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# METABOLISM OF ISOPRENALINE AFTER AEROSOL AND DIRECT INTRABRONCHIAL ADMINISTRATION IN MAN AND DOG

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1 Administration of isoprenaline by aerosol inhalation to man results in over 80% being metabolized to the sulphate conjugate.

2 The majority of an inhaled dose is probably swallowed since the metabolic pattern resembles that after an oral dose.

3 Isoprenaline, administered in aqueous solution directly into the bronchial tree in both man and dog, is rapidly *O*-methylated to 3-*O*-methyl isoprenaline, which is subsequently conjugated with sulphate.

4 3-*O*-Methylation is the main metabolic pathway for the small part of an inhaled dose which does enter the bronchial tree.

## Introduction

Isoprenaline (3,4-dihydroxyphenyl-2-isopropyl-amino-ethanol) is widely used in the treatment of bronchial asthma and is most commonly administered in microcrystalline form from a pressurized aerosol. Proper use of the canister during inspiration produces a therapeutic effect within 2 minutes. However, the majority of the dose can be recovered from the mouth and pharynx and presumably would be swallowed (Paterson, Conolly, Davies & Dollery, 1968). In this series of experiments, we have studied the metabolism of isoprenaline, after inhalation or direct administration of an aqueous solution into the bronchial tree, in order to determine the role of the lung in the metabolism of isoprenaline.

## Methods

### *Materials*

(±)-Isoprenaline-[7-$^3$H]-hydrochloride, 1800 mCi/mM in sterile aqueous solution, was made on request by the Radiochemical Centre, Amersham, UK.

(±)-Isoprenaline-[7-$^3$H]-sulphate was prepared in microcrystalline form. It was then loaded into pressurized aerosol containers, which were identical to those commercially available, by Riker Laboratories. The canisters were designed to deliver, at each firing, 500 $\mu$g (50 $\mu$Ci) of isoprenaline sulphate in freon propellant forming a 5 $\mu$m particle diameter.

### *Analytical procedures*

Heparinized blood samples were immediately cooled and centrifuged at 4°C to prevent formation of 3-*O*-methyl isoprenaline by red cell catechol-*O*-methyl transferase (Axelrod & Cohn, 1971) which has been observed in this laboratory to occur with isoprenaline. Ascorbic acid (1 mg/ml) was added as an antioxidant to plasma, which was stored at 4°C before analysis.

Urine samples were acidified to pH 1 with hydrochloric acid immediately after each collection and were stored at 4°C.

Total radioactivity was estimated by counting 1 ml samples of plasma or urine added directly to Instagel (Packard Instrument Ltd) in a Packard 3375 Tricarb Liquid Scintillation Spectrometer. All counts were corrected for quenching from a calibration curve, constructed using the external, high energy, compound radioactive source of the counter.

Quantitative measurements of isoprenaline before and after acid or enzyme hydrolysis were made by a modification of the method of Weil-Malherbe (Weil-Malherbe & Bone, 1952). Samples of plasma or urine (1 or 2 ml) were adjusted to pH 8.4 and diluted with 2 ml of buffer. The buffer (pH 8.4) was made freshly for each analysis and

consisted of 0.2 M sodium acetate with 1 mg/ml EDTA and 1 mg/ml ascorbic acid. The samples were passed through a glass column (0.55 cm bore) packed with a slurry of 0.5 g aluminium oxide (Brockmann Grade 1) in the acetate buffer. After washing the column with 5 ml acetate buffer followed by 5 ml water, the isoprenaline was eluted with 5 ml 0.2 N hydrochloric acid. Isoprenaline was estimated by counting the total acid fraction in 10 ml Instagel. The recovery from the column (84% ± 2.6) was checked with a solution of authentic isoprenaline at each analysis. The identity of the material in the acid eluate was confirmed by paper chromatography in two solvent systems (Conolly, Davies, Dollery, Morgan, Paterson & Sandler, 1972).

Quantitative measurements of 3-*O*-methyl isoprenaline were made following elution from cation exchange resin prepared in the hydrogen form (Smith & Weil-Malherbe, 1962). Amberlite resin CG-50, 200 mesh, (0.5 gm) was packed in 0.55 cm bore glass columns and 1-2 ml of plasma or urine at pH 6 were passed down the column. Isoprenaline was eluted with 5 ml 2 N boric acid (Mattock & Wilson, 1965) and 3-*O*-methyl isoprenaline was eluted with 5 ml 4 N ammonium hydroxide followed by 3 ml water. The total ammonium fraction eluted was counted in 10 ml Instagel. The recovery from the column (93% ± 0.8) was checked with [$^3$H]-3-*O*-methyl isoprenaline prepared enzymatically by incubating [$^3$H]-isoprenaline and S-adenosyl methionine with 150,000 x *g* supernatant of rat liver homogenate. The identity of the 3-*O*-methyl isoprenaline was also checked by chromatography in two solvent systems (Conolly *et al.*, 1972).

Hydrolysis of conjugates was carried out chemically and enzymatically. For acid hydrolysis, samples of urine or plasma were adjusted to pH 1.0 with hydrochloric acid and were refluxed for 20 minutes. Plasma proteins were first precipitated with 0.4 N perchloric acid. For enzyme hydrolysis, 1 ml samples of plasma or urine were diluted with 1 ml acetate buffer, adjusted to pH 5.5 and incubated at 37°C for 15 h with β-glucuronidase 5.8 units (Sigma) or aryl sulphatase 0.26 units/β-glucuronidase 0.52 units (Boehringer Mannheim).

*Human studies*

*(a) Inhalation.* Three subjects (one asthmatic and two normal volunteers) were given approximately 500 μg (50 μCi) [$^3$H]-isoprenaline from the specially prepared pressurized aerosol canister. In two subjects blood samples from the brachial artery were taken at 0.5, 1, 2 and 4 min after the dose. Urine was collected at 2 h intervals for the first 8 h and then at 12 h intervals thereafter.

*(b) Direct intrabronchial instillation.* Two male patients, undergoing genito-urinary surgery, agreed to join the study when its nature had been explained to them. Anaesthesia was induced with intravenous thiopentone and maintained with halothane. Endotracheal intubation was carried out under Scoline relaxation. A sterile solution of isoprenaline (2 μg, 30 μCi) in 1 ml was instilled into the bronchial tree through a fine polythene catheter passed to approximately 25 mm beyond the end of the endotracheal tube. The catheter was washed through with a further 1 ml of sterile saline.

Venous blood samples were collected at intervals during the surgery and for up to 16 h in one subject. Urine was collected for 48 hours.

*Animal studies*

Two female greyhound dogs, each weighing 25 kg, were anaesthetized with intravenous thiopentone and endotracheal tubes were passed. They were respired as needed and aqueous isoprenaline was instilled into the bronchial tree as described in the human studies. A bladder catheter allowed frequent urine sampling. After the experiments, the endotracheal tubes and catheters were removed and the dogs allowed to recover from anaesthesia. They were kept overnight in metabolism cages so that urine could be collected for 24 hours. Venous blood was sampled at intervals throughout the study.

## Results

*Administration of isoprenaline by pressurized aerosol to man*

Radioactivity could not be detected in the blood samples taken from the brachial arteries in the first few minutes after inhalation in two subjects.

The urinary recovery of radioactivity ranged from 54% to 84% of the dose. In all subjects the greatest excretion rate was between 2 and 3 h after inhalation, when 15% of the dose was excreted (Figure 1).

The excreted metabolites were similar in the three subjects studied. The major metabolite was the sulphate conjugate of isoprenaline, which accounted for over 80% of the total radioactivity recovered in the urine, while 10% was the sulphate conjugate of 3-*O*-methyl isoprenaline (Table 1). Less than 5% of the total radioactivity was

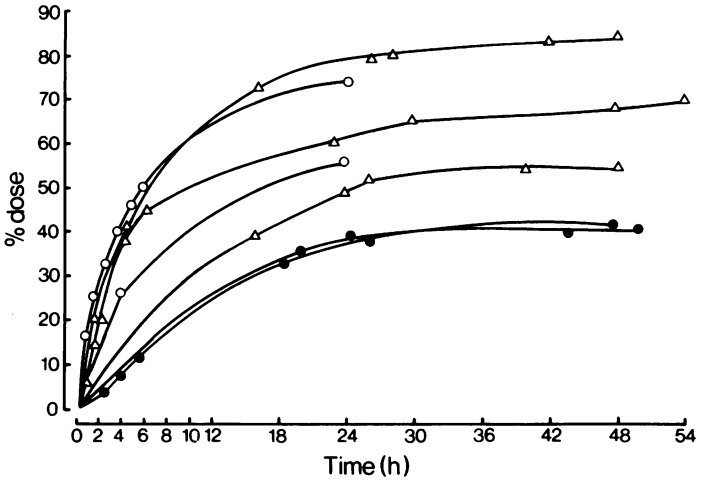


**Fig. 1** Cumulative excretion of total radioactivity in urine. (△) Man, after administration of isoprenaline from pressurized aerosol; (●) man, after administration of aqueous isoprenaline solution directly into the bronchial tree; (○) dog, after administration of aqueous isoprenaline solution directly into the bronchial tree.

unchanged isoprenaline and less than 4% appeared as 3-*O*-methyl isoprenaline.

*Direct instillation of aqueous isoprenaline into the bronchial tree in man*

Peak plasma levels of total radioactivity were reached 2 h after the dose and then a slow decline was seen during the following 12 hours. Analysis of the plasma (Fig. 2) showed that the major component (51% total radioactivity) was the sulphate conjugate of 3-*O*-methyl isoprenaline, while 11% was the sulphate conjugate of isoprenaline. Free 3-*O*-methyl isoprenaline accounted for 5% of the total radioactivity and less than 10% was unchanged isoprenaline. A fraction (20%) remained unidentified even after exhaustive acid and enzymic hydrolysis and acid extraction.

The urinary recovery of radioactivity in 48 h was 35% and 41% of the dose (Figure 1). The pattern of metabolites reflected that of the plasma and was similar in the two subjects studied, the

**Table 1** Urine analysis after aerosol inhalation and intrabronchial dosing in man

| *Subject* | *C.D.* | *D.D.* | *S.M.* | *L.N.* | *J.S.* |
|---|---|---|---|---|---|
| Dose (μg) | 480 | 230 | 200 | 2.0 | 2.0 |
| Route administration | Aerosol | Aerosol | Aerosol | Intrabronchial solution | Intrabronchial solution |
| % Dose recovered | 84 | 70 | 54 | 41 | 35 |
| Duration collection (h) | 48 | 54 | 48 | 48 | 50 |
| Metabolites in pooled urine as % total radioactivity recovered | | | | | |
| Isoprenaline | 3.5 | 1.7 | 5.6 | 8.6 | 8.8 |
| Sulphate conjugate of isoprenaline | 86.1 | 94.9 | 81.9 | 14.9 | 6.3 |
| 3-*O*-methyl isoprenaline | 1.2 | 0.8 | 4.1 | 12.1 | 6.2 |
| Sulphate conjugate of 3-*O*-methyl isoprenaline | 6.1 | 4.2 | 9.8 | 48.2 | 59.7 |
| Unidentified | 3.1 | 0 | 0 | 16.2 | 19.0 |

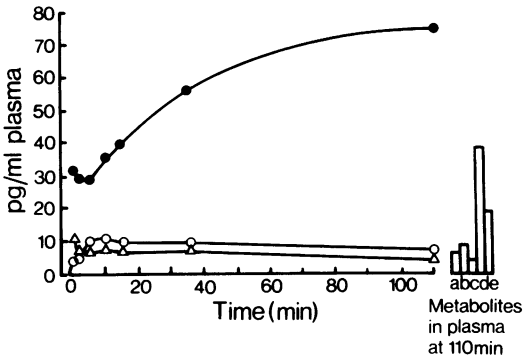


**Fig. 2** Metabolites in plasma after administration of aqueous isoprenaline solution directly into the bronchial tree in man. (●) Total radioactivity; (○) unchanged isoprenaline; (△) free 3-*O*-methyl isoprenaline. (a) Unchanged isoprenaline; (b) sulphate conjugate of isoprenaline; (c) free 3-*O*-methyl isoprenaline; (d) sulphate conjugate of 3-*O*-methyl isoprenaline; (e) unidentified fraction.

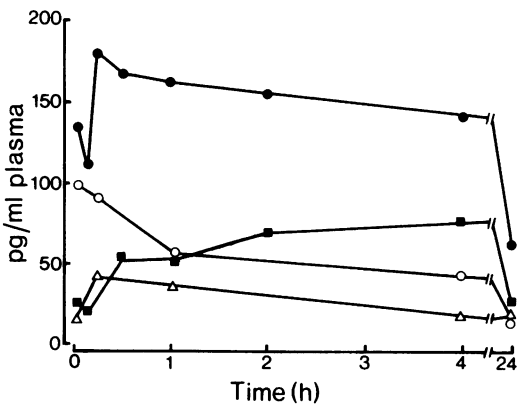


**Fig. 3** Metabolites in plasma after administration of aqueous isoprenaline solution directly into the bronchial tree in the dog. (●) Total radioactivity; (○) unchanged isoprenaline; (△) free 3-*O*-methyl isoprenaline; (■) sulphate conjugate of 3-*O*-methyl isoprenaline.

major component being the sulphate conjugate of 3-*O*-methyl isoprenaline, which accounted for 48% and 60% of the total radioactivity (Table 1). The remaining radioactivity was distributed between free 3-*O*-methyl isoprenaline, free isoprenaline and the sulphate conjugate of isoprenaline, while in both subjects a fraction (16% and 19%) of the total radioactivity remained unidentified.

*Direct instillation of aqueous isoprenaline into the bronchial tree in dog*

The peak plasma level occurred about 40 min after the dose and then a slow decline in total radioactivity was observed (Figure 3). The metabolites in plasma were the same as those seen in man with the sulphate conjugate of 3-*O*-methyl isoprenaline becoming, after 1 h, the major component (Figure 3).

The dogs excreted 56% and 74% of the dose in urine over 24 hours. Approximately 45% of the total radioactivity in urine was the sulphate conjugate of 3-*O*-methyl isoprenaline and 25% remained unconjugated (Table 2). Unchanged isoprenaline or the sulphate conjugate of isoprenaline together accounted for less than 17% of the total radioactivity. As with the human studies about 15% total remained unidentified after several investigations.

**Table 2** Urine analysis after intrabronchial dosing in dog

| Dog | *A* | *B* |
|---|---|---|
| Dose (μg) | 15 | 8 |
| % Dose recovered | 56 | 74 |
| Duration collection (h) | 24 | 24 |
| Metabolites in pooled urine as % total radioactivity recovered | | |
| Isoprenaline | 16.6 | 8.5 |
| Sulphate conjugate of isoprenaline | 0 | 6.5 |
| 3-*O*-methyl isoprenaline | 23.5 | 26.0 |
| Sulphate conjugate of 3-*O*-methyl isoprenaline | 43.8 | 45.0 |
| Unidentified | 16.1 | 14.0 |

## Discussion

Isoprenaline was extensively metabolized in all of the studies since unchanged drug accounted for about 10% or less of the total radioactivity in plasma and urine. The pathways for metabolism were *O*-methylation and sulphate conjugation. In these and other studies (Conolly *et al.*, 1972) we have no evidence for acidic metabolites arising from attack by monoamine oxidase. However, in these studies, when the drug was given directly into the bronchial tree, a small but significant fraction of radioactivity remained unidentified. This possibly represents non-enzymic degradation products of isoprenaline.

We have shown that when isoprenaline is given to man by aerosol inhalation, the major excreted metabolite is isoprenaline sulphate. However, when the drug is instilled directly into the bronchial tree, 3-*O*-methyl isoprenaline sulphate is the predominant metabolite.

The explanation for this difference lies in the observation by Paterson (Paterson *et al.*, 1968) that most of an inhaled dose is deposited in the mouth and pharynx and is probably swallowed. The pattern of metabolism described here for the inhaled drug is identical with that reported by Conolly *et al.* (1972) for an oral dose. The swallowed isoprenaline is probably converted to its sulphate conjugate in the gut wall. We have shown (unpublished results) that everted sacs of dog duodenum conjugate isoprenaline with sulphate. Boström, Brömster, Nordenstam & Wengle (1968) have also demonstrated the occurrence of phenol and steroid sulphokinases in mucosa of human gastrointestinal tract. The fate of the small proportion of the inhaled isoprenaline, which reaches the airways and exerts the pharmacological effect is probably similar to that of drug introduced directly into the airways down an endotracheal tube.

Isoprenaline introduced directly into the bronchial tree was metabolized by catechol-*O*-methyl transferase (COMT) to form 3-*O*-methyl isoprenaline, much of which was then conjugated with sulphate. Lung tissue has been shown to contain COMT (Axelrod, 1959; Axelrod & Cohn, 1971) and we have reported recently (Briant, Blackwell, Williams, Davies & Dollery, 1973) that *O*-methylation of isoprenaline and isoetharine occurs in the isolated perfused dog lung. In addition, we were able to demonstrate that about 30% of the isoprenaline was *O*-methylated in its first pass from the airways into the circulation.

Thus the isoprenaline which enters through the lung, *in vivo*, will probably be *O*-methylated in the lung or by erythrocytes, which also contain COMT (Axelrod & Cohn, 1971). Subsequent conjugation with sulphate probably occurs at a site other than lung as we were unable to detect conjugated metabolites in the isolated lung preparations.

Our results suggest that the lung plays a minor role in the absorption and metabolism of a so called 'inhaled' dose of isoprenaline. However, *O*-methylation by the lung of that small proportion of inhaled isoprenaline which reaches the airways may be important in limiting the cardiac effects of the drug administered by this route.

We are unable to comment on whether *O*-methylation in the lung also reduces the bronchodilator activity of isoprenaline since we do not know whether metabolism occurs before or after reaching the receptors in bronchial smooth muscle.

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