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Inhibition of mouse epidermal 12-lipoxygenase by 2,3,4-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861)

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2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) strongly inhibited epidermal lipoxygenase activity which was determined by the formation of [^{14}C]12-hydroxy-5,8,10,14-eicosatetraenoic acid by incubating [^{14}C]arachidonic acid with cytosol fraction of epidermal homogenate of CD-1 mice. AA861 failed to inhibit epidermal cyclooxygenase activity. The present results indicate that AA861 is a potent inhibitor of 12-lipoxygenase in mouse epidermis (IC50 1·9 μM).

Most of the known lipoxygenase inhibitors, such as phenidone (Blackwell & Flower 1978), 3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline (BW755C) (Higgs et al 1979) and 5,8,11,14-eicosatetraynoic acid (ETYA) (Hamberg & Samuelsson 1974), also inhibit cyclooxygenase activity to varying extents. Lipoxygenase inhibitors without cyclooxygenase inhibition are required for studying the biochemical and physiological actions of different arachidonic acid metabolites. Recently, several lipoxygenase inhibitors without cyclooxygenase inhibition, such as NDGA (Hamberg 1976), 15-HETE (Vanderhoek et al 1980), nafazatrom (Honn & Dunn 1982), baicalein (Sekiya & Okuda 1982), esculetin (Sekiya et al 1982), caffeic acid (Nakao et al 1982) and 5,6-methanoleukotriene A_4 (Koshihara et al 1982), have been reported. Newly synthesized 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) is one of these drugs and it was reported to selectively inhibit 5-lipoxygenase of guinea-pig peritoneal leukocytes without inhibiting 12-lipoxygenase from bovine platelets and cyclooxygenase from bovine vesicular gland (Yoshimoto et al 1982). However, the potency and selectivity of lipoxygenase inhibitors may vary with species of animal or type of tissues and cells.

Recently, we investigated the effects of various lipoxygenase inhibitors on mouse epidermal lipoxygenase activity (Kato et al 1983) because we had proposed the hypothesis that the lipoxygenase product(s) of arachidonic acid is involved in the mechanism of 12-*O*-tetradecanoylphorbol-13-acetate-induced skin tumour promotion and ornithine decarboxylase induction (Kato et al 1983; Nakadate et al 1982a, b, c). Therefore, we investigated the effect of AA861 on mouse epidermal lipoxygenase and cyclooxygenase activities, and found that AA861 inhibits mouse epidermal 12-lipoxygenase and is not a selective 5-lipoxygenase inhibitor.

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Methods

[1-^{14}C]Arachidonic acid (52·9 mCi mmol^{-1}) was obtained from New England Nuclear, Boston, MA. AA861 was kindly supplied by Dr S. Terao, Takeda Chemical Industries, Ltd, Osaka, Japan. 12-Hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) and 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) were synthesized using rat platelets (Hamberg & Samuelsson 1974) and rabbit peritoneal polymorphonuclear leukocytes (Borgeat & Samuelsson 1979), respectively. Female CD-1 mice (Charles River, Atsugi, Japan), 7–8 weeks, were shaved of dorsal hair at least 2 days before they were used, and only those mice in a resting phase of the hair cycle were used. The 105 000*g* supernatant fraction of epidermis homogenized in 100 mM Tris-HCl buffer (pH 7·5) containing 1 mM EDTA was used for lipoxygenase assay (Kato et al 1983). [^{14}C]Arachidonic acid (final 3·6 μM) was added to the reaction tubes containing an aliquot (500 μl, about 1 mg protein) of supernatant and calcium ion (final 2 mM) which were preincubated for 5 min with AA861, to initiate the reaction which was carried out for 10 min at 37 °C and terminated by the addition of 2·0 ml of a solvent mixture consisting of 15 parts ethyl acetate, 2 parts methanol and 1 part 0·4 M citric acid. The products formed from [^{14}C]arachidonic acid during the incubation were extracted by the above organic solvent and analysed by silica gel thin-layer chromatography (tlc) using the solvent system: benzene–ether–ethanol–acetic acid (50 : 40 : 2 : 0·2, by vol.) as described by Kato et al (1983). The 105 000*g* particulate fraction of epidermis, which was homogenized in 100 mM Tris-HCl buffer (pH 8·0) containing 1 mM EDTA, 2 mM glutathione and 2 μM haemoglobin, was used for cyclooxygenase assay. The incubation, like that for lipoxygenase assay, was without addition of calcium ion. The products from [^{14}C]arachidonic acid were analysed by silica gel tlc as for the lipoxygenase assay. The solvent system used was ethyl acetate–n-hexane–acetic acid–water (57 : 26 : 6 : 60, by vol., upper layer).

Results and discussion

The cytosol fraction of mouse epidermis effectively converts [^{14}C]arachidonic acid to lipoxygenase products

(Hammarstrom et al 1979: Kato et al 1983). The main lipoxygenase product is 12-HETE in mouse epidermis (Hammarstrom et al 1979; Kato et al 1983) as it is in man (Hammarstrom et al 1979) and guinea-pig (Ruzicka et al 1983) epidermis; the 5-lipoxygenase product, 5-HETE was not detected (Kato et al 1983). The production of [^{14}C]12-HETE was markedly inhibited by the addition of AA861 (Figs 1, 3). The 50% inhibition of 12-lipoxygenase, which was estimated by measuring the formation of [^{14}C]12-HETE from [^{14}C]arachidonic acid, was observed with AA861 at 1·9 μM.

When [^{14}C]arachidonic acid was incubated with 105 000*g* particulate fraction of mouse epidermis and labelled products were analysed by tlc, a radioactive peak which overlayed that for authentic prostaglandin E_2 (PGE_2) was obtained (Fig. 2). The production of this metabolite was inhibited by 1 μM of indomethacin (data not shown). Thus, the epidermal cyclooxygenase activity was estimated by measuring the formation of [^{14}C]PGE_2. As clearly shown in Figs 2 and 3, AA861 failed to inhibit cyclooxygenase activity. Yoshimoto et al (1982) also reported that bovine vesicular gland cyclooxygenase was not affected by AA861 and our result is consistent with this.

Yoshimoto et al (1982) reported that AA861 fails to inhibit bovine platelet 12-lipoxygenase and even at 100 μM AA861 only partially inhibits porcine leukocyte and rat lung 12-lipoxygenase. In the present study, AA861 strongly inhibited epidermal lipoxygenase and its IC50 value was 1·9 μM. Neichi et al (1983) reported that esculetin showed inhibitory effects on 5- and 12-lipoxygenases of cloned mastocytoma cells, although it was originally reported as a selective inhibitor of 12-lipoxygenase in platelets (Sekiya et al 1982). Vanderhoek et al (1980) also reported that the endogenous

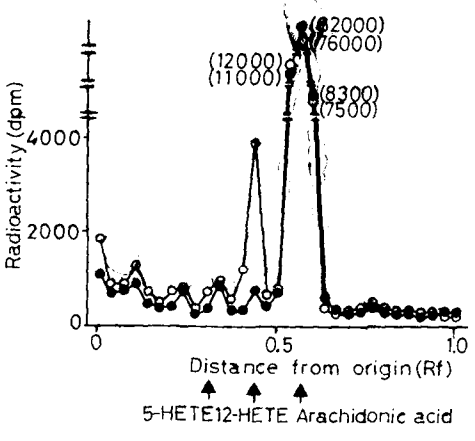

FIG. 1. Thin-layer chromatogram of products formed from [^{14}C]arachidonic acid during the 10 min incubation at 37 °C with 105 000*g* supernatant fraction of mouse epidermal homogenate in the presence (●) or absence (○) of AA861 (10 μM). Migrating positions of authentic arachidonic acid, 5-HETE and 12-HETE are also shown. Details are given in Methods.

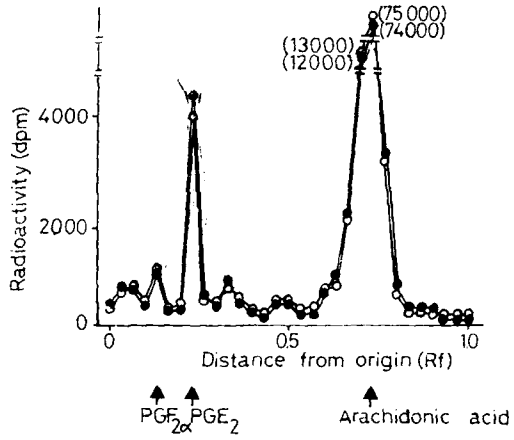

FIG. 2. Thin-layer chromatogram of products formed from [^{14}C]arachidonic acid during the 10 min incubation at 37 °C with 105 000*g* particulate fraction of mouse epidermal homogenate in the presence (●) or absence (○) of AA861 (10 μM). Migrating positions of authentic arachidonic acid, PGE_2 and $PGF_{2\alpha}$ are also shown. Details are given in Methods.

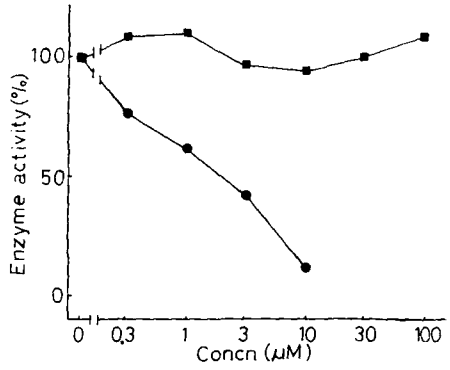

FIG. 3. Effects of AA861 on epidermal lipoxygenase and cyclooxygenase. Details are given in Methods. The results are expressed as the percent formation of [^{14}C]12-HETE (for lipoxygenase activity) and [^{14}C]PGE_2 (for cyclooxygenase activity) from [^{14}C]arachidonic acid. Enzyme activities in the absence of AA861 are shown to be 100%. Each point represents the mean of duplicate determinations. ●, lipoxygenase; ■, cyclooxygenase.

lipoxygenase inhibitor 15-HETE inhibited 5-lipoxygenase of rabbit and human polymorphonuclear leukocytes, but enhanced 5-HETE and leukotriene B_4 production in PT-18 mast/basophil cells (Vanderhoek et al 1982). In addition, it was reported that the formation of 5-HETE by rabbit polymorphonuclear leukocytes was not blocked by ETYA (Borgeat et al 1976; Vanderhoek et al 1980), however ETYA has been shown to inhibit 5-HETE production by human neutrophils and eosinophils (Goetzl 1980; Goetzl et al 1980).

Since the inhibition of mouse epidermal 12-lipoxygenase by AA861 was extremely potent compared with other reported 12-lipoxygenase systems (Yoshimoto et al 1982), the present study clearly demonstrates that AA861 is not always a selective

inhibitor of 5-lipoxygenase. The potency and selectivity of lipoxygenase inhibitors may be altered in accordance with animal species or types of tissues and cells, or experimental conditions.

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Effect of dopaminergic drugs on striatal acetylcholine concentration

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Striatal acetylcholine concentration was determined after administration of varying doses of apomorphine, (+)-PPP and (−)-PPP to rats. (+)-PPP at 3 and 10 mg kg^{-1} is a dopamine agonist, whereas (−)-PPP at 0·3 and 3 mg kg^{-1} is a dopamine antagonist in the striatum.

Dopamine (DA) is an inhibitory neurotransmitter in the striatum (McLennan & York 1967). Treatment with dopamine agonists like (−)-dopa, apomorphine, trivastal, bromocriptine, and lergotrile increases the concentration of acetylcholine (ACh) in the striatum of rats by their inhibitory effect on the intrastriatal cholinergic neurons (Consolo et al 1974; Sethy & VanWoert 1974a, b; Sethy 1979). On the other hand, DA antagonists such as chlopromazine, haloperidol, pimozide, metoclopramide, and molindone decrease striatal ACh concentration by blocking the inhibitory action of DA on the cholinergic neurons (McGeer et al 1974; Sethy & VanWoert 1974a, b, c; Sethy 1976, 1979).

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Recently, the existence of dopamine autoreceptors on the presynaptic terminals of nigrostriatal dopaminergic neurons has been demonstrated and they may serve to regulate the influence of DA neurons on post-synaptic follower cells (Roth 1979). Thus, autoreceptors located on nigrostriatal dopaminergic neurons may affect the activity of follower intrastriatal cholinergic neurons. If presynaptic DA receptors are different from their post-synaptic counterparts, then a specific agonist of autoreceptors may reduce dopamine transmission, leading to an increase in release of ACh and subsequently a decrease in ACh concentration, just like the DA antagonist. Apomorphine, at small doses (DiChiara et al 1978), and (+)- and (−)-PPP (Hjorth et al 1983) have been shown to be autoreceptor agonists of the nigrostriatal dopaminergic system in the rat. Administration of these drugs may *decrease* dopamine transmission, which in turn may reduce ACh concentration in the striatum. The results of such investigations are now described.