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Pharmacokinetic Studies of Taurine in Bovine Purkinje Fibers

STEVEN I. BASKIN, PAUL T. ZAYDON, ZEBULON V. KENDRICK, TOBI C. KATZ, AND PAUL L. ORR

SUMMARY Taurine (2-aminoethane sulfonic acid) is found in high concentrations in the heart, particularly in Purkinje fibers. We studied the transport of taurine in Purkinje fibers that were excised rapidly from the heart and placed in a vessel containing oxygenated Krebs-Henseleit solution (37°C). After equilibration, 4.4X10^-4 M radiolabeled taurine[^14C] was added to the bath. A computer compartmental analysis of the uptake and efflux indicated the presence of two pools for uptake—a pool with a rapid kinetics K1 (t_1/2 = 0.80 min) and K2 (t_1/2 = 176.30 min). These studies suggest that Purkinje fibers have the capacity to transport taurine rapidly. Michaelis-Menten procedures showed the presence of a high affinity and a low affinity transport process. Guanidinotaurine, at a 10:1 ratio, had no appreciable effect on taurine uptake, but 3-aminopropane phosphonic acid decreased taurine uptake by 42.7%. Ouabain and acetylstrobilphonytin (10^-5 M) inhibited taurine uptake (K_1) by 34% and 73%, respectively. The inhibition of the rapid component of taurine uptake suggests that K_1 is an energy-linked process possibly requiring Na^+-K^+-ATPase. Taurine uptake in a calcium-free medium was decreased by 58%. Verapamil (6 X 10^-5 M) decreased taurine uptake by 42%, Tetrodotoxin (3.4 X 10^-5 M) decreased taurine uptake by 51%. The requirement of calcium and sodium for taurine uptake suggests an important relationship between taurine, calcium, and sodium in the function of fibers in the cardiac conducting system. Circ Res 47: 763-769, 1980

THE acidic amino acid, taurine, exists in relatively high concentrations in the hearts of animals (Scharff and Wool, 1964) and humans (Grosso and Bressler, 1976). The rat heart, in vivo, retains taurine for a greater period of time than any other tissue (Awapara, 1976; An and Fromogeot, 1960), suggesting a high affinity of this organ for this substance. Taurine has been proposed to play a role in the maintenance of cardiac and erythrocyte osmolarity (Fugelli and Zacharriassen, 1976; Vislie and Fugelli, 1975), to be antiarrhythmic in isoproterenol- and digoxin-induced arrhythmias (Novelli et al., 1969; Read and Welty, 1963), and to have a role in regulating the cardiac rhythm of the dog (Chazov et al., 1974), the transmembrane ionic currents of guinea pig ventricle (Diacono and Dietrich, 1976), and positive inotropic activity (Guidotti et
al., 1971; Dietrich and Diacono, 1971). In addition, the taurine concentration was increased approximately 2-fold in the myocardium of patients with congestive failure (Huxtable and Bressler, 1974) and dogs with experimentally induced cardiac failure (Newman et al., 1977; Peterson et al., 1973).

The effect of taurine on ion movements is not understood. An action on cations, such as calcium (Dolara et al., 1973a, 1973b) and potassium (Read and Welty, 1965), as well as on anions, such as chloride (Hosli et al., 1975), has been proposed as a possible means through which taurine could exert an effect.

Our earlier studies (Kocsis et al., 1976) indicated that taurine is concentrated preferentially in Purkinje fibers, rather than in other cardiac tissues. Since taurine’s high concentration in cardiac Purkinje fibers may explain some of its effects on cardiac rhythm and contractility in both physiological and pathological states, we performed pharmacokinetic studies on taurine in isolated Purkinje fiber bundles.

Methods

General

In all experiments, bovine (steer) hearts were obtained from a local abattoir, immediately packed in ice, and bathed in Krebs-Henseleit (K-H) solution, pH 7.4. The composition of the K-H in mM was as follows: 118 mM NaCl, 4.8 mM KCl, 1.0 mM KH2PO4, 1.2 mM MgSO4, 2.5 mM CaCl2, 11.1 mM glucose, 27.2 mM NaHCO3, vigorously gassed for 15 minutes with 95% O2-5% CO2. Purkinje fiber bundles (approximately 2-3 mm long and 0.5-1 mm wide) were excised from the endocardium, tied off at one end using 5-0 nylon suture [negligible amounts of 14C-taurine (less than 0.1%) was found to be bound to the nylon suture].

The preloaded terminal Purkinje fiber bundles were blotted dry, all of the 5-0 nylon suture removed, and the fiber bundles were weighed to the nearest 0.1 mg.

Calculations

Statistical analyses were performed on a CDC Cyber 174 computer using the statistical package for the social sciences. For all data with multiple groups, a one-way analysis of variance was performed, followed by the post hoc Scheffé test (α = 0.05) using the harmonic mean of the sample sizes. Neither the Bartlett Box-F nor Cochran C-test for homogeneity of variance was significant; therefore, the hypothesis of equal variances (an assumption of the ANOVA) was not rejected (Weiner, 1971). The asymptote of uptake was determined by a statistical program (Olivetti Underwood, Program number 4.35).

Compartmental analysis was performed by a Hewlett Packard 2000 computer program developed by Dr. Leslie Bailey of the Dalhousie University, Halifax, Nova Scotia, Canada.

Rate of Uptake of 14C-Taurine in Purkinje Fibers

Uptake of taurine was determined by bathing Purkinje fiber bundles in K-H solution containing 4.4 X 10^-6 M radiolabeled taurine[14C]. This solution was maintained in a jacketed Pyrex bath at 37°C and gassed continuously with 95% O2/5% CO2. Non-beating Purkinje fiber bundles were bathed for 0.083, 0.25, 0.5, 1, 3, 5, 10, 15, and 20 minutes. Fiber bundles were seen not to be beating by microscopic visualization. Five different fiber bundles were excised and used for each time period tested. On removal from the bath, the Purkinje fiber bundles were blotted dry, all of the 5-0 nylon suture removed, and the fiber bundles were weighed immediately. Fiber bundles were digested overnight in 0.1 ml NCS tissue solubilizer and counted for 10 minutes using 10 ml of Scintiverse scintillation fluid in a Packard liquid scintillation counter.

Efflux of 14C-Taurine in Preloaded Purkinje Fibers

Purkinje fiber bundles were bathed in K-H containing 4.4 X 10^-6 M 14C-taurine at 37°C for 15 minutes to load them. This preloaded fiber bundle was placed in a jacketed Pyrex bath which contained 3.0 ml of K-H. After 30 seconds the K-H was drained and 3.0 ml of fresh K-H was added and at 1 minute this was drained and 3.0 ml of fresh K-H was added over a time course of 0.5, 1, 3, 5, 10, 15, 20, and 30 minutes to yield nine 3.0-ml K-H washout samples for each Purkinje fiber bundle used.

Each K-H washout sample was counted as two 1.5-ml portions (to facilitate operation in the liquid scintillation counter). Each portion received 10 ml of Scintiverse and was similarly assayed for radioactive taurine. At the end of each experiment the Purkinje fiber bundle was weighed, digested, and assayed as described in “Rate of Uptake of 14C-Taurine in Purkinje Fibers.”
Concentration of Taurine vs. Uptake

To examine the effects of concentration on uptake, 14C-taurine solutions of the following molar concentrations were used: $6.6 \times 10^{-7}$, $6.6 \times 10^{-6}$, $6.6 \times 10^{-5}$, $6.6 \times 10^{-4}$, and $6.6 \times 10^{-3}$. Fiber bundles were prepared as before in the manner described in "Rate of Uptake" and incubated for 30 seconds in one of the above 14C-taurine solutions contained in a jacketed Pyrex bath. Fiber bundles were removed, blotted dry, and immediately weighed. Fiber bundles were digested and were counted for 10 minutes as previously described.

Inhibition of Taurine Uptake

Ouabain, acetylstrophanthidin, 3-amino propane phosphonic acid, and guanidinotaurine were examined for their inhibitory effect on taurine uptake. Ouabain and acetylstrophanthidin were chosen to determine whether K$_i$ (fast rate component) was an extracellular component. Guanidinotaurine and 3-amino propane phosphonic acid were employed to determine which chemical may inhibit the taurine uptake process.

Quiescent Purkinje fibers were bathed for 30 seconds in K-H containing ouabain ($10^{-5}$ M) and taurine ($4.4 \times 10^{-6}$ M). After 30 seconds had elapsed, the radioactivity in each fiber was measured according to the procedure previously mentioned. Because of the slow onset of action of ouabain and thus the less than maximal effect at 30 seconds, we also used acetylstrophanthidin, a glycoside-like compound, which is known to exhibit a more rapid onset of action than ouabain. A solution containing acetylstrophanthidin ($10^{-5}$ M) and taurine ($4.4 \times 10^{-6}$ M) was prepared and fibers were bathed in this solution for 30 seconds and the radioactivity was measured.

A 3-amino propane phosphonic acid/taurine solution was prepared at a ratio of $4.4 \times 10^{-5}:4.4 \times 10^{-6}$ (10:1). Fibers were bathed in this solution for 30 seconds and counted for 10 minutes according to the taurine uptake procedure. Guanidinotaurine ($4.4 \times 10^{-6}$ M) was prepared in the same manner as was 3-amino propane phosphonic acid. Fibers were bathed for 30 seconds in this solution and counted for 10 minutes as before.

To determine the effects of extracellular calcium on taurine uptake, Purkinje fibers were placed in a radiolabeled taurine ($4.4 \times 10^{-6}$ M) K-H solution in which the CaCl$_2$ was replaced by an equimolar amount of sucrose for the 15-minute equilibration period. The fibers were bathed for 30 seconds in the radiolabeled taurine K-H solution and the radioactivity measured as described previously.

Verapamil Inhibition of Taurine Uptake

Verapamil, $6 \times 10^{-6}$, $6 \times 10^{-7}$, $6 \times 10^{-8}$, and $6 \times 10^{-9}$ M (final concentration) was added to the K-H bath containing 14C-taurine/taurine ($4.4 \times 10^{-6}$ M) and its effect on taurine uptake was determined.
Table 1  Kinetics of Taurine in Bovine (Steer) Fibers

<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>$K_i$ (mol)</th>
<th>$t_{1/2}$ (min)</th>
<th>$K_{2f}$ (mol)</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake</td>
<td>5</td>
<td>1.66 ± 0.05</td>
<td>0.42 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>9.89 ± 0.20</td>
</tr>
<tr>
<td>Efflux</td>
<td>5</td>
<td>0.86 ± 0.20</td>
<td>0.80 ± 0.13</td>
<td>3.93 ± 0.76</td>
<td>176.31 ± 37.38</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM.
* Kinetics determined by compartmental analysis.
† $K_i$ and $K_{2f}$ and kinetic rate constants for uptake and efflux.
‡ $t_{1/2}$ is the half-time for uptake and efflux.

Discussion

Our studies indicate the following: (1) bovine Purkinje fibers have the ability to rapidly take up and release taurine in vitro; (2) the rapid uptake of taurine may be explained by an active transport to an intracellular compartment. This is suggested, although not proven, by the inhibition of the uptake component ($K_i$) by ouabain and acetylstrophanthidin; (3) the sulfonic acid moiety of taurine seems to be involved in the uptake of this molecule, whereas the amino group has no effect on taurine uptake at the concentrations employed; (4) the uptake of taurine is calcium and sodium dependent.

Compartmental analysis of uptake and efflux yielded data which demonstrated a rapid and slow component for both uptake and efflux of taurine in < 0.05). Figure 5 shows the effect of selected concentrations of verapamil on inhibition of $^{14}$C-taurine when fibers were bathed for 60 seconds. At a $6 \times 10^{-6}$ M concentration, verapamil reduced taurine uptake by 42%.

Discussion

Our studies indicate the following: (1) bovine Purkinje fibers have the ability to rapidly take up and release taurine in vitro; (2) the rapid uptake of taurine may be explained by an active transport to an intracellular compartment. This is suggested, although not proven, by the inhibition of the uptake component ($K_i$) by ouabain and acetylstrophanthidin; (3) the sulfonic acid moiety of taurine seems to be involved in the uptake of this molecule, whereas the amino group has no effect on taurine uptake at the concentrations employed; (4) the uptake of taurine is calcium and sodium dependent.

Compartmental analysis of uptake and efflux yielded data which demonstrated a rapid and slow component for both uptake and efflux of taurine in
bovine Purkinje fibers. The existence of two kinetic compartments is not unlike the high and low affinity compartments found for taurine kinetics in the central nervous system (Lahdesmäki and Oja, 1973). High and low affinity uptake rates were found in Purkinje fibers and have also been reported in various regions of the central nervous system (Lombardini, 1978). The uptake and efflux phenomena observed in this in vitro study are consistent in terms of time parameters with the proposed anti-arrhythmic role of this compound in the heart. Rapid uptake and efflux rates in Purkinje fibers of endogenous taurine, a known anti-arrhythmic substance (Chazov et al., 1974), are proposed to prevent redistribution of electrolytes, possibly calcium or potassium, which may lead to rhythm disturbances. This process may be more prominent in a partially depolarized state. Hutter and Noble (1961) and

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**Figure 3** The effect of taurine concentration on taurine uptake in bovine Purkinje fibers. Purkinje fibers were bathed for 30 seconds in one of the five taurine concentrations ranging from $6.6 \times 10^{-3}$ to $6.6 \times 10^{-1}$ M. Total radioactivity was counted and plotted as the inverse (1/μ) vs. the inverse of the taurine bath concentration (1/C). Using Michaelis-Menton methods, two uptake systems are indicated: a low $K_m$ (4.19 $\times 10^{-6}$ mol) high affinity and a high $K_m$ (7.57 $\times 10^{-3}$ mol) low affinity process.

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**Figure 4** The effect of various agents on taurine uptake in bovine Purkinje fibers. Purkinje fibers were bathed in $4.4 \times 10^{-6}$ M taurine, following methods described in text. Bars indicate mean value for taurine uptake (dpm/mg wet weight) of each treatment. Vertical lines through each bar indicate the standard error of the mean. The asterisk indicates significance compared to control ($P < 0.05$).

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**Figure 5** The effect of verapamil on taurine uptake in bovine Purkinje fibers. Verapamil at the indicated final concentrations was added to $^{14}$C-taurine/taurine 4.4 $\times 10^{-6}$ M solution and the Purkinje fibers bathed for 60 seconds. The dpm/mg dry weight is indicated on the ordinate; increasing verapamil concentrations are indicated on the abscissa. Asterisks indicate significance ($P < 0.05$) following methods described in text. Vertical lines indicate standard error of the mean. Numbers of experiments at each concentration in parentheses.
Carmeliet, (1961) have demonstrated that anion conductance (e.g., chloride) is more important in partially depolarized cardiac tissue than in control tissue. Taurine is known to antagonize, directly, chloride fluxes (Hosli et al., 1975). These two facts taken together suggest that taurine may be more important in affecting electrical activity in a partially depolarized state than in the completely repolarized state.

Ouabain and acetylstrophanthidin (which acts more rapidly) were used to study the possibility that the fast uptake component (K_i) was not due to accumulation in the extracellular space. In the presence of ouabain, taurine uptake (K_i) was reduced by 34%, thereby indicating an energy-linked process because the glycosides are known to inhibit Na^+, K^+ -ATPase. Acetylstrophanthidin depressed uptake by an even greater degree (P < 0.05) than ouabain (73%), which supports the view that rapid uptake occurs from an extracellular site.

The possible existence of high and low affinity uptake processes was studied by observing the change in taurine uptake as a function of its concentration in the surrounding medium. The data, when plotted on a double reciprocal graph (Fig. 3), suggest two uptake processes; a high affinity process (K_m = 4.19 X 10^{-6} mol) and a low affinity process (K_m = 7.56 X 10^{-3} mol). The tissue concentrations and high affinity kinetic properties may suggest that taurine could be a modulator of ionic current or chemical transmitter in Purkinje fibers (Lähdesmäki and Oja, 1973). Data provided in this paper and elsewhere (Baskin et al., 1979) would not yet allow taurine to be considered a chemical transmitter. The V_max and K_m for bovine left ventricular cardiac muscle compare favorably, although these values are one order of magnitude greater than that found in rat heart (Chubb and Huxtable, 1978).

To characterize further the transport of taurine, some structural analogs of taurine were examined. Our objective was to see if we could inhibit taurine accumulation by cardiac Purkinje fibers. Substitution of the sulfonic acid moiety of taurine by using 3-amino propane phosphonic acid inhibited uptake to a much greater degree than did substitution of the amino moiety using guanidinotaurine. These structural differences suggest a specificity for taurine uptake by these fibers.

Removal of the calcium from the medium markedly inhibited taurine uptake by Purkinje fibers. In other studies performed in our laboratory, direct chemical interaction between calcium and taurine was investigated using a calcium ion electrode, and insignificant chelation of calcium by taurine was found (Baskin and Finney, 1979). This finding has confirmed those of others (Dolara et al., 1978; Izumi et al., 1978). Dolara and his associates (1973b) found that taurine could inhibit the rate of loss of intracellular calcium from isolated guinea pig atria. These experiments substantiate the interaction between taurine and calcium in cardiac tissue. Calcium currents previously have been proposed to be related to taurine in both neuronal (Benjamin and Quastel, 1977; Hilton, 1977) and cardiac systems (Dolara et al., 1978).

Tetrodotoxin’s (3.4 X 10^{-5} M) effect on taurine uptake at 30 seconds in Purkinje fibers may suggest that taurine uptake is dependent on sodium inward current in Purkinje fibers. Unlike the other inhibitors examined, tetrodotoxin was found to be without effect at longer time periods (i.e., 10 minutes). The reason for this is uncertain at this time. Our experiments do not completely rule out the influence of other ionic species (i.e., chloride and potassium on taurine uptake. Verapamil (Naylor and Szeto, 1972; Watanabe and Besch, 1974) has been studied extensively for its effects on ion movements, principally calcium, in the heart. Our results (Fig. 5) indicate that when taurine and verapamil are both present in the bathing medium (K-H, containing calcium, as above), verapamil reduces taurine uptake in a dose-dependent fashion (P < 0.05). This finding, along with the observation that a marked reduction of taurine uptake occurs in the calcium-free medium, indicates a calcium dependency for uptake. The increased taurine concentration and efflux (Crass and Lombardini, 1978) observed during arrhythmias and oxygen deficit, respectively, may reflect an endogenous means by which arrhythmias might be prevented.

It is possible that taurine exerts its multiple physiological functions through more than one molecular mechanism. As has been postulated for cardiac glycosides, the positive inotropic effects of taurine have been claimed to be a result of an action on calcium (Iwata and Fujimoto, 1976), whereas the antiarrhythmic effects may be mediated by an action on potassium (Read and Welty, 1965; Fujimoto, 1977). Thus, taurine at a lower concentration may act through an interaction with calcium; however, at higher concentrations it may interact with potassium.

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