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Does adenosine release taurine in the A1-receptor-rich hippocampus?

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Abstract
Purpose. To determine whether taurine would be protective in the hippocampus (rich in A1 receptors but devoid of the A2 receptor effector adenylate cyclase, which mediates the release of taurine) by determining whether taurine is released by adenosine in the non-ischemic hippocampus.
Methods. Microdialysis of probes, inserted stereotactically into the bilateral hippocampi of five nonischemic brains of anesthetized rabbits and combined with high-performance liquid chromatography, was used to evaluate the effects of adenosine on taurine concentration in the interstitial fluid.
Results. Sequential increase of the dialytic artificial cerebrospinal fluid concentration of adenosine (to 1×10^{-4} M, 5×10^{-4} M, 1×10^{-3} M, 5×10^{-3} M, and 1×10^{-2} M) linearly and significantly ($P < 0.0001$) increased taurine concentration in the interstitial fluid.
Conclusion. Adenosine delivered by microdialysis into the interstitial fluid of nonischemic rabbit hippocampus induces release of taurine in a dose-dependent manner, suggesting involvement of additional mechanisms besides the already known A2-receptor-induced release. Taurine can be anticipated to have protective effects in the hippocampus as well.

Key words: Taurine release, A1-rich hippocampus, Adenosine

Introduction

Adenosine is released by ischemia, hypoxia, and K^+-induced depolarization [1]. The flooding of the interstitial fluid of all areas of the ischemic or concussive brain, regardless of whether it is rich in adenosine A1 or A2 receptors, with potentially harmful (glutamate) as well as protective (GABA and taurine) amino acids [2–5] has been attributed mainly to the shift of amino acids from intra- to extracellular compartments secondary to membrane depolarization or permeability changes [6].

Protective effects of adenosine in the CNS have been ascribed mostly to G-protein-coupled cell-surface A1 receptors and include mainly inhibition of the ischemia-induced release of excitatory amino acids, decrease of the Ca^2 influx, and increased conductance of K^+ and Cl^- channels, leading to membrane hyperpolarization [7–9].

Taurine (2-aminoethane sulfonic acid, chemically and metabolically stable), an inhibitory and protective β-amino acid, is released by the activation of β-receptors and A2 receptors via intracellular accumulation of cyclic AMP [10–12] as well as by activation of receptors for NMDA, quinolinic acid or kainate, kappa-opiate, and serotonin [13].

Radioligand studies have shown the hippocampus to be an area rich in A1 receptors [7,14,15] but reportedly to be lacking in adenosine-sensitive adenylate cyclase, the effector enzyme of A2 receptors [16], which could place it at risk of not benefiting from the taurine-mediated protection induced by adenosine.

Knowing whether taurine would be released by adenosine delivered directly in the nonischemic hippocampus may have practical implications for developing protective strategies for the CNS in general, including the vulnerable hippocampus.

Materials and methods

Experiments were performed on five Japanese white rabits (weight, 2.5–3.0 kg). After induction of anesthesia with 5% isoflurane, anesthesia was maintained with 1.5% isoflurane in a gas mixture of 65–70% N_2O and 25–35% O_2 via tracheotomy. The animals were kept paralyzed with pancuronium ($0.1 \cdot mg \cdot h^{-1}$). Ventilation was adjusted to obtain an end-expiratory CO_2 concentration of 4.6–5% by continuous monitoring of end–tidal O_2

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and CO_2 (Respina, Sanei IH26, Tokyo, Japan). The rectal temperature was maintained at 38 ± 0.5°C by means of an automatically controlled heating water coil jacket. The arterial blood pressure was continuously recorded from the descending thoracic aorta or the femoral artery.

The skull was exposed after mounting the animal on the stereotaxic apparatus (Narishige, Tokyo, Japan), and two burr holes on the right and left were made at the appropriate locations for insertion of two hippocampal dialyzer probes, one in each side of a single animal, according to the coordinates given in the atlas of Girgis and Chang [17]: posterior, 3.5mm; lateral, 4.5mm; height or depth, 4.5mm relative to the bregma or dura mater.

The basic technique of microdialysis has been reported elsewhere [18]. After implantation of the probe, artificial CSF perfusate having the composition ($mEq \cdot l^{-1}$) Na^+ 146, K^+ 4, Cl^- 110, Ca^{2+} 3, Mg^{2+} 1.3, HCO_3^- 15, lactate$^-$ 28, and ($mg \cdot l^{-1}$) glucose 650 was perfused at the rate of $1 \mu l \cdot min^{-1}$ and collected for periods of 22min in a tube containing 10μl of HCl pH 2.0 using a refrigerated automatic fraction collector. Starting with the fifth collection period, i.e., once steady-state levels were observed, three collection periods were used as basal control. Then the adenosine concentration of the artificial CSF perfusate was changed sequentially to 1×10^{-4}M, 5×10^{-4}M, 1×10^{-3}M, 5×10^{-3}M, and 1×10^{-2}M each for three collection periods, the first period being discarded from computation. The NaCl of the artificial CSF was then substituted with one containing KCl ($100 mmol \cdot l^{-1}$) for two collection periods, the second period being used for computation, followed by three periods with the basic artificial CSF fluid perfusion to corroborate return to baseline levels.

Taurine was measured after derivatization [19] by high-performance liquid chromatography (HPLC). The mobile phase was pumped at the rate of $1 ml \cdot min^{-1}$, its composition being adjusted to be able to measure taurine and GABA in the same run of each collection period (methanol 28–30% in 0.1M pH 6.0 NaH_2PO_4/$NaHPO_4$ buffer, with EDTA added for a final concentration of 1×10^{-5}M). An auto-sampling injector (Model 231–401, Gilson Medical, Villiers le Bel, France) and HPLC with an MA-ODS column coupled to an electrochemical detector (EICOM, Kyoto, Japan), potential set at +750mV against an Ag/AgCl reference electrode, completed the system. The taurine assay (internal control) detection limit was 1×10^{-13}mol.

The responses to the varying concentrations of adenosine were normalized to the baseline concentration for each animal and expressed as mean ± percentile change of the recovered analyte collected during a 22-min period. For statistical analysis (Stat View Ver 4 software, Abacus Concepts, Berkeley, CA, USA), the paired Student's t-test was used, and $P < 0.05$ was considered significant. The dose dependency was assessed by regression correlation analysis. Only data from probes with no substantial difference in the relative recovery values before and after their use (8–12%) were analyzed. Taurine data from seven hippocampi of five animals were usable.

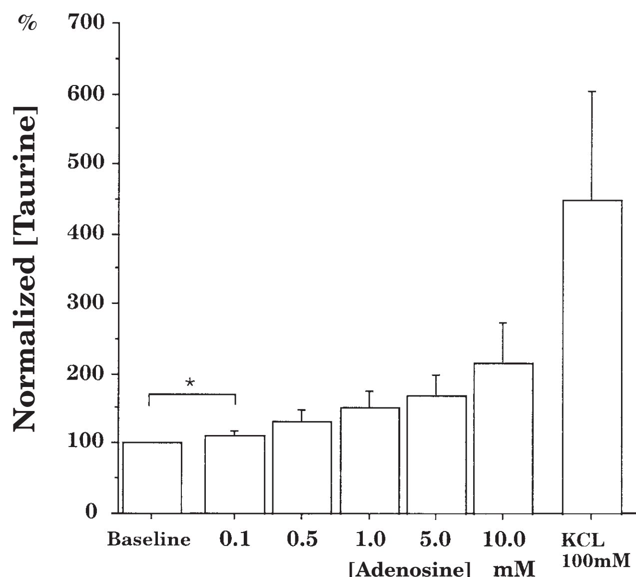

Fig. 1. Normalized [taurine] changes. Increasing concentrations of adenosine in the perfused CSF significantly increaseed (*P = 0.001) the [taurine] in the collected dialyzate even at the lowest concentration. Depolarization by perfusion of the dialytic probe with CSF solution containing 100mM of K^+ induced a far greater release of taurine into the interstitial space than adenosine

Results

Adenosine induced a significant and dose-dependent linear increase of taurine release in the hippocampus perfused with normally oxygenated blood through all studied concentrations, the linearity being similar for the small and the high concentrations. Depolarization by KCl induced almost a doubling of the response elicited by CSF containing 10mM adenosine (Figs. 1 and 2).

Discussion

Recognizing the limitations of using adenosine instead of specific A1 or A2 agonists, because studies with simultaneous delivery of adenosine antagonists, adenosine transport inhibitors, or adenosine deaminase inhibitors have not been completed, and because of

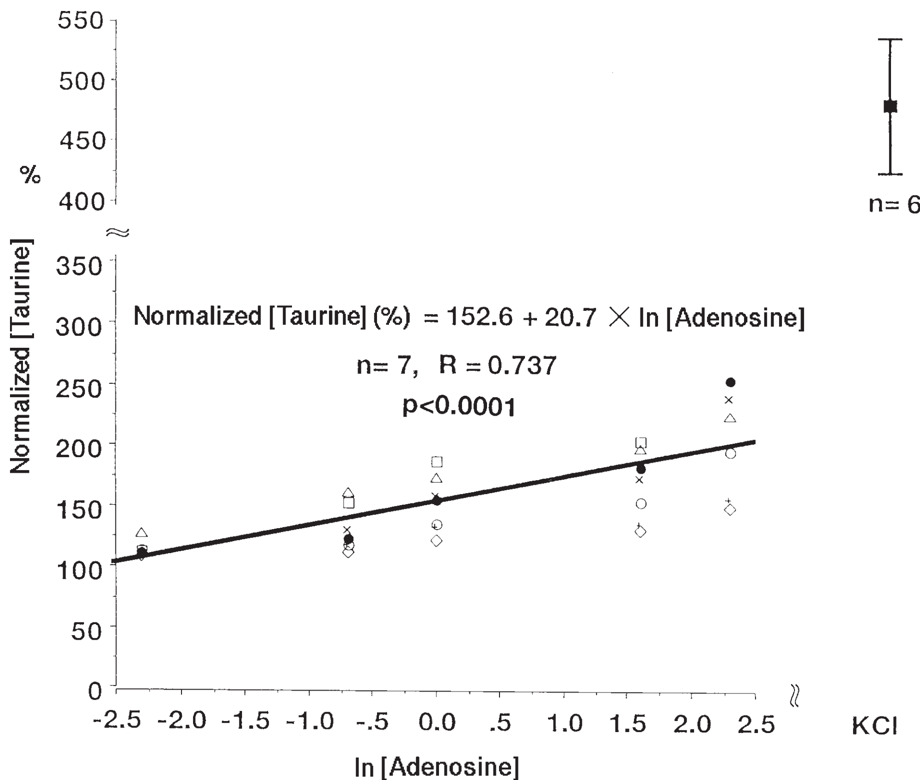

Fig. 2. Dose-response curve of the [taurine] changes in the non-ischemic hippocampus of in vivo preparations. Linearity of the dose dependency is apparent ($P < 0.0001$) when changes normalized to the basal level (100) of taurine (*vertical axis*) are regressed against the increasing concentrations of adenosine in the dialytic perfusate, expressed in logarithmic scale (*horizontal axis*)

the inability to perform radioisotope tracing, the study does not permit us to identify accurately the specific mechanism(s), i.e., metabolic or receptor mediated (and if so, which).

Nevertheless, the following observations can be made: Taurine release, reportedly mediated by β-receptors and adenosine A2 receptors via intracellular accumulation of cyclic AMP in glial cell cultures [10–13], was induced by adenosine in the in vivo hippocampus, which has been reported to be an area without adenosine-sensitive adenylate cyclase [16]. The similar dose-dependent response within ranges of adenosine for both A1 and A2 receptors. Taken together, these observations suggest the possible existence of another mechanism not mediated by surface A2 receptors or cyclic AMP, in agreement with the documented release of taurine induced by A1 agonists as well, though of less magnitude than A2 agonists, by an undetermined mechanism not involving cAMP [11,20].

K^+ depolarization evoked a considerably greater release of taurine than that induced by adenosine. It might reflect the shift of the high intracellular concentration of taurine by the loss of the membrane retention function caused by the depolarization, similar to the ischemic shift [6]. The fact that K^+ (depolarization) and adenosine (hyperpolarization) have opposite effects on the membrane potential and the different extents of release suggest the operation of two different mechanisms.

Taurine increases Cl^- permeability and hyperpolarizes, like adenosine, when applied directly to neurons [20,21]. Taurine and its precursor homotaurine (3-aminopropane sulfonic acid) are amino acids known to have marked calcium-modulating, anti-epileptic, and antioxidant properties [20,22–25], and hence protective effects.

Two hypothesis can be postulated:

a) Marked inhibitory effects from activation of the intracellular P site of the G protein have been reported [26,27], although the mechanism and what is involved are unknown. Whether adenosine acts directly or via these P sites on the influx β-transporting mechanism, on the efflux L-system for taurine [28–30], or other cellular membrane mechanisms is yet to be elucidated.

b) On the one hand, the hippocampus has been reported by Prémont et al. [16] not to have adenosine-sensitive adenylate cyclase and yet to be equally active in taking up labeled adenosine as areas of the brain expressing adenosine-sensitive adenylate cyclase. On the other hand, the capacity of the enzyme *S*-adenosyl-homocysteinase to sequestrate large amounts of adenosine has been reported by Newman [31]. The linearity of the dose-dependent response is compatible with the metabolic hypothesis: the adenosine delivered into the interstitial space is taken up by cells and bound by the *S*-adenosyl-homocysteinase for recycling to *S*-

adenosyl-methionine or to conversion to cysteine [20,25,32,33] and eventually to taurine.

The study does not answer the question whether taurine was formed de novo or whether preexisting taurine was released from glial or neuronal cells, but regardless of the mechanism(s), and whether or not surface A2 receptors are involved, our data seem to suggest an intracellularly governed phenomenon and offer the view that taurine-mediated protective mechanisms induced by adenosine start to operate before adenosine is transported or leaked out of the cell, hence not requiring cell-surface adenosine A2 receptors to explain the occurrence in all CNS areas and other tissues, whether rich in A1 or A2 receptors. It raises the corollary that the administration of exogenous taurine should be protective for all CNS areas, including the hippocampus and other organs, by mimicking or even amplifying many of the adenosine protective actions without the severe hypotension that systemic administration of sufficient dosages of adenosine would induce, which has been corroborated in preliminary studies with a spinal cord ischemia model.

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