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UPTAKE OF [^{3}H]TAURINE INTO MYOCARDIAL MEMBRANES

FLAVIA FRANCONI, FLAVIO MARTINI, NICOLETTA MANGHI, ALESSANDRO GALLI, FEDERICO BENNARDINI and ALBERTO GIOTTI

Istituto Interfacoltà di Farmacologia e Tossicologia della Università di Firenze, Viale G B. Morgagni, 65, 50134 Firenze, Italy

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Abstract—In the ventricular sarcolemma of guinea-pig heart two uptake systems are present, a high affinity and a low affinity one. The uptake is Na^+, K^+, Mg^{2+} dependent and Ca^{2+} independent. In the absence of Mg^{2+} only one uptake system is present. β-alanine, hypotaurine, homotaurine and guanidethylsulphonate inhibit the uptake of [^{3}H]taurine; isethionic acid increases it.

Taurine is a sulfonic amino acid that constitutes fifty per cent of the free amino acid pool in the heart [1], and its levels are elevated in various cardiac conditions suggesting that it has a possible pathophysiological role [2, 3]. This amino acid prevents the development of epinephrine or digoxin induced premature ventricular contraction in dogs [4, 5]. Perfusion with potassium-free solution induces a fibrillation in isolated guinea-pig heart which could be blocked by the administration of K^+ + taurine but not by K^+ alone [6]. Moreover, taurine has a positive inotropic effect on guinea-pig hearts [7, 8] correlated with modified calcium kinetics in the hearts [9]. Kulakowski *et al.* [10] reported that in rat heart sarcolemma "two taurine receptors" are present. It is well known that taurine exerts different actions in rat and guinea-pig hearts; in fact in the former it has a negative inotropic effect, in the latter a positive one [8].

It therefore seemed interesting to us to study taurine interactions with guinea-pig heart sacolemma.

MATERIALS AND METHODS

Cardiac ventricular sarcolemma was isolated from male guinea-pig heart. The heart was washed to remove the blood and placed in ice cold 0.25 M sucrose. The membranes were prepared from ventricles as described by Kidwai *et al.* [11] using a sucrose gradient. The sarcolemma obtained by this method was in the form of vesicles [11]. The purity of membranes was examined by determining the activities of several enzyme markers. The (Na^+ – K^+)ATPase was measured according to Sulakhe and Dhalla [12], the succinate dehydrogenase according to King [13] and glucose-6-phosphatase as in Kidwai *et al.* [11]. The activities of these enzymes in the sarcolemma preparations were the following: (Na^+ + K^+)ATPase, 7 Pi μmoles/mg protein/min; glucose-6-phosphatase, 0.28 Pi μmoles/mg protein/min; and succinate dehydrogenase, 0.28 μmoles succinate/mg protein/min.

The membranes were suspended in Tris–maleate buffer 10 mM, pH 7, containing 1.2 mM $MgSO_4$, 4.8 mM KCl, 1.25 mM $CaCl_2$ and 120 mM NaCl. The membranes were stirred for 30 min at 0° to obtain an homogeneous suspension.

In some experiments the membranes were disrupted by hypo-osmotic shock in water and then centrifuged and resuspended in the standard incubation buffer.

The incubation mixture was shaken for 30 min at 20°. NaCl, KCl, $CaCl_2$ were substituted with choline chloride; magnesium sulphate with sodium sulphate*. The incubation was terminated by rapid dilution with 2.5 ml of ice cold incubation buffer and by subsequent rapid vacuum filtration of the diluted incubate through Whatman GF/C filters. The filters were then quickly washed by vacuum filtration with 3 ml of ice cold buffer three times. The entire filtration procedure took less than 20 sec.

After drying, filters were placed directly into triton/toluene [14] and then counted in a Packard liquid scintillation spectrometer (Packard Instruments).

Separate incubations were carried out in each assay in the presence of a high concentration (0.7 M) of unlabelled taurine to assess unspecific binding. Data from competitive experiments were graphically analyzed with Scatchard analysis as Rosenthal [15] and Feldman [16]. Membrane protein concentrations were determined using the method of Weichselbaum [17]. [^{3}H]Taurine (sp. act. 10.5 Ci/mM) was purchased from the Radiochemical Centre, Amersham.

RESULTS

The incubation time selected for uptake assay was 30 min, since at that time it was maximum as shown in Fig. 1. [^{3}H]Taurine uptake was temperature-dependent, with its highest value at 20° (Fig. 2)†, and showed a pH optimum at 7 (Fig. 3). The uptake was Ca^{2+}-independent and Na^+, K^+ and Mg^{2+}-dependent. The absence of Na^+ markedly reduced the uptake while Mg^{2+} and K^+ withdrawal increased it (Table 1). Also in magnesium-free medium the

* So the variation of sodium concentration was less than one per cent.

† The experiments shown in the temperature curve were carried out in April and May. This could explain the difference in values obtained as compared with other experiments performed in winter time.

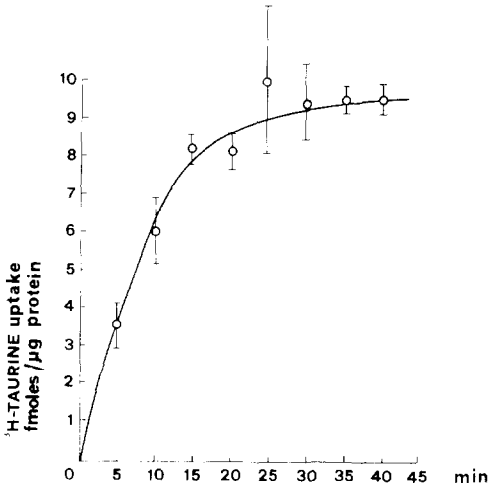

Fig. 1. Uptake of [^{3}H]taurine to ventricular sarcolemma as a function of incubation time. Results (means ± S.E.) are from 5 experiments performed in triplicate.

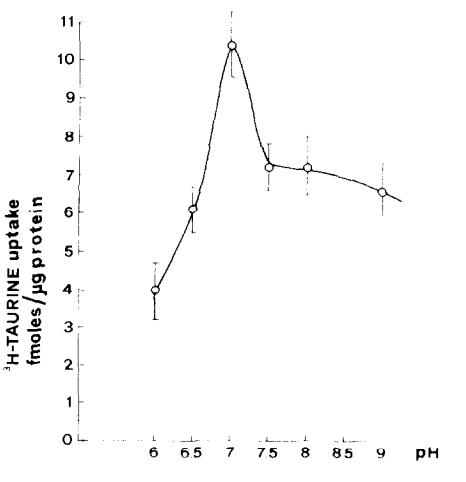

Fig. 3. Uptake of [^{3}H]taurine to myocardial membranes with varying pH. Results (means ± S.E.) are from 5 experiments performed in triplicate.

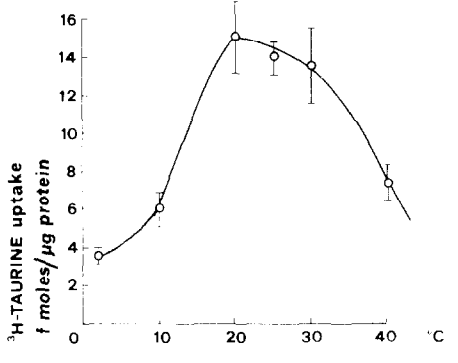

Fig. 2. Uptake of [^{3}H]taurine to ventricular sarcolemma as a function of temperature. Results (means ± S.E.) are from 5 experiments performed in triplicate.

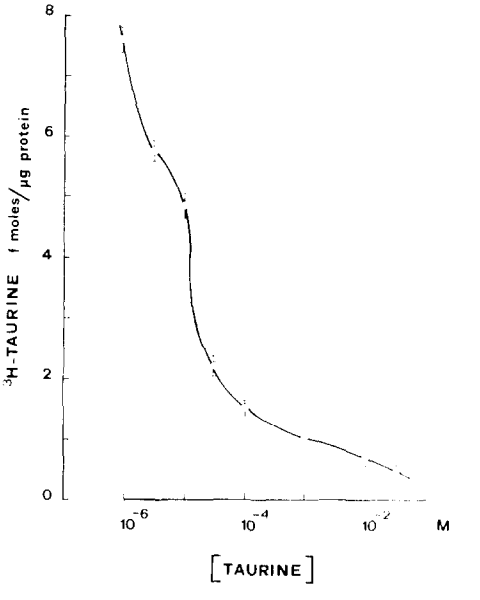

Fig. 4. Inhibition of [^{3}H]taurine uptake to guinea-pig heart membranes by increasing concentrations of taurine. The points of the curves are means ± S.E. of 5–9 experiments performed in triplicate.

Table 1. Effect of various incubation conditions on [^{3}H]taurine transport*

Incubation conditions	fmoles of [^{3}H]taurine/µg protein
Control	9.64 ± 0.12 (25)
Choline Cl replaced NaCl	0.75 ± 0.05 (5)
Choline Cl replaced $CaCl_2$	11.28 ± 1.98 (6)
Choline Cl replaced KCl	15.81 ± 1.6 (5)
Na_2SO_4 replaced $MgSO_4$	15.42 ± 1.07 (6)
Hypo-osomotic shock	2.79 ± 0.48 (5)

* The transport of [^{3}H]taurine was measured under different conditions. Each value is the mean ± S.E.; the number of experiments is shown in parentheses. The experiments were performed in triplicate.

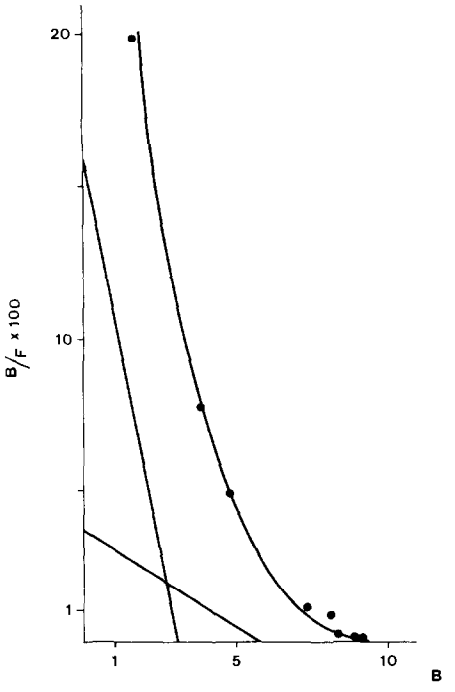

Fig. 5. Scatchard plot of inhibition curve. Data were plotted as a Scatchard plot. The K_m's and B_{max}'s were obtained by subjecting the Scatchard plot to the graphic analysis of Rosenthal [15] and Feldman [16].

uptake was statistically increased ($P \leq 0.001$) while in the calcium-free medium the uptake was equal to the control (Table 1).

The uptake was markedly decreased (65%) when the membranes were submitted to hypo-osmotic shock. This value was similar to the one obtained when the incubation was performed at 2°. Competition by cold taurine for [^{3}H]taurine uptake produced the curve shown in Fig. 4.

The Scatchard analysis is best described by hyperbole, indicating the presence of two uptake systems with different affinities and capacities (Fig. 5).

The number of high and low affinity sites in the control experiments was 3.05 and 5.76 fmoles/μg protein respectively (Table 2). In the absence of Ca^{2+} the apparent K_m's were 1.8×10^{-5}M and 1.3×10^{-6}M and the number of the two uptake systems was 2.8 for the high affinity type and 6.15 for the low one (Table 2). In the absence of K^+, two uptake systems were also detected, but here the apparent K_m's were smaller than those in the control experiments and the number of the uptake sites in this case was 4.92 fmoles/μg protein for the high affinity and 9.98 fmoles/μg protein for the low one. In the case of the inhibition curve in the absence of Mg^{2+}, there was present only one uptake system with an apparent K_m of 1×10^{-5}M (Table 2). The uptake of the drug into ventricular membranes was modified by taurine analogues (Table 3). In fact, β-alanine at both concentrations tested almost completely abolished uptake, while hypotaurine, homotaurine and guanidethylsulphonate only partially inhibited it. Of the taurine analogues tested, only isethionic acid was able to increase the uptake in a statistically significant manner (Table 3). The absence of Ca^{2+}, Mg^{2+} and K^+ did not affect the uptake pattern of these analogues.

DISCUSSION

Recent studies with isolated plasma membrane vesicles have largely confirmed the observations with intact cells on Na^+-coupled transport of amino acids [18–20].

Kennedy and Voaden [21] have demonstrated that taurine uptake in the retina is Na^+-dependent and Hruska *et al.* [22] have reported similar findings for rat brain synaptosomes.

Our experiments, reported here, have demonstrated that taurine is taken up into isolated guinea-pig ventricle sarcolemma by a Na^+-dependent transport; also time–course suggests an uptake process. The marked inhibition by hypo-osmotic shock further supports the presence of uptake systems.

The K_m derived from Scatchard analysis for high affinity transport closely corresponds to the values observed in the rat brain synaptosomes [22]. The K_m of low affinity corresponds to K_m calculated in the rat heart and kidney slices [23]. The uptake of [^{3}H]taurine was increased by removal of K^+ and Mg^{2+}, while Hruska *et al.* [22] have found that the removal of K^+ reduces the taurine uptake in rat brain synaptosomes. The removal of Ca^{2+} only slightly increased (Table 1) the uptake, while in the rat retina the substitution of Ca^{2+} with choline chloride causes a reduction in the taurine uptake, but the substitution of Mg^{2+} does not modify this process in the same organ [24].

Borg *et al.* [25] also show that the uptake of taurine in a calcium-free medium is significantly lower in the neural and glial cells, and further evidence shows that this uptake in the small intestine of the guinea-pig is Na^+- and Ca^{2+}-dependent [26].

Table 2. K_m's and B_{max}'s calculated from Scatchard analysis in absence of Mg^{2+}, Ca^{2+}, K^+

	K_m	B_{max} (fmoles/μg protein)
Standard incubation	1.5×10^{-5}M	5.76
buffer	1.94×10^{-6}M	3.05
Without K^+	6.5×10^{-6}M	9.98
	7.6×10^{-7}M	4.92
Without Mg^{2+}	1×10^{-5}M	14.66
Without Ca^{2+}	1.8×10^{-5}M	6.15
	1.65×10^{-6}M	2.81

Table 3. Effect of some analogues on [^{3}H]taurine binding

Analogues		Per cent binding
None		100
β-Alanine	10 mM	7.67 (6)
	1 mM	6.04 (5)
Hypotaurine	10 mM	28.94 (5)
	1 mM	-
Homotaurine	10 mM	17.07 (6)
	1 mM	29.05 (5)
Guanidoethylsulphonate	10 mM	23.55 (5)
	1 mM	27.7 (6)
Isethionic acid	10 mM	121 (6)*
	1 mM	141 (6)†

Membranes were incubated in the presence of the analogues. [^{3}H]taurine (0.5 μM) was added and incubation continued for 30 min at 20°.

Numbers in brackets indicate number of experiments; each experiment was performed in triplicate.

* $0.01 \geq P \geq 0.002$.

† $0.01 \geq P \geq 0.001$.

We observed the smallest apparent K_m in the absence of K^+. In the absence of Mg^{2+}, on the other hand, only one system seemed to be present with a K_m equal to that of the low-affinity system in the control. Uptake in the absence of the same ion was higher than in the control experiments suggesting that the uptake systems are modified quantitatively and qualitatively by this absence. All these data lead to the conclusion that the ions markedly affected [^{3}H]taurine uptake processes.

Specificity of uptake was demonstrated by the fact that taurine analogues were able to inhibit it (Table 3), while isethionic acid increased it. These data, obtained on guinea-pig heart, do not agree with those of Kulakowski *et al.* [10]. In fact these authors measured the binding of [^{3}H]taurine to rat sarcolemma in an iso-osmotic medium and they found that β-alanine increased the binding and isethionic acid inhibited it. Guinea-pig and rat hearts apparently also have different pH optima. These differences support the observation of different actions exerted by taurine in the same organs of the two animals [7].

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