Abstract  Following a delay of 45 min, stimulation of the CD95 (Fas/Apo-1)-receptor in Jurkat T-lymphocytes leads to the release of the osmolyte taurine, an event coinciding with apoptotic cell shrinkage. The present study has been performed to elucidate the cellular mechanisms involved in CD95-induced taurine release as compared to swelling-induced taurine release, and to explore whether taurine modifies apoptotic DNA fragmentation and cell shrinkage. Taurine release stimulated by osmotic cell swelling is insensitive to the tyrosine kinase inhibitor herbimycin A and the caspase inhibitor z-Val-Ala-Asp(OMe)-fluoromethylketone (zVAD) but is blunted in the absence of extracellular Ca²⁺. Conversely, the Ca²⁺ ionophore ionomycin stimulates taurine release. However, the taurine release following CD95 stimulation is not paralleled by an increase of cytosolic Ca²⁺ and not inhibited by complexation of extracellular Ca²⁺. None of herbimycin A, the phosphatase inhibitor vanadate, spingomyelinase or Lck⁵⁶ deficiency prevent CD95-induced taurine release. In contrast, the caspase inhibitor zVAD, but not the caspase inhibitor Ac-Tyr-Val-Ala-Asp-chloromethylketone (YVAD), almost abolishes CD95-induced taurine release. Both caspase inhibitors blunt CD95-induced cell shrinkage and DNA fragmentation, zVAD being more effective than YVAD. Preloading of the cells with 40 mM taurine but not with 40 mM mannitol significantly inhibits CD95-induced DNA fragmentation (by 28%) and apoptotic cell shrinkage (by 25%). In conclusion, CD95-receptor triggering leads to caspase-dependent stimulation of cellular taurine release, which facilitates, but is not sufficient for, the triggering of apoptotic DNA fragmentation and cell shrinkage.

Key words  Apoptosis · Caspases · Cell death · DNA fragmentation · Osmolytes

Introduction  Stimulation of the CD95-receptor leads to apoptosis of Jurkat T-lymphocytes [29], an event paralleled by cell shrinkage [13, 30]. The apoptotic cell volume decrease results in part from cellular loss of Na⁺ and K⁺ ions [1, 3, 4]. Moreover, CD95-receptor stimulation has been shown to be paralleled by activation of the cell volume regulatory Cl⁻ channels ORCC [25]. Cell shrinkage is considered to be important for induction of cell death [1, 3, 4, 19, 22] even though moderate osmotic cell shrinkage may interfere with receptor-mediated apoptosis by negative feedback [10].

Recently, we have shown that stimulation of the CD95-receptor leads to release of taurine [16]. Since taurine serves as an organic osmolyte in a variety of cells [15, 23, 24] including lymphocytes [9], its release is expected at least to contribute to apoptotic cell shrinkage. Indeed following stimulation of the CD95-receptor, taurine release immediately precedes apoptotic cell shrinkage and DNA fragmentation [16]. The taurine release is not due to non-specific leakage through the cell membrane, since the amino acid leucine is not released following CD95 triggering [16]. In contrast to the almost immediate activation of the Cl⁻ channel ORCC [28], taurine release occurs with a delay of some 45–60 min. Several Cl⁻ channel inhibitors (NPPB, glibenclamide, AZT, tamoxifen) failed to inhibit CD95-induced taurine release [16]. However, CD95-induced taurine release and DNA fragmentation were blunted by lowering the temperature to 23°C. In a similar fashion to CD95-stimulated Jurkat cells, cerebellar granule neurons release taurine prior to apoptotic cell death [20].

The present study has been performed to elucidate the cellular mechanisms involved in CD95-induced taurine release and to explore whether taurine contributes to the survival or death of CD95-stimulated Jurkat cells. Be-
sides testing for the effect of inhibitors of anion channels, cation channels and the permeability transition pore, we tested for the roles of Ca\textsuperscript{2+}, caspases, tyrosine kinases and sphingomyelinase in the stimulation of taurine release. For comparison, the effects of tyrosine kinases, Ca\textsuperscript{2+} and caspases on swelling-induced taurine release have been tested. The results show that only the caspase inhibitor z-Val-Ala-Asp(OMe)-fluoromethylketone (zVAD) prevented CD95-induced taurine release. The role of taurine in CD95-induced cell death has been studied by loading the cells with taurine prior to CD95-receptor triggering, which indeed proved to reduce CD95-induced DNA fragmentation.

**Materials and methods**

Studies were performed on human leukemic Jurkat T-lymphocytes (ATCC, Rockville, Md., USA), Lck\textsuperscript{56}-deficient JCaM1.6 cells (ATCC), JCaM1.6 cells retransfected with Lck\textsuperscript{56} (a gift from Dr. A. Weiss, University of California, San Francisco, Calif., USA), EBV-infected B-cells deficient of acid sphingomyelinase (ASM–) (derived from B-cells of a patient with Niemann-Pick-Disease type 1) as well as ASM-deficient B-cells retransfected with ASM. All cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 10 mM HEPES, pH 7.4, 2 mM L-glutamine, 1 mM sodium pyruvate, 1% non-essential amino acids, 100 units/ml penicillin, 100 µg/ml streptomycin, and 50 µM β-mercaptoethanol. All experiments were performed at 37°C.

Prior to loading, the cells were washed with HEPES-buffered saline. To load the cells with \textsuperscript{[3}H\textsuperscript{}]taurine, 37 kBq/ml (1 µCi/ml) experiments were performed at 37°C. For determination of DNA fragmentation the cells were fixed by adding 50% (v/v) ethanol and subsequently incubated for 30 min at room temperature, washed in phosphate-buffered saline, exposed to 50 µg/ml RNase A (Sigma) for 30 min and then to 5 µg/ml propidium iodide (Sigma) for 20 min. Propidium iodide staining was then determined utilizing FACS analysis. To identify any influence of intracellular taurine on DNA fragmentation, cells were exposed for 64 h to 40 mmol/l taurine prior to stimulation of the CD95-receptor. For comparison, cells were exposed to 40 mmol/l mannitol.

FACS analysis (FACS Calibur, Becton Dickinson) of [Ca\textsuperscript{2+}]; in single cells was performed after loading the cells at 37°C with 4.5 µM fura-red-AM for 30 min and 0.5 µM fluo-3-AM for 10 min in the presence of 0.015% pluronic acid (Molecular Probes, Eugene, Ore., USA). Excitation was performed with an argon laser and emission was acquired as FL-1/FL-3 at 20°C. Calibration was performed for each experimental condition using 30 M ionomycin and varying external Ca\textsuperscript{2+} concentrations. Ca\textsuperscript{2+} concentrations were approximated as described elsewhere [21].

Data are expressed as arithmetic means ±SEM and statistical analysis was made by paired or unpaired t-test, where appropriate.

![Fig. 1 Effect of CD95(Fas/Apo-1)-receptor stimulation on taurine uptake in Jurkat cells exposed to isotonic extracellular fluid (top) or hypertonic extracellular fluid (addition of 30 mM NaCl, bottom). Means ±SEM, n=4-40](image)