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EFFECT OF ELASTIN PEPTIDES ON HUMAN MONOCYTES: Ca^{2+} MOBILIZATION, STIMULATION OF RESPIRATORY BURST AND ENZYME SECRETION

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The effect of elastin peptides (Kappa-elastin) was investigated on human monocytes. The data presented here indicate that elastin peptides increase the intracellular Ca^{2+} level measured by Quin 2 fluorescence and mediate the release of β-glucuronidase and elastase. The O_2 consumption and H_2O_2 release were stimulated in a dose-dependent manner. The early rise of cAMP was followed by a return to the original level at 30 min and by a concomittant increase of cGMP level. The action of elastin peptides on intracellular calcium level and cGMP levels may well be related to its previously demonstrated chemotactic activity. These activities may well play a role in the modifications of the extracellular matrix following elastin degradation as observed in atherosclerosis, emphysema and aging.

The degradation of elastin by elastase type enzymes was shown to play an important role in several pathological processes (1-9). All these enzymes although of different nature (10-12) are able to hydrolyse elastin. The released peptides were shown to have a variety of biological properties (13-16) and among them a chemotactic effect on monocytes and fibroblasts (17).

The chemotactic peptide receptors were shown to be coupled to the phosphoinositide specific phospholipase C, through a guanine nucleotide regulatory protein (18). This receptor activation involves hydrolysis of phosphoinositides followed by generation of inositol triphosphate and diacylglycerol. The inositol triphosphate is believed to induce the release of Ca^{2+} from an intracellular storage and as a consequence the level of intracellular free Ca^{2+} is increased (19). The chemotactic effect, the release of enzymes, the production of free radicals, the modulation of cyclic nucleotides involved in cell activation are dependent on the increase of cytosolic Ca^{2+} (20-22).

Recently, we have demonstrated (23) that elastin peptides stimulate Ca^{2+} uptake and inhibit Ca^{2+} extrusion by monocytes, skin fibroblasts and arterial smooth muscle cells, acting inversly as formyl peptides (ex:

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N-Formyl-Methionyl-Leucyl-Phenylalanine (FMLP)).
Therefore, we investigated the effects of elastin peptides on cytosolic free Ca^{2+} level,on production of biologically active oxygen species (BAOS), on enzyme release and on intracellular cyclic nucleotides concentration .

MATERIALS AND METHODS

PATIENTS: Monocytes were obtained from 20 middle-aged healthy male subjects (age: 25-52 years) after informed consent. The selection of the subjects was based on the following criteria: good physical and mental health confirmed by clinical, radiological and biological examinations.

MONOCYTES were separated by Ficoll-Hypaque gradient centrifugation according to Kumagai et al (24) without modifications (25).

KAPPA ELASTIN (Elastin peptides) was prepared from bovine ligamentum nuchae elastin by alkaline hydrolysis in ethanol (26).

EXPERIMENTAL CONDITIONS: All incubations were performed in a CO_2 incubator (CO_2 5%, air 95%, humidity 95% at 37°C) and the cells were prepared for all measurements by suspending them in phosphate buffered saline. Final densities were 2.5×10^6 cells/ml for βglucuronidase determination and 10^7 cells/ml for elastase determination. For these determinations, cells were preincubated for 30 min with 10 ng/ml cytochalasin B (Calbiochem) following by 60 min incubation with various concentrations of elastin peptides. After incubation, cells were centrifuged and enzyme activity determined in the medium.

βGLUCURONIDASE ACTIVITY was determined according to Brittinger et al (27) using phenolphtalein glucuronide (Serva) as substrate. The released phenolphtalein was determined spectrophotometrically after 18 hours incubation.

ELASTASE ACTIVITY DETERMINATION was carried out according to the method of Hornebeck et al (28) with slight modifications. Briefly, to 1,0 ml of the 1/4 dilution of culture supernatant, 20 µl of N-Succinyl-trialanine-paranitroanilide in dimethylsulfoxide (5 mg/ml) were added and further diluted in 0, 1M Tris-HCl, pH 8,6. After 15 hours incubation, the change in absorbance at 410 nm was determinated (29).

LACTATE DEHYDROGENASE ASSAY was carried out according to the method of Dioguardi et al (30).

O_2 CONSUMPTION was measured with Clark type oxygen electrode in 3 ml cell suspension containing 5×10^6 monocytes under constant stirring at 37°C. The preincubation time for equilibration with air was 30 min. The measurement was carried out according to Tanabe et al (31).

H_2O_2 PRODUCTION was determined according to the method of Pick and Keisari (32) based on the H_2O_2 mediated and horseradish peroxidase dependent oxydation of phenol red. For these determinations, 2×10^6 monocytes/ml were used in the presence of various concentrations of elastin peptides after 30 min of incubation.

DETERMINATION OF cAMP and cGMP LEVELS: This was carried out before and after the addition of 1 µg/ml of kappa-elastin at 0, 15, 30, 60, 120 min of incubation. For the cyclic nucleotides determinations, the cells were prepared as described by Stabinsky et al (33). The determinations were made according to the instructions enclosed in the radioimmunoassay kit (Amersham).

MEASUREMENT OF CYTOSOLIC FREE Ca^{2+} CONCENTRATION BY QUIN 2: Fluorescence of Quin

2-loaded cells was measured by the method of Tsien et al (34-35). Monocytes (10^7 cells per ml) incubated for 20 min at 37°C with Quin 2 acetoxymethylester (50 µM) in RPMI, then diluted ten-fold with buffer and incubated for an additional 60 min. The cells were centrifuged at 1000 g for 5 min and suspended in Hank's buffer at a concentration of 5×10^6 cells/ml. One ml of this cell suspension was centrifuged at 5000 g in a microcentrifuge and resuspended with 2,0 ml of buffer which contains or not 1 mM $CaCl_2$ and then transfered to a 1 cm cuvette. Fluorescence intensities (F) were measured in a Hitachi fluorescence spectrophotometer using an excitation

wavelength of 339 nm and an emission wavelength of 492 nm. The total Quin 2 present in the cells was measured by the addition of digitonin (Serva). Fmin is obtained by setting $(Ca^{2+})_i$ to 1nM by adding 2mM EGTA and Tris to take the pH above 8,3. The kappa elastin at various concentrations was added and the fluorescence was followed for 8 min. The level of intracellular free Ca^{2+} was computed by the equation $(Ca^{2+})_i = Kd(F-Fmin)/(Fmax-F)$.

RESULTS

Addition of kappa elastin to cells enhanced very rapidly the cytosolic free calcium concentration, measured by Quin 2. The most important increase was obtained by 1 µg/ml of kappa elastin (Fig. 1). The measurement was continued until the level of Ca^{2+} returned to the original value. As the kappa elastin concentration was increased, the recovery of the original Ca^{2+} concentration was delayed. This recovery time was 4 min with 0,5 µg/ml of kappa elastin and 6 min with 1 µg/ml.

We determined also with various concentrations of kappa elastin the β glucuronidase and elastase release from monocytes (Table 1). Kappa elastin stimulated the release of both enzymes from monocytes significantly

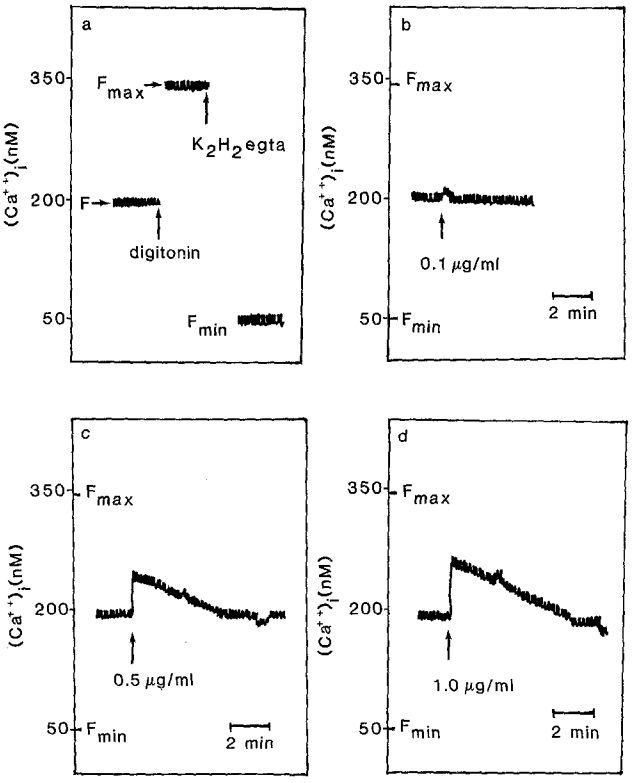

Figure 1
Effect of kappa elastin on cytosolic $(Ca^{2+})_i$ measured by Quin 2 fluorescence.
a) Fluorescence of Quin 2 loaded monocytes. Digitonin (50 ug/ml) is added to determine the maximal calcium dependent fluorescence (Fmax). Egta (2mM) is added to quench the calcium dependent fluorescence (Fmin).
Fluorescence of Quin 2 loaded monocytes following additions of b) 0,1 ug/ml; c) 0,5 ug/ml and d) 1,0 µg/ml of kappa elastin. Measurements performed as described in Materials and methods.

Table 1

Effect of kappa elastin on the β glucuronidase and elastase release from monocytes. Monocytes are preteated for 30mn with cytochalasin B. Each value represent the mean ± SEM of five experiments. Experiments were done in triplicate.

Incubation (60min)	enzymes released into the medium		
	Lactate dehydrogenase % of total activity	β glucuronidase n mol phenolphthaline / 19 hours / 10^6 cells	elastase n mol pNA / 18 hours / 10^7 cells
Cytochalasin B 5 µg / ml	< 5%	non detected	non detected
+ KE 0.1 µg/ml	< 10%	82.4 ± 4.36	12.8 ± 0.58
0.5 µg/ml		75.8 ± 3.25	14.5 ± 0.79
1.0 µg/ml		91.3 ± 4.63	13.6 ± 0.64

already at the lowest concentration tested. The release was significant as compared to control ($p < 0,001$). Kappa elastin even at the lowest concentration tested was already able to mediate the release of these enzymes from monocytes.

The production of H_2O_2 as well as O_2 consumption were also determined (Table 2). The oxygen consumption and H_2O_2 production were markedly stimulated ($p < 0,001$) in a dose dependent manner, maximal stimulation being obtained with 0,5 µg/ml of kappa elastin. Higher concentration did not further stimulate the oxygen metabolism of the cells.

The intracellular concentration of cyclic nucleotides was also determined in monocytes in the presence of 1 µg/ml kappa elastin The cAMP concentration was increased, with a peak at the 15th min, during the first 30 min of incubation. After the 30th min, it returned to the initial level. By contrast, the cGMP level showed a progressive increase with a more marked increase after the 30th min of incubation (Fig. 2).

Table 2

Effect of kappa elastin on the O_2 consumption and H_2O_2 production Each value represent the meen ± SEM of five experiments. Experiments were done in tripicate.

Experimental group	O_2 consumption µ mol / 10min / 10^7 cells	produced H_2O_2 µ mol / 30min / 10^7 cells
control	320 ± 14	217 ± 8
KE 0.1 µg / ml	1680 ± 62	1086 ± 34
0.5 µg / ml	1890 ± 64	1215 ± 32
1.0 µg / ml	1815 ± 48	1137 ± 41

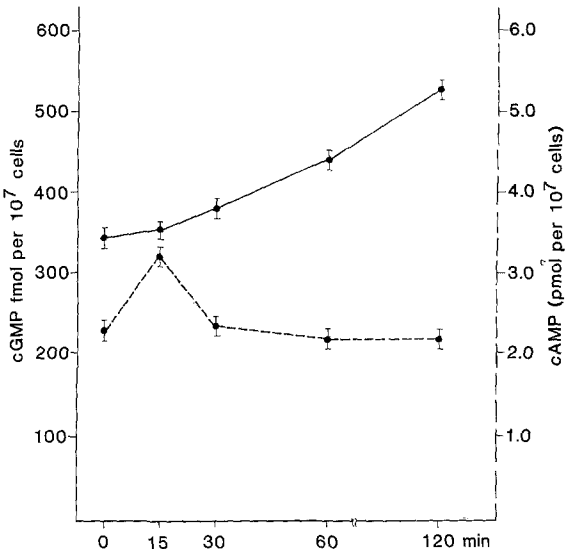

Figure 2
cAMP and cGMP levels in monocytes after the addition of kappa elastin (1 µg/ml). The determinations were carried out at 0, 15, 30, 60 and 120 min of incubation.

—— o —— cGMP -----o------ cAMP

DISCUSSION

Elastin peptides were shown to exhibit several biological activities: induction of immunoarteriosclerosis when injected to rabbits (13-16), chemotactic activity for monocytes and fibroblasts (17), cells which play an important role in the pathogenesis of arteriosclerosis (36-38).

Our results further confirm the chemotactic action of kappa elastin and try to elucidate the intracellular mechanisms. As it was already demonstrated for the chemotactic peptide FMLP, at the level of signal transduction, the major effect is apparently the mobilization of Ca^{2+} (39-40). Kappa elastin also stimulates the increase of cytoplasmic free Ca^{2+} as determined by Quin 2 fluorescence. This effect is due to the enhancing of Ca^{2+} influx as shown before (23), but possibly also to the liberation of Ca^{2+} from intracellular storage sites.This effect could be mediated by the stimulation of the phosphatidylinositol breakdown involving the formation of inositol triphosphate and diacylglycerol, as it is occuring in the case of other chemotactic peptides (41). This suggests that kappa elastin may have a receptor site on the monocyte membrane, coupled positively to a guanine nucleotide regulatory (N) protein. This contention is supported by the stimulation of cGMP formation, although cAMP level is also transiently elevated after stimulation by kappa elastin, as in the case of other chemoattractant receptor stimulation.

The elevation of cytosolic Ca^{2+} has been proposed to regulate the stimulation of phagocytic cells by formyl peptides, ionophore A 23187,...(39,42), and this could well be the case for kappa elastin also. In fact we found that kappa

elastin caused a marked elevation of BAOS production measured by the increase of oxygen consumption, chemiluminescence (data not shown) reflecting the O^{-}_{2} production and H_2O_2 release. This BAOS stimulation was dose dependent. The βglucuronidase and elastase release was also markedly stimulated but in a dose independent manner. This elastase release could further amplify the effect of kappa elastin by inducing the production of new peptides at the site of enzyme release.

These results suggest that the rapid rise of intracellular free Ca^{2+} could play an important role in the cell activation during kappa elastin stimulation of monocytes by the increased production and release of metabolically active oxygen species and lysosomal enzymes. These effects of kappa elastin on monocytes could further explain some of the biological effects obtained during its injection to rabbits (13-16) and suggest that monocytes could play an important role in the biological alterations induced by elastin peptides at sites of elastin degradation in lung tissue (1-3), arterial wall (4-6) or skin (7-9).

REFERENCES

1. Crystal, R.G. (1976) The biochemical basis of pulmonary function, Marcel Dekker Inc., New York/Bâle.
2. Bignon, J., and Robert, L. (1978) Rev. Med. 19, 764-778.
3. Lafuma, C., and Robert, L. (1982) Cellular biology of the lung, pp. 293-309, Plenum Press, NewYork/London.
4. Robert, L., and Robert, A.M. (1980) Frontiers of matrix biology: Biology and pathology of elastic tissues, Vol 6, pp 130-173, S. Karger, Bâle.
5. Robert, L., Chaudière, J., and Jacotot, B. (1984) Regression of atherosclerosis in humans, NATO ASI Series, Vol. 79, pp 145-173, Life Sciences.
6. Robert, L. (1982) The coronary artery, pp. 391-416, Billing and Sons Ltd., Guildford, London.
7. Robert, L. (1980) Précis de physiologie cutanée, pp. 155-173, Edition de la Porte Verte, Paris.
8. Frances, C., and Robert, L. (1984) Int. J. Dermatol. 23, 166-179.
9. Takemori, K., Ikeda, S., Naito, K., and Ogawa, H. (1985) Br. J. Dermatol. 112, 533-538.
10. Bourdillon, M.C., Hornebeck, W., Soleilhac, J.M., and Robert, L. (1984) Biochem. Soc. Trans. 12,876.
11. De Cremoux, H., Hornebeck, W., Jaurand, M.C., Bignon, J., and Robert, L. (1978) J. Pathol. 125, 172-177.
12. Janoff, A. (1972) Amer. J. Pathol. 68, 579-591.
13. Robert, L., Stein, F., Pezess, M.P., and Poullain, N. (1967) Arch. Mal. Coeur 60, 233-241.
14. Robert, L., Robert, B., and Robert, A.M. (1970) Exp. Gerontol. 5, 339-356.
15. Robert, A.M., Grosgogeat, Y., Reverdy, V., Robert, B., and Robert, L. (1971) Atherosclerosis 13, 427-449.
16. Jacob, M.P., Hornebeck, W., Lafuma, C., Bernaudin, J.F., Robert, L., and Godeau, G. (1984) Exp. Mol. Pathol. 41, 171-190.
17. Senior, R.M., Griffin, G.L., and Mecham R.P. (1980) J. Clin. Invest. 66, 859-862.
18. Berridge, M.J., and Irvine, R.F. (1984) Nature 312, 315-321.
19. Reynolds, E.E., and Dubyak, G.R. (1985) Biochem. Biophys. Res. Commun. 130, 627-632.
20. Plescik, M.T., Babich, M., and Rush, M.E. (1983) J. Biol. Chem. 258, 10913-10918.
21. O'Flynn, K., Linch, D.C., and Tatham, P.E. (1984) Biochem J. 219, 661-666.
22. Gilman, A.G. (1984) Cell 36, 577-579.
23. Jacob, M.P., Fülöp, T., Foris, G., and Robert, L. (1986) Proc. Natl. Acad. Sci. USA, submitted for publication.
24. Kumagai, K., Itoh, K., Hinume, S., and Tade, M. (1979) J. Immunol. Methods 29, 17-25.
25. Fülöp, T.Jr., Foris, G., Worum, I., and Ledvey, A. (1984) Int. Arch. Allergy Appl. Immunol. 74, 76-79.24.
26. Kornfeld-Poullain, N., and Robert, L. (1968) Bull. Soc. Chim. Biol. 50, 759-771.
27. Brittinger, G.S., Hirschorn, R., Douglas, S.D., and Weissmann, G. (1968) J. Cell Biol. 37, 394-401.

28. Hornebeck, W., Potazman, J.P., De Cremoux, H., Bellon, G., and Robert, L. (1983) Clin. Physiol. Biochem. 1, 265-292.
29. Fülöp, T. Jr., Komaromi, I., Foris, G., Worum, I., and Leövey, A. (1986) Immunobiology 171, 302-310.
30. Dioguardi, N.L.A., Agostai, A., Fiorelli, G., and Lomante, A. (1963) J. Lab. Clin. Med. 61, 713-720.
31. Tanabe, A., Kobayashi, Y., and Usui, T. (1983) Experientia 39, 604-606.
32. Pick, E., and Keisari, Y. (1980) J. Immunol. Methods 38, 161-170.
33. Stabinsky, Y., Bar-Shavit, Z., Fridkin, M., and Goldman, R. (1980) Mol. Cell. Biochem. 30, 71-77.
34. Tsien, R.Y., Pozzan, T., and Rink, T.J. (1982) Nature 295, 68-71.
35. Tsien, R.Y., Pozzan, T., and Rink, T.J. (1982) FEBS Lett. 148, 21-26.
36. Ross, R. (1986) N. Engl. J. Med. 314, 488-500.
37. Pawlowski, N.A., Abraham, E.L., Pontier, S., Scott, W.A., and Cohn, Z.A. (1985) Proc. Natl. Acad. Sci. USA. 82, 8208-8212.
38. Fülöp, T. Jr., Hauck, M., Kebesy, D., Foris, G., Worum, I., and Leövey, A. (1986) Immunology, submitted for publication.
39. Stickle, D.F., Daniele, R.P., and Holian, A. (1984) J. Cell. Physiol 121, 458-466.
40. Smolen, J.E., Eisenstat, B.A., and Weissmann, G. (1982) Biochim. Biophys. Acta 717, 422-431.
41. Verghese, M.W., Smith, C.D., Charles, L.A., Jakoi, L., and Snyderman, R. (1986) J. Immunol. 137 271-275.
42. Onozaki, K., Takenawa, T., Homma, Y., and Hashimoto, T. (1983) Cell. Immunol. 75, 242-254.