that of the normal subjects and to the Scatchard plots from other patients with insulin resistance (*1-10, 13, 14*). Further analysis of the data indicated that the decrease binding observed in the diabetic subjects was associated with a decreased number of receptor sites per cell as compared to the normal subjects. Erythrocytes from normal subjects had approximately 50 percent more insulin binding sites than those from patients with adult-onset diabetes mellitus, whereas the monocytes from normal subjects reported by Olefsky and Reaven (*7*) also had approximately 50 percent more sites than those from patients with chemical diabetes.

Although the physiological significance of the decrease in receptor number is unclear, it is possible that this decreased number of receptors in the diabetic subjects is in part responsible for the cellular metabolic deficits that enhance the morbidity associated with this insulin resistant state.

In summary, these studies demonstrate that insulin binding to erythrocytes is decreased in patients with adult-onset diabetes. This defect of insulin binding is associated with a decrease in the number of insulin receptor sites per cell. The erythrocyte is a readily available cell for the evaluation of cellular insulin receptor activity. Human erythrocytes may also be useful for studying the actions of other hormones at the cellular level.

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References

1. J. A. Archer, P. Gorden, J. Roth, *J. Clin. Invest.* **55**, 166 (1975).
2. R. S. Bar, P. Gorden, J. Roth, *ibid.* **58**, 1123 (1976).
3. J. M. Olefsky, *ibid.*, **57**, 1165 (1976).
4. ———, *Diabetes* **25**, 1154 (1976).
5. H. Beck-Nielsen, O. Pedersen, J. P. Bagger, N. S. Sorensen, *Acta Endocrinol. (Copenhagen)* **83**, 565 (1977).
6. J. H. Amatruda, J. N. Livingston, D. H. Lockwood, *Science* **188**, 264 (1975).
7. J. M. Olefsky and G. M. Reaven, *Diabetes* **26**, 680 (1977).
8. ———, *J. Clin. Endocrinol. Metab.* **43**, 232 (1976).
9. S. Goldstein, M. Blecher, R. Binder, P. V. Perrino, L. Recant, *Endocrinol. Res. Commun.* **2**, 367 (1975).
10. L. C. Harrison *et al.*, *J. Clin. Endocrinol. Metab.* **44**, 206 (1977).
11. K. K. Gambhir, J. A. Archer, L. Cruz, M. Sanders, in preparation.
12. J. A. Archer, P. Gorden, J. R. Gavin, III, M. A. Lesniak, J. Roth, *J. Clin. Endocrinol.* **36**, 627 (1973).
13. R. S. Bar, W. R. Lewis, M. M. Rechler, L. C. Harrison, C. Siebert, J. Podskalny, J. Roth, M. Muggeo, *N. Engl. J. Med.* **298**, 1164 (1978).
14. C. R. Kahn, J. S. Flier, R. S. Bar, J. A. Archer, P. Gorden, M. M. Martin, J. Roth, *ibid.* **294**, 739 (1976).
15. R. H. Schwartz, A. R. Bianco, B. S. Handwerger, C. R. Kahn, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 474 (1975).
16. A. Thorsson and R. L. Hintz, *N. Engl. J. Med.* **297**, 908 (1977).
17. R. D. Fussganger, C. R. Kahn, J. Roth, P. DeMeyts, *J. Biol. Chem.* **251**, 2761 (1976).
18. J. M. Olefsky, P. Jen, G. M. Reaven, *Diabetes* **23**, 565 (1974).
19. J. R. Gavin, III, J. Roth, P. Jen, P. Freychet, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 747 (1972).
20. M. M. Rechler and J. M. Podskalny, *Diabetes* **25**, 250 (1976).
21. I. D. Goldfine and G. J. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1427 (1976).
22. B. I. Posner, D. Raquidan, Z. Josefsberg, J. J. M. Bergeron, *J. Cell Biol.* **75**, 3302 (1978).
23. K. K. Gambhir, J. A. Archer, C. Bradley, *Diabetes* **27**, 701 (1978).
24. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
25. A. Boyum, *Scand. J. Clin. Invest. (Suppl)* **21**, 7 (1968).
26. K. K. Gambhir, J. A. Archer, L. Carter, *Clin. Chem.* **23**, 1590 (1977).
27. G. E. Moore, E. Ito, K. Ulrich, A. A. Sanderberg, *Cancer* **19**, 713 (1966).

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Angiotensin Convertase Activities in Human Alveolar Macrophages: Effects of Cigarette Smoking and Sarcoidosis

Abstract. *Angiotensin I convertase activity has been found in human alveolar macrophages from normal volunteers and patients with pulmonary sarcoidosis. This activity is higher in the alveolar macrophages from smokers than from nonsmokers, and is even more elevated in sarcoid patients. The activity can be detected with both angiotensin I and bradykinin analogs and appears to require protein synthesis, but the enzyme is not secreted by alveolar macrophages in culture.*

The activity of angiotensin I convertase (ACE) in the serum is increased in patients with the granulomatous disorder, sarcoidosis (*1-5*). The enzyme ACE is well recognized as a vascular endothelial cell ectoenzyme (*6*), and is also present in sarcoid granulomata (*7*). The cell types present in granulomata in general include the tissue macrophage and its close relative, the epithelioid cell (*8*). One type of tissue macrophage, the pulmonary alveolar macrophage (AM) of the rabbit does not normally exhibit ACE activity, but such activity may be induced when the cells are cultured in the presence of $10^{-7}M$ dexamethasone (*9*). We confirmed this observation and obtained similar results with the addition of beclamethasone dipropionate ($10^{-7}M$) to the culture medium (*2*). In the experiments described here, we examined the ACE activities of human AM's from both healthy subjects (cigarette smokers and nonsmokers) and patients with sarcoidosis to determine whether such cells contribute to the increased serum ACE activity in sarcoidosis.

The ACE activities in the cells from the three groups of subjects are shown in Fig. 1. In Fig. 1A, the ACE activity is expressed per milligram of cell protein. On this basis, when compared with the activity in nonsmokers, the ACE activity is increased in the cells from both smokers and sarcoidosis patients ($P < .05$). Although the lavage cells from all three groups contained from 1 to 2 percent polymorphonuclear leukocytes, the cell populations in these groups differed. The cells from smokers were 98 ± 2 percent AM [mean ± 1 standard deviation (S.D.)]. However, in nonsmokers and patients with sarcoidosis, the AM comprised 88 ± 8 percent and 77 ± 10 percent, respectively. The remaining cells were lymphocytes as judged by morphologic examination of cells treated with Wright's stain and the failure of these cells to stain with nonspecific esterase stains. The differential counts by these two methods were in close agreement. Since we could not detect ACE activity in a lymphocyte-rich- (75 percent) human peripheral blood mononuclear cell preparation (separated by Ficoll-Hypaque gradient), we have expressed the ACE activity on the basis of 10^7 AM (Fig. 1B). These data indicate that, compared with AM's from nonsmokers, the ACE activities in AM's from smokers and patients with sarcoidosis are, respectively, three and five times greater ($P < .01$).

To define further the characteristics of the ACE activity in AM's, we used SQ 20881 (H-<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH), a specific inhibitor of angiotensin convertase (*10*). With hippuryl-l-histidyl-l-leucine as substrate, SQ 20881 ($10^{-5}M$) totally inhibited the activity of the macrophage enzyme. A single enzyme acts as a dipeptidyl carboxypeptidase catalyzing both the conversion of angiotensin I to angiotensin II and the hydrolysis of bradykinin (*11, 12*). Since the hippuryl-l-histidyl-l-leucine substrate

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is an angiotensin I analog, its cleavage indicates an ability to convert angiotensin I to active angiotensin II. To determine whether this AM enzyme likewise has features of a more generalized dipeptidyl carboxypeptidase, we measured this activity with *p*-[^{3}H]benzoyl-Phe-Ala-Pro-OH, a carboxy-terminal analog of bradykinin potentiating peptide 5a (*13, 14*). Assays of AM activity with this substrate were performed in 0.05*M* Hepes [4-(2-hydroxyethyl)-1-piperazine ethane sulfonate] buffer, *p*H 8.0, and 0.15*M* NaCl at 37°C for 1 hour. The reaction product was extracted in toluene and counted in Biofluor. Such assays correlated with activities measured with hippuryl-l-histidyl-l-leucine compound and showed > 90 percent inhibition with the relatively specific ACE inhibitor SQ 14225 [1-(D-3-mercapto-2-methyl-3-oxopropyl)-L-proline] at $10^{-5}M$ concentration. These results indicate that the ACE activity in AM's has some features of the peptidyl dipeptidase (kininase II), and is therefore probably a true angiotensin convertase.

Evidence for the synthesis and secretion of ACE was sought with cultures of AM's from 11 smokers and 3 patients with sarcoidosis. First, the intracellular specific ACE activity persisted essentially unchanged for up to 5 days in the cultured cells which were morphologically all macrophages. This persistence suggests, but does not prove, the active synthesis of ACE. Second, in three experiments with AM's from smokers, cycloheximide (15 μg/ml) sharply decreased the cellular ACE content and also enhanced LDH (lactate dehydrogenase) release into the medium. Thus, both cell viability and ACE content depend on protein synthesis. Third, no ACE activity could be detected in the medium in eight experiments in which the percentage of LDH released was ≤ 25 percent. There were detectable amounts of ACE in the media of five cultures in which the percentage of LDH was > 35. Thus, either ACE appears to be a cellular enzyme whose release reflects cell turnover rather than secretion, or the rate of destruction of ACE under these conditions exceeds its secretion rate.

These data establish that ACE activity is present in human AM's, cells present in lung granulomata, including sarcoidosis. Unlike rabbit AM's, human AM's "normally" contain ACE activity. This activity increases five times in AM's lavaged from sarcoid patients. This may be another manifestation of macrophage activation, a characteristic of granulomata in general (*15, 16*) and possibly also in sarcoid granulomata. The increase in ACE activity in the AM's of cigarette smokers is of interest and is also compatible with macrophage activation. Such activation of AM's in the human cigarette smoker is suggested by heightened glucose oxidation (*17*), the increased adherence of these cells, the increased secretion of lysozyme (*18*), and a report of increased elastase secretion (*19*).

We suggest that the increase in serum ACE in sarcoidosis derives from two factors, namely the increased ACE activity of the macrophage and the increased numbers of macrophages present in sarcoidosis, a disease characterized by multisystem granulomata. The failure of smokers to show increased serum ACE activity in the presence of increased macrophage ACE activity is presumably due to two factors, the largely intra-alveolar, as opposed to intrapulmonary, location of the macrophages and the absence, in the "normal" smoker, of systemic granulomata characteristic of sarcoid.

We offer two interpretations. First, we have already suggested that ACE of AM's has features of the peptidyl dipeptidase capable of degrading bradykinin. Second, the accumulation of ACE in the serum of patients with sarcoidosis may reflect the turnover of macrophages in the granuloma. The biologic significance of the ACE in AM's and its enhanced activity in smokers and patients with sarcoid is not yet clear. However, a number of oligopeptides (bradykinin and arginine vasopressin) regulate prostaglandin biosynthesis by releasing the prostaglandin precursor, arachidonic acid, from several cultured cell lines such as fibroblasts (*20*) and renomedullary cells (*21*). Heightened prostaglandin biosynthesis is a well-recognized feature of immunologically activated macrophages (*22*). The possible role of ACE in regulating macrophage function and prostaglandin metabolism requires further study.

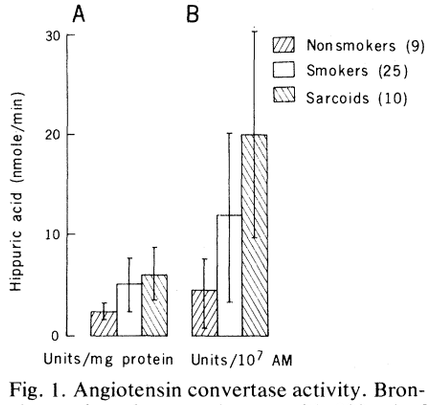

Fig. 1. Angiotensin convertase activity. Bronchoscopic pulmonary lavage with 200 ml of sterile saline was performed on 34 normal volunteers (25 cigarette smokers and nine nonsmokers) and on ten patients with biopsy-proved pulmonary sarcoidosis (two smokers and eight nonsmokers). The recovered lavage fluid (100 to 120 ml) was centrifuged at 2000 rev/min, the supernatant decanted and the cells resuspended. The cells were counted by means of differential staining with Wright's stain and a nonspecific esterase stain (*23*); portions of the remaining cells were sonicated (0°C, 30 seconds) and assayed both for ACE activity, by the method of Lieberman with hippuryl-l-histidyl-l-leucine as substrate, and for total protein (Folin reagent) (*AM*, alveolar macrophage). Data are expressed as mean ± 1 S.D. In patients with sarcoidosis, the data include both smokers and nonsmokers. Exclusion of the two smokers does not affect the statistical difference between the sarcoidosis patients and either control group.

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References and Notes

1. J. Lieberman, *Am. J. Med.* **53**, 365 (1975).
2. J. B. L. Gee, P. T. Bodel, S. K. Zorn, L. M. Hinman, C. A. Stevens, *Lung* **155** (No. 3) (1978).
3. B. L. Fanburg, M. D. Schoenberger, B. Bachus, G. L. Snider, *Am. Rev. Respir. Dis.* **114**, 525 (1976).
4. S. K. Zorn, M. R. Littner, C. E. Putman, J. B. L. Gee, *Clin. Res.* **24**, 552a (1976).
5. K. Ashutosh and J. F. H. Keighley, *Thorax* **31**, 552 (1976).
6. U. S. Ryan and J. W. Ryan, in *Metabolic Functions of the Lung*, Y. S. Bakhle and J. R. Vane, Eds. (Dekker, New York, 1977), pp. 197–232.
7. E. Silverstein, J. Friedland, H. S. Lyons, A. Gurin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2137 (1976).
8. D. L. Boros, *Prog. Allergy* **24**, 183 (1978).
9. J. Friedland, C. Setton, E. Silverstein, *Science* **197**, 64 (1977).
10. M. A. Ondetti, B. Rubin, D. W. Cushman, *ibid.* **196**, 441 (1977). The abbreviations are as follows: <Glu, pyroglutamic acid; Trp, tryptophan; Pro, proline; Arg, arginine; and Glu, glutamine.
11. H. Y. T. Yang, E. G. Erdos, Y. Levin, *Biochim. Biophys. Acta* **214**, 374 (1970).
12. F. E. Dorer, J. R. Kahn, K. E. Lentz, M. Levine, L. T. Skeggs, *Circ. Res.* **34**, 824 (1974).
13. J. W. Ryan, A. Chung, L. C. Martin, U. S. Ryan, *Tissue Cell* **10**, 555 (1978).
14. U. S. Ryan, E. Clements, D. Habliston, J. W. Ryan, *ibid.*, p. 535.
15. Q. N. Myrvik and J. D. Acton, in *Respiratory Defense Mechanisms II*, J. D. Brain, D. F. Proctor, L. M. Reid, Eds. (Dekker, New York, 1977), pp. 1023–1051.
16. J. B. L. Gee and A. S. Khandwala, in *ibid.*, pp. 927–981.
17. J. D. Harris, E. W. Swenson, J. E. Johnson III, *J. Clin. Invest.* **49**, 2086 (1970).
18. L. M. Hinman, C. A. Stevens, R. A. Matthay, H. Y. Reynolds, J. B. L. Gee, *Am. Rev. Respir. Dis.* **117** (pt 2), 67 (1978).
19. R. J. Rodriguez, R. R. White, R. M. Senior, E. A. Levine, *Science* **198**, 313 (1977).
20. S. L. Hong and L. Levine, *J. Biol. Chem.* **251**, 5814 (1976).
21. R. M. Zusman and H. R. Keiser, *ibid.* **252**, 2069 (1977).
22. S. Murota, M. Kawamura, I. Monita, *Biochim. Biophys. Acta* **528**, 507 (1978).
23. L. T. Yam, C. Y. Li, W. H. Crosby, *Am. J. Clin. Pathol.* **55**, 283 (1971).

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