Large Scale Purification of Hog Renin  
Physicochemical Characterization

PIERRE CORVOL, CLAUDINE DEVAUX, TAKETOSHI ITO, PHILIPPE SICARD, 
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SUMMARY Renin was purified from 47 kg of hog kidney to produce enough enzyme for enzymatic and physicochemical characterization. The procedure included extraction at pH 3.5 in the presence of protease inhibitors, two ammonium sulfate precipitations, ion exchange chromatography on Sepharose-hexamethylene diamino-pepsatin gel, gel filtration, and isoelectric focusing. Renin, 2.3 mg, with a specific activity of 1,100 GU/mg of protein was obtained with about 70,000-fold purification and 16% overall recovery. The purity criteria were: (1) a single band on sodium dodecyl sulfate (SDS)-gel electrophoresis, (2) same retardation factor on polyacrylamide gel electrophoresis for renin activity, protein, and glycoprotein coloration. Renin was characterized by its stability at approximately -20°C, pH 6.5; its molecular weight on SDS-gel electrophoresis, 36,800; its relative mobility on polyacrylamide gel electrophoresis at pH 7.8; its isoelectric point, 5.15; its amino acid composition, which revealed that renin is a glycoprotein; and its Michaelis constant on tetradecapeptide substrate at pH 6.6, K_m = 7.7 x 10^{-6} M.

THE IMPORTANCE of the renin-angiotensin system in the pathogenesis of hypertension remains under debate, despite numerous studies in both experimental and human hypertension. Besides studies on the physiology of the renin-angiotensin system, much work has been devoted to the biochemistry of this system and has been recently reviewed by Skeggs et al.1 However, even though the structure of the pressor peptide, angiotensin II, has been known for 20 years, renin itself has never been purified to the stage of homogeneity in quantities sufficient to perform physicochemical characterization. The need for such a study is underlined by the recently reported evidence for a proform of renin2 for circulating activators and inhibitors of renin,3,4 for isorenin,5 and for pseudorenin.6 The suggested complexity of the biochemistry of renin prompted us to purify hog renin.

Twenty-two years elapsed between the first historical purification of hog renin by Haas et al.7 and the preparation of pure renin by Murakami and Inagami.8 The main problems encountered during the preparation of this enzyme were due to (1) its very low concentration in the kidney and (2) its lack of stability when the purity increases.7-9 Definite improvement was achieved by the use of affinity chromatography for renin purification10-13 since this method combines a very high increase of specific activity with a high recovery.

The purpose of this study was therefore (1) to prepare hog renin from a large enough quantity of kidneys to produce several milligrams of the enzymatic and physicochemical properties of highly purified renin.

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Methods

PURIFICATION PROCEDURE

Renin (EC 3.4.99.19) was extracted from 47 kg of hog kidney. Kidneys were frozen and thawed twice at room temperature and once at 37°C. Fat and inner medulla were removed and the meat was minced in a Waring Blender. Renin was then purified in a seven-step procedure. All the operations were run at 4°C except when otherwise stated. The tissues were suspended in 47 liters of a solution containing: 9% NaCl, 1% ethylenediaminetetraacetic acid, 0.348% phenyl methane sulfonyl fluoride, and 1% sodium tetrathionate. The mixture was brought to pH 3.5 with HCl, homogenized at room temperature for 1 hour, and filtered with PrimiSil 511.

Step 1
The tissues were suspended in 47 liters of a solution containing: 9% NaCl, 1% ethylenediaminetetraacetic acid, 0.348% phenyl methane sulfonyl fluoride, and 1% sodium tetrathionate. The mixture was brought to pH 3.5 with HCl, homogenized at room temperature for 1 hour, and filtered with PrimiSil 511.

Step 2
A second filtration was performed on Celite 545, and the volume was reduced to 17.5 liters by ultrafiltration.

Step 3
Proteins were precipitated at pH 3.5 by the addition of solid ammonium sulfate, 400 g/liter, in order to get a final molarity of 2.5 M; this precipitation was repeated, and after the second centrifugation, proteins were dissolved in 4.7 liters of phosphate buffer, 5 mM, pH 7.0, and dialyzed against the same buffer in a Travenol hemodialyzer.

Step 4
The solution was applied to a diethylaminoethyl (DEAE)-cellulose column (DE 52; Whatman). Stepwise elution was performed with 5 mM, 20 mM, 40 mM, and 100 mM phosphate buffer, pH 7.0. Renin was eluted with 2.6 liters of 100 mM phosphate buffer, pH 7.0.
Step 5
After the molarity of the phosphate buffer was adjusted to 0.2 M, the solution was applied to a column of Sepharose-hexamethylenediamino-pepsatin gel. The preparation of the affinity gel, its characteristics, and the elution conditions have been reported.14 Renin eluted with 0.1 M acetic acid (2.25 liters) was collected on 225 ml of saturated sodium acetate in order to provide a pH of 4.8–5.0.

Step 6
After concentration by ultrafiltration, renin was further purified on a Sepharose-acrylamide AcA 44 (LKB) column. Upward elution was developed in phosphate buffer, 0.1 M, pH 6.5 (Fig. 1).

Step 7
Final purification of renin was obtained by isoelectrofocusing between pH 3 and 6 according to the original method of Vesterberg and Svensson.16 Renin focused at pH 5.15. Ampholines were separated from renin by filtration on Sephadex G-25 in phosphate buffer 0.1 M, pH 6.5. (Fig. 2)

RENIN ASSAYS

Radioimmunoassay
Renin was tested by measuring the amount of angiotensin I generated during the enzymatic reaction by radioimmunoassay as already described.17 The sensitivity of the assay was 5 pg of angiotensin I. 125I-Angiotensin I was measured in a Packard Tri Carb scintillation spectrometer model 3330 with a counting efficiency of 50%. The calibration in terms of Goldblatt Units (GU) was established with the MRC standard hog renin (65/119) from the Division of Biological Standards, Holly Hill, London.

Fluorimetric Assay
Highly purified renin (step 7) was tested by its ability to hydrolyze the tetradecapeptide substrate generating a free NH2 group which is detected by a fluorimetric assay.18 The method was adapted for hog renin according to the following procedure: 0.01 GU of renin was incubated for 1 hour at 37°C with increasing concentrations (0.86–17.30 × 10⁻⁶ M) of tetradecapeptide (Beckman) in 1 ml of phosphate-citrate buffer, 0.02 M, pH 6.6. The reaction was stopped by immersion in a boiling water bath. After the tubes were cooled, the pH was raised by the addition of 1 ml of citrate-phosphate buffer 0.1 M, pH 7.3. The fluorescence was measured in a Jobin-Yvon spectrophotofluorimeter model Bearn at 396–493 nm after the addition of 0.2 ml of a solution containing 30 mg of fluorescamine (Roche) per 100 ml of dioxane. It was verified that angiotensin I was effectively liberated from tetradecapeptide by the following experiment: an excess of highly purified renin (0.2 GU) was incubated with 140 pmol of tetradecapeptide. After 2 hours of incubation, all the substrate was converted into angiotensin I measured by radioimmunoassay.

Bioassay
The pressor activity of renin was tested by Drs. Haas and Goldblatt in four unanesthetized dogs in their laboratory.

CHARACTERIZATION OF PURE RENIN

Polyacrylamide Gel Electrophoresis
Polyacrylamide gel electrophoresis was performed in Buchler equipment. Acrylamide and bisacrylamide (Sigma) were recrystallized from acetone. Two types of experiments were performed.

SDS Gel. The gels were prepared according to the method of Weber and Osborn.19 Renin, 35 μg, was either incubated overnight at 37°C with 1% SDS (Merck) and 1% mercaptoethanol (Aldrich) or directly applied to the gel. When the electrophoresis was completed, the gels were stained overnight with 0.1% amido black (Baker) in 7.5% acetic acid.20 Destaining was performed in 7.5% acetic acid under agitation for 24 hours. Calibration curve for molecular weight determination was constructed with the following proteins as standards: albumin (Sigma), mol wt 68,000; ovalbumin (Miles), mol wt 43,000; pepsin (Sigma), mol wt 35,000; chymotrypsinogen (Sigma), mol wt 25,700; and trypsin (Sigma), mol wt 23,000. Each protein was applied in duplicate during the same experiment. Toluidine blue was used as a tracking dye and the retardation factor (Rf) obtained for each protein was plotted against the logarithm of the molecular weight.

Gel Electrophoresis in Multiphasic Buffer System. Electrophoresis was performed at 4°C in multiphasic system D (bis Tris/HCl/cacodylic acid/N-Tris-methyl-2-aminoethanesulfonate), operative at pH 7.8, according to the method of Rodbard and Chrambach.21 The separation gel contained 10% of total acrylamide with a cross-linking of 2% of bisacrylamide. After electrophoresis, the gels were either stained or sliced in 33 slices, 1.2 mm thick, for renin assay; each gel slice was cluted overnight in phosphate buffer, 0.2 M, pH 6.5, at 4°C and tested for renin activity. Two methods were used for staining the gels: amido black

![Figure 1](https://example.com/figure1.png)

**Figure 1** Gel filtration of hog renin. Sixty-three milligrams of step 5 renin containing 8570 Goldblatt units (GU) were applied to a 2.5 × 93-cm column of Sepharose-acrylamide AcA 44. Upward elution was developed in phosphate buffer, 0.1 M, pH 6.5, with a constant flow rate of 0.36 ml/min. Fractions (2.8 ml) were collected for renin ( ) and protein determinations ( ).

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(as described above) for protein determination and Schiff coloration for detection of glycoproteins according to the method of Zaccharius et al.\textsuperscript{22}

**Amino Acid Analysis**

Step 7 renin (100 \(\mu g\)) was hydrolyzed at 100°C in 6 N HCl for 16 hours. Amino acid composition was determined in Unichrome Beckman acid auto-analyzer. Results were expressed in g/100 g of proteins.

**Immunodiffusion**

Immunodiffusion was studied by Ouchterlony's method.\textsuperscript{23} Antisera were raised in rabbits by repeated multiple intraepidermic injections with 40 GU of step 5 renin mixed with an equal volume of complete Freund's adjuvant. Rabbits were reinjected 4 times, every 3 or 4 weeks, with the same renin mixed with an equal volume of incomplete Freund's adjuvant.

**Stability Experiments**

Renin of step 6 and 7 was stored at -20°C in phosphate buffer, 0.1 M, pH 6.5, in aliquots in order to avoid repeated freezing and thawing of the total sample. The enzyme was reasayed 4 months later. An attempt was made to lyophilize step 6 renin. Renin (50 \(\mu l\)) was added to 1 ml of 15% mannitol in four glass vials and submitted to lyophilization. The powder was dissolved in water and tested for renin activity.

**Results**

**RENIN PURIFICATION**

The overall purification of hog renin is described in detail in Table 1. The enzyme was purified about 70,000-fold with a 16% total recovery. The most efficient step was the affinity chromatography procedure, which provided a 100-fold purification with a 67% recovery. Even after affinity chromatography, renin was not pure, since a major contaminant (mol wt 32,000) was partly separated on gel filtration (Fig. 1). However, there was a single symmetric renin peak. Final purification was obtained by isoelectric focalization. Renin focused at pH 5.15 (Fig. 2) but the elution peak was not quite symmetric. The shoulder on the left part of this peak suggests the presence of a slightly more acidic renin.

Renin of step 5 had a specific activity of 136 GU/mg protein. The same renin activity was found when tested either in vitro by radioimmunoassay against MRC standard hog renin or in vivo by increase in blood pressure in dogs. Renin of step 7 had a specific activity of 1100 GU, determined in vitro.

**PHYSICOCHEMICAL CHARACTERIZATION OF RENIN**

**Molecular Weight**

Step 7 provided a single band in SDS gel with the same \(R_o\) of 0.365 whether the enzyme was previously reduced or not (Fig. 3). From the standard calibration curve (Fig. 4) a molecular weight of 36,800 \(\pm\) 2,600 (SD) was obtained for renin. On this basis, 1 GU corresponds to 24 pmol of hog renin.

**Polyacrylamide Gel Electrophoresis**

The relative mobility of step 7 renin was studied by polyacrylamide gel electrophoresis in a multiphasic buffer system. The same \(R_o\) (\(R_o\) 0.47) was found for protein staining and renin activity. However two very faint bands

**Table 1 Renin Purification from 47 kg of Hog Renin**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total proteins (mg)</th>
<th>Total renin (GU)*</th>
<th>Specific activity (GU/mg protein)</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crude extract</td>
<td>960,000</td>
<td>15,600</td>
<td>0.01625</td>
<td>1.0</td>
</tr>
<tr>
<td>2. Celite filtration</td>
<td>245,000</td>
<td>21,000</td>
<td>0.0857</td>
<td>5.3</td>
</tr>
<tr>
<td>3. Ammonium sulfate precipitation</td>
<td>110,000</td>
<td>20,000</td>
<td>0.182</td>
<td>11.4</td>
</tr>
<tr>
<td>4. DEAE-cellulose</td>
<td>9.540</td>
<td>13,000</td>
<td>1.360</td>
<td>85.4</td>
</tr>
<tr>
<td>5. Affinity chromatography</td>
<td>63.0</td>
<td>8,600</td>
<td>136.1</td>
<td>8,409</td>
</tr>
<tr>
<td>6. Ultrogel ACA 44</td>
<td>9.72</td>
<td>7,700</td>
<td>794.2</td>
<td>49,027</td>
</tr>
<tr>
<td>7. Electrophoresing and Sephadex G 25</td>
<td>2.30</td>
<td>2,520</td>
<td>1,097.3</td>
<td>67,737</td>
</tr>
</tbody>
</table>

* GU = Goldblatt units.
Characterization of pure hog renin on sodium dodecyl sulfate (SDS)-gel electrophoresis. 35 μg of step 7 renin were applied to SDS gel after overnight incubation with 1% β-mercaptoethanol and 1% SDS. Electrophoresis was run under a constant current of 8 mA. Toluidine blue was used as a tracking dye. Proteins were stained with amido black. A single band was obtained with a retardation factor of 0.365.

Amino Acid Composition

The amino acid composition of renin is given in Table 2. About 3% of glucosamine residues was detected.

Immunodiffusion

One line of precipitation was detected by double immunodiffusion in the case of step 7 renin, whereas at least two precipitin lines were found for step 4 renin.

Kinetic Study on Tetradecapeptide Substrate

Renin (step 7) was incubated at various values of pH (from 4.0 to 7.5) with tetradecapeptide substrate, and the amount of tetrapeptide (Leu-Val-Tyr-Ser) generated was measured by fluorimetric assay. Corrections were made for the blank value due to the terminal free amino groups of the tetradecapeptide at this pH to determine its Michaelis constant (Km). Results were plotted according to the Lineweaver-Burk representation and a Km of $7.7 \times 10^{-6}$ M was found (Fig. 5).

Stability

The stability of highly purified renin was tested, and in each experiment the activity of MRC hog renin was used as an internal standard. Each assay was performed in triplicate and represented the mean of six radioimmunological determinations. The between-assay reproducibility of the radioimmunoassay was ±8%.

Purified renin was reassayed after 4 months of storage at -20°C in phosphate buffer (0.1 M, pH 6.5). Ninety-one

Molecular weight

![Molecular weight diagram](image)

**Figure 4** Electrophoretic mobility [retardation factor (Rf)] as a function of molecular weight of the proteins. Thirty-five micrograms of five standard proteins were run in duplicate on SDS-gel electrophoresis. The regression line was calculated according to the least square method. From the renin Rf (0.365), a molecular weight of 36,800 was determined.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Amount (g/100 g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>12.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.8</td>
</tr>
<tr>
<td>Serine</td>
<td>8.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.9</td>
</tr>
<tr>
<td>Proline</td>
<td>4.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.3</td>
</tr>
<tr>
<td>Valine</td>
<td>4.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.1</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Table 2** Amino Acid Composition of Hog Renin
The affinity gel is due to (1) the ability of pepstatin to bind protein contaminants can be detected. In the present study, a major protein contaminant (mol wt 32,000) was found. Although this protein was not identified, it was demonstrated to be devoid of cathepsin activity. The lack of complete specificity of the pepstatin affinity gel is due to (1) the ability of pepstatin to bind to other proteins, since this pentapeptide is not a specific inhibitor of renin and (2) the fixation of some proteins by hydrophobic binding. Attempts made to reduce the hydrophobicity of the arm have been unsuccessful up to now. Two more conventional methods were necessary to obtain extremely purified renin, i.e., a gel molecular sieving and a separation by isoelectric focusing.

The seven-step procedure (Table I) allowed us to obtain 2.3 mg of renin from 47 kg of hog kidney with an overall recovery of 16%. A specific activity of 1100 GU/mg protein, tested against standard MRC hog renin, was obtained for this renin. It can be compared to the specific activities of 780 GU/mg enzyme obtained by Haas et al. and of 1225 GU/mg protein quoted by Skeggs et al. On the other hand, Poulsen et al. obtained a specific activity of 234 GU/mg protein in purifying hog renin by affinity chromatography on a (D-Leu) octapeptide inhibitor of renin. Similar direct comparisons cannot be made with renin preparations from other groups since no measurement of renin had been made with an international standard. Perhaps an indirect approximate comparison can be undertaken on the basis of the purification factor from the renin crude extract. The purification factor of 68,000 in this study can be compared with that of 56,000 reported by Haas et al. and of 14,000 and 1,000 given by Peart et al. and Rubin, respectively. From the study of Murakami and Inagami a purification of 42,000 can be calculated from the kidney extract. The authors found a 180,000-fold purification from the whole kidney which should be compared to 246,000 in this study.

The extremely high degree of renin purity was established by the two following criteria: (1) a single protein band on SDS-polyacrylamide gel electrophoresis and (2) an identical relative mobility in multiphasic buffer polyacrylamide gel electrophoresis for protein staining, glycoprotein staining, and renin activity.

The physicochemical characterization of renin has been performed by several physicochemical and enzymatic methods. The molecular weight of renin calculated from SDS-polyacrylamide gel electrophoresis (36,800) is slightly lower than that of 40,000 reported by the literature. However, the previous determinations have always been made by molecular sieving. This apparent slight discrepancy could be due to the presence of carbohydrate residues in the molecule, since Andrews has shown that many glycoproteins have a too low elution volume on gel filtration that can account for an overestimation of their molecular size. Renin apparently behaves as a single polypeptide chain, since its molecular size is not altered by the reduction of the disulfide bridges by mercaptoethanol. The isoelectric point of renin is 5.15, in good agreement with the results of Rubin and our own on semipurified renin.

The amino acid analysis showed the presence of all amino acids that can be detected by the Beckman automatic amino acid analyzer. The presence of cysteine or cystine was not detected. Moreover, at least 3% of glucosamine was detected in the molecule after 16 hours of hydrolysis. That renin is a glycoprotein was confirmed by the positive Schiff reaction on polyacrylamide gel, at the same Rf as that of renin activity.
The stability of renin is very satisfactory, since more than 90% of the activity was found after 4 months of storage at ~20°C. It contrasts with the previous reports of renin instability after prolonged storage.5,9 This improvement of renin stability might be due to the inhibition of proteases during the extraction and the first steps of purification, and also to the reduction in the number of purification procedures.

The enzymatic activity of renin was determined with the tetradecapeptide substrate by a simple and reproducible new method. The assay is based on the fluorescence of the tetrapeptide Leu-Val-Tyr-Ser which is liberated from the tetradecapeptide substrate. Under these conditions, the optimum pH for renin is 6.6, in agreement with that found by Montague et al.29 The Michaelis constant is 7.7 \times 10^{-4} M. This value is within the same order of magnitude as that previously reported by Skeggs et al.30 with purified renin on a tetradecapeptide substrate.

In extracts of hog kidney, several other types of renin have been reported according to their charges or their molecular size. The presence of several forms of renin of the same molecular size with slightly different charge has already been demonstrated on DEAE-cellulose29 and by isoelectric focusing.29 In this study, a microheterogeneity of the renin molecule is likely, since the isoelectric focusing presented a shoulder on the acidic part of the renin peak, suggesting the presence of a more acidic renin. This was further assessed by the presence of two faint bands with higher mobility on polyacrylamide gel electrophoresis. The same molecular weight can be estimated for these proteins since there is a symmetric peak on gel filtration and a single band on SDS-gel electrophoresis. These more acidic forms of renin might be originally present in the kidney or they might have been generated during the purification. Although proteases were inhibited during the acidification process, it is not possible to exclude a partial proteolysis. Alternatively, a relative desialation of the protein might occur at an acidic pH.

The presence of a higher molecular weight renin in hog kidney has been reported by several authors.31,32 This renin might be bound to another protein.28 This type of renin could not be detected in our preparation either by gel filtration or by SDS-gel electrophoresis. The acidification process at pH 3.5 cannot account for the presence of renin with a molecular weight of only 40,000 since higher molecular forms of renin have been reported in both unacidiﬁed and acidified extracts.31

Finally, the renin purified in this study can be easily differentiated from the pseudorenin described by Skeggs et al.8 The present enzyme has an optimum pH of 6.6 on the tetradecapeptide substrate, whereas no reaction occurred at the pH of 4.5 optimal for pseudorenin. Furthermore, in contrast with recent experiments of Day and Reid28 on brain renin, no cathepsin-like activity was detected in our preparation.

**Addendum**

Since this manuscript was submitted for publication, T. Inagami and K. Murakami reported the isolation and characterization of pure hog renin (J Biol Chem 252: 2978-2983, 1977). Similar physicochemical characteristics were found (glycoprotein, molecular weight, isoelectric point). The difference of specific activity reported in the present study (1100 GU/mg protein) and in their report (2000 GU/mg protein) could be due to differences in the pH of the renin assay and to the use of impure substrate from different species. This discrepancy emphasizes the need for a new standardization of renin in a pure system.

**Acknowledgments**

We thank Dr. Haas and Dr. Goldblatt for their continuing interest in our work and for testing our purified renin in their trained unanesthetized dogs. We also express our gratitude to Professor Uncea and Dr. Anyagi for their most generous gifts of pepstatin and we wish to thank Dr. Frenoy for performing the amino acid analysis. The gift of standard hog renin by Medical Research Council is gratefully acknowledged.

**References**


Relationship between Acetylstrophanthidin-Induced Aftercontractions and the Strength of Contraction of Canine Ventricular Myocardium

GREGORY R. FERRIER

SUMMARY  Contractile activity was recorded from isolated canine ventricular muscle exposed to acetylstrophanthidin (AS), 0.5 to 2 x 10^-7 g/ml. Development of the positive inotropic effect of AS was accompanied by the appearance of aftercontractions (AC) coupled to the driven responses. The amplitudes of AC increased with the number of preceding beats. Test beats occurring during the ascending limb of AC were potentiated and the potentiation increased with the amplitude of the AC. Beats falling during the descending limb decreased in strength as the amplitude of the AC increased. AS eliminated the frequency dependence of restitution. However, staircase phenomena persisted and were then found to be dependent on the phase relationship of each beat to underlying AC. These findings suggest an important role for AC in the inotropic actions of digitals and in determining strength-interval relationships of ventricular muscle treated with digitals.

CARDIAC GLYCOSIDES and aglycones can induce oscillations in both electrical and mechanical activity of isolated cardiac tissues. Oscillatory afterpotentials (OAP) in ventricular and atrial specialized conducting tissues may provide an important mechanism underlying certain digitalis arrhythmias.1 I have recently shown that OAP induced in canine Purkinje tissues by acetylstrophanthidin (AS) are accompanied by aftercontractions (AC).2 Also in that study, I observed AS-induced AC in ventricular muscle, most frequently in the absence of OAP, but occasionally accompanied by this electrical event. AC with or without electrical correlates have been observed previously in cardiac tissues, usually in response to low temperature and elevated calcium concentrations.3 The studies of Reiter4 are notable because AC were induced by dihydro-ouabain in combination with low temperature (25°C) and elevated calcium concentrations. Also of special importance to the present study are the observations of Braveny et al.4 and Posner and Berman.2 Braveny et al. studied AC induced in guinea pig atria by low temperature and high rate or elevated calcium concentrations. Recorded weights over a wide range. Biochem J 96: 595-606, 1965


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