Modulation of AV nodal and Hisian conduction by changes in extracellular space

KEITH G. LURIE,1 ATSUSHI SUGIYAMA,2 SCOTT McKNITE,1 PAUL COFFEEN,1 KEITARO HASHIMOTO,3 AND SHIGERU MOTOMURA3

1Cardiac Arrhythmia Center, University of Minnesota, Minneapolis, Minnesota 55455; 2Department of Pharmacology, Yamanashi Medical Center, 409-38 Yamanashi; and 3Department of Pharmacology, Hiroasaki Medical Center, 036 Hiroasiki, Japan

Lurie, Keith G., Atsushi Sugiyama, Scott McKnite, Paul Coffeen, Keitaro Hashimoto, and Shigeru Motomura. Modulation of AV nodal and Hisian conduction by changes in extracellular space. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H953–H960, 1999.—Previous studies have demonstrated that the extracellular space (ECS) component of the atrioventricular (AV) node and His bundle region is larger than the ECS in adjacent contractile myocardium. The potential physiological significance of this observation was examined in a canine blood-perfused AV nodal preparation. Mannitol, an ECS osmotic expander, was infused directly into either the AV node or His bundle region. This resulted in a significant dose-dependent increase in the AV nodal or His-ventricular conduction time and in the AV nodal effective refractory period. Mannitol infusion eventually resulted in Wenckebach block (n = 6), which reversed with mannitol washout. The ratio of AV nodal to left ventricular ECS in tissue frozen immediately on the development of heart block (n = 8) was significantly higher in the region of block (4.53 ± 0.61) compared with that in control preparations (2.23 ± 0.35, n = 6, P < 0.01) and donor dog hearts (2.45 ± 0.18, n = 11, P < 0.01) not exposed to mannitol. With lower mannitol rates (10% of total blood flow), AV nodal conduction times increased by 5–10% and the AV node became supersensitive to adenosine, acetylcholine, and carbachol, but not to norepinephrine. We conclude that mannitol-induced changes in AV node and His bundle ECS markedly alter conduction system electrophysiology and the sensitivity of conductive tissues to purinergic and cholinergic agonists.

Although the electrophysiology of the atrioventricular (AV) node and His bundle region of the heart has been studied extensively over the past several decades, much less is known about the unique biochemical features of this amalgam of modified cardiac muscle and nerve cells (9–11, 16, 19). Hampered previously by complex anatomy, cellular heterogeneity, and microscopic size, over the past several years, new microanalytic tools have been developed to study anatomically complex regions of the heart, including the AV nodal region. We used these techniques to measure regional differences in extracellular space between conductive and adjacent contractile tissues (10). In studies in rat and rabbit hearts, we observed that the extracellular space component of the AV nodal region was 2.5 times larger than the extracellular space component in the adjacent contractile tissue. On the basis of wet-to-dry weight tissue ratios and the serum concentration of extracellular markers, we estimated that ∼30% of the contractile myocardium and 70% of the AV nodal region of the beating heart was composed of extracellular space.

Given the existence of such marked regional differences in extracellular space, we have speculated that changes in extracellular space induced by endogenous processes such as ischemia and aging or exogenous hyperosmotic agents such as mannitol would alter AV nodal conduction characteristics (10). In the present study, we tested this hypothesis in a well-established blood-perfused canine AV nodal preparation (7, 8, 14, 15, 18). The canine blood-perfused AV nodal preparation provides an opportunity to study electrophysiological, biochemical, and anatomic differences between different regions of the heart in a single preparation. With this physiological preparation, perfusion can be selectively directed to either the AV node or the His bundle region, and we evaluated the electrophysiological and biochemical effects of mannitol. When injected into the coronary circulation of the preparation, this osmotically active agent moves passively from the vasculature into the interstitium but does not move intracellularly. The results support the hypothesis that alterations in extracellular space in the AV nodal region dramatically alter the electrical properties of this region.

METHODS

Blood-Perfused Canine Preparation

All experiments were performed according to the guidelines of the Committee for Animal Experimentation at the University of Minnesota. The canine blood-perfused AV nodal preparation has been well described previously (7, 8, 15, 18). In brief, the coronary arteries were cannulated in an explanted canine heart, which we will refer to as the “preparation” heart. Only vessels that supply blood to the sinus node. AV node, His bundle, and interventricular septum remained patent; all others were ligated. Atrial and ventricular muscle subserved by the ligated coronary arteries was removed. The preparation was trimmed such that the sinus node and AV nodal region were visualized. Endocardial electrodes were gently attached to the region adjacent to the sinus node. His bundle, and right ventricular septal region. Bipolar electrograms were recorded continuously. The preparation was paced as needed. This preparation received oxygenated blood from a “donor” dog, which was previously anesthetized and treated with heparin as also previously described (15). The temperature of the preparation was maintained at 37.0 ± 0.5°C. Perfusion pressure was maintained at 120 mmHg. The preparation time varied between 45 min and 1.5 h. After reperfusion, the preparations typically fibrillated for ∼30–45
min, at which point in time normal sinus rhythm was restored. After sinus rhythm was restored in these preparations, the preparation heart remained constant, from an electrophysiological standpoint (8, 14, 15), for >4 h.

The coronary anatomy of the dog is unlike that of some other species in that the first septal perforator artery, a branch of the left anterior descending coronary artery, provides most of the blood to the His-Purkinje system. Similarly, the circumflex coronary artery provides most of the blood supply to the AV nodal region. Blood flow was measured in each coronary artery using electromagnetic flow probes (Howell Instruments, Camarillo, CA) attached to the perfusion cannula. This enabled instantaneous measurement of flow in each of the three coronary arteries throughout the experiments as previously described (14, 15). With this model, injections of mannitol and other cardioactive agents were directed selectively into one particular region of the conduction system.

Mannitol was prepared as a 20% (wt/vol) solution of either normal saline or Tyrode solution, depending on the experiment. It was infused at different rates of the total coronary blood flow (10–50%) into either the circumflex or first septal coronary artery.

Effects of Mannitol on AV Nodal and Hisian Conduction and Extracellular Space

Electrophysiology studies. Once preparations had returned to a normal sinus rhythm, pacing was performed at a cycle length of 400 ms with a pulse width of 0.5 ms and an amplitude of 1.0 V delivered via a quadrupolar electrode attached to the right atrial tissue near the sinus node. The atrio-Hisian (AH), Hisian-ventricular (HV), and R-R intervals were recorded continuously as previously described (14). Under basal conditions, the Wenckebach block cycle length and the effective refractory period (ERP) at a pacing cycle length of 400 ms were determined. The sensitivity to acetylcholine was also evaluated in each preparation as previously described (14). After the baseline electrical characteristics of the preparation were obtained, mannitol (20% wt/vol) was infused at varying percentages (10–50%) of the total blood flow, depending on the specific experimental protocol. In studies designed to examine the potential to induce Wenckebach block with infusions of mannitol, the mannitol infusions were initiated at 10% of the total blood flow to a specific coronary artery. The infusion rate was adjusted every 10–15 s depending on the overall blood flow to the target coronary artery. After 5 min, the infusion rate was increased sequentially every 5 min to 20, 25, 30, 40, and 50% of the total coronary artery blood flow until Wenckebach block was observed. The ERP was determined at the different infusion rates of mannitol until block occurred. When heart block was induced, the mannitol infusion was immediately turned off.

In studies designed to examine the sensitivity of the AV node to purinergic, cholinergic, and adrenergic agents before and after mannitol, mannitol was infused into the circumflex artery as described above at 10% of the total blood flow. After 5 min, once the AV nodal conduction time stabilized, a dose-response curve to either adenosine, carbachol, or norepinephrine was obtained. There was a minimum of 3 min between each drug infusion. When the same preparation was used for more than one agonist, we waited 10 min before using a second agonist.

Biochemical studies. In preparations in which extracellular space was measured, 200 mg/kg of inulin (1 g/10 ml normal saline solution at 37°C) was infused into the jugular vein of the donor dog 15 min before initiation of the mannitol infusion. Blood samples were obtained every 3 min from the arterial blood perfusing the preparation heart to evaluate inulin concentrations over time as previously described (10). Inulin levels, measured in the serum from the donor dog, achieved a stable equilibrium between 10 and 15 min after venous infusion into the donor dog. In these control heart preparations, no mannitol was administered. In controls, 20 min after inulin was injected into the donor dogs, the AV node and His bundle region were rapidly excised and plunged into tetrafluoroethane (Stephens Scientific, Riverdale, NJ) previously cooled to −80°C in liquid nitrogen. In experiments in which mannitol was administered, the preparations were rapidly removed just at the time that heart block was observed electrophysiological, and these preparations were rapidly frozen as described above. The frozen preparations were stored at −40°C until biochemical measurements were performed.

Inulin was measured in serum and tissue as previously described (10). Tissue inulin was assayed in portions of the right atrium, AV node, His bundle, and left ventricle in freeze-dried microdissected sections. The discrete regions of the conduction system were localized by using both anatomic landmarks and a stain for acetylcholinesterase activity as previously described (5, 10, 19, 21).

Biochemicals were obtained from Sigma Chemical (St. Louis, MO), and enzymes used for the enzymatic measurement of inulin were obtained from Boehringer Mannheim (Indianapolis, IN). Animals were obtained from class B breeders through Laboratory Animal Medicine at the University of Minnesota. Mongrel dogs (10–25 kg) of either gender were used in these studies.

Statistical Analysis

Data were recorded and analyzed as previously described (7, 14). All data were expressed as means ± SE. Statistical significance within a parameter was evaluated by one-way repeated-measures ANOVA. When a P value was <0.05 by ANOVA, the intervention was judged as having affected the parameter. In this case, the statistical significance between the control and a value at a particular time point after the intervention (for example, mannitol infusion) was determined by contrasts for mean values comparison, and a P value <0.05 was considered significant. The statistical significance between groups was evaluated by one-way factorial ANOVA followed by multiple-comparison tests with Bonferroni-Dunn. A P value of <0.05 was considered statistically significant.

RESULTS

Electrophysiology Studies

The effects of mannitol on the ERP and AV nodal conduction times from six preparations are shown in Table 1. The mean AV conduction time for the six preparations before mannitol infusion was 148 ± 4.2 ms, and the mean AH interval was 112.2 ± 3.6 ms. These values are similar to those we have previously reported (14, 15). With incremental increases in the infusion rate of mannitol, both the refractoriness of the AV node and the AH interval increased in parallel. The differences in the ERP with increasing concentrations of mannitol were statistically significant. There were fewer data points with the higher mannitol infusion rates, because AV block was observed in 50% of the preparations with 30% mannitol infusion rates.
A representative tracing of the AV conduction time from a single preparation during the administration of mannitol is shown in Fig. 1. The right atrium was paced at a cycle length of 400 ms. After infusion of mannitol at 10% of the baseline blood flow, AV nodal conduction time began to increase. As the conduction time continued to be prolonged, there was the appearance of two regular but distinct AV conduction time intervals (Fig. 1, arrow) just before the onset of Wenckebach block. At that point, the mannitol infusion was turned off and AV nodal conduction was restored. Although 1:1 AV conduction was rapidly restored with mannitol washout, the time required for AV nodal conduction times to return to premannitol values varied. However, this kind of heart block was reproducible from one preparation to the next and was reproducible within the same preparation. The median amount of mannitol solution (2 g/10 ml normal saline or Tyrode solution) needed to generate heart block was 25% (range 10–50%) of the coronary artery blood flow. Full reversibility of the mannitol effect was dependent on the number of times the preparation was exposed to mannitol and the overall quality of the preparation at the time of the initial mannitol infusion.

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### Table 1. Effects of mannitol on AV conduction

<table>
<thead>
<tr>
<th>Mannitol infusion, % total blood flow</th>
<th>AH</th>
<th>ERP</th>
<th>FRP</th>
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</thead>
<tbody>
<tr>
<td>Baseline values, ms</td>
<td>112.2 ± 3.6</td>
<td>263.7 ± 7.7</td>
<td>336.0 ± 6.1</td>
</tr>
<tr>
<td>10</td>
<td>2.8 ± 0.08</td>
<td>3.8 ± 2.2</td>
<td>1.0 ± 0.6</td>
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<tr>
<td>20</td>
<td>6.5 ± 2.1</td>
<td>12.8 ± 4.4</td>
<td>5.0 ± 1.5</td>
</tr>
<tr>
<td>25</td>
<td>17.7 ± 3.5</td>
<td>23.0 ± 4.7</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>30</td>
<td>22.2 ± 4.1</td>
<td>43.3 ± 2.6</td>
<td>21.6 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. AH, atrio-His interval (preparations were paced at a cycle length of 400 ms); ERP, effective refractory period; FRP, functional refractory period. Mannitol (2 g/10 ml Tyrode solution) was infused at increasing concentrations of total blood flow directly into circumflex artery. Baseline values represent mean values obtained over 5 min before initial mannitol infusion. Data demonstrating difference between baseline values and those observed after mannitol infusion (change from baseline values) were obtained by measuring largest change from baseline values during successive mannitol infusions. A total of 6 atrioventricular (AV) nodal preparations were studied. *P < 0.01, significant change from baseline value.

### Results

Results from a representative experiment demonstrating the relationship between AV nodal refractoriness and mannitol infusion rates (Fig. 2) highlight the effects of mannitol in these preparations. With an increase in infusion of mannitol from 0 to 30% of total blood flow in the circumflex coronary artery, which supplies blood to AV node, relationship between A1A2 and A2H2 shifted upward and to the right in a consistent and characteristic fashion. A1 is the atrial pacing cycle length (400 ms), and A2 is the premature atrial stimulus. A1A2 is the coupling interval between A1 and A2. A2H1 is the recorded atrio-His interval following the delivered premature atrial impulse (A2).

Infusion of mannitol selectively into either the AV nodal or first septal artery reproducibly resulted in either reversible AV nodal block or reversible infra-
Hisian block. When mannitol was infused into the first anterior septal artery of the left anterior descending coronary artery selectively to the His bundle region, infra-Hisian block could be selectively induced without changing the AV nodal conduction properties (Table 2). Figure 3 is an example of electrograms from a typical experiment in which infra-Hisian block was induced with mannitol and then readily reversed as the mannitol was washed out. Invariably, 1:1 conduction was restored within <2 min after the mannitol infusion was terminated.

### Biochemical Measurements

Measurements of extracellular space were performed in both the preparation hearts as well as in hearts from the donor dogs. For these experiments, inulin was infused into the donor dog, and, after serum inulin levels had reached an equilibrium (20 min after the

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**Table 2. Effects of mannitol on HV conduction**

<table>
<thead>
<tr>
<th></th>
<th>AH</th>
<th>HV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline values, ms</td>
<td>117.5 ± 8.8</td>
<td>38.5 ± 1.1</td>
</tr>
<tr>
<td>Mannitol infusion, % total blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (5 min)</td>
<td>2.3 ± 1.0</td>
<td>6.5 ± 1.4*</td>
</tr>
<tr>
<td>20 (5 min)</td>
<td>2.0 ± 0.8</td>
<td>10.0 ± 2.9*</td>
</tr>
<tr>
<td>20 (10 min)</td>
<td>4.8 ± 3.6</td>
<td>22.0 ± 8.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. AH and His-ventricular (HV) values shown during mannitol infusion represent the change from baseline values without mannitol. Mannitol (2 g/10 ml Tyrode solution) was infused into His bundle region at increasing concentrations of total blood flow for different time intervals. Results from 4 preparations are shown. HV intervals reported after mannitol were obtained by measuring longest HV interval just before onset of complete HV block. *P < 0.05, significant change from baseline value.
The relationship between structure and function is vital to understanding the mechanism of impulse transduction through the AV nodal region of the heart. Results from the present study performed in dog hearts confirmed our previous observations made in rat and rabbit hearts that the extracellular space component of the AV nodal region is approximately 2.5 times larger than the corresponding extracellular space in adjacent contractile muscle (5, 10). The current results also demonstrated two new basic physiological observations. The first is that infusion of mannitol selectively into the AV node-to-left ventricular extracellular space increased significantly with the mannitol infusions.

Table 3 and Fig. 4 also demonstrate the changes observed in the extracellular space of conductive and contractile tissues from preparations (n = 8) infused with mannitol (2 g/10 ml normal saline solution) until development of Wenckebach block. These data reflect the extracellular space measurements at the time of heart block. In the mannitol-infused preparations, the AV node-to-left ventricular extracellular space ratio was markedly increased after the mannitol treatment. The ratio between AV nodal and left ventricular extracellular space increased from 2.23 ± 0.35 in control preparations to 4.53 ± 0.61 in mannitol-treated preparations (P < 0.001) and was associated with electrophysiological evidence of heart block.

Mannitol-Induced Supersensitivity to Adenosine and Acetylcholine

We next examined the relationship between the responsivity of the AV node to cholinergic, purinergic, and adrenergic agonists before and after mannitol infusion. In these studies, mannitol was infused at 10% of baseline blood flow. As shown in Fig. 5, mannitol infusion resulted in a significant increase in the sensitivity of the AV node to the negative dromotropic properties of acetylcholine, adenosine, and carbachol. The adenosine and carbachol concentration curves were shifted to the left when preparations were treated with an infusion of mannitol (2 g/10 ml Tyrode solution) at an infusion rate of 10% of the total coronary flow. In these studies the AV nodal tissue was significantly more sensitive to lower concentrations of adenosine and carbachol when injected into the AV nodal artery during infusion of mannitol when compared with injection of Tyrode solution alone. Evaluation of the effective dose of agonist resulting in a 20-ms increase in the AV conduction time (ED20) revealed that mannitol decreased ED20 from >300 to 100 µg with adenosine, from 0.35 to 0.17 µg with carbachol, and from 0.31 to 0.24 µg with acetylcholine (Fig. 5). In contrast, there was no change in sensitivity of the AV node to the adrenergic agonist norepinephrine. The AH interval decreased in a characteristic fashion when norepinephrine (3–30 µg) was injected in the absence or presence of a concurrent infusion of mannitol at 10% of the total blood flow.

**DISCUSSION**

The relationship between structure and function is vital to understanding the mechanism of impulse transduction through the AV nodal region of the heart. Results from the present study performed in dog hearts confirmed our previous observations made in rat and rabbit hearts that the extracellular space component of the AV nodal region is approximately 2.5 times larger than the corresponding extracellular space in adjacent contractile muscle (5, 10). The current results also demonstrated two new basic physiological observations. The first is that infusion of mannitol selectively into the AV node-to-left ventricular extracellular space increased significantly with the mannitol infusions.
nodal artery resulted in a prolongation of both the AV nodal conduction time and ERP and eventually resulted in the development of reversible heart block. Similar findings were observed with selective infusion of mannitol into the His bundle region. Measurement of the extracellular space component of the AV node at the time of block demonstrated that heart block in these preparations was associated with a significant increase in the extracellular space component of the AV node compared with that of control preparations. These observations suggest that modulation of the extracellular space component of the AV node is associated with alterations in AV nodal conduction velocity, AV nodal refractoriness, and the development of reversible heart block.

The second new observation from these studies is that infusion of concentrations of mannitol sufficient to slow AV nodal conduction but not create AV block resulted in a marked supersensitivity of the AV nodal region to cholinergic and purinergic agonists. These findings support the hypothesis that the sensitivity of the AV nodal region to selective agonists may be enhanced by small shifts in extracellular space. The mechanism underlying the increased sensitivity to cholinergic and adenosinergic agonist remains speculative. Mannitol may alter ion channel activity such as the chloride swell channel (4) and/or potassium channel (17, 22), gap junction integrity, changes in repolarization, or receptor-G protein-receptor coupling, or it may induce stretch or alter the metabolism of agonists or secondary messengers such as acetylcholine and adenosine. In the present study, selective infusion of the AV nodal artery with a mannitol-containing solution resulted in selective AV nodal block with maintenance of intact His-Purkinje conduction. Similarly, infusion of the same mannitol solution selectively into the His bundle via the first septal perforator artery resulted in selective infra-Hisian block with maintenance of intact AV nodal conduction. These results demonstrate that the interstitial space surrounding the AV nodal cells and the His bundle region are anatomically distinct spaces. Moreover, processes that selectively alter the extracellular space within each respective region can induce regionally specific heart block. Heart block within each of these regions could be mediated by distinct extracellular space-related mechanisms and may have different clinical implications.

Fig. 5. Mannitol infusion at 10% of total blood flow to AV nodal region resulted in a marked increase in sensitivitiy of AV node to negative dromotropic properties of ACh (n = 4 experiments), adenosine (n = 4), and carbachol (n = 2), with no change in sensitivity of preparation to norepinephrine (NE; n = 4). Adenosine, ACh, and carbachol dose-response curves were shifted upward and leftward when preparations were treated with an infusion of mannitol (2 g/10 ml Tyrode solution) at an infusion rate of 10% of total coronary blood flow. Tyrode solution infusion alone had no significant effect on AH interval. *P < 0.05 compared with control values and preparations exposed to Tyrode solution alone. AVB, AV block; ratios in parentheses are proportion of preparations that developed heart block at a given agonist concentration.

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distinct regions could be rapidly induced and was rapidly reversible on washout of the mannitol solution.

Taken together, these results suggest that modulation of extracellular space within the cardiac conduction system is a tightly regulated process. At present, little is known about the basic physiological regulatory processes governing extracellular space in the conduction system (23). We hypothesize that agents or processes that result in an increase in extracellular space, such as infusions of hyperosmolar agents, ischemia, and probably aging, will result in an increased propensity for heart block. For example, in patients with inferior myocardial infarctions, who often develop reversible AV nodal block, an increased extracellular space within the AV nodal region secondary to ischemia may result in an increased sensitivity of that region to both adenosine and acetylcholine (4, 6). A similar effect of hyperosmolar agents has been previously observed in the atria, where infusion of mannitol and hypertonic saline resulted in the development of atrial fibrillation and increased sensitivity to purinergic and cholinergic agents (3). In view of the present observations, we believe that the regulation of AV nodal conduction, even in the absence of ischemia, may be in part modulated by subtle changes in extracellular space.

Whereas induction of AV nodal heart block with mannitol infusion is associated with an increase in the extracellular space component of the AV node, this correlation may be by association and not causal. In these studies we were unable to examine potential changes in the intracellular volume of AV nodal cells under control conditions and after mannitol. Thus it is possible that rapid cell shrinkage, rather than, or in addition to, extracellular space expansion, was involved in the development of AV nodal block in these experiments. In addition, it is possible that endogenous adenosine was not metabolized as rapidly in the presence of mannitol or an increased extracellular space and that the observed AV block was a consequence of altered adenosine metabolism. These questions remain under investigation. Another potential limitation of these observations is that we tested only one osmotically active agent, mannitol, in the current studies. However, we have created reversible heart block with both mannitol and sorbitol infusions using a rat heart Langendorff preparation (unpublished observations), and similar kinds of reversible conduction system block have been reported in patients who received hypertonic contrast agents during cardiac catheterization procedures (23). Thus it is likely that the results in the current study are due not to a unique property of mannitol but rather to the osmotic effects of this agent.

Finally, the animal model itself could be criticized. For example, a potential limitation of the present studies relates to changes in the regulation of extracellular space in the AV nodal preparation itself. The present results, demonstrating that the AV node-to-left ventricular extracellular space ratio is ~2.5, are identical to results we have produced previously in rat and rabbit hearts frozen immediately after they were removed from the chest, in the absence of subsequent blood perfusion as in the present experiments (10). In addition, similar results were observed in studies in the control donor dog hearts and in the canine blood-perfused AV nodal preparations in the present experiments, demonstrating that the processes that control extracellular space appear to remain intact in the blood-perfused AV nodal preparations. Although no model is perfect, we chose the blood-perfused canine model because of the stability of the preparation and because we could infuse drugs directly and selectively into each of the respective coronary arteries (7, 14, 15). This is one of the only ex vivo models in which one can selectively infuse drug into either the AV node or the His bundle region.

In other parts of the body, for example, the brain and spinal cord, alterations in extracellular space can result in dramatic functional abnormalities. Changes in the extracellular space in the hippocampus can result in seizure activity (1, 2, 12, 20). This activity is tightly linked to cellular osmolality, and restoration of the extracellular space component to control values results in cessation of seizure activity. We speculate that similar processes may help to regulate impulse conduction velocity within different regions of the cardiac conduction system and, perhaps, throughout the contractile myocardium. Disorders of cardiac conduction may result, in part, from disorders in extracellular space regulation. The processes that modulate extracellular-intracellular fluid balance remain poorly understood yet vital to the normal physiology of the heart (11). In pathological states, and with cell dropout associated with aging in particular, the importance of extracellular space homeostasis may be more important than previously recognized. The present experiments serve to underscore the potential critical role that the regulation of extracellular space plays in the beat-to-beat impulse transduction in the heart. A better understanding of these processes may lead to pharmacological therapies for certain types of cardiac arrhythmias.

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REFERENCES