J. Soc. Cosmet. Chem., 25, 61–66 (February 1974)

The Presumptive Role of Amino Acid Derivatives and Catecholamines in the Etiology of Vitiligo*

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Presented May 25, 1972, 14th International Congress of Dermatology, Venice

Synopsis—Based on the hypothesis that vitiligo could be a phenomenon of the same type as chemically-induced leukoderma, an attempt was made to find some physiological or physiopathological *p*-hydroxyphenyl derivatives which could be responsible for MELANOGENESIS INHIBITION. Thus, the inhibitory effect of many derivatives of TYROSINE and also sympathomimetic substances on a DOPA-meloanocyte system was tested. The main result was that ADRENALINE (epinephrine) was found to be a strong inhibitor of the Dopa reaction whereas noradrenaline (norepinephrine) was found to have no such effect. Other strong inhibitors were *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenylcinnamic acid, which could be metabolites of aromatic amino acids.

Introduction

In previous research on the mode of action of the hydroquinone derivatives in the phenomenon of depigmentation, it was concluded that these substances were active as competitive inhibitors of tyrosinase (1). In effect, after four applications of *p*-ethoxyphenol (or monoethylether of hydroquinone or MEH) the melanocytes of the basal layer of guinea pig skin had already exhausted their reserves of melanin granules, but at this stage the Dopa-reaction was still positive. This clearly demonstrates that in this first step the melanocytes do not produce any more melanin unless the inhibitory action is arrested.

When the treatment is prolonged (10–20 days), melanocytes are no longer found, that is, the Dopa reaction is negative and the repigmentation *in vivo*

*Work supported by Grant 3.606.71, Swiss National Fund for Scientific Researches.
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can no longer produce itself. Therefore, a vitiligo-like lesion is artifically produced. The examination of guinea pig skin treated with MEH under electron microscopy confirmed the death of the melanocytes. In effect, Frenk (2) has demonstrated that in the first stage the melanocytes show important changes and then rapidly die. Furthermore, this author confirmed that this toxic effect is specifically directed against the melanocytes and that neither the keratinocytes nor the cells of Langerhans are influenced by this treatment; these facts were previously established by our experiences *in vivo* and through examination under light microscope (3). With our present knowledge of vitiligo, it can be verified that, apart from small changes probably due to the absence of protection against ultraviolet light, vitiligo and areas of leukoderma produced artificially by phenol derivatives are similar, if not identical, lesions.

Consequently, it seems logical to examine the following hypothesis: "Vitiligo could be due to the inhibitory action on the tyrosine-tyrosinase system by a phenol derivative; for example, a substance produced in the body (physiological or pathological) or a metabolite of such a substance. This product could then provoke the blockage of the melanogenesis (by competition), and after some days of inhibitory action, the death of the melanocytes. The presence of the inhibitory substance at the level of the melanocytes could be a consequence of a pathological phenomenon, likewise localized and confined in time."

In effect, only several days of inhibition of the melanogenesis can provoke the disappearance of the melanocytes. Therefore, a transitory pathological phenomenon could be the source of a vitiligo lesion. At the nonprogressive stage, vitiligo could be no more than a sequela of such a pathological phenomenon. This hypothesis could equally explain the relatively limited results that have been obtained in the study of vitiligo.

The artificial inhibitors previously studied were derivatives of phenol with "para" substituents. It is normal, therefore, to try to discover in which group of substances in the organism an inhibitor of this type could be produced pathologically. Thus, we have considered two families of compounds which could play an important role in this field; tyrosine derivatives on one hand and adrenaline derivatives on the other. It is clear that other related chemical families should also be studied.

Experimental

A modification of the method of Ijima and Watanabe (4), namely, the inhibition of the Dopa recation, was used. The modification consisted of the use of unfixed fresh tissue, frozen to −80°C quickly after the biopsy and cut by the cryostat to 8–10 μm. The competitive inhibitors are mixed with the Dopa solution according to a molar ratio "substrat (Dopa)/inhibitor" from 1/1 to 1/½₂ by successive dilution of the inhibitor.

$$1/1 \text{ corresponding to } \frac{5.10^{-6} \text{ mole/ml Dopa}}{5.10^{-6} \text{ mole/ml inhibitor}}$$

$$1/1/32 \text{ corresponding to } \frac{5.10^{-6} \text{ mole/ml Dopa}}{0.156\ 10^{-6} \text{ mole/ml inhibitor}}$$

The dilutions were made in a buffered phosphate solution (Soerensen) of pH 7.35 with traces of copper sulphate. The pH is adjusted, if necessary, after the addition of the inhibitor.

For testing, the standardized tissue used was the nipple of the male colored guinea pig. Verification was also made with normal human skin. The reaction is performed on slides in damp boxes; incubation time is 2½ hours at 37°C.

It is important to test pure chemical substances and not the pharmaceutical forms of these products because of the common presence in the latter of preservative agents which can interfere with the Dopa-reaction.

Results and Discussion

It must be emphasized that the method of Ijima and Watanabe (4) is not recommended in the study of all inhibitors of melanogenesis but, after our experiences, we have found it to be adequate and to give accurate results with competitive inhibitors. The results are shown in Table I.

Derivatives of Tyrosine and L-Dopa

It was found that MEH, which is very active *in vivo,* is already an inhibitor at a molecular concentration 1/16 of that of Dopa. Moreover, we have been able to show that two substances which are derivatives of tyrosine present an inhibitory activity of the same order (1/16 and 1/8). These substances are *p*-hydroxycinnamic acid and *p*-hydroxyphenylpyruvic acid. It is conceivable that under the influence of a pathological phenomenon (to be determined) these degradation products of tyrosine could be liberated at the level of the melanocytes.

In this aromatic amino acid family, the presence of one or two hydroxyphenolic groups does not seem to play an important role in inhibitory activity. On the other hand, as long as the molecule possesses a free amino function no inhibitory effect could be found.

Epinephrine and Derivatives

The substances of this family are also either physiological or chemical derivatives of tyrosine and Dopa. The results found in this group are very surprising in view of the strong inhibitory action of adrenaline (epinephrine) in comparison with the complete inactivity of noradrenaline (norepine-

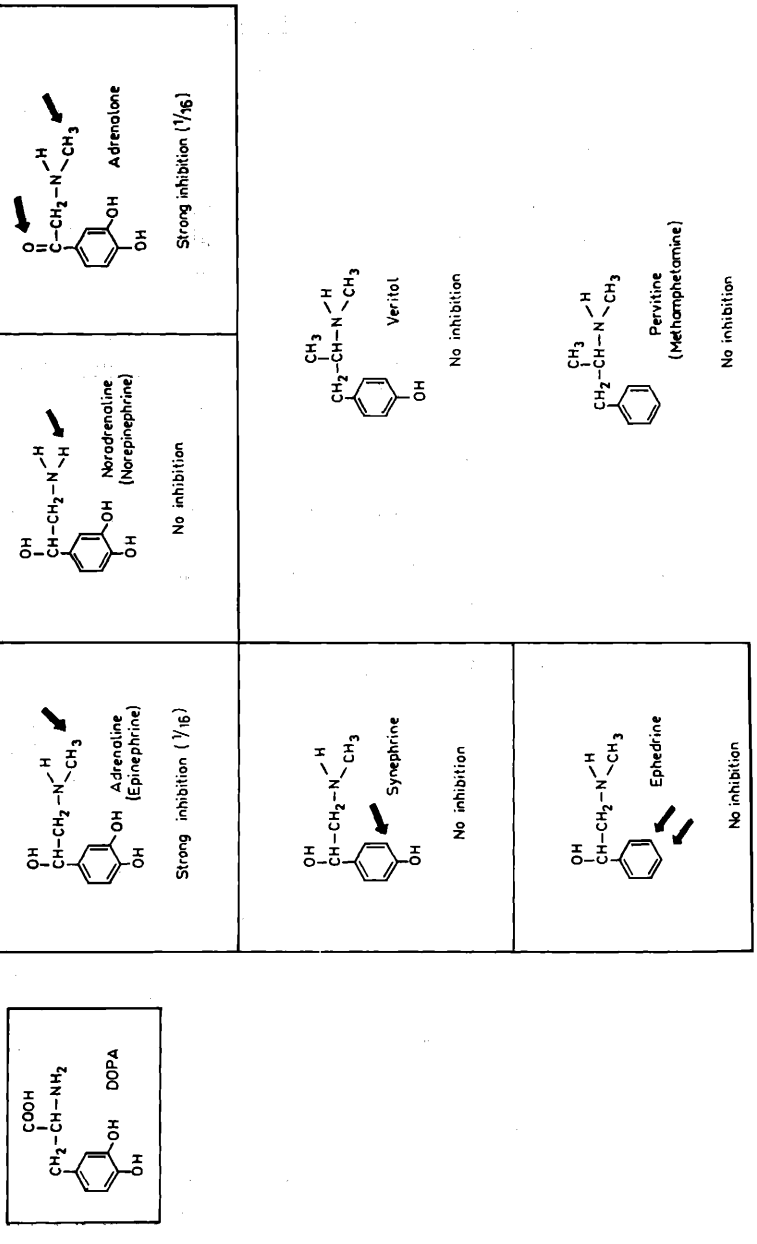

Figure 1. Dopa-oxidase inhibitory activity of epinephrine and related products

Table I
Inhibitory Effect on a Dopa-Melanocyte System

Inhibitors	Diln. Limit[a]	Inhib. Effect[b]
Tyrosine and Related Products		
Tyrosine	>1	0
Tyramine	>1	0
p-Hydroxyphenylpropionic acid	½	+
p-Hydroxyphenylpyruvic acid	⅛	++
p-Hydroxycinnamic acid	1/16	+++
p-Hydroxyphenylacetic acid	>1	0
p-Hydroxymandelic acid	>1	0
p-Ethylphenol	⅛	++
p-Ethoxyphenol (MEH)	1/16	+++
p-Hydroxybenzoic acid	½	+
Methyl-*p*-hydroxybenzoate	>1	0
p-Hydroxybenzaldehyde	>1	0
p-Hydroxybenzyl alcohol	>1	0
Dopamine	>1	0
3,4-Dihydroxyphenylpropionic acid	½	+
3,4-Dihydroxyphenylacetic acid	½	+
Phenylpyruvic acid	>1	0
Adrenaline and Related Products		
Adrenaline	1/16	+++
Noradrenaline	>1	0
Adrenalone	1/16	+++
Synephrin®[c]	>1	0
Veritol	>1	0
Ephedrine	>1	0
Pervitine®[d]	>1	0

[a]Ratio at which the activity of the inhibitor is still detectable, e.g., ⅛ = molar concentration of inhibitor 8 times inferior to that of Dopa.
[b]0 = nil, + = weak, ++ = fair, +++ = strong.
[c]Winthrop Laboratories, New York, N.Y.
[d]Temmler, Germany.

phrine). In this category of substances, the inhibitory effect seems bound with the presence of two hydroxyphenolic groups and the substitution of the amino group (i.e., the effects of adrenaline, noradrenaline, adrenalone, synephrine). This can be seen in Fig. 1.

If we take into account only the strongest inhibitors of the Dopa reaction found in this work, we can see that three of them correlate and strengthen the starting hypothesis. Indeed, *p*-hydroxyphenylpyruvic acid as well as *p*-hydroxycinnamic acid may be the result of a defect of the metabolism of tyrosine. However, the inhibitory effect of adrenaline in relation to the lack of the inhibitory power of noradrenaline may be of greatest significance.

A few years ago, Shelley and Öhman (5) found that a simple injection of epinephrine in rats produced a spot of white hair at the site of injection. They concluded that intense vasoconstriction was the reason for this selective damage of the melanocytes. But at the same time, the injection of nor-

epinephrine did not produce the whitening of the rat hair. These results are too similar to ours not to make a correlation between them.

(Received February 14, 1973)

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