Kinetics of rate-dependent slowing of intraventricular conduction by the class Ib antiarrhythmic agent tocainide *in vivo*

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- 1 The effects of the class I antiarrhythmic agent, tocainide, on intraventricular conduction were assessed in guinea-pigs, anaesthetized with pentobarbitone sodium 60 mg kg⁻¹, i.p.
- 2 After electrical ablation of the sinus node, heart rate was controlled by atrial pacing. His bundle electrograms were recorded by means of an epicardial bipolar electrode.
- 3 During continuous stimulation, comparison of HV intervals measured at a cycle length of 475 ms, with HV intervals measured at a cycle length of 250 ms yielded the following results: 25.26 ± 0.64 ms versus 25.02 ± 0.70 ms (NS), at baseline, 26.65 ± 0.80 ms versus 29.88 ± 1.13 ms (*P* < 0.001) after i.v. administration of 30 mg kg⁻¹ tocainide, and 28.04 ± 0.64 ms versus 36.24 ± 1.31 ms (*P* < 0.001), after addition of 20 mg kg⁻¹ tocainide. Thus, tocainide caused HV intervals to increase in a strictly rate-dependent fashion.
- 4 In order to characterize the rate-dependent class I activity of tocainide in terms of its binding kinetics to sodium channels, fractional sodium channel block was estimated from drug induced reductions of intraventricular conduction velocity ($\Delta\theta$). On abruptly changing the drive cycle length from 500 ms to 250 ms, $\Delta\theta$ reached a new steady state with rate constants of 1.23 ± 0.09 beat⁻¹ and 1.28 ± 0.09 beat⁻¹, after administration of 30 mg kg⁻¹ and addition of 20 mg kg⁻¹ tocainide, respectively. At a basic drive cycle length of 250 ms $\Delta\theta$ recovered with time constants of 250.29 ± 23.32 ms and 183.04 ± 8.03 ms after administration of 30 mg kg⁻¹ and addition of 20 mg kg⁻¹ tocainide, respectively.
- 5 The experimentally determined kinetic parameters were implemented into a mathematical model that assumes drug binding to sodium channels in terms of a periodical two-state process. Rate-dependent reductions in conduction velocity during continuous stimulation after administration of tocainide were closely approximated by steady state reductions in sodium channel availability as calculated on the basis of the aforementioned model.
- 6 In agreement with previously published *in vitro* studies, our data, obtained *in vivo*, confirm the classification of tocainide as a class I antiarrhythmic agent with fast onset and offset kinetics. The kinetic parameters obtained *in vivo* can be used in order to predict steady state reductions in conduction velocity at a wide range of frequencies.

Keywords: Tocainide; lignocaine; antiarrhythmic drugs; bundle of His; rate-dependent; mathematical models

Introduction

Class I antiarrhythmic drugs have been shown to reduce sodium channel availability in a rate-dependent fashion (Heistracher, 1971; Campbell, 1983b). Mathematical models have been developed which describe the rate-dependent nature of sodium channel block by class I antiarrhythmic drugs in terms of a restriction of drug binding to certain conformational channel states (Hondeghe & Katzung, 1977; Starmer *et al.*, 1984; Starmer & Grant, 1984; Starmer, 1986). With regard to the kinetics of binding to the high-affinity state, class I antiarrhythmic agents can be subdivided into agents with 'fast' and 'slow' onset-offset kinetics (for review see Campbell, 1992). On the basis of experimental data and theoretical models it has recently been suggested that class Ib drugs, which exhibit fast onset-offset kinetics (e.g. lignocaine, tocainide and mexiletine) are likely to cause less proarrhythmic events than slow kinetic agents (Starmer *et al.*, 1991; Nesterenko *et al.*, 1992; Campbell, 1983a). The present study was designed to investigate the use-dependent action of the class Ib antiarrhythmic drug, tocainide, an oral congener of lignocaine, on intraventricular conduction velocity *in vivo*. To date, the rate-dependent class I action of tocainide has only been characterized *in vitro* (Oshita *et al.*,

1980; Borchard *et al.*, 1985; Kodama *et al.*, 1987). Since both antiarrhythmic and proarrhythmic actions of class I agents are mediated via depression of conduction velocity on a multicellular level (Wald *et al.*, 1980; Spinelli & Hoffman, 1989; Nesterenko *et al.*, 1992), the characterization of rate-dependent drug effects on intraventricular conduction *in vivo* may be of considerable clinical relevance.

Furthermore, we wished to determine whether rate-dependent depression of conduction by tocainide *in vivo* can be characterized on the basis of an established theoretical model of periodical ligand binding to sodium channels (Starmer & Grant, 1984; Starmer *et al.*, 1984; Starmer, 1986). Because the onset-offset kinetics of rate-dependent suppression of conduction by sodium channel blockers depends upon fibre orientation (Turgeon *et al.*, 1992), we chose to assess conduction time within the well defined physiological conduction pathway of the His-Purkinje system. For this purpose we used the recently described method of epicardial His bundle recording in the guinea-pig (Todt & Raberger, 1992; Todt *et al.*, 1992a).

Methods

The animals used in this study were handled in accordance with the animal welfare regulations of the University of

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Vienna. The method and the experimental protocol were approved by the Animal Subjects Committee of the University of Vienna and by the Austrian Ministry of Science.

Guinea-pigs of either sex (300–500 g, $n = 11$) were anaesthetized with pentobarbitone sodium (60 mg kg⁻¹, i.p.) and respired artificially by a small animal ventilator (Type 874072, Braun Melsungen AG, FRG). Using needle electrodes, standard limb leads I–III were recorded by means of Grass 7P4F EKG preamplifiers and Grass model 7DAG driver amplifiers (Grass Instrument Co., Quincy, Mass., U.S.A.). The right jugular vein was dissected and a catheter was inserted for drug application. Following sternotomy the heart was exposed and sinus rhythm was abolished by application of high frequency alternating current (1.8 MHz; Siemens Radiotom 612) to the junction of the Superior vena cava with the right atrium. Thereafter heart rate was controlled by bipolar pacing of the left atrium with a Medtronic Model 5328 programmable stimulator and a Grass S48 Stimulator. The cycle length of stimulation was set at the value of spontaneous sinus rhythm before ablation of the sinus node. The electrical activity of the bundle of His was recorded by means of an epicardial bipolar electrode which was placed in the aortic-right atrial groove (Todt & Raberger, 1992; Todt *et al.*, 1992a). The electrode was connected to a Gould model 11 G412301 amplifier (Gould Inc., Cleveland, Ohio, U.S.A.) using a filter setting of 30 Hz–20 kHz. Both His bundle and standard limb ECG recordings were stored on magnetic tape (portable instrumentation recorder model 17-6500-00, Gould Inc., Cleveland, Ohio, U.S.A.). For measurement of HV intervals, the recordings were displayed on a digital storage oscilloscope (OS 4200, Gould Inc., Cleveland, Ohio, U.S.A.).

HV intervals were measured from the H-deflection in His bundle electrograms to the earliest peak of the R wave in any of the recorded leads. Usually the earliest R-peak was registered in the His-bundle electrogram. This measurement technique encompasses the ventricular activation time (MacFarlane & Veitch Lawrie, 1989) and thus allows assessment of the time span from the beginning of activation of the specialized ventricular conduction system until ventricular activation reaches its maximum. No measurements were performed if any change of the conduction pathway was indicated by alterations of the QRS vector in standard limb leads I–III.

Atrial stimulation for assessment of steady state conduction slowing (protocol 1)

The right atrium was continuously paced at selected cycle lengths between 200 ms and a cycle length 10 ms below the cycle length of spontaneous AV nodal rhythm. HV interval measurements were performed on beats 11–15 of each train and the mean value of all 5 measurements was calculated.

Atrial stimulation for assessment of onset of conduction slowing (protocol 2)

Eight basic drive beats, paced at a cycle length of 500 ms, were followed by a rapid train of 11 beats, paced at a cycle length of 250 ms.

Atrial stimulation for assessment of recovery from conduction slowing (protocol 3)

After a basic drive train of 8 depolarizations at a cycle length of 250 ms, an extrastimulus was introduced at a coupling interval of 225 ms. Thereafter the coupling interval was increased by 25 ms steps until interference of the spontaneous pacemaker was encountered. Because the excitation wave elicited by atrial stimulation has to pass the AV node before activating the ventricular conduction system, AV nodal accommodation may cause differences between the cycle length of stimulation (S_1S_2) and the cycle length of ven-

tricular activation (H_1H_2 intervals). Differences between S_1S_2 and H_1H_2 were usually encountered only at coupling intervals of less than 275 ms. If present, such disparities were compensated by adjustment of coupling intervals to yield the desired H_1H_2 interval. The same applies to drug-induced changes in AV conduction. Subsequently the terms cycle length, activation rate, coupling interval etc. refer to activation of the ventricular conduction system.

Drug administration

The stimulation protocol was performed during a drug-free baseline period and following cumulative i.v. infusion of tocainide (30 mg kg⁻¹ and 20 mg kg⁻¹, each over 30 min). The protocol was started 10 min after the end of each drug administration. The interval between administration of the two doses was 60 min.

Data evaluation

Percentage sodium channel block, b , was calculated from measurements of HV intervals by the formula

$$[\text{eq 1}] \quad b = \Delta\theta = [1 - (HV_b/HV_d)] \cdot 100$$

where HV_b denotes the mean HV interval at baseline and HV_d indicates the HV interval during a given activation following drug administration (Packer *et al.*, 1989). Under the assumption of a stable conduction pathway, eq 1 yields the drug induced fractional reduction of conduction velocity within the His-Purkinje system ($\Delta\theta$).

The onset rate constant of block (λ^*) was estimated by fitting $\Delta\theta$ of every depolarization following an abrupt decrease in cycle length to the single exponential equation (Packer *et al.*, 1989):

$$[\text{eq 2}] \quad b_n = b_{ss} + (b_0 - b_{ss})e^{-n\lambda^*}$$

where b_n is the block associated with the n^{th} stimulus, b_{ss} is the steady state block during the rapid train, and b_0 is the initial block prior to the abrupt beginning of rapid pacing.

In order to calculate the time constant of recovery from block, τ_{rec} , the $\Delta\theta$ values of each extrastimulus after the basic train were plotted against the respective coupling interval. The time course of recovery from steady state block at a cycle length of 250 ms is then given by

$$[\text{eq 3}] \quad b(t) = (b_{250} - r_\infty) \cdot (e^{-(t-250)/\tau_{rec}}) + r_\infty$$

where b_{250} is the amount of block at a coupling interval of 250 ms and r_∞ denotes the final level of block that is approached if the recovery pause was extended for infinite time.

Analytical procedure

Starmer developed a theoretical model of drug binding to sodium channels by assuming a periodical two-state binding process (Starmer, 1986). Accordingly, sodium channels are considered to switch between high affinity and low affinity states during the cardiac cycle. Thus, steady state block (b_{ss}), which is present if heart rate is maintained stable during a prolonged period of time, can be described by:

$$[\text{eq 4}] \quad b_{ss} = \frac{a_\infty(1 - e^{-\frac{t_h}{\tau_{ss}}})e^{-\frac{t_l}{\tau_{ss}}} + r_\infty(1 - e^{-\frac{t_l}{\tau_{ss}}})}{1 - e^{-\lambda^*}}$$

where t_h and t_l are the durations of high and low affinity states, respectively. a_∞ and r_∞ denote the fractional block that would be achieved if channels were held for infinite time in high and low affinity states, respectively. Since tocainide has been shown to bind to both activated and inactivated sodium channels (Borchard *et al.*, 1985; Kodama *et al.*, 1987), t_h roughly corresponds to the duration of the action

potential (APD) and t_r corresponds to the interval between action potentials.

τ_{on} , the time constant of drug binding during the high affinity state, can be calculated from the relationship:

$$[\text{eq 5}] \quad \lambda^* = \frac{t_a}{\tau_{on}} + \frac{t_r}{\tau_{rec}}$$

where λ^* and τ_{rec} are determined from eq 2 and 3.

a_∞ was calculated by the following approach: during a rapid train according to protocol 2, the amount of block at the end of any action potential ($b_{n(\text{APD})}$) is given by

$$[\text{eq 6}] \quad b_{n(\text{APD})} = (a_\infty - b_n)(1 - e^{-\text{APD}/\tau_{on}}) + b_n$$

where b_n is the initial block at depolarization. $b_{n(\text{APD})}$ can also be estimated by a modification of eq 3:

$$[\text{eq 7}] \quad b_{n(\text{APD})} = (b_{n+1} - r_\infty) * (e^{-(\text{APD}-250)/\tau_{rec}}) + r_\infty$$

Once $b_{n(\text{APD})}$ has been determined from eq 7, a_∞ can be calculated from eq 6. In the present study $b_{n(\text{APD})}$ was calculated from the $\Delta\theta$ values of beats 10 ($=b_n$) and 11 ($=b_{n+1}$) of the rapid train according to protocol 2.

r_∞ is estimated from eq 3.

Because APD of the His-Purkinje system cannot be measured *in vivo*, we estimated APD by means of the equation:

$$[\text{eq 8}] \quad \text{APD} = 0.045 * \text{CL} + 152$$

where CL = cycle length. This equation was derived by linear regression analysis of previously published plots relating APD in isolated guinea-pig Purkinje fibres to cycle lengths between 300 ms and 1000 ms ($r = 0.96$) (Aomine, 1989). Although the relationship between cardiac repolarization and cycle length becomes hyperbolic if cycle lengths >1000 ms are included (Aomine, 1989; Surawicz, 1992), linear regression can be fitted equally well to the relationship if only the range of cycle lengths pertinent to this study are considered (Todt *et al.*, 1992b).

Statistics

Unless otherwise stated, data are given as mean $\pm$ s.e.mean. Statistical comparisons were performed using Student's *t* test for paired data with Bonferroni adjustment for multiple comparisons (Wallenstein *et al.*, 1980).

Results

Steady state decrease in conduction velocity

At baseline, steady state HV intervals were independent of changes in stimulation cycle length (25.26 ± 0.64 versus 25.02 ± 0.70 ms, NS, at stimulation cycle lengths of 475 and 250 ms, respectively). After administration of tocainide the rate of spontaneous AV-nodal rhythm decreased, thus allowing the assessment of lower frequencies than at baseline. The longest cycle length that could be achieved in all animals both during baseline and after tocainide was 475 ms (rate = 126.3 beats min^{-1}). At this cycle length HV intervals were only moderately prolonged when compared to the respective baseline values (26.65 ± 0.80 ms, NS, and 28.04 ± 0.64 ms, $P < 0.001$, after administration of 30 mg kg^{-1} and subsequent addition of 20 mg kg^{-1} tocainide, respectively). At a cycle length of 250 ms (rate = 240 beats min^{-1}) both doses of tocainide caused very pronounced increases in HV intervals when compared to the respective baseline values (29.88 ± 1.13 ms, $P < 0.001$, and 36.24 ± 1.31 ms, $P < 0.001$, after administration of 30 mg kg^{-1} and subsequent addition of 20 mg kg^{-1} tocainide, respectively). At both dosages of tocainide, HV intervals measured at cycle lengths of 250 ms were significantly longer than HV intervals measured at cycle lengths of 475 ms ($P < 0.001$).

The relationship between drive cycle length and $\Delta\theta$ values at all assessed cycle lengths is shown in Figure 1. The drug-induced increase in $\Delta\theta$ was tonic at long cycle lengths but became strongly rate-dependent at short cycle lengths. The longest cycle length that caused $\Delta\theta$ values to increase significantly was 400 ms (rate = 150 beats min^{-1}) and 475 ms (rate = 126.3 beats min^{-1}) after 30 mg kg^{-1} and after additional 20 mg kg^{-1} , respectively. Increasing the dosage of tocainide had little effect upon $\Delta\theta$ values at long cycle lengths but induced an extensive additional increase in $\Delta\theta$ at short cycle lengths.

Onset of conduction slowing

Although in some animals spontaneous AV-nodal rhythm interfered with sustained pacing at a cycle length of 500 ms

Figure 1 Relationship between sodium channel block (b), as reflected by conduction slowing ($\Delta\theta$), and cycle length (rate) during continuous stimulation. The points connected by dashed lines are measurements after 30 mg kg^{-1} tocainide; the points connected by dotted lines indicate measurements after addition of 20 mg kg^{-1} tocainide. Symbols indicate the level of significance when compared with baseline values: (▼): $P < 0.001$; (■): $P < 0.05$; (●): NS; (○): no comparison (because of interference of AV-nodal rhythm with stimulated rhythm at baseline).

Figure 2 Onset of sodium channel block (b), as reflected by conduction slowing ($\Delta\theta$), during a rapid train paced at a cycle length of 250 ms. The beats of the rapid train are numbered consecutively (abscissa scale). The rapid train was preceded by 8 basic stimuli paced at a cycle length of 500 ms (not shown). The points fitted by dashed lines are measurements after 30 mg kg^{-1} tocainide, the points fitted by dotted lines indicate measurements after addition of 20 mg kg^{-1} tocainide. Symbols indicate the level of significance when compared with baseline values: (▼): $P < 0.001$; (●): NS.

(protocol 1), such interference was not encountered during the short 8-beat basic drive train before abruptly decreasing the cycle length to 250 ms. Figure 2 shows the effect of a sudden change of cycle length from 500 ms to 250 ms on $\Delta\theta$ values after tocainide. Tocainide induced a small but non-significant increase in $\Delta\theta$ at a cycle length of 500 ms. As the cycle length was abruptly reduced to 250 ms, $\Delta\theta$ values increased to a new steady state within 2–3 beats. Although the amount of steady state block at a cycle length of 250 ms was dose-dependent, the time course of onset of block was similar for the two applied doses of tocainide. Thus, the calculated rate constants of drug induced onset of $\Delta\theta$ are in the same order of magnitude (Table 1).

Recovery from conduction slowing

An original registration demonstrating the recovery from tocainide-induced HV-prolongation is presented in Figure 3. Figure 4 shows the effect of administration of tocainide on the time course of recovery from drug-induced increase in $\Delta\theta$. Recovery of $\Delta\theta$ exhibited a monoexponential time course that allowed estimation of τ_{rec} and r_{∞} (Table 1). τ_{rec} was found to be in the same order of magnitude (<300 ms) following both doses of tocainide. r_{∞} was unaffected by 30 mg kg⁻¹ tocainide but exhibited a slight increase following addition of 20 mg kg⁻¹ tocainide.

Comparison with model predictions

In order to test whether the rate-dependent steady state increase in $\Delta\theta$ after tocainide can be predicted by a mathematical model of periodical two-state ligand binding to sodium channels (eq 4), the model parameters τ_{on} and a_{∞} were calculated according to eqs 5, 6 and 7. APD within the

His-Purkinje system cannot be measured *in vivo* and was therefore estimated by means of eq 8, which describes the relationship between APD and cycle length under drug free conditions *in vitro*. However, the administration of tocainide has been shown to shorten APD (Oshita *et al.*, 1980). In order to take such a potential shortening of APD into consideration, we calculated the APD-dependent model parameters τ_{on} and a_{∞} , as well as predicted steady state values of $\Delta\theta$ under the assumption of various degrees of shortening of APD (Table 2). The calculated values of steady state block were only negligibly changed by the assumption of drug-induced reductions in APD. This is the case because reductions in steady state $\Delta\theta$, as would be predicted if only APD was shortened in eq 4, are counteracted by concomitant increases in the calculated value of a_{∞} . Figure 5 shows the predicted curves, relating $\Delta\theta$ to steady state cycle length. Again, for each dosage, separate curves were calculated by assuming drug induced shortening of APD by 0%, -5%, -10%, -15%, -20%. Because these assumptions of APD shortening had little influence on shape and position of the calculated relationship between $\Delta\theta$ and steady cycle length, the respective curves in Figure 5 are superimposed, giving the impression of a single curve for each dosage of tocainide. Also shown in Figure 5 are the experimentally determined steady state values of $\Delta\theta$ at selected cycle lengths. Clearly, measured values of $\Delta\theta$ are in good agreement with the predicted relationship between $\Delta\theta$ and steady state cycle length. This is also indicated by the residual sum of squares (SS) of predicted values and experimental data, presented in Table 2.

Discussion

It has been recently shown that the slow binding kinetic properties of quinidine and procainamide can be characterized from measures of impulse propagation within the framework of a mathematical model suggesting periodical drug binding to Na⁺-channels (Packer *et al.*, 1989; Villemaire *et al.*, 1992). The present study provides an *in vivo* analysis of rate-dependent conduction slowing induced by tocainide, which exhibits fast kinetic binding properties *in vitro* (Oshita *et al.*, 1980; Borchard *et al.*, 1985; Kodama *et al.*, 1987).

Table 1 Binding kinetics of tocainide as determined from measurements of HV intervals

	λ^* (beat ⁻¹)	τ_{rec} (ms)	r_{∞} ($\Delta\%$)
Tocainide 30 mg kg ⁻¹	1.23 ± 0.09	250.29 ± 23.32	0.00 ± 0.63
+ Tocainide 20 mg kg ⁻¹	1.28 ± 0.09	183.04 ± 8.03	5.79 ± 0.36

Values are mean $\pm$ s.e.mean.

Figure 3 Original registration of epicardial His Bundle electrograms at baseline (a) and following cumulative administration of 30 mg kg⁻¹ and 20 mg kg⁻¹ tocainide (b). Shown are the two last beats of a basic train consisting of 8 beats, paced at a cycle length of 250 ms, and one extra beat after a longer coupling interval which is adjusted for differences in AH-intervals to yield a 500 ms interval between the His activations before and after the pause. Measurements of HV intervals are indicated by dotted lines. Clearly, introduction of a 500 ms pause has no effect upon HV intervals at baseline. Administration of tocainide prolongs HV intervals during the rapid train. After the pause, HV prolongation recovers partially.

Figure 4 Time course of recovery from sodium channel block (b), as reflected by conduction slowing ($\Delta\theta$). A conditioning train of 8 depolarizations at a cycle length of 250 ms was followed by a test stimulus at variable coupling intervals. $\Delta\theta$ of the test depolarization is displayed for each coupling interval. The points fitted by dashed lines are measurements after 30 mg kg⁻¹ tocainide, the points fitted by dotted lines indicate measurements after addition of 20 mg kg⁻¹ tocainide. Symbols indicate the level of significance when compared with baseline values: (▼): $P < 0.001$; (◆): $P < 0.01$; (■): $P < 0.05$; (●): NS; (○): no comparison (because of interference of AV-nodal rhythm with stimulated beats at baseline).

Table 2 Binding kinetics of tocaïnide as calculated from a mathematical model that assumes drug binding to sodium channels in terms of a periodical two-state process

APD ($\Delta\%$)	Tocainide (30 mg kg ⁻¹)					+ Tocainide (20 mg kg ⁻¹)				
	0	- 5	- 10	- 15	- 20	0	- 5	- 10	- 15	- 20
τ_{on} (ms)	185.25	182.75	180.05	177.12	173.94	201.59	202.67	203.88	205.26	206.82
a_{∞} ($\Delta\%$)	28.82	30.49	32.30	34.28	36.43	57.26	61.65	66.61	72.26	78.74
b_{200} ($\Delta\%$)	22.38	22.37	22.36	22.34	22.33	41.85	41.82	41.79	41.76	41.72
b_{250} ($\Delta\%$)	16.92	16.92	16.92	16.92	16.92	30.47	30.46	30.46	30.46	30.46
b_{475} ($\Delta\%$)	5.96	5.98	5.99	6.01	6.03	12.03	12.05	12.07	12.10	12.12
b_{800} ($\Delta\%$)	1.65	1.66	1.67	1.68	1.69	6.91	6.92	6.93	6.94	6.95
SS	38.3	38.78	39.34	39.97	40.69	55.59	56.05	56.6	57.23	57.97

Model parameters (τ_{on} , a_{∞}) and values of rate-dependent steady state block are calculated under assumptions of variable degrees of drug induced reductions of APD. b_{200} , b_{250} , b_{475} , b_{800} denote calculated block values at steady state cycle lengths of 200, 250, 475 and 800 ms respectively. SS = residual sum of squares between all measured steady state values of $\Delta\theta$ and predicted values.

Figure 5 Comparison of experimentally determined steady state values of sodium channel block (b), as reflected by conduction slowing ($\Delta\theta$), after 30 mg kg⁻¹ tocaïnide (○) and additional 20 mg kg⁻¹ (▽), with respective predictions as calculated from eq 4 (dotted lines). Analogously to the data presented in Table 3, steady state reductions in $\Delta\theta$ are predicted under the assumption of drug-induced reductions in APD by 0%, -5%, -10%, -15% and -20%. The respective predicted curves are superimposed. However, the deviations of the predicted curves from each other are negligible, thus giving the impression of a single curve for each dosage of tocaïnide. The residual sum of squares (SS) of predicted values and experimental data are presented in Table 2.

In our model, the chosen procedure of atrial pacing following ablation of the sinus node not only allows the assessment of HV-conduction at slow rates without interference with sinus rhythm, but also of HV intervals at activation rates pertinent to the frequency pattern of ventricular tachycardia in man. Because of the reported difficulties in achieving selective His bundle pacing *in vivo* (Williams *et al.*, 1976), we did not attempt to pace directly the bundle of His. Furthermore, in small animals like the guinea-pig, atrial frequencies higher than 200 beats min⁻¹ are conducted in a 1:1 pattern to the ventricles thus eliminating the need to pace the bundle of His in order to assess HV conduction at high rates. By contrast, *in vitro* techniques, such as microelectrode impalements of the papillary muscle are limited with regard to the assessment of high frequencies by the increasing mechanical activity of the preparations (Weirich & Antoni, 1988). On the other hand, resting membrane potential, an important modulator of sodium channel block, can only be controlled *in vitro*. In the present study we avoided pacing at cycle lengths <200 ms in order to exclude phase 3 block. Nevertheless, any characterization of the dynamics of sodium channel block from data obtained *in vivo* is limited by the

inability to exclude shifts in resting membrane potential, which contribute to drug binding.

Tocainide depresses HV-conduction in a strictly rate-dependent manner, pronounced depression of intraventricular conduction only occurring at short cycle lengths. Increasing the dosage caused an increase in rate-dependent block as well as a shift in significant conduction slowing to longer cycle lengths. As illustrated in Figure 1, a substantial increase in dosage of tocaïnide may produce little extra increase in block at long cycle lengths, but may be associated with a very profound additional depression of conduction velocity at short cycle lengths. Thus, as the dosage of tocaïnide is increased, the amount of additional conduction slowing that may be encountered during a period of tachycardia appears to be essentially unpredictable if only cycle lengths of >500 ms (rates of <120 beats min⁻¹) are considered.

The steady state relationship between $\Delta\theta$ and heart rate was approximately linear for both dosages (Figure 1). These dynamics of frequency-dependent steady state block are in excellent agreement with previously published calculations using *in vitro* kinetic data (Weirich & Antoni, 1990).

Steady state $\Delta\theta$ after tocaïnide was reached within two beats of a rapid train at a cycle length of 250 ms. In guinea-pig ventricular muscle, the rate-dependent reduction of V_{max} induced by tocaïnide has been reported to occur with an onset rate constant of 0.277 AP⁻¹ (Campbell, 1983a), which is lower than the values of 1.23 (30 mg kg⁻¹ tocaïnide) and 1.28 beat⁻¹ (30 + 20 mg kg⁻¹ tocaïnide) obtained in the present study. This difference may be accounted for by the longer APD within the His-Purkinje system than in ventricular muscle. Upon cessation of continuous stimulation, steady state block induced by tocaïnide recovered rapidly. The recovery time constants that were found in the present study reasonably agree with those reported *in vitro* (Oshita *et al.*, 1980; Borchard *et al.*, 1985; Kodama *et al.*, 1987) and, thus, confirm the classification of tocaïnide as a fast sodium channel blocker within the novel framework of the 'Sicilian gambit' (τ_{rec} < 300 ms; Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991). An additional slower recovery time constant of 2.1–3.8 s was found by Oshita *et al.* (1980) in guinea-pig isolated papillary muscle. Because of the limited range of coupling intervals that can be assessed *in vivo*, estimation of time constants >1 s would have required an extrapolation far beyond the measured data points and might have been associated with considerable variance. We, therefore, decided to apply a monoexponential fit to the recovery data. Furthermore, from visual inspection of the recovery curves, the contribution of a second (slower) time constant to the overall amount of conduction slowing was regarded as only minimal.

As a major finding of our study, the relationship between drug-induced slowing of conduction and cycle length closely follows the model of a two-state process of drug binding to

Na⁺-channels proposed by Starmer (1986). Thus, depression of conduction occurring during continuous stimulation over a wide range of cycle lengths can be predicted, if a limited number of kinetic parameters is provided (λ^* , τ_{rec} , a_∞ , r_∞). Increasing the dosage of tocainide induced a pronounced increase in rate-dependent block although the parameters λ^* and τ_{rec} remained in the same order of magnitude. Thus, within the framework of the described mathematical model, the dose-dependent increase in frequency-dependent block resulted predominantly from a substantial increase in a_∞ (and to a lesser extend in r_∞). In the clinical setting r_∞ could be approximated by the steady state decrease in conduction velocity at normal (low) heart rates. Using the mathematical

approach described in the methods section (eqs 6 and 7), a_∞ can be estimated from reductions in conduction velocity measured during a short period of rapid stimulation. Thus, once the values of λ^* and τ_{rec} of an agent exhibiting class I action have been determined *in vivo*, steady state reductions of conduction velocity can be predicted for a large range of frequencies using a simple stimulation protocol (protocol 2).

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