A STUDY OF THE DISPOSITION OF α -METHYLDOPA IN NEWBORN INFANTS FOLLOWING ITS ADMINISTRATION TO THE MOTHER FOR THE TREATMENT OF HYPERTENSION DURING PREGNANCY

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- 1 The nine infants participating in this study were born to mothers who received continuous therapy with α -methyldopa (0.75–2.0 g/day) for several weeks extending to the time of delivery.
- 2 The concentration of free and total (free plus conjugated) α -methyldopa was determined by gas chromatography—mass spectrometry in amniotic fluid, umbilical cord plasma and maternal plasma at delivery; also in urine collected over timed intervals from neonates during the first days after birth.
- 3 The results indicate that α -methyldopa administered to the mother is present in the infant at birth at a level comparable to the maternal level and persists for some days. The ratio of conjugated to free drug increases with time after birth.
- 4 The excretion of free and conjugated α -methyldopa in the urine indicated that the drug is slowly eliminated in the neonate by excretion in the urine and apparently by metabolism, mainly to the sulphate conjugate.
- 5 The concentration of free and conjugated α -methyldopa in amniotic fluid tended to be higher than in umbilical cord plasma but lower than in neonatal urine, conjugated drug predominated.

Introduction

α -Methyldopa (Aldomet) has been used for the treatment of hypertension during pregnancy for many years and no serious adverse effects to the fetus have been described (Hans & Kopelman, 1964; Kincaid-Smith, Bullen & Mills, 1966; Leather, Humphreys, Baker & Chadd, 1968; Redman, Beilin & Bonner, 1976; 1977). However, no studies of the disposition of α -methyldopa in pregnant women, or in the babies born to them appear to have been reported.

The mean features of the disposition and metabolism of α -methyldopa have been established in normal and hypertensive adults (Buhs, Beck, Speth, Smith, Trenner, Cannon & Laragh, 1964; Prescott, Buhs, Beattie, Speth, Trenner & Lasagna, 1966; Au, Dring, Grahame-Smith, Isaac & Williams, 1972; Kwan, Faltz, Breault, Baer & Totaro, 1976). The results of investigations conducted with radiolabelled α -methyldopa have shown that after intravenous dosage most of the administered radioactivity is excreted in the urine within 24 h and little excretion occurred beyond this time. The absorption of an oral dose of α -methyldopa is limited and highly variable. The major metabolite in man is α -methyldopa 3-O sulphate (Buhs *et al.*, 1964) and of the radioactivity

excreted in urine, about 70% is associated with free and sulphate conjugated α -methyldopa (Au *et al.*, 1972). The rate of elimination is fairly rapid; Kwan *et al.* (1976) report the mean half-life to approximate 106 min in twelve normal adult subjects.

In preliminary studies using a fluorimetric method of assay, the free and sulphate conjugated α -methyldopa concentrations in umbilical cord plasma were shown to be similar to the concentrations in maternal plasma at delivery; α -methyldopa was also shown to be present in amniotic fluid at a relatively high concentration and at low levels in early milk (Jones & Cummings, 1978). Using a gas chromatography-mass spectrometric method of assay, an investigation has now been made of the rate of elimination of α -methyldopa in babies born to mothers who received the drug for treatment of hypertension during their pregnancy, by determination of the amount of α -methyldopa and its sulphate conjugate excreted in neonatal urine collected during the first few days after birth. The concentration of free and conjugated α -methyldopa was also determined in umbilical cord and maternal plasma at delivery and at a few subsequent times in the plasma of certain of the new born infants.

Methods

The babies participating in these studies were born to mothers who had received α -methyldopa (0.75–2.0 g/day) for several weeks of continuous therapy extending to the time of delivery. Details of the babies are given in Table 1.

Collections of urine from the babies were made using urine bags (Abbot Laboratories, Queensborough, UK) and each sample was drained into individual containers. The period over which the urine samples were collected, the time after delivery and the urine volume were recorded and the samples stored at -20°C until analysed. Maternal and umbilical cord blood samples (10 ml) were collected into heparinized tubes at the time of delivery. Neonatal blood samples were collected by heel prick into heparinized containers. After the addition of sodium metabisulphite (0.5 mg/ml) the plasma was separated and stored at -20°C until analysed.

These studies were conducted with the approval and under the rules of Northwick Park Hospital and Clinical Research Centre, Harrow, Ethical Committee.

Urine samples from one of the babies (CR) was analysed for total (free plus conjugated) α -methyldopa by gas chromatography-mass spectrometry as described by Setchell, Jones, Lawson & Cummings (1978). This method of assay has since been modified to separately determine free and total α -methyldopa (Setchell, Lawson, & Cummings, 1978) and this latter method has been used for all other determinations of α -methyldopa in urine, plasma and amniotic fluid.

Method of assay

Small volumes of plasma, urine or amniotic fluid (20–100 μl) and added internal standard (α -trideuteriomethyldopa) were subjected to reverse phase chromatography on small columns of Lipidex 5000 (Packard-Becker, Groningen, Holland) to remove interfering substances. Unconjugated α -methyldopa was separated from its 3-O-sulphate conjugate by chromatography on the lipophilic ion-exchange gel, diethyl aminohydroxypropyl Sephadex LH-20 the more acidic sulphate conjugate being retained. The *n*-butyl ester of α -methyldopa was formed by reacting with *n*-butanol saturated with hydrochloric acid at 100°C . This treatment when applied directly to plasma or urine brings about the simultaneous hydrolysis and derivatization of the α -methyldopa 3-O-sulphate, so that total α -methyldopa was determined. The drug was measured as the *n*-butylester-pentafluoropropionate derivative by selected ion monitoring in the electron impact mode of the ion at m/e 542 and the corresponding ion at m/e 545 for the internal standard.

Gas chromatography was with a Varian 2700 gas chromatograph fitted with a glass column (2m $\times$ 0.4 cm) packed with 3% OV-1 on Chromabsorb Q and operated isothermally at 195°C . The gas chromatograph was combined with a Varian MAT-731 double focusing mass spectrometer.

The α -methyldopa concentration in urine, plasma and amniotic fluid was determined as total (free plus conjugated) and as free (unconjugated)- α -methyldopa; the concentration of the conjugated drug was calculated by subtraction of the free

Table 1 Details of infants participating in the studies

Infants	Sex	Gestational age (weeks)	Birth weight (kg)	Condition at birth	Apgar score at	
					1 min	5 min
LO	F	38	2.35	Normal	8	—
HA	M	38	3.54	Normal	8	10
HN	M	39	3.66	RDS	6	8
CR	M	38	1.82	Hypoglycaemia	4	7*
AU	M	32	1.42	RDS Required ventilation	4	7*
CA	F	32	2.10	Normal	7	9
PA	M	31	1.24	RDS	5	7
HL	M	30	1.16	RDS	1	8
WI	M	27	0.94	RDS	8	9

*Apgar score at 10 min

RDS—respiratory distress syndrome

concentration from the total concentration. With the exception of the few cases where only total α -methyldopa was determined, the results are expressed in terms of free and conjugated α -methyldopa.

Results

The concentration of α -methyldopa in the maternal and umbilical cord plasma are given in Table 2, together with the dosage schedule of the mother and the last dose-delivery time interval. The maternal and umbilical cord plasma concentrations of free α -

methyldopa are in fair agreement, but the corresponding levels of conjugated α -methyldopa differ appreciably; the α -methyldopa concentrations in plasma collected from the neonates at various times up to about 120 h after birth are given in Table 3.

The rate of excretion of free and conjugated α -methyldopa in the urine of five neonates was calculated from the amounts excreted over the period of urine collection, these results are plotted on a logarithmic scale against time after birth in Figure 1; the urine output during each collection period is also given. The corresponding results for the rate of total α -methyldopa excretion in one other infant (CR) are given in Figure 2.

Table 2 The concentration of free and conjugated α -methyldopa in umbilical cord and maternal plasma at delivery and in amniotic fluid

Infant	Maternal dose (mg)	Time after last dose (h before delivery)	α -Methyldopa concentration (ng/ml)					
			Cord plasma		Maternal plasma		Amniotic fluid	
			Free	Conjugated	Free	Conjugated	Free	Conjugated
LO	250/6 h	5.3	374	533	384	2194	—	—
HA	500/12 h	4.0	300	255	245	333	423	1233
HN	250/6 h	—	583	1114	618	2925	—	—
CR	250/6 h	7.0	870	500	—	—	—	—
AU	500/6 h	4.0	34	120	—	—	—	—
CA	250/6 h	11.0	(1802)*		(2291)†		—	—
WI	500/8 h	14.0	265	367	369	53	939	1581
FR	500/6 h	—	—	—	—	—	200	1980
SU	500/12 h	28.0	—	—	—	—	216†	1364†

*Total α -methyldopa only determined.

†Obtained by amniocentesis 1 week before delivery

Table 3 The α -methyldopa concentration in the plasma of infants during the neonatal period

Infant	Time after birth (h)	α -Methyldopa concentration (ng/ml)		
		Free	Conjugated	Total
WI	11	96	356	452
	35	88	193	281
HN	16	135	466	601
	25	787	1893	2680
HL	121	88	—	—
	26	30	265	295
HA	50	348	916	1264
LO	50	17	288	305
CA	17	—	—	4876*
PA	40	—	—	1387*
	67	—	—	1076*
	112	—	—	163*

*Total α -methyldopa only determined

Figure 1 The excretion of free (○, △) and conjugated (●, ▲) α -methyldopa in the urine of five infants born to women who received the drug during their pregnancy. Urine output (■) is given in the lower graph. The initial values for infant HA are uncertain as a small loss of urine sample is suspected.

The rate of excretion of both free and conjugated α -methyldopa shows considerable variation between subjects and also with time in individual infants. The results from two infants (CR, HA, Figure 1 and 2) show a definite peak in the rate of excretion at around 12 and 20 h respectively and there is a suggestion of a maximum in the rate of excretion at between these times in the results from other infants; this might be due to developing kidney function.

The rates of excretion of both the α -methyldopa and the α -methyldopa-3-O-sulphate (Figure 1) show a slow decline with time, continuing for at least 40 h after birth and in one baby (CR) α -methyldopa excretion was detectable for more than 96 h (Figure 2).

Certain of these results (Figure 1 and 2) show

marked fluctuations between consecutive urine collections, for which no definite explanation can be offered, possible contributing factors are considered in the discussion.

Although some parallelism is apparent in the two sets of results (Figure 1), there is a tendency for the ratio of conjugated to free α -methyldopa to increase with time (Figure 3).

The cumulative amount of free and conjugated α -methyldopa excreted in the urine collections are given in Table 4. However, as it was possible to obtain an apparently complete collection of urine over the whole period of drug excretion only in baby WI, these results underestimate the amount of drug present in the infant at birth.

The concentrations of free and conjugated α -

Figure 2 The excretion of total (free plus conjugated) α -methyldopa in the urine of one neonate after dosage to the mother. Joined points represent results in consecutive urine collections, single points represent results in isolated urine collections.

methyldopa in amniotic fluids (Table 2) tend to be higher than those in umbilical cord plasma and lower than in urine (Figure 1), in all cases there is a predominance of conjugated α -methyldopa.

Discussion

Most of the infants participating in these studies were to some extent premature, or of less than normal body weight for their gestational age (Table 1) and required treatment in the Special Care Unit for some time after birth; two of the neonates survived for only

Figure 3 The change with time after birth of the ratio of conjugated to free α -methyldopa excreted in the urine of neonates.

a few days (HL, WI). They cannot therefore be considered normal babies.

The results (Table 2) show α -methyldopa and its conjugate to be present in umbilical cord plasma. The concentration of free α -methyldopa in umbilical cord plasma and in maternal plasma at delivery, were

Table 4 The total amount of free and conjugated α -methyldopa excreted in the urine of neonates over the period of urine collection

Neonate	Period of urine* collection (h after birth)	α -Methyldopa in urine (μ g)			
		Free	Conjugated	Total	$\frac{\text{Conjugated}}{\text{Free}}$
LO	5-27	29	84	113	3.0
HA	12-64	56	136	192	2.4
HN	6-12	44	176	220	4.1
AU	17-45	97	476	573	4.9
CA	5-23	89	369	459	4.1
HL	22-31	18	219	237	12.0
WI	13-52	45	223	269	4.9

*Time of first and last urine collection to nearest hour.

similar indicating a fairly rapid distribution across the placenta, but the corresponding concentration of conjugated α -methyldopa differed appreciably. This raises the question of the origin of the conjugated drug in the fetus, is it formed by fetal enzyme systems or is it transferred across the placenta from the mother as was previously postulated (Jones & Cummings, 1978)? Most drug substances present in the maternal plasma will transfer across the placenta to the fetus to some extent (Horning, Butler, Nowlin & Hill, 1975), therefore some placental transfer appears likely as the levels of conjugated α -methyldopa in certain of the maternal plasmas are relatively high. However, the definite indications that conjugation of α -methyldopa occurs in the neonate, for example, increasing ratio of the conjugated to free α -methyldopa in the urine of the neonates with time after birth (Figure 3), suggest that the fetus at or near term may well be able to conjugate α -methyldopa in the same way.

The nature of the conjugate of α -methyldopa in the neonatal urine and plasma and the extent to which other metabolites of the drug are formed, was not determined in these studies. Buhs *et al.*, (1964) provided evidence that in the adult conjugation of α -methyldopa was predominantly by sulphation to give the α -methyldopa-3-O-sulphate. Since sulphation is the best developed of the conjugating systems in the newborn (Gladtko & Heimann, 1975; Dutton, 1978) it would appear reasonable to suppose that this is also the principal conjugate in the neonate.

The average half-life of α -methyldopa in adults, calculated from the post-absorption plasma concentration decay curves after oral dosage, has been reported to be 1.77 ± 0.21 h (Kwan *et al.*, 1976) and 2.1 ± 0.7 h (Barnett, Bobik, Carson, Korman & McLean, 1977) in normal and hypertensive subjects respectively. In the two infants (FR, WI) for whom an approximate value can be calculated from the urinary data a half-life of 15 h and 9 h respectively was obtained for α -methyldopa excretion; a half-life of 20 h was calculated from the plasma data of infant PA (Table 3). While in adults, most of an oral or intravenous dose is eliminated within 24 h, (Au *et al.*, 1972) the rate of elimination of α -methyldopa in the newborn as exemplified by excretion of free drug in the urine (Figure 1) is comparatively slow, with excretion of both free and conjugated drug continuing for at least 40 h and in one infant (CR), continuing for more than 4 days. α -Methyldopa was detected in the plasma of neonates up to 120 h after birth.

These results would seem to be consistent with elimination of α -methyldopa in the newborn being by excretion in the urine and by conjugation, with the rate of excretion of the conjugated drug being similar to the rate of elimination of α -methyldopa. The

average renal clearance of the free and conjugated drug are stated to be equal by Kwan *et al.* (1976). Under these conditions the ratio of metabolite to free drug would continue to increase for a relatively long period of time before approaching a constant value. Similarly high ratios of conjugated to free α -methyldopa and a relatively long half-life have been reported in adult patients with renal insufficiency who received the drug (Myhre, Brodwall, Stenbaek & Hansen, 1972b), possibly suggesting that the high ratio and prolonged half-life in the neonates might be due to immature renal function.

The total amounts of drug determined in the urine collected from the neonates (Table 3) might provide an indication of the body load of the drug at birth. However, complete collections of urine over the whole period of α -methyldopa excretion were not usually obtained. Also, Myhre, Brodwall, Stenbaek & Hansen, (1972a) and Kwan *et al.* (1976) report the incomplete recovery of intravenous doses of α -methyldopa from the urine of adults and have suggested that an extra-renal route of elimination might exist. Thus, the amounts of α -methyldopa determined in the urine provide an uncertain indication of the amount of drug in the newborn infant. The body load of α -methyldopa at birth has been estimated from the body weight and umbilical cord plasma concentration of the drug (Table 2) assuming that the free and conjugated α -methyldopa is distributed throughout the extra-cellular water at these concentrations; the extracellular water in the neonate being taken to equal 40% of the birth weight (Rane & Wilson, 1976). These estimates of the amount of free and conjugated α -methyldopa in the infants at birth vary between 130–850 μ g (mean 442 μ g) and 275–1630 μ g (mean 692 μ g) respectively. They probably underestimate the free α -methyldopa and overestimate the α -methyldopa sulphate, since Myhre *et al.* (1972b) reported the volume of distribution of free drug to be in excess of the extracellular water volume in adults, while Myhre *et al.* (1972a) state that about 50% of the α -methyldopa sulphate in plasma is bound to protein, so that the whole of the plasma concentration will not distribute throughout the extracellular water.

The marked fluctuation in the amounts of both free and conjugated α -methyldopa excreted in consecutive urine collections from individual infants (Figure 1), is not readily explained and may be due to a combination of factors. For example, incomplete bladder emptying on micturition is usual in neonates according to Osborn, Du Mont, Beecroft & Ayres (1977) and could contribute to an apparent variation in drug output. There is a suggestion of some correlation between the urine volume and the amount of α -methyldopa excreted (Figure 1), but a consistent relationship is not apparent. Kwan *et al.* (1976) have

suggested that enterohepatic circulation of α -methyldopa occurs to some extent, however, since the fluctuations involve both free and conjugated α -methyldopa almost equally, this appears an unlikely explanation. While obvious leakage or loss from the collecting bag was always reported, small losses or errors arising during collection and measurement of the volume of the small urine samples can not be excluded. Random errors attributable to the analytical method were relatively very small and are unlikely to contribute to a significant extent.

The concentration of α -methyldopa in amniotic fluid samples (Table 2) tends to be higher than the corresponding maternal or umbilical cord plasma concentration but very appreciably lower than the concentration in early neonatal urine. Assuming that the drug in the amniotic fluid results from its excretion in the fetal urine, it would seem that drug is also removed from the amniotic fluid possibly by

swallowing by the foetus or via the fetal lungs and transference to the mother. The relatively high ratio of conjugated and free α -methyldopa might indicate preferential transfer of the free drug.

The results which have been obtained in these studies indicate that α -methyldopa administered to the mother is present in the infant at birth at a level comparable with the maternal level and persists for some days after birth. Whether the α -methyldopa concentration is sufficient to significantly depress the blood pressure is uncertain at the present, but is undergoing investigation.

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