a form of P-450 whose activities are coupled with aryl hydrocarbon hydroxylation is preferently induced.

It is interesting to note that the induction of drug metabolism by rifampicin in man shows a resemblance, with respect to changes in *in vivo* parameters [1] and in *in vitro* parameters [29]. The pig therefore seems to be a good animal model for studying the mechanism of induction by rifampicin. Especially this species is useful for further *in vivo* and *in vitro* studies in which the induction by the antibiotic and other inducers (phenobarbital, 3-methylcholanthrene) is compared, for instance with respect to the induction of metabolic pathways of hexobarbital, antipyrine and other test substances. The purification of pig microsomal cytochrome P-450 will be a useful tool [18].

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Inhibition of aromatic amino acid decarboxylase and depletion of biogenic amines in brain of rats treated with α-monofluoromethyl *p*-tyrosine: similitudes and differences with the effects of α-monofluoromethyldopa

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Monofluoromethyldopa (MFMD) is a selective, irreversible inhibitor of aromatic amino acid decarboxylase (AADC) [1, 2], so potent that the decarboxylation of Dopa and 5-hydroxytryptophane (5-HTp) become rate-limiting in the biosynthesis of catechol- and indoleamines, respectively [3, 4]. In our attempts to restrict AADC inhibition and amine depletion to catecholaminergic neurons, we decided to take advantage of the substrate specificity and the neuronal localization of tyrosine hydroxylase (TH), the first and normally rate-limiting enzyme of catecholamine biosynthesis. For this purpose, we needed a compound which did not inhibit AADC directly but was transformed by the catalytic action of TH into an AADC inhibitor of the potency of MFMD. We wish to report that α-monofluoromethyl *p*-tyrosine (MFMT) has the required properties.

Materials and methods. MFMD, MFMT and its methyl ester (MFMT-Me) were synthesized in our laboratory. α-Methyl *p*-tyrosine, methyl ester (MT-Me) and Dopa were obtained from Sigma Chemical Co.; haloperidol used was

a pharmaceutical preparation (®Haldol: Jannsen-Lebrun). [1-^{14}C]Dopa was obtained from the Radiochemical Centre (Amersham, Bucks., U.K.).

AADC was partially purified from hog kidney; it was assayed using [1-^{14}C]Dopa as substrate by measuring the release of $^{14}CO_2$ [5]. The same assay was used to determine AADC activity in tissue homogenates.

Male Sprague–Dawley rats were used in the animal work. The methyl ester of MFMT was used for *in vivo* work as the tyrosine derivatives are extremely insoluble. The drug (free base) was dissolved in 1% asorbic acid and was given orally. MT-Me and haloperidol were given i.p. The animals were decapitated at appropriate times; the brains were divided sagitally. One half was immediately homogenized in 9 volumes of 50 mM phosphate (pH = 7.4; 10 μM pyridoxal phosphate; 10 mM mercaptoethanol) and served for AADC determination. The other half was frozen at −80° until homogenized in 0.2 N $HClO_4$ for the determination of catechols and indoles by reversed-phase HPLC with electrochemical detection [6].

Results and discussion. There was no time-dependent decrease of AADC activity upon addition of 100 μM–1 mM MFMT to a partially purified preparation of the enzyme (hog kidney). Under the same conditions, 10 μM MFMD inhibited AADC by over 90% within 5 min (Fig. 1). However, after oral administration of MFMT-Me to rats there was a time- and dose-dependent decrease of AADC activity in the brain. The effects on other organs will be discussed elsewhere. The progress of the inhibition was much slower than with MFMD. Figure 2 compares the effects of a dose of 100 mg/kg of MFMD and MFMT-Me on rat brain AADC activity as a function of time after administration. Whereas the inhibition by MFMD reached a plateau within 6 hr, that by MFMT-Me was much slower and reached its maximum around 24 hr. These findings suggest that MFMT-Me is transformed *in vivo* into an efficient AADC inhibitor. This inhibitor is most probably MFMD as the presence of this compound in the brain of rats treated with MFMT-Me could be demonstrated (HPLC with electrochemical detection). Indeed, MFMT is a substrate of TH partially purified from hog adrenals: at 100 μM, the rate of hydroxylation of MFMT was approximately 1/30th that of tyrosine. Full kinetics will be published elsewhere.

The decrease of AADC activity was influenced by agents known to inhibit or stimulate TH activity. Administration of MT-Me before MFMT-Me and then at 6 hr intervals until death resulted in a significant reduction of AADC inhibition (Fig. 3a). On the contrary, when animals were pre-treated with haloperidol at a dose known to increase

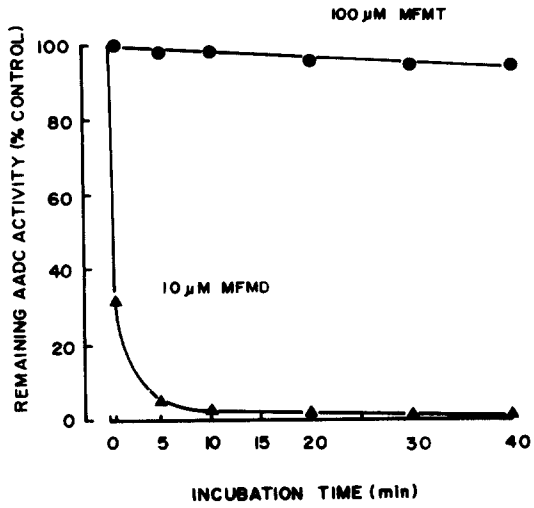

Fig. 1. Time-dependent inhibition of partially purified hog kidney AADC by MFMD and MFMT. A preparation of hog kidney AADC was incubated either with MFMD (10 μM) or MFMT (100 μM). At the indicated time intervals, aliquots were withdrawn and assayed for remaining enzyme activity under the conditions described in the text. The methyl esters of MFMD or MFMT had no effect on AADC activity *in vitro* (not shown).

TH activity rapidly [7], MFMT-Me produced a greater inhibition than in naïve animals (Fig. 3b).

MFMT-Me decreases the brain concentration of norepinephrine and dopamine in a time and dose-dependent manner even though AADC is not totally inhibited. In order to prove that the amine depleting effects are restricted to catecholaminergic neurons even after prolonged administration, MFMT-Me was given daily to rats for six consecutive days. Figure 4 represents the changes in biogenic amine metabolism 24 hr after the last dose. AADC activity was decreased in a dose-dependent manner; however, even in the high-dose group there was still significant enzyme activity remaining. Despite the presence of AADC activity, Dopa was accumulated at all doses and the concentration increased with the dose used. This tends to rule out that amine depletion is achieved by inhibition of TH. Dopamine depletion became significant at the dose 25 mg/kg; at the highest dose this depletion was *ca* 50%.

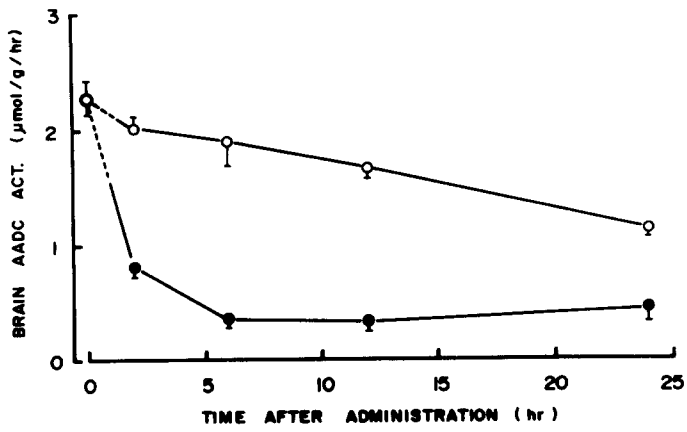

Fig. 2. Inhibition of brain AADC by MFMD and MFMT-Me as a function of time in rats. MFMD (full circles) and MFMT-Me (open circles) were given at a dose of 100 mg/kg to rats by gavage. The animals were killed at indicated times and brain AADC activity was determined as indicated in Materials and Methods. Each value is the mean ±S.E.M. of five animals.

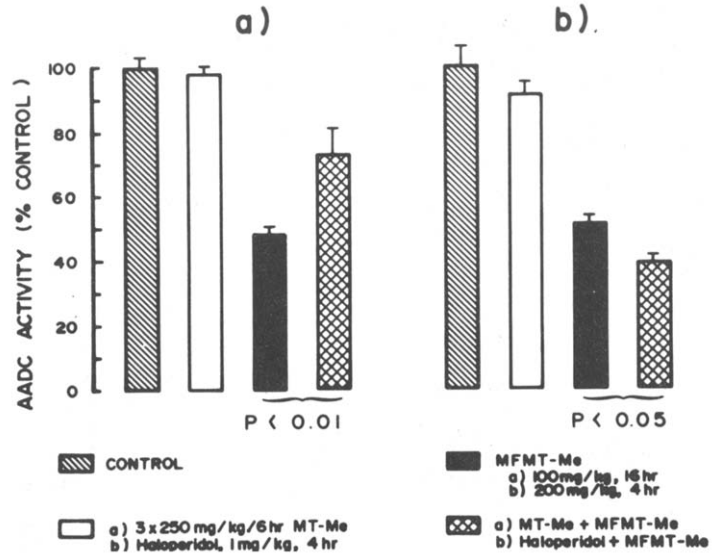

Fig. 3. Effects of factors influencing tyrosine hydroxylase activity on AADC inhibition by MFMT-Me in rat brain. (a) Rats were injected i.p. with MT-Me (250 mg/kg) or saline 30 min before oral administration of MFMT-Me (100 mg/kg). The animals were given two other doses of MT-Me (or saline) at 6 hr intervals. They were killed by decapitation 16 hr after MFMT-Me administration. (b) Animals were pretreated with one i.p. injection of 1 mg/kg haloperidol. Thirty minutes later, MFMT-Me (200 mg/kg) was given orally. The animals were killed 4 hr after MFMT-Me administration. AADC activity was measured in brain homogenates. Each value is the mean ±S.E.M. of five animals. Control values are the same as in Fig. 1.

The decrease of Dopac, the main metabolite of dopamine, approximately paralleled that of dopamine. The increase of Dopac in the 10 mg/kg group was not due to the dopamine-releasing effect of MFMT-Me as such an increase was not seen a short time after administration of 10–20 times higher doses of the tyrosine analogue (not shown). Norepinephrine was already depleted by 50% in the small dose group. Multiplying the daily dose by five did not produce a greater norepinephrine depletion. In agreement with our starting postulate, there was no effect of MFMT-Me on serotonine metabolism: no accumulation of the serotonine precursor 5-HTp, no decrease of 5-HT or of its metabolite 5-hydroxyindole acetic acid (5-HIAA).

A few points need comment. When the depletion of

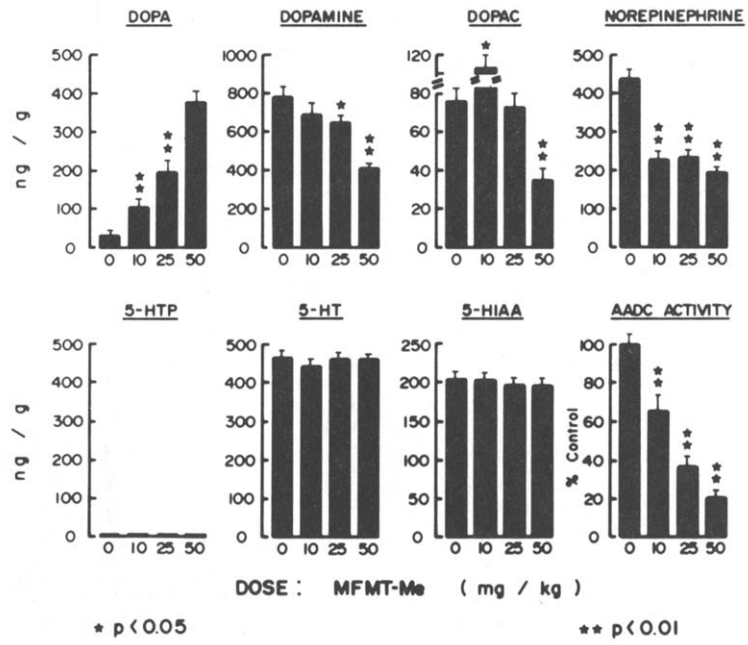

Fig. 4. Dose-dependent effects of MFMT-Me on biogenic amine metabolism in rat brain after sub-acute administration. MFMT-Me was given daily by gavage to groups of rats at doses of 10, 25 and 50 mg/kg for six consecutive days. Twenty-four hours after the last dose, the animals were killed; one half of the brain was used for AADC determinations, the other half for measurements of catechols and indoles. Each value represents the mean ±S.E.M. of five animals.

catecholamines is achieved by inhibition of TH by MT-Me, the concentration of dopamine and norepinepherine decrease in parallel [8]. As seen in Fig. 4, MFMT-Me depletes norepinephrine before dopamine. *In vitro* at least, neither MFMT-Me nor MFMT inhibits dopamine β-hydroxylase at concentrations up to 1 mM. To explain the preferential depletion of norepinephrine by MFMT-Me, we would like to suggest that the ratio between the AADC and TH activities is smaller in noredrenergic neurons than in dopaminergic neurons. Less inhibition of AADC activity is therefore necessary before the decarboxylation of Dopa becomes rate-limiting in the synthesis of norepinephrine. Depletion of catecholamines by MFMT-Me is found with significant AADC remaining—this was not the case with MFMD [3]. A reasonable explanation is that MFMD indiscriminately inhibits AADC whether it is functional in amine synthesis or not, while MFMT which needs activation by TH will inhibit AADC in those sites where TH-activity is the most elevated, i.e. in sites of active amine synthesis. Therefore MFMT should be selective for neuronal AADC and could possibly be used as a marker for catecholaminergic neurons.

In conclusion, MFMT, or better MFMT-Me, has a number of advantages over MFMD as a means to regulate catecholamine synthesis and hopefully to study the pharmacological consequences thereof: selective depletion of catecholamines over indoleamines, selectivity at low doses for the norepinephrine system, sensitivity to factors regulating TH activity. We believe that this represents the first attempt to use an enzyme to generate a suicide inhibitor for an enzyme coming later in the normal catabolic cascade.

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Reversibility of protein synthesis inhibition by quassinoid antineoplastic agents in a rabbit reticulocyte system

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Bruceantin, a quassinoid now in phase II clinical trials, was first isolated from *Brucea antidysenterica* and was shown to have potent antitumor activity in several animal screens [1, 2]. The antitumor activity of bruceantin is presumed to be due to its strong inhibition of eukaryotic protein synthesis, although it does inhibit DNA synthesis to a lesser extent [3]. Detailed studies have shown that bruceantin binds reversibly to the peptidyl transferase center of eukaryotic ribosomes and inhibits peptide bond formation [4]. More recently, the structurally related compound brusatol has been obtained from *Brucea javonica* [5, 6] and has been shown to possess potent antitumor activity [5, 7]. Brusatol inhibits protein synthesis by the same mechanism as bruceantin in both rabbit reticulocytes [8] and P-388 cells [7].

Liao *et al.* [3] originally classified these compounds as irreversible inhibitors of protein synthesis. Their data conflict with the later observations by Fresno *et al.* [4] that quassinoids bind reversibly to the ribosome. Since the question of reversibility of inhibition by potential therapeutic agents is of practical interest, the experiments in this paper were designed to resolve this issue.

Materials and Methods

[^{3}H]Leucine (125 Ci/mmole) was obtained from Amersham-Searle. [^{14}C]Trichodermin (20.4 mCi/mmole, 2.66 mM) was obtained from Research Triangle Institute (Research Triangle Park, NC) on special contract. The [^{14}C]trichodermin was synthesized from trichodermol (supplied by Dr. W. O. Godfredson, Leo Pharmaceutical Products) and [1-^{14}C]acetic anhydride as previously described by Barbacid and Vazquez [9]. Bruceantin and brusatol were prepared as described previously [5, 6]. Both compounds were initially dissolved in ethanol–acetone (1:1). After removal of the acetone under a stream of nitrogen, each drug was diluted to a 200–500 μM stock solution in 2.5% ethanol and stored in small aliquots at −20°. ATP, GTP, and creatine phosphate were obtained from Sigma. Creatine phosphokinase was obtained from Boehringer–Mannheim.

Rabbit reticulocyte lysates were prepared from New Zealand minikin rabbits weighing 1–2 kg as described by Hardesty *et al.* [10]. Dialysis of lysates was carried out against buffer D (5 mM Tris–Cl, pH 7.6; 1 mM 2-mercaptoethanol; 1 mM $MgCl_2$). The 80 S run-off ribosomes were prepared from the lysate essentially as described by Falvey and Staehelin [11].

Endogenous protein synthesis in rabbit reticulocyte lysates was carried out in an assay containing: 2/3 vol. of lysate, 5 mM Tris–Cl (pH 7.6), 78 mM KCl, 1.4 mM $MgCl_2$, 7.8 mM creatine phosphate, 0.3 mg/ml creatine phosphokinase, 15 μM hemin, 0.1 mM ATP, 0.05 mM GTP, 0.1 mM each of nineteen amino acids, 0.6 mM 2-mercaptoethanol, and 50 μCi [^{3}H]leucine (125 Ci/mmole). Incubation was at