Seminalplasmin

An endogenous calmodulin antagonist

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Seminalplasmin, a strongly basic protein isolated from bull semen, was found to antagonize with high potency and extraordinary specificity the function of calmodulin. Calmodulin antagonism is the result of an interaction between the two proteins, which is mainly determined by electrostatic forces. The stimulation of Ca^{2+}-transporting ATPase and phosphodiesterase by calmodulin was half-maximally inhibited at approx. 0.1 μM-seminalplasmin. However, the basal activity of calmodulin-dependent enzymes was not significantly altered by seminalplasmin over the concentration range investigated.

Seminalplasmin is a small basic protein that has been isolated from bull seminal plasma (Reddy & Bhargava, 1979). The protein was shown to inhibit bacterial and fungal RNA synthesis and therefore growth (Reddy & Bhargava, 1979). Purification to homogeneity by using h.p.l.c. enabled determination of seminalplasmin's amino acid sequence (Theil & Scheit, 1983). Seminalplasmin is composed of 48 amino acids and has an M_r of 6385. Owing to the random distribution of hydrophobic amino acids, seminalplasmin is highly water-soluble and also shows no tendency to self-aggregate in aqueous medium (Theil & Scheit, 1983). It is rich in the amino acids lysine and arginine [11 residues (Theil & Scheit, 1983)], which determine its isoelectric point of 9.8 (Reddy & Bhargava, 1979).

The function of calmodulin, the major Ca^{2+} receptor in eukaryotic cells (Cheung, 1980), can be inhibited by a variety of chemically unrelated substances that share one common feature: they are all cationic amphiphiles (Gietzen et al., 1982). Interaction of antagonists with the anionic amphiphile calmodulin very likely occurs via ionic and hydrophobic interactions (Gietzen et al., 1982). Those substances exhibiting calmodulin antagonism also include basic peptides such as opioid peptides (Sellinger-Barnette & Weiss, 1982; Malencik & Anderson, 1982), hormones (Malencik & Anderson, 1982), and insect venoms (Katoh et al., 1982; Barnette et al., 1983), all of which possess a certain degree of hydrophobicity and net positive

charge at physiological pH. Since seminalplasmin's structural features are similar to those of the above-mentioned peptides, we investigated whether this endogenous protein could also antagonize calmodulin's activity. A preliminary account of this work was presented as a poster at the Spring Meeting of the German Pharmacological Society held at Mainz, March 12–15, 1985 (Gietzen & Galla, 1985).

Materials and methods

Seminalplasmin was isolated as described by Theil & Scheit (1983) and calmodulin by using the method of Kakiuchi et al. (1981). A calmodulin–Sepharose conjugate was prepared by coupling 10 mg of purified calmodulin/g of CNBr-activated Sepharose 4B in accordance with the manufacturer's instructions. Ca^{2+}-transporting ATPase from human erythrocytes and cyclic nucleotide phosphodiesterase from bovine brain were prepared by standard procedures (Gietzen et al., 1982; Gietzen, 1983).

ATPase and phosphodiesterase activities were determined at 37°C by measuring the rate of P_i liberation as reported by Stewart (1974), with slight modifications (Lanzetta et al., 1979). Briefly, phosphodiesterase activity was assayed by coupling the phosphodiesterase reaction with the 5'-nucleotidase reaction and measuring the P_i produced within 30 min (Butcher & Sutherland, 1962; Wang & Desai, 1977). The assay mixture (final vol.

1 ml) consisted of 25 mM-Hepes, pH 7.5, 3 mM-magnesium acetate, 1.2 mM-cyclic AMP and 0.1 mM-$CaCl_2$. The reaction of Ca^{2+}-transporting ATPase was monitored over a period of 10 min. The assay medium for Ca^{2+}-transporting ATPase contained, in a final incubation volume of 1 ml, 30 μg of erythrocyte membrane protein/ml, 25 mM-Mops, pH 7.0, 100 mM-KCl, 0.25 mM-ouabain, 10 mM-NaN_3, 1 mM-ATP, 2 mM-$MgCl_2$ and 36 μM-Ca^{2+} [as a 0.4 mM-Ca^{2+}/Mg^{2+}/EDTA buffer (Wolf, 1973)]. Ca^{2+}-free controls contained 0.4 mM-Mg^{2+}/EGTA instead of the Ca^{2+}/Mg^{2+}/EDTA buffer. 'Ca^{2+}-transporting ATPase activity' refers to the difference in activity obtained in the presence and absence of Ca^{2+}. Before the reaction was started with the respective substrate (ATP, cyclic AMP), enzymes were first preincubated at 37°C for 10 min with an inhibitor and additionally for 10 min in the presence or absence of calmodulin.

Results and discussion

The stimulation of brain phosphodiesterase and erythrocyte Ca^{2+}-transporting ATPase by calmodulin and antagonism of the activation by seminalplasmin is demonstrated in Figs. 1(a) and 1(b). In the absence of seminalplasmin, as documented by the points on the ordinates, phosphodiesterase and Ca^{2+}-transporting ATPase could be maximally stimulated by calmodulin 10-fold or 5-fold respectively above the basal enzyme activity ('basal' activity of a enzyme was defined as that activity determined in the absence of added calmodulin). As can be seen, seminalplasmin specifically antagonized the calmodulin-induced stimulation of both phosphodiesterase (Fig. 1a) and Ca^{2+}-transporting ATPase with an I_{50} value (concentration producing 50% inhibition) of approx. 0.1 μM. However, the basal activities of these enzymes were not significantly inhibited over the investigated concentration ranges.

The inhibitory actions of seminalplasmin on the calmodulin-sensitive enzymes phosphodiesterase and Ca^{2+}-transporting ATPase were not caused by Ca^{2+} chelation, since increasing the concentration of Ca^{2+} had no influence on the effect of seminalplasmin (results not shown). By contrast, the inhibition of Ca^{2+}-transporting ATPase provoked by seminalplasmin could be completely reversed by increasing concentrations of calmodulin (Fig. 2). The inhibitory effect of 0.15 μM-seminalplasmin was more pronounced as compared with 0.04 μM-seminalplasmin, as can be seen from the parallel-shifted dose–effect curves (ATP ase activity versus calmodulin concentration), and consequently higher concentrations of calmo-
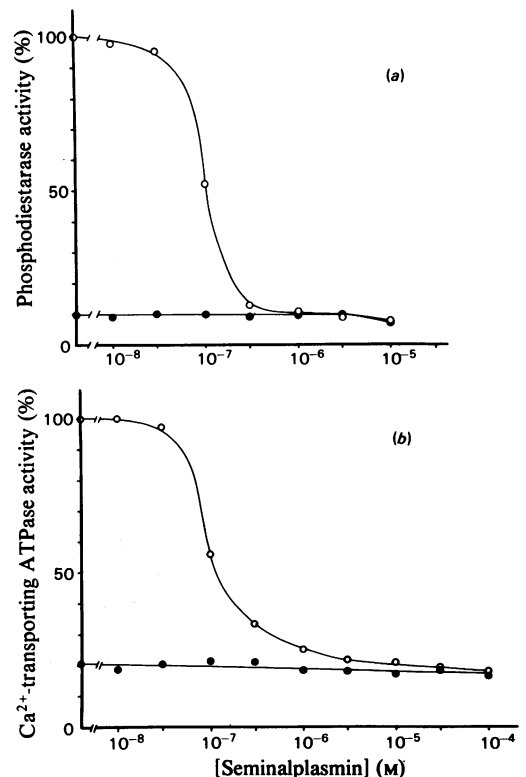

Fig. 1. *Effects of seminalplasmin on phosphodiesterase (a) and Ca^{2+}-transporting ATPase (b)*

Basal calmodulin-independent (●) and calmodulin (30 nM)-activated (○) enzyme activities were determined in the absence and presence of various concentrations of seminalplasmin. ATPase (100% activity = 80 nmol/min per mg of protein and phosphodiesterase (100% activity = 1 μmol/min per mg of protein) activities are related to the calmodulin-stimulated enzymes in the absence of inhibitor. Each point represents the mean of six determinations.

dulin were required to antagonize the effects of 0.15 μM- than of 0.04 μM-seminalplasmin.

Further evidence for the involvement of calmodulin in the inhibition of calmodulin-stimulated enzymes by seminalplasmin was obtained from binding studies with a calmodulin–Sepharose conjugate. Seminalplasmin strongly interacts with calmodulin, since binding occurs not only in the presence of Ca^{2+}, but also in its absence (results not shown). This is in contrast with other calmodulin antagonists that bind to calmodulin in a Ca^{2+}-dependent manner (Gietzen et al., 1982; Sellinger-Barnette & Weiss, 1982; Malencik & Anderson, 1982) and whose interaction with calmodulin is dependent on a Ca^{2+}-induced exposure of a hydrophobic site on calmodulin (LaPorte et al.,
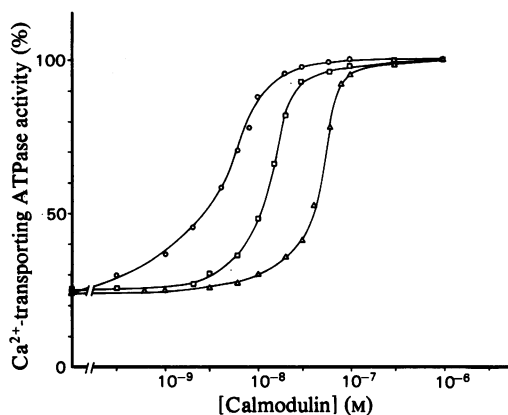
Fig. 2. *Effect of seminalplasmin on calmodulin-induced stimulation of Ca^{2+}-transporting ATPase*
The increase in ATPase activity induced by various concentrations of calmodulin was determined in the absence of seminalplasmin (○) or in the presence of 0.04 µM-(□) and 0.15 µM-(△) seminalplasmin. Points on the ordinate represent the basal Ca^{2+}-transport ATPase activity. Each point is the mean of four determinations.

Table 1. *Inhibition of calmodulin-sensitive Ca^{2+}-transporting ATPase by various compounds*
The influence of various compounds on erythrocyte Ca^{2+}-transporting ATPase activity was determined in the absence (basal activity) and presence (activated) of 30 nM-calmodulin. The I$_{50}$ value is the concentration of agent producing 50% inhibition of either the basal or the calmodulin-stimulated ATPase. Note that the concentrations of compound 48/80 are given as µg/ml, as indicated by *, which corresponds to µM when assuming an average M_r of 1000 for the mixture of constituents of which compound 48/80 is composed. Abbreviation used: W-7, N-(6-amino-hexyl)-5-chloro-1-naphthalenesulphonamide.

	I$_{50}$ (µM; *µg/ml)	
Compound	Calmodulin-activated ATPase	Basal ATPase
W-7	42	1100
Calmidazolium	0.35	
Melittin	0.1	70
Compound 48/80	0.85*	>700*
Seminalplasmin	0.1	>100

1980), Ca^{2+}-independent binding of seminalplasmin to calmodulin and elution of seminalplasmin from the calmodulin–Sepharose column with high-ionic-strength (500 mM-NaCl) buffer (results not shown) indicates that the interaction is predominantly of electrostatic nature. Thus the binding of seminalplasmin to calmodulin provides a molecular basis for its inhibitory actions on calmodulin-stimulated enzymes.

Table 1 summarizes the effect of several outstanding calmodulin antagonists that were tested under identical experimental conditions on both basal and calmodulin-activated Ca^{2+}-transporting ATPase. It is evident that seminalplasmin and melittin are extremely potent antagonists of Ca^{2+}-transporting ATPase's activation by calmodulin. So far only mastoparan has been shown to be more potent when directly compared with melittin (Barnette *et al.*, 1983). When calculating the ratio of the I$_{50}$ value for basal ATPase to the I$_{50}$ value for calmodulin-dependent ATPase activity, a measure is obtained of the specificity of a substance to inhibit the calmodulin-induced stimulation (Gietzen, 1983). For seminalplasmin a ratio of more than 1000 may be calculated, which means that this substance is more specific by at least a factor of 1000 in antagonizing the calmodulin-dependent as opposed to the basal fraction of Ca^{2+}-transporting ATPase activity. Thus seminalplasmin is even more specific in this respect when compared with melittin, and at least as specific with respect to compound 48/80 (a condensation

product of N-methyl-p-methoxyphenethylamine with formaldehyde).

Although it has been produced that seminalplasmin might act as an antimicrobial agent in seminal fluid (Reddy & Bhargava, 1979), its physiological role remains to be clearly established. Considering the high potency and exceptional specificity of seminalplasmin as a calmodulin antagonist, together with its relatively high concentrations (≥25 µM) in seminal plasma, the question arises as to whether the physiological role of seminalplasmin is related to that of calmodulin.

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