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THE IDENTIFICATION OF TAURINE RECEPTORS FROM RAT HEART SARCOLEMMA

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Summary. Two sets of taurine receptors on rat heart sarcolemma have been identified. The high affinity taurine receptors ($Kd=3.5x10^{-4}M$) show a non-cooperative binding profile while the low affinity taurine receptors exhibit positive cooperativity. Taurine binding to the membrane exhibits a typical bell shaped pH profile with maximum binding occurring at pH 8.0. The maximum temperature for binding is 24°C. The effect of various taurine analogues on the receptors was investigated. It was found that binding is prevented by hypotaurine and inhibited to a lesser degree by isethionic acid and cysteine sulfinic acid, while β-alanine was found to increase taurine binding. The effect of several hydrolytic enzymes was also examined and it was shown that several proteases and phospholipase C inhibit binding. The results indicate that the taurine receptors are membrane bound proteins in a phospholipid environment.

Taurine (2-aminoethanesulfonic acid) is an amino acid present in high concentrations in the heart, where it constitutes as much as fifty percent of the free amino acid pool (1). It has been shown to be an effective anti-arrhythmic agent against epinephrine- and digoxin- induced premature ventricular contractions (2). In addition, taurine has been shown to exert a positive inotropic effect on isolated guinea pig heart and to potentiate the inotropic effect of the steroid, strophanthin-K (3,4). These observed effects of taurine appear to be mediated through changes in ion flux (5-9).

Since taurine is physiologically and pharmacologically important to the heart, it is of considerable relevance to determine the mode of taurine action. Huxtable (10) has proposed that the interaction of taurine with membranes results in structural changes which alter ion flux. In this paper, we present evidence for two sets of taurine binding sites located on the cardiac ventricular sarcolemma.

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Methods and Materials: Cardiac ventricular sarcolemma was isolated from hearts of male Wistar rats (240-280 gm). The hearts were perfused at 37°C with Krebs-Henseleit buffer, pH 7.4, to remove blood from the coronary system and the ventricles. The membranes were prepared from the isolated ventricles as described for skeletal muscle by Sulakhe et al. (11) except for one minor modification. The final membrane pellet was suspended in 10 mM Tris-maleate buffer, pH 7.4, containing 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.8 mM KCl, 1.25 mM $CaCl_2$ and 120 mM NaCl to approximate normal physiological conditions.

Purity of the membrane isolate was monitored by assaying several enzyme markers. The sarcolemma preparation was shown to contain high activity of Na^+-K^+ ATPase (12), while exhibiting both low succinate dehydrogenase (13) and glucose-6-phosphatase activities (14).

Sarcolemma protein concentration was adjusted to 300-500 µg/ml Tris-maleate buffer containing the appropriate ions. The final protein concentration was determined according to the method of Lowry (15), with bovine serum albumin serving as the standard.

Taurine binding was performed utilizing a Millipore Filtration System. Aliquots (60 to 100 µg protein/assay tube) of the sarcolemma protein suspension were incubated in Tris-maleate buffer, pH 7.4, at 24°C with [^{14}C]-taurine. After one hour incubation, the reaction was terminated by the addition of 3.0 ml cold buffer. The membrane suspensions were filtered by vacuum filtration on cellulose acetate filters (0.2 µm pore size) and washed three times with 3 ml volumes of the cold buffer. The membrane filters were placed in 10 ml triton X-100 liquiflour and radioactivity was determined on a Hewlett-Packard β-liquid scintillation counter. Non-specific activity was determined by preincubation of the sarcolemma with 0.7 M unlabeled taurine prior to the addition of [^{14}C] -taurine. [^{14}C]-taurine, obtained from New England Nuclear, was shown to be radiochromatographically pure.

All hydrolytic enzymes were obtained from Sigma.

RESULTS: Specific taurine binding to cardiac ventricular sarcolemma was determined over a taurine concentration range of 1.75×10^{-4}M to 7.0×10^{-3}M. Binding was corrected for non-specific interactions using the procedure of Cuatracasas (16) and the resulting specific binding profile is seen in Figure 1A. A Scatchard plot (Fig. 1B) of the data revealed what we interpret as two sets of taurine binding sites. This interpretation is in agreement with Wagner (17), who observed a similar Scatchard plot for GMP binding to polylysine. The high affinity binding sites exhibit a non-cooperative binding pattern and are characterized by a dissociation constant for taurine of 3.5×10^{-4}M. From the Scatchard plot, it was determined that these high affinity receptors bind 0.5 nmoles taurine/mg protein. A second set of taurine binding sites was observed at taurine concentrations in excess of 1mM. The Scatchard plot of the data indicates that taurine binding to the low affinity binding sites exhibits positive cooperativity.

Taurine binding was shown to depend upon both temperature and pH. The

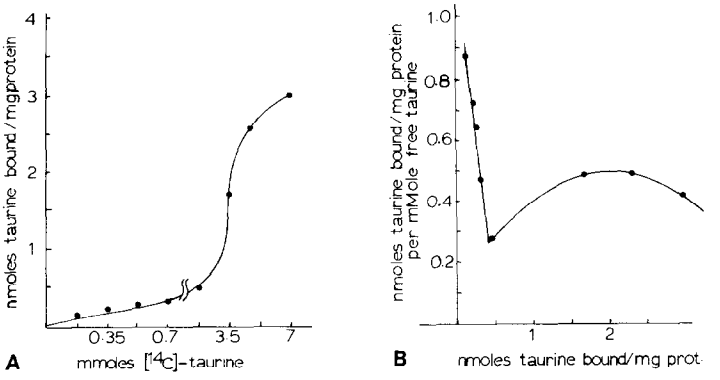

Figure 1A Taurine binding isotherm for cardiac ventricular sarcolemma. Membranes (60-100 µg protein) were incubated with 1.75×10^{-4} -7 x 10^{-3}M [^{14}C]-taurine in Tris-maleate buffer maintained at pH 7.4 and 24°C and containing the appropriate physiological ions (see Methods). The reaction was terminated after one hour incubation by the addition of 3 ml cold buffer. The solution was immediately passed through cellulose acetate filters (0.2µm). The filters were washed and then counted for radioactivity. Specific activity is expressed as n moles taurine bound per mg protein.

Figure 1B Scatchard plot of the binding data from Figure 1A.

pH binding profile was shown to be bell shaped with maximum binding occurring at pH 8.0 (Fig. 2A). The optimum temperature for binding was found to occur at 24°C (Fig. 2B).

Various taurine analogues were investigated to assess the specificity of the taurine receptors. Equal molar concentrations of β-alanine, isethionic acid, hypotaurine, and cysteine sulfinic acid were preincubated with heart cell membranes prior to the addition of [^{14}C]-taurine. Table IA shows the effect of the various analogues on taurine binding. It is seen that while isethionic acid and cysteine sulfinic acid partially inhibit the taurine receptors, hypotaurine completely blocks taurine binding. By contrast, β-alanine is the only analogue found to increase taurine binding.

To characterize the nature of the taurine binding sites, the effects of various hydrolytic enzymes on the integrity of the receptors were examined (Table IB). Preincubation of the membranes with either proteolytic enzymes or phospholipase C significantly decreased taurine binding, indicating the importance of both proteins and phospholipids in maintaining the integrity of

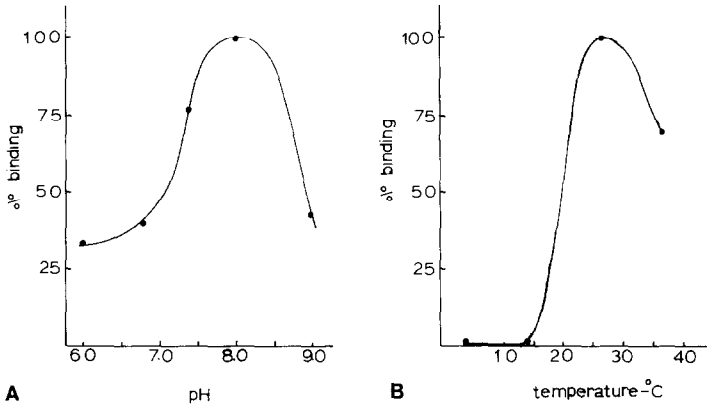

Figure 2A The effect of pH on taurine binding. Aliquots of membrane (60-100µg prot./assay) were incubated with 3.5 x 10^{-3} [^{14}C]taurine in a medium of varying pH and constant temperature at 24°C. Samples were analyzed for binding as described in Figure 1A.

Figure 2B The effect of temperature on taurine binding. Membranes (60-100µg protein/assay) were incubated with 3.5 x 10^{-3}M taurine at the desired temperatures. Samples were analyzed for binding as described in Figure 1A.

TABLE IA

Effects of Taurine Analogues on the Binding of Taurine to Cardiac Ventricular Sarcolemma

Taurine Analogue	% Control Taurine Binding
β-alanine	124
Isethionic Acid	57
Hypotaurine	0
L-Cysteine Sulfinic Acid	74

IA.
Membranes were incubated with 1mM β-alanine, isethionic acid, hypotaurine or cysteine sulfinic acid for 10 minutes prior to the addition of 1 mM [^{14}C]-taurine. Control samples not containing taurine analogues were run concurrently. Binding was determined by methods described in Figure 1A.

TABLE IB

Effects of Proteolytic Enzymes on Taurine Binding to Cardiac Ventricular Sarcolemma

Enzyme	% Decrease In Taurine Binding
Chymotrypsin	73
Pepsin	65
Trypsin	87
Neuraminadase	<10
β-Glucosidase	36
Phospholipase C	100

IB.
Membranes were preincubated with 0.25 units neuraminadase, 100 µg chymotrypsin, 100 µg pepsin, 100 µg trypsin, 2 units phospholipase C, or 4.5 units β-glucosidase for 60 min. at 24°C. Controls not containing hydrolytic enzymes were incubated under the same conditions as the hydrolytic enzymes. Following preincubation, the sarcolemma were centrifuged and suspended in 1.8 ml buffer to yield a protein concentration of 80-120 µg protein/ml buffer. Aliquots were examined for [^{14}C]-taurine binding as described in Figure 1A

the membrane receptors. However, neither neuraminadase nor β-glucosidase were found to appreciably inhibit taurine binding.

Discussion. In this paper we have identified two distinct sets of taurine receptors located on the cardiac ventricular sarcolemma. This is particularly evident from the binding isotherm and the Scatchard plot. Since the K_d of these taurine binding sites is of the same order of magnitude as the K_m for taurine uptake by other biological tissue, the high affinity receptors may be associated with the β-amino acid transport system (18, 19).

A low affinity taurine receptor was observed at a taurine concentration in excess of 1 mM. This is the same taurine concentration range necessary to elicit certain physiological and pharmacological effects of taurine (3,4,6). We have seen that taurine binding to these receptors exhibits positive cooperativity. Since allosterism is by definition associated with conformational changes, the data imply that binding to the low affinity sites induces conformational changes in the cardiac ventricular sarcolemma. These data are consistent with Huxtable's model relating taurine binding to associated conformational changes (10).

It is our contention that taurine binds to protein receptors rather than interacting with membrane phospholipids as suggested by Huxtable (10). We base our hypothesis on five lines of evidence. First, taurine binding is saturable, suggesting the receptor is a protein. Second, specificity is a fundamental property of proteins and these receptors exhibit varying degrees of specificity for different taurine analogues. Third, since allosteric control characteristically occurs through proteins, and the low affinity taurine binding is characterized by positive cooperativity, it follows that these receptors may be proteins. Fourth, taurine binding is inhibited by proteases. Finally, the pH and temperature profiles are characteristic of proteins.

The identification of two sets of taurine receptors on cardiac ventricular sarcolemma should provide a rational basis for further investigation into the physiological and pharmacological effects of taurine. It remains to be determined if the low taurine affinity receptors are indeed the same sites deemed necessary for the observed pharmacological effects of taurine. In

addition, further characterization and isolation of the taurine receptor proteins should be investigated.

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