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KALLIKREIN ACTIVATION OF A HIGH MOLECULAR WEIGHT ATRIAL PEPTIDE

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SUMMARY: Mammalian atrial extracts contain bioactive peptides that exert profound effects upon renal function and isolated smooth muscle preparations. Gel filtration chromatography of rat atrial extract separates the activity into two peaks having apparent molecular weights of 20,000 to 30,000 and less than 10,000. Mild proteolyic treatment (trypsin 1 U/ml) of the high molecular weight fraction enhances the smooth muscle relaxant activity of this fraction and concomitantly reduces the apparent molecular weight of this fraction to less than 10,000. In this report we show that urinary and submaxillary kallikrein enhances the activity of rat atrial extracts in a similar fashion. Pretreatment of the high molecular weight fraction with either kallikrein (1 μg/ml) enhances the smooth muscle relaxant activity of this fraction. Similar treatment of the low molecular weight fraction had no effect. The enhancement of the bioactivity of the high molecular weight substance(s) by the kallikreins was abolished by aprotinin but was unaffected by soybean trypsin inhibitor. These results suggest that exogenous addition of tissue kallikrein activates a high molecular weight peptide by limited proteolysis. Analysis of the kallikrein-treated high molecular weight peptide fraction by gel filtration indicates that the biological activity comigrates with the low molecular weight peptides present in the original atrial extract.

Cardiac myocytes of the atria but not of the ventricles possess granules typical of protein secretory cells (1). The granularity of the atria is enhanced by water deprivation and sodium deficiency (2). Associated with the atrial granules are bioactive peptides that are natriuretic, diuretic, and spasmolytic (3,4). Gel filtration chromatography of atrial extracts showed the bioactivity to have apparent molecular weights of 20,000 to 30,000 and less than 10,000 (4). Recently, we and others have reported the amino acid sequence of several bioactive peptides from rat (5,6) and human (7) atrial extracts, ranging from 21 to 28 residues in length. We have named the two low molecular weight peptides that we have purified and characterized, atriopeptin

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I and II (5). The fact that the high and low molecular weight fractions possessed similar biological activity suggested to us that both might be structurally related. Indeed, mild proteolytic digestion with trypsin of the high molecular weight substance enhanced its biological activity and resulted in the appearance of substances which co-migrated with the low molecular weight peptides (8). We have termed the less active high molecular weight substance atriopeptigen (5). Since the structure(s) of the peptide(s) that are finally secreted by atrial myocytes is unknown at present, we have considered the possibility that the components of the high as well as low molecular weight fraction might be found in blood. Further processing of the high molecular weight components possibly takes place at sites other than the heart. Since the atrial peptides have such profound effects upon renal salt and water excretion we examined whether tissue kallikreins, endogenous serine proteases involved in the regulation of these renal functions (9), might enhance the bioactivity of the atrial peptides.

MATERIALS AND METHODS

Preparation of atrial extract. Six hundred frozen rat atria (Biotrol, Indianapolis, IN) were homogenized in 10 volumes of phosphate buffered saline containing phenylmethysulfonyl fluoride and pepstatin (Sigma) (each 1 μg/ml) and centrifuged (500 x g 10 min). The supernatant was boiled for 10 min, then chilled and centrifuged (10,000 x g 10 min). This supernatant was made 0.5 M acetic acid and centrifuged again (27,000 x g 10 min). The acid supernatant (in 300 ml aliquots) then was applied to a Sephadex G-15 column (8x36 cm) and eluted with 0.5 M acetic acid (600 ml/hr). The protein fraction from this column was lyophilized, taken up in 0.5 M acetic acid, and 40 ml aliquots applied to a Sephadex G-75 column (5x90 cm) eluted with 0.5 M acetic acid (96 ml/hr). The high molecular weight fraction was collected between 40 and 55 ml and the low molecular weight fraction between 72 and 90 ml.

Smooth Muscle Bioassay. This technique was performed as previously described (4). Spiral strips of rabbit thoracic aorta and chick rectum were continuously superfused at 10 ml/min with oxygenated Krebs-Henseleit medium (37°). In order to detect relaxant substances a resting tone was induced by infusing norepinephrine (aorta) or cabachol (chick rectum) at 2 x 10^{-8} M.

Preparation of Urinary and Submaxillary Kallikrein. These enzymes were purified to homogeneity by ammonium sulfate fractionation, DEAE-cellulose, CM-cellulose, Sephadex G-100 and aprotinin affinity chromatography as described previously (10,11). Enzyme activities were 75 α-N-tosyl-L-arginine methyl ester (TosArgOMe) esterase units/mg of protein (10,11).

RESULTS

Smooth muscle relaxant activity of rat atrial extract was measured using isolated rabbit aorta and chick rectum bioassay strips. The dose-response curves (Figs 1 and 2) for smooth muscle relaxation of the high molecular

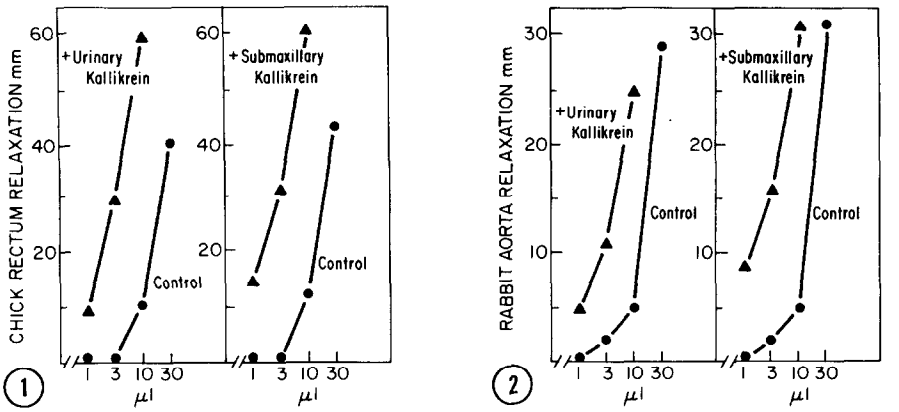


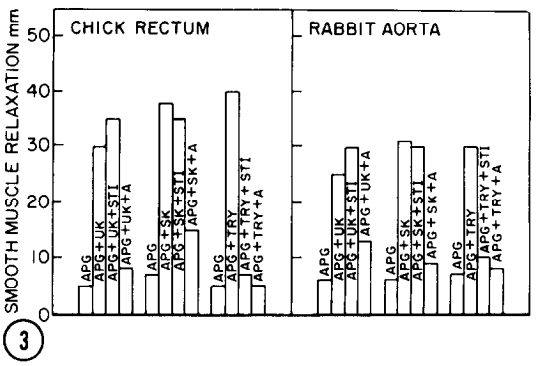
Figure 1. Quantitative comparison of the chick rectum relaxant response of atriopeptigen activated by tissue kallikreins treatment. Atriopeptigen was incubated either in the presence (Δ) or absence (O) of the kallikreins (1 μg/ml for 60 min at 24°C). Following the incubation period various doses (1-30 μl) of atriopeptigen was tested upon the assay tissues. The experiment was repeated 3 times with similar results obtained.

Figure 2. Quantitative comparison of the rabbit aorta relaxant response of atriopeptigen activated by tissue kallikreins treatment. The Protocol for this experiment is as indicated in the legend of Fig. 1.

weight fraction (atriopeptigen) show that the activity of this fraction is increased by treatment with either rat urinary or submaxillary kallikrein at 1 μg/ml for 60 min at 25°C. The kallikreins had no effect upon low molecular weight (atriopeptin) biological activity (data now shown). The kallikreins also did not have direct effects on rabbit aorta and chick rectum smooth muscle tone.

The activation of the atriopeptigen by the tissue kallikreins was blocked only by aprotinin, whereas trypsin activation was suppressed by both soybean trypsin inhibitor and aprotinin (Fig. 3). These results are consistent with the specificity pattern of inhibition of the proteolytic action of tissue kallikreins (9).

Previously, we have shown that trypsin activation of atriopeptigen is associated with the appearance of substances that resemble the atriopeptins on gel filtration (8). Analysis of the kallikrein-treated atriopeptigen by gel filtration (Sephadex G-75) shows the kallikrein activation is similar in this respect (Fig. 4). In this experiment, a preparation of atriopeptigen (derived from 200 rat atria) was equally divided and was incubated at 24°C either in



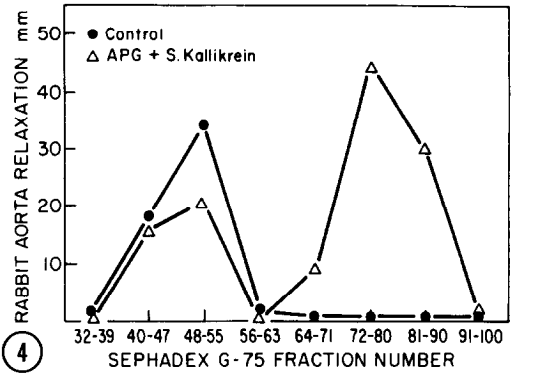


Figure 3. Effect of protease inhibitors on the activation of atriopeptigen by kallikrein and trypsin digestion. Atriopeptigen was incubated with either kallikreins (1 μg/ml) or trypsin (1 u/ml) for 60 min at 24°C. The protease inhibitors aprotinin and soybean trypsin inhibitor in 10-fold excess were preincubated with the proteases for 30 min at 37°C in PBS, pH 7.2. The experiment was repeated 2 times with similar results obtained. Abbreviations employed: APG-atriopeptigen, UK-urinary kallikrein, SK-submaxillary kallikrein, Try-trypsin, STI-soybean trypsin inhibitor, A-aprotinin.

Figure 4. The analysis of atriopeptigen by Sephadex G-75 chromatography after kallikrein activation. A preparation of atriopeptigen (derived from 200 rat hearts) was equally divided and incubated for 60 min at 24°C either in the presence (Δ) or absence (O) of submaxillary kallikrein (1 μg/ml). The atriopeptigen (±kallikrein) was subjected to gel filtration chromatography and the column fractions analyzed by bioassay. Abbreviations employed: APG-atriopeptigen, S. Kallikrein-submaxillary kallikrein.

the presence or absence of submaxillary kallikrein (1 μg/ml). The control and kallikrein-treated atriopeptigen were passed through a Sephadex G-75 column and the biological activity monitored by bioassay. The control incubation shows a single high molecular weight (20,000-30,000) peak of vasorelaxant activity; however, following kallikrein-treatment the high molecular weight peak is reduced and a large low-molecular weight (<10,000) peak of vasorelaxant activity appears. Similar results were obtained when the chick rectum was utilized as the bioassay (data not shown).

DISCUSSION

The results of the present study strongly suggest that the high molecular weight biologically active fraction of atrial extracts (atriopeptigen) may be processed by tissue kallikreins in a similar manner as it is by our trypsin model system (8). Activation of biological activity (spasmolytic activity) by the tissue kallikreins is accompanied by the production of low molecular

weight bioactive species and is blocked by aprotinin but insensitive to trypsin inhibitor.

Tissue kallikreins have been shown to be involved in the regulation of salt and water excretion (9). These serine proteases are localized in plasma, salivary ducts, and the late distal nephron and early collecting duct (12). Micropuncture studies indicate that these segments are also possible sites of action for the atrial peptides (13). The kallikreins apparently are situated proximal to the sites of action of the atrial peptides and might possibly be involved in the processing of atriopeptigen by the distal nephron. Kallikrein catalyzed cleavage may also take place in submaxillary gland, which recently has been suggested to contain atrial peptides (14). Kallikreins have been shown to convert kininogen to kinin (9), proinsulin to insulin (15) and prorenin to renin (16). The physiological signficance of the involvement of tissue kallikreins in the metabolism of atrial peptides will have to await the elucidation of the nature of products directly secreted from atrial myocytes in vivo.

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REFERENCES

1. Jamieson, J.D., and Palade, G.E. (1964) J. Cell Biol. 23, 151-172.
2. deBold, A.J. (1979) Proc. Soc. Exp. Biol. Med. 161, 508-511.
3. deBold, A.J., Borensetin, H.B., Veress, A.T., and Sonnenberg, H. (1981) Life Sci. 28, 89-94.
4. Currie, M.G., Geller, D.M., Cole, B.R., Boylan, J.G., Wu, Y., Holmberg, S.W., and Needleman, P. (1983) Science 221, 71-73.
5. Currie, M.G., Geller, D.M., Cole, B.R., Siegel, N.R., Fok, K.F., Adams, S.P., Eubanks, S.R., Galluppi, G.R. and Needleman, P. (1984) Science 223, 67-69.
6. Flynn, T.G., deBold, M.L., and deBold, A.J. (1983) Biochem. Biophys. Res. Comm. 117, 859-865.
7. Kangawa, K., and Matsuo, H. (1984) Biochem. Biophys. Res. Comm. 118, 131-139.
8. Currie, M.G., Geller, D.M., Cole, B.R., and Needleman, P. Proc. Natl. Acad. Sci. U.S.A., in press.
9. Margolius, H.S. (1984) Ann. Rev. Physiol. 46, 309-326.

10. Chao, J. and Margolius, H.S. (1979) Biochem. Pharm. 28, 2071-2079.
11. Chao, J., Woodley, C., Chao, L., and Margolius, H.S. (1983) J. Biol. Chem. 258, 15173-15178.
12. Tomita, K., Hitoshi, E. and Fuminori, S. (1981) Pflugers Arch. 389, 91-95.
13. Briggs, J.P., Steipe, B., Schubert, G. and Schnermann, J. (1982) Pflugers Arch 395, 271-276.
14. Cantin, M., Gutkowska, J., Milne, R.W., Thibault, G., Garcia, R., and Genest, J. (1983, Sept.) Paper presented at the Council for High Blood Pressure Research 37th Annual Fall Conference and Scientific Sessions.
15. Ole-Moi Yoi, O., Seldin, D.C., Spragg, J., Pinkus, G.S., Austen, K.F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 3612-3616.
16. Sealy, J.E., Atlas, S.A., Laragh, J.H., Ozas, N.B., and Ryann, J.W. (1978) Nature 275, 144-145.