CONCISE REPORT

Immunocytochemical Demonstration of γ-Melanocyte Stimulating Hormone-Like Immunoreactivity in Human Neutrophilic Granulocytes

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Using the indirect immunohistochemical approach the occurrence of γ-melanocyte stimulating hormone (MSH)-like immunoreactivity in neutrophilic granulocytes is described.

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P
eptides, which are common to both the neuroendocrine cells as well as the nervous tissue have recently been found also in a variety of other cells. For instance, certain blood cells and cells of the immune system can produce and/or store such peptides. Human polymorphonuclear leucocytes (PMN) and monocytes (MNC) contain immunoreactivity to vasoactive intestinal polypeptide (VIP), somatostatin, glucagon, and gastrin/cholecystokinin. Under appropriate stimulation human lymphocytes and leucocytes can also produce corticotropin (ACTH), thyrotropin (TSH), and endorphins. In addition, VIP has been found to be present in mast cells and platelets. In the present study, we report the occurrence of γ-melanocyte stimulating hormone (γ-MSH) or a γ-MSH-like peptide in human PMN cells.

MATERIALS AND METHODS

Blood smears were prepared from venous blood of healthy adult male and female volunteers. The freshly prepared smears were immediately fixed either in ice-cold buffered formol-acetone or 4% paraformaldehyde for five minutes and washed in phosphate buffered saline (PBS) for 15 minutes. The indirect immunofluorescence method was used to demonstrate γ-MSH-like immunoreactivity.

Antibodies to synthetic γ-MSH1-11 (no. 83C) were raised in rabbits. Although the antibodies used showed no apparent crossreactivity in radioimmunoassay tests, such reactions cannot be excluded in the histochemical situation and therefore terms such as "γ-MSH-like immunoreactivity," "γ-MSH immunoreactive," etc. have been preferred in the text (see below). The smears were incubated with γ-MSH antibodies diluted 1:200 or 1:400 in 0.01 mol/L PBS containing 0.3% Triton X-100 for 16 hours at 4°C in a humid atmosphere, washed in ice-cold PBS for 15 minutes with three changes, and incubated with tetramethylrhodamine-isothiocyanate isomer R (TRITC)-labeled goat anti-rabbit antiserum (Boehringer Mannheim Biochemicals, West Germany) diluted 1:80 for 30 minutes at 37°C. After rinsing in PBS for 15 minutes the slides were mounted in glycerine-PBS (10:1) containing 0.1% para-phenylenediamine. The slides were studied and photographed with a Zeiss fluorescence microscope followed by further staining with routine hematoxylin-eosin stains.

RESULTS

Under fluorescence microscopy numerous cells showing γ-MSH-like immunoreactivity were seen. The cytoplasm of the cells was finely granular and the lobulated nucleus was unstained (Fig 1A). Occasionally some weak nuclear staining was seen in smears fixed in 4% paraformaldehyde instead of formol-acetone. To confirm that the strongly γ-MSH immunoreactive cells were neutrophilic granulocytes the same smears were restained with hematoxylin-eosin (Fig 1B). Erythrocytes, platelets, and other WBCs did not show immunoreactivity to γ-MSH. Finally, preincubation of the γ-MSH antiserum with α- or β-MSH peptide did not diminish the immunoreaction.

In this context, it may be mentioned that Lundberg et al.12 using the same antiserum on different rat tissues, found a very complex crossreactivity situation: preabsorption of the γ-MSH antiserum with NPY (neuropeptide tyrosine), APP (avian pancreatic polypeptide), APP31-36, BPP (bovine pancreatic polypeptide), PYY (peptide YY) and FMRF-amide (molluscan cardioexcitatory neuropeptide) markedly reduced or abolished most of the γ-MSH staining of nerve fibers of brain, sympathetic ganglia and vas deferens and of colonic endothelial cells. The γ-MSH staining of pancreatic endocrine cells was markedly reduced by APP, BPP, and PYY preabsorption, but not using preabsorption with NPY, APP31-36, or FMRF-amide. In radioimmunoassay, the γ-MSH antiserum did not crossreact to any significant extent (<0.1%) with NPY, APP, or FMRF-amide. In 85 times higher concentrations the antiserum did not recognize 125I-NPY. However, in the present investigation no evidence for such crossreactivities mentioned above could be found.

DISCUSSION

γ-MSH is a newly discovered peptide produced by the pituitary. It is derived from the same large precursor molecule, pro-opiomelanocortin (POMC) as α-MSH, β-MSH, ACTH, β-lipotropin, and β-endorphin. Previous immunohistochemical studies have demonstrated γ-MSH-like immunoreactivity in the pituitary, hypothalamus, and human skin. The present results demonstrate, that also human PMN cells contain immunoreactivity to a γ-MSH-like substance. The origin of the peptide in human PMN cells is unclear. Smith et al. have shown that the POMC gene may also be expressed in human leucocytes resulting in ACTH and endorphin production by leucocytes. It remains to be elucidated if human leucocytes are also capable of producing...
γ-MSH, which is coded by the same gene. However, it is possible that the peptide may have been absorbed by the PMN cells, since leucocytes possess specific binding sites for several neuropeptides.18

The physiological role of γ-MSH is still poorly known. In general, the MSH peptides are supposed to control pigmentation,19 although their role in humans is not as well established. Inflammatory reactions in the skin often lead to abnormal skin pigmentation of unknown cause.2 Inflammation is also accompanied by PMN cell migration through the vessel walls. Perhaps, the γ-MSH–like peptide in the PMN cells could be responsible for the pigmentary changes in inflammation. Further studies are needed to confirm this suggestion.

Recent studies have indicated that the nervous and immunologic systems interact with each other. Blood cells containing peptide hormones have been proposed to be the link between these systems.18 Thus, the presently observed PMN neutrophilic cells containing a γ-MSH–like hormone may act as local modulators between the nervous system and the immune system.

ACKNOWLEDGMENT

For a generous supply of γ-MSH antiserum the authors are grateful to Prof L. Terenius, Department of Pharmacology, Uppsala University, Sweden. For skilful technical assistance we thank S. Nilsson and S. Soltesz-Mattisson.

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