CHAPTER 2

Melanocortins and Pigmentation

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It all began in 1916, the story not only for the melanocortins and pigmentation but also for the entire field of pituitary endocrinology. Two independent papers appeared in *Science* by two young biologists, Philip E. Smith in California *(1)* and Bennett M. Allen in Kansas *(2)*. They described a way to ablate the pituitary glands of tadpoles without killing the animals, and they observed that the tadpoles so treated were light in color. Soon after these reports it was found that injections of pituitary extracts into tadpoles and frogs would turn them dark *(3)*. Before this apparently simple achievement, it was thought that the pituitary gland was necessary for life. No investigator had been able to destroy or remove that gland from any animal and keep it alive. Ten years later Smith *(4)*, in another major success, reported his procedure for the ablation of the hypophysis in rats. He opened the door for intense research on the role of the pituitary gland in mammalian systems. It should not be a surprise that a change in color of tadpoles marked the beginning of pituitary endocrinology. It was essential that one be able to see and measure a change with visible light. There were no spectrophotometers or other equipment to monitor the metabolic processes that occurred outside the visible range after the destruction or removal of a gland or the injection of extracts from glands into animals.

While impressive advances were being made in the basic biologic and medical sciences, there were numerous questions in clinical medicine regarding disorders of pigmentation. Some people had defects from birth—albinism, piebaldism, large nevi, and so on. Others had conditions that were acquired—local or generalized hyperpigmentation or hypopigmentation, or both. Both acquired conditions occur in adrenal insufficiency or Addison's disease. In this paper I will be concerned mostly with the darkening that occurs in patients with loss of adrenal function from any cause (idiopathic atrophy, tuberculosis, metastatic cancer, removal of the adrenals, etc.). In this disorder there is hyperpigmentation of the exposed areas (face, hands, arms) the body folds and

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creases (axillae, groin, palms), pigmented nevi, the oral cavity, and sites of recent scars. People of medium dark color and who tan well can become extremely dark. Replacement therapy with relatively low doses of cortisone, 37.5 mg daily, is usually sufficient to get the patient back to his or her original color.

What caused the darkening? It was generally assumed that the darkening that occurred in tadpoles and frogs minutes after the injection of pituitary extracts was totally unrelated to what happens in human beings. It was assumed—wrongly—that it takes days or weeks for human being to darken following adrenalectomy. It should have been realized that under the proper conditions human beings do have the capacity to darken quickly. For example, some people of medium dark complexion can darken within 24 hours after exposure to strong sunlight. Injection of melanocyte-stimulating hormone (MSH) can darken someone in 2 or 3 days.

In the early 1950s efforts were being made to isolate MSH from the pituitary gland. Smith had also previously identified an adrenocorticotropic principle when he demonstrated that adrenal atrophy following hypophysectomy could be reversed by implantation of pituitaries. At about the same time the Armour Laboratories began to market adrenocorticotropic hormone (ACTH) for clinical use. It was found that Armour ACTH was a potent darkening agent for tadpoles and frogs. In addition, patients receiving ACTH for several weeks were turning dark. Some investigators were beginning to conclude that ACTH was the major darkening peptide in the pituitary. But when α- and β-MSH were isolated, they proved to be more potent than ACTH in darkening frog skin, with no ability to stimulate the adrenal glands. Armour produced their ACTH from whole bovine pituitary glands while we isolated MSH from bovine posterior pituitary glands, which we knew included cells of the intermediate lobe. Armour changed their method and began to separate physically the anterior lobes from the posterointermediate lobes. When their commercial ACTH came only from the anterior lobes the darkening stopped. Something other than ACTH caused the darkening. Their first ACTH product was contaminated with MSH and it was the MSH that was the offending agent. Injections of MSH into human subjects made them dark *(5–9)*.

The five peptides α-, β-, and γ-MSH, ACTH, and β-lipotropin that are part of the precursor molecule proopiomelanocortin (POMC) are referred to as *melanocortins*. They are peptide hormones and neuropeptides that together with their receptors participate in the control of an amazing array of processes, including pigmentation, adrenocortical steroidogenesis, energy homeostasis, inflammation, and others. Most is known about α-MSH and ACTH and their receptors. It appears that γ-MSH has no role in pigmentation. We do not know whether β-MSH can be processed from its parent peptide β-lipotropin or from

POMC, nor do we know much about β-lipotropin and pigmentation. Even though the sequence of the 13 amino acids that make up α-MSH is acetylated via an *N*-acetyltransferase to give α-MSH. The N and C-terminal ends of ACTH are free, but they are blocked in α-MSH—by an acetyl group at the N-terminus and by an amide group at the C-terminus. On the dispersion of melanin granules in frog melanocytes α-MSH is 30 times more potent than ACTH but only about five times more potent than *N*-acetylated ACTH made in the laboratory. We do not know whether or not there is an *N*-acetyltransferase for ACTH. If *N*-acetyl-ACTH were made either under normal or abnormal conditions it would be a potent darkening peptide. We know from limited clinical studies that synthetic α-MSH and an *N*-acetyl 23-amino acid ACTH can produce striking hyperpigmentation *(6,7)*. We also know that there was no darkening of a patient who received 1 mg (~100 units) of ACTH daily for 21 d but marked darkening in another patient who received 24 mg (approx 2400 units) of ACTH daily for 8 d *(8)*. On an equimolar basis α-MSH produced more rapid darkening than ACTH. The darkening of a patient with an unknown disorder *(11)* as well as those with primary biliary cirrhosis *(12)* may have been due to high levels of α-MSH.

Now that POMC has been found to be present in keratinocytes, melanocytes and other cells far from the pituitary gland we need to know whether or not it can be processed in these locations to produce the melanocortins, α-MSH and ACTH. This is particularly important, since hypophysectomy does not produce the depigmentation in mammals that it does in amphibians. What *N*-acetyl and des-*N*-acetyl forms exist? If they are produced, do they affect pigmentation locally? What defects occur in the receptors for α-MSH and ACTH?

Under normal conditions is there a role for MSH-ACTH on pigmentation? Probably yes. The melanocortins may serve to prime pigment cells so that, when needed, the cells can produce pigment. Such is the case of tanning of skin in response to exposure to ultraviolet light to protect against further photo damage.

In the past few years several growth factors have been found to be potent mitogens for melanocytes in culture *(13)*. Most of the pigment cells for these studies came from the foreskins of newborns. The factors include basic fibroblast growth factor (bFGF), endothelin-1, hepatocyte growth factor/scatter factor and stem-cell factor. These growth factors are generally more potent as mitogens for melanocytes in culture than α-MSH and ACTH. We don't know if the MSH-ACTH receptors of neonatal human pigment cells are modified in the culturing process so that they become less active to MSH-ACTH *(14)*. As stated above, we do know that α-MSH and ACTH injected into adults produce marked darkening. It is tempting to give these growth factors to people with vitiligo to see if the loss of melanocytes can be stopped and the proliferation of new cells increased.

Another area of investigation that is opening up concerns melanocytes and nitric oxide (NO). Human melanocytes express the neuronal form of nitric oxide synthase (NOS) and they are sensitive to NO produced via inducible NOS from Langerhans cells (E.A. Lerner, personal communication). We need to understand the interactions of the melanocortins, growth factors, and NO on pigment cells.

Summary

The melanocortins α-,β-,and γ-MSH, ACTH and β-lipotropin are neuropeptides processed from the precursor molecule POMC in different cells of the pituitary gland. POMC is also present in other cells including keratinocytes but the processing in these cells is still unknown. An *N*-acetyltransferase catalyzes the acylation of deacetyl MSH to α-MSH. Both α-MSH and ACTH can bring about the darkening of human beings. If ACTH could be acetylated in vivo it would be much more potent darkening agent than free ACTH but still not as potent as α-MSH.

What started off as a quest to explain the hyperpigmentation seen in patients with adrenal insufficiency led to the isolation of the melanocortins and their receptors. This knowledge together with the advances made on growth factors and nitric oxide will in turn be the basis for explaining the mechanism of many disorders of pigmentation.

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