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Stereospecific Interaction of Tocainide with the Cardiac Sodium Channel

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SUMMARY

The antiarrhythmic action of type I antiarrhythmic drugs may be mediated via binding of the drugs to a receptor associated with the cardiac sodium channel. This suggested that the effects of type I drugs might be stereospecific. We measured the effect of the tocainide stereoisomers (which have stereospecific antiarrhythmic effects) on conduction time and on radioligand binding to the cardiac sodium channel. The concentration-dependent effects of the individual enantiomers of tocainide on interventricular conduction time measured during constant rate ventricular pacing at 350 msec were assessed in 47 isolated perfused rabbit heart preparations. Significant increases ($p < 0.05$) in conduction time occurred for both *R*-(−)-tocainide (75 μM, 10 ± 5 msec) and *S*-(+)-tocainide (150 μM, 4 ± 1 msec). *R*-(−)-Tocainide was more potent than the *S*-(+)-tocainide in prolonging conduction time ($p < 0.05$). This stereospecific prolongation of conduction time suggested a stereospecific interaction with the sodium channel. The affinities of the enantiomers for the channel were measured with a radioligand binding assay using [^{3}H]batrachotoxinin benzoate and freshly isolated cardiac myocytes. Both enantiomers inhibited [^{3}H]batrachotoxin benzoate binding, but the IC_{50} (±SD) values were different: *R*-(−)-tocainide 184 ± 8 μM; *S*-(+)-tocainide, 546 ± 37 μM ($p < 0.003$). Tocainide isomers are stereospecific in terms of prolonging conduction time and in binding to the sodium channel. The stereospecific electrophysiologic effects of tocainide may result from binding to a receptor associated with the cardiac sodium channel.

The antiarrhythmic action of the type I antiarrhythmic drugs generally is thought to be mediated via blockade of the fast sodium channel which may be reflected in depression of the inward sodium current (2), depression of the maximum upstroke velocity of the action potential (3), and prolongation of conduction time. A number of models have been developed to explain the interaction between antiarrhythmic drugs and the cardiac sodium channel (e.g., Refs. 3 and 4). These models invoke the binding of type I antiarrhythmic drugs to specific sites or receptors associated with sodium channels (5). Stereospecificity is one characteristic feature of receptor binding. If type I drugs do bind to a receptor, then they should have stereospecific effects on sodium channel blockade.

Tocainide, a type I agent, is a racemic mixture of *R*-(−)- and *S*-(+)-isomers which Byrnes *et al.* (6) have demonstrated to have a stereospecific antiarrhythmic effect of tocainide in a chloroform-induced ventricular fibrillation model. *R*-(−)-Tocainide was 3-fold more effective than the *S*-(+)-isomer. This suggested that tocainide might interact in a stereospecific fashion with the cardiac sodium channel. We have determined the effect of the stereoisomers of tocainide on two measures of sodium channel blockade: conduction time and radiotoxin binding. The first hypothesis tested in this study was that the effects of tocainide on prolongation of conduction time would be stereospecific. The effects on conduction time of the individual enantiomers of tocainide were therefore assessed in 47 isolated perfused heart preparations. The second hypothesis tested was that binding of tocainide to the sodium channel would also be stereospecific. We used an assay which measures the binding of [^{3}H]BTXB, a sodium channel-specific toxin, to freshly isolated rat cardiac myocytes (7–9). This assay has been used to identify a receptor associated with the sodium channel for the type I agents (10). The affinity of tocainide for the channel was estimated from its ability to inhibit the binding of BTXB to the channel.

Materials and Methods

Electrophysiologic Measurement

Preparation. New Zealand White rabbits were given heparin (1000 units intravenously) and anesthetized with sodium pentobarbital (30 mg/kg). The heart was excised and the aorta cannulated. The heart was perfused retrogradely in a non-recirculating system with Krebs-Henseleit solution (37°), saturated with 95% O_2/5% CO_2 (pH 7.4) containing (in mmol/liter): NaCl 127, KCl 2.3, KH_2PO_4 1.5, $MgSO_4$ 0.6, $CaCl_2$ 2.7, $NaHCO_3$ 25, and glucose 5.6. Each isolated heart prep-

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ABBREVIATIONS: BTXB, batrachotoxinin benzoate; MEM, minimal essential medium; BSA, bovine serum albumin.

aration was enclosed in a humidified cylinder maintained at 37°. The perfusion rate was adjusted to maintain a perfusion pressure of 30–40 mm Hg (approximately 18 ml/min) using a constant pressure pump (MX International Inc., Aurora, CO). The perfusion pressure was monitored and recorded with an Electronics for Medicine VR16 recorder.

Protocol. Baseline electrophysiologic measurements were made 15 min after beginning perfusion with Krebs-Henseleit solution (11). Electrophysiologic measurements were then made 15 min after the addition of either *R*-(−)-tocainide, *S*-(+)-tocainide, or saline. Every sixth heart was selected to be treated with vehicle (saline) alone, and the drugs were assigned randomly. Near-maximum electrophysiologic effects were seen after 15 min of infusion of tocainide; however, occasionally, some increase appeared after 15 min. Because of this time-dependent increase in activity, sequential interventions in a single preparation were not done and a separate drug treatment was given to each individual preparation. The treatments were saline or a single concentration of *R*-(−)-tocainide or of *S*-(+)-tocainide over a period of 15 min.

Electrophysiologic studies. Epicardial monophasic action potentials were recorded simultaneously from the right and left ventricular free walls using bipolar suction electrodes (ABO Trading, Tullv., 1138, S-430, 41 Kullavik, Sweden) and techniques described by Olsson *et al.* (12). This technique has been validated by comparison to transmembrane recordings of action potential duration by Hoffman *et al.* (13). Monophasic action potential catheters were held 1—2 mm from the epicardial surface in a sleeve. When the electrophysiologic measurements were being taken, the catheters were advanced and held in position on the epicardium by suction (−25 mm Hg) for not more than 3 min. Following each recording the catheters were withdrawn 1—2 mm from the epicardial surface. Subsequent recordings were made at the same epicardial site. The monophasic action potential signals were recorded by a DC coupled Electronics for Medicine VR16 recorder with frequency response to 1000 Hz and stored on a Nicholet 4094 digital memory oscilloscope. Recordings were made following 1 min of ventricular pacing at a cycle length of 350 msec. Stimuli were introduced at twice diastolic threshold and a 2-msec pulse width. Bipolar silver-silver chloride electrodes with an interelectrode distance of 5 mm were placed in the right ventricular pulmonary outflow tract, and a second pair was placed on the left ventricular lateral free wall. The ventricular pacing sites were constant throughout the experiment. This cycle length was chosen because the effect of tocainide on conduction time is markedly frequency dependent, with its maximal effect in the range of 250–400 msec (14). The method for measuring conduction time is outlined in Fig. 1. Five complexes were measured for each recording and the mean was reported for each preparation. These five measurements were within 1.6 msec of each other, indicating a high degree of reproducibility.

Advantages and limitations. There are both advantages and disadvantages of our *ex vivo* myocardium model for measuring conduction time. This isolated perfused heart model allows the ability to control potassium, sodium, and pH of the perfusate, all of which can alter electrophysiologic actions of antiarrhythmic drugs. As well, electrophysiologic studies *in vivo* normally require the use of a general anesthetic, which can independently alter the sodium channel. A potential limitation in the study is the use of suction monophasic action potential electrodes. When suction is applied, local myocardium may be injured. The level of suction used was the minimal amount necessary to maintain position of the electrodes on the myocardium, and the maximum duration of suction was less than 3 min. Furthermore, no local injury was seen at the catheter application sites with the nitroblue sodium dye technique. Another technical limitation is the method used to measure conduction time in the study. Although the sites of the pacing electrodes and monophasic action potential electrodes were constant throughout the study, we cannot exclude the possibility that drug-related changes in conduction time could indicate a change in the route of propogation of the impulse rather than a true change in conduction velocity. To minimize the likelihood of detecting a change in conduction time that reflected only a change in propagation route, we paced the heart in two ways. First, we paced the right ventricle and measured the time delay from the onset of the right ventricular free wall monophasic action potential record to the onset of the left ventricular monophasic action potential record. Second, we paced the left ventricle and measured the time delay from the onset of the left ventricular free wall monophasic action potential record to the onset of the right ventricular monophasic action potential record. If concentration response changes with right and left ventricular pacing (when very different routes or propagation would be used) produced similar changes in conduction time delays that such a change would likely reflect change in conduction time delays that such a change would likely reflect change in conduction velocity. We avoided measurements of the time from stimulus artifact to upstroke of monophasic action potential as an index of conduction time as changes in latency rather than changes in conduction time could alter such measurements.

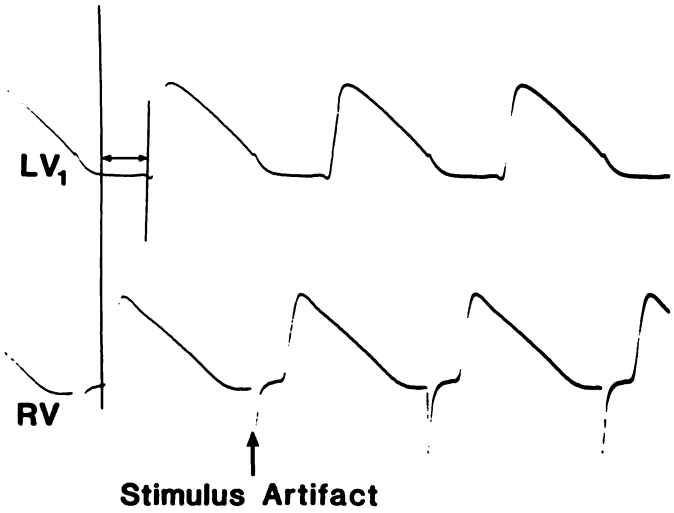

Fig. 1. An example of the measurement of conduction time. During right ventricular pacing, the conduction time was measured as the time delay from the upstroke of the right ventricular monophasic action potential signal to the upstroke of the left ventricular monophasic action potential signal.

Vital staining. Nitroblue tetrazolium was added to the perfusate to identify injured tissue at the sites of suction electrode placement (15). No local injury was seen.

Radioligand Binding Studies

Myocyte preparation. Cardiac myocytes were isolated from adult male Sprague-Dawley rats (200–250 g) using the method of Kryski *et al.* (16). Rats were killed by cervical dislocation and the heart was rapidly removed. The aorta was cannulated and the heart was perfused retrograde in a Langendorff perfusion apparatus. The heart was perfused and later incubated with a series of solutions which were equilibrated with 95% O_2/5% CO_2 at 37°. The solutions were based on Joklik's minimal essential medium supplemented with 1.2 mM $MgSO_4$ and 1 mM DL-carnitine (MEM). They included a rinse solution (MEM), a digestion solution [MEM with 0.1% (w/v) fatty acid-free BSA and 0.1% collagenase], a calcium solution (MEM with 1 mM $CaCl_2$ and 1% fatty acid-free BSA), and an incubation solution (MEM with 50 μM $CaCl_2$ and 1% dialyzed BSA). First, the heart was perfused at 20° for 5 min with rinse solution; then it was perfused for 20 min at 37° with digestion solution. The ventricles were then removed, minced with scissors, and rinsed at 37° for 15 min with calcium solution. Calcium solution was then removed by aspiration and the tissue pieces were incubated at 37° for 15 min with digestion solution in a shaking water bath. Dispersed cells were decanted into a plastic centrifuge tube and the residual tissue was shaken again with digestion solution. This resulted in almost total dispersion of the heart. The pooled myocytes

were then filtered through a 185-μm silkscreen mesh, collected by gentle centrifugation, and rinsed with incubation solution. The cells were again collected by gentle centrifugation and resuspended in incubation solution.

This method routinely yielded about 100 mg (dry weight) of myocytes, which corresponds to 2×10^7 cells (16, 17). The cells were 82–92% viable rod-shaped cells which excluded trypan blue. The cells maintained a resting membrane potential of −75 to −80 mV, and have been characterized metabolically by Kryski *et al.* (16).

Radioligand binding. Myocytes (6×10^6) in 50 μl of incubation buffer were incubated with 1.3 μM sea anemone toxin II, 13 nM [^{3}H]BTXB (50 Ci/mmol), and 0.13 mM tetrodotoxin for 45–60 min at 37° (see Ref. 18 for a review of sodium channel toxins). Tetrodotoxin was added to prevent depolarization induced by sodium influx; without tetrodotoxin, no specific binding is observed (9, 19). Various concentrations of tocainide enantiomers were included in the incubations. Assays were done in parallel with tubes containing 0.2 mM aconitine to define nonspecific binding. Concentration-response curves for both stereoisomers were performed on common preparations of cells and toxins. Reactions were terminated by adding 10 ml of KHS buffer (Krebs-Henseleit-BSA; 127 mM NaCl, 2.33 mM KCl, 1.30 mM KH_2PO_4, 1.23 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 50 μM $CaCl_2$, 1% BSA) equilibrated with 95% O_2/5% CO_2 and incubated at 37° for 1 min, then filtered through a Whatman GF-C 24-mm filter and washed five times with 5 ml of rinse buffer (25 mM Tris-Cl, pH 7.4, 130 mM NaCl, 5.5 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM glucose, 50 μM $CaCl_2$.) The filters were then dried and counted in Econofluor scintillation fluid. The retained radioactivity represents [^{3}H]BTXB bound to the myocyte.

Materials

Collagenase was purchased from Cooper Biomedical, [^{3}H]BTXB was from New England Nuclear, and tetrodotoxin, aconitine, sea anemone toxin II, and albumin were from Sigma Chemical Co. Tocainide stereoisomers were provided by Astra Pharmaceutical.

Statistical Analysis

Continuous data for conduction times are presented as msec change from baseline, $\bar{x} \pm$ SE. One-way analysis of variance was used to calculate the significance of concentration-dependent electrophysiologic changes of the *R*-(−)- and *S*-(+)-isomers compared to the change seen with saline. Duncan's Multiple Range Test was then used to ascertain at what concentrations significant changes were observed. The differences between the dissociation constants of the individual enantiomers were assessed using the paired *t* test.

Results

Electrophysiology. A total of 47 preparations was studied; 8 received vehicle (saline) treatment alone; 18 received *S*-(+)-tocainide, and 21 received *R*-(−)-tocainide. No significant change from baseline was noted during vehicle treatment in conduction time (Table 1).

Table 1 and Fig. 2 compare the effects of conduction time of various concentrations of the individual enantiomers of tocainide. *R*-(−)-Tocainide was more potent than *S*-(+)-tocainide in prolonging conduction time. *R*-(−)-Tocainide prolonged conduction time at both pacing sites at concentrations of 75 μM and greater, whereas *S*-(+)-tocainide had a smaller but significant effect only at the maximum concentration tested.

Radioligand binding. The affinity of the tocainide stereoisomers for the cardiac sodium channel was studied by measuring the ability of the isomers in various concentrations to inhibit the binding of BTXB to cardiac myocytes (10). Typical results are shown in Fig. 3. In this example, the IC_{50} values for *R*-(−)-tocainide and *S*-(+)-tocainide are 176 μM and 520 μM, respectively. The mean IC_{50} values (±SD) for three experiments are 184 ± 8 μM for *R*-(−)-tocainide and 546 ± 37 μM for *S*-(+)-tocainide ($p = 0.003$). These results demonstrate that tocainide stereospecifically inhibits BTXB binding to the sodium channel, with *R*-(−)-tocainide being greater than 3-fold more potent than *S*-(+)-tocainide.

Discussion

The antiarrhythmic action of type I antiarrhythmic drugs may be mediated via binding of the drugs to a receptor associated with the cardiac sodium channel. The presence of a putative receptor suggested that the effects of type I drugs might be stereospecific. We have confirmed this by demonstrating that tocainide (which has stereospecific antiarrhythmic effects) also has stereospecific effects both on an electrophysiologic model and a radioligand model of sodium channel blockade.

Electrophysiologic Model

The data in Table 1 and Fig. 2 demonstrate that *R*-(−)-tocainide is significantly more potent than *S*-(+)-tocainide in prolonging conduction time in the intact *ex vivo* rabbit myocardium. This demonstrates that tocainide stereospecifically prolongs conduction time, a measurement thought to reflect sodium channel blockade. The changes in conduction time measured in this study likely reflect changes in conduction velocity. Conduction time was measured in two ways. When the right ventricle was paced, the time delay was measured from the onset of the right to the onset of the left ventricular monophasic action potential record. Similarly, when the left ventricle was paced, the time delay was measured from the onset of the left to the onset of the right ventricular monophasic action potential record. Therefore, two pacing techniques were used to assess two differing routes of propagation. Independent of whether the right or left ventricles were being paced, similar concentration-response relationships were noted. This suggests that route of propagation does not alter whether conduction time is prolonged by tocainide. It is difficult to conceive of a drug effect which would stereospecifically alter route of propagation. Our method of measurement of conduction time consists of assessing the time delay between propagated beats. We avoided measurement of conduction time as the delay from stimulus artifact to onset of activation because such measures would include possible change in latency. Significantly, *R*-(−)-tocainide is more potent both as an antiarrhythmic drug and in prolonging conduction time, indicating that the two may be related.

Stereospecificity of action is one of the persuading lines of evidence that local anesthetics and class I antiarrhythmic agents bind to specific receptor sites associated with sodium channels. Akerman *et al.* (20) demonstrated that several experimental local anesthetics (e.g., RAC 109, RAC 322, and RAC 535) exhibited significant stereospecificity in their ability to block action potential propagation in a variety of neural preparations. The stereospecific block by RAC 109 and RAC 421 in squid axons is markedly frequency dependent (21). Furthermore, RAC 109 exhibits stereospecific inhibition of [^{3}H]BTXB binding to nerve sodium channels. Thus, stereospecificity is a well established feature of local anesthetic effect on nerve sodium channels.

There is also evidence of stereospecific effects of these drugs on cardiac tissue. Akerman *et al.* (20) showed that the RAC 109 enantiomers differed in their ability to suppress ouabain-in-

TABLE 1

Effect of tocainide stereoisomers on conduction time

The effects of tocainide stereoisomers on conduction time measured during constant rate ventricular pacing at 350 msec are presented. Baseline data, those recorded during drug treatment, and resultant change in conduction time from baseline are shown. The changes seen at various concentrations of tocainide isomers are compared to the change in conduction time seen with saline (by analysis of variance and Duncan's Multiple Range Test). When pacing the left ventricle (LV), the conduction time was measured as the time delay from the upstroke of the LV monophasic action potential signal to the upstroke of the right ventricle (RV) monophasic action potential signal. During RV pacing, the conduction time was measured as the time delay from the upstroke of the RV monophasic action potential signal to the upstroke of the LV monophasic action potential signal.

Concentration (μM)	LV pacing						RV pacing					
	Baseline	*S*-(+)	Δ*S*-(+)	Baseline	*R*-(−)	Δ*R*-(−)	Baseline	*S*-(+)	Δ*S*-(+)	Baseline	*R*-(−)	Δ*R*-(−)
Saline	30 ± 3	30 ± 2	0 ± 1	30 ± 3	30 ± 2	0 ± 1	32 ± 10	32 ± 9	0 ± 13	32 ± 10	32 ± 9	0 ± 3
19	30 ± 3	32 ± 4	2 ± 2	31 ± 4	32 ± 7	1 ± 3	36 ± 14	30 ± 10	−6 ± 4	30 ± 17	32 ± 20	2 ± 2
37.5	30 ± 6	31 ± 9	1 ± 2	34 ± 4	39 ± 7	5 ± 2	28 ± 11	31 ± 7	3 ± 3	36 ± 3	39 ± 7	3 ± 3
75	32 ± 3	33 ± 6	1 ± 2	23 ± 4	33 ± 10	10 ± 5*	30 ± 12	35 ± 10	5 ± 5	31 ± 16	40 ± 20	9 ± 3*
100	27 ± 3	28 ± 5	1 ± 3	25 ± 6	36 ± 5	11 ± 1*	32 ± 10	32 ± 14	0 ± 6	24 ± 4	36 ± 8	12 ± 3*
150	28 ± 3	33 ± 7	4 ± 3*	30 ± 4	37 ± 7	7 ± 1*	30 ± 9	33 ± 11	3 ± 2	24 ± 3	32 ± 2	8 ± 4*

* Significantly different, concentration-dependent change (relative to the changes seen with saline), $p < 0.05$, as assessed by analysis of variance.

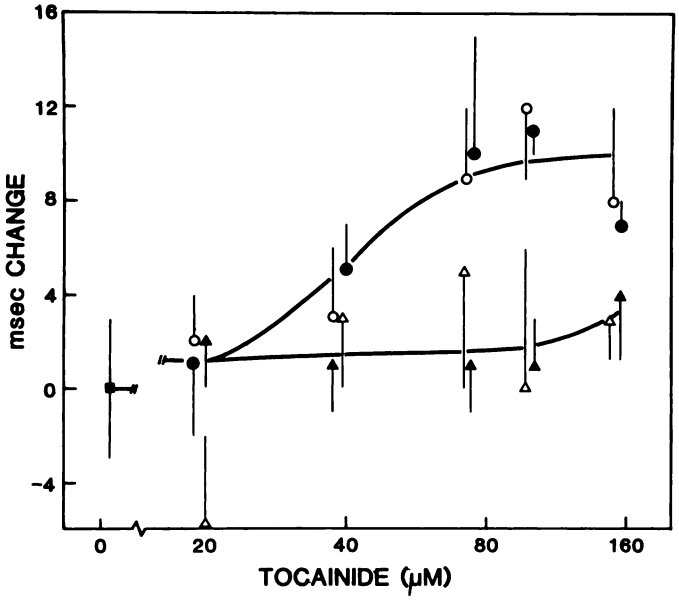

Fig. 2. Concentration-dependent changes in conduction time (± standard error) in msec with the enantiomers of tocainide relative to that seen with vehicle alone. Conduction time when pacing the left ventricle is shown as: ○, *R*-(−)-tocainide; △, *S*-(+)-tocainide. Conduction time when pacing the right ventricle is shown as: ●, *R*-(−)-tocainide; ▲, *S*-(+)-tocainide. Data are presented as the mean; for standard deviation and significance see Table 1.

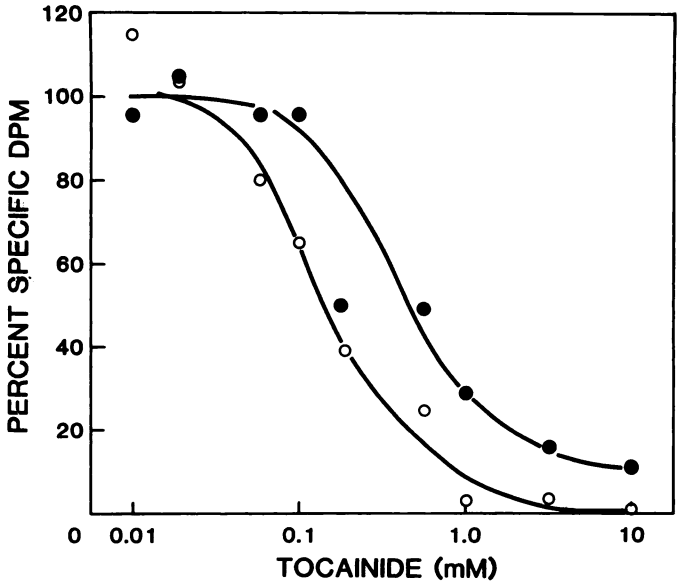

Fig. 3. Effect of tocainide stereoisomers on the binding of [^{3}H]BTXB to myocytes. Binding of [^{3}H]BTXB was measured in the presence of various concentrations of *R*-(−)-tocainide (○) and *S*-(+)-tocainide (●). Results are expressed as per cent of maximum specific binding.

duced ventricular tachycardia in dogs. RAC 109 also exhibits stereospecific blockade of the inward sodium current in voltage-clamped guinea pig myocytes (22). As well, the tocainide isomers exhibit stereospecific effects at 20–25° on the myocardial sodium channel.[1] In contrast, Mirro *et al.* (Ref. 23, Table 3) showed that in dog Purkinje fibers the stereoisomers of quinidine stereospecifically depressed V_{max}. Quinidine (10 μM) depressed V_{max}, whereas quinine (10 μM) did not. Together, these electrophysiologic models of sodium channel activity—both nerve and heart—are consistent with the notion that antiarrhythmic drugs interact with a stereospecific binding site associated with the sodium channel.

Radioligand Model

The radioligand binding experiments more directly measure the binding of antiarrhythmic agents to a receptor associated with the sodium channel. In this assay we measured the binding of [^{3}H]BTXB, a sodium channel-specific toxin, to freshly isolated rat cardiac myocytes. The BTXB is thought to bind to a specific site on cardiac sodium channels because the binding occurs only in the presence of sea anenome toxin II (a second sodium channel-specific toxin), is inhibited by aconitine (a sodium channel-specific toxin known to inhibit BTXB binding to nerve channels), is reversible, and is saturable (7–9). We have also shown that type I antiarrhythmic agents inhibit the binding of BTXB to myocytes at concentrations similar to their effective serum concentrations and with the same rank order of potency (10). This indicates that this assay can be used to measure the affinities of type I antiarrhythmic agents for specific binding sites associated with sodium channels.

In this study we have used this radioligand assay to compare the affinities of the stereoisomers of tocainide for the channel. The results showed the respective IC_{50} values of the stereoisomers to be 184 μM for *R*-(−)-tocainide and 546 μM for *S*-(+)-tocainide. This demonstrates that tocainide stereospecifically binds to the cardiac sodium channel, and that *R*-(−)-tocainide

[1] C. W. Clarkson, personal communication.

binds more than 3-fold more tightly than *S*-(+)-tocainide. Thus, there is a striking similarity in the stereospecificity of *R*-(−)- and *S*-(+)-tocainide in antiarrhythmic action *in vitro*, in prolonging conduction time, and in binding to the sodium channel. With the data of Clarkson (22), this strongly suggests that type I antiarrhythmic agents bind to a stereospecific receptor associated with the cardiac sodium channel.

The stereospecific interaction of tocainide isomers with the cardiac sodium channel may partly explain the broad range of total concentrations of tocainide associated with a therapeutic effect. Notably, Sedman *et al.* (24) have shown marked interindividual and intraindividual variability in the concentration of *S*-(+)- to *R*-(−)-enantiomers during chronic tocainide therapy in patients with ventricular arrhythmias (range from 1.3:1 to 4:1, respectively). In that study (24), the amount of the *R*-(−)-enantiomer present at any total concentration varied by more than 2-fold. It has also been reported that differences exist in the individual enantiomer pharmacokinetic characteristics, in particular, their metabolic profiles (25). This suggests that disease states which alter hepatic or renal function could further change the enantiomer ratio. Therefore, interindividual and intraindividual differences in concentration of the stereoisomers could account for sizeable differences in electrophysiologic and therapeutic effects.

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