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Local Effects of Synthetic Leukotrienes (LTC_4, LTD_4, LTE_4, and LTB_4) in Human Skin

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The local effects of intracutaneous injections into humans of 1–3 nmol of five products of arachidonic acid metabolism, leukotrienes (LT) C_4, D_4, E_4, and B_4 from the 5-lipoxygenase pathways and prostaglandin (PG) D_2 from the cyclooxygenase pathway, were assessed clinically and histologically. In equimolar concentrations, LTC_4, LTD_4, and LTE_4 elicited erythema and wheal formation, in which a wheal with central pallor was present up to 2 hr, and the erythema persisted as long as 6 hr. PGD_2 elicited a wheal that lasted up to 1 hr and erythema that lasted up to 2 hr. The dermal vascular sites affected by LTD_4 and PGD_2 included capillaries, superficial and deep venules, and arterioles. LTB_4 elicited a transient wheal and flare, followed in 3–4 hr by induration that was characterized by a dermal infiltrate comprised predominantly of neutrophils. The combination of LTB_4 and PGD_2 elicited tenderness and increased induration associated with a more intense neutrophil infiltration. Thus, the products of the 5-lipoxygenase pathway of arachidonic acid metabolism in nanomole amounts can induce cutaneous vasodilation with edema formation and a neutrophil infiltrate, and these responses are enhanced by a cyclooxygenase pathway product, PGD_2.

The oxidative products of arachidonic acid appearing with physiologic and pathobiologic perturbations of cellular membrane phospholipids include the prostaglandins, thromboxane, and the leukotrienes. Prostaglandin (PG) D_2, the predominant product of arachidonic acid metabolism in human mast cells in vitro [1,2] and in vivo [3,4], and leukotrienes (LT) C_4, D_4, and E_4 [5–9], recognized as components of slow reacting substance of anaphylaxis (SRS-A), are considered among the mediators of acute allergic processes.

Knowledge of the cutaneous effects of the LT components of SRS-A is limited to observations of alterations in venular permeability in guinea pigs [6,10,11] and rats [11], but not rabbits [11], and the morphologic demonstration of dilated superficial and deep blood vessels in monkey skin [12]. PGD_2, a metabolite of the cyclooxygenase pathway of arachidonic acid, can produce vasodilation in rat skin and elicit a wheal and erythema reaction in human skin [13]. LTB_4, a potent chemoattractant [14] that elicits a neutrophil infiltrate in monkey skin [15], is inactive in the rabbit skin unless combined with a vasodilating prostaglandin [16]. Inasmuch as there is no information on the cutaneous effects in humans of the leukotrienes alone or in combination with PGD_2, the intracutaneous injection of LTC_4, LTD_4, LTE_4, PGD_2, and LTB_4 alone and in some combinations was assessed by clinical and histologic parameters.

MATERIALS AND METHODS

LTC_4, LTD_4, LTE_4, PGD_2, and LTB_4 were prepared as previously described [8,17–20] and stored frozen at a concentration of 25 μg/ml in 0.1 M phosphate buffer (pH 6.8):ethanol (4:1, v/v) under argon until the day of use. Immediately before injection these substances were dried under argon and then redissolved in 0.1 ml of a sterile solution containing 0.15 M NaCl with 2% ethanol. LTC_4 (1.0 nmol/site), LTD_4 (1.0 nmol/site), LTE_4 (1.0 nmol/site), PGD_2 (3.0 nmol/site), LTB_4 (1.6 nmol/site), and vehicle alone or in various combinations were coded and injected intradermally into the ventral aspects of the forearms of 3 adult male volunteers after informed consent was obtained. Clinical

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Abbreviations:
LT: leukotriene
PG: prostaglandin
SRS-A: slow reacting substance of anaphylaxis

manifestations were recorded, and the reactions were quantitated by measuring their greatest diameter in millimeters at 7 time points from 10 min to 6 hr; neither the subject nor the recorder had specific knowledge of the material administered to a site.

Preliminary dose-response studies were conducted with LTD_4 (0.1, 0.2, and 1.0 nmol/site) in each of the 3 subjects in order to determine the single amount and the times of observation to be used for further studies. Although each of the three amounts of LTD_4 elicited erythema and wheal formation, the persistence of these manifestations was substantially greater at 1.0 nmol/site, and thus this amount was used for further studies of LTC_4, LTD_4, and LTE_4.

Skin biopsy specimens were obtained from sites injected with vehicle at 2 or 6 hr; with PGD_2 at 0.5, 2 and 6 hr; with LTD_4 at 2 hr; with LTB_4 at 6 hr; and with LTB_4 plus PGD_2 at 6 hr. Trephine biopsy specimens measuring 4 mm were taken from a skin site after the periphery at the four quadrants had been injected with 1% lidocaine without epinephrine. The tissue was bisected and fixed for 18 hr at 4°C in solution composed of 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% $CaCl_2$ in 0.1 M cacodylate buffer, pH 7.4. This fixed tissue was washed in the same buffer, postfixed in osmium tetroxide, and embedded in Epon. One-micrometer sections were prepared and stained with Giemsa diluted 1:10 in 2% Na borate solution and examined with a Leitz Dialux 20 microscope at magnifications up to ×1000 [21–23]. The cells were counted, and the number of infiltrating cells per total biopsy specimen was determined by subtracting the cells in the specimens from sites receiving vehicle alone from the total cells; results were expressed as net cells/mm^2 of 1-μm thick tissue sections.

Neutrophils in the dermis were defined by morphologic criteria including multilobed nuclei and a finely granular blue-gray cytoplasm. Activation of endothelial cells of the venules was defined by enlargement of both cytoplasmic and nuclear mass and by the presence of a prominent nucleolus in a nucleus with loose chromatin and basophilic staining of the nucleoplasm.

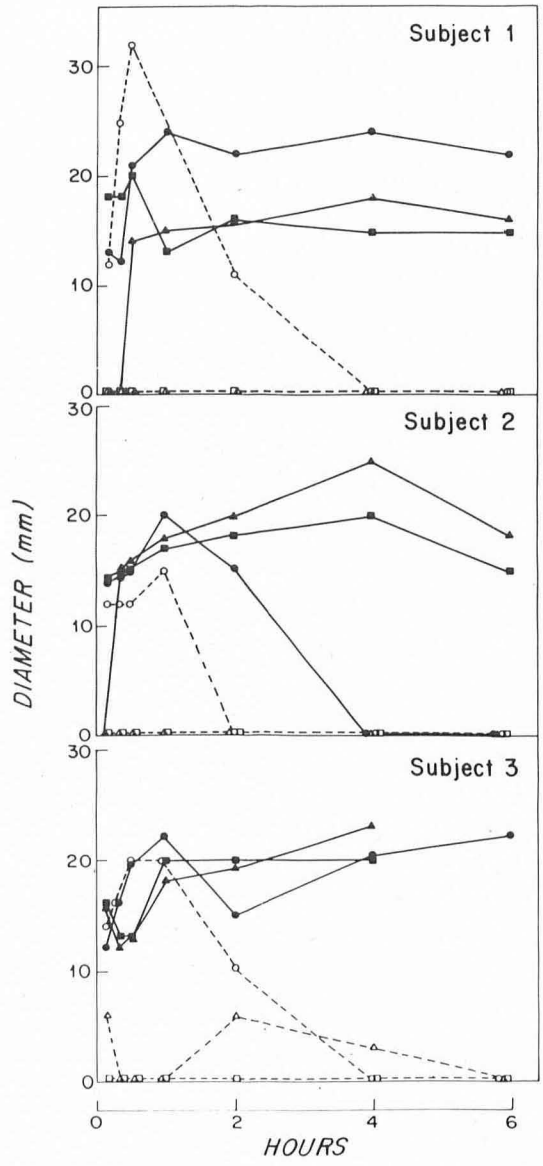

FIG 1. Erythema occurring after the intracutaneous injections of LTC_4 (■), LTD_4 (●), LTE_4 (▲), LTB_4 (Δ), PGD_2 (○), and saline vehicle (□) in 3 subjects. The initial observations were made at 10 min to minimize the nonspecific effects of injection. The greatest diameter of the clinical lesions is depicted in mm on the ordinate.

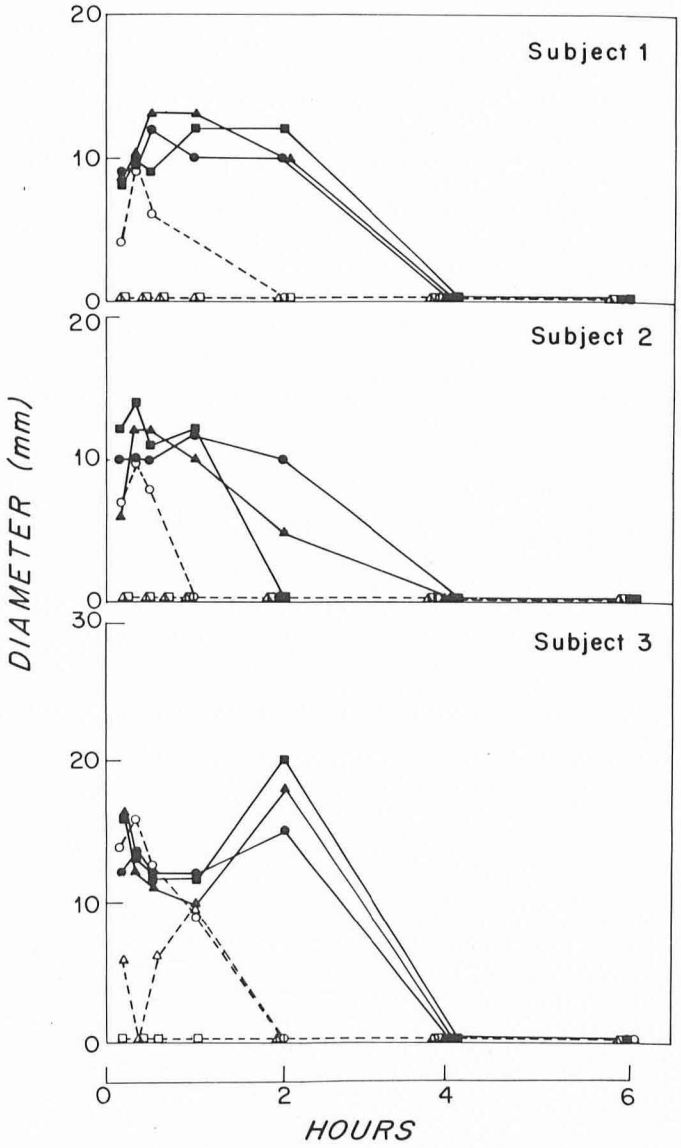

FIG 2. Wheal formation occurring after the intracutaneous injections of LTC_4 (■), LTD_4 (●), LTE_4 (▲), LTB_4 (Δ), PGD_2 (○), and saline vehicle (□) in 3 subjects. The greatest diameter of the clinical lesions is depicted in mm on the ordinate.

RESULTS

Clinical Cutaneous Responses

Each of the three SRS-A leukotrienes, LTC_4, LTD_4, and LTE_4 (1.0 nmol/site), elicited erythema that blanched with pressure and wheal formation in each subject (Figs 1, 2). Transient burning was noted in each subject after the injections of LTD_4 and LTE_4, but not after LTC_4. After the injections of LTC_4, LTD_4, and LTE_4, the center of the wheal was pale and the erythema was less intense than it was at the periphery and beyond the wheal. The lack of erythema over the wheal was

more prominent with LTC_4 and LTD_4 than with LTE_4. The erythema appeared by 10 min, peaked at 1 hr, and was not dissipated by 4 hr; wheal formation was noted at 10 min, peaked at 1 hr, and was dissipated by 4 hr. The injection of the saline vehicle did not elicit a wheal or flare reaction during the period of observation from 10 min to 6 hr.

PGD_2 (3.0 nmol/site) elicited erythema that blanched with pressure and a wheal (Figs 1, 2) without occurrence of symptoms. The erythema over the wheal was of the same intensity as that occurring beyond the wheal. Erythema appeared at 10 min, peaked at 30–60 min, and was dissipated by 4 hr. A wheal appeared at 10 min, peaked at 30 min, and was dissipated by 2 hr.

LTB_4 (1.6 nmol/site) elicited transient erythema that blanched with pressure and wheal formation in only 1 of 3 subjects (Figs 1, 2). LTB_4 caused induration as a firm 3-mm papule without tenderness in the same subject at 6 hr. The combination of LTB_4 and PGD_2 produced a prolonged erythema of 6 hr duration and a wheal that became tender at 2 hr and indurated as a firm 5-mm papule by 4 hr in each subject.

Histologic Alterations

Skin biopsy specimens obtained 2 hr after the injection of LTD_4 (1.0 nmol/site) (Fig 3) in each of 3 subjects exhibited dermal edema and marked and uniform dilation of capillaries and superficial and deep venules with activation of endothelial cells, as well as dilation of some arterioles. Biopsy specimens obtained 0.5 (Fig 4) and 2 hr after the injection of PGD_2 (3.0 nmol/site) exhibited dermal edema and marked and uniform dilation of capillaries and superficial and deep venules with activation of endothelial cells, as well as dilation of some arterioles. By 6 hr a diminution in the degree of dilation of the superficial and deep venules was noted. LTD_4 at 2 hr elicited 12.8 neutrophils/mm^2 in 1 of 3 subjects (Table I, Fig 3). Although PGD_2 elicited a transient perivenular neutrophil infiltrate at 0.5 hr in each of 3 subjects, ranging from 32.6 to 295.4 cells/mm^2, neutrophils were not noted at the 2-hr time point.

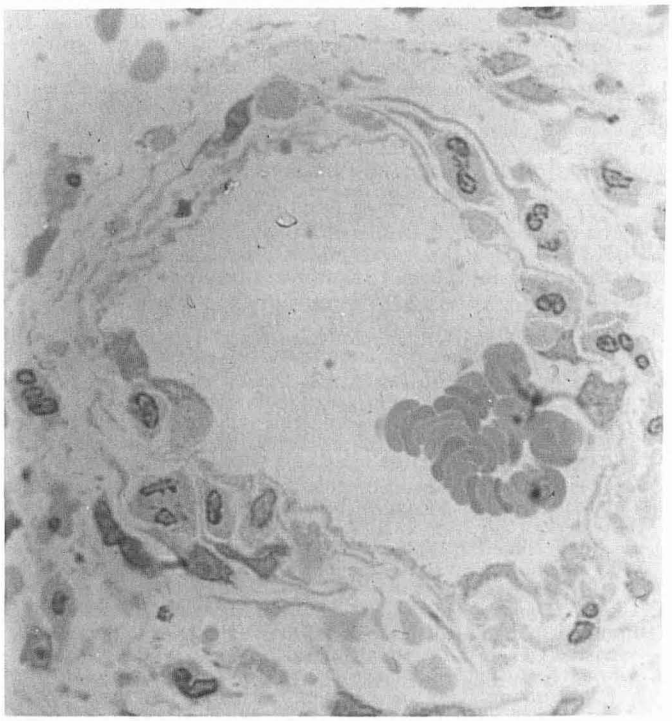

FIG 3. Histologic response occurring after the intracutaneous injection of LTD_4 (1.0 nmol/site) at 2 hr in subject 3. Note erythrocytes within dilated dermal venule and perivenular neutrophils (1-μm thick Epon-embedded specimen; Giemsa; × 252).

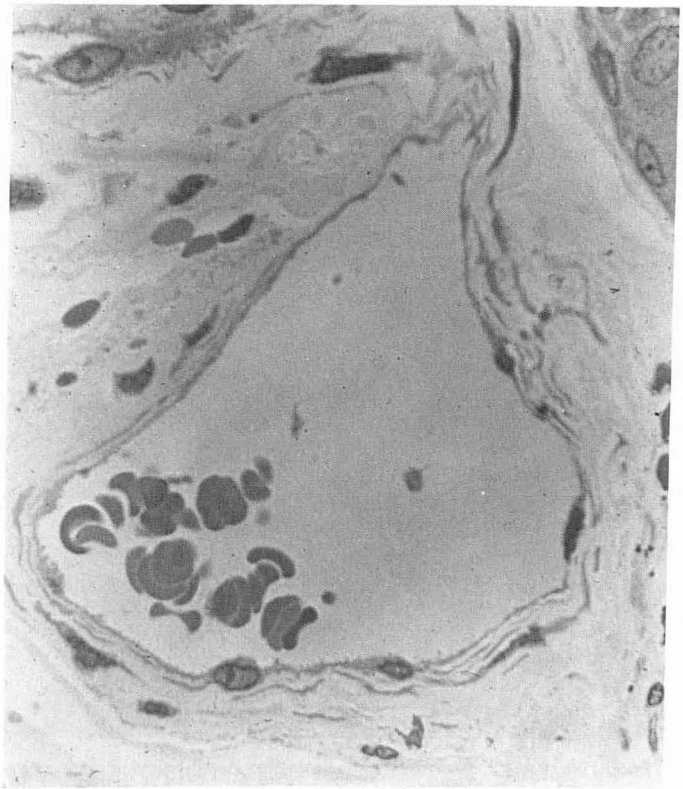

FIG 4. Histologic response occurring after the intracutaneous injection of PGD_2 (3.0 nmol/site) at 0.5 hr in subject 3. Note erythrocytes within dilated dermal venule and perivenular neutrophils (1-μm thick Epon-embedded specimen; Giemsa; × 252).

TABLE I. *Quantitation of infiltrating cells in skin*

Molecule	Time after injection (hr)	Neutrophils (net cells/mm^2)		
		Subject 1	Subject 2	Subject 3
LTD_4	2	0	0	12.8
PGD_2	0.5	295.4	170.7	32.6
PGD_2	2	0	0	0
PGD_2	6	0	38.4	663.9
LTB_4	6	169.3	44.7	440.1
$LTB_4 + PGD_2$	6	651.0	905.0	513.3

At the 6-hr time point neutrophils were again present in 2 of 3 subjects, ranging from 38.4 to 663.9 neutrophils/mm^2.

Biopsy specimens obtained at 6 hr after the injection of LTB_4 (1.6 nmol/site) (Fig 5) exhibited perivenular infiltrating neutrophils in each subject, ranging from 44.7 to 440.1 cells/mm^2 (Table I) without dilation of superficial and deep venules and arterioles. The combination of LTB_4 and PGD_2 evoked a synergistic increase in neutrophil numbers in 2 of 3 subjects. Fibrin was deposited in interstitial array in 2 of 3 subjects after the injection of LTB_4 (Fig 5) and was not increased after the combination with PGD_2. Dermal edema was not observed.

DISCUSSION

The pathobiologic effects occurring after the intracutaneous administration of the oxidative products of arachidonic acid metabolism known to be generated by immediate-type hypersensitivity reactions differed in the character of the erythema and wheal responses to PGD_2 and the 6-sulfidopeptide leukotrienes. The administration of 1.0 nmol of LTC_4, LTD_4, and LTE_4 and 3.0 nmol of PGD_2 elicited an erythema and wheal response that was well developed by 10 min (Figs 1, 2), the point at which the wheal associated with injection of the control site had subsided. The wheal elicited by the 6-sulfidopeptide

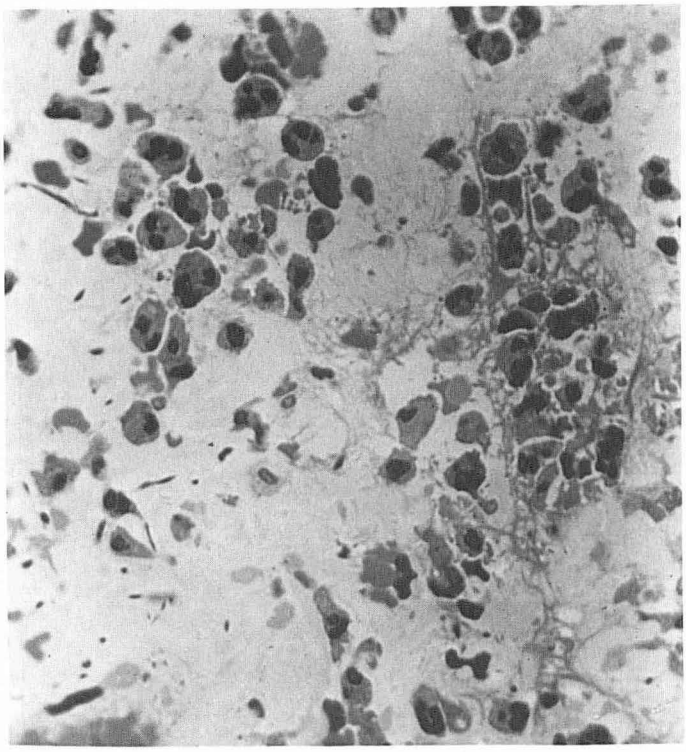

FIG 5. Histologic response occurring after the intracutaneous injection of LTB_4 (1.6 nmol/site) at 6 hr in subject 1. Note neutrophil infiltration and fibrin deposition (1-μm thick Epon-embedded specimen; Giemsa; × 196.9).

leukotrienes exhibited a pale center as compared to the erythema at its periphery and to the erythema extending beyond the wheal. The wheal persisted for more than 2 hr, and the erythema continued to be prominent at 6 hr. The erythema and wheal response to PGD_2 differed in that the intensity of the erythema was uniform over and about the wheal, and the duration of both the erythema and wheal was less than one-half that observed with the 6-sulfidopeptide leukotrienes.

The basis for the differences in clinical morphology in the lesions elicited by PGD_2 as compared to the 6-sulfidopeptide leukotrienes was not apparent either from studies of biopsy specimens carried out at the peak of wheal formation, 0.5 hr for PGD_2 and at 2 hr for LTD_4, or from a comparison at 2 hr when the response elicited by PGD_2 had subsided. The degree of dermal edema was compatible with the magnitude of the clinical response, and the microvascular changes consisting of dilation of capillaries and superficial and deep venules and some arterioles were not different in intensity or uniformity (Figs 3, 4). The constriction of the cutaneous microvasculature in guinea pig skin occurring after the injection of 6-sulfidopeptide leukotrienes [6,10,11] has been demonstrated by a decreased local blood flow measured by isotopic and dye extravasation techniques and is associated with an increase in venular permeability, as indicated by the leakage of protein-bound blue dye. These findings are compatible with the visual observation that the application of 6-sulfidopeptide leukotrienes to the hamster cheek pouch causes constriction of terminal arterioles followed by extravasation of protein-bound fluorescent dye into the surrounding tissues [24]. The clinical wheal and dermal edema observed in human skin reflect the augmentation of venular permeability. The erythema relates to the dilation of the microvasculature, and the presumptive arteriolar constriction would account for the central pallor of the leukotriene-induced wheal, and would not be expected to be maintained in the biopsy specimen.

The prevailing view that vasodilation and augmentation of venular permeability are prerequisites for the in vivo chemotactic response to a chemotactic factor [25] is supported by the synergistic interaction between LTB_4 and PGD_2 in 2 of 3 subjects. Although 3.0 nmol of PGD_2 did not elicit a clinical response that was maintained at 6 hr (Figs 1, 2) as noted in an earlier study [13], the microvasculature was still dilated in biopsy specimens. LTB_4 (1.6 nmol) alone caused a transient wheal in a single subject (Fig 2) and the development of a nontender, indurated, 3.0-mm papule at 6 hr; when combined with PGD_2, LTB_4 caused a tender, indurated 5.0-mm papule that appeared at 4 hr in each subject. The clinical synergy between LTB_4 and PGD_2 was supported by morphologic findings in 2 of 3 subjects. In these subjects, quantitative neutrophil counts (Table I) were increased 4- and 10-fold over the additive effect of each agent alone. In the third subject, the clinical synergy was not confirmed by a quantitative increase in neutrophil number. The PGD_2-initiated neutrophil accumulations were subclinical as compared to the effects of LTB_4, possibly because only the effects of the latter agonist included fibrin deposition. Inasmuch as PGD_2 has been demonstrated to be a chemokinetic factor in vitro [26], it could be argued that its combination with the chemotactic factor LTB_4 was synergistic in terms of the neutrophil as a target cell, in addition to providing the necessary alterations in the microvasculature. Moreover, these interactive effects may have clinical importance in that the inhibition of the cyclooxygenase pathway by nonsteroidal anti-inflammatory agents could be beneficial or detrimental depending on the responses of cells to the altered array of products produced.

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3,4-Dihydroxybenzylamine: An Improved Dopamine Analog Cytotoxic for Melanoma Cells in Part Through Oxidation Products Inhibitory to DNA Polymerase

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The dopamine analog 3,4-dihydroxybenzylamine (3,4-DHBA), a novel antitumor agent, was shown to inhibit directly DNA polymerase in cells of the deeply pigmented murine melanoma, S-91A, permeabilized to nucleotides by lysolecithin. In contrast, levodopa and dopamine did not inhibit DNA polymerase in permeabilized cells in the absence of exogenous tyrosinase. Analysis using isolated DNA polymerase showed that the inhibitory activity of the ortho dihydroxy compounds was totally dependent upon enzymatic activation. The enzymatic activation of the ortho derivative 3,4-DHBA by tyrosinase results in two reactive species: a semiquinone intermediate and a less reactive quinone. Inhibition of DNA polymerase by activated 3,4-DHBA was shown by dialysis and kinetic studies to involve an irreversible reaction which occurs at two inhibitor interaction sites as determined by a Hill plot analysis. Double-stranded DNA protected the enzyme from inhibition by 3,4-DHBA, suggesting that the inhibitory sites are at or near the template-initiator binding site.

Levodopa, dopamine, and the therapeutically superior analog, 3,4-dihydroxybenzylamine (3,4-DHBA) have been shown to be effective antitumor agents in a variety of experimental melanoma tumors [1–3]. Recently, preliminary studies have suggested that these agents are capable of inhibiting replication in human melanoma and are in a clinical trial [4,5].

The initial studies with these drugs revealed that they inhibited thymidine incorporation in vitro, while having minimal effects on RNA or protein synthesis [1,2]. It appeared that the immediate cellular effect of these catechols was a direct and selective inhibition of DNA synthesis. Further studies demonstrated that these drugs, in the presence of the polyphenol oxidase, tyrosinase (which catalyzes the oxidation of tyrosine to melanin in melanocytes), are capable of inhibiting sulfhydryl-dependent DNA polymerases [3,4]. We have recently reported a detailed analysis with the sulfhydryl-dependent enzyme, reverse transcriptase (RT), which showed that active oxidation by tyrosinase produces a more reactive intermediate—semiquinone—than either the completely reduced or oxidized forms of 3,4-DHBA [6]. The findings of Mason et al [7], and more recently, Felix and Sealy [8], which demonstrate by electron spin resonance that *o*-benzosemiquinones are formed by tyrosinase oxidation of catechols, support this concept of a highly reactive intermediate inhibitory species.

We have extended our studies of the inhibitory action of 3,4-DHBA to the enzyme, DNA polymerase α, which is likely to be

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Abbreviations:
3,4-DHBA: 3,4-dihydroxybenzylamine
DTE: dithioerythritol
IC_{50}: concentration resulting in 50% inhibition of enzyme
RT: reverse transcriptase