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INCORPORATION OF L-DOPA, L-α-METHYLDOPA AND DL-ISOPROTERENOL INTO GUINEA PIG HAIR

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Abstract. Incorporation of L-dopa, L-α-methyl dopa and DL-isoproterenol into the hair of black pigmented guinea pigs was measured by a ^{3}H-tracer method. These drugs were found to be incorporated into the hair and part of the radioactivity was found in isolated melanin. In an experiment with an albino guinea pig, incorporation of L-dopa into hair was demonstrated, indicating an independence of the incorporation phenomenon from active melanogenesis. The findings are in conformity with the concept that these drugs gain access to melanocytes through the arteriovenous or lymphatic circulation, enter melanosomes, interact with the melanosomal protein and melanin, and reside ultimately in the melanosomal granule in the hair fiber. The findings suggest a pathway which may influence the biological activity of these and related drugs.

Numerous adrenergic drugs are related structurally and chemically to L-tyrosine and L-dopa, the precursors of melanin in skin and hair of mammals. The question arises, whether the oxidation and polymerization systems in melanogenesis are strictly specific to these compounds and, if not, whether adrenergic drugs can gain access to melanocytes and become incorporated into melanin. There is considerable presumptive evidence suggesting that some adrenergic drugs can enter the melanogenesis pathway. For example, the chemical and biochemical feasibility of such reactions has been indicated in studies showing formation of "melanin"-like pigments from catecholamines such as noradrenaline or adrenaline via either oxidation by chemical oxidants or by catalysis of oxidation by various tyrosinases of plant and animal origin (1, 3). In addition, melanoma tissue slices have been shown to catalyse the oxidation and polymerization of various compounds related to L-tyrosine and L-dopa when L-dopa is present as a cofactor (5). Furthermore, probably the best evidence indicating non-specificity of melanogenesis comes from a recent investigation of Yu & Scott (7) who tested 45 compounds related to L-tyrosine and L-dopa for ability to form pigment in melanocytes of freshly plucked hair of humans and guinea pigs. These authors found that about one-third of the compounds tested, when incubated at 0.1–10 mM concentrations, may be converted to pigment, depending on the source and color of the hair.

We have studied the incorporation in vivo of adrenergic drugs into melanin and non-melanin components of the hair of black pigmented guinea pigs and an albino. We focused our attention on hair because it is convenient to monitor and because melanogenesis in hair may serve well as a model for melanogenesis in other tissues. The data from experiments involving L-dopa, L-α-methyldopa and DL-isoproterenol are reported herein.

MATERIALS AND METHODS

English smooth haired guinea pigs with black-pigmented hair, skin, and eyes and an albino guinea pig of the same type were used. The radioactive drugs included: L-dopa-^{3}H(L-(-)-3,4-dihydroxyphenylalanine) (ring-labelled, ICN), L-α-methyldopa-3-^{3}H(L-(-)-3,4-dihydroxyphenyl-β-methylalanine) (Amersham-Searle), and D,L-isoproterenol-7-^{3}H (DL-(2-3′,4′-dihydroxyphenyl -2-hydroxy-*N*-isopropylethylamine)) (New England Nuclear). Aqueous solutions of the drugs were administered intraperitoneally. Each animal was given a total of 1 mCi radioactivity per 1.26 μmoles drug; the dose was administered to the pigmented animals in five equal portions, one on each of the first 5 days and to the albino animal in four equal portions, one on each of the first 4 days. The weights of the pigmented animals used in the L-dopa, α-methyldopa, DL-isoproterenol experiments and the albino used in the L-Dopa experiment were 405, 160, 370, and 260 g, respectively. The animals were housed in separate cages to avoid cross-contamination. Extrafollicular hair (100–200 mg) was sampled daily during the initial part of the experiment by cutting or shaving from numerous areas of the body in a random manner. The daily hair samples were washed in

0.01 M HCl in a Buchner funnel and dried with acetone and air. For total radioactivity in intact hair, 50 mg of the washed hair was combusted in a Packard TriCarb Sample Oxidizer 305. The tritiated water was collected and the ^{3}H counted in Packard "Instagel" with a Packard TriCarb Scintillation Counter. In addition, 50 mg samples were placed in sealed vials containing 1.0 ml of 1.0 M HCl and hydrolysed for 5 days at 100°C in a constant temperature heating block. The acid supernatant was changed daily for the first 3 days, each change involving a centrifugation for 30 min at 1 000 *g*; separate experiments showed that this results in the removal of all acid-soluble radioactivity. Insoluble matter remaining after 5 days of hydrolysis was operationally defined as "melanin" (3); it was collected by centrifugation for 30 min at 1 000 *g* and washed and recentrifuged fourtimes with deionized water, then transferred to tared filter paper and air dried. After drying, the residues were weighed and analysed for ^{3}H by the method described above for intact hair. In preliminary experiments, we found that the above method was more consistent and reliable than measuring the non-melanin (acid-supernatant) fraction by direct scintillation spectrometry and the melanin by combustion analysis. To exclude the possibility that the radioactivity of the hair represented an exchange of ^{3}H not involving incorporation of the drug, a control experiment with L-dopa-^{14}C was carried out; the results (not detailed here) indicated the same type of incorporation and time course phasing as was found with L-dopa-^{3}H.

The hair in the L-dopa-^{3}H experiment was monitored for 40 days in an attempt to define the average follicular cycle with respect to incorporation of radioactivity in the hair and isolated melanin; a final hair sample was taken after 68 days. The α-methyldopa-^{3}H and L-dopa-^{3}H (albino experiments were terminated earlier (27–28days).

The animal in the isoproterenol experiment unfortunately died of pneumonia after 15 days; the data accumulated up to that point are included in this report because the experiment was continued long enough to show definitive results.

RESULTS

Tritium incorporation into the extrafollicular hair of the pigmented animals after intraperitoneal injections of L-dopa-^{3}H, L-α-methyl-dopa-^{3}H, and DL-isoproterenol-^{3}H is shown in Fig. 1 in terms of specific radioactivity of the hair and also of isolated "melanin". Fig. 2 describes the experiment in which L-dopa-^{3}H incorporation into extrafollicular hair of an albino guinea pig was measured; specific radioactivity of melanin is not reported for this animal since no insoluble matter (melanin) remained after the hydrolysis. The hair of the pigmented animal given L-dopa-^{3}H and terminated at day 68 contained 23 116 c.p.m./g hair and 13 000 c.p.m./g melanin.

DISCUSSION

The tritium incorporation into the extrafollicular hair of the pigmented animals given L-dopa-^{3}H, L-α-methyldopa-^{3}H, and DL-isoproterenol-^{3}H (Fig.

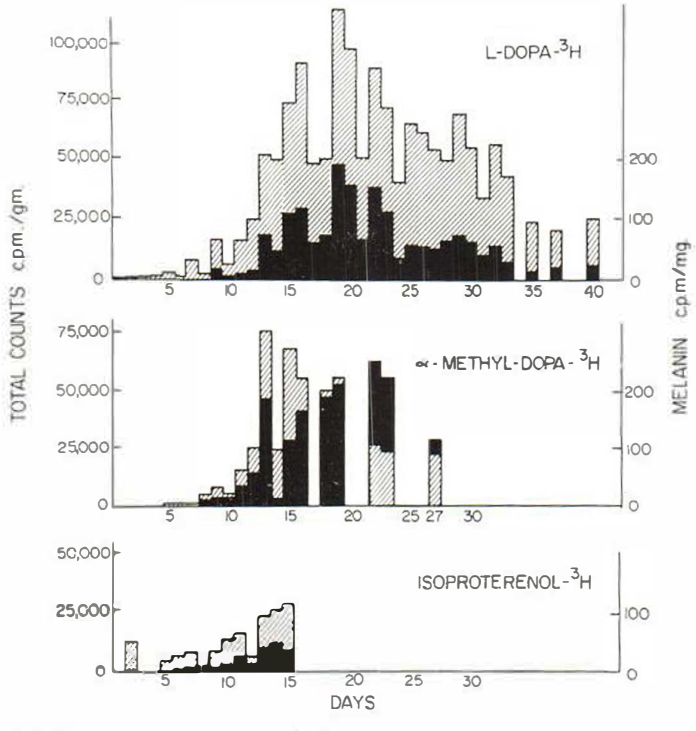

Fig. 1. Time course of the incorporation of L-dopa-^{3}H, L-α-methyl dopa-3-^{3}H, and DL-isoproterenol-7-^{3}H into pigmented guinea pig whole hair (□) and isolated melanin (■).

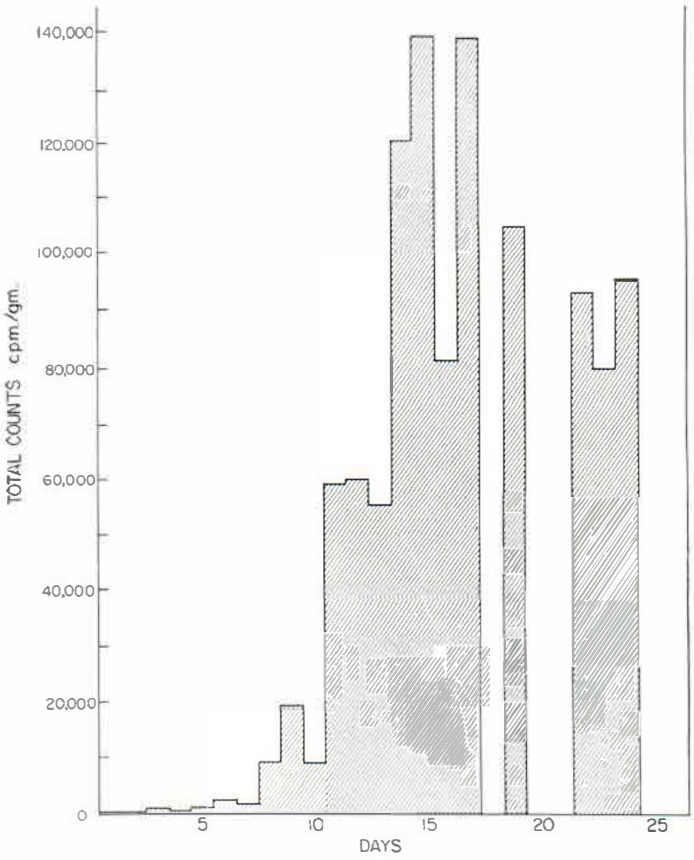

Fig. 2. Time course of the incorporation of L-dopa ^{3}H into non-pigmented guinea pig whole hair.

1) clearly shows that the three drugs are incorporated into the structure of the hair and reside in both melanin and non-melanin fractions. The wide fluctuations in radioactivity were anticipated, since the random samples included hair in different stages of their growth cycle. The results are best explained as being (and in complete conformity with) what would be expected from monitoring hair for a substance which gains access to the melanosome in the melanocyte, associates with both the protein and melanin components of the melanosomes and is then carried into the hair shaft by hair bulb cellular activity. The results do not exclude, however, the possibility of some uptake of drugs into keratinocytes and this will therefore be tested in future experiments. The magnitude of the specific activities found in each case should not be construed as indicating relative accessibility or relative rate of polymerization of the three drugs, since there was no attempt to adjust the amount and radioactivity of the three drugs to the same level with respect to animal weight; our major concern in this series was to maximize as far as possible the radioactive dose to establish definitively whether or not the drugs are incorporated. The experiment with the albino guinea pig given L-dopa-^{3}H clearly shows that incorporation into hair *per se* does not depend on the presence of melanin. Although there are alternative explanations, it is quite possible that the pathway of incorporation into the hair of the albino is similar to that of the pigmented animal, except that the possibility of its association with melanin is excluded. In support of the concept of association of L-dopa with the protein of the melanosome in the non-pigmented as well as the pigmented hair, Takahashi & Fitzpatrick (6) have demonstrated association of L-dopa with the protein of the melanosomal fraction of mouse melanoma; they found release of L-dopa during acid hydrolysis of isolated melanosomal protein.

We have found in separate experiments that the non-melanin radioactivity resulting after the acid hydrolysis of hair is dialysable but we have not characterized it further. We do not assume implicitly

that the melanin-associated radioactivity represents polymerized drug; we are attempting to ascertain whether the drugs under study are biosynthetically incorporated into melanin, or merely absorbed. We have found that melanin from hair and skin of control animals exhibits an absorptive capacity for this class of compounds (4) and our data suggest that both enzyme oxidation of the drugs and absorption take place. Blois (2) has found that melanin has a strong absorptive capacity for a variety of drugs but did not study drugs in relation to the catecholamines.

The above study raises many additional questions. Assuming that the drug affinity shown for hair melanin also occurs with cutaneous melanin as well as other tissues, as our preliminary data indicate (4), then would prolonged use of the drug alter the function of the melanin particularly during pre- or postnatal development? We are now performing long-term experiments to determine this. The radioactivity in the hair of the pigmented animal at day 68 suggests either a storage of radioactivity in the melanocyte or gradual transport of the labelled drug from other tissues to the melanocyte during the preceding time period.

The three drugs studied are incorporated into the hair from either the arteriovenous or lymphatic circulations. The results of the studies of α-methyldopa-^{3}H and isoproterenol-^{3}H disposition in vivo underline and magnify what must have been inferred from the data of Yu & Scott's (7) in vitro experiment with plucked hair. We estimate from our experiments that each of the drugs studied was present in the environment of the melanocyte at concentrations not exceeding 1×10^{-6} M compared with the 0.1×10^{-3} M to 10.0×10^{-3} M concentrations used in Yu & Scott's (7) experiment with plucked hair. Therefore, the intact melanocyte appears far more efficient in utilizing the substrate than the melanocyte of the plucked hair. One of the numerous questions which arise about drug disposition concerns the relationship between drug structure and drug incorporation. In separate studies of other drug series (to be reported elsewhere), we have found that the presence of the phenolic or catecholic group may not be required for incorporation, since amphetamine (α-methylphenylethylamine) which contains a phenyl group has been found to be incorporated. On the other hand, aspirin (acetylsalicylic acid) is not incorporated. This latter finding suggests a specific structural requirement for incorporation to take place. Numerous compounds must be tested to extrapolate towards a general rule regarding structure and incorporation relationships.

Our studies also suggest that hair may be a useful and sensitive indicator of the history of consumption by humans of certain drugs, since the average hair cycle of the human falls within the 2–6 year range with outside limits as long as a decade or more. To exploit this phenomenon further, the drug derivative deposited in the hair must first be characterized and a sensitive method developed for its analysis in hair.

These experiments indicate a pathway for adrenergic drugs not previously considered in metabolic studies. It is a minor pathway, affecting not more than 0.1% of the administered dose, but it should not be ignored, especially since incorporation has been found in other melanin-containing tissues (4), suggesting that internal pockets of long-lasting drug deposits may exist with unknown consequences to its host.

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