e.g., pyridoxal-P and the pyruvate in the pyruvoyl coenzymes [15].

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Inhibitory effects of spermine and spermidine on muscle calpain II

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Summary. The muscle enzyme calpain II, in contrast to muscle calpain I, was markedly inhibited by millimolar concentrations of the polyamines spermine and spermidine. These compounds and the calpain inhibitor calpastatin had synergistic inhibitory effects on calpain II. These results suggest that the polyamines may have possible regulatory effects on the in vivo activity of calpain II enzymes.
Key words. Calpain; calcium-activated neutral proteinase; spermine; spermidine.

Polyamines such as spermine and spermidine are the most abundant intracellular organic cations in eucaryotes, and their concentrations, which can be changed by a number of factors [1, 2], may be as high as 10 mM in different cell compartments and at certain phases of the cell cycle [3]. Interest has recently centered on their possible roles as modulators of cellular processes through their effects on intracellular calcium levels [4] and the activities of calcium-dependent enzyme systems [5] and calmodulin [6]. An interesting group of calcium-dependent enzymes is the calpains [7] (calcium-activated neutral proteinases) which are intracellular thiol proteinases containing calmodulin-like sequences [8]. The calpains have been implicated in a number of important intracellular processes, and like the calmodulins, their activities can be inhibited by a number of pharmacological agents [9–11]. Because of the effects of spermine and spermidine on calmodulin, and the fact that calpains are sensitive to effectors of calmodulin, we have investigated the effects of spermine and spermidine on different calpains in order to determine if in vivo calpain activity may be affected by the polyamines.

Materials and methods

Calpain I was prepared from chicken gizzard smooth muscle using Reactive Blue 2-Sepharose Cl-6B chromatography [12], after which active fractions from this column were fractionated on a Pharmacia FPLC MonoQ column in 1 mM dithiothreitol, 1 mM [ethylenebis (oxyethylenenitrilo)] tetraacetic acid, 0.01% NaN_3, 50 mM Tris, pH 8.0. Pure enzyme was eluted at approximately 0.35 M when the column was developed with a 0–0.5 M NaCl gradient. Calpain II was purified from chicken gizzard [13] and from mixed skeletal hamster muscle [14]. Calpastatin was obtained from chicken gizzard smooth muscle, and after heat treatment [9], active fractions were purified to homogeneity on a 5 × 60-cm Superose 12 column of a Pharmacia FPLC system. Assays of calpains were performed at the minimal Ca^{2+} concentrations necessary for maximum activity (5 μM for calpain I and 2.5 mM for calpain II) with purified desmin as the substrate for calpain I [15] and azocasein as the calpain II substrate [16]. In experiments on calpain in the presence of calpastatin, an amount of calpastatin was used in the assays which gave approximately half maximal inhibi-

tion of the calpain[9]. The results reported for the enzyme assays in the presence of the polyamines are the averages of triplicate determinations performed on at least two separate enzyme preparations.

Results

When the activity of chicken gizzard calpain I was measured in the presence of concentrations of the polyamines up to 80 mM, it was found that both spermine and spermidine had very little inhibitory effect on the ability of the enzyme to degrade desmin (fig. 1 A). For calpain II from smooth (fig. 1 B) and striated (results not shown) muscle sources, it was found that the polyamines had much stronger inhibitory effects on this enzyme than they had on calpain I. With calpain II from both muscle sources, spermine displayed a more pronounced inhibitory effect than spermidine. At a concentration of 20 mM spermine, calpain II was inhibited to about 55% of control activity. When chicken gizzard calpain II activity was assayed in the presence of both calpastatin and the polyamines, it was found that the inhibitory effect of the combination of calpastatin and polyamine was much greater than the sum of the individual inhibitory effects (fig. 2), and at 20-mM concentrations, the inhibitory effects of each polyamine were more than doubled in the presence of calpastatin.

Discussion

These studies show that, like calmodulin, the biological activities of calpain II enzymes can be inhibited in the presence of millimolar concentrations of spermine and spermidine. In contrast to the effects of polyamines on calpain II, these compounds had little or no inhibitory effect on calpain I. As these enzymes have identical small subunits[8], the differences in polyamine effects suggest that the polyamines interact with the unique large subunits of the calpains in different fashions. The differential effect of the polyamines on calpain I and II is of interest as it may indicate that the possible regulatory effects of the polyamines on intracellular protein degradation by calpains are exerted on the calpain II enzyme, which cleaves a number of unrelated proteins, rather than on

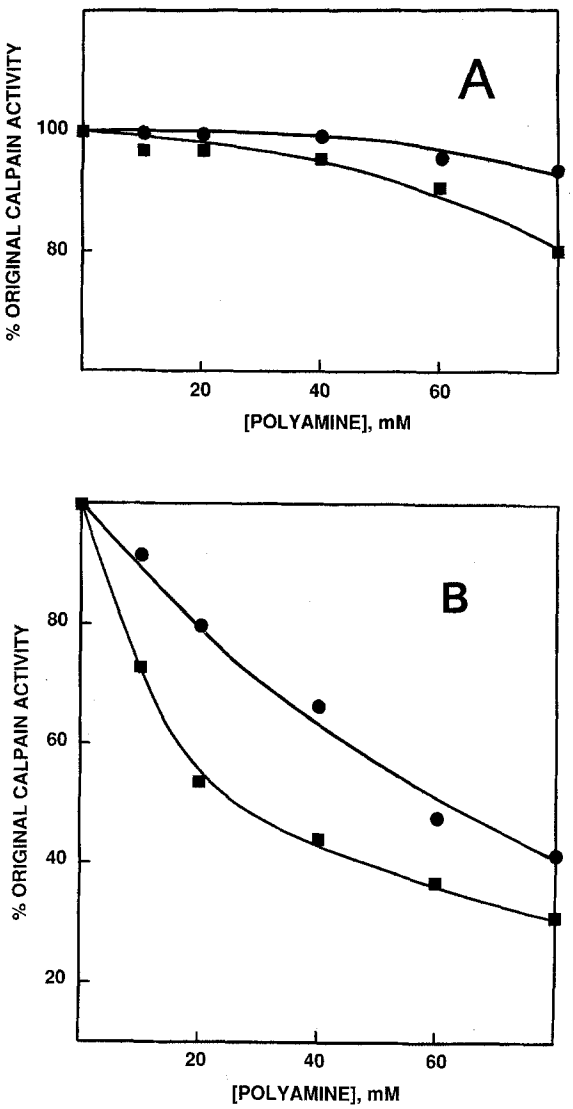

Figure 1. Effects of spermine and spermidine on chicken gizzard calpain I and calpain II activities. In *A*, the activity of calpain I at 5 μM Ca^{2+} is shown in the presence of various concentrations of the polyamines, and in *B*, the enzyme activity of calpain II at 2.5 mM Ca^{2+} is shown. Results are expressed in terms of the level of enzyme activity measured in the absence of polyamines. Spermine (■—■), spermidine (●—●).

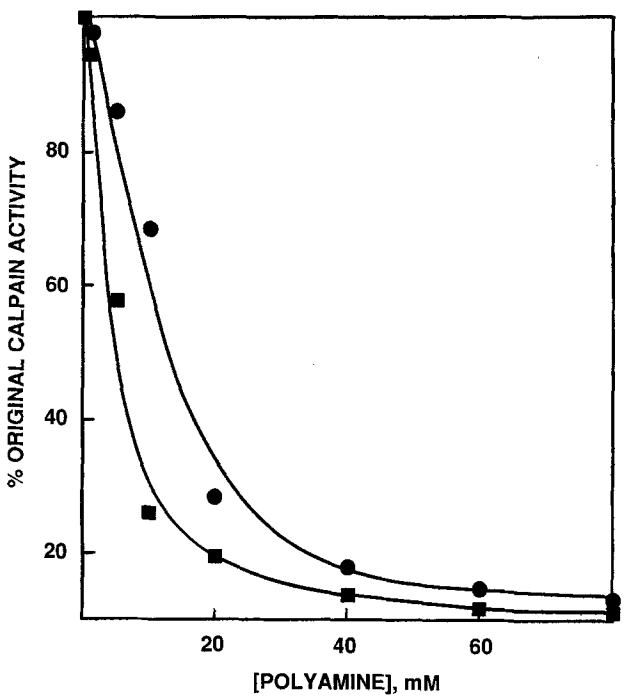

Figure 2. Effects of spermine and spermidine on chicken gizzard calpain II in the presence of chicken gizzard calpastatin. Activities were determined at 2.5 mM Ca^{2+}, and results are expressed as a percentage of the activities of control (no polyamine) samples containing a constant amount of calpastatin which gave half maximal inhibition of the calpain under the assay conditions. Spermine (■—■), spermidine (●—●).

calpain I which has a specificity for intermediate filament proteins[12,15]. For calpain II, the effectiveness of inhibition by the polyamines is less than that reported for calmodulin. Concentrations of spermine required to give a 50% inhibition of calmodulin effects on target enzymes were about 1 mM[6], whereas our studies indicate that for a 50% inhibition of the calpain II enzymes, the spermine concentration needed is about 25 mM. Spermidine is a less effective inhibitor of calpain II than spermine as concentrations of about 50 mM are required for 50% inhibition. The inhibitory effects of the polyamines on calpain II were synergistically increased in the presence of the endogenous calpain inhibitor calpastatin, with the concentration of spermine required for 50% inhibition being lowered to about 6 mM in the presence of calpastatin. It is therefore possible that the in situ inhibitory effectiveness of the polyamines is of physiological importance, particularly in those tissues which have high calpastatin: calpain II ratios[7]. Further work will be needed to identify the regions of the calpain molecule which are involved in polyamine binding, and to explain the reasons for the differences in inhibitory effectiveness of the polyamines on calpains I and II.

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In vivo inactivation of transglutaminase during the acute acrylamide toxic syndrome in the rat

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Summary. The activity of liver and brain transglutaminase is rapidly lost following i.p. injection of acrylamide (50–200 mg/kg). Other enzymes investigated were not modified by the treatment, with the exception of brain enolase.
Key words. Transglutaminase; in vivo inactivation; acrylamide; neurotoxicity.

Professional and experimental exposure to acrylamide leads to severe poisoning, with morphological and functional changes in the central nervous system, the liver and the gonads[1–4]. The nervous alterations have been thoroughly investigated because of their prominence in the clinical picture[5], but their pathogenesis is still unclear. Interference of the chemical with the energy metabolism of the neuron and with axoplasmic transport has been reported[6–8].

Quite recently, we discovered that acrylamide inactivates purified erythrocyte transglutaminase in a calcium-dependent way[9]. Independent data suggested that this enzyme is of critical importance for the release of granules of secretion in the central nervous system and in the endocrine pancreas[10–12]. These considerations led us to investigate the effects of acrylamide on transglutaminase in vivo to evaluate the possibility that the enzyme is involved in the pathogenesis of the toxic syndrome. The data we report here document that the enzyme is effectively inactivated in vivo by treatments which induce acute toxicity, and that its sensitivity to the chemical compares favorably with that of other proteins whose sensitivity to acrylamide was previously described.

Materials and methods

Analytical grade chemicals and enzyme reagents were obtained from Sigma or Boehringer. Acrylamide was purchased from Merck and recrystallized from ethyl acetate before use. Stock solutions were prepared in phosphate buffered saline and the concentration measured spectrophotometrically[13]. Male Wistar rats (b.wt 200–250 g), maintained on standard rat diet with free access to water, were injected i.p. with acrylamide at the specified dosages; control rats received an equivalent amount of vehicle. At the required time, rats were killed by decapitation and the liver and brain were removed and homogenized with a teflon-glass Potter-Elvehjem apparatus in 6