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Role of taurine uptake on the induction of long-term synaptic potentiation

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Abstract

Taurine application in the CA1 area of rat hippocampal slices induces a long-lasting potentiation of excitatory synaptic transmission that has some mechanistic similitude with the late phase of long-term potentiation (L-LTP). Previous indirect evidence such as temperature and sodium dependence indicated that taurine uptake is one of the primary steps leading to the taurine-induced synaptic potentiation. We show that taurine-induced potentiation is not related to the intracellular accumulation of taurine and is not impaired by 2-guanidinoethanesulphonic acid, a taurine transport inhibitor that is a substrate of taurine transporter. We have found that taurine uptake in hippocampal synaptosomes was inhibited by SKF 89976A, a GABA uptake blocker that is not transportable by GABA transporters. SKF 89976A prevents the induction of synaptic potentiation by taurine application. This effect is neither mimicked by nipecotic acid, a broad inhibitor of GABA transporters that does not affect taurine uptake, nor by NO-711, a specific and potent inhibitor of GABA transporter GAT-1. In addition, L-LTP induced by trains of high-frequency stimulation is also inhibited by SKF 89976A, and taurine, at a concentration that does not change basal synaptic transmission, overcomes such inhibition. We conclude that taurine induces synaptic potentiation through the activation of a system transporting taurine and that taurine uptake is required for the induction of synaptic plasticity phenomena such as L-LTP.

Introduction

Taurine is one of the most abundant free amino acids in the brain, where it seems to act as an organic osmolyte involved in cell volume regulation (Solís *et al*., 1988; Nagelhus *et al*., 1993; Pasantes-Morales & Schousboe, 1997). In addition, taurine can activate $GABA_A$ (Zhu & Vicini, 1997; del Olmo *et al*., 2000a) and strychnine-sensitive glycine receptors (Flint *et al*., 1998; Hussy *et al*., 2001; Mori *et al*., 2002) with less affinity than their respective agonists.

We identified another taurine action that consists of a long-lasting potentiation of both synaptic efficacy and axon excitability in the CA1 area of rat hippocampal slices (Galarreta *et al*., 1996) that we named LLP-TAU (del Olmo *et al*., 2000b). This kind of potentiation was independent of $GABA_A$ and ionotropic glutamate receptor activation (Galarreta *et al*., 1996; del Olmo *et al*., 2000b), but similar to LTP induction, LLP-TAU needs extra- and intracellular calcium presence to be generated (del Olmo *et al*., 2000b, c). The phenomenon of LLP-TAU lasts for more than 3 h (Galarreta *et al*., 1996; del Olmo *et al*., 2003), and, based on pharmacological experiments, it appears that the activation of cAMP-dependent protein kinase (PKA) and the synthesis of proteins are required for its maintenance (del Olmo *et al*., 2003). These molecular mechanisms are known to be required for the late phase of LTP or L-LTP (Frey *et al*., 1988, 1993; Huang *et al*., 1996). In fact, we have shown (del Olmo *et al*., 2003) that the persistence of both L-LTP and LLP-TAU occludes mutually, indicating that common mechanisms are involved in the late phase of both potentiation processes.

Given that LLP-TAU induction was severely impaired when taurine was applied in a low-sodium perfusion solution or at low bath temperature, we proposed that taurine has to be taken up to induce LLP-TAU (Galarreta *et al*., 1996). This early suggestion could not be properly evaluated because the unique substance known to inhibit taurine transport, 2-guanidinoethanesulphonic acid (GES), was also able to induce a long-lasting potentiation of synaptic transmission (Galarreta *et al*., 1996).

Recently, Chepkova *et al*. (2002) have reported that taurine also induces a long-lasting enhancement of corticostriatal synaptic transmission that is blocked by 1 mM GES. By contrast, 10 mM GES induces synaptic potentiation similarly to taurine. The same group (Sergeeva *et al*., 2003) also reported that taurine evokes synaptic potentiation in the hippocampus but not in the striatum of knockout mice for the taurine transporter (TAUT). They also found that the synaptic potentiation induced by taurine in the hippocampus and striatum were pharmacologically distinct: 1 mM nipecotic acid, a broad inhibitor of GABA transporters (Borden, 1996), inhibits LLP-TAU in hippocampus but not in striatum, and vice versa with 1 mM GES. These observations encouraged us to use these pharmacological tools to assess the role of taurine uptake in LLP-TAU induction and also to investigate whether taurine has any physiological meaning in L-LTP.

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Materials and methods

The care and use of animals was carried out in accordance with the European Communities Council Directive (86/609/ECC).

Slice preparation

Experiments were performed on 400-μm-thick transverse hippocampal slices obtained with standard methods from male and female Sprague–Dawley rats (200–250 g). Animals were decapitated after anaesthesia with halothane, and the brain quickly removed and dropped into ice-cold Krebs-Ringer bicarbonate (KRB) solution containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 2.5 KCl, 1 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$ and 11 glucose. This solution was pregassed with 95% O_2 and 5% CO_2. The hippocampi were sliced by a manual tissue chopper and placed in a holding chamber for at least 3 h at room temperature (20–25 °C). A single slice was transferred to a submersion-type recording chamber, where it was continuously perfused (1.7–2 mL/min) with standard KRB solution. Experiments were carried out at 31–32 °C.

Solutions

Drugs applied by addition to the standard perfusion solution included: D,L-2-amino-5-phosphopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), ethylene glycol bis-(b-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA) (±) nipecotic acid, NO-711 hydrochloride, picrotoxin and taurine from Sigma; GES from Toronto Research Chemicals (North York, Canada); CGP 55845 and SKF 89976A hydrochloride from Tocris (London, UK). The drugs were prepared as stock solutions, stored frozen in the dark and diluted to their final concentrations in the perfusion solution immediately before use. Stock solutions of AP5 (30 mM), CNQX (20 mM), NO-711 (25 mM), picrotoxin (5 mM) and SKF 89976A (25 mM) were prepared in distilled water. Stock solution of CGP 55845 (2 mM) was prepared in dimethyl sulphoxide (final concentration 0.1%). Taurine, GES and nipecotic acid were dissolved directly in the perfusion solutions at their final concentrations. To prevent epileptiform discharges in those experiments in which the $GABA_A$ receptors were blocked with picrotoxin, a cut was made between CA1 and CA3 areas, and the concentrations of Ca^{2+} and Mg^{2+} were increased to 4 mM. The osmolarity of the perfusion solutions was tested by a micro-osmometer (Advanced Instruments Mod.3MO, Norwood, MA, USA).

Recording of evoked synaptic potentials

Evoked field excitatory postsynaptic potential (fEPSP) and presynaptic fibre volley (FV) were recorded in CA1 stratum radiatum with tungsten microelectrodes (1 MΩ) connected to an AI-401 amplifier (Axon Instruments, Foster City, CA, USA) plugged to a CyberAmp 320 signal conditioner (Axon Instruments). These field responses were evoked by stimulating Schaffer collateral-commisural fibres with biphasic electrical pulses (20–60 μA; 40–100 μs; 0.033 or 0.066 Hz) delivered through bipolar tungsten insulated microelectrodes (0.5 MΩ) placed in CA1 mid-stratum radiatum. Electrical pulses were supplied by a pulse generator A.M.P.I. Mod. Master 8 (Jerusalem, Israel).

In some experiments two independent groups of axons were stimulated by placing two sets of stimulation electrodes on opposite sides of the recording electrode. The independence of both pathways was assessed by the lack of paired-pulse facilitation between them, i.e. when two stimuli were applied one on each pathway at 50-ms interval and the second response was identical to that evoked by an unpaired pulse applied 10 s after the paired stimulation. The two independent pathways were stimulated alternately every 15 s. Induction of L-LTP was achieved by applying three trains of high-frequency stimulation (100 Hz/1 s) at 10-min intervals at twice the pulse duration applied during baseline stimulation (HFT).

Intracellular recordings of neurons located in the stratum pyramidale of the CA1 region were obtained while perfusing standard KRB with glass micropipettes filled with 3M K-acetate (60–80 MΩ). The micropipettes were connected to an Axoclamp-2B amplifier (Axon Instruments) used in bridge mode. Only cells with a stable resting membrane potential (V_m) more negative than −60 mV and an apparent input resistance (R_{in}) greater than 30 MΩ were studied. The R_{in} of the neurons was calculated from the voltage deflection produced by hyperpolarizing current pulses (0.1–0.3 nA) of 100 ms duration.

Electrophysiological data analysis

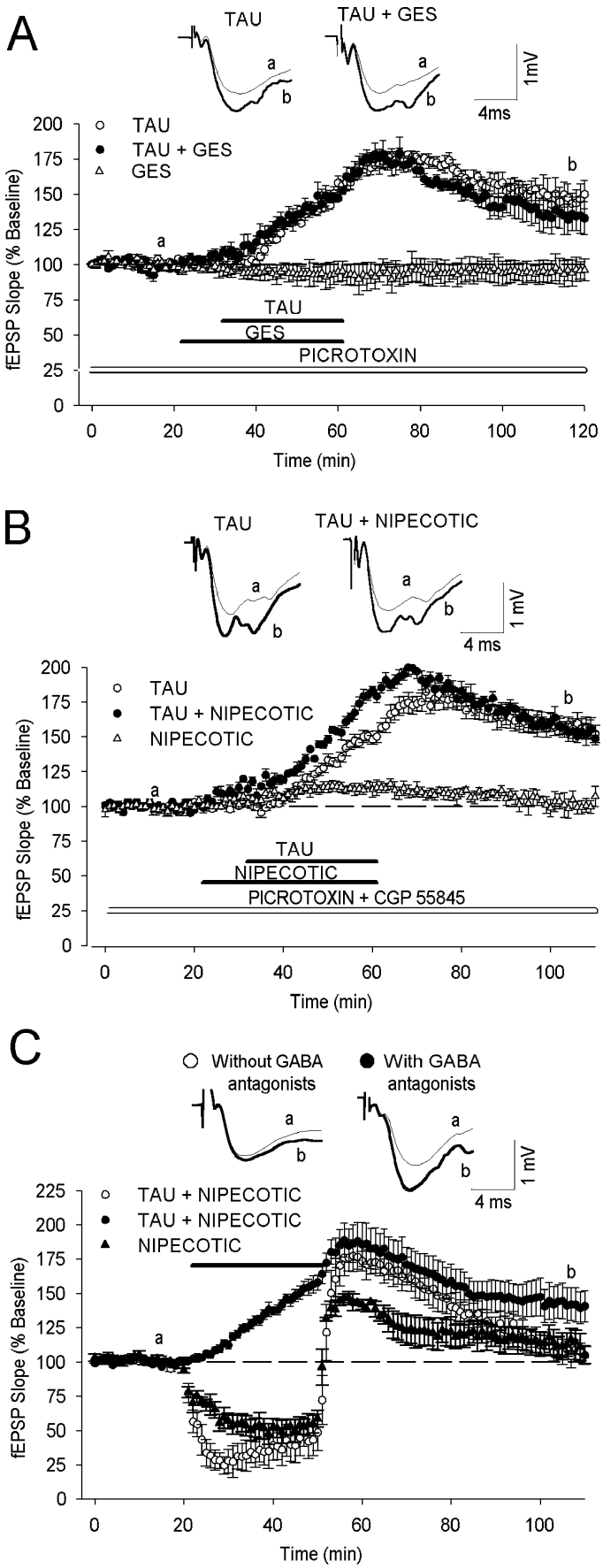
Evoked responses were digitized at 25–50 kHz using a Digidata 1320A board (Axon Instruments), and stored on a Pentium III IBM-compatible computer using pCLAMP 8.0.2 software (Axon Instruments). The amplitude of the presynaptic FV was measured from the baseline to the negative peak of the FV. The synaptic strength was calculated using the initial rising slope phase of the fEPSP to avoid contamination of the response by the population spike. We considered as baseline the mean value of the signal, taken in a window of 2–5 ms preceding the stimulus artefact. We also used pCLAMP-8.0.2 software for these calculations. Traces shown are averages of eight consecutive responses. Data were normalized with respect to the mean values of the responses during the final 10 or 20 min of the baseline period in standard medium, in intra- or extracellular recording experiments, respectively.

Synaptosomal uptake

To obtain the synaptosomal fraction, the hippocampus was dissected in cold (4 °C) KRB, and then transferred to ten volumes of a 0.32 M sucrose solution. The hippocampi were homogenized with the help of a Teflon pestle. The homogenate was centrifuged at 1000 ***g*** for 10 min at 4 °C. The obtained supernatant was centrifuged at 17 000 ***g*** for 55 min at the same temperature. The resulting pellet was gently resuspended in ten volumes of KRB solution. Synaptosomal uptake determinations, performed in KRB, were made in triplicate, by adding the synaptosomes (about 1 mg protein) to tubes containing the compound under study and 50 μM ^{3}H-taurine (made by adding 1 μCi ^{3}H-taurine [1,2-^{3}H]taurine, 32 Ci/mmol, Amersham, to 50 μM taurine) or 10 mM taurine. Incubation lasted 5 min (50 μM ^{3}H-taurine) or 10 min (10 mM taurine) at 37 °C (0 °C for controls). Preliminary experiments showed uptake to be linear at these incubation conditions. In the experiments with 50 μM ^{3}H-taurine, at the end of the incubation period, each tube content was rapidly filtered (GF/B filters) and washed twice with KRB solution under vacuum. ^{3}H-taurine content in each filter was determined using a scintillation counter (LKB-Wallac, Sweden). Nonspecific uptake represented about 25% of total uptake. The SKF 89976A concentration required to produce 50% inhibition of ^{3}H-taurine uptake (IC_{50}) was estimated by computerized nonlinear regression analysis (GraphPad Software, San Diego, CA). In the experiments with 10 mM taurine, the incubation was ended by microcentrifuging at 13 000 ***g*** for 90 s at 2 °C, and the tubes were washed three times by resuspension and recentrifugation under the same conditions. Finally, each tube received 100 μL of 5-sulphosalicylic acid (15%) and was subsequently processed for amino acid analysis.

Amino acid analysis

In some experiments, at the end of the perfusion period, the slice was collected in a tube containing 200 μL of 5-sulphosalicylic acid (15%)



where they were sonicated to extract the amino acids. Analysis of taurine content in these samples was performed with a reverse-phase HPLC system (Beckman) after derivatization with o-phthalaldehyde. Protein concentration in slices was determined by the method of Lowry.

Statistical analysis

Data are expressed as means ± SEM. Mean values of fEPSP slope or FV amplitude given throughout the text correspond to averages of 5-min periods. Statistical differences were assessed by one- or two-way analysis of variance followed by two-tailed *t*-tests; a value of $P < 0.05$ was considered statistically significant.

Results

LLP-TAU is not inhibited by either GES or nipecotic acid

GES, in addition to inhibiting taurine transport, can interact with $GABA_A$ receptors (Herranz *et al*., 1990) acting as an agonist (Mellor *et al*., 2000). Therefore, to study how GES-inhibition of taurine uptake affects LLP-TAU induction without the interference of $GABA_A$ receptor activation, we used a perfusion medium with 100 μM picrotoxin, an antagonist of this type of receptor. The presence of picrotoxin did not impair the induction of LLP-TAU evoked by bath application of 10 mM taurine for 30 min (145 ± 10% of baseline, at 1 h of taurine washout; $n = 5$), as we previously reported (Galarreta *et al*., 1996). In another group of experiments ($n = 6$) we perfused 1 mM GES 10 min before and during taurine application. The presence of GES did not affect either the time course or the magnitude of the taurine-induced increase in fEPSP slope (Fig. 1A). After 1 h of taurine and GES washout, the fEPSP potentiation (141 ± 11%) was not statistically different ($P > 0.05$) from control experiments in the absence of GES. Application of 1 mM GES for 40 min ($n = 4$) had no significant effect ($P > 0.05$) on baseline fEPSP slope during either its application (95 ± 6% at 40 min) or its washout (95 ± 8% at 1 h).

Two rat GABA transporters, GAT-2 and GAT-3, are also able to transport taurine with low affinity (IC_{50} 1.3 and 2.9 mM, respectively) when they were expressed in COS-7 cells (Borden, 1996). Because we applied 10 mM taurine to induce LLP-TAU, we wondered whether taurine uptake throughout GAT-2 and/or GAT-2 contributes to the generation of synaptic potentiation. To answer this question, we applied taurine in the presence of 1 mM nipecotic acid (Fig. 1B), which at this concentration inhibits GAT-1, GAT-2 and GAT-3 rat

FIG. 1. Neither GES nor nipecotic acid interfere with LLP-TAU induction. The upper traces, here and in subsequent figures, show averaged representative fEPSPs (eight consecutive responses) recorded at the times indicated by the letters on the graph during one of the experiments used for the graph. (A) fEPSP slopes normalized to the mean value of the 20-min baseline period and expressed as the mean ± SEM. Picrotoxin (100 μM), a $GABA_A$ antagonist, was present throughout the experiments. Substances were applied during the time period indicated by horizontal bars. Taurine (10 mM) was bath applied for 30 min in the absence (open circles; $n = 5$) or in the presence of 1 mM GES (filled circles, $n = 6$). GES did not affect synaptic transmission in the presence of picrotoxin (open triangles; $n = 4$). (B) The experiments were carried out in the continuous presence of picrotoxin and CGP 55845. Open circles correspond to the experiments ($n = 6$) in which taurine was applied in the presence of GABA antagonists. Nipecotic acid (1 mM) did not impair LLP-TAU induction (filled circles; $n = 5$). The application of just nipecotic acid (1 mM for 30 min) induced a small reversible increase in fEPSP slope (open triangles; $n = 5$). (C) Co-application of taurine and 3 mM nipecotic acid in a standard solution induced a potentiation that slowly declined back toward baseline (open circles; $n = 5$) but evoked LLP-TAU when they were applied in the continuous presence of GABA receptor antagonists (filled circles; $n = 7$). Filled triangles ($n = 4$) represent the time course of changes in the fEPSP induced by the application of 3 mM nipecotic acid alone in the absence of GABA receptor antagonists.

transporters (Borden, 1996). These experiments were carried out in the continuous presence of $GABA_A$ and $GABA_B$ antagonists to prevent the activation of these receptors by the endogenous GABA released by nipecotic acid (Solís & Nicoll, 1992).

Nipecotic acid perfusion evoked a small potentiation ($113 \pm 3\%$, $P < 0.01$, compared with baseline values, $n = 5$; Fig. 1B) that returned to baseline values during the washout of the uptake antagonist ($101 \pm 5\%$ at 1 h of washout). The presence of 1 mM nipecotic acid ($n = 5$) increased significantly the progressive fEPSP enhancement elicited during taurine perfusion ($149 \pm 4\%$ in control slices vs. $177 \pm 5\%$ in nipecotic acid, at 30 min of taurine perfusion, $P < 0.001$). However, the level of potentiation remaining 60 min after taurine and nipecotic acid washout was statistically indistinguishable from that obtained with taurine alone ($147 \pm 7\%$ in control slices vs. $148 \pm 6\%$ in nipecotic acid, $P > 0.05$). Additionally, in another group of slices ($n = 7$; Fig. 1C) we observed that taurine application in the presence of a higher concentration of nipecotic acid (3 mM) did not reduce LLP-TAU significantly ($142 \pm 12\%$ at 60 min of taurine washout, $P > 0.05$ compared with LLP-TAU in the absence of nipecotic acid). The perfusion of 3 mM nipecotic acid alone for 30 min ($n = 3$), in the presence of GABA receptor antagonists, enhanced fEPSP to $120 \pm 5\%$, a level similar to that found with 1 mM nipecotic acid. This potentiation decayed back toward baseline values after the washout of nipecotic acid ($104 \pm 3\%$). These results are not compatible with a contributing role of GABA transporters in LLP-TAU induction. Interestingly, when the experiments were carried out in the absence of GABA receptor antagonists ($n = 5$; Fig. 1C), the concomitant application of nipecotic acid and taurine caused a profound depression of fEPSP (to $43 \pm 12\%$ of baseline values at 30 min of application) that was followed by a slowly decaying potentiation after the washout of both substances ($108 \pm 7\%$ at 60 min of taurine washout, $P < 0.05$ compared with LLP-TAU in the presence of GABA receptor antagonists). This sequence of fEPSP changes was similarly produced when 3 mM nipecotic acid without taurine was perfused in the absence of GABA receptor antagonists (depression to $55 \pm 6\%$ at 30 min of nipecotic acid perfusion, rebound to $142 \pm 3\%$ at 8–12 min of washout that decayed to $113 \pm 9\%$ at 60 min of washout; $n = 4$; Fig. 1C, filled triangles). These results indicate that GABA receptor activation during the application of nipecotic acid could affect the synaptic potentiation induced by taurine.

All these data are difficult to reconcile with the hypothesis that taurine transport is involved in LLP-TAU induction. This hypothesis, however, could still be feasible considering that GES does not block taurine transporter activity but that it is, in fact, transported by taurine carrier (Huxtable *et al.*, 1979; Barakat *et al.*, 2002). Consequently, the intracellular accumulation of taurine is reduced in the presence of GES because this substance competes with taurine for the carrier (Huxtable *et al.*, 1979). It is therefore worth investigating whether the intracellular accumulation of taurine is involved in LLP-TAU induction.

Intracellular accumulation of taurine does not affect LLP-TAU

Endogenous taurine content of hippocampal slices perfused in a standard KRB for 110 min was 18.8 ± 2.0 nmol/mg protein ($n = 21$; Fig. 2A). Taurine application (10 mM for 30 min) significantly increased taurine levels to 74.5 ± 12.1 nmol/mg protein ($P < 0.001$, $n = 7$) in the slices collected 1 h after taurine washout. In another group of slices in which a second taurine application was carried out 75 min after the first, there was no additional accumulation of taurine (72.9 ± 9.0 nmol/mg protein; $n = 5$; Fig. 2A).

We carried out another group of experiments ($n = 6$), following this sequence of taurine application, in which fEPSPs were recorded (Fig. 2A). The first application of taurine elicited an fEPSP potentiation of $137 \pm 6\%$ measured 60 min after taurine washout, a value similar to that previously reported (Galarreta *et al.*, 1996; del Olmo *et al.*, 2000b). Thereafter, the stimulus strength was reduced to match the size of the fEPSP slope to baseline values. A second application of taurine, which reduced fEPSP slope as in the first taurine application, only evoked a small transitory potentiation ($101 \pm 5\%$ at 1 h after taurine washout; Fig. 2A). This result could be due to the fact that there was no additional accumulation of intracellular taurine during the

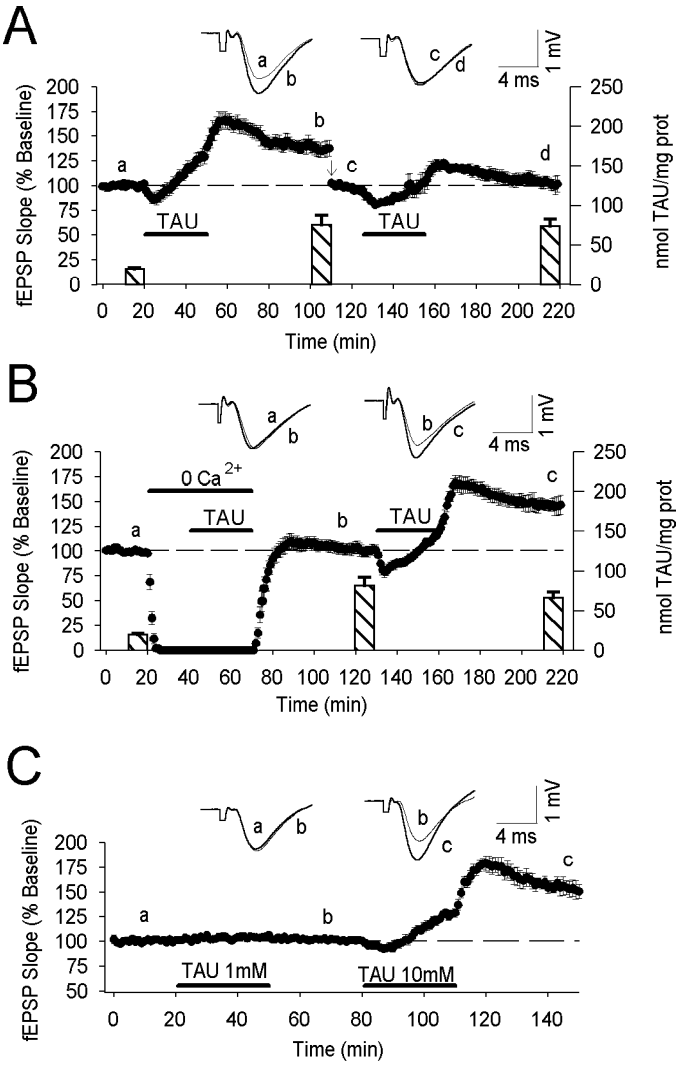


FIG. 2. The enhancement of intracellular taurine levels does not preclude the subsequent induction of LLP-TAU. (A) Filled circles represent temporal evolution of fEPSP slope changes induced by two applications of taurine (10 mM, 30 min) in a standard perfusion solution ($n = 6$). The arrow indicates the moment after the first application of taurine, when the stimulus strength was reduced to return fEPSP slope to baseline values. Bars represent the concentration of taurine measured in three independent groups of slices under the following conditions: 110 min of perfusion in KRB ($n = 21$), after 1 h of the first application of taurine ($n = 7$) and after 1 h of a second application of taurine ($n = 5$) as in the experiments plotted in the graph. (B) In another group of slices ($n = 5$), the first application of taurine was performed in the nominal absence of extracellular calcium (0 Ca^{2+}) whereas the second was performed in a standard KRB. Bars represent the concentration of taurine found in slices treated as follows: basal levels of taurine correspond to the value depicted in A after 1 h of a taurine application in calcium-free solution ($n = 6$) and after 1 h of the second application of taurine in standard solution ($n = 4$). (C) The induction of LLP-TAU by the application of 10 mM taurine for 30 min was not affected by the previous perfusion of 1 mM taurine for 30 min ($n = 5$), which increased nearly to three times taurine levels in the slices.

second application or, alternatively, it could simply be that the synaptic potentiation induced by taurine was saturated.

To distinguish between these two possibilities, we performed a group of experiments in which the first application of taurine was carried out in the nominal absence of extracellular Ca^{2+} (a solution without Ca^{2+} containing 6 mM Mg^{2+} and 100 μM EGTA), an experimental situation blocking LLP-TAU induction (del Olmo *et al*., 2000b) but allowing the accumulation of taurine as in normal conditions (81.4 ± 10.6 nmol/mg protein; $n = 6$; Fig. 2B). Effectively, taurine perfusion in a calcium-free medium did not induce LLP-TAU (100 ± 5%, at 1 h post-taurine application; $P > 0.05$; $n = 5$) whereas a subsequent application in a standard solution evoked a potentiation of 146 ± 5% measured 1 h after taurine washout ($P < 0.001$ compared with baseline values; Fig. 2B). Taurine levels at the end of these experiments (65.4 ± 7.8 nmol/mg protein; $n = 4$) were statistically indistinguishable ($P > 0.05$) from the amount of taurine found in those slices treated with two applications of taurine in standard medium (Fig. 2A).

Another strategy to assess the relevance of intracellular taurine levels on LLP-TAU induction was to perfuse taurine at 1 mM, a concentration that did not cause significant changes in fEPSP slope (103 ± 2%, at 30 min after taurine washout; $P > 0.05$; $n = 5$; Fig. 2C) but that greatly increased taurine concentration (53.9 ± 4.5 nmol/mg protein, analysed in a group of eight independent slices). The accumulation of taurine did not alter the subsequent induction of normal LLP-TAU evoked by 10 mM taurine application (149 ± 10%, at 1 h after taurine washout; $P > 0.05$ compared with LLP-TAU in Fig. 2A).

Together, these results indicate that LLP-TAU induction does not require a net gain of intracellular taurine, although this could be related to the process of taurine transport.

SKF 89976A inhibits taurine uptake in synaptosomes

To evaluate the above hypothesis it would be helpful to have a substance able to block taurine transport without being itself transported. GES, the only known taurine transport inhibitor, is not valid because, as we pointed out before, it is a substrate of taurine transporters. Because the cloned taurine and GABA transporters present significant homologies (Smith *et al*., 1992) and there is a group of agents blocking GABA carriers (Krogsgaard-Larsen *et al*., 1987), we considered the possibility that some of these substances could also inhibit taurine transporters. To assess this hypothesis we assayed whether one of these agents, SKF 89976A (Larsson *et al*., 1988), affected ^{3}H-taurine uptake in hippocampal synaptosomes. We found that SKF 89976A inhibited taurine uptake with an IC_{50} of 0.6 mM (Fig. 3A), a value about 1000-fold less than that inhibiting GAT-1 (Borden *et al*., 1994). The inhibition of ^{3}H-taurine uptake caused by 1 mM GES (68 ± 3%) was statistically indistinguishable ($P > 0.05$) from that evoked by an equimolar concentration of SKF 89976A (60 ± 6%). However, 1 mM nipecotic acid, which at this concentration inhibits all GABA transporters (Borden, 1996), but which presents a low affinity for taurine transporter ($IC_{50} = 2$ mM; Borden *et al*., 1995), only reduced ^{3}H-taurine uptake by 19 ± 2%, an inhibitory effect significantly smaller ($P < 0.01$) than that produced by either GES or SKF 89976A. Thus, SKF 89976A-sensitive taurine uptake in hippocampal synaptosomes is more likely to be mediated through taurine transporters than through GABA transporters.

Taurine transporters currently cloned present high-affinity kinetics (K_m of 4.5 and 40 μM; Liu *et al*., 1992; Smith *et al*., 1992). However, taurine concentrations in the millimolar range are required for LLP-TAU induction (Galarreta *et al*., 1996). To assess the effect of SKF 89976A on the low-affinity uptake of taurine, we investigated how this drug affected the accumulation of 10 mM taurine in synaptosomes.

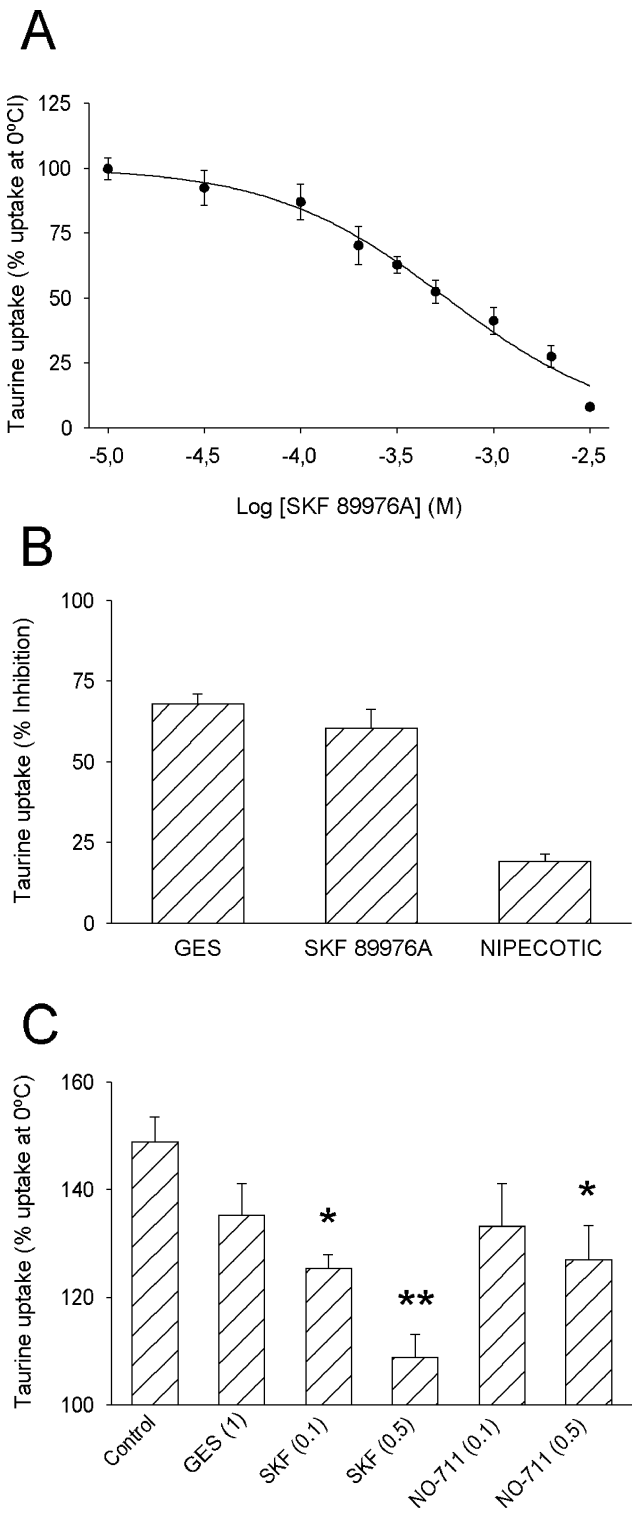


FIG. 3. SKF 89976A inhibits taurine uptake to hippocampal synaptosomes. Uptake assays were carried out as described in 'Materials and methods'. (A) Effect of SKF 89976A on ^{3}H-taurine uptake. Points correspond to the mean ± SEM of three independent experiments, each in triplicate. The data fit the equation: $Y_i = Y_{min} + (Y_{max} - Y_{min})/(1 + 10)^{(Xi\ -\ Log\ IC50)}$ yielding an IC_{50} of 0.6 mM. (B) Comparison of inhibition against ^{3}H-taurine uptake of 1 mM GES ($n = 3$), 1 mM SKF 89976A ($n = 6$) and 1 mM nipecotic acid ($n = 3$). GES and SKF 89976A were equipotent whereas nipecotic acid inhibits much less ($P < 0.01$). (C) Effect of 1 mM GES ($n = 5$), 0.1 and 0.5 mM SKF 89976A ($n = 4$ and 12, respectively), and 0.1 and 0.5 mM NO-711 ($n = 4$ in both cases) on taurine accumulation by synaptosomes incubated in 10 mM taurine for 10 min ($^*P < 0.05$, $^{**}P < 0.0001$; compared with control, $n = 12$).

Endogenous taurine content of synaptosomes (55.0 ± 5.0 nmol/mg protein; $n = 4$) was not modified significantly by the presence of 0.5 mM SKF 89976A (56.3 ± 2.8 nmol/mg protein; $n = 3$). As shown in Fig. 3C, taurine uptake in control conditions ($149 \pm 5\%$; $n = 12$) was inhibited to $125 \pm 3\%$ and $109 \pm 4\%$ when the synaptosomes were incubated in the presence of 0.1 mM ($n = 4$) and 0.5 mM ($n = 12$) SKF 89976A, respectively, indicating that this substance markedly blocks the low-affinity transport of taurine. However, 1 mM GES ($n = 5$) or 0.1 mM NO-711 ($n = 4$), the latter a potent and selective nontransportable blocker of GAT-1 ($IC_{50} = 0.38$ μM; Borden *et al.*, 1994), reduced taurine uptake, although not significantly, to $135 \pm 6\%$ and $133 \pm 8\%$, respectively. A higher concentration of NO-711 (0.5 mM) was able to reduce ($P < 0.05$) taurine uptake significantly, to 127 ± 6 ($n = 4$), similar to the effect of 0.1 mM SKF 89976A. Therefore, these results demonstrate that SKF 89976A is more potent than the prototypic TAUT antagonist, GES, in inhibiting the low-affinity transport of taurine in synaptosomes. Additionally, they indicate that this taurine uptake does not seem to be mediated via GAT-1 transporter.

SKF 89976A inhibits LLP-TAU induction

The following group of experiments was performed to examine whether LLP-TAU could be induced in the presence of this new taurine uptake inhibitor. Again, taurine application in a standard solution induced, at first, a small transitory depression of fEPSP slope, followed by a persistent potentiation ($148 \pm 2\%$ at 30 min after taurine washout; $P < 0.001$ compared with baseline; Fig. 4A, open circles). However, fEPSP potentiation was completely blocked when taurine was concomitantly applied with 0.5 mM SKF 89976A

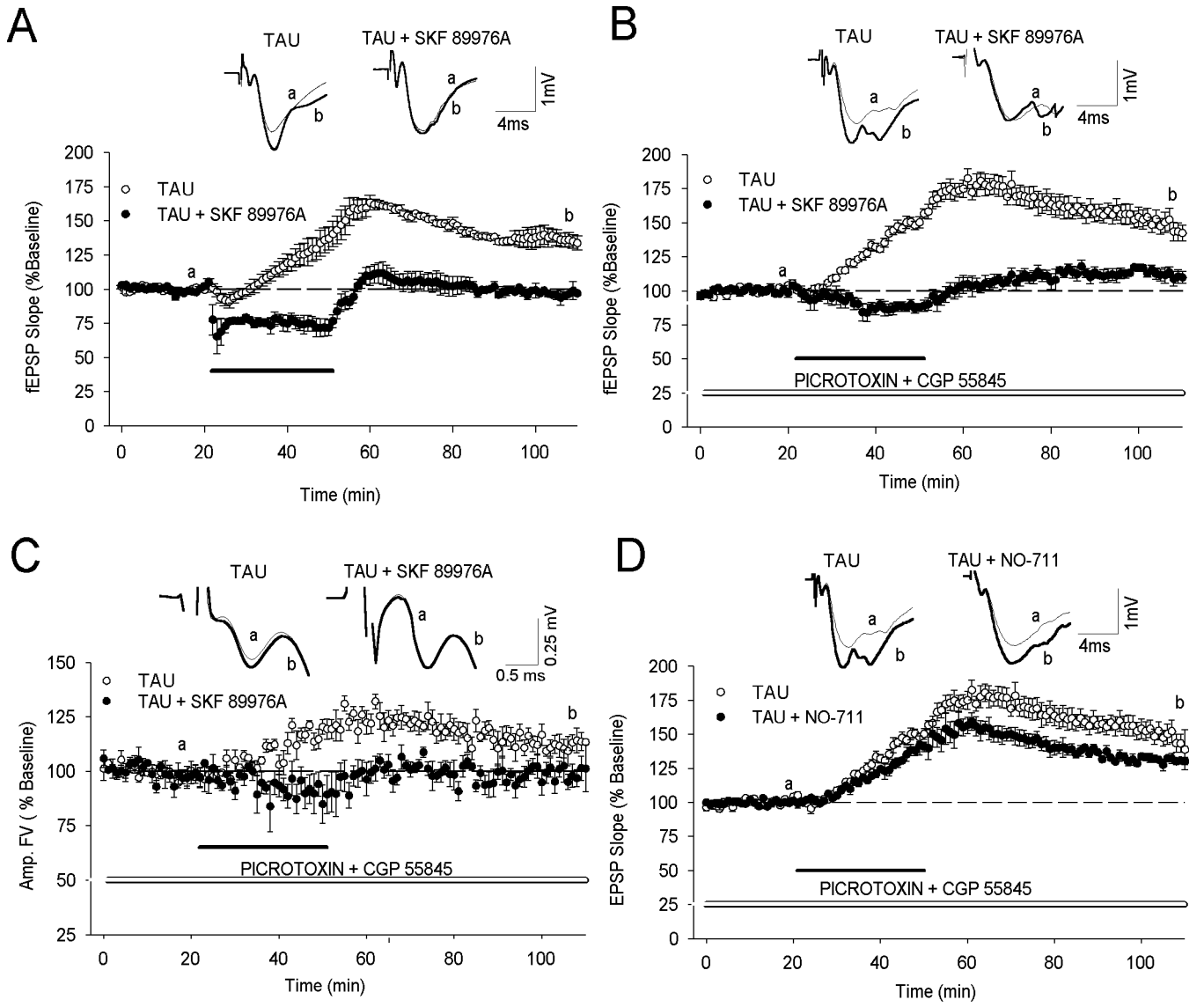


FIG. 4. SKF 89976A (0.5 mM) but not NO-711 (0.1 mM) inhibits LLP-TAU induction. (A) Taurine application in standard solution evoked LLP-TAU (open circles; $n = 4$), whereas the co-application of taurine and SKF 89976A only induced a reversible depression of fEPSP without eliciting LLP-TAU (filled circles; $n = 4$). (B) Open circles correspond to the experiments ($n = 6$) shown in Fig. 1B, in which taurine was applied in the continuous presence of GABA receptor antagonists, picrotoxin and CGP 55845. Under these experimental conditions, SKF 89976A was still able to inhibit LLP-TAU induction, although the fEPSP decrement evoked during the co-application of taurine and SKF 89976A was greatly reduced (filled circles; $n = 5$). (C) Summary FV amplitude values from some of the experiments depicted in B in which this potential could be recorded and measured with certainty (six control experiments, open circles; and four SKF 89976A experiments, filled circles). (D) LLP-TAU induction was not significantly affected by the presence of 0.1 mM NO-711 (filled circles; $n = 4$). Open circles correspond to taurine perfusion experiments shown in Fig. 1B.

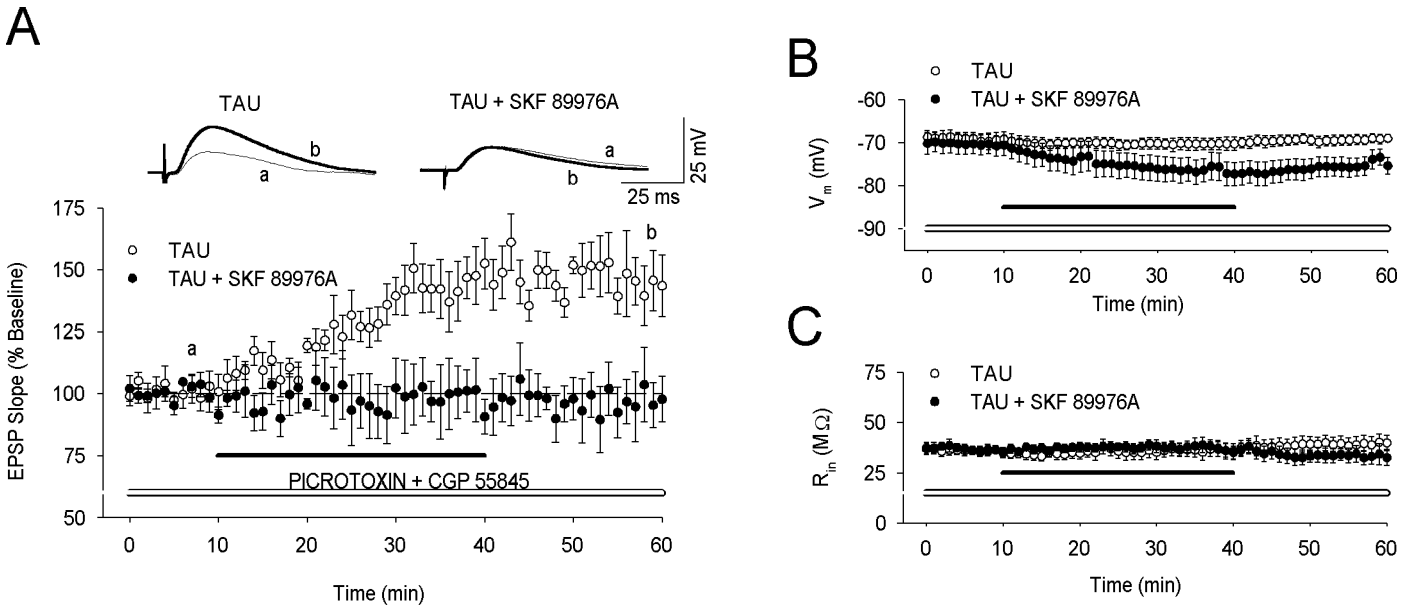


FIG. 5. SKF 89976A also inhibits intracellularly recorded LLP-TAU. In the presence of GABA receptor antagonists, 10 mM taurine application (open circles; $n = 5$) evoked EPSP potentiation (A) without changes in either V_m (B) or R_{in} (C). However, co-application of taurine and 0.5 mM SKF89976A (filled circles; $n = 5$), did not induce significant changes of either EPSP (A) or R_{in} (C), but slowly hyperpolarized resting membrane potential (B).

($104 \pm 7\%$ at 30 min after taurine washout; $P > 0.05$ compared with baseline; Fig. 4A, filled circles). Under this experimental condition, taurine only evoked a sustained depression of fEPSP slope ($73 \pm 6\%$ at 30 min of taurine application compared with $132 \pm 9\%$ in control conditions, $P < 0.001$) that recovered to baseline value when standard perfusion solution was resumed. Because the inhibition of GABA uptake by SKF 89976A could increase interstitial levels of GABA that might activate GABA receptors (Isaacson *et al.*, 1993), we carried out another set of experiments ($n = 5$) to exclude the possibility that the inhibition of taurine-induced potentiation by SKF 89976A was due to activation of GABA receptors. Both $GABA_A$ and $GABA_B$ receptors were inhibited by 100 μM picrotoxin and 2 μM CGP 55845, respectively. The depression of fEPSP elicited during taurine and SKF 89976A perfusion appears to be partially due to activation of GABA receptors because it was significantly reduced in the presence of picrotoxin and CGP 55845 ($88 \pm 3\%$ vs. $73 \pm 6\%$ in the absence of antagonists, $P < 0.05$). By contrast, both antagonists did not affect the induction of LLP-TAU ($147 \pm 7\%$, at 1 h of taurine washout; Fig. 4B, open circles). Under these experimental conditions, SKF 89976A was still able to reduce LLP-TAU induction significantly ($111 \pm 5\%$, at 1 h of taurine washout; $P < 0.01$ vs. experiments without SKF 89976A; Fig. 4B, filled circles). Taurine application, in the presence of GABA antagonists, also induced a long-lasting increase in FV amplitude ($120 \pm 4\%$, at 30 min of taurine washout; $n = 6$; Fig. 4C) as previously reported (Galarreta *et al.*, 1996; del Olmo *et al.*, 2000b, 2003). Such a taurine-induced FV potentiation was completely prevented in the experiments performed in the presence of SKF 89976A ($99 \pm 4\%$, at 30 min of taurine washout; $n = 4$; $P < 0.05$ vs. experiments without SKF 89976A; Fig. 4C).

To determine whether the inhibitory effect of SKF 89976A on LLP-TAU was due to the blockade of GAT-1 transporter, we investigated the induction of LLP-TAU in the presence of 0.1 mM NO-711, a compound inhibiting GAT-1 with an IC_{50} of 0.38 μM (Borden *et al.*, 1994). This GABA transport blocker reduced, but not significantly ($n = 4$; $P > 0.05$), the potentiation induced by taurine ($131 \pm 3\%$, at 1 h of taurine washout; Fig. 4D, filled circles).

We also performed a series of intracellular recordings in CA1 pyramidal layer, to verify that the taurine-induced potentiation of the extracellularly recorded fEPSP and its inhibition by SKF 89976A mirror changes of synaptic potentials in individual neurons, and also to examine whether V_m and R_{in} are modified under our experimental conditions. In the presence of GABA antagonists, 10 mM taurine application enhanced EPSP slope to $145 \pm 10\%$ and $140 \pm 7\%$ at 30 min of taurine perfusion and 20 min of taurine washout, respectively ($n = 5$, $P < 0.01$ compared with basal values; Fig. 5A). This intracellularly recorded LLP-TAU, similar to that recorded with extracellular electrodes, was completely inhibited when taurine was applied in the presence of 0.5 mM SKF 89976A ($98 \pm 10\%$ and $100 \pm 8\%$ at 30 min of taurine perfusion and 20 min of taurine washout, respectively; $n = 5$, $P < 0.01$ compared with their respective values in the absence of the drug). Neither V_m (Fig. 5B, open circles) nor R_{in} (Fig. 5C, open circles) were significantly affected during taurine perfusion or its washout. However, taurine in the presence of SKF 89976A induced a slowly developing hyperpolarization of the resting membrane potential (-70.5 ± 2.3 mV) that stabilized after 20–25 min to average values of 5.8 ± 0.7 mV, and then partially returned back to baseline values during the washout of both substances (Fig. 5B, filled circles). These changes in V_m were not accompanied by significant modifications in R_{in} values (Fig. 5C, filled circles).

These results provide support for the hypothesis that a taurine uptake process, independent of GAT-1 transporters, triggers LLP-TAU induction.

SKF 89976A inhibits L-LTP

The prediction that endogenous taurine might have a role in LTP is based on two facts: first, the enduring phase of both LLP-TAU and L-LTP requires the activation of PKA and protein synthesis; and second, both phenomena occlude mutually (del Olmo *et al.*, 2003). Because we have found that SKF 89976A is able to block LLP-TAU induction (see Figs 4 and 5), such a prediction can be now examined. To test this prediction, we examined in a group of slices ($n = 7$) the magnitude of L-LTP that could be induced in the presence of $GABA_A$ and $GABA_B$ antagonists (picrotoxin and CGP 55845, respectively). We stimulated

two independent pathways (alternately every 15 s) in the stratum radiatum contacting on the same population of cells. One pathway (test pathway) received the tetanization protocol of HFT, which evoked an fEPSP potentiation of $157 \pm 8\%$ of baseline values at 2 h after the trains, whereas the pathway that did not receive HFT (control pathway) did not suffer any significant change during the experiment ($92 \pm 4\%$ at 2 h after trains, Fig. 6A). This L-LTP was similar to that previously obtained in our laboratory in the absence of GABA antagonists (see del Olmo *et al*., 2003, their figure 7).

In another group of slices ($n = 8$) when HFT stimulation was applied in the presence of 0.1 mM SKF 89976A, the potentiation level attained by the individual trains was reduced and the resulting LTP decayed to pretetanization values about 1 h after the last train ($102 \pm 6\%$ and $101 \pm 6\%$, at 1 h and 2 h, respectively; $P > 0.05$ compared with baseline; Fig. 6B). The synaptic responses evoked by control pathway stimulation was not affected during SKF 89976A perfusion ($103 \pm 3\%$ after 40 min of drug perfusion, $P > 0.05$ compared with baseline, Fig. 6B, open circles), indicating that this substance does not affect basal synaptic transmission. Furthermore, SKF 89976A does not appear to affect post-tetanic potentiation because it did not change significantly ($P > 0.05$) the relative potentiation measured during a 3-min period after the trains (Fig. 6, C2). This result indicates that SKF 89976A does not change the amount of released glutamate during high-frequency stimulation

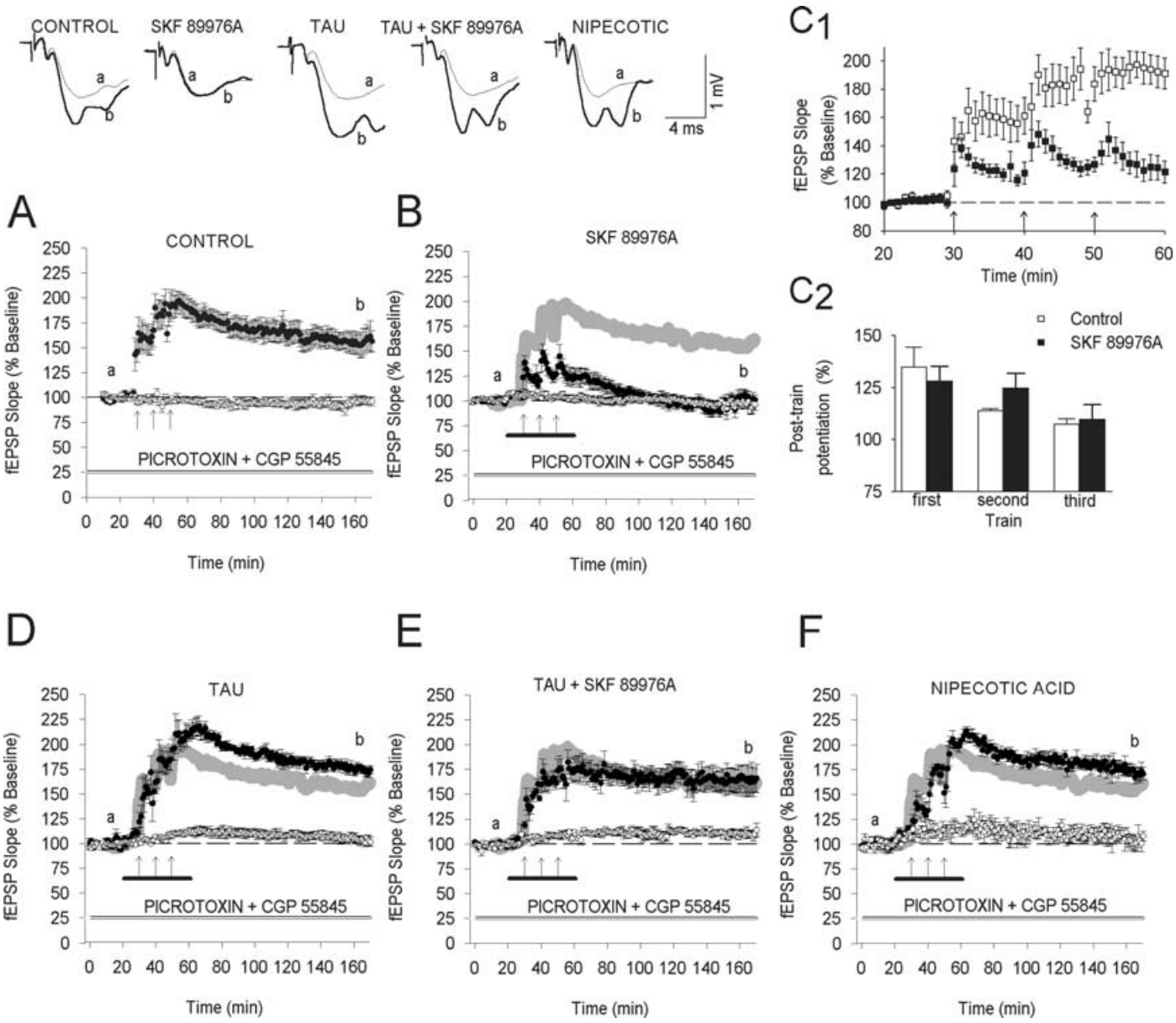


FIG. 6. L-LTP is inhibited by SKF 89976A. In these experiments two independent pathways contacting over the same population of cells were alternately stimulated (open and filled circles represent untetanized and tetanized pathways, respectively). These experiments were performed in the continuous presence of GABA receptor antagonists. (A) Application of three trains of high-frequency stimulation (arrows) induced L-LTP only in the tetanized pathway ($n = 7$). (B) In another group of slices ($n = 8$) the tetanization protocol was applied in the presence of 0.1 mM SKF 89976A (black horizontal bar), which prevented L-LTP induction. The thick grey trace represents the control potentiation depicted in A for comparison. (C_1) The potentiation level induced during the three-trains protocol in control (open squares) and SKF 89976A (filled squares) conditions from the experiments depicted in A and B, respectively. (C_2) The potentiation attained in the first 3-min period after every train that was normalized to the minute previous to every train. (E) The presence of 2 mM taurine overcomes the inhibitory action of 100 μM SKF 89976A on L-LTP induction ($n = 5$). L-LTP was slightly enhanced when the three tetani were applied in the presence of 2 mM taurine (D) or 1 mM nipecotic acid (F).

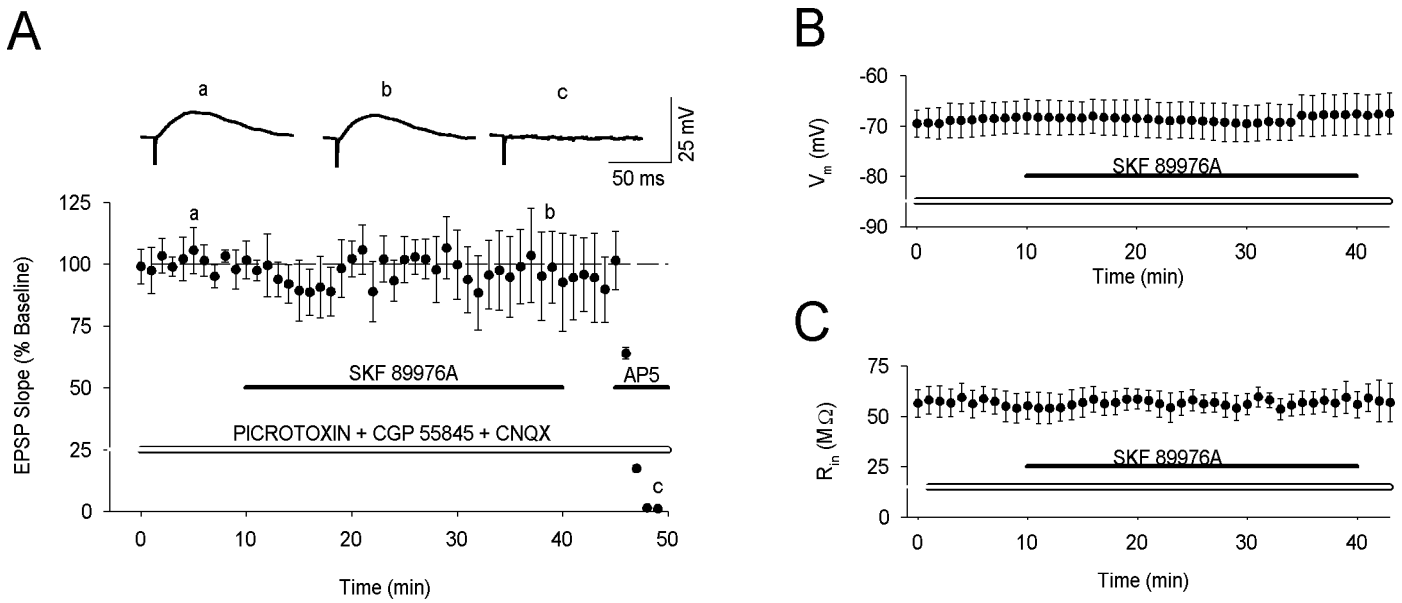


FIG. 7. SKF 89976A (0.1 mM) does not affect the pharmacologically isolated NMDA-EPSP. Intracellular recordings ($n = 5$) in the CA1 pyramidal layer in the continuous presence of GABA receptor antagonists and the AMPA receptor antagonist, CNQX, revealed that SKF 89976A (0.1 mM, 30 min) did not affect significantly either NMDA-EPSP (A), V_m (B) or R_{in} (C). At the end of the experiments, the perfusion of AP5 (50 μM), an NMDA antagonist, completely blocked synaptic responses (A), confirming that they were mediated by NMDA receptor activation.

To establish further that taurine transport is required for L-LTP induction, we tried to displace the blocking effect of SKF 89976A on L-LTP with exogenous taurine. In a first group of slices ($n = 5$), we observed that the application of HFT stimulation in the presence of 2 mM taurine induced a potentiation of 199 ± 3%, 187 ± 3% and 174 ± 4% measured at 30 min, 1 h and 2 h after the last tetanus application, respectively. These were slightly but significantly higher than those obtained in control conditions (166 ± 8%, 159 ± 10% and 157 ± 8%, measured at the same times, $P < 0.05$; Fig. 6D). Moreover, under these experimental conditions, the control independent pathway developed a small nonsignificant potentiation (111 ± 5% vs. 104 ± 5% in the control pathway of control experiment, at 30 min post HFT; $P > 0.05$). In the next group of slices ($n = 5$), we showed that the presence of 2 mM taurine during HFT stimulation overcomes the blocking effect of SKF 89976A on L-LTP induction (170 ± 9%, 162 ± 10% and 164 ± 13% vs. 114 ± 7%, 102 ± 6% and 101 ± 6% in SKF 89976A without taurine, at 30 min, 1 h and 2 h after the last tetanus, respectively; $P < 0.001$; Fig. 6E, filled circles). These results provide support for the idea that the uptake of endogenous taurine is necessary for L-LTP induction.

In another group of slices ($n = 5$), the tetanization protocol was applied in the presence of 1 mM nipecotic acid (Fig. 6F), yielding a potentiation of 186 ± 3% and 170 ± 6% at 1 h and 2 h after the last train, respectively, a result clearly different from that obtained in the presence of SKF 89976A (Fig. 6B). This indicates that GABA transporters do not mediate the effect of SKF 89976A on L-LTP.

Finally, to eliminate the possibility that the impairment of LTP by SKF 89976A was caused by an unknown interaction of this drug with NMDA receptors, whose activation is normally required for LTP induction (Bliss & Collingridge, 1993), we carried out intracellular recordings from pyramidal neurons ($n = 5$; Fig. 7) in which the NMDA component of EPSP was pharmacologically isolated in the presence of the AMPA antagonist CNQX (20 μM), and the GABA antagonists picrotoxin (100 μM) and CGP 55845 (2 μM). Under these experimental conditions, the EPSP-NMDA amplitude (3.8 ± 0.3 mV), resting membrane potential (−68.3 ± 3.3 mV) and cell input resistance (55.4 ± 8.2 MΩ) were not significantly modify during 30 min of SKF 89976A perfusion (4.2 ± 0.7 mV, −69.2 ± 3.5 mV, 57.0 ± 9.0 MΩ, respectively; $P > 0.05$). At the end of the experiments, the NMDA nature of the EPSP was confirmed by completely inhibiting this synaptic potential with AP5 (50 μM), an NMDA antagonist.

Discussion

Based on experimental evidence, it has been suggested that taurine has to be taken up to elicit a long-lasting increase of synaptic efficacy in the hippocampus and striatum (Galarreta *et al*., 1996; Chepkova *et al*., 2002; Sergeeva *et al*., 2003). The investigation of this claim has been hindered by the lack of pharmacological tools to interact with taurine transporters. In the present study we have found a GABA transport antagonist, SKF 89976A, that was also effective at blocking taurine uptake. With this substance we have been able to inhibit the induction of LLP-TAU. In addition, the induction of L-LTP evoked by HFT stimulation was also blocked by SKF 89976A. Such a blockade was displaced by the concomitant application of taurine.

Relevance of taurine transport in LLP-TAU induction

Based on the fact that during the slicing procedure there is a large leakage of taurine from the intracellular compartment (about 70%, Hamberger *et al*., 1982), we proposed (Galarreta *et al*., 1996) that the replenishment of endogenous taurine pools during taurine perfusion, via taurine transporters, could be the basis of LLP-TAU induction.

We can now conclude that intracellular accumulation of taurine is not by itself the factor contributing to the induction of LLP-TAU in the hippocampus, because we have shown that taurine application, under some circumstances, can recover preslicing levels of taurine, without evoking synaptic potentiation. Furthermore, LLP-TAU induction was not affected when taurine was applied in the presence of the taurine transport antagonist GES (1 mM), a result similar to that recently reported by Sergeeva *et al*. (2003). By contrast, taurine application in the striatum, by a process inhibited by the glycine receptor

antagonist strychnine, induces a long-lasting synaptic potentiation that is blocked by 1 mM GES (Chepkova *et al*., 2002). This inhibitory effect could be explained by considering that GES behaves as an antagonist on glycine receptors (Sergeeva *et al*., 2002). Glycine receptor activation does not seem to be required for the induction of LLP-TAU in the hippocampus because taurine application in the continuous presence of 10 μM strychnine elicited a normal LLP-TAU (our unpublished results).

As a consequence, these data are difficult to reconcile with the hypothesis that taurine uptake is required for LLP-TAU induction in the hippocampus. Nevertheless, GES, although competing with taurine for its transporter, does not block the function of taurine transporter. In fact, GES is a transportable substrate of taurine carrier (Huxtable *et al*., 1979) that also induces transport current (Barakat *et al*., 2002), and that at a concentration of 10 mM can evoke synaptic potentiation in the hippocampus (Galarreta *et al*., 1996) and striatum (Chepkova *et al*., 2002).

Taking these properties into account it could be proposed that LLP-TAU generation requires the activity of a carrier transporting taurine but not the intracellular accumulation of this amino acid.

Identity of the transporter involved in hippocampal LLP-TAU induction

Two distinct high-affinity brain taurine transporters, one from rat (TAUT1; Smith *et al*., 1992) and another from mouse (TAUT2; Liu *et al*., 1992), have been cloned. Heller-Stilb *et al*. (2002) have deleted exon 1 of the taurine transporter gene, obtaining knockout mice deficient in both TAUT1 and TAUT2. These mice display very low taurine levels in several brain structures, including hippocampus and striatum, and low ability to elicit LLP-TAU in striatum but not in hippocampus (Sergeeva *et al*., 2003). These data, together with the absence of an effect by the TAUT antagonist GES on hippocampal LLP-TAU (Fig. 1A; Sergeeva *et al*., 2003), strongly indicate that taurine evokes synaptic potentiation in the hippocampus by a process independent of TAUT activation. Sergeeva *et al*. (2003) proposed that a GABA transporter is the taurine uptake system participating in mouse hippocampal LLP-TAU based on: (i) 1 mM nipecotic acid, a blocker of GAT-1, GAT-2 and GAT-3 transporters (IC_{50} values <0.2 mM; Borden *et al*., 1994) inhibited hippocampal LLP-TAU; (ii) taurine can be taken up by GAT-1, GAT-2 and GAT-3 (IC_{50} of 32.3 mM, 1.3 mM and 2.9 mM to inhibit GABA uptake, respectively; Borden *et al*., 1995); and (iii) LLP-TAU was induced by applying 10 mM taurine, a concentration high enough to activate GAT-2 and GAT-3 transporters.

Unlike Sergeeva *et al*. (2003), we could not block the induction of hippocampal LLP-TAU with 1 or 3 mM nipecotic acid when both $GABA_A$ and $GABA_B$ receptors were inhibited. However, when the experiments were carried out in the absence of GABA antagonists, as in Sergeeva *et al*. (2003), the concomitant application of both taurine and 3 mM nipecotic acid induced a decaying LLP-TAU that was partially explained by a nipecotic acid-induced potentiation of unknown origin (see Fig. 1C, filled triangles). Consequently, the most plausible explanation for the discrepancy between both sets of results is that the activation of GABA receptors, by the GABA released via the carrier-mediated release of GABA stimulated by nipecotic acid (Solís & Nicoll, 1992), could have prevented the induction of LLP-TAU in those experiments conducted in the absence of GABA receptor antagonists. Presumably, $GABA_B$ but not $GABA_A$ receptors would be the receptors mediating this inhibitory effect because LLP-TAU induction is not affected by the activation of $GABA_A$ receptors (Galarreta *et al*., 1996). In addition, we cannot exclude that species differences (rat vs. mouse) in transporter structure might account for the disparity of nipecotic acid effect found in both laboratories.

Therefore, our results showing the lack of effect of nipecotic acid on rat hippocampal LLP-TAU is difficult to reconcile with the hypothesis implicating the identified GABA transporters on this type of potentiation.

In view of the reduced levels of taurine found in the hippocampus of TAUT knockout mice (Sergeeva *et al*., 2003), it could be concluded that a taurine transport system other than TAUT does not exist. Alternatively, a deficiency in the taurine supply into the brain caused by the reduction of TAUT at the blood–brain barrier, where this transporter seems to be functionally expressed (Kang *et al*., 2002), could partially explain the low taurine levels in brain structures of TAUT knockout mice. Thus, the existence of an unidentified taurine transport system is still possible. In fact, the accumulation of ^{3}H-taurine in hippocampal neurons does not correspond with the localization of TAUT (Pow *et al*., 2002), indicating that other taurine transport system must be participating in taurine uptake. Low-affinity uptakes of taurine with K_m values in the millimolar range have been reported in several brain structures (Debler & Lajtha, 1987; Hanretta & Lombardini, 1987; Oja & Saransaari, 1996), although it is not known whether they correspond to molecular entities still not identified or to a different kinetic state of the already known taurine transporters. Identified TAUTs present high-affinity kinetics (K_m of 4.5 and 40 μM; Liu *et al*., 1992; Smith *et al*., 1992), which saturate at 1 mM taurine, a concentration that does not evoke LLP-TAU (Fig. 2C and Galarreta *et al*., 1996).

Therefore, LLP-TAU induction is more compatible with the participation of a low-affinity taurine transport system. In this sense, we found in hippocampal synaptosomes that SKF 89976A, a known blocker of GABA transporters, inhibited more potently the low-affinity taurine uptake than did GES, the prototypical TAUT blocker, and it was more effective in inhibiting low-affinity than high-affinity taurine uptake. Interestingly, 0.5 mM SKF 89976A completely blocked both the uptake of 10 mM taurine in synaptosomes and the induction of hippocampal LLP-TAU. The fact that we observed LLP-TAU induction in the presence of NO-711, a selective blocker of GAT-1 transporter, argues strongly against the possibility that SKF 89976A via this type of transporter inhibits LLP-TAU induction.

We present here data showing that taurine (in the presence of GABA antagonists) can induce synaptic potentiation without changing either V_m or R_{in}, as in the previous work (Galarreta *et al*., 1996; del Olmo *et al*., 2000b). This result is not compatible with the existence of an electrogenic transport of taurine underlying LLP-TAU induction. However, this hypothesis could still be feasible considering that if such an electrogenic transporter were located far away in apical dendrites, the intracellular recording in soma may not have been sensitive enough to see changes in basic membrane electrical properties. By contrast, the concomitant application of taurine and SKF 89976A, in the presence of GABA receptor antgonists, induced a membrane hyperpolarization without changing R_{in}. We have not explored the origin of this hyperpolarization, but the possibility that this membrane potential change could have affected negatively LLP-TAU induction appears unlikely because taurine perfusion, in the absence of $GABA_A$ antagonists, evoked a hyperpolarization of similar magnitude (del Olmo *et al*., 2000a) and LLP-TAU (Galarreta *et al*., 1996).

Taking these results and the above considerations, we propose the idea that the process of taurine uptake via an unidentified transport system, displaying low affinity for taurine, is an essential step to induce LLP-TAU.

The role of taurine in the induction of LTP

Taurine does not appear to affect either the basic electrical properties of the neurons (Galarreta *et al*., 1996) or the binding of glutamate to NMDA and non-NMDA receptors (Pullan *et al*., 1987; Chen *et al*., 2001). However, taurine application, by calcium-dependent mechanisms (del Olmo *et al*., 2000b, c), induces a perdurable potentiation of synaptic efficacy requiring PKA activation and protein synthesis, a process that is also accompanied by cAMP response element binding protein phosphorylation (del Olmo *et al*., 2003). This signalling pathway is similar to that involved in L-LTP (Bourtchuladze *et al*., 1994; Impey *et al*., 1996). In fact, synaptic potentiation induced by HFT stimulation and that by taurine occlude one another (del Olmo *et al*., 2003), which provides support for the assumption that taurine might bypass the requirement for tetanic stimulation to trigger the downstream signalling cascade leading to L-LTP.

We have now shown that L-LTP induction was prevented when tetanization was applied in the presence of SKF 89976A (Fig. 6B). This inhibitory effect was not mimicked by nipecotic acid, that as with SKF 89976A is an inhibitor of GABA transport, indicating that GABA transporters do not play an essential role in L-LTP induction. We have experimentally excluded the possibility that this effect of SKF 89976A could be due to changes in V_m, R_{in} or to interactions with NMDA, $GABA_A$ or $GABA_B$ receptors. The possibility that SKF 89976A could inhibit L-LTP by interfering with any crucial step of its signalling mechanisms is not easy to exclude. However, our result showing that the concomitant application of taurine overcomes SKF 89976A block of LTP induction supports the view that taurine transport is an essential step participating in some point of the signalling cascade leading to L-LTP.

The physiological activation of glutamate receptors could stimulate taurine release from a neuronal or glial compartment to the interstitial space, where it might be taken up to a different cellular compartment from where it was initially released. This hypothetical scenario is partially supported by a number of studies showing that various agonists of glutamate receptors massively stimulate taurine release (Menéndez *et al*., 1989, 1993; Koyama *et al*., 1994; Oja & Saransaari, 2000). However, it is as yet unknown whether activation of glutamate receptors during tetanization induces taurine release.

The extracellular concentration of taurine implicated in the induction of L-LTP must be much less than that needed to evoke LLP-TAU, because it was inhibited by 0.1 mM SKF 89976A, a concentration that was ineffective at inhibiting LLP-TAU evoked by 10 mM taurine (data not shown). Moreover, the blocking effect of SKF 89976A on L-LTP was displaced by 2 mM taurine, a concentration that only induced minor changes in basal synaptic transmission. In view of these data, it could be argued that taurine at a low millimolar range, although insufficient to trigger a persistent potentiation of synaptic transmission, could be mediating or modulating LTP induced by tetanization. Such a concentration of taurine might be reached under physiological conditions, taking into account that the basal extracellular concentration of taurine has been estimated at 20 μM in the rat hippocampus by using microdialysis (Lerma *et al*., 1986), and that the activation of glutamate receptors of NMDA type *in vivo* (Menéndez *et al*., 1989) and in hippocampal slices (Menéndez *et al*., 1993) increases extracellular taurine levels more than six-fold. In addition, this figure probably represents a fraction of the actual concentration of taurine attained where it is released, considering the small volume of the interstitial space and the existence of uptake systems.

The precise molecular targets of taurine that govern LTP induction still need to be identified. In fact, we cannot rule out completely the possibility that taurine uptake into a neuronal or glial compartment might induce the release of another molecule that finally potentiates synaptic transmission. An intriguing possibility is that taurine could be regulating the phosphorylation state of key mediators of LTP generation. Some evidence for this proposal comes from the experiments showing that taurine inhibits the phosphorylation of specific proteins catalysed by protein kinase C in cortical synaptosomes (Li & Lombardini, 1991), stimulates tyrosine phosphorylation of extracellular signal-regulated protein kinase 2 in osteoblasts (Park *et al*., 2001) and enhances cAMP response element binding protein phosphorylation in hippocampal slices (del Olmo *et al*., 2003).

In summary, with the help of a new taurine uptake inhibitor, we have presented evidence that taurine uptake, mediated by a taurine transport system, is required for the induction of LTP. This, in turn, highlights the physiological relevance of this amino acid in the mechanisms of synaptic plasticity.

Acknowledgements

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Abbreviations

AP5, D,L-2-amino-5-phosphopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione disodium; EGTA, ethylene glycol bis-(b-aminoethyl ether) N,N,N′,N′-tetraacetic acid; fEPSP, field excitatory postsynaptic potential; FV, fibre volley; GAT, GABA transporters; GES, 2-guanidinoethanesulphonic acid; HFT, trains of high frequency stimulation; KRB, Krebs–Ringer bicarbonate; LLP-TAU, long-lasting potentiation induced by taurine; LTP, long-term potentiation; L-LTP, late phase of long-term potentiation; PKA, cAMP-dependent protein kinase; R_{in}, apparent input resistance; TAUT, taurine transporter; V_m, resting membrane potential.

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