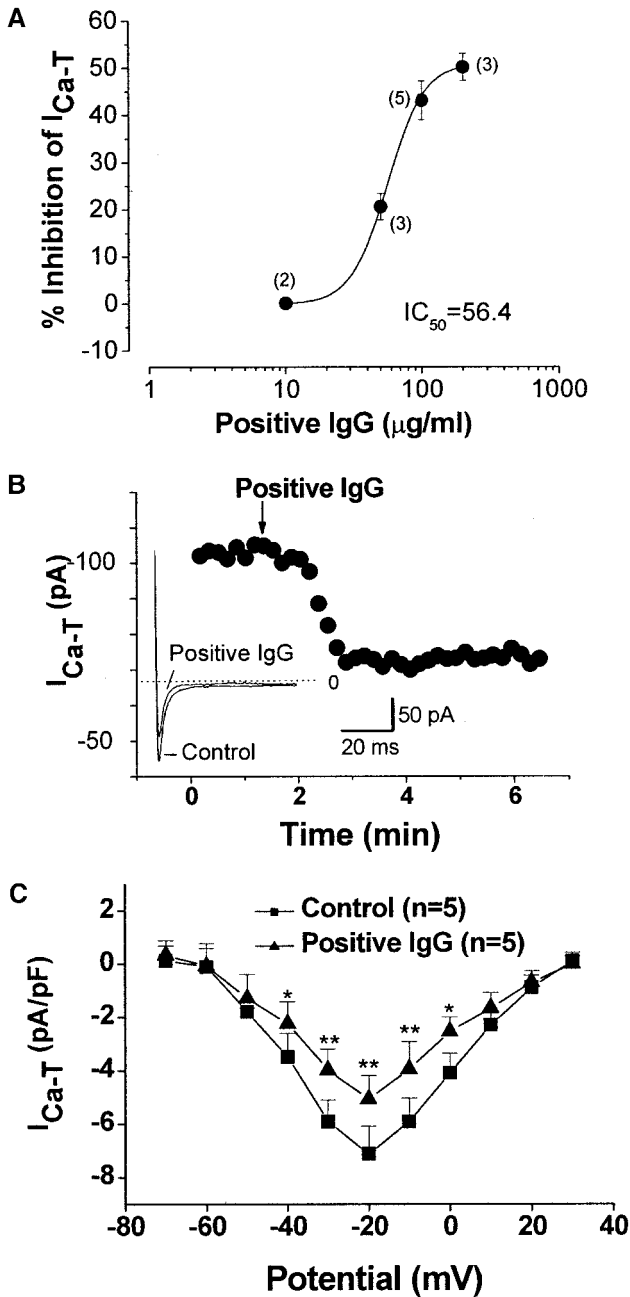


Figure 2. Effect of positive IgG on $I_{Ca,T}$. A, Dose-response relation for the effect of positive IgG on $I_{Ca,T}$ at -40 mV from a holding potential of -80 mV in single SA node cells before and during application of positive IgG ($100 \mu\text{g/mL}$). Original traces during control and positive IgG are shown in the inset. B, Time course of peak $I_{Ca,T}$ at -40 mV from a holding potential of -80 mV in single SA node cells before and during application of positive IgG ($100 \mu\text{g/mL}$). Original traces during control and positive IgG are shown in the inset. C, Averaged current density-voltage relations of peak $I_{Ca,T}$ in response to various depolarizing pulses from a holding potential of -80 mV before (control, $n=5$) and during application of positive IgG ($100 \mu\text{g/mL}$, $n=5$). * $P<0.05$; ** $P<0.01$.

bradycardia at irregular firing intervals (about 78 bpm, Figure 5B). After 2 minutes of superfusion with positive IgG, further bradycardia (about 66 bpm) was observed (Figure 5C). After 10 minutes superfusion with Tyrode's solution, only partial recovery was seen (Figure 5D). During positive IgG application, the AP amplitude was reduced (from 84.3 ± 7.9 to 71.5 ± 12.6 mV; $P<0.05$, $n=5$), the slope of phase 4 was also

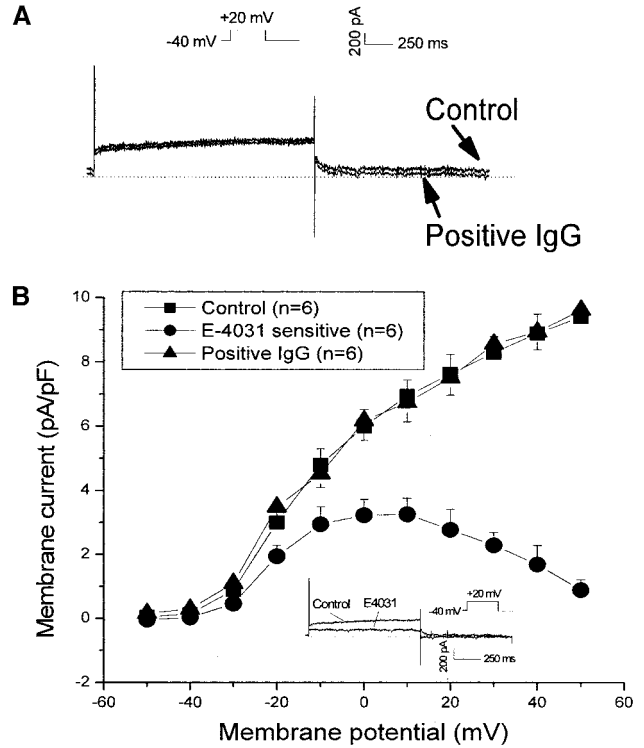


Figure 3. Effect of positive IgG on I_K . A, Lack of effect of positive IgG on the outward current, I_K . Superimposed original traces elicited at 20 mV from -40 mV before (control) and during application of positive IgG ($100 \mu\text{g/mL}$) from a single SA node cell. B, Averaged current density-voltage relations under control condition, E-4031-sensitive current ($5 \mu\text{mol/L}$ E4031), and application of positive IgG ($n=6$ for each group). Current amplitude was measured at the end of depolarization pulse. E-4031-sensitive currents were obtained by subtracting currents after application of E-4031 from control (before E-4031). Representative original traces recorded from a single SA node cell at 20 mV from -40 mV are shown in the inset.

reduced (from 62.8 ± 4.2 to 41.9 ± 6.9 mV/sec; $P<0.05$, $n=5$) without significant change in the maximum diastolic potential (MDP; control -63.8 ± 2.6 mV versus -58.0 ± 7.0 mV; $P=NS$, $n=5$). In contrast, superfusion of SA node myocytes ($n=3$) with negative IgG did not alter the spontaneous AP rate, AP amplitude, phase 4 slope, or MDP (AP rate, 164.3 ± 10.5 versus 162.5 ± 12.5 bpm; MDP, 164.3 ± 10.5 versus 162.5 ± 12.5 mV; phase 4 slope, 61.5 ± 5.5 to 62.4 ± 9.6 mV/sec; $P=NS$, $n=5$; MDP, -62.5 ± 4.5 versus -61.8 ± 6.6 mV, respectively).

Discussion

In the present study, we found that maternal antibodies from mothers of children with CHB decreased $I_{Ca,L}$ and $I_{Ca,T}$, two important currents to spontaneous cardiac pacing, without altering I_K and I_f in rabbit SA node cells. In addition, positive IgG caused sinus bradycardia in single SA node myocytes. These effects were not seen with normal IgG from healthy mothers with healthy children. This is the first study that provides a functional basis for sinus bradycardia associated with CHB, and points to the important role of both $I_{Ca,T}$ and $I_{Ca,L}$ in this sinus bradycardia.

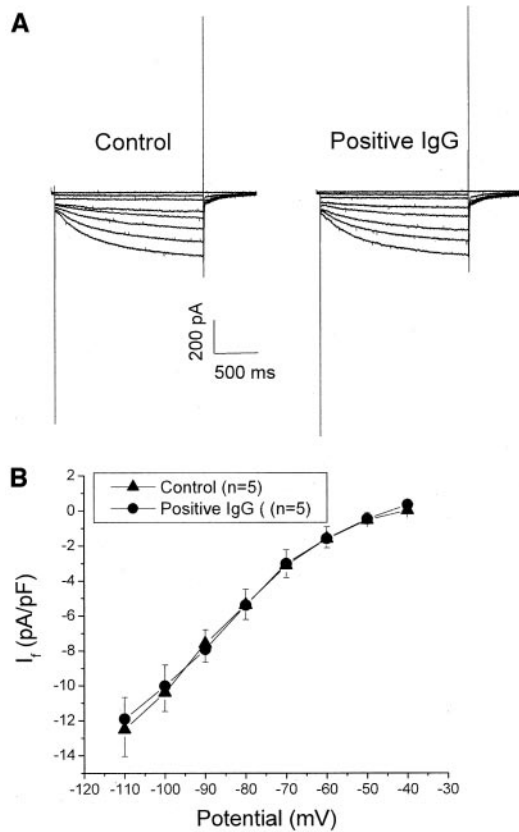


Figure 4. Effects of positive IgG on I_t . A, Original current traces from a single SA node cell elicited by hyperpolarization to test potentials between -40 and -110 mV in increments of 10 mV from a holding potential of -40 mV before (control, $n=5$) and during application of positive IgG ($n=5$, $100 \mu\text{g/mL}$). B, Averaged current density-voltage relations of I_t before and during application of positive IgG ($100 \mu\text{g/mL}$) to various potentials from a holding potential of -40 mV.

Bradycardia and SA Node Involvement

Because abnormalities of the AV node are the hallmark of autoantibody-associated CHB, the AV node rather than the SA node was the main focus of previous publications⁵⁻⁸ even during routine clinical diagnosis of CHB.⁹ Although Garcia et al,¹³ using isolated rabbit heart, and our group, using Langendorff perfused isolated rabbit⁵ and human fetal hearts,⁷ have also observed significant sinus bradycardia in their models, this bradycardia was not emphasized and its electrophysiological basis have not been investigated. Unexpectedly, we also observed high incidence of sinus bradycardia in an experimental mouse model of CHB developed by passive transfer of human autoantibodies into pregnant mice⁶ and in by directly immunization of female mice or rabbits with SSA/Ro antigen,^{7,8} suggesting a possible involvement of SA node. This observation is further supported by clinical data by Brucato et al,⁹ demonstrating sinus bradycardia in children born to mothers seropositive to SSA/Ro antibodies. This novel finding is of clinical importance because it is only recently that clinicians caring for infants with CHB have begun focusing their attention on sinus bradycardia in addition to AV node conduction abnormalities. Indeed, human fetal autopsies^{14,15} showed calcification of the SA node,

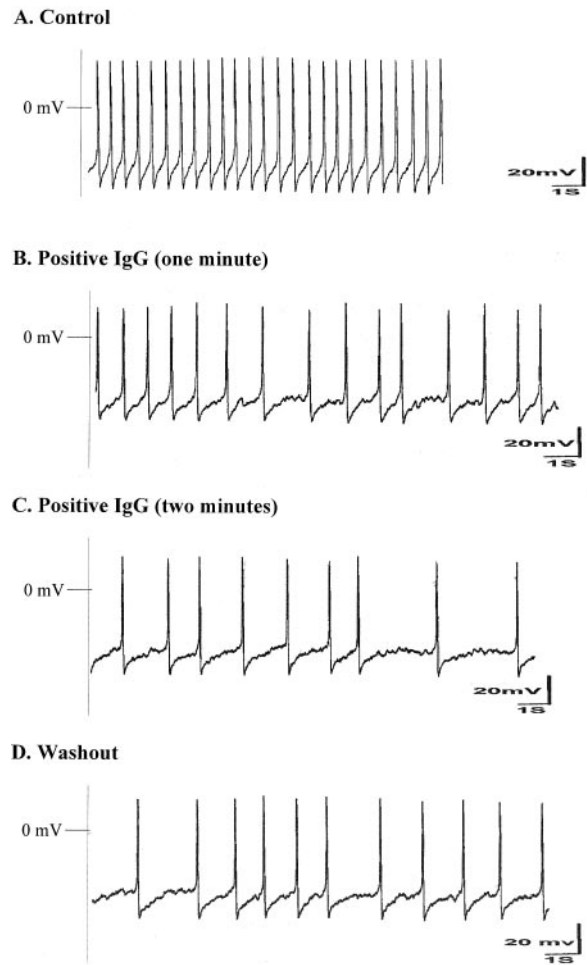


Figure 5. Effects of positive IgG on action potentials from SA node myocytes. A, Control action potentials (APs) recorded from an SA node myocyte in Tyrode's solution at 35°C . B, APs recorded during the application of positive IgG ($100 \mu\text{g/mL}$) for 1 minute. C, APs recorded during the application of positive IgG for 2 minutes. D, APs recorded during washout with Tyrode's solution for 10 minutes.

further suggesting that the SA node may be affected. Because circulating maternal autoantibodies are directed against intracellular autoantigens, hypotheses have been proposed that intracellular SSA/Ro and SSA/La proteins are being trafficked to the cell surface during development by the induction of stress proteins, hormonal influences, viral infection, or apoptosis.¹⁶⁻¹⁹ The mechanisms by which these events alter SA node pacemaker activity remain unclear.

Effect of Positive IgG on the Membrane Currents and Action Potential in SA Node Cells

In general, the total IgG levels of CHB patients are higher than that from healthy individuals. The level of IgG in CHB cord sera at the time of delivery varied from 500 to 1500 mg/dL,²⁰ which corresponds to 5 to 15 mg/mL. To study the role of antibodies from mothers with CHB children on currents involved in spontaneous pacing, we used the concentration of IgG (80 to $100 \mu\text{g/mL}$), which we have previously shown to inhibit Ca channels in single ventricular myocytes^{7,21} and determined dose-response curves as shown

in Figures 1A and 2A. Higher concentrations of 800 to 1200 $\mu\text{g}/\text{mL}$ were required to induce complete AV block in whole human fetal and rabbit heart perfused in a Langendorff fashion. Because the serum specimens from the infants were obtained after birth, ie, weeks later after CHB manifestation in the fetus, the exact concentration of IgG at that time is not known.

Single SA node cells were voltage-clamped to assess the effect of positive IgG on the four major time-dependent ionic currents involved in diastolic depolarization, I_K , $I_{\text{Ca,L}}$, $I_{\text{Ca,T}}$, and I_f . Our data show that both $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$ are reduced significantly by positive IgG in the single rabbit SA node cells. This is consistent with the observed inhibition of phase 4 diastolic depolarization in SA node cells, suggesting that the negative chronotropic effect of positive IgG is derived, at least in part, from reduction of both $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$.

In the present study, we showed $I_{\text{Ca,L}}$ inhibition by positive IgG, suggesting that the L-type Ca^{2+} channel is a target for maternal antibodies in SA node cells. The consequences of this inhibition may account for the sinus bradycardia. Because we show that positive IgG reduces $I_{\text{Ca,L}}$ without shifting I - V relations or activation curve (voltage dependence was unchanged), the underlying mechanism for positive IgG-induced reduction of $I_{\text{Ca,L}}$ may not be due to changes in channel gating. Indeed, we have previously demonstrated that positive IgG inhibited ventricular $I_{\text{Ca,L}}$ by reducing open times and increasing closed times at the single channel level in both human fetal⁷ and rat²¹ heart. We proposed that these data may, in part, explain the basis of the whole-cell $I_{\text{Ca,L}}$ inhibition by positive IgG in the present study. However, the exact mechanism by which positive IgG affects $I_{\text{Ca,L}}$ gating in the rabbit SA node is yet to be determined.

T-type Ca^{2+} channels are usually present in the SA cells and Purkinje fibers of the heart. The physiological role of the T-type Ca^{2+} channel is not completely understood, and believed to be involved in the pacemaker activity. Indeed, in vivo studies have shown that sinus bradycardia can be induced in conscious rats²² and in anesthetized dogs²³ by mibefradil alone, a selective T-type Ca current blocker. Similar dose-dependent decrease in heart rate has been also reported in human.²⁴ Therefore, both $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$ in SA node cells may contribute to the negative chronotropic effect of maternal antibodies to Ro/La.

It is noteworthy that normal IgG lacking anti-Ro/SSA and anti-La/SSB antibodies did not affect both Ca^{2+} channels (L- and T-types), indicating that it is unlikely that other unidentified components of IgG may contribute to the effects. However, because we did not use affinity-purified antibodies in this study, we cannot completely rule out the contribution of unidentified components of IgG to our observations.

We have previously shown that positive IgG did not alter the transient outward K current, I_{to} , the inward rectifier K current, I_K , and the fast Na current, I_{Na} .²¹ Because time-dependent currents are absent after the administration of E-4031 plus nifedipine, the instantaneous I - V relation represents a background current. This background current could be a mix of some time-independent current that have been suggested to be involved in SA node pacemaking activity, ie, Na^+ - K^+ pump, Na^+ - Ca^{2+} exchanger. However, we did not see

any effect on the net current after applying positive IgG, indicating that this time-independent background current may not be involved in bradycardia associated with CHB. Altogether, positive IgG seems to selectively affect Ca^{2+} channels (L- and T-types) but not other currents such as I_f , I_K , I_{to} , and I_{Na} suggesting specificity to Ca^{2+} channels.

Potential Significance

In rabbit SA node cells, $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$ are important currents in late phase of diastolic depolarization.¹⁰ In the present study, we found that both $I_{\text{Ca,L}}$ and $I_{\text{Ca,T}}$ were reduced by positive IgG in SA node cells. Our observations provide direct evidence for the ionic mechanism of the negative chronotropic action of maternal antibodies to SSA/Ro and SSB/La proteins associated with CHB. The findings raise the possibility that sinus bradycardia which often precedes AV block may indicate the potential for AV conduction abnormalities. The findings also provide new insights to the pathogenesis of CHB and potentially to the therapeutic management of a disease considered irreversible and for which currently available therapies are refractory.

Acknowledgments

This study was supported by an NIH grant (HL-55401) and VA Medical Research Funds (Merit Grant Award and REAP grant) to M.B. IgGs were kindly provided by Dr Jill Buyon through the Research Registry for Neonatal Lupus (AR-4220). We would like to thank the animal laboratory staff for their assistance.

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