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The metabolism of atropine in man

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A metabolic pattern of atropine in man, based on the detection of radiolabelled products in urine by high performance liquid chromatography after administration of [^{3}H]atropine sulphate to a normal volunteer is proposed. Noratropine (24%), atropine-*N*-oxide (equatorial isomer) (15%), tropine (2%) and tropic acid (3%) appear to be the major metabolites, while 50% of the administered dose is excreted as apparently unchanged atropine. No conjugates were detectable. Evidence that atropine is present as (+)-hyoscyamine was found, suggesting that stereoselective metabolism of atropine probably occurs.

To date, the metabolism of atropine ((±)-hyosycamine) in man still remains partly a matter of conjecture. The low plasma concentrations attainable after tolerable doses is the primary reason for the lack of absolute identification of the resultant metabolites. Gosselin et al (1960) found that approximately 50% of a given dose was excreted unchanged, the ester bond remained largely intact, and the tropic acid portion of the molecule was unaltered. They concluded, therefore, that the metabolites seen must be components in which the tropine portion of the molecule had been modified. Kalser & McLain (1970) detected small quantities of $^{14}CO_2$ (3 and 1·4%) in the exhaled air from two volunteers who had received [*N*-methyl-^{14}C]atropine.

Their conclusion was that atropine was probably partly *N*-demethylated to form noratropine. Conflicting evidence on the formation of glucuronides has also been reported (Gosselin et al 1960; Kalser & McLain 1970).

Based upon this earlier research and theoretical considerations, we postulated that noratropine, atropine-*N*-oxide, tropine and tropic acid would be the most likely metabolites in man. These compounds were therefore synthesized from radioactive atropine, and retention volumes in an HPLC reversed phase system were compared with the retention volumes of radioactive products in the urine of a normal volunteer who had received an intravenous dose of atropine sulphate together with [^{3}H]atropine sulphate.

Materials and methods

Thin-layer chromatography (TLC). Separations of synthesized products were achieved by means of TLC on silica gel 60 F_{254} plates (Merck 5715), using the following systems as mobile phases: System A: ethyl acetate–isopropanol–ammonia (conc) (45 : 35 : 15 v/v); System B: 70% ethanol–ammonia (conc) (95 : 5 v/v).

Detection was by inspection with ultraviolet irradiation at 254 nm, or by spraying the plates sequentially with Dragendorff's reagent and 5% copper chloride solution. R_F values are in Table 1.

Table 1. R_F values of atropine and its postulated metabolites.

	System A	System B
Atropine	0·61	0·43
Noratropine	0·48	0·34
Atropine-*N*-oxide (equatorial)	0·34	0·66
Atropine-*N*-oxide (axial)	0·41	0·71
Tropine	0·27	0·07

High performance liquid chromatography (HPLC). HPLC was based upon a modification of the method described by Fell et al (1979). The mobile phase consisting of acetonitrile (Merck 800015), aqueous tetrabutyl ammonium hydrogen sulphate (Regis Chem. Co. L1683) (20 mM) and sodium acetate (Merck 6268) (50 mM) (25 : 75 v/v) at pH 5, was pumped through a Radial Pak C18 (10 μm) cartridge (8 mm × 10 cm high density polyethylene column packed with octadecylsilane-bonded porous silica) held in an RCM-100 radial compression unit (Waters) at a rate of 0·75 ml min^{-1} by means of a solvent delivery system consisting of a Gow Mac pump and Bourdon Tube Pulse Dampener. Eluted compounds were detected either by an ultraviolet detector (Waters Associates, model 450) at 230 nm, or by fractionating the eluate into 10-drop aliquots, and counting radioactivity in a Packard 3385 scintillation spectrometer after the addition of Instagel (Packard Instruments) scintillation fluid.

Mass spectra (MS) were recorded using a Varian Mat CH5 double focussing mass spectrometer in the electron impact mode. Nuclear magnetic resonance studies were performed on an 80 MHz Bruker Pulse Fourier Transform NMR spectrometer. Circular dichroism spectra were recorded using a Jasco 20 Spectropolarimeter.

Preparation of postulated metabolites. Authentic specimens of unlabelled and radioactively labelled metabolites were required, and were synthesized from atropine sulphate (Merck 1575) spiked with [^{3}H]atropine sulphate (3·6 Ci mmol^{-1}, Nuclear Research Centre, Negev), as starting material. The identities of the synthesized compounds were determined by nuclear magnetic resonance and mass spectrometry.

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Atropine-N-oxides. A modification of the method described by Phillipson & Handa (1975) was used. To 200 mg of atropine base and 200 ng of [^{3}H]atropine sulphate in 20 ml of cold $CHCl_3$, was added 500 mg of *m*-chloroperbenzoic acid. The solution was allowed to stand at 0 °C for two days. The $CHCl_3$ was evaporated to dryness and sufficient 10% K_2CO_3 was added to dissolve the residue (final pH7). By TLC (systems A and B), three components were found, corresponding to atropine, atropine-*N*-oxide (equatorial isomer) and atropine-*N*-oxide (axial isomer) as described by Phillipson & Handa (1975). A 25 µl sample was subjected to HPLC analysis and fractionated as before.

Noratropine. Noratropine was obtained by oxidative demethylation of atropine with $KMnO_4$ by modifications to the method of Phillipson et al (1976) as previously described (Van der Meer & Hundt 1983).

Tropine and tropic acid. Atropine sulphate (3 mg) and [^{3}H]atropine sulphate (200 ng) were dissolved in 3 ml sodium hydroxide solution (1 M) and allowed to stand at 95 °C overnight. The solution was cooled, neutralized to pH 7 (1 M H_2SO_4) and examined by TLC and HPLC. TLC showed two spots corresponding in R_F to authentic tropine and atropine. Radioactivity detection of the HPLC eluate showed three components which were identified, in order of increasing retention volume as tropine, atropine and tropic acid. UV detection after HPLC showed only two of these compounds, since tropine has very weak UV absorbance at 230 nm.

Identification of metabolites in human urine. Urine was collected from a normal volunteer over various times (see Table 2) after he had given consent to the administration of 2 mg of atropine sulphate and 100 µCi [^{3}H]atropine sulphate. This study was approved by the Ethics and Isotope Committee of the Medical Faculty of the University of the Orange Free State, Bloemfontein. The dose was administered by intravenous infusion in isotonic saline (10 ml) over 10 min at a rate of 1 ml min^{-1}. Total radioactivity, corrected for quenching, was measured in 100 µl aliquots of the collected urine specimens and is presented in Table 2. (Counting error at 99% confidence level was 1%.)

Aliquots (100 µl), of urines U2 to U5 (see Table 2) were chromatographed by HPLC and histogram plots of activity vs fraction number were plotted and are depicted in Fig. 1A. Total radioactivity for each peak was calculated to quantify the various fractions, and the results are presented in Table 3. The identity of each peak was established by reference to an HPLC chromatogram of the authentic compounds (Fig. 1B).

Deconjugation. In an attempt to identify any conjugates which may be present and which may not have appeared after direct injection of urine onto the HPLC column, the pH of 20 ml of urine U2 was adjusted to 5·6 by the addition of acetate buffer (0·1 M, 2 ml) and 50 µl of glucuronidase/arylsulphatase (Boehringer 127698) was added. The solution was incubated at 31 °C for 24 h before 100 µl was examined as before by HPLC. The activity of each peak was calculated relative to the total amount of radioactivity injected in order to detect quantitative changes. No additional peaks were detected, and the relative activity of each peak was identical to its corresponding peak before reaction with the enzyme.

Table 2. Analysis of total radioactivity excreted in urine of a normal volunteer.

Sample no.	Collection period (h)	Vol (ml)	Activity (dpm 100 µl^{-1})	% excreted
U1	0–2	4·5	17988	0·4
U2	2–4	355	19020	30·0
U3	4–6	272	17240	21·0
U4	6–9	313	10217	14·0
U5	9–13	419	5555	10·0
U6	13–22	512	2223	5·0
U7	22–23	284	374	0·5
U8	23–24	241	172	0·2

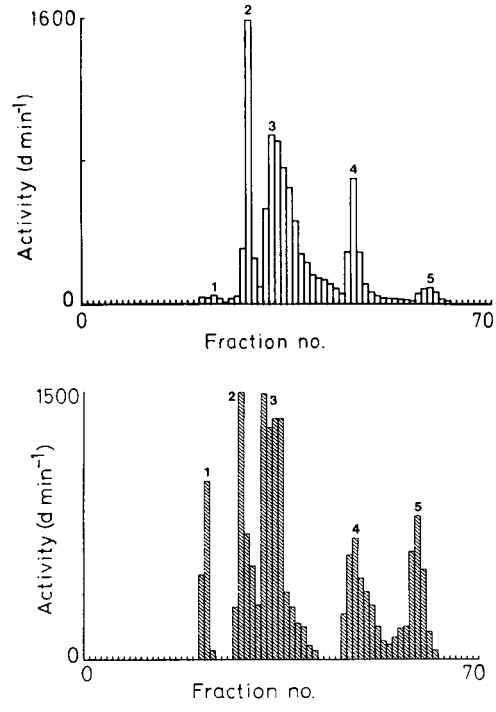

FIG. 1. A, HPLC separation and radioactivity detection of [^{3}H]atropine and metabolites in the urine (specimen U2) of a normal volunteer. Key: 1, tropine; 2, noratropine; 3, atropine; 4, equatorial *N*-oxide; 5, tropic acid. B, HPLC separation and radioactivity detection of [^{3}H]atropine and synthesized tritiated metabolites. Key: as A, except 5, axial *N*-oxide and tropic acid.

Table 3. Analysis of radioactivity in fractionated urine specimens of a normal volunteer.

Peak no.	Fraction nos	Compound	Counts min^{-1} per peak U2	U3	U4	U5	% total radioactivity (T) U2	U3	U4	U5	Mean %
1	21–25	Tropine	70	160	110	110	0·6	2	2	3	2
2	27–31	Noratropine	1880	2330	1762	906	17	27	31	28	24
3	32–45	Atropine	7134	4512	2918	1590	65	52	51	49	57
4	46–50	Atropine-*N*-oxide	1460	1456	774	558	13	17	14	17	15
5	58–63	Tropic acid	352	266	172	62	3	3	3	2	3
Total radioactivity (T)/100 µl			10896	8724	5736	3226					

Stereoselective metabolism in man. The possibility that only one isomer of atropine was being selectively metabolized was investigated as follows. Patient 1, admitted to hospital after accidentally ingesting an unknown amount of an organophosphate pesticide (demeton-S-methyl), was treated with large doses of atropine by means of a continuous infusion. Over 8 h he received 67 mg of atropine sulphate and passed 1750 ml of urine which was collected for analysis. The pH of 100 ml of this urine was adjusted to 7·2 and adsorbed onto five Extrelut columns (Merck 11737).

Similarly one 20 ml portion of urine from the same normal volunteer who had received 100 µCi [^{3}H]atropine sulphate was similarly treated to obtain radiolabelled metabolites for tracer purposes. The alkaloids were eluted from each column with chloroform (3 × 40 ml/column), and the combined $CHCl_3$ eluates were evaporated to dryness on a rotary evaporator to yield 40 mg residue, which was redissolved in 500 µl of the HPLC mobile phase.

This solution was chromatographed by HPLC as five 100 µl samples, and the fraction corresponding to atropine was in each case collected for further analysis. An aliquot of this fraction, subjected to TLC, showed one alkaloidal spot which corresponded to atropine. In control experiments, 200 ml of drug-free urine, as well as 200 ml of the same urine spiked with 7·66 mg of atropine sulphate, were processed in the same manner.

The optical activity of the fractions collected was investigated in each case by obtaining circular dichroism spectra. The spectrum (Fig. 2) for the atropine obtained from the urine of patient I was a mirror image of the spectrum obtained from authentic (−)-hyoscyamine, i.e. it was (+)-hyoscyamine.

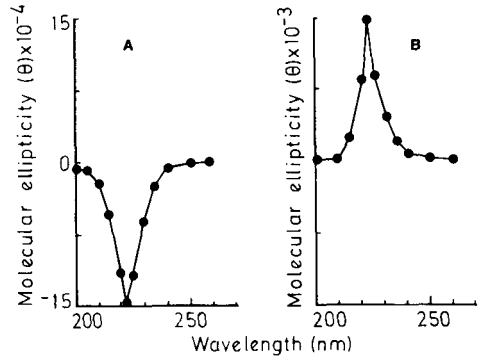

FIG. 2. Circular dichroism spectra of (A), (−)-hyoscyamine and (B), (+)-hyoscyamine extracted from the urine of a normal volunteer.

Results and discussion

Most of the work on atropine metabolism in man, has so far concentrated on finding chromatographic evidence for glucuronide formation and for the presence of the hydrolysis products tropine and tropic acid. Paper chromatography was employed almost exclusively and a limited number of solvent systems was used. Because of its toxicity, only very low doses of atropine could be administered to volunteers, which precluded the isolation of metabolites in sufficient quantities for identification by means of physical methods such as mass spectrometry and nuclear magnetic resonance.

This study used [^{3}H]atropine sulphate in an attempt to detect metabolites which may be present in very low concentrations. Based upon previous work and theoretical considerations, several compounds postulated as being possible metabolites were synthesized and characterized chromatographically using a suitably modified HPLC method previously described (Fell et al 1979) as well as two TLC systems with silica gel stationary phases.

The coincidence of retention volumes of products from the urine of a volunteer with those of the authentic synthesized compounds can be considered to be strong evidence of the identity of the metabolites of atropine in man.

Atropine has been reported to be excreted unchanged in man to the extent of approximately 50% (Kalser & McLain 1970; Gosselin et al 1960), which together with our own similar findings suggested that the excreted, unmetabolized atropine could be (+)-hyoscyamine. Such stereoselective metabolism has been shown to occur with other racemic mixtures such as (±)-ephedrine (Axelrod 1955b), (±)-amphetamine (Axelrod 1955a), (±)-methadone (Axelrod 1956) and (±)-3-methoxy-*N*-methylmorphinan (Axelrod 1956; Takemori & Mannering 1958).

Qualitatively, five components were detected in the

urine of a single volunteer to whom atropine sulphate and [^{3}H]atropine sulphate had been administered. Based upon chromatographic retention data, the compounds were identified in order of increasing retention volume as tropine, noratropine, atropine, atropine-*N*-oxide (equatorial isomer) and tropic acid. Tropine and tropic acid were present as minor metabolites, accounting for only about 2–3% of the administered dose. Besides (+)-atropine (50%), noratropine (24%) and the equatorial isomer of atropine-*N*-oxide (15%) were found to be the major metabolites. Incubation of urine with glucuronidase/arylsulphatase failed to produce either qualitative or quantitative changes in the chromatographic pattern thereby ruling out the existence of glucuronide or sulphate conjugates.

It would appear that normal man probably metabolizes stereoselectively the (−)-hyoscyamine enantiomer of atropine and excretes the biologically inactive (+)-enantiomer unchanged. This being the case, the excreted products should be the optically active compounds (+)-hyoscyamine, (−)-norhyoscyamine, (−)-hyoscyamine-*N*-oxide and (−)-tropic acid as well as tropine.

REFERENCES

Axelrod, J. (1955a) J. Biol. Chem. 214: 753–763

Axelrod, J. (1955b) J. Pharmacol. Exp. Ther. 114: 430–438

Axelrod, J. (1956) Ibid. 117: 322–330

Fell, A. F., Johnstone, S. J. K., Smith, G. (1979) J. Pharm. Pharmacol. 31 (Suppl.): 20 P

Gosselin, R. E., Gabourel, J. D., Wills, J. H. (1960) Clin. Pharmacol. Ther. I: No. 5, 597–603

Kalser, S. C., McLain, P. L. (1970) Ibid. II: No. 2, 214–227

Phillipson, J. D., Handa, S. S. (1975) Phytochemistry 14: 999–1003

Phillipson, J. D., Handa, S. S., Gorrod, J. W. (1976) J. Pharm. Pharmacol. 28: 687–691

Takemori, A. E., Mannering, G. J. (1958) J. Pharmacol. Exp. Ther. 123: 171–179

Van der Meer, M. J., Hundt, H. K. L. (1983) J. Pharm. Pharmacol. 35: 408