SCIENCE PAPERS

AN INVESTIGATION OF THE METABOLISM OF NEOSTIGMINE IN PATIENTS WITH MYASTHENIA GRAVIS

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Evidence of two metabolic products of neostigmine has been obtained by paper chromatography of urinary extracts from patients receiving the drug. One product has been identified as *m*-hydroxyphenyltrimethylammonium bromide and the relevance of this is discussed in relation to the therapeutic effect of oral neostigmine in the treatment of myasthenia gravis. A method is described for extracting neostigmine and related compounds from urine by precipitation with bromine water.

WHILE studying the urinary excretion of neostigmine in patients with myasthenia gravis it was found that up to 67 per cent of the drug was excreted unchanged when given by intramuscular injection but only about 5 per cent when administered orally (Nowell, Scott and Wilson, 1962). This evidence suggested that after oral administration the drug is metabolised; one probable metabolite being *m*-hydroxyphenyltrimethylammonium bromide, attempts were made to identify this and any related substances in the urine of myasthenic patients.

EXPERIMENTAL AND RESULTS

Procedure

Urine (100 ml.) was evaporated to dryness and the residue extracted with absolute ethanol (2 × 10 ml.). The extract was centrifuged, evaporated to dryness and while still warm was dissolved in distilled water (2 ml.). To this solution bromine water (8 ml.) was added and after thorough mixing, the orange-yellow precipitate* was centrifuged off, and washed with 2 ml. water. The precipitate was then warmed with 50 per cent (v/v) methanol (5 ml.), centrifuged and extracted once more with 50 per cent methanol (1 ml.). The combined supernatant methanol extracts were passed through a column of Amberlite CG 50 resin (12·0 × 0·7 cm.), buffered at pH 6·86 with 0·2M phosphate buffer, containing 15·76 g. anhydrous Na_2HPO_4 and 15·11 g. $NaH_2PO_4.2H_2O$ per litre, and suspended in 50 per cent methanol. The column was washed with 50 per cent methanol (10 ml.) and then with water (10 ml.). It was eluted with 0·2N HCl (50 ml.) and the eluate evaporated to dryness. The residue was extracted with absolute ethanol (5 ml.), the extract was centrifuged and again evaporated to dryness. The residue was re-extracted with abolute ethanol (1 ml.) and 0·5 ml. of this solution was chromatographed on Whatman 541 paper using butanol : ethanol : water : acetic acid (8 : 2 : 3 : 0·25) as running solvent (Nowell, Scott and Wilson, 1962).

* The reaction involves the perhalides of quaternary ammonium nitrogen compounds and of brominated phenols.

Detection of Neostigmine and Related Compounds in the Urine of Patients with Myasthenia Gravis treated with Neostigmine

Urine was collected from six patients with myasthenia gravis before and after administration of a dose of neostigmine. Three of the patients were given 30 mg. neostigmine bromide by mouth and three were injected intramuscularly with doses of 2·5 mg. neostigmine methylsulphate. Each specimen of urine was extracted and chromatographed by the procedure described above.

With patients given intramuscular neostigmine, urine samples collected 2 and 4 hr. after administration yielded a spot with the same R_F value as neostigmine (0·50–0·53). This was absent in an 8 hr. sample. Each sample of urine also yielded three spots between R_F 0·2 and 0·35 but as these were present before treatment and also in normal urine, they were considered not to be derived from neostigmine.

By contrast, after oral administration of the drug, no neostigmine was detected in any samples of urine but a spot appeared at R_F 0·45-0·46 in specimens collected at 4, 8 and 12 hr. after treatment. Another spot was also detected at R_F 0·37–0·38 in the 8 and 12 hr. samples. The fact that these two spots gave a blue colour with iodoplatinate and had an alkaline reaction with bromocresol purple (0·1 per cent) in acetone/ethanol, 9:1, (Gordon and Hewel, 1955) suggested that they were quaternary nitrogen bases.

The substance at R_F 0·45–0·46 produced a characteristic blue colour with iodoplatinate which was different from the colour of all the other spots, but was identical with that obtained from an authentic specimen of *m*-hydroxyphenyltrimethylammonium bromide which when chromatographed alone had an R_F value of 0·48–0·51; when mixed with neostigmine and chromatographed, neostigmine was detected in its usual position (R_F 0·50–0·53) while the spot for the *m*-hydroxytrimethylammonium bromide appeared about 2 cm. lower, at R_F 0·45–0·46. This shift in position was also obtained when it was chromatographed in the presence of NaCl, the shift being independent of the amount of salt added. When *m*-hydroxyphenyltrimethylammonium bromide was added to normal urine a spot with the characteristic blue colour was obtained at R_F 0·45–0·46; suggesting that the spot seen at these R_F values after oral neostigmine was probably due to *m*-hydroxyphenyltrimethylammonium bromide. This conclusion was supported by the results of paper electrophoresis; similar extracts of urine from myasthenic patients treated with oral neostigmine produced a spot with the same mobility as that obtained from normal urine to which *m*-hydroxyphenyltrimethylammonium bromide had been added.

Discussion

The results of these experiments have shown that neostigmine is metabolised in the body, and that after oral administration two derivatives are excreted in the urine. One of these is probably *m*-hydroxyphenyltrimethylammonium bromide, the other (R_F = 0·37–0·38) has not

been identified, but is apparently a quaternary nitrogen compound also. The extent of this metabolic change cannot at present be measured quantitatively. The method of estimating neostigmine in urine, by formation of a complex with bromophenol blue, is not applicable to *m*-hydroxyphenyltrimethylammonium bromide because this forms a complex only at a concentration of about 1 mg./ml. which is much higher than would occur at any time in the blood or urine of patients receiving the normal therapeutic doses of neostigmine.

Work is at present in progress to determine the sites in the body where neostigmine is metabolised to *m*-hydroxyphenyltrimethylammonium.

These findings may provide some explanation for the apparent anomaly that after intramuscular injection, neostigmine is excreted unchanged in the urine but no unchanged drug can be detected after oral administration, although in each case satisfactory relief of signs and symptoms occurs. There is adequate evidence for the anticurare action of *m*-hydroxyphenyltrimethylammonium bromide (Cowan, 1938; Randall, 1950; Randall and Lehmann, 1950; Riker and Wescoe, 1950a; Riker and Wescoe, 1950b; Artusio, Riker and Wescoe, 1950; MacFarlane, Pelikan and Unna, 1950). The last mentioned authors have also shown that this substance effectively relieves the symptoms of myasthenia gravis; they reported that after an intravenous injection of 5 mg. *m*-hydroxyphenyltrimethylammonium bromide the therapeutic effect was equivalent to that produced by 0·4 mg. neostigmine, but was much more rapid in onset.

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