Dose-Dependence of 4-Aminopyridine Plasma Concentrations and Electrophysiological Effects in Dogs
Potential Relevance to Ionic Mechanisms In Vivo

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Background—Previous investigators have administered 4-aminopyridine (4AP) to dogs to evaluate the role of transient outward current ($I_{to}$) in vivo; however, plasma concentrations of 4AP were not measured, and it is therefore uncertain which cardiac ion channels were blocked at the concentrations achieved.

Methods and Results—We applied high-performance liquid chromatography to measure 4AP concentrations produced by intravenous 4AP administration to dogs. A previously described dose regimen produced plasma concentrations that increased during the maintenance infusion but never exceeded 250 μmol/L and caused significant mortality. Whole-cell patch-clamp experiments on isolated canine myocytes showed that even the maximum 4AP concentrations achieved in vivo failed to alter ventricular $I_{to}$ and had very small effects on atrial $I_{to}$; however, concentrations achieved in vivo had a strong inhibitory effect on the dog ultrarapid delayed rectifier ($I_{Kur.d}$), present only in atrial cells. We designed a loading and maintenance infusion regimen to produce stable 4AP plasma concentrations. At concentrations in the range of 25 and 50 μmol/L, 4AP had no effect on ventricular refractory period but increased atrial refractoriness significantly, consistent with the results of voltage clamp studies.

Conclusions—The interpretation of previous studies using intravenous 4AP administration to inhibit $I_{to}$ in dogs in vivo needs to be reevaluated in light of the fact that the infusion regimens used produce plasma concentrations that are inadequate to affect ventricular $I_{to}$. Our findings also support the concept that selective inhibition of ultrarapid delayed rectifier current can prolong atrial refractory periods without affecting ventricular refractoriness. (Circulation. 2000;101:1179-1184.)

Key Words: ion channels ■ antiarrhythmic agents ■ electrophysiology ■ action potentials ■ heart diseases

Over the past 10 years, there has been an explosion of knowledge regarding the ionic currents controlling repolarization in various species, as well as their molecular determinants and pharmacological properties.1 One of the challenges in cardiac electrophysiology has been to link these fundamental mechanisms identified in studies with isolated cells to in vivo electrical phenomena. A potential approach to determining the ionic mechanisms governing electrophysiological processes in the intact heart is to administer selective channel blocking drugs in vivo and to measure the resulting electrophysiological alterations. This approach was used by del Balzo and Rosen, who infused 4-aminopyridine (4AP) into morphine/chloralose-anesthetized, open-chest dogs to evaluate effects on T-wave memory.2 They found that ventricular pacing caused substantial T-wave alterations during subsequent sinus rhythm, alterations that were prevented by the infusion of 4AP. The results were interpreted as indicating an important role of transient outward current ($I_{to}$) in T-wave memory. Similarly, Tachibana et al3 used 4AP infusion in dogs to evaluate the possible role of $I_{to}$ in the ventricular proarrhythmic effects of flecainide. Because 4AP appeared to abolish flecainide proarrhythmia, $I_{to}$ was considered to play a central role in mediating the proarrhythmic action of flecainide-induced $I_{to}$ inhibition. The interpretation of these experiments assumes that 4AP infusion produced sufficient plasma concentrations in vivo to inhibit $I_{to}$; however, the plasma concentrations achieved were unknown.

The approach of administering K⁺ channel blockers in vivo to study ionic mechanisms requires knowledge of the resulting plasma drug concentrations in relationship to blocking concentrations for specific ionic currents. The present experiments were designed to (1) determine the plasma concentrations achieved by infusing 4AP in vivo to dogs, (2) design a loading and maintenance infusion regimen of 4AP that maintains stable plasma concentrations over time, (3) determine the effects of 4AP infusion on canine atrial and
ventricular effective refractory period (ERP), and (4) relate any changes observed to 4AP-induced changes in ionic currents.

Methods

Protocols
We performed preliminary experiments with methods and 4AP infusion techniques similar to those described by del Balzo and Rosen. Tachibana et al used a similar approach with doses that were ~25% lower. We chose the del Balzo-Rosen infusion rates because the Tachibana study had yet not been published at the time that we designed our experiments. Because del Balzo and Rosen used a larger 4AP dose than Tachibana et al, our results reflect the 4AP concentrations achieved in vivo with previously described dose regimens. In initial experiments, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (100 mg/kg IV), and 4AP was infused as an initial dose of 1.6 mg/kg followed by an infusion of 0.25 mg · kg⁻¹ · min⁻¹. All dogs handled in this fashion developed clinical muscle contractions during 4AP infusion that made experimentation difficult. We therefore elected to paralyze the dogs with pancuronium 0.1 mg/kg IV initially, followed by subsequent doses of 0.1 mg/kg each hour. The use of the paralytic agent necessitated (for humane purposes) both loading (30 mg/kg) and maintenance (10 mg · kg⁻¹ · h⁻¹) doses of intravenous pentobarbital to ensure continuous and adequate general anesthesia. This form of anesthesia was therefore used instead of morphine/chloralose in all subsequent in vivo studies. All experiments described in the present article were performed with continuous-infusion pentobarbital anesthesia.

In the first series of in vivo experiments, 4AP was infused intravenously at a dose of 1.6 mg/kg followed by 0.25 mg · kg⁻¹ · min⁻¹. Blood samples were collected at the end of the initial loading dose (given over 2 minutes) and at 5, 10, 15, 20, 30, 40, 50, and 60 minutes thereafter for subsequent high-performance liquid chromatography (HPLC) assay of plasma 4AP concentration. On the basis of the results of these initial studies, we designed a loading and maintenance infusion of 4AP to produce and maintain stable plasma drug concentrations and tested it in a series of 5 dogs. We then used this approach to produce 2 plasma concentration levels of 4AP in dogs and evaluated the effects of 4AP infusion on atrial and ventricular ERP in 6 additional dogs. Finally, we applied patch-clamp techniques to determine the effects of 4AP on drug-sensitive ionic currents.

Methods for In Vivo Electrophysiological Study

General surgical methods (with cardiac access provided via a right thoracotomy) and instrumentation were as described in detail previously. To prevent interference from autonomic reflexes and direct neural release of neurotransmitters, β-adrenergic receptor blockade was produced by administration of intravenous nadolol (0.5 mg/kg initially, followed by 0.25 mg/kg every 2 hours) and atropine (1 mg every 2 hours). ERP was measured in duplicate with the extrastimulus technique. Square-wave, 2-ms twice-threshold pulses were applied via bipolar hook electrodes in the right atrial appendage or right ventricular free wall. A single extrastimulus was applied after every 15 basic stimuli, with the coupling interval reduced by 10-ms decrements until failure to capture indicated the ERP.

HPLC Assay

HPLC assay was performed with general methods similar to ones we have described in detail previously. In brief, aliquots (0.5 mL) of plasma, with 0.1 mL of 1N NaOH added, were placed in 15-mL tubes containing 10 μg of procainamide as the internal standard. Ethyl acetate (2.5 mL) was then added to the tubes, which were agitated vigorously for 30 seconds. After 10 minutes of centrifugation at 3000 rpm for 10 minutes, 2 mL of the upper organic phase was transferred to Reactivils and evaporated to dryness with a stream of pure nitrogen gas. The residue was dissolved in 500 μL of mobile phase, and 25 μL was introduced into the injection loop. The mobile phase consisted of equal quantities (by volume) of acetonitrile and 0.03 mmol/L potassium dihydrogen phosphate aqueous solution, with the addition of 2 mL/L glacial acetic acid and 0.006 mmol/L l-octane sulfonic acid (Na’ salt). Separation was carried out on a Spherisorb ODS 10-μm reverse-phase column from Chromatography Sciences coupled to a Waters 501 HPLC solvent delivery module and a Waters 481 UV visible detector. The compounds (4AP and the internal standard, procainamide) were detected at a wavelength of 263 nm, and the retention times for 4AP and procainamide were 3.5 and 4.5 minutes, respectively, with complete baseline separation. A standard curve was performed for each set of sample determinations, with concentrations obtained from the linear standard curve without extrapolation. The coefficient of variation ranged from 1.7% to 2.7%.

Cell Isolation and Patch Clamp Methods

Canine atrial and ventricular myocytes were isolated with methods previously described in detail. Whole-cell patch-clamp was used to record the 4AP-sensitive currents Ic and ICa.S by methods previously described in detail. Blood samples were collected at the end of the initial loading dose, followed by 0.1 mg · kg⁻¹ · h⁻¹ doses of intravenous pentobarbital to ensure continuous and adequate general anesthesia. This form of anesthesia was therefore used instead of morphine/chloralose in all subsequent in vivo studies. All experiments described in the present article were performed with continuous-infusion pentobarbital anesthesia.

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Results

Plasma Concentrations Resulting From 4AP Infusion In Vivo

The previously described 4AP infusion regimen resulted in mean concentrations that averaged 14.1 μmol/L at 5 minutes into the maintenance infusion, and increased slowly thereafter to reach a maximum in the range of 225 μmol/L after 60 minutes (Figure 1A). Because of the instability of the plasma concentrations achieved by this dose regimen and substantial lethality (3 of 6 dogs died before receiving the full dose), we designed a dose regimen to achieve and maintain stable drug concentrations and administered it to 5 additional dogs. This regimen, consisting of an initial dose of 1 mg/kg, followed by 0.1 mg · kg⁻¹ · min⁻¹ for 20 minutes and then 0.05 mg · kg⁻¹ · min⁻¹ for 40 minutes, resulted in stable plasma concentrations in the range of 20 to 30 μmol/L from 5 minutes after the end of the loading dose until the end of the observation period at 60 minutes (Figure 1B).
Electrophysiological Effects of 4AP Infusion

We applied the loading and maintenance approach to study the effects on atrial and ventricular ERP of stable 4AP concentrations in the range of 25 (dose indicated above) and 50 (additional 1 mg/kg load, doubled maintenance doses) μmol/L. After baseline values were obtained, we infused 4AP according to the regimen shown in Figure 1B (dose 1) and repeated the ERP measurements. We then gave an additional 1-mg/kg loading dose, followed by 0.2 mg·kg⁻¹·min⁻¹ for 20 minutes and then 0.1 mg·kg⁻¹·min⁻¹ (dose 2). The resulting electrophysiological effects are shown in the Table. Neither dose significantly altered ventricular ERP. Dose 1 increased atrial ERP slightly but not significantly, whereas dose 2 produced significant increases on the order of 25% to 30% in atrial ERP.

Concentration-Response Relations for 4AP Inhibition of Ionic Currents

To assess the potential ionic mechanisms of the electrophysiological effects of 4AP in vivo, we studied the effects of 4AP on the ionic currents known to be sensitive to the compound: \( I_{\text{Na}} \) and \( I_{\text{Kur.d}} \) in canine atrial myocytes \(^9,^{10} \) and \( I_{\text{Na}} \) in canine ventricular cells. \(^11 \) Figure 2 (top) shows examples of dog atrial and ventricular \( I_{\text{Na}} \) before and after the addition of 4AP at various concentrations. Corresponding concentration-response curves, shown at the bottom, indicate that relatively large concentrations of 4AP are needed to inhibit \( I_{\text{Na}} \). The mean 50% inhibitory concentration (IC\(_{50}\)) averaged 1486±261 μmol/L for ventricular cells (\( n = 4 \)) and 471±97 μmol/L for atrial cells (\( n = 3 \)). Concentrations of 4AP in the range of the largest 4AP concentrations achieved by intravenous infusion in vivo, 200 μmol/L, reduced ventricular \( I_{\text{Na}} \) by an average of 10±4% (\( P=\text{NS} \) versus control) and atrial \( I_{\text{Na}} \) by 25±4% (\( P=0.02 \) versus control). Figure 3 shows the effect of several concentrations of 4AP on \( I_{\text{Kur.d}} \) in a representative myocyte (left), as well as the 4AP concentration-response curve for \( I_{\text{Kur.d}} \) inhibition (right). \( I_{\text{Kur.d}} \) was much more sensitive to 4AP than was \( I_{\text{Na}} \) with an IC\(_{50}\) of 5.3±0.7 μmol/L (\( n = 8 \)). At a concentration of 50 μmol/L, in the range of concentrations produced by dose 2 for the in vivo studies, 4AP reduced \( I_{\text{Kur.d}} \) in atrial myocytes by an average of 99±1% at +40 mV. At the same concentration (50 μmol/L), ventricular \( I_{\text{Na}} \) was reduced by 1±1% (\( P=\text{NS} \)) and atrial \( I_{\text{Na}} \) by 3±6% (\( P=\text{NS} \)).

Discussion

We measured the plasma concentrations produced by a previously described 4AP infusion protocol in dogs. The plasma concentrations changed over time and reached a maximum just over 200 μmol/L, a concentration that had no significant effect on \( I_{\text{Na}} \) in canine ventricular myocytes and a very small effect on \( I_{\text{Na}} \) in canine atrial myocytes. In contrast, 4AP concentrations as low as 50 μmol/L completely inhibited \( I_{\text{Kur.d}} \) in atrial myocytes. A 4AP dose regimen was developed to produce and maintain stable plasma concentrations. With this dose regimen, 4AP significantly altered canine atrial but not ventricular ERP in vivo, in general agreement with effects on corresponding drug concentrations on ionic currents in vitro.

Relationship to Previous Studies of 4AP Pharmacokinetics

Relatively few published studies have evaluated the effects of 4AP on cardiac electrophysiology in vivo. Del Balzo and Rosen used 4AP infusion to evaluate the potential role of \( I_{\text{Na}} \) in T-wave memory in the dog. They estimated that their infusion regimen would result in blood concentrations of 3 mmol/L, assuming no loss from the intravascular compartment. \(^2 \) Our direct measurements of plasma concentrations are not consistent with this assumption and indicate that the infusion regimen reported by del Balzo and Rosen produces
much lower concentrations, averaging \( \approx 80 \mu \text{mol/L} \) at the end of the loading dose, \( 50 \mu \text{mol/L} \) at 5 minutes, and \( 225 \mu \text{mol/L} \) after 60 minutes of maintenance infusion. In fact, the concentrations achieved were well below the threshold for \( I_\text{to} \) inhibition in ventricular myocytes.

Uges et al.\(^{12}\) studied the pharmacokinetics of intravenous 4AP in humans. Their pharmacokinetic analysis indicated a serum drug clearance of \( 0.61 \text{ L h}^{-1} \text{ kg}^{-1} \). On the basis of the well-known pharmacokinetic relationship \( \text{IR} = C_\text{ss} \cdot \text{Cl} \),\(^{13}\) where \( \text{IR} \) is drug infusion rate, \( C_\text{ss} \) is steady-state drug concentration in the blood, and \( \text{Cl} \) is drug clearance from the blood, the pharmacokinetic parameters calculated by Uges et al. would predict a steady-state concentration of \( 265 \mu \text{mol/L} \) with a 4AP maintenance infusion rate of \( 0.25 \text{ mg kg}^{-1} \text{ min}^{-1} \), as used by del Balzo and Rosen. This concentration is very similar to the concentrations (in the range of \( 225 \mu \text{mol/L} \)) that we measured after 60 minutes of maintenance infusion with the del Balzo-Rosen infusion protocol.

Uges et al. found that 4AP concentration-time data were best fit by a triexponential model in 5 patients and a biexponential model in 4 others. Mean central compartment volume estimates ranged from 0.0708 to 0.78 L/kg, which would predict a drug concentration between \( 240 \) and \( 22 \mu \text{mol/L} \), respectively, after a loading dose of \( 1.6 \text{ mg/kg} \) 4AP, as used by del Balzo and Rosen. The concentrations at the end of a \( 1.6-\text{mg/kg} \) 4AP load in our dogs varied from 31 to 111 \( \mu \text{mol/L} \), with a mean of \( 80 \mu \text{mol/L} \), quite consistent with predictions based on the pharmacokinetic analysis of Uges et al.\(^{12}\)

### Novelty and Potential Significance

The present study is the first of which we are aware to measure the plasma 4AP concentrations achieved in dogs by intravenous infusion regimens that have been applied to study the role of \( I_\text{to} \) in cardiac electrophysiological phenomena in intact animals. Our results indicate that such infusion regimens do not result in plasma drug concentrations that are sufficient to inhibit ventricular \( I_\text{to} \) to any significant extent.

The work of del Balzo and Rosen\(^{2}\) has been widely interpreted as reflecting the role of \( I_\text{to} \) in ventricular repolarization, and particularly T-wave memory. Tachibana et al.\(^{3}\) recently described an effect of intracoronary flecainide to induce ST alternans and ventricular tachyarrhythmias in anesthetized dogs. In some experiments, they administered intravenous 4AP (1.2 mg/kg load followed by 0.17 mg \( \text{kg}^{-1} \text{ min}^{-1} \)) and found that the effects of flecainide were attenuated. On the basis of the response to 4AP, they concluded that their results pointed to a central role for a 4AP-sensitive current such as \( I_\text{to} \) in flecainide proarrhythmia. The doses of 4AP administered by Tachibana et al. were \( \approx 75\% \) of those used by del Balzo and Rosen, and according to our data would have been most unlikely to directly inhibit outward currents in canine ventricular myocytes. In fact, assuming coronary flow rates in the range of \( 100 \text{ mL/min} \),\(^{14}\) the proarrhythmic flecainide infusion rate (\( 100 \mu \text{g kg}^{-1} \text{ min}^{-1} \)) that they administered into the left anterior descending coronary artery would have been expected to produce concentrations on the order of 20 \( \mu \text{mol/L} \) (\( \approx 100 \mu \text{mol/L} \)) in the coronary blood flow. Flecainide reduces \( I_\text{to} \) effectively at concentrations in the range of \( 10 \mu \text{mol/L} \); thus, the flecainide concentrations achieved by...
Tachibana et al (=100 μmol/L) would in themselves have been expected to have a very strong inhibitory effect on $I_{\text{ur}}$. Other mechanisms that may account for the effects of 4AP noted by del Balzo and Rosen and Tachibana et al include interactions with cardiac autonomic innervation. Furukawa et al observed cardiac effects of 4AP that they interpreted to be a consequence of activation of parasympathetic ganglionic neurotransmission. Neuronal K$^+$ channels may be very sensitive to 4AP, and altered autonomic nervous system tone is well known to have important effects on cardiac repolarization. 4AP appears to increase sympathetic and parasympathetic outflow to the heart. Acetylcholine can have profound transmurally heterogeneous effects on ventricular repolarization that depend on the magnitude of $I_{\text{ur}}$ and are enhanced in the presence of sympathetic agonists. Thus, 4AP could perhaps act indirectly via the autonomic nervous system to suppress T-wave changes related to alterations in $I_{\text{ur}}$. At the moment, there is no direct proof for this notion, and it remains speculative. In any case, our data suggest that direct inhibition of cardiac $I_{\text{ur}}$ is unlikely to have mediated the effects of 4AP infusion previously observed.

Ultrarapid delayed rectifier currents ($I_{\text{ur}}$) have been observed in cardiac tissues from a wide variety of species. They have been noted to play a role in repolarizing human and canine atrial myocytes and mouse ventricular myocytes. The human $I_{\text{ur}}$ is absent in human ventricular cells, and it has been suggested that $I_{\text{ur}}$ may be an interesting target for atrial-selective antiarrhythmic drugs. In the present study, we observed $I_{\text{ur,at}}$ in canine atrial myocytes and found no evidence of its presence in dog ventricle. As shown in the Table, we found that 4AP is capable of increasing atrial ERP in the dog in vivo without affecting ventricular ERP. Because $I_{\text{ur,at}}$ is the main outward K$^+$ current affected by 4AP at the concentrations achieved by the modified loading and infusion regimen, these observations are consistent with the notion that selective inhibition of ultrarapid delayed rectifiers can produce atrial-selective refractoriness prolongation in vivo. To the best of our knowledge, this is the first such demonstration in the literature.

Our findings make it unlikely that the observations of del Balzo and Rosen regarding the effects of 4AP on T-wave memory in vivo were due to actions on $I_{\text{ur}}$; however, this does not exclude a potentially important role for $I_{\text{ur}}$ in T-wave memory. Subsequent studies have shown that 3 mmol/L 4AP suppresses changes in action potential difference signals induced by altered activation pattern in canine ventricular slabs studied in vitro. Epicardial cells from the left ventricular base of dogs subjected to pacing at the left ventricular apex to induce T-wave alterations in sinus rhythm (T-wave memory) showed a decreased phase I notch, along with a decreased $I_{\text{ur}}$ density, altered $I_{\text{ur}}$ voltage dependence, slowed $I_{\text{ur}}$ recovery, and decreased Kv4.3 mRNA levels. Thus, strong suggestive evidence remains for a role of $I_{\text{ur}}$ in T-wave memory. Further work would be useful to establish more precisely the role of $I_{\text{ur}}$, as well as that of other ionic currents and factors, such as changes in cell coupling and myocardial structural remodeling, in T-wave memory.

We noted quite different 4AP potencies for inhibition of canine atrial $I_{\text{ur}}$ (IC$_{50}$ of 0.47 mmol/L) compared with ventricular $I_{\text{ur}}$ (1.49 mmol/L). Canine atrial and ventricular $I_{\text{ur}}$ are both believed to be composed of Kv4.3 subunits. The molecular basis for the difference in 4AP sensitivity between canine atrial and ventricular $I_{\text{ur}}$ is an interesting issue that merits further study.

### Potential Limitations

Cell isolation can produce artifacts by affecting cardiac ion channels; however, we have found that $I_{\text{ur}}$ is relatively unaffected by the cell isolation method. It could be argued that myocardial tissue concentrations of a drug like 4AP may not be the same as plasma concentrations. Although this may be true, the concentration of a drug in the extracellular solution space is equivalent to plasma concentrations of free (ie, non–protein-bound) drug. Because 4AP shows negligible protein binding, plasma concentrations are probably equivalent to the concentration in the extracellular space with which the cell membrane is in equilibrium. Therefore, the effects of a given 4AP concentration applied to single cells via the extracellular (bath) solution should be equivalent to the effects of a corresponding plasma concentration in vivo.

### Conclusions

Our study shows that previously described 4AP infusion regimens produce inadequate plasma drug concentrations to significantly inhibit ventricular $I_{\text{ur}}$. Given the toxicity of these infusion regimens, it is unlikely that sufficient 4AP can be given intravenously to block cardiac $I_{\text{ur}}$ in vivo. Thus, the interpretation of the results of previous studies using intravenous 4AP in vivo needs to be reevaluated, and caution should be used in designing subsequent studies. Our data also suggest that selective inhibition of atrial ultrarapid delayed rectifier currents can produce atrial-specific ERP prolongation.

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### References

2. del Balzo U, Rosen MR. T wave changes persisting after ventricular pacing in canine heart are altered by 4-aminopyridine but not by lidocaine: implications with respect to phenomenon of cardiac “memory.” Circulation. 1992;85:1464–1472.


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