

A NOTE ON TRANSMEMBRANE POTENTIAL IN DERMAL MELANOPHORES OF THE FROG AND MOVEMENT OF MELANIN GRANULES

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SUMMARY

1. Transmembrane potentials were measured and distribution of melanin granules observed in dermal melanophores of the isolated frog's web.

2. In normal Ringer solution, melanin granules were aggregated in the cell bodies and the transmembrane potentials were usually in the range 70–90 mV.

3. Addition of alpha-melanocyte stimulating hormone (α -MSH) to the bathing solution resulted in dispersion of the melanin granules into the processes of the cells, without any accompanying change in membrane potentials. Altering the membrane potentials by changing the external K^+ concentration had no effect on melanin migration.

4. It is concluded that migration of melanin granules in these cells is unrelated to cell membrane polarization.

INTRODUCTION

The major regulatory factors concerned with the movement of melanin granules within dermal and epidermal pigment cells of the frog appear to be hormonal, although other variables such as skin moisture and temperature exert some influence (Hogben, 1924). Direct nervous innervation of the pigment cells is not involved (Snell & Kulovich, 1967). Experimentally, pigment dispersion may be produced by injection of alpha melanocyte-stimulating hormone (α -MSH). The mechanism whereby such hormones control pigment migration is not known. One possibility is that dispersion is associated with depolarization of the cell membrane in a way analogous

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to excitation-contraction coupling in muscle cells. The present experiments were undertaken to determine whether administration of a α -MSH produced any such changes in membrane potential of dermal melanophores.

METHODS

A triangular area of skin was removed from the dorsal surface of the web between the second and third toe of a pithed frog (*Rana pipiens*) and placed in a small chamber (0.6 ml. capacity). The skin was pinned out with glass needles to a sheet of polyethylene in the bottom of the chamber, with the inner surface uppermost, and bathed in Ringer solution of the following composition (mm): NaCl, 115; KCl, 2.0; CaCl_2 , 1.8; NaHCO_3 , 2.4. The solution was dripped continuously through the chamber at a rate of about 2.5 ml./min, the level being maintained at a constant height by suction. A reference electrode of sintered Ag-AgCl was imbedded in one wall of the chamber.

Cells were penetrated with glass micropipettes filled with 3 M-KCl and having resistances of 35–45 M Ω . These were connected by a second Ag-AgCl electrode to the input of a unity gain amplifier of high input impedance mounted on a micromanipulator. The output of the amplifier was connected to one differential input of a cathode ray oscilloscope. The other input was connected to the indifferent electrode, which was usually earthed. Penetration of a cell resulted in a sudden deflexion of the oscilloscope beam, the magnitude of the deflexion indicating the membrane potential of the cell. Deflexions which were slow in onset as the pipette penetrated the tissue, or which could be graded by slight adjustments of the vertical position of tip of the pipette, were not accepted. As soon as a potential was measured the micropipette was withdrawn from the cell. Attempts to maintain penetrations for more than a few minutes were usually unsuccessful. On the other hand, successive penetrations of the same cell could be made without apparent deterioration of its membrane potential. Usually ten to twenty different cells were penetrated and their membrane potentials noted. The bathing solution was then replaced by an identical solution to which α -MSH had been added in a concentration of 20,000 u./ml. Membrane potentials were then measured in twenty to thirty more cells before returning to the control solution.

The amount of pigment dispersion produced by the hormone was assessed visually according to an index similar to that described by Hogben & Sloane (1931). On this scale, number 1 indicates complete aggregation of the melanin granules into the cell body and number 5 complete dispersion throughout the body and processes of the cell (Fig. 1). In most of the skin samples, the granules were well aggregated (No. 1 on the index) within an hour after excision. After addition of the hormone they dispersed at varying rates, and an attempt was made to penetrate cells in which the dispersion was more or less average for the skin at that particular moment. Small clusters of cells in which the granules were not aggregated at the beginning of the experiment and which showed only small changes in index on addition of the hormone were avoided.

RESULTS

Because of their melanin content, dermal melanophores could be seen easily under the dissecting microscope and could be penetrated with a micropipette with only moderate difficulty. Usually as the pipette tip was advanced toward a cell there were one or two sudden deflexions of the oscilloscope beam which probably indicated the penetration of other cells in the dermis. The apparent resting potentials of these cells were of the order of 35–40 mV. Penetration of a melanophore resulted in a much larger

deflexion, usually of the order of 70–80 mV (Table 1). The results of a typical experiment are presented in Fig. 2 in which the membrane potential and the index of dispersion of the melanin granules in individual cells are plotted against time after the beginning of the experiment. Initially, the average membrane potential was about 73 mV and the melanin was fully aggregated. Addition of α -MSH resulted in gradual dispersion of the

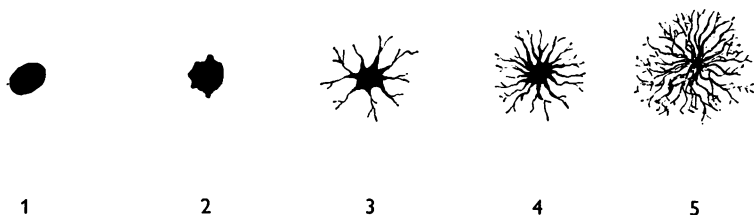


Fig. 1. Drawings of dermal melanophores of the frog's web at different stages in the dispersion of melanin granules. Numbers give dispersion index, which is a modification of index of Hogben & Sloane (1931); see Snell & Kulovich (1967).

melanin during the next 20 min with no significant change in average membrane potential. Thirty minutes later dispersion was, on the average, somewhat greater, with still no change in membrane potential. Washing out the hormone was followed by a gradual aggregation of the melanin. A second application of α -MSH was somewhat less effective in producing dispersion, and again resulted in no change in membrane potential.

The results of three other experiments were essentially the same as those described above: migration of melanin granules was unaccompanied by any change in membrane potential. The results are summarized in Table 1. In Exp. IV, the level of K^+ in the bathing solution was altered to determine whether the resulting change in membrane potential would itself produce pigment migration or alter in any way the effect of α -MSH. A five fold increase in external K^+ concentration produced a decrease in membrane potential from an average of 92.5 mV to an average of about 53 mV, which is close to the change of about 40 mV expected for a potential dependent solely on the K^+ concentration ratio across the membrane. No migration of the pigment accompanied the depolarization and addition of the hormone produced the usual dispersion, without any further alteration in potential. Similarly, hyperpolarizing the cells by washing the skin with Ringer solution to which no KCl had been added had no effect on melanin migration. The membrane potentials averaged about 124 mV after 'K⁺-free' solution was introduced into the chamber, and declined to 99 mV over the next 50 min, presumably because of loss of internal K^+ from the cells. α -MSH had no detectable effect on the potential, and again produced the usual dispersion of the melanin granules.

TABLE 1. Membrane potentials of dermal melanophores in presence and absence of α -MSH. Figures are mean membrane potentials in mV \pm s.d. Numbers in brackets are number of cells sampled. Exp. IV *b* was done in Ringer solution containing 5 times the normal concentration of K⁺; IV *c* in solution to which no K⁺ had been added

Experiment	Control	α -MSH	Control	α -MSH
I	73.3 \pm 6.3 (20)	65.2 \pm 7.0 (20)	55.0 \pm 6.7 (20)	54.2 \pm 6.7 (20)
II	72.9 \pm 5.3 (10)	75.8 \pm 5.2 (30)	78.5 \pm 4.6 (20)	77.3 \pm 4.2 (20)
III	84.9 \pm 5.3 (20)	79.2 \pm 5.9 (30)	78.5 \pm 4.3 (40)	—
IV <i>a</i>	93.0 \pm 3.5 (10)	92.5 \pm 5.4 (22)	92.0 \pm 5.6 (10)	—
<i>b</i>	53.1 \pm 3.5 (10)	52.5 \pm 2.5 (10)	—	—
<i>c</i>	123.5 \pm 4.2 (10)	109.0 \pm 3.1 (10)	99.0 \pm 2.7 (5)	—

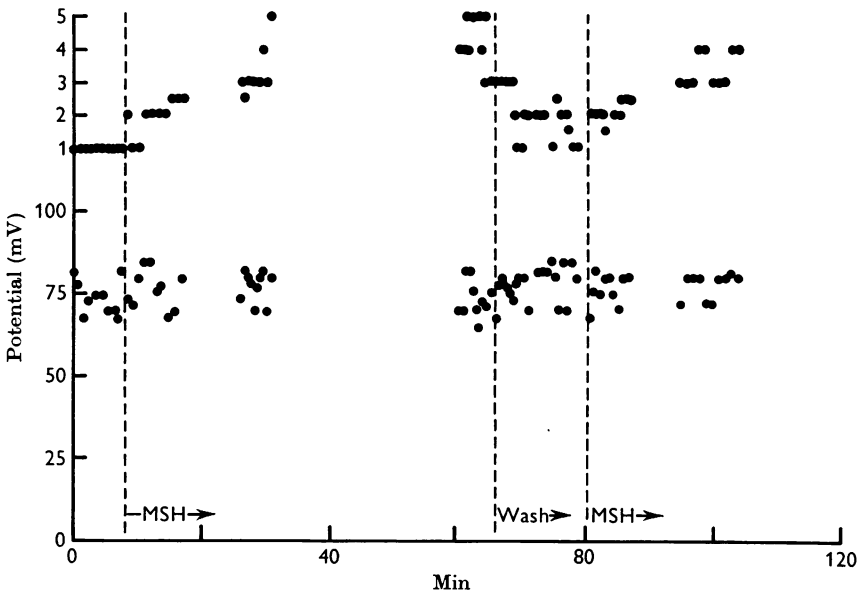


Fig. 2. Effect of α -MSH on dispersion of melanin granules (upper scale on ordinate) and membrane potential (lower ordinate). Abscissa is time in minutes after beginning of experiment. Dispersion by MSH is unassociated with any change in membrane potential.

DISCUSSION

The present results indicate that dispersion of melanin granules into the processes of dermal melanophores is in no way related to the membrane potential of these cells. Migration is not accompanied by changes in membrane potential and altering the membrane potential of the cells has no effect on migration. This implies that there is no net flux of ions across the membrane during the action of α -MSH, or, in other words, no change in permeability of the membrane to any major ion not in equilibrium across it. Other changes in membrane properties cannot be ruled out,

but if such changes occur, they must be relatively independent of membrane potential.

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