Pharmacokinetics and metabolism of (−)-α-[2-^{3}H]acetylmethadol (LAAM) in the monkey: evidence for a new metabolite

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Because of its properties of effective suppression of opiate withdrawal symptoms and longer duration of action, (—)-α-acetylmethadol (LAAM), a synthetic congener of methadone has practical therapeutic advantages over methadone in treatment and rehabilitation programs for chronic opiate users (Jaffe, Schuster & others, 1970; Levine, Zaks & others, 1973). Previous (Sung & Way, 1954; McMahon, Culp & Marshall, 1965) and recent studies (Billings, Booher & others, 1973; Billings, McMahon & Blake, 1974; Nickander, Booher & Miles, 1974) have shown that some of the pharmacological activity and long duration of action of LAAM may be due to its biotransformation to (−)-α-acetylnormethadol and (−)-α-acetylbisnormethadol. Methadol and normethadol have also been reported (Kaiko & Inturrisi, 1973) as metabolites of LAAM in addicts. This study deals with the pharmacokinetics and metabolism of LAAM in the monkey and demonstrates that aromatic hydroxylation of (—)-α-acetylbisnormethadol and partial conjugation of this metabolite is an important and significant metabolic pathway of LAAM in the monkey. In addition, evidence for the presence of acetylnormethadol and conjugated metabolites of methadol and normethadol has also been obtained. The metabolite, *p*-hydroxyacetylbisnormethadol has further been observed in the plasma, cerebrospinal fluid and brain of monkeys administered a 2 mg kg^{-1} oral dose of (—)-α-[2-^{3}H] acetylmethadol.

Female monkeys (*M. mulatta*) (3–5 kg) were catheterized with Bardex-Foley indwelling catheters (size 8 and 12 Fr.) transferred to a restraining chair and either injected subcutaneously or orally administered a 2 mg kg^{-1} (free base) dose of (—)-α-[2-^{3}H]acetylmethadol (sp. act. 10 μCi mg^{-1}, radiochemical purity > 95%). Urine and blood were collected at 1 h intervals up to 6 h. Thereafter, urine and faeces were collected in metabolism cages. Distribution of LAAM in tissues and selected anatomic areas of the cns was studied 4 and 6 h post-administration of a 2 mg kg^{-1} oral dose. Assay of extracted LAAM and total radioactivity was according to Misra, Bloch & Mulé (1975).

The results on excretion of LAAM and total radioactivity are summarized in Table 1. Pooled urine from each monkey given a 2 mg kg^{-1} oral dose of [^{3}H]-LAAM was chromatographed on Amberlite XAD-2 (5 × 18 cm) and adsorbed LAAM and metabolites eluted with 1 litre of methanol using previous techniques (Misra, Mulé & others, 1973; Misra, Bloch & others, 1974). The eluate, concentrated to a small volume *in vacuo*, was separated into methanol-soluble and insoluble fractions and these fractions separately chromatographed on Gelman preparative i.t.l.c. (silica gel sheets 20 × 20 cm) with ethyl acetate–methanol–conc. ammonia (17:2:1 v/v). Thus, the eluted metabolites were separated into non-polar and polar fractions. Non-polar fraction on sequential i.t.l.c. in solvent systems, n-hexane–ethyl acetate–conc. ammonia (85:15:0·1 v/v), n-hexane–ethyl acetate–ether–conc. ammonia (70:25:5:0·1 v/v), benzene–ethyl acetate–methanol–conc. ammonia (80:20:1·2:0·1v/v) provided evidence on co-chromatography for the presence of unmetabolized LAAM, acetyl normethadol and a major unknown metabolite (approximately 80% of the total radioactivity in the non-polar fraction). Larger amounts of unknown metabolite were purified by preparative i.t.l.c. The unknown radioactive metabolite showed the presence of a phenolic (Folin-Ciocalteau and $FeCl_3$/ferricyanide reagents) and primary amine group (ninhydrin and *p*-nitroaniline reagents). It was differentiated from acetylbisnormethadol and bisnormethadol by its different t.l.c. mobility characteristics and different retention time (8·98 min), acetylbisnormethadol (5·27 min) on gas chromatography (20 in column 10% UCW-982 on 80–100 mesh chromosorb W-HP, $\frac{1}{8}$ in o.d. stainless steel; column, injector and flame ionization detector temperatures 210, 250, 300° respectively; carrier gas helium).

The polar metabolite fraction was enzymatically hydrolysed in M/15 phosphate buffer with glusulase at pH 5·5–6·5 for 24 h and the hydrolysate chromatographed on Amberlite XAD-2 (2 × 20 cm) and adsorbed metabolites eluted with methanol. The residue from the eluate on i.t.l.c. with benzene–ethyl acetate–methanol–conc. ammonia (80:20:0·5:0·1 v/v) provided evidence for the presence of α-methadol (50%), nor-

Table 1. *Excretion of [^{3}H]LAAM and its metabolites in four female monkeys, two after a single 2 mg kg^{-1} oral and two after a subcutaneous (s.c.) injection of the drug.*

	Mean % of administered dose* excreted in 3 weeks					
	Oral			s.c.		
	Urine	Faeces	Total	Urine	Faeces	Total
Free extractable drug	2·3 3·5	19·5 15·1	21·8 18·6	4·6 3·0	15·2 25·5	19·8 28·5
Total radioactivity	17·9 26·9	27·6 28·4	45·5 55·3	23·2 19·6	39·2 33·5	62·4 53·1

* Major excretion of free extractable drug and its metabolites occurred in urine within 24 h and in faeces within 48 h, but significant and measurable radioactivity continued to be excreted for several weeks both in oral and subcutaneous experiments.

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methadol (15%) by co-chromatography and one major unknown polar metabolite (35%). Enzymic hydrolysis with β-glucuronidase (pH 6·8) and glusulase showed glucuronide conjugation was a major and sulphate conjugation a minor route. The hydrolysate gave strong positive tests for phenolic and primary amine groups. Gas chromatographic-mass spectrometric analysis of the unknown metabolite obtained from non-polar and hydrolysed polar fractions showed a spectrum relatively weaker compared to standard acetylbisnormethadol but having distinct peaks due to M^+ ions at 341, a definitive but not intense peak at M-44 due to CH_3–CH–NH_2 ion and a major peak at M-92 possibly due to a C_6H_4O ion. The observed differences in the mass fragmentation patterns of the *p*-hydroxy metabolite and standard acetylbisnormethadol could arise from the presence of coloured impurities in the methanol eluate of the metabolite from i.t.l.c. (silica gel) sheets. These observations provide additional tentative evidence for the presence of free and conjugated *p*-hydroxyacetylbisnormethadol as new metabolites of LAAM in the monkey. Methadol and normethadol observed as conjugated metabolites in this study have earlier been shown (Nickander & others, 1974) to possess pharmacological activity and *N*-acetylation of normethadol was an important metabolic pathway in the rat (Sullivan, Due & McMahon, 1973). Our study provides evidence for the presence of acetylnormethadol, free and conjugated metabolites of *p*-hydroxyacetylbisnormethadol (major), an unidentified *p*-hydroxy metabolite with a tertiary amine group, conjugated methadol and normethadol as urinary metabolites in the monkey. *p*-Hydroxybisnormethadol was previously shown by Misra & Mulé (1972) to be a metabolite of methadone and to persist along with methadone for prolonged periods in the brains of rats injected with a single dose of methadone.

The maximum peak concentrations of LAAM (73–79 ng ml^{-1}) occurred in plasma 2–6 h after oral administration and these values declined to 5–11 ng ml^{-1} 48 h after administration. Peak concentrations after subcutaneous injection were comparatively higher (117–249 ng ml^{-1}) and were sustained between 1–6 h and these declined to 3–10 ng ml^{-1}, 48 h after injection. The approximate half-lives of free extractable drug in plasma from two monkeys after a single 2 mg kg^{-1} oral dose were 12 and 17 h, respectively, those after subcutaneous injection were 15 and 8 h, respectively. In a monkey administered a 2 mg kg^{-1} oral dose of LAAM on alternate days (3 times a week) for 5 weeks followed by the same dose of [^{3}H]LAAM, the $T_{\frac{1}{2}}$ of free LAAM in the plasma was 8 h and peak concentrations occurred earlier in this chronically treated monkey (Table 2). In both acutely and chronically treated monkeys, free LAAM was not detectable in plasma 48 h after administration. Lower $T_{\frac{1}{2}}$ values in the plasma after chronic treatment indicated a faster rate of metabolism of LAAM. Total radioactivity values

Table 2. *Distribution of extractable free (−)-α-acetylmethadol and total radioactivity in plasma of acute* and chronically-treated† female monkeys after 2 mg kg^{-1} (free base) oral administration of (−)-α-[2-^{3}H]acetylmethadol.*

	0·5 h	1 h	2 h	4 h	6 h	8 h	24 h	48 h	96 h
Acute									
Extractable free drug	3 0	21 4	72 30	73 79	48 49	— —	20 16	11 5	0 0
Total radioactivity	83 5	80 18	171 156	121 218	117 146	— —	96 84	83 64	— —
Chronic									
Extractable free drug	—	—	134	122	84	76	9	3	0
Total‡ radioactivity	—	—	305	295	215	209	103	80	50

* Data represent mean values (ng ml^{-1} of plasma) from 2 determinations for each sample obtained from 2 female monkeys for acute study and one chronically-treated monkey at different times.
† A 2 mg kg^{-1} oral dose of non-radioactive acetylmethadol was administered to the female monkey on alternate days, three times a week (on Monday, Wednesday and Friday) for 5 weeks, followed by a 2 mg kg^{-1} oral dose of (−)-α-[2-^{3}H]acetylmethadol.
‡ Radioactivity at 1, 2 and 3 weeks was 41, 44 and 16.

in plasma (Table 2), comprising acetylbisnormethadol, *p*-hydroxyacetylbisnormethadol and conjugated metabolites, declined slowly and significant detectable amounts of radioactivity were present even 3 weeks after the administration of [^{3}H]LAAM (Table 2). The t.l.c. mobility characteristics of plasma extracts at later times (1, 3 weeks) provided suggestive evidence for the persistence of *p*-hydroxyacetylbisnormethadol and only traces of acetylbisnormethadol.

Furthermore, t.l.c. studies on extracts of plasma, cerebrospinal fluid and brains of monkeys 6 h post-administration of a single 2 mg kg^{-1} oral dose of [^{3}H]LAAM provided tentative evidence for the presence of unconjugated free *p*-hydroxy metabolites, acetylnormethadol, acetylbisnormethadol, methadol and normethadol in addition to unmetabolized LAAM. The concentration (ng g^{-1}) of extractable free drug in selected anatomic areas of the cns of the animals 6 h post-administration of 2 mg kg^{-1} oral dose of [^{3}H] LAAM were as follows: temporal cortex (grey) 191; temporal cortex (white) 156; cerebellum, 157; spinal cord, 119; hypothalamus, 170; thalamus, 163; medulla, 126; pons, 137; mesencephalon, 150; caudate nucleus, 187.

Earlier work in man (Billings & others, 1974) has shown that even though the plasma and urinary concentrations of acetylnormethadol and acetylbisnormethadol were roughly similar in 3 men, the pharmacological response to LAAM was very variable. Our unpublished studies in monkeys chronically-treated with 2 mg kg^{-1} oral dose of LAAM, three times a week for 25 weeks have also shown this variable response to LAAM and two out of five monkeys on different occasions have shown severe depression even after such prolonged treatment, while others did not show such intense effects. Those severely depressed did not respond promptly to a 1 mg kg^{-1} (s.c.) injection of

nalorphine. The possibility therefore exists that this variable response to LAAM in monkeys could be due to the differential rates of formation of free and conjugated *p*-hydroxyacetylbisnormethadol or some other metabolite.

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Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotoninergic inhibition of feeding

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A number of recent studies suggest that an inhibitory serotoninergic system is involved in the control of food intake. In rats intraventricular injection of 5-hydroxytryptamine (5-HT) has been found to inhibit food intake, an effect which is blocked by the 5-HT antagonist cyproheptadine (Kruk, 1973). Furthermore, cyproheptadine has been reported to increase body weight, food intake and subjective feelings of hunger in man (Silverstone & Schuyler, 1975) and body weight in rats (Gosh & Parvarty, 1973). In addition, a large body of evidence indicates that the anorectic properties of fenfluramine are mediated by direct or indirect activation of serotoninergic neurons, providing further support for the concept of 5-HT mediated inhibition of feeding (Kruk, 1973; Jesperson & Scheel-Kruger, 1973; Blundell, Latham & Lesham, 1973; Clineschmidt, 1973; Samanin, Ghezzi & others, 1972; Funderbunk, Hazelwood & others, 1971; Clineschmidt, McGuffin & Werner, 1974; Ghezzi, Samanin & others, 1973; Garratini, Bizzi & others, 1975). Blundell & Lesham (1975) recently reported that the 5-HT precursor, 5-hydroxytryptophan (5-HTP) has anorectic properties in rats and potentiates the catecholaminergic mediated (Cole & Gay, 1974) anorectic effects of amphetamine, but not the serotoninergically mediated anorectic effects of fenfluramine. These results led the authors to propose that there exists "An inhibitory serotoninergic system for feeding activated by serotoninergic agonists." Whilst this conclusion is in accord with the results of the studies considered above, there is little evidence that the reported anorectic effects of intraventricularly injected 5-HT and peripherally administered 5-HTP are mediated directly by serotoninergic systems rather than by some non-specific disruption of the neurochemical systems controlling food intake. Peripheral administration of 5-HTP results in the formation of 5-HT in many areas of the brain due to the widespread occurrence of the enzyme L-amino acid decarboxylase. It has been shown that 5-HTP may displace catecholamines from intraneuronal granules of catecholaminergic neurons (Fuxe, Butcher & Engel, 1971). Since catecholamines are universally acknowledged to be involved in the control of feeding, it is conceivable that the reported anorectic effects of 5-HTP are due to a general disruption of control of food intake, and that they do not directly involve 5-HT neurons.

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