Peptides, Vol. 11, pp. 1305–1307. © Pergamon Press plc, 1990. Printed in the U.S.A.

0196-9781/90 $3.00 + .00

BRIEF COMMUNICATION

ACTH(1–24) Stimulates the Migration of Human Monocytes In Vitro

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Received 8 January 1990

GENEDANI, S., M. BERNARDI, M. G. BALDINI AND A. BERTOLINI. *ACTH(1–24) stimulates the migration of human monocytes in vitro*. PEPTIDES **11**(6) 1305–1307, 1990.—In view of the increasing evidence that a variety of stresses can influence immune responses, the direct effect of adrenocorticotropic hormone on the migration of human monocytes was studied in vitro. ACTH(1–24) significantly increased the number of migrating cells when placed in the same or the opposite compartment of the chemotaxis chamber, maximum activity being obtained at 10^{-14} and 10^{-8} M. The results indicate that ACTH(1–24) directly and potently stimulates the migration of human monocytes by means of a chemokinetic effect.

ACTH Chemokinesis Monocytes Neuropeptides Immune system Immunomodulating peptides

A rapidly growing body of anatomical, physiological, neurochemical and psychological data clearly indicates not only that a vital link exists between the immune system and the central nervous system (CNS) but also that these two systems communicate with each other (19). In fact, it is now widely recognized that the communication between the immune and nervous systems is bidirectional, rather than unidirectional: the CNS interacts with, and can modulate, the immune system, just as, conversely, the immune system can regulate nervous (and endocrine) functions (2, 3, 14, 19).

An abundance of anecdotal data, recently confirmed by rigorous, extensive clinical and epidemiological evidence, suggests that a variety of stresses, both major and minor, may adversely affect the resistance of the human organism to infections and possibly to malignancies (13, 15, 19). The humoral response to stressful situations involves the release of CRF, pro-opiomelanocortin (POMC)-derived peptides (endorphins, Met-enkephalin, ACTH, α-, β- and γ-MSH), and adrenal gland products (corticosteroids, adrenaline, Met-enkephalin). The influence of corticosteroids on the immune system is well known (12), and the possible role of opioid peptides in the immune response is being extensively studied at present, albeit with intriguing and contradictory results (3, 5, 14). On the other hand, and rather surprisingly, the possible, direct role of ACTH as an immunomodulating peptide has received scant attention (1,3). Accordingly, we set out to study the effect of ACTH on the migration of human monocytes, in vitro.

METHOD

In Vitro Locomotion of Human Monocytes

Human peripheral blood was obtained from healthy volunteers and mononuclear cells separated by sedimentation over Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) (4). For each experiment a pool from three donors was used to minimize individual differences. The cells were removed from the interface, washed in PBS, resuspended in minimal essential medium (Serva Feinbiochemica, Heidelberg/New York), to which 1% BSA (Boeringher Biochemia Robin, Milano, Italy) had been added, and diluted to a final concentration of 1.5–2 millions/ml. Migration was studied by using blind-well chemotaxis chambers (Nuclepore, Pleasanton, CA) in which the upper and lower compartments were separated by a 5-μm pore polycarbonate polyvinylpyrrolidone free (PVPF) filter to allow the cells to migrate actively through the pores (22). A suspension of three–four hundred thousand monocytes in a volume of 200 μl was placed in the upper compartment, and different concentrations of ACTH(1–24) or of ACTH(1–39) were placed either in the lower or in the upper, or in both the upper and lower compartments. After 90 minutes of incubation at 37°C, the migrated cells adhering to the distal part of the filter were fixed in 70% ethanol and stained with Wright's Giemsa. The number of migrating cells was assessed microscopically, five fields being counted in duplicate using an optical image analyzer (Tesak VDC 501); the monocytic nature of the cells

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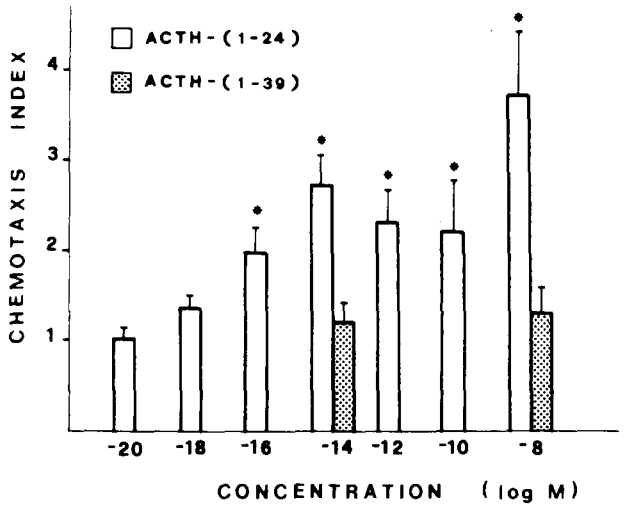

FIG. 1. Human monocytes locomotion induced by different concentrations of ACTH(1–24) and of ACTH(1–39). Data are means ± SE of 5–7 experiments. *$p<0.01$ versus medium alone. Kruskal-Wallis followed by Wilcoxon test.

was confirmed by morphological examination. Control data were obtained by measuring the migration of cells in the presence either of medium alone or of the synthetic chemotactic peptide formyl-Met-Leu-Phe (f-MLP).

Drugs

Formyl-Met-Leu-Phe (f-MLP) and ACTH(1–39) were supplied by Sigma Chemical Company, St. Louis, MO; ACTH(1–24) was kindly donated by Ciba-Geigy (Basel, Switzerland). The drugs were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10^{-3} M and then, just before use, diluted to the appropriate concentration in medium; in the control experiments DMSO was added to the medium at the same final concentration.

Data Analysis

Statistical evaluation of the results was performed by ANOVA followed by a multiple comparison test. The data, expressed as a chemotactic index (the ratio between migration to the test attractant and medium alone), were analyzed by Kruskal-Wallis analysis of variance followed by Wilcoxon multiple comparison test.

RESULTS

As shown in Fig. 1, when ACTH(1–24) was placed in the lower compartment of the chemotaxis chamber, it significantly increased the number of migrating cells starting from the concentration of 10^{-16} M, two peaks of activity being obtained at 10^{-14} and 10^{-8} M ($p<0.01$). On the other hand, ACTH(1–39) had no significant effect, either at a final concentration of 10^{-14} or of 10^{-8} M. The number of monocytes migrating in the presence of the medium alone (plus DMSO) in the lower compartment of the chemotaxis chamber (random migration) was 24.93 ± 3.06, while in the presence of f-MLP the number rose to 227.33 ± 12.49.

In order to distinguish between true chemotaxis and chemokinesis, a "checkboard" analysis was performed in which ACTH(1–24), at the two more active concentrations, was placed in both the upper and lower compartments (16, 21, 23). As shown in Table 1, at either concentration ACTH(1–24) was able to stimulate the migration of monocytes whether there was a positive gradient (peptide only in the lower well), no gradient (peptide both in the upper and lower wells), or even a negative gradient (peptide only in the upper well), thus indicating that ACTH stimulates monocyte migration by chemokinesis.

DISCUSSION

The present data show that ACTH(1–24) stimulates the migration of human monocytes, in vitro, at concentrations in the femtomolar range. The fact that the migratory response of monocytes is greater when ACTH(1–24) is placed in the upper compartment than when it is placed in the lower one indicates that ACTH acts by chemokinesis rather than by chemotaxis.

Adrenocorticotropic hormone (ACTH) was previously thought to act on the immune system exclusively through glucocorticoids. Now, however, it is recognized that ACTH can directly modulate immune responses (3). Lymphocytes and macrophages synthesize pro-opiomelanocortin (POMC) in both an inducible and constitutive fashion (6, 7, 10); the mRNA for POMC has been indentified in these cells (6, 11, 20), and lymphocytes behave quite akin to pituitary corticotrophs with respect to control of the POMC gene by the classic stimulators (CRF, AVP) and inhibitors (glucocorticoid hormones) of POMC production (17). Different stimuli elicit different posttranslational POMC processing by leukocytes, with the concurrent or prevalent production [besides ACTH(1–39) and β-endorphin] of ACTH(1–24 to 26) and α- and γ-endorphin (7,8). Moreover, cells of the immune system (mouse spleen monocytes, human mononuclear lymphocytes) have both high and low affinity receptors for ACTH (9,18). Finally, ACTH directly enhances the growth and differentiation of human B lymphocytes (1).

TABLE 1

CHEMOKINETIC EFFECT OF ACTH(1–24) ON HUMAN MONOCYTES, IN VITRO

		ACTH(1–24) (log M) in the Upper Chamber		
		0	−8	−14
ACTH(1–24) (log M) in the Lower Chamber	0	24.93 ± 3.06	98.63 ± 15.24	117.60 ± 7.45‡
	−8	82.11 ± 11.26*	126.23 ± 5.51†	103.93 ± 11.47‡
	−14	66.85 ± 8.53*	113.93 ± 7.77†	99.45 ± 12.74‡

Number of cells/field (mean ± SE; 3–7 experiments/dose). The cells migrating towards the control chemotactic peptide (f-MLP, 10^{-8} M) were 227.33 ± 12.49.

*$p<0.05$ versus medium alone. †$p<0.05$ versus ACTH(1–24) 10^{-8} M in the lower chamber. ‡$p<0.05$ versus ACTH(1–24) 10^{-14} M in the lower chamber. ANOVA followed by multiple comparison test.

All those data strongly support the idea that ACTH plays a direct, physiological role in the immune response, and our present finding that ACTH(1–24) stimulates monocyte migration at subpicomolar concentrations further strengthens such a claim. The fact that ACTH(1–39) is ineffective is not surprising. Such disparate behavior on the part of different ACTH molecules has been described for other functions of the immune system: for example, ACTH(1–39) inhibits antibody synthesis and lymphokine production, and suppresses the lymphokine-mediated macrophage activation, whereas ACTH(1–24), ACTH(18–39) and ACTH(1–13) (α-MSH) do not (3). Similarly, complex and often paradoxical and contradictory effects on the immune system have been obtained with opioid peptides (3, 5, 14, 19). However, our data show that one of the ACTH molecules that is normally synthesized by immune cells in response to different stimuli (7) directly and potently stimulates the migration of human monocytes, and lend further support to the argument that this peptide plays a physiological role in the immune response.

ACKNOWLEDGEMENTS

This work has been supported in part by grants from the Ministero della Pubblica Istruzione, Roma. We thank Dr. Paola Sacerdote for her helpful suggestions.

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