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α-Methyladrenaline is a central metabolite of α-methyldopa

P. M. BEART*, P. R. ROWE, W. J. LOUIS, *University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria, 3084, Australia*

α-Methylnoradrenaline and α-methyldopamine, two metabolites of α-methyldopa (L-α-methyl-3,4-dihydroxyphenylalanine), are considered to mediate most of the pharmacological actions of this widely used antihypertensive drug. α-Methylnoradrenaline, in particular, is believed to be responsible for the hypotensive action of α-methyldopa in brain regions controlling blood pressure (Henning & Rubenson 1971). Another possible metabolite, α-methyladrenaline has been the subject of little attention, but might be responsible for the depletion of central adrenaline observed after α-methyldopa treatment (Beart et al 1981) and hence contribute to the antihypertensive action of the drug. This communication concerns the possible formation of α-methyladrenaline within the central nervous system.

Initially, the synthesis of α-methyladrenaline was examined in-vitro using crude brain homogenates as a source of phenylethanolamine-*N*-methyltransferase (PNMT, EC 2.1.1.28). Freshly dissected rat hypothalami and medulla oblongatae were homogenized in 20 volumes of ice cold 5 mM Tris-maleate pH 7·4 containing 0·2% Triton X-100, and the homogenates were centrifuged at 10000 *g* for 10 min. Portions of the supernatants were employed as the enzyme preparations in the assay of Moore & Phillipson (1975), and α-methylnoradrenaline and noradrenaline (both 160 μM, saturating concentration for noradrenaline) were tested in parallel experiments (Beart et al 1979). ^{3}H-*N*-Methylated reaction products were isolated by alumina adsorption (Henry et al 1975) and determined by scintillation spectrometry. α-Methylnoradrenaline was transformed to α-methyladrenaline by enzyme preparations of both brain regions, but was a less effective substrate than noradrenaline. The α-methyl analogue was 48 ± 8% (mean ± s.e.m., n = 4) as active as noradrenaline using the crude rat hypothalamic extract and the observed activities for α-methylnoradrenaline and noradrenaline were 26 ± 6 (4) and 53 ± 10 (4) pmol product mg^{-1} wet wt h^{-1} respectively. α-Methylnoradrenaline was found to be a less effective substrate when the rat medullary preparation was employed and was only 25 ± 4% (4) as effective as noradrenaline itself: activities with α-methylnoradrenaline and noradrenaline were 32 ± 7 (4) and 123 ± 20 (4) pmol product mg^{-1} wet wt h^{-1} respectively. In a further study employing partially purified, dialysed beef adrenal medulla PNMT (Henry et al 1975) α-methylnoradrenaline gave 33 ± 12% (3) of the activity found with noradrenaline.

In further experiments, Sprague-Dawley rats (female, 150–200 g) were treated subcutaneously with α-methyldopa (Aldomet ester HCl, 2 × 40 mg kg^{-1} free acid for 4 days) and killed 1 h after the final injection. Freshly dissected hypothalami were homogenized in 10 volumes of ice-cold 0·1 M aqueous perchloric acid containing 0·05% EDTA, 0·05% sodium metabisulphite and 100 ng ml^{-1} of the internal standard, dihydroxybenzylamine. The homogenates were adjusted to pH 8·5 and the catechols were isolated from an alumina column (50 mg) in 250 μl of 0·1 M aqueous perchloric acid containing 0·05% EDTA.A 150 μl portion of this eluate was analysed by high pressure liquid chromatography with electrochemical detection (Rowe et al 1980) employing a μBondapak C_{18} column (Waters) and a mobile phase of 70 mM sodium phosphate containing 2 mM heptane-1-sulphonic acid, 0·1 mM EDTA, 7% methanol at final pH 3·95 or 3·80. At pH 3·80, authentic α-methyladrenaline had a retention time of 9.98 min and could be separated from the other catechols and the internal standard, dihydroxybenzylamine (Fig. 1a, b). The chromatograms of the extracts from the hypothalami of the α-methyldopa-treated rats contained a peak possessing a retention time identical to that of authentic α-methyladrenaline (Fig. 1b). Extensive studies revealed that no other substances, including 3,4-dihydroxyphenylethylglycol, 4-hydroxy-3-methoxyphenylethylglycol, vanillylmandelic acid, 3-methoxytyramine, homovanillic acid, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, yielded a peak on the chromatograms with a retention time similar to that of α-methyladrenaline. The concentration of α-methyladrenaline was estimated to be 4·8 ± 0·3 (9) ng g^{-1} wet wt, while those of α-methylnoradrenaline, α-methyldopamine and α-methyldopa were 3850 ± 580 (4), 870 ± 120 (4) and 5520 ± 1170 (4) ng g^{-1} wet wt respectively. The α-methyldopa treatment reduced the concentration of noradrenaline in the hypothalamus to 7 ± 1% (4) of control level ($P < 0.001$), while the concentration of dopamine was not significantly altered (data not shown). Under the chromatographic conditions employed in this study adrenaline possessed a retention time almost identical to that of α-methylnor-

* Correspondence.

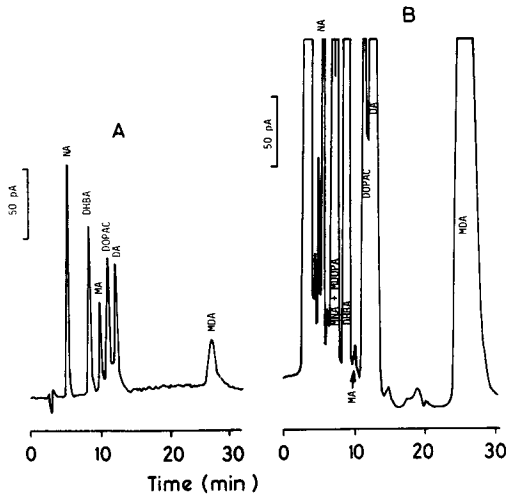

FIG. 1. A. Chromatogram of authentic noradrenaline (NA), dihydroxybenzylamine (DHBA), α-methyladrenaline (MA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA) and α-methyldopamine (MDA). One ng of each catechol at + 0.7 V (carbon paste and Ag/AgCl electrodes) with a flow rate of 1 ml min^{-1}. B. Chromatogram of hypothalamic extract (30 mg wet wt tissue) showing peaks corresponding to authentic NA, α-methylnoradrenaline (MNA), α-methyldopa (MDOPA), DHBA, MA, DOPAC, DA and MDA. Recorder sensitivity and details as for A.

adrenaline, but hypothalamic adrenaline is likely to represent < 10% of the α-methylnoradrenaline concentration reported above especially since the administration of α-methyldopa produces a 50–70% depletion of hypothalamic adrenaline (Beart et al 1981).

Such results suggest that α-methylnoradrenaline may be a substrate for brain PNMT, and indicate that α-methyladrenaline can be formed from α-methyldopa within the central nervous system. α-Methyladrenaline has previously been reported to be present in appreciable amounts only in peripheral tissues with high PNMT activity (e.g. adrenal gland; Muscholl 1972), and there appear to have been no reports of its existence in tissues possessing low (e.g. brain) or no PNMT activity. The concentration of α-methyladrenaline found in whole hypothalamus represented < 5% of the adrenaline concentrations found in individual hypothalamic nuclei (Beart et al 1979), and thus α-methyladrenaline is unlikely to be responsible for the 50–70% depletion of adrenaline seen after α-methyldopa (Beart et al 1981). α-Methyladrenaline would appear to differ from α-methylnoradrenaline and α-methyldopamine in that the large accumulation of these α-methylamines is considered to be responsible for the replacement of noradrenaline and dopamine.

Since α-methylnoradrenaline has been recently reported to be a poor substrate for brain PNMT in-vitro, although still apparently methylated by 'nonspecific' methyltransferases (Fuller & Hemrick-Luecke 1983), further experiments were performed to examine the route of α-methyladrenaline synthesis in-vivo. Rats, treated with α-methyldopa (as above), were concurrently injected with the PNMT inhibitor, SK & F 64139 (intraperitoneally, 2 × 20 mg kg^{-1} for 3 days) or saline, and hypothalamic α-α-methyladrenaline was determined as described. α-Methyladrenaline was present in the extracts of the hypothalami of both groups of rats and the concentration was not significantly altered by the administration of SK & F 64139 (data not shown). Since this schedule of SK & F 64139 will effect an almost complete inhibition of brain PNMT activity (see Fuller et al 1981), the in-vivo synthesis of α-methyladrenaline after the administration of α-methyldopa probably occurs via the action of 'non-specific' methyltransferases (Saavedra et al 1973; Fuller & Hemrick-Luecke 1983). Further investigations are needed to establish the pharmacological actions of this central metabolite of α-methyldopa.

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