Canine Electrocardiographic and Cardiac Electrophysiologic Changes Induced by Procainamide

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SUMMARY
Microelectrode technics were used to study the effects of procainamide (PA) on electrophysiologic properties of canine Purkinje fibers (PF) perfused in an extracorporeal circuit with arterial blood of donor dogs. Donor electrocardiogram (ECG) and blood pressure (BP) and electrophysiologic properties of the isolated PF were simultaneously monitored. PA, 25 mg/kg, was administered as a single intravenous injection and plasma PA concentrations (PPAC) were determined spectrophotometrically. A decrease in PP automaticity was noted within 5-10 min of PA injection. Within 20 min, when PPAC was 10 µg/ml, amplitude and maximal rate of rise of phase 0 of the AP and conduction velocity (CV) decreased and alterations in AP duration and refractoriness were noted. BP and ECG rate were unchanged but the QRS complex had widened. At PPAC = 10-20 µg/ml (induced by further PA infusion) the aforementioned changes were accentuated. At PPAC > 20 µg/ml membrane responsiveness was significantly depressed. These experiments suggest that therapeutic PA levels rapidly decrease PF automaticity. Slowing of conduction commences later, simultaneously with changes in QRS duration. Within the same time span and range of plasma levels there are alterations in AP duration and refractoriness. Membrane responsiveness is not significantly depressed until PPAC is well into the clinically toxic range.

Additional Indexing Words:
Automaticity Plasma concentration of procainamide Blood perfusion Plasma potassium concentration Ventricular conducting system Purkinje fiber action potential characteristics Microelectrode technics QRS duration

Procainamide is a mainstay of cardiac antiarrhythmic therapy. Its effects on the electrocardiogram and especially changes that reflect drug toxicity have been studied extensively. Procainamide plasma levels also have been the subject of much clinical research. It has been established that a concentration of 4-10 µg/ml encompasses the usual range of therapeutic plasma levels although lower concentrations may be associated with cessation of clinical arrhythmias. Concentrations higher than 10 µg/ml most often are associated with cardiac toxicity.

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The effects of procainamide on the electrophysiologic properties of isolated cardiac tissues have been documented. Hoffman, in studies on canine Purkinje fibers, and Szekeres and Papp, in studies on rabbit atrium, have determined that procainamide decreases the automaticity of isolated cardiac tissues and slows conduction velocity. The latter change appears to accompany a loss in action potential amplitude and decrease in maximal slope of phase 0 of the action potential. However, for these changes to develop in isolated Purkinje fibers, concentrations of procainamide in Tyrode solution of at least 10-30 μg/ml were required.

The current study was undertaken to see if we could correlate findings for the three areas mentioned: clinical electrocardiographic effects of procainamide, therapeutic and toxic plasma concentrations, and electrophysiologic effects of procainamide on cells of the cardiac conducting system. To permit these correlations, we used a method developed in this laboratory for perfusion of isolated cardiac tissues with arterial blood from a donor animal.

Methods

Nine experiments were conducted utilizing 18 mongrel dogs weighing 20–30 kg. For each experiment, one dog was anesthetized with sodium pentobarbital, 30 mg/kg. The heart was rapidly removed and the Purkinje fiber bundles were excised from right or left ventricle and pinned to the wax bottom of a Lucite perfusion chamber with a volume of 4.5 ml. They were then perfused with a modified Tyrode solution containing (in mM/liter) NaCl, 137; NaHCO₃, 12; dextrose, 5.5; KCl, 4; NaH₂PO₄, 1.8; MgCl₂, 0.5; CaCl₂, 2.7. The Tyrode solution, warmed to 36–37°C in a glass heat exchanger, was pumped (Buchler Instruments, Polystaltic Pump) into the chamber at a constant flow rate which varied in different experiments from 10 to 13 ml/min. Extracellular driving stimuli were delivered to the preparation by means of Teflon-coated bipolar silver wires. The stimulus strength was 1–1.5 times threshold and duration was 1 msec. Cycle length in each experiment was set to match donor heart rate; the range for the series was 500–630 msec.

The Purkinje fibers were impaled with glass capillary microelectrodes filled with 3M KCl and having tip diameters of less than 1 μ and resistances of 10–30 Mohms. The recordings from those impalements which could be maintained throughout an experiment could be included in this study. The microelectrodes were coupled by a 3M KCl interface to an Ag-AgCl bar leading to a Bioelectric Instruments NF-1 amplifier. The output was displayed on the upper beam of a Tektronix RM 565 oscilloscope and was led into a Tektronix Type 0 operational amplifier used to electronically derive the first derivative of the input signal. This signal was displayed on the lower beam of the RM 565 oscilloscope, permitting calibration and monitoring of maximal rate of rise of phase 0, using technics previously described. For studies of conduction, time for passage of the propagated action potential between two intracellular microelectrodes was recorded at rapid sweep speeds to facilitate accurate measurement. The method used for online determination of time intervals has been described previously.

To study the effect of procainamide on the membrane responsiveness a premature stimulus (S₂) at least 50 ms or twice threshold was introduced at various levels of membrane potential during phases 3 and 4. Maximal slope of phase 0 of premature action potentials (R₂) originated at various times during the repolarization phase of the basic action potential (R₁) was plotted as a function of the membrane voltage at which the R₂ arose. The effective refractory period was studied by impaling one intracellular microelectrode in a Purkinje fiber approximately 3 mm from the stimulus site and the second microelectrode in a Purkinje fiber 4–10 mm from the proximal site and distal to the area of maximal action potential duration or gate. The earliest premature response (R₂) elicited at the proximal microelectrode site which propagated across the area of maximal action potential duration to the distal microelectrode site was considered to define the end of the effective refractory period.

To initiate blood perfusion the second (donor) dog was anesthetized with sodium pentobarbital and heparinized with 3 mg/kg sodium heparin. A femoral artery and vein were cannulated and arterial blood pumped into the tissue chamber in place of Tyrode solution. Effluent from the chamber was pumped through an air trap and infused into the femoral vein. Previous studies have shown the stability of this method and the viability of both the intact animal and the isolated tissues during blood perfusion. The differences between control values in Tyrode solution and blood have also been reported previously.

Plasma electrolytes (Na, K, Cl, CO₂, Ca, and P) measured periodically during these studies (flame photometer or autoanalyzer) showed little variation. The range of values for plasma potassium concentration for the series was 3.2–4.3 mEq/liter. The variation in any one experiment did not vary by more than 0.6 mEq/liter.
exceed 0.6 mEq/liter. Donor and chamber blood pHi and O2 saturation did not vary significantly in any experiment. The ECG of the donor animal was monitored continuously as was arterial pressure, utilizing a Statham manometer. The Purkinje fiber preparations were permitted to stabilize for ½ hour during blood perfusion. At this point, control readings of action potential amplitude, maximal slope of phase 0, action potential duration measured to 50, 75, and 100% repolarization, effective refractory period, conduction time (between two intracellular microelectrodes), and slope of phase 4 of the action potential (reflecting automaticity) were made. In two experiments the preparations were permitted to beat spontaneously until a stable rate and rhythm had been attained. These were used to further study the effects of procainamide upon automaticity. All results were recorded on Polaroid film.

Procainamide (Ponestyl, E. R. Squibb) was then administered as a single 25-mg/kg intravenous injection to the donor animal. Infusion time was 1–2 min. Plasma procainamide levels were determined spectrophotometrically utilizing the method of Mark et al.9 Samples of donor arterial blood were drawn at intervals of 1–15 min after the initial injection and values for procainamide concentration were correlated with changes in the aforementioned electrophysiologic parameters as well as with changes in the donor electrocardiogram and arterial pressure. In three experiments, chamber blood was collected at the same time as donor arterial blood. Differences in procainamide concentration in the simultaneous specimens were less than 0.5 μg/ml. For induction of procainamide toxicity, a 5-mg/min procainamide infusion was started, 90–120 min after the initial rapid injection. Plasma procainamide concentrations were measured as described above, and the same parameters were studied.

**Results**

**Plasma Concentration of Procainamide**

Following administration of a single intravenous injection of 25 mg/kg procainamide there was a rapid fall in plasma concentration until, after 20 min, the plasma level reached the range of 5–10 μg/ml (fig. 1). Thereafter the decline was far more gradual, procainamide concentration not falling below 5 μg/ml until approximately 3 hours after the initial injection. Except for alterations in automaticity, which will be described below, within the first 20 min there were no changes in the electrocardiogram of the donor or the electrophysiologic properties of the isolated tissues. When plasma level was within 5–10 μg/ml (i.e., therapeutic range) changes in electrical activity of the donor heart and isolated tissues did develop and stabilize. The subsequent infusion of procainamide at a rate of 5 mg/min caused a progressive increase in plasma procainamide concentration (fig. 1) and further change in electrical activity of both donor heart and isolated Purkinje fibers.

In one experiment the rapid procainamide infusion was followed by a fall of 25 mm Hg in systolic and 10 mm Hg in diastolic pressure. Pressure returned to control within 10 min. The progression of electrophysiologic events in this experiment was the same as in the others, where only minor variations in arterial pressure occurred.

**Action Potential Characteristics**

Results of a representative experiment relating plasma procainamide concentration to changes in action potential characteristics are shown in figure 2. Panel A shows the control

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**Figure 1**

Plasma procainamide concentration. **Abscissa:** time in minutes. **Ordinate:** procainamide concentration in μg/ml. **Inj.** = time of an i.v. injection of 25 mg/kg; **Inf.** = time during which a constant infusion of procainamide at a rate of 5 mg/min was maintained; **shaded area** = the therapeutic plasma concentration range of 5–10 μg/ml. See text for discussion.

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CHANGES INDUCED BY PROCAINAMIDE

Effect of plasma procainamide concentration on action potential characteristics. Purkinje fiber at 36.5°C, cycle length 630 msec. (Center panel is duplication of figure 1.) (A) Control. (B) PA concentration = 9 μg/ml; (C) PA concentration = 6 μg/ml; (D) PA concentration = 24 μg/ml. Amp = amplitude; V_max = maximal slope of phase 0 of the AP; APD = action potential duration to full repolarization. See text for discussion.

Figure 2

Action potential and maximal slope of phase 0 prior to procainamide administration. Figure 2B, recorded 20 min later, demonstrates a slight decrease in action potential amplitude and maximal slope of phase 0 as well as alteration of the voltage-time course of repolarization. These changes in action potential characteristics progressed and then stabilized over the next 30 min. Figure 2C, recorded at the 90-min mark, demonstrates the steady state that was attained for action potential amplitude, maximal slope of phase 0, and voltage-time course of repolarization. After 90 min, a constant infusion of procainamide was commenced. The attainment of toxic concentrations accentuated the previously mentioned alterations in the action potential. This is seen in figure 2D, which was recorded 55 min after onset of the procainamide infusion.

Changes in the amplitude of action potentials recorded from 14 fibers during seven experiments are displayed graphically in figure 3. The upper ordinate is action potential amplitude in mv; the lower, plasma procainamide concentration. Mean action potential amplitude and range were unchanged for approximately 20 min following the initial procainamide injection despite the high plasma concentrations achieved during this period. Twenty to 30 min after the injection, amplitude decreased and then remained stable within the new range for as long as the 5–10 μg/ml plasma concentration was maintained. As additional procainamide was infused,
producing an increasing elevation in plasma concentration, there was a further decrease in action potential amplitude. The same type of relationship to procainamide concentration was found for values of maximal slope of phase 0. On the other hand, the 10–20 μg/ml range of procainamide concentration had no demonstrable effect on resting membrane potential.

**Action Potential Repolarization**

The changes in repolarization induced by procainamide are depicted in two representative experiments (fig. 4). The purpose of these experiments is to show the variability of procainamide effect that may occur at different plasma potassium concentrations (K⁺o). The action potentials in figure 4A, when plasma concentration was 7–8 μg/ml and donor K⁺o = 4 mEq/liter show there was no change in action potential duration measured to full repolarization but a decreased action potential duration measured to 50 and 75% repolarization. In the same experiment, when procainamide concentration was 14–15 μg/ml action potential duration at 50% repolarization was even shorter, while at 75% and 100% repolarization action potential duration had prolonged.

**Figure 3**

Effect of plasma procainamide concentration on action potential amplitude. Abscissa: time in minutes. Upper ordinate: Mean (dark line) and range (shaded area) of action potential amplitude recorded from 14 impalements; temperature 36–37°C; cycle length 500–630 msec. Lower ordinate: Procainamide plasma concentration (μg/ml). Inj. = 25 mg/kg procainamide injection; Inf. = 5 mg/min procainamide infusion. See text for discussion.

**Figure 4**

Effect of procainamide on action potential duration and slope of repolarization. (A) Purkinje fiber perfused with blood (plasma potassium concentration = 4 mEq/liter. (B) Purkinje fiber perfused with blood (plasma potassium concentration = 3.2 mEq/liter). 50% = APD to 50% repolarization; 75% = APD to 75% repolarization; 100% = APD to 100% repolarization.

These changes thus altered the slopes of phases 2 and 3 of repolarization, increasing the first and decreasing the second, although overall action potential duration did not prolong until a toxic procainamide plasma concentration had been attained.

Figure 4B shows results from an experiment in which plasma K⁺o of the donor dog was 3.2 mEq/liter. Here, when procainamide concentration was 7–8 μg/ml, there was a prolongation of action potential duration at 50, 75, and 100% repolarization which resulted from a decrease in the slopes of phases 2 and 3. When procainamide concentration attained a value of 14–15 μg/ml there was a further increase in the action potential duration at 100% repolarization. However, in relation to the trace recorded at a plasma concentration of 7–8 μg/ml, action potential duration at 50 and 75% shortened. Again, the net effect of procainamide was to make the overall slope of
repolarization more gradual. It should be apparent from these two examples that the perfusate K\textsuperscript{+} serves to modify the effect of procainamide upon repolarization.

**Effective Refractory Period**

Changes in the effective refractory period caused by procainamide were measured in seven experiments, one of which is depicted in figure 5. The controls are shown in A. In figure 5B, 25 min after the initial injection of procainamide, action potential duration was prolonged from 230 to 270 msec, whereas effective refractory period increased from 200 to only 210 msec. This ratio between action potential duration and effective refractory period was maintained for the next 70 min as shown in figure 5C. Note that only the alternate R\textsubscript{2} in C propagates distally. This alternation could be due to a variation in refractoriness from beat to beat or an intermittent change in membrane responsiveness. In figure 5D, toxic concentrations of procainamide were attained. Action potential duration was now 325 msec and effective refractory period, 250 msec. In this instance, conduction of each R\textsubscript{2} to the distal site occurred but was slower than that in figure 5A and B. In every instance where effective refractory period was studied this variable changed in the same direction as action potential duration although tending to lag behind in magnitude of change. In three additional experiments where membrane responsiveness was evaluated no change occurred during maintenance of therapeutic procainamide plasma concentrations, but the curve was depressed at toxic levels.

**Conduction Time and QRS Duration**

Conduction time in isolated Purkinje fiber bundles and QRS duration of the donor electrocardiogram were studied in seven experiments, one of which is depicted in figure 6. No change in overall heart rate occurred following procainamide injection. However, within 40 min QRS duration had prolonged from a control of 35 to 45 msec. Nearly simultaneous with the QRS alteration there was a prolongation in conduction time between the two recording sites in the isolated Purkinje fiber bundle, from a control value of 2.3 msec to 3.6 msec. In each experiment, procainamide brought about a change in QRS duration that correlated well in timing with the change in Purkinje fiber conduction.

**Automaticity**

The slope of phase 4 was recorded in seven experiments in which the Purkinje fibers were driven by an external stimulus. In every instance this variable decreased within 5–10 min after the initial injection of procainamide, suggesting that automaticity would have been decreased. One of the two additional experiments, in which Purkinje fibers were permitted to beat spontaneously during blood perfusion, is depicted in figure 7. Within 4 min after injection of procainamide, spontaneous rate had decreased from a control of 60/min

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**Figure 5**

Effect of procainamide on effective refractory period. Temp = 36–37°C, cycle length 630 msec. ERP (effective refractory period) is defined as earliest premature response (R\textsubscript{p}) elicited at proximal Purkinje fiber site propagated to a distal microelectrode. (A) Control. (B) 25 min after a 25 mg/kg procainamide injection. (C) After 85 min. (D) 50 min after start of a 5 mg/min procainamide infusion. Onset of R\textsubscript{p} marked with arrows. See text for discussion.

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Figure 6
Effect of procainamide on the electrocardiogram and on conduction time in an isolated Purkinje fiber bundle. Temp = 36° C; cycle length 630 msec. The left panels show donor ECG, the right show conduction time (CT) between two microelectrodes impaled at different sites in a Purkinje fiber bundle. (A) Control. Heart rate (upper trace) = 100/min; QRS duration (lower trace) = 35 msec; interelectrode CT = 2.3 msec. (B) Recorded 40 min after an intravenous injection of PA, 25 mg/kg (plasma concentration = 7 μg/ml); heart rate 100/min; QRS duration = 45 msec; interelectrode CT = 3.6 msec. See text for discussion.

(fg. 7A) to 46/min (fig. 7B). At 120 min (fig. 7C) rate had fallen to 40/min. These changes in rate were associated with a decrease in the slope of phase 4 depolarization (fig. 7C). Action potential amplitude, which initially decreased slightly, then increased markedly as is seen in figure 7C. The donor electrocardiogram (bottom) showed only a decrease in heart rate from 150 to 135 beats/min during this time.

Discussion
The two etiologies for ventricular arrhythmias most generally accepted are increased automaticity and altered conduction.10 Therapeutic concentrations of procainamide invariably decreased automaticity and depressed conduction during our experiments. Changes in conduction in isolated tissue and in electrocardiographic QRS duration occurred over a 20–40-min period. Automaticity, on the other hand, decreased within 5–10 min of drug administration. Although plasma levels were higher at the 5–10-min mark than they were at 20–40 min (fig. 1), it is probable that tissue concentrations of procainamide had not yet achieved as high a level at the earlier time as they did later on when conduction was being affected. The suggestion, then, is that a lower tissue concentration of procainamide is required to depress automaticity than to depress conduction. One may speculate that cardiac arrhythmias which respond to procainamide rapidly after intravenous administration might be due to aberrations in automaticity; those that respond more slowly might result from abnormal conduction.

The relationship between plasma concentration of drug and time is further demonstrated by comparing the electrophysiologic responses that occurred within 5 min after an initial injection and following subsequent elevation of plasma levels to the toxic range utilizing a procainamide infusion. Drug concentration per se is no different at the 5- and the 140-min mark, 20 μg/ml in both instances. Yet at 5 min
only automaticity was changing. At 140 min action potential amplitude, maximal slope of phase 0, and conduction had all decreased markedly. Obviously, the only difference in the parameters measured here was that adequate time for equilibration of tissue and plasma concentrations had not occurred at the 5-min mark and did occur during the subsequent procainamide infusion until toxicity was seen. One view concerning the etiology of reentrant arrhythmias is that they result from unidirectional block in a depressed portion of the distal conducting system and retrograde conduction of impulses through this segment. By prolonging conduction time, as shown in figure 6, propagation through such segments might be abolished by procainamide, and the arrhythmia stopped. Procainamide-induced alterations in conduction in these experiments invariably followed a decrease in action potential amplitude and maximal slope of phase 0. Two events might explain the drug-induced loss of action potential amplitude. One is a decrease in resting membrane potential which, however, was not demonstrated in these experiments. The other is a decrease in action potential overshoot which invariably occurred. It has been demonstrated that decreasing the extracellular sodium available to cardiac cells will result in a decrease of AP amplitude and maximum slope of phase 0 without any change in resting membrane potential. Hence, it may be that procainamide exerts its effect upon the action potential in part by altering the influx of sodium into the cell during phase 0 depolarization. The ensuing decrease in maximal slope of phase 0 could then explain the procainamide-induced prolongation of conduction time in Purkinje fiber and ventricular muscle.

The effects of procainamide on repolarization were consistent insofar as the slope of phase 3 repolarization was concerned, but were variable in terms of action potential duration. In every instance the slope of phase 3 became more gradual. In experiments in which plasma potassium was in the lower physiologic range, there was more of a tendency toward prolongation of action potential duration measured at 50, 75, and 100% repolarization. In the higher potassium range, the action potential duration at 50 and 75% decreased, while at 100% repolarization it either remained unchanged or decreased slightly with therapeutic concentrations of procainamide, and prolonged when toxic concentrations were attained. The mechanisms behind these changes are currently being investigated.

The same direction of change noted for action potential duration was seen in evaluating effective refractory period. However, the latter did not necessarily keep pace with the former so that especially at higher drug concentrations the change in action potential duration was greater than that in effective refractory period. The slope of repolarization that was seen in any instance apparently depended on a combination of procainamide concentration and plasma potassium concentration. In the lower potassium range, due to increases in action potential duration and effective refractory period, refractoriness was prolonged and a premature beat that was conducted under control circumstances could no longer be conducted. At higher potassium concentrations lesser alterations in refractoriness and action potential duration were seen. To what extent premature beats occurring at the same membrane voltage might fail to be conducted is uncertain as membrane responsiveness was not significantly changed within the therapeutic concentration range. These observations concerning effective refractory period and membrane responsiveness suggest that altered response to premature impulses following procainamide may not be as consistent a mechanism for abolition of arrhythmias as are changes in conduction and automaticity.

At toxic plasma procainamide concentrations the decreases in action potential amplitude, maximal slope of phase 0, and conduction time were more marked than in the therapeutic range. Of greater interest here is the fact that action potential duration to 100%
repolarization was prolonged in all experiments by toxic plasma concentrations of procainamide due to an altered slope of phase 3. This change was accompanied by a marked decrease in membrane responsiveness which could lead to decremental conduction in Purkinje fibers that previously conducted normally. This supports the theory that drug-induced decremental conduction may be a major mechanism for production of arrhythmias due to procainamide toxicity.13

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