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FURTHER STUDIES ON THE INHIBITION OF MONOAMINE SYNTHESIS BY MONOFLUOROMETHYLDOPA

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1 α-Monofluoromethyldopa (MFMD, RMI 71963), a potent and selective enzyme-activated irreversible inhibitor of aromatic L-amino acid decarboxylase produces a substantial and long-lasting decrease in the catecholamine content of mouse brain, heart and kidney.

2 Single doses of MFMD reduce the 5-hydroxytryptamine concentration of mouse brain without altering the tryptophan concentration.

3 In animals treated with MFMD, peripheral but not brain noradrenaline is restored within 1 h to control levels by an intraperitoneal injection of dopamine.

Introduction

Aromatic L-amino acid decarboxylase (AADC EC 4.1.1.26) catalyses the formation of dopamine from L-DOPA and of 5-hydroxytryptamine (5-HT) from L-5-hydroxytryptophan (5-HTP) (Bartholini & Pletscher, 1975). We have recently found that DL-α-monofluoromethyldopa (MFMD, RMI 71963) is a potent enzyme-activated, irreversible inhibitor of AADC: when administered to mice it greatly decreases the concentrations of dopamine, noradrenaline and 5-HT in brain, heart and kidney (Jung, Palfreyman, Wagner, Bey, Ribéreau-Gayon, Zraïka & Koch-Weser, 1979a). The present studies were undertaken to characterize further the monoamine-depleting effects of MFMD as functions of dose and duration of treatment. We also searched for selectivity in the action of MFMD, in terms of central versus peripheral depletion and catecholamine versus indoleamine depletion.

Part of this work has been presented in preliminary form to the British Pharmacological Society (Jung, Palfreyman, Ribéreau-Gayon, Wagner & Zraïka, 1979b).

Methods

Male CD_1 Albino mice (20 to 30 g) from Charles River, France were used. Animals were housed in groups and had free access to food and water. They were maintained on a 12 h light–dark cycle at an ambient temperature of 23°C.

MFMD was dissolved in 0.9% w/v NaCl solution (saline) and administered intraperitoneally. All control animals received an equivalent volume of vehicle.

Preparations of tissues

Animals were killed by decapitation. The tissues were rapidly dissected, weighed and immediately frozen in liquid nitrogen. Within 24 h the tissues were homogenized (Potter–Elvehjem or Polytron) in 2 ml 0.4 N $HClO_4$ containing 0.05% (w/v) disodium edetate (EDTA), 0.1% (w/v) sodium metabisulphite and α-methyldopa (usually 250 ng/ml) as internal standard. After centrifugation (10,000 *g* for 10 min at 4°C) the catechols (MFMD, L-DOPA, dopamine, noradrenaline, adrenaline and dihydroxyphenylacetic acid (DOPAC)) in an aliquot of the supernatant were analysed following adsorption on and elution from alumina. In some experiments a further aliquot of the 0.4 N $HClO_4$ supernatant was used for determination of 5-HT and tryptophan.

Methods of assay

The catechols MFMD, DOPA, dopamine, noradrenaline and DOPAC, were determined by reversed-phase ion-pair HPLC with electrochemical detection as described in detail by Wagner, Palfreyman & Zraïka (1979).

For the determinations of 5-HT and tryptophan a μBondapak C_{18} column (300 mm long; 3.5 mm i.d.) was used. The eluent was a 87/13 (v/v) mixture of a citrate–phosphate buffer (ionic strength 0.02 M) with methanol and containing octane sulphonic acid (OSA,

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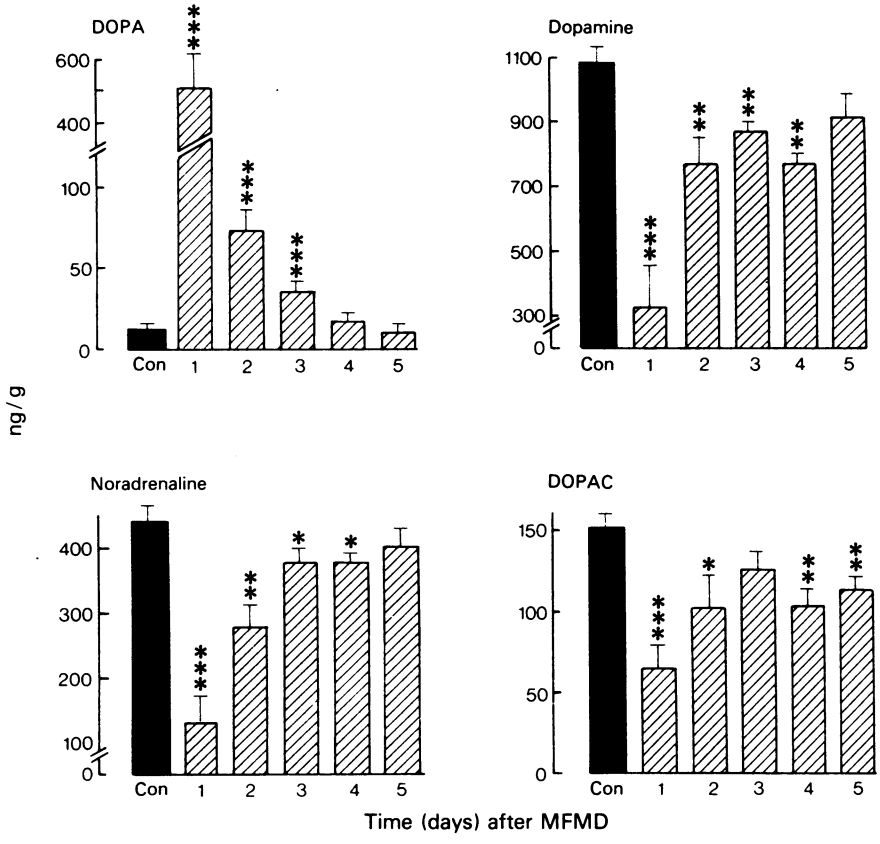

Figure 1 Time course of effect of monofluoromethyldopa (MFMD) on catechol concentrations in mouse brain. Groups of 5 mice were injected i.p. with MFMD (100 mg/kg i.p.) and killed 1, 2, 3, 4 and 5 days later. A further group of 5 mice injected (i.p.) with saline served as controls (Con). Catechols were measured in brain extracts as detailed in the methods. Values shown in ng/g; vertical lines show s.e. mean. In this and subsequent figures statistical significance is shown by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Student's t test, two-tailed).

2.6×10^{-3} M) and EDTA (5×10^{-5} M), pH 3.18. Flow rate was 1 ml/min and electrode working potential was set at +0.9 volt versus a Ag/AgCl reference electrode. Using these conditions, we obtained the following retention times (min): 13.3 for 5-HTP, 13.8 for dopamine, 31 for 5-HT and 33 for tryptophan. However, only 5-HT and tryptophan could be measured, because unidentified substances in the tissue extract interfered with dopamine and 5-HTP.

The AADC activity of extracts of brain, heart and kidney was determined by the $^{14}CO_2$ trapping method as used previously (Palfreyman, Danzin, Bey, Jung, Ribéreau-Gayon, Aubry, Vevert & Sjoerdsma, 1978) using L-1-[^{14}C]-DOPA as substrate (Radiochemical Centre Amersham).

Chemicals

L-DOPA, noradrenaline, adrenaline, dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), α-methyldopa, L-5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and L-tryptophan were purchased from Sigma (St Louis, MO). All other reagents were analytical grade from Merck (Darmstadt, Germany). DL-α-Monofluoromethyldopa (MFMD, RMI 71963) was synthesized in our centre (Bey, 1978).

Results

Time-course of effect of a single dose of monofluoromethyldopa on concentrations of DOPA, catecholamines and dihydroxyphenylacetic acid in whole brain of mice

Mice were injected intraperitoneally (i.p.) with MFMD 100 mg/kg and killed 1, 2, 3, 4 or 5 days later. Figure 1 shows that the concentrations of dopamine,

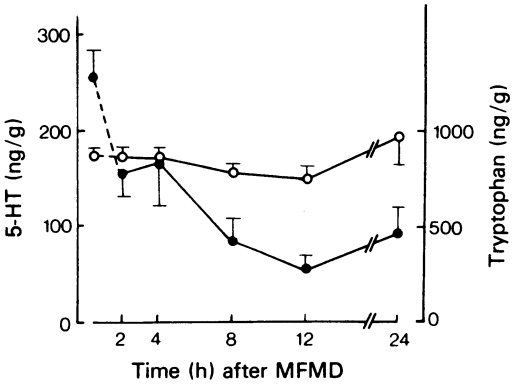

Figure 2 Time course of effect of monofluoromethyldopa (MFMD) on 5-hydroxytryptamine (5-HT) and tryptophan concentrations in mouse brain. Groups of 5 mice were injected with MFMD (250 mg/kg i.p.) and killed 2, 4, 8, 12 and 24 h later. A group of 5 mice injected with saline served as control. 5-HT (●) or tryptophan (○) was determined in brain extracts as detailed in the methods. Values are shown in ng/g; vertical lines show s.e. mean. All values of 5-HT are significantly different ($P < 0.05$) from control. Tryptophan concentrations do not differ significantly ($P > 0.05$) from control.

noradrenaline and DOPAC were significantly reduced 24 h after the injection of MFMD and slowly returned towards control levels which dopamine and noradrenaline attained by the 5th day after injection. DOPA concentrations, on the other hand, were significantly increased for 3 days.

Time-course of effect of a single dose of monofluoromethyldopa on tryptophan and 5-hydroxytryptamine concentrations in whole brain of mice

Figure 2 shows the effect of a single dose of MFMD 250 mg/kg (i.p.) on tryptophan and 5-HT concentrations of mouse brain at 2, 4, 8, 12 and 24 h after injection. 5-HT concentration decreased progressively during the first 12 h and remained depressed at 24 h. Tryptophan concentration was not significantly altered.

Effect of small repeated doses of monofluoromethyldopa on the catechol concentrations and aromatic amino acid decarboxylase activity of brain, heart and kidney of the mouse

Mice injected with MFMD at doses of 0.25, 1, 5 and 25 mg/kg every 12 h for 3 days and killed 6 h after the last dose showed a dose-related diminution in the catecholamine content of brain, heart and kidney, and a dose-related inhibition of AADC activity (Figure 3). DOPA concentrations were elevated in a dose-related manner in the three tissues.

Effect of an intraperitoneal injection of dopamine on the depletion of catecholamines produced by monofluoromethyldopa

Mice were injected twice, 12 h apart, with MFMD 100 mg/kg (i.p.). Twelve hours after the second dose of MFMD they received dopamine (50 mg/kg i.p.) or saline. One, 2, 3, or 4 h later the mice were killed and the catechol content of brain and heart determined. The intraperitoneal injection of dopamine restored the noradrenaline content of the heart to control levels (Figure 4). Dopamine concentrations were significantly elevated above those found in control hearts. As expected, only a very small proportion of the injected dopamine penetrated the brain (Figure 4).

Discussion

MFMD is the first inhibitor of AADC potent enough to make decarboxylation the rate-limiting step in catecholamine and 5-HT synthesis and to deplete tissue monoamines (Jung *et al.*, 1979a). From the relative activities of tyrosine hydroxylase and AADC (Jung *et al.*, 1979a) it is possible to calculate that AADC activity in mouse brain must be 99% inhibited before it becomes rate-limiting. MFMD does not inhibit tyrosine hydroxylase (Jung *et al.*, 1979a). Two days after MFMD administration the AADC activity of whole brain has returned to 12% of control values (Jung *et al.*, 1979a), but catecholamines in the brain remain reduced for up to 4 days. Preliminary experiments (data not shown) suggest that AADC activity recovers more slowly in synaptosomes than in crude homogenates from MFMD-treated animals. Since synthesis of catecholamines occurs mainly in the nerve endings, this may explain the slow recovery of the catecholamine content.

A single dose of MFMD 250 mg/kg, chosen to inhibit the brain enzyme completely in less than 1 h (unpublished), causes a substantial reduction in the 5-HT content of the brain which is maximal 12 to 24 h after injections. Brain tryptophan content is not altered (Figure 2) but the concentration of 5-HTP is markedly elevated (unpublished). This strongly suggests that MFMD does not modify the synthesis of 5-HT by reducing the availability of its natural precursor, tryptophan or by inhibiting its hydroxylation. Thus inhibition of AADC slows the decarboxylation of both DOPA and 5-HTP and non-selectively depletes both catecholamines and the indoleamine.

A possible approach to selective depletion of peripheral catecholamines would be to administer small doses of MFMD repeatedly. Jung *et al.* (1979a) found

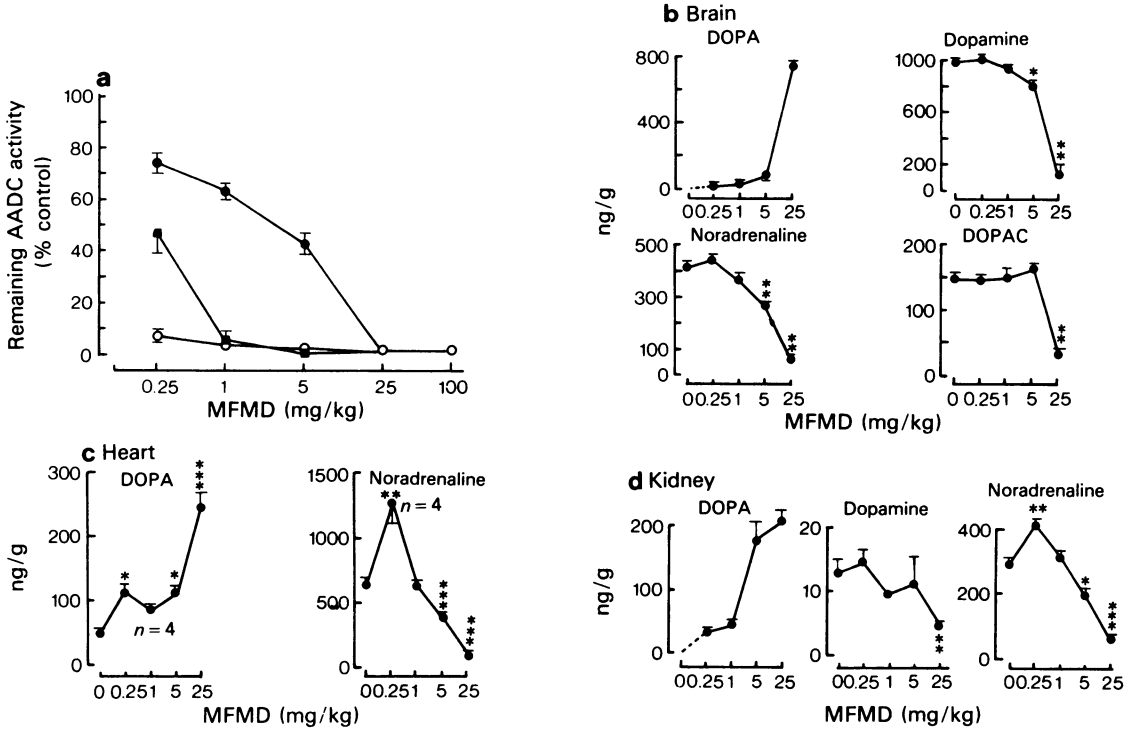

Figure 3 Effects of small, repeated (i.p.) injections of monofluoromethyldopa (MFMD) on the aromatic amino acid decarboxylase (AADC) activity and catecholamine content of brain, heart and kidney of the mouse. Groups of 5 mice were injected intraperitoneally with MFMD 0.25, 1, 5 or 25 mg/kg every 12 h for 3 days. Animals were killed 6 h later and the AADC activity was measured in half the brain and heart and in one kidney by the $^{14}CO_2$ trapping method. The other half of the brain and heart and the remaining kidney were assayed for catecholamines. A group of 5 mice injected i.p. with saline served as controls. (a) Shows the AADC activity as a function of control activity: brain (●); heart (■) and kidney (○). Control activities were brain: 64 ± 1.3 nmol g^{-1} h^{-1}; heart 27.8 ± 1.3 nmol g^{-1} h^{-1} and kidney 100 ± 1.5 nmol g^{-1} h^{-1}. (b) Shows the catecholamine content of brain, (c) of heart and (d) of kidney. Results are expressed as ng/g; vertical lines show s.e. mean.

that small doses (0.25 to 2.5 mg/kg i.p.) of MFMD inhibited the AADC activity of kidney and heart without markedly affecting brain AADC. This preferential inhibition of the enzyme in peripheral tissues is retained when the compound is given repeatedly. Unfortunately, this is not reflected in catecholamine depletion and repeated doses of MFMD high enough to reduce catecholamines in the heart also do so in the brain. The non-selective depletion of catecholamines in the face of a relatively selective effect on AADC probably reflects the fact that almost complete inhibition of AADC is a prerequisite for transmitter depletion.

In an attempt to replenish selectively the peripheral stores of catecholamines depleted by MFMD treatment, we administered dopamine (which does not readily cross the blood–brain barrier) intraperitoneally to MFMD-treated mice. Dopamine restored concentrations of noradrenaline in the heart but not in the brain to the level found in saline-treated control animals. Fozard, Palfreyman, Spedding, Wagner & Woodward (1979) have recently shown that an intravenous infusion of as little as 500 μg/kg dopamine not only replenishes heart noradrenaline stores but also restores the impaired sympathetic nervous activity of MFMD-treated rats to control levels. Through coadministration of MFMD and dopamine it is clearly possible to deplete brain catecholamines while retaining peripheral sympathetic function.

Considerable effort has been directed towards synthesizing inhibitors of the rate-determining enzymes in catecholamine and 5-HT biosynthesis i.e. tyrosine and tryptophan hydroxylase (E.C. 1.14.3.2. and E.C. 1.14.16.4. see Levitt, Spector, Sjoerdsma & Udenfriend, 1965 and Green & Grahame-Smith, 1975) and several examples now exist (see Musacchio, 1975; Green & Grahame-Smith, 1975). Many of these inhibitors effectively lower catecholamine and 5-HT

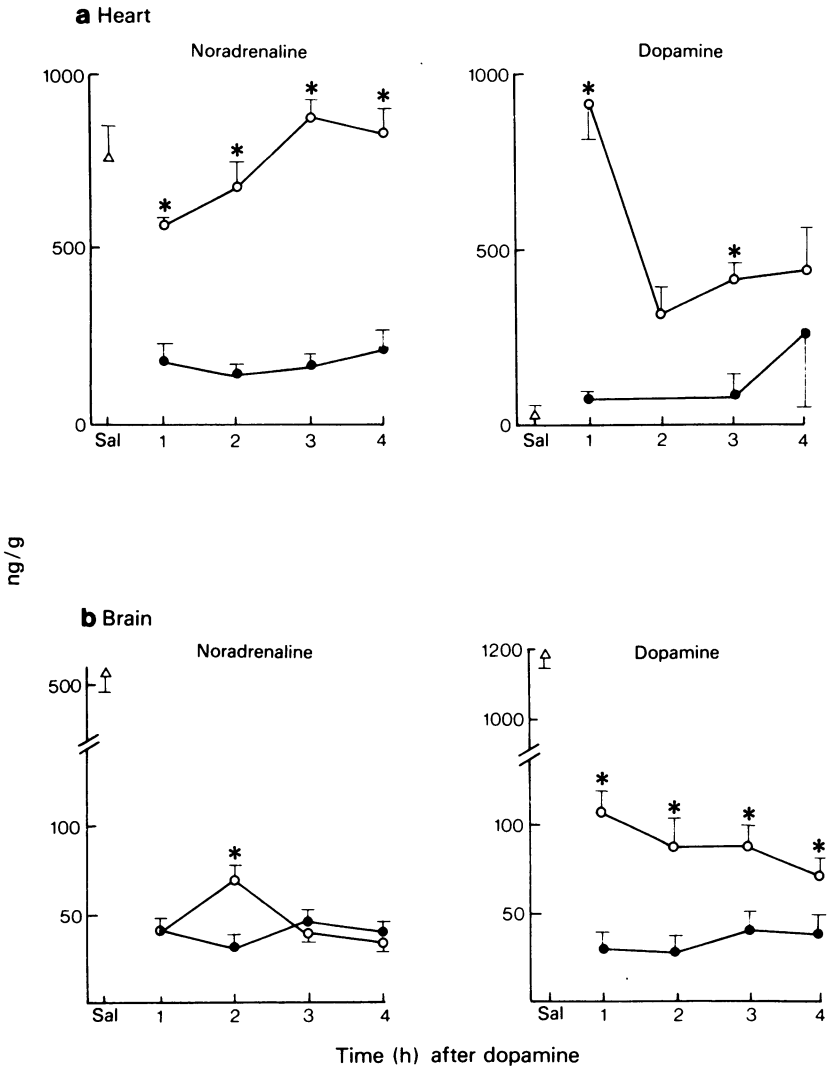

Figure 4 Effect of an (i.p.) injection of dopamine on the depletion of catechols produced by monofluoromethyldopa (MFMD). Groups of 5 mice were injected (i.p.) twice, 12 h apart, with MFMD (100 mg/kg): 12 h after the second dose, dopamine (50 mg/kg) or saline were administered (i.p.). A further group of 5 mice injected three times with saline (Sal) served as undepleted controls. Mice were killed 1, 2, 3 and 4 h after the last injection and catechols determined in brain and heart. (●) MFMD plus saline; (○) MFMD plus dopamine; (△) saline plus saline. Note the scale is broken in the brain. Values are in ng/g; vertical lines show s.e. mean. * $P < 0.05$ compared with MFMD.

concentrations in various tissues including brain, but they suffer from two major drawbacks. Most are competitive inhibitors with a relatively short duration of action and the hydroxylase enzymes are subject to feedback control and are readily induced by a variety of agents and physiological manipulations (see Thoenen, 1975). MFMD differs from these compounds in that it is irreversible (Jung *et al.*, 1979a; Kollonitsch, Patchett, Marburg, Maycock, Perkins, Doldouras, Duggan & Aster, 1978) and that it inhibits an enzyme which is subject to much less stringent feedback control than the hydroxylase enzymes.

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