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Potentiation of Mitochondrial Ca^{2+} Sequestration by Taurine

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ABSTRACT. The effects of taurine (2-aminoethanesulphonic acid) and its analogues, 2-aminoethylarsonic acid, 2-hydroxyethanesulphonic (isethionic) acid, 3-aminopropanesulphonic acid, 2-aminoethylphosphonic acid, and *N*,*N*-dimethyltaurine, were studied on the transport of Ca^{2+} by mitochondria isolated from rat liver. Taurine enhanced Ca^{2+} uptake in an apparently saturable process, with a K_m value of about 2.63 mM. Taurine behaved as an uncompetitive activator of Ca^{2+} uptake, increasing both the apparent K_m and V_{max} values of the process. This effect was not modified in the presence of cyclosporin A (CsA). *N*,*N*-Dimethyltaurine also stimulated Ca^{2+} uptake at higher concentrations, but there was no evidence that the process was saturable over the concentration range used (1–10 mM). Aminoethylarsonate was a weak inhibitor of basal Ca^{2+} uptake, but inhibited that stimulated by taurine in an apparently competitive fashion (K_i = 0.05 mM). The other analogues had no significant effects on this process. Taurine either in the presence or the absence of CsA had no effect on Ca^{2+} release induced by 200 nM ruthenium red. Thus, the mechanism of taurine-enhanced Ca^{2+} accumulation appears to involve stimulation of Ca^{2+} uptake via the uniport system rather than inhibition of Ca^{2+} release via the ion (Na^+/Ca^{2+} and/or H^+/Ca^{2+}) exchangers or by taurine modulating the permeability transition of the mitochondrial inner membrane. Overall, these findings indicate an interaction of taurine with an as yet unidentified mitochondrial site which might regulate the activity of the uniporter. The unique role of taurine in modulating mitochondrial Ca^{2+} homeostasis might be of particular importance under pathological conditions that are characterised by cell Ca^{2+} overload, such as ischaemia and oxidative stress. BIOCHEM PHARMACOL **58**;7: 1123–1131, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. rat liver mitochondria; taurine; Ca^{2+} transport

Mitochondria have been implicated in the maintenance of the Ca^{2+} "set-point" in cells [1–3], where control of Ca^{2+} levels plays a significant role in enzymatic regulation and energy production [4–8]. Pathological conditions that result in increased tissue Ca^{2+} concentrations, such as ischaemia, oxidative stress, and excito- and neurotoxicity, involve impairment of the continued ability of Ca^{2+}-regulating organelles, such as sarcolemma, mitochondria, and sarcoplasmic reticulum, to provide long-term control of cellular Ca^{2+} levels [9–12]. The subsequent increase in cytoplasmic Ca^{2+} is widely considered to be a critical initiating event in the development of damage in cells destined to die. Although the mechanisms responsible for cell injury under these conditions are not yet fully clarified, excessive free cytosolic Ca^{2+} may lead to uncoupling of mitochondrial oxidative phosphorylation [13] with consequently decreased ATP synthesis [14]. The resulting inactivity of ATP-dependent pumps would lead to membrane depolarisation and further cellular Ca^{2+} influx in a regenerative and self-potentiating mechanism. In this context, removal of Ca^{2+} to the extracellular space and/or uptake into organelles, including mitochondria, work to restore the normal cytoplasmic Ca^{2+} concentration, as occurs during the early stages of recirculation after short-term cerebral ischaemia [15].

Taurine is a naturally occurring amino acid present in high (mM) concentrations in mammalian tissues included liver, where its content is in the range of 3.5–60 μmol/g wet weight (for review see [16]). Despite extensive studies, there is still controversy about its role(s). One of its possible functions concerns modulation of Ca^{2+} transport. In many tissues, taurine has been shown to have marked effects on the kinetics of Ca^{2+} movement across the membranes of both cellular and subcellular preparations. A system with a relatively high affinity (15–20 μM) for uptake of Ca^{2+} from cytosol is stimulated by taurine in several membrane preparations, including cardiac sarcolemma, retinal rod outer segments, disk membrane, and brain synaptosomes [17–19]. In heart, the normalising action of taurine on both low and high levels of intracellular Ca^{2+} has been ascribed to direct modulation of Ca^{2+} channels and/or indirect effects oper-

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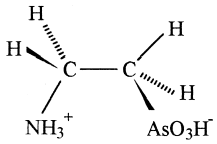
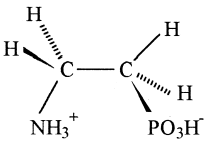
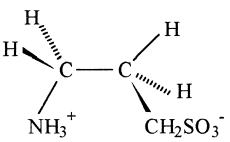


Aminoethylarsonic acid

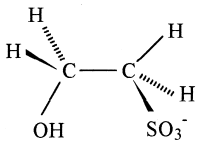


Aminoethylphosphonic acid

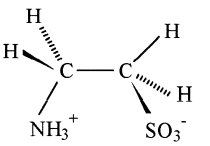


Aminopropanesulphonic acid

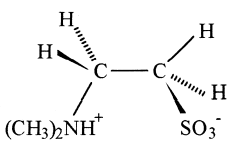
FIG. 1. Structures of the compounds studied.



Isethionic acid



Taurine



***N,N*-Dimethyltaurine**

ating via Na^+/Ca^{2+} exchange [20, 21]. In addition to its ability to modulate cellular Ca^{2+} transport, taurine has also been reported to influence protein phosphorylation [19], but no clear relationship between these two processes has yet been established. In the present study, we used isolated, functionally intact mitochondria from rat liver as a model with which to investigate the effects of taurine and some structurally related analogues on Ca^{2+} transport. An understanding of the influence of taurine on these processes might be of importance in pathological and toxicological conditions characterised by loss of Ca^{2+} homeostasis. A preliminary account of part of this work has been published [22].

MATERIALS AND METHODS

Materials

The structures of taurine and the taurine analogues used in this work are represented in Fig. 1. 2-Aminoethylarsonic acid was prepared by periodate oxidation of 2-[(2-hydroxyethyl)amino]ethylarsonic acid, itself made by treating 2-chloroethylarsonic acid with ethanolamine [23]. *N,N*-Dimethyltaurine was prepared by adding 2-chloroethyl(dimethyl)amine as its hydrochloride (69.4 mmol) to sodium bisulphite (70 mmol) in 200 mL of water in a round-bottomed flask fitted with a water-cooled condenser. This was boiled under reflux for 2 hr, cooled to room temperature, and strongly acid ion-exchange resin was added to it. The solution was rotary evaporated to remove SO_2 and added to a column containing more of the same resin. The compound was eluted with two column volumes of 2 M acetic acid and shown to be Na^+-free by the flame test. The solution was concentrated by rotary evaporation and *N,N*-dimethyltaurine was crystallised from 50% aqueous ethanol and recrystallised from the same (yield = 6.23 g [58%]). Elemental analysis gave C, 30.8; H, 7.2; N, 9.0%; $C_4H_{11}NO_3S$ requires C, 31.4; H, 7.2; N, 9.1%. A better yield might well have been obtained if sulphite rather than bisulphite had been used, i.e. if the pH had been raised, as sulphite is a better nucleophile. 2-Hydroxyethanesulphonic (isethionic) acid, 3-aminopropanesulphonic acid, 2-aminoethylphosphonic acid, RR*, and all the other reagents were purchased from Sigma Chimica.

Mitochondrial Preparation

Male Wistar rats (200–250 g) were lightly anaesthetised with a mixture of Ketavet® and Rompun® and rapidly exsanguinated. Livers were rapidly removed and homogenised in a medium containing 250 mM sucrose, 5 mM Tris, 1 mM EGTA, at pH 7.4, and mitochondria were separated by differential centrifugation [24]. After centrifugation, the mitochondrial pellet was resuspended in 1–2 mL of a medium containing 150 mM KCl, 25 mM sucrose, 5 mM Mg^{2+}, 2.7 mM HEPES (KSH), pH 7.2. The mitochondrial protein content was determined by the biuret method [25] using BSA as standard.

Determination of Mitochondrial Ca^{2+} Transport

Ca^{2+} UPTAKE. Ca^{2+} uptake was determined spectrophotometrically in two different media by using antipyrylazo III

* *Abbreviations:* RR, ruthenium red; CsA, cyclosporin A; TPMP, triphenylmethylphosphonium; $K_{0.5}$, apparent affinity constant; and $\Delta\Psi$, membrane potential.

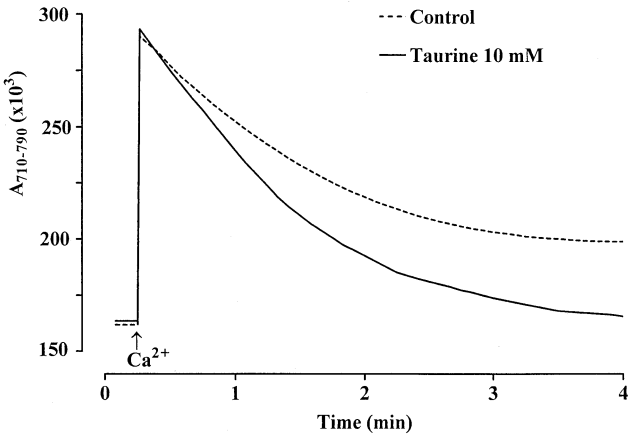


FIG. 2. Kinetics of Ca^{2+} uptake by rat liver mitochondria in two different media (medium 1: 195 mM mannitol, 65 mM sucrose, 3 mM HEPES, pH 7.2; medium 2: 92.5 mM KCl, 18.5 mM K^+MOPS (3-(*N*-morpholino)propanesulphonic acid, 7.5 mM Na^+pyrophosphate, pH 7.2). The reaction containing 1 mg/mL of mitochondrial protein was initiated by adding 50 μM $CaCl_2$ (arrow). Two successive calibrating additions of 50 μM $CaCl_2$ were made at the end of the experiment. Uptake (in nmol/min/mg of mitochondrial protein) was determined spectrophotometrically from the differential absorbance changes ($A_{710-790}$) using the Ca^{2+} indicator antipyrylazo III.

as indicator [26]. In one case, mitochondria (2 mg of protein) were suspended in a medium (medium 1) consisting of 1.9 mL of buffer (195 mM mannitol, 65 mM sucrose and 3 mM HEPES, pH 7.2) containing 250 μM antipyrylazo III to which had been added 13 μM rotenone and 10 mM succinate. In the other case, mitochondria (2 mg of protein) were suspended in 2 mL of experimental medium (medium 2), consisting of 1.9 mL of buffer (92.5 mM KCl, 18.5 mM K^+MOPS (3-(*N*-morpholino) propanesulphonic acid), 7.5 mM Na^+pyrophosphate, pH 7.2) containing 250 μM antipyrylazo III, to which had been separately added 0.1 mL of 1 mM Mg^{2+}-ATP, creatine phosphokinase (20 μg/mL), and 5 mM phosphocreatine [27]. When CsA was present in the experimental media, this was at a concentration of 0.5 μM. Both experimental media were equilibrated at 30°, with stirring, before being placed in a 1-cm path-length cuvette. Changes in Ca^{2+} concentration were monitored with time, using a Shimadzu UV-160 spectrophotometer or a Hewlett Packard diode-array spectrophotometer, by measuring the absorbance at 710 nm and subtracting the absorbance at 790 nm ($A_{710-790}$). The experiment was started by adding 50 μM $CaCl_2$ to the cuvette; this caused an initial rapid increase in absorbance because of the formation of the Ca^{2+}-antipyrylazo III complex. This was followed by a time-resolvable decrease in absorbance as a result of the transport of Ca^{2+} into the mitochondria and its consequent disappearance from the extramitochondrial solution (see Fig. 2). At the end of the experiment, after the absorbance decrease had stabilised, the response of the system was checked by determining the change in absorbance following the addition of successive portions of $CaCl_2$. It was found that the absorbance difference was only strictly linearly correlated to Ca^{2+} concentration at concentrations below about 60 μM. However, three successive additions of 10 μM $CaCl_2$ at the end of the experiment gave increases none of which were significantly different (results not shown, N = 8 in each case) from one-fifth of the change induced by the 50 μM addition at the start of the experiment. The presence of ATP and pyrophosphate, both Ca^{2+}-chelating agents, in medium 2 decreased the free Ca^{2+} concentration, as measured with antipyrylazo III, by approximately 40%. However, since the standard 50 μM Ca^{2+} concentration used was greatly in excess of the K_m value for the uptake process, this would have an effect of less than 10% on the initial velocity (see below). Compounds to be tested were present in the antipyrylazo-containing buffers.

It has been reported that under de-energised conditions, the presence of pyrophosphate might sensitise mitochondria to the permeability transition, and increase matrix volume and swelling; however, when mitochondria are incubated in the presence of ATP, this phenomenon is greatly reduced [28]. Although ATP was present in the pyrophosphate-containing medium (medium 2), experiments to measure mitochondrial swelling were carried out to assess whether mitochondria underwent any permeability transition under these conditions. Mitochondrial swelling was measured by the method of Broekemeier *et al.* [29] by monitoring the decrease in light-scattering of a mitochondrial suspension. Following preparation in KSH (as described above), the mitochondria were suspended in the pyrophosphate-containing medium and placed in a 3-mL stirred cuvette at 30°. The final suspension, containing 1 mg/mL of mitochondria, was monitored spectrophotometrically at 540 nm in a Shimadzu UV-160 spectrophotometer. After the attenuance had stabilised, Ca^{2+} was added. The results showed that under the experimental conditions of either medium 1 or 2, the addition of 50 μM Ca^{2+} did not cause detectable swelling, either in the absence or presence of taurine and taurine analogues (data not shown).

Ca^{2+} RELEASE. Mitochondria were loaded with Ca^{2+} by incubation with 100 μM $CaCl_2$ for a period of 5 min. They were then treated with 200 nM RR. This concentration of RR was chosen on the basis of the results of direct titration of Ca^{2+} uptake by mitochondria (1 mg/mL). The results (data not shown) indicated that 200 nM RR completely inhibited the mitochondrial Ca^{2+} uptake stimulated by 100 μM Ca^{2+}. Addition of RR to inhibit the mitochondrial Ca^{2+} uniporter caused an increase of Ca^{2+} in the medium, due to efflux of Ca^{2+} from mitochondria [30]. This was accompanied by a time-resolvable increase in $A_{710-790}$ as a result of Ca^{2+} complexation by antipyrylazo III. Loading mitochondria with 100 μM Ca^{2+} in pyrophosphate-containing medium (medium 2) may induce pore opening, causing swelling which is prevented by adding CsA. For this reason, the Ca^{2+} efflux experiments performed in medium 2 were carried out in the presence of CsA (0.5 μM).

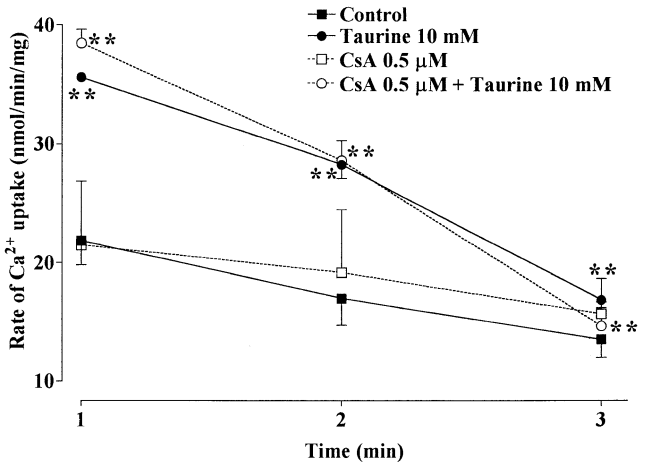


FIG. 3. Effects of taurine on the rate of Ca^{2+} uptake by rat liver mitochondria. The time–course of the rates of Ca^{2+} uptake, measured in terms of differential absorbance changes ($A_{710-790}$) in antipyrylazo III per min at the times indicated, was determined in medium 1 (see Methods). Values shown represent the means ± SEM from 3 to 5 separate experiments, each performed in duplicate. Statistical significance with respect to control values for $^{**}P < 0.01$ was determined by Student's *t*-test for unpaired data, followed by Welch's *t*-test.

Taurine was added immediately before RR and the rates of Ca^{2+} release were determined after 2, 4, and 6 min.

Measurement of Membrane Potential

The mitochondrial $\Delta\Psi$ was determined from the distribution of the lipophilic cation $TPMP^+$ across the mitochondrial inner membrane [31]. The concentration of $TPMP^+$ in the extramitochondrial medium was continuously monitored with a $TPMP^+$-selective electrode [32] in an open thermostatically controlled (30°) and stirred chamber. $\Delta\Psi$ was then calculated from Nernst's equation, and corrections due to the binding of $TPMP^+$ to the mitochondrial membranes were made as described by Brand *et al.* [33]. Mitochondria (1 mg/mL of mitochondrial protein) were suspended in 3 mL of medium containing mannitol 195 mM, sucrose 65 mM, HEPES 3 mM (pH 7.2), and 13.3 μM rotenone, in the absence or presence of taurine (10 mM). Additions of $TPMP^+$ were made to calibrate the $TPMP^+$ electrode, giving a final concentration of 5 μM $TPMP^+$. After the calibration, 10 mM succinate (K^+ salt) was added, followed by additions of Ca^{2+} at final concentrations of 10, 20, 30, and 50 μM.

Statistics

The mean and SEM values from 3 to 5 separate experiments were determined and the percentage differences between these and controls were then calculated. The statistical significance of the differences was determined by Student's *t*-test for unpaired data followed by Welch's *t*-test.

RESULTS

Mitochondrial Ca^{2+} Influx Rate

Figure 3 shows the effects of 10 mM taurine on the rate of Ca^{2+} uptake by rat liver mitochondria determined in medium 1. Taurine also induced a significant and equivalent increase in the rate of Ca^{2+} uptake in medium 2 (data not shown). The percent changes (mean ± SEM) over controls were 62.8 ± 2.3 in medium 1 and 64.5 ± 6.5 in medium 2. When these experiments were repeated in the presence of 0.5 μM CsA, the trends in Ca^{2+} influx were similar in both media, and the effect of taurine over the respective (CsA-containing) controls were 78.9 ± 2.94 and 76.5 ± 9.0 in medium 1 and medium 2, respectively. These values were not significantly different from those observed in the absence of CsA. When calculated in terms of cumulative mitochondrial Ca^{2+} uptake over time, the data from Fig. 3 indicate that in the absence of taurine, about 50 per cent of added Ca^{2+} (100 nmol) was accumulated by 1 mg of mitochondrial protein within 3 min, whereas in the presence of taurine this quantity increased to 80%. Similar effects were observed in medium 2 (data not shown). The effects of taurine were similar either in the presence or absence of 0.5 μM CsA (medium 1: 80.7 ± 1.7 and 81.7 ± 3.7 nmol, respectively; medium 2: 75.9 ± 4.6 and 77.1 ± 8.2 nmol, respectively), indicating that mitochondrial pore opening did not occur and was not involved in the action of taurine on Ca^{2+} fluxes.

Figure 4 shows the effects of different concentrations of

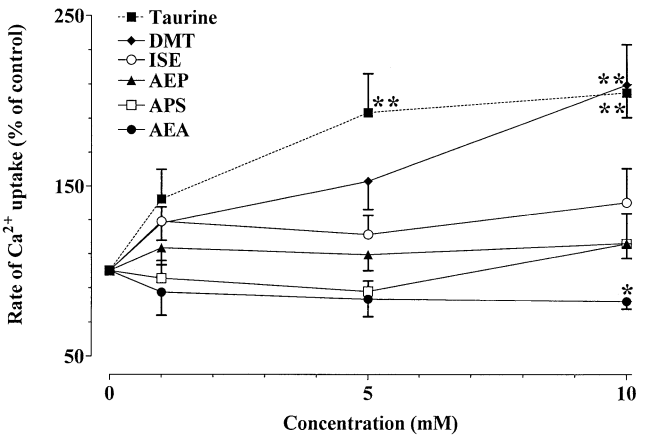


FIG. 4. Effects of taurine and analogues on the initial rate of Ca^{2+} uptake by rat liver mitochondria. Initial rates of Ca^{2+} uptake, measured in terms of differential absorbance changes ($A_{710-790}$) in antipyrylazo III per min following 50 μM Ca^{2+} addition, were determined in medium 2 (see Methods) in the absence or presence of taurine, *N,N*-dimethyltaurine (DMT), isethionic acid (ISE), aminoethylphosphonic acid (AEP), aminopropanesulphonic acid (APS), or aminoethylarsonic acid (AEA). The rate of Ca^{2+} uptake in the absence of taurine or the analogues was 20.350 ± 0.331 nmol/min/mg of mitochondrial protein (N = 27). Values shown are percentage means ± SEM above control (100%) from 3 to 5 separate experiments, each determined in duplicate. Statistical significance with respect to control values for $^{*}P < 0.05$ and $^{**}P < 0.01$ was determined by Student's *t*-test for unpaired data, followed by Welch's *t*-test.

taurine and some taurine analogues on the initial rates of Ca^{2+} uptake by rat liver mitochondria determined in medium 2. Taurine, in the range of concentrations used (1–10 mM), induced a significant ($P < 0.01$) increase in the rate of Ca^{2+} uptake, which approached a plateau at about 10 mM taurine. The taurine analogues studied differed in their effects on Ca^{2+} uptake. In the 1–10 mM range, isethionic acid, aminopropanesulphonic acid, and aminoethylphosphonic acid had no statistically significant effects, whereas *N*,*N*-dimethyltaurine had a stimulating effect only at a high concentration; at 10 mM this effect was similar to that produced by the same concentration of taurine. In contrast to the behaviour of taurine, the kinetics of *N*,*N*-dimethyltaurine-induced Ca^{2+} uptake did not appear to be saturable in the concentration range used. Among the compounds examined, only aminoethylarsonic acid inhibited the process: the effect was small, the Ca^{2+} uptake rate being lowered by 8.0 ± 1.4% of control at a concentration of 10 mM.

As shown in Fig. 5a, aminoethylarsonate competitively inhibited the effects of taurine in stimulating the transport of Ca^{2+}. The apparent K_m value for the stimulation of Ca^{2+} uptake by taurine in the absence of inhibitor, corresponding to the taurine concentration that increased the velocity of Ca^{2+} uptake by 50% of maximum stimulation, was approximately 2.63 mM. A K_i for aminoethylarsonate of approximately 0.05 mM was determined by the method of Dixon [34], by plotting the dependence of the reciprocal of the stimulation of the initial velocities of Ca^{2+} uptake against the aminoethylarsonate concentration at a series of fixed taurine concentrations (Fig. 5b).

In order to evaluate whether a change in affinity of the uniporter may account for the effect of taurine on Ca^{2+} uptake, the dependence of transport on Ca^{2+} concentration was determined. As shown in Fig. 6, the rates of uptake increased with increasing Ca^{2+} in a process that was almost saturated at a Ca^{2+} concentration of 12.5 μM. Thus, as discussed earlier, the reduction in free Ca^{2+} concentration as a result of chelation in medium 2 would have only a small effect on the velocity observed in the presence of a 50 μM Ca^{2+} concentration used in the previous experiments. By plotting the dependence of the reciprocal of Ca^{2+} uptake against the reciprocal of Ca^{2+} concentration (see inset of Fig. 6), the V_{max} and $K_{0.5}$ of the Ca^{2+} uniporter process could be determined. These data indicated that the presence of taurine (10 mM) increased both V_{max} and $K_{0.5}$ (from 29.8 ± 4.11 nmol/min/mg and 10.37 μM, respectively, in the absence of taurine to 50.9 ± 7.5 nmol/min/mg and 17.32 μM in the presence of 10 mM taurine).

Mitochondrial Ca^{2+} Efflux

Possible interactions of taurine with the RR-insensitive Ca^{2+} efflux system of liver mitochondria were determined using mitochondria that had been loaded with 100 μM Ca^{2+}. The Ca^{2+} uniporter was then inhibited by addition of 200 nM RR. As shown in Fig. 7, where results in medium 1 and in pyrophosphate-containing medium (medium 2) in the presence of 0.5 μM CsA are shown, the rates of Ca^{2+} release did not vary significantly in the interval following RR addition (range 2.2 ± 0.3–3.45 ± 0.5 nmol/min/mg). Furthermore, in both media, the presence of 10 mM taurine did not significantly affect the rates of Ca^{2+} efflux.

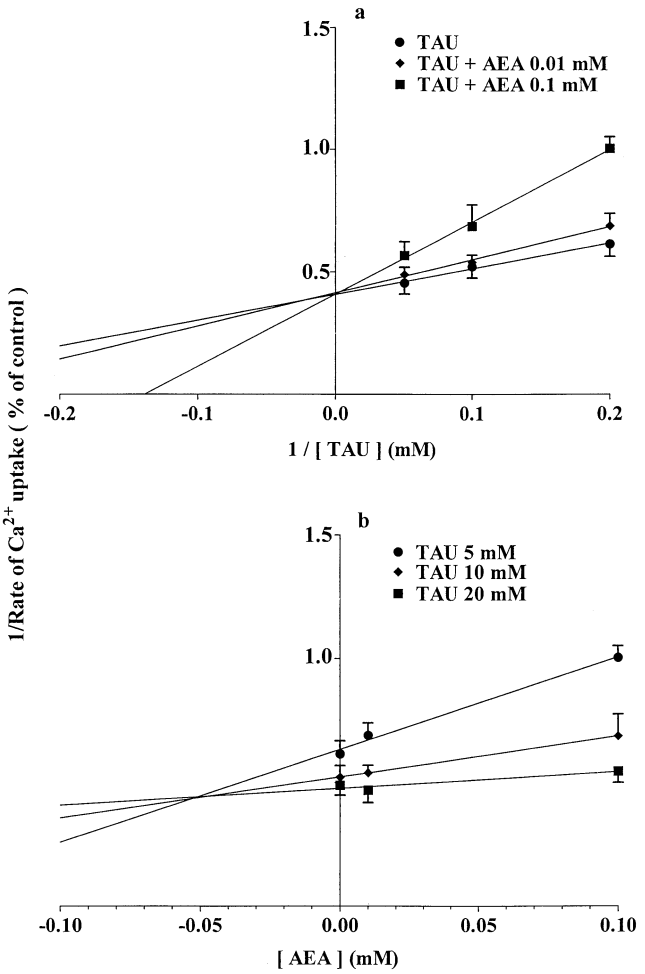


FIG. 5. Antagonism by aminoethylarsonate (AEA) of taurine (TAU)-stimulated Ca^{2+} transport in rat liver mitochondria. (a) Double-reciprocal plot to indicate the mechanism of inhibition. (b) Dixon [30] plot of the data to facilitate calculation of the K_i value. Values shown represent the means ± SEM from 3 to 5 separate experiments, each performed in duplicate.

Effect of Taurine on Membrane Potential

In order to characterise further the mechanism by which taurine stimulated mitochondrial Ca^{2+} uptake, the effects of taurine on mitochondrial $\Delta\Psi$ were studied under the same conditions as those used to determine Ca^{2+} uptake in medium 1. The distribution of $TPMP^+$ was measured both in the steady state before Ca^{2+} and during Ca^{2+} uptake. As shown in Fig. 8, the addition of 50 μM Ca^{2+} induced a transient drop in $\Delta\Psi$ that slowly recovered to nearly the initial value as the Ca^{2+} uptake became complete. The presence of 10 mM taurine did not significantly modify $\Delta\Psi$

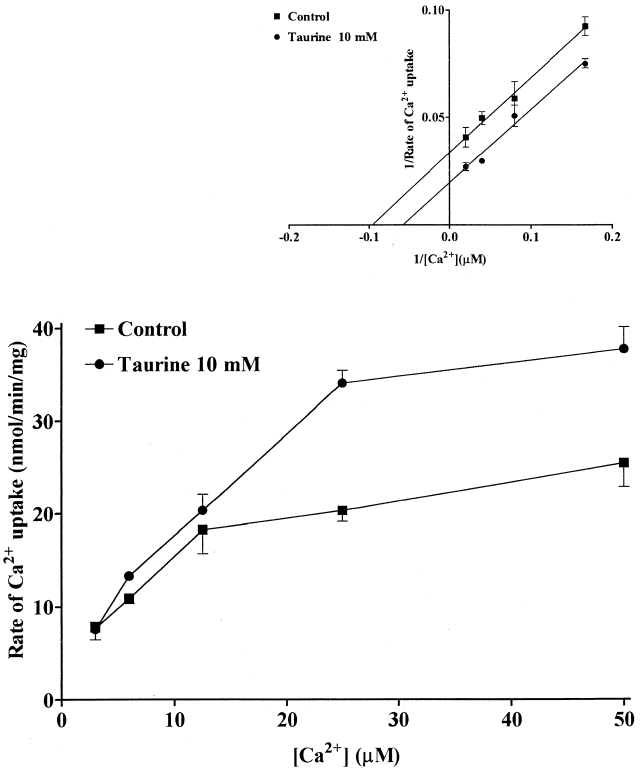


FIG. 6. Ca^{2+} concentration dependence of Ca^{2+} conductance of the mitochondrial inner membrane; effect of taurine. Ca^{2+} uptake, measured in terms of differential absorbance changes ($A_{710-790}$) in antipyrylazo III per min following Ca^{2+} addition, was determined in medium 1. Values represent the means ± SEM from 3 to 5 separate experiments, each performed in duplicate. Statistical significance with respect to control values for *P < 0.05 and **P < 0.01 was determined by Student's *t*-test for unpaired data, followed by Welch's *t*-test. The inset represents the dependence of the reciprocal of Ca^{2+} uptake against the reciprocal of Ca^{2+} concentration.

values, either in the steady state or during Ca^{2+} uptake. Lower external concentrations of Ca^{2+} induced smaller transient falls in $\Delta\Psi$ which, similarly to those observed with 50 μM Ca^{2+}, were not significantly affected by taurine (data not shown).

DISCUSSION

Just as taurine has been shown to increase high (15–20 μM)-affinity Ca^{2+} uptake by different membrane preparations [14–16], the present work shows that it stimulates Ca^{2+} uptake by rat liver mitochondria. The effect was relatively large, since 10 mM taurine increased the rate of uptake almost twofold. The initial rate of Ca^{2+} uptake by mitochondria was shown to depend on the taurine concentration in a saturable process. This suggests that taurine acts by reversibly forming a complex. This cannot be a complex with Ca^{2+}, since taurine has a very low affinity for this cation [35]. The effect of taurine was specific, since replacement of the sulphonic group with a phosphonic (aminoethylphosphonate) or an arsonic (aminoethylarsonate) group, replacement of its amino group with a hydroxyl group

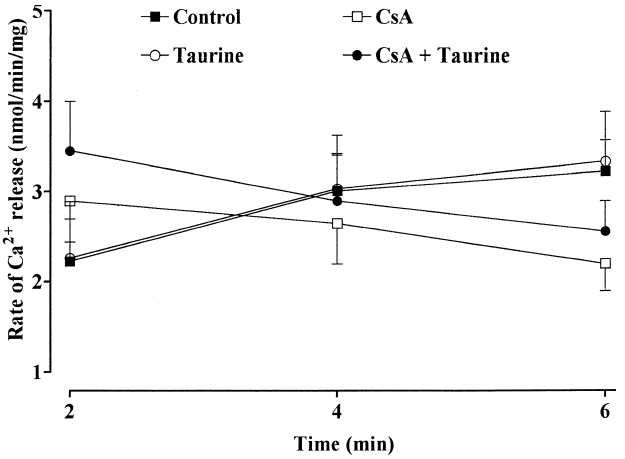


FIG. 7. Effect of taurine on the rate of Ca^{2+} release by rat liver mitochondria. Ca^{2+} efflux stimulated by 200 nM RR was determined in Ca^{2+} (100 μM)-preloaded mitochondria in the presence or absence of 10 mM taurine either in medium 2 in the presence of 0.5 μM CsA or in medium 1. Rates of Ca^{2+} efflux, measured in terms of differential absorbance changes ($A_{710-790}$) in antipyrylazo III per min at the times indicated, represent the means ± SEM from 3 to 5 separate experiments, each performed in duplicate. None of the values were significantly different from the respective control values (as determined by Student's *t*-test for unpaired data, followed by Welch's *t*-test).

(isethionate), or elongation of the carbon chain between these two groups, as in aminopropanesulphonate, abolished the stimulation of Ca^{2+} uptake. Although *N*-substituted *N*,*N*-dimethyltaurine did stimulate Ca^{2+} uptake, it did so only at higher concentrations and the effect was not saturable over the concentration range used. These results are at variance with those of Dolara *et al.* [36], who reported that both taurine and isethionate stimulate Ca^{2+} accumulation by rat liver mitochondria. However, those effects were observed only at very high concentrations of Ca^{2+} (10 mM), with no statistically significant effect of either com-

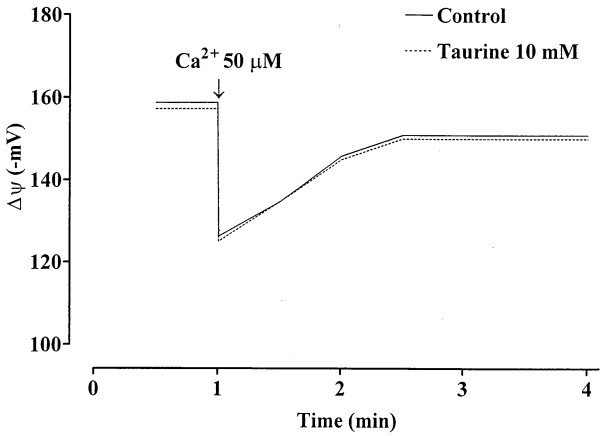


FIG. 8. Effect of taurine on mitochondrial $\Delta\Psi$ (−mV). The measurements were performed both in the steady state before Ca^{2+} addition and during Ca^{2+} uptake in medium 1, in the presence or absence of 10 mM taurine. After calibration, 10 mM succinate (K^+ salt) was added, followed by addition of 50 μM Ca^{2+} (see Methods).

pound being found when Ca^{2+} concentrations in the range 1–5 mM were used. Aminoethylarsonate not only failed to stimulate Ca^{2+} uptake, but antagonised the stimulatory effects of taurine in an apparently competitive process, with a K_i that was some two orders of magnitude lower than the apparent K_m for taurine. These data might indicate a common interaction site for aminoethylarsonate and taurine, thus further substantiating the specificity of the stimulatory effect of taurine on this process.

Analysis of the kinetics of Ca^{2+} uptake indicates that taurine acts as an "uncompetitive activator" of Ca^{2+} transport, increasing both the V_{max} and $K_{0.5}$ of the uniporter process [37]. These opposing effects on $K_{0.5}$ and V_{max} result in the stimulatory effects of taurine being dependent on the Ca^{2+} levels in the medium. For Ca^{2+} concentrations within the range of 3–12.5 μM, taurine has little effect on the rate of Ca^{2+} uptake, whereas the rate is increased at higher Ca^{2+} concentration. Thus, the effects of taurine enhance the total buffering capacity of the cell under conditions of Ca^{2+} overload. Such behaviour might contribute to the apparently paradoxical effects of taurine in increasing Ca^{2+} availability under conditions of low Ca^{2+}, but protecting against Ca^{2+} overload under conditions of high Ca^{2+} availability [16]. In this respect, the behaviour of taurine resembles that of spermine [38, 39]. However, although spermine and other polyamines increase the extent of Ca^{2+} accumulation, they also decrease the steady-state velocity of Ca^{2+} uptake [30, 38, 39]. Two spermine binding sites with dissociation constants of 0.5 and 4.7 mM have been found to modulate Ca^{2+} transport in liver mitochondria [39]. Although these values are similar to K_m for taurine, the different responses make it unlikely that one or both of the spermine sites is involved in the actions of the latter compound. However, the possibility that other physiologically occurring compounds may interact with the taurine site, perhaps at lower concentrations, cannot be excluded.

As has been shown, for example, in the case of glucagon, the rate of Ca^{2+} uptake can be affected not only by changes in the kinetic parameters of the Ca^{2+} uniporter itself but also by a change in $\Delta\Psi$, which provides the major component of the driving force for this uptake process [40]. Indeed, proton translocation by the respiratory chain may easily become the rate-limiting step for the process of Ca^{2+} uptake [41, 42]. In the present study, initial rates were determined which should depend only on the initial mitochondrial $\Delta\Psi$. Furthermore, measurement of $TPMP^+$ distribution under conditions identical to those used in the Ca^{2+} uptake experiments showed that taurine did not modify $\Delta\Psi$, either under resting conditions (before Ca^{2+} addition) or during Ca^{2+} uptake, indicating that the stimulation of uptake by taurine primarily reflects a direct effect on the transport system rather than an indirect effect operating through alterations in $\Delta\Psi$. The polyamines spermine and spermidine have also been reported to increase the total Ca^{2+} accumulation, but have little or no effect on $\Delta\Psi$ [30, 38, 39]. However, as our experiments were conducted at a physiological resting $\Delta\Psi$ of about 160 mV and Ca^{2+} uptake lowered it to about 126 mV, the possibility that taurine might have more profound effects at that lower initial membrane potential remains to be investigated.

Since mitochondrial influx and efflux of Ca^{2+} may be occurring simultaneously, as indicated by the relatively low rates as compared to those seen with Ca^{2+}-depleted mitochondria [43], an apparently increased accumulation of this ion might result from either stimulated uptake or inhibition of Ca^{2+} extrusion [4]. The observation [22] that taurine might have an uncoupling effect under the state 4 condition might also reflect an increased rate of Ca^{2+} cycling across the mitochondrial membrane. Although a specific effect of taurine was also observed on state 3 respiration in the presence of ADP [22], a condition whose rate is known to be jointly controlled by the electron transport chain and by the phosphorylating system, there was no direct effect on state 3 uncoupled respiration, which is largely controlled by the electron transport chain [44]. Thus, it appears that taurine may act at the level of the phosphorylating system, which is mainly limited by the adenine nucleotide translocase and the F_0–F_1 ATP synthase complex.

Loading mitochondria with Ca^{2+} in the presence of pyrophosphate might induce opening of the permeability transition pore in the mitochondrial inner membrane with release of accumulated Ca^{2+}, a process that is blocked by low concentrations of CsA [12]. Since 0.5 μM CsA did not modify the effects of taurine on the measured rate of Ca^{2+} accumulation, such a permeability transition did not occur under the experimental conditions used. Furthermore, the stimulatory effects of taurine were also seen in assays that did not contain pyrophosphate (medium 1). Thus, the increased Ca^{2+} uptake was not the result of taurine modulating the pore opening. The possibility that the apparently increased Ca^{2+} uptake in the presence of taurine might result from effects on Ca^{2+} efflux through an ion (Na^+/Ca^{2+} or H^+/Ca^{2+}) exchanger may be ruled out on the basis of the data showing that taurine did not change the rate of Ca^{2+} efflux stimulated by RR. Finally, since there were no significant differences between the Ca^{2+} uptake values determined in medium 1 and medium 2, the possibility that phosphate ions, derived from pyrophosphate hydrolysis, were affecting the observed Ca^{2+} accumulation may be excluded. Thus, under the conditions used in the present work, pyrophosphate did not affect Ca^{2+} transport and the mechanism of taurine-enhanced Ca^{2+} uptake was independent of the presence of either phosphate or pyrophosphate. Taken together, these data strongly support the view that stimulation of Ca^{2+} uptake by taurine resulted from stimulation of influx rather than inhibition of efflux. It has recently been reported that taurine decreases the fluidity of biological membranes, but only when co-administered with Ca^{2+}, suggesting that an interaction between taurine and Ca^{2+} affects membrane lipid fluidity [45]. Although the nature of this interaction is not yet clear, a change in the dynamics of mitochondrial membranes might

be invoked to explain how taurine modifies Ca^{2+} transport through them. Thus, taurine might alter the interaction of the mitochondrial Ca^{2+} uniporter with the lipid bilayer, thereby affecting its kinetic behaviour.

In isolated hepatocytes, taurine was shown to prevent lipid peroxidation [46] and to protect against the cytotoxicity of different compounds such as hydrazine, carbon tetrachloride, and 1,4-naphthoquinone [47]. In rats, a reduction of liver taurine significantly increased the hepatotoxicity of carbon tetrachloride [48], whereas treatment with taurine protected the liver against carbon tetrachloride-induced lipid peroxidation and concomitantly reduced intracellular Ca^{2+} accumulation [49], suggesting a correlation between the effect of taurine on Ca^{2+} and its protective effect. Changes in intracellular sequestration of Ca^{2+} have also been suggested to explain the protective effect of taurine on galactosamine-induced hepatic necrosis [50]. The effects of mitochondrial Ca^{2+} accumulation may be particularly beneficial for the maintenance of normal cell function in pathologies characterised by cytoplasmic accumulation of Ca^{2+}, as may occur during oxidative stress, excitotoxicity, and hypoxia/ischaemia–reperfusion. The observation that taurine counteracts the Ca^{2+} influx and overload in brain tissue that results from hypoxia suggests that such damage may indeed be improved by taurine supplementation [51]. Furthermore, taurine has been reported to protect dopaminergic neurones against the neurotoxicity of the mitochondrial inhibitor 1-methyl-4-phenylpyridinium ion (MPP^+) [52]. Since the mitochondrial inhibitory action of MPP^+ may, in turn, lead to the production of oxygen radicals and impaired Ca^{2+} homeostasis [53], the present results suggest that the protection afforded by taurine may be related to its effects on mitochondrial function.

In conclusion, we have shown that taurine potentiates the initial rate of Ca^{2+} uptake in mitochondria and that this may be due to its interaction with a specific recognition site in the mitochondrial membrane. This apparently specific role of taurine in modulating mitochondrial Ca^{2+} homeostasis may be important in pathological conditions characterised by cell Ca^{2+} overload, such as ischaemia and oxidative stress.

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