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Metabolism of isoprenaline in dog and man

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Summary

1. The metabolism of isoprenaline has been studied in man and dog following intravenous and oral or intra-duodenal administration.

2. Intravenous isoprenaline was excreted largely unchanged in urine in both species. Only one-third of the radioactivity in urine was in the form of the *O*-methyl metabolite.

3. After oral doses in man or intraduodenal doses in dogs, plasma radioactivity was almost entirely as conjugated isoprenaline and this metabolite accounted for more than 80% of radioactivity in urine.

4. Catechol-*O*-methyl transferase may be less important than $Uptake_2$ in limiting the pharmacological action of isoprenaline.

5. Pharmacological response (heart-rate increase) was related to plasma concentration of isoprenaline only after rapid intravenous injections. In dogs, following prolonged infusion or intraduodenal doses, heart rate returned to base-line values when plasma concentrations of isoprenaline were high.

Introduction

Isoprenaline (3,4-dihydroxyphenyl-2-isopropylamino-ethanol) is widely used in the treatment of bronchial asthma. We were prompted to study its pharmacology and metabolism after a reported increase in the number of asthma deaths (Speizer, Doll & Heaf, 1968) especially in young people, was tentatively linked to the use of pressurized aerosols of adrenaline-like bronchodilators including isoprenaline. Although there has been extensive investigation into the pharmacology and metabolism of the catecholamines noradrenaline and adrenaline (Axelrod, 1966), little attention has been given to their structural isomer, isoprenaline. Hertting (1964) found that in the rat 92% of an intravenous dose of (±)-isoprenaline-7-^{3}H was excreted in 8 hours. Free and conjugated isoprenaline and 3-*O*-methyl isoprenaline were found in the urine and conjugated 3-*O*-methyl isoprenaline in bile. There was no evidence of deaminated products of isoprenaline, presumably because the isopropyl group attached to the nitrogen atom prevented metabolism by monoamine oxidase (MAO). Indeed, the extent of *O*-methylation of the (+)-isomer of isoprenaline was assumed by Sjoerdsma (1961) to be an index of catechol-*O*-methyl transferase (COMT) activity in hypertensive patients.

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The fate of isoprenaline has also been studied in the dog following intravenous and oral doses (Conway, Minatoya, Lands & Shekosky, 1968). Intravenously administered isoprenaline was reported to be inactivated by conversion to 3-*O*-methyl isoprenaline by COMT whilst orally administered drug was largely conjugated with sulphate during absorption.

In this paper the fate of the drug following both intravenous and oral administration in man and dog is reported. In addition, the relationship between pharmacological response and plasma levels of the drug was also studied. Preliminary reports of these studies have been given (Davies, Morgan, Conolly, Paterson, Sandler & Dollery, 1969; Morgan, Sandler, Conolly, Davies, Paterson & Dollery, 1969).

Methods

Materials

(±)-Isoprenaline-7-^{3}H hydrochloride 1,500 mCi/mM was made on request by the Radiochemical Centre, Amersham, U.K. It was diluted with nonradioactive carrier to prepare doses with specific activities ranging from 0·01 to 8·66 μCi/μg for oral and intravenous studies. 3-*O*-Methyl isoprenaline was kindly supplied by Drs. H. Klupp (C. H. Boehringer) and G. Hertting (University of Vienna).

Alumina (aluminium oxide, neutral X 'Camag' M.F.C. 100–240 mesh) was obtained from Hopkins & Williams Limited, and treated according to the method of Crout (1961). Cation exchange resin (Amberlite CG 50 (H) Type 1 100–200 mesh) was obtained from British Drug Houses Limited.

Animal preparations

Mongrel dogs of either sex, weighing between 12 and 25 kg were used. The animals were anaesthetized with thiopentone, and anaesthesia was maintained with pentobarbitone. Respiration was maintained with a Palmer pump, with minute volume settings appropriate to the dogs' size. Arterial blood pH, Pa_{O_2} and Pa_{CO_2} remained within normal physiological limits throughout the studies. Blood pressure (1 mmHg≡1·333 mbar) was measured with a Consolidated Electrodynamics Strain gauge attached to a PE catheter inserted into the femoral artery, the signal being fed to a Devices M4 recorder. Heart rate was measured either on a Neilson type instantaneous rate meter, or on a specially designed digital rate meter (Emons & Conolly, 1971). Drugs were administered through a PE 160 catheter inserted into a brachial vein and blood samples were taken from an arterial catheter.

In appropriate experiments the bile duct was cannulated, with a small angiocath, the cystic duct and the distal common bile duct being ligated. The portal vein was cannulated with an angiocath behind the head of the pancreas. Oral isoprenaline administration was simulated by injecting the drug through a soft portex catheter tied into the duodenum with a purse string suture.

Human studies

The patients studied were asthmatics over the age of 45 attending Hammersmith Hospital. Studies were done in the supine position. Heart rate was monitored by means of an ECG triggered rate meter. Blood pressure was monitored with a

sphygmomanometer, taking the 1st and 4th phase of the Korotkoff sounds to represent systolic and diastolic pressure levels.

Isoprenaline was taken by mouth in an aqueous solution. For intravenous studies, specially prepared sterile solutions of isoprenaline were injected or infused into an antecubital vein via a scalp vein needle.

Analytical procedures

Blood samples were heparinized and centrifuged immediately and a 1 ml aliquot of plasma was counted in a Packard Liquid Scintillation Spectrometer with a dioxane based scintillator. Total radioactivity in urine and bile samples was similarly measured. Daily faecal samples were homogenized and diluted to 1,000 ml and 0·1 ml aliquots were ignited in plastic bags in an atmosphere of oxygen (Gupta, 1968). The tritiated water so formed was dissolved in a dioxan-based scintillator injected into the bag after combustion. An aliquot of the scintillator fluid was taken and counted. All counts were corrected for quenching by means of a calibration curve constructed by utilizing the external radioactive source of the scintillation spectrometer. Qualitative examination of samples of plasma, bile or urine for unchanged drug or metabolites was carried out by paper and cellulose thin layer chromatography in two solvent systems: (a) butanol:acetic acid:water (4:1:1); (b) secondary butanol:pH 3·9 buffer (4:1) the buffer being composed of water:pyridine:acetic acid (100:10:41).

Isoprenaline was detected by spraying with 0·44% potassium ferricyanide in 0·1 M phosphate buffer (pH 7·4) and 3-*O*-methyl isoprenaline was visualized with 0·25% w/v diazotized *p*-nitroaniline. Radioactivity was detected on the developed chromatograms with an automatic 4 π strip scanner (Packard Instrument Company) or by cutting the paper into 2 cm strips and counting the radioactivity eluted with methanol in a liquid scintillation spectrometer. The Rf values of the radioactive materials were compared with those of authentic isoprenaline and 3-*O*-methyl isoprenaline added to samples and visualized as described above.

Quantitative measurements of isoprenaline concentrations were made by a method based on the extraction procedure of Weil-Malherbe & Bone (1952). Plasma or urine samples, 2 to 4 ml in volume, were taken before and after hydrolysis. 10% w/v ethylene diaminetetra-acetic acid (EDTA) (0·2 ml) and 5% w/v ascorbic acid (0·2 ml) and non-radioactive isoprenaline (100 μg) and 3-*O*-methyl isoprenaline (100 μg) were added as carriers and the samples were carefully adjusted to pH 8·4.

The samples were passed through a glass column (0·55 cm internal diameter) packed with alumina (0·5 g) slurried in 0·2 M sodium acetate buffer (pH 8·4, 1–2 ml). This acetate buffer was prepared by mixing solutions of 0·2 N sodium acetate (50 ml), 10% w/v EDTA (1 ml) and 5% w/v ascorbic acid (1 ml) and adjusting to pH 8·4. The column was washed with acetate buffer (4·0 ml) and water (4·0 ml). Isoprenaline was eluted with 0·2 N HCl (5·0 ml) and water (5·0 ml) and an aliquot of the acid eluate was counted to determine the content of radioactive isoprenaline.

Recovery for this column procedure was determined each time by adding a known amount of radioactive isoprenaline to duplicate samples of plasma or urine and all estimations were corrected for these recoveries. The recovery was

$63 \pm 1{\cdot}3\%$. The identity of the material in the acid eluate from these columns was confirmed by paper chromatography in two solvent systems.

Quantitative estimation of 3-*O*-methyl isoprenaline in plasma and urine was carried out by a procedure based on the method of Ruthven & Sandler (1965) for metadrenalines, after isoprenaline had been adsorbed on alumina.

The effluent from the alumina column was adjusted to pH 6·5 and passed down a cation exchange resin Amberlite CG 50 column (bore 10 mm, bed 5 cm). The column was washed with water (10 ml) 4% w/v boric acid (10 ml) (Mattock & Wilson, 1965) and water (10 ml) and the radioactive 3-*O*-methyl isoprenaline was then eluted with 4 N NH_4OH (10 ml). An aliquot (1 ml) of the ammonia eluate was counted to determine the radioactive 3-*O*-methyl isoprenaline.

The column recovery of 3-*O*-methyl isoprenaline ($86{\cdot}6 \pm 2{\cdot}9\%$, 6 estimations) was determined by adding a known amount of ^{14}C 3-*O*-methyl isoprenaline as internal standard, followed by double-labelled counting. All estimations were corrected for this recovery. The identity of the 3-*O*-methyl isoprenaline was also checked by chromatography in two solvent systems.

Hydrolysis

Hydrolysis of suspected conjugates of isoprenaline and its metabolites was carried out chemically and enzymatically. For acid hydrolysis, urine or plasma samples were adjusted to pH 0·9 with 3 N HCl and refluxed for 20 minutes. For enzymic hydrolysis 1 ml samples of plasma or urine were adjusted to pH 5·5 with acetate buffer and were incubated at 37° C for 15 h with glusulase (0·26 units sulphatase + 0·52 units β-glucuronidase) or β-glucuronidase (5·8 units).

Results

Intravenous administration in man

Isoprenaline given by rapid intravenous injection to normal subjects produced a dose-related increase in heart rate, which declined with a half-life ranging from 10 to 67 s (mean 29·1 seconds).

In one subject tritiated isoprenaline was given as a slow intravenous injection in a dose of 0·063 μg/kg over 1 minute. The heart rate increased by 15 beats/min and returned to normal after 8 minutes. Figure 1 shows that plasma radioactivity declined in a biphasic manner, during the first more rapid phase the half-life of plasma radioactivity was approximately 5 min and during the second and slower phase it was more than 2·5 hours. Analysis of plasma radioactivity for 6·5 h after dosing revealed that unchanged isoprenaline was always the major component, accounting for over 80% of the total. The remaining radioactivity was 3-*O*-methyl isoprenaline, which declined with a half-life similar to the second phase of the decay curve.

One patient received an intravenous infusion of isoprenaline (0·44 μg base/kg) over a period of 30 minutes. Heart rate increased by 30 beats/min during the infusion and returned to normal with a half-life of 10 min at the end of the infusion. Fractionation of two plasma samples from this patient showed that the major radioactive component in plasma, at least at the early time, was isoprenaline. Twenty-seven minutes after the end of the infusion a considerable concentration of 3-*O*-methyl isoprenaline was found in plasma (Table 1).

Some plasma samples from both patients were hydrolyzed but there was no change in the pattern of distribution of radioactivity confirming the absence of labelled conjugates of isoprenaline or 3-*O*-methyl isoprenaline in plasma (Table 1).

Urine was collected for 24 h from both patients receiving intravenous isoprenaline and analysed for unchanged drug and metabolites (Table 1). An average of 73% of the radioactive dose appeared in 24 h by which time excretion of radioactivity had virtually ceased. More than 60% of the radioactivity was excreted in the

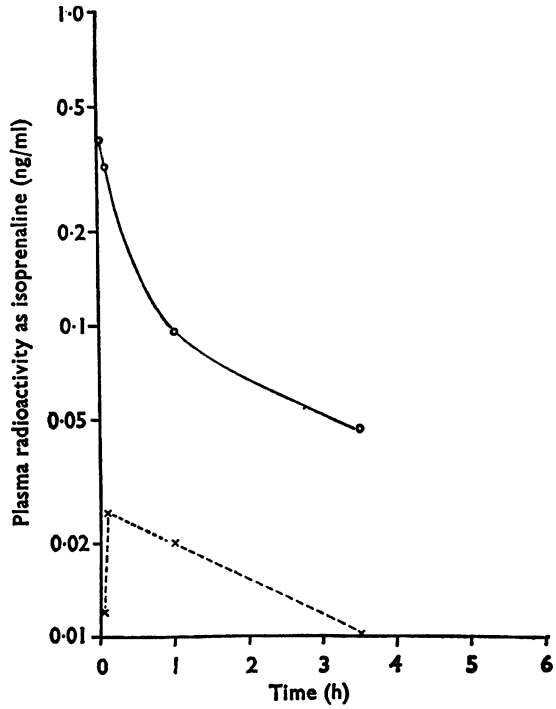

FIG. 1. Time course of plasma concentrations of free isoprenaline (○——○) and 3-*O*-methyl isoprenaline (×----×) in man following an intravenous dose of 0·063 μg/kg.

TABLE 1. *Isoprenaline and metabolites in plasma and urine after intravenous dosing in man*

			Plasma			Urine (% dose)		
Subject	Dose (μg/kg)	Period of injection	Time of sample (min)	Isoprenaline* (ng/ml)	3-*O*-methyl isoprenaline* (ng/ml)	Time of sample (h)	Isoprenaline*	3-*O*-methyl isoprenaline*
1	0·063	1 min	1	0·39 (100)	0·012 (100)	0– 1·3	5·4 (95)	0·9 (100)
			5	0·32 (100)	0·025 (100)	1·3– 1·5	14·4 (95)	5·3 (70)
			60	0·095	0·020	1·5– 3·5	12·9 (100)	5·1 (21)
			210	0·046	0·01	3·5–10·5	12·0 (100)	12·2
						10·5–15	2·2	1·2
						Total	46·9	24·7
2	0·44	30 min	34	0·66 (100)	—	0–0·5	5·8 (100)	1·1 (78)
			57	0·32 (100)	0·24 (100)	0·5–4	8·7 (100)	2·6 (43)
						4–7	19·9 (100)	23·6 (5)
						7–22	5·3 (100)	7·9 (4)
						Total	39·7	35·2

* Figures in parentheses indicate the percentage of each compound in the unconjugated state.

first 8 hours. Unchanged isoprenaline accounted for more than 60% of the urinary radioactivity. The remainder was present as free or conjugated 3-*O*-methyl isoprenaline and there was no evidence of acidic metabolites after hydrolysis. The pattern of distribution of radioactivity was the same whether the drug was given by injection or by slow infusion.

Intravenous administration in dogs

In dogs larger doses of the drug (0·27–0·64 μg base/kg) were given intravenously to produce a greater increase in heart rate so that the relationship between the rate of decline of tachycardia and fall in plasma isoprenaline could be studied more accurately. Following intravenous doses of 0·27 to 0·64 μg/kg the heart rate in anaesthetized dogs showed increases ranging from 22 to 80 beats above resting rates and then declined with a half-life ranging from 0·5 to 2·5 minutes. Plasma radioactivity declined biphasically and in these studies the similarity in the rates of decline of heart rate and the initial fall of radioactivity was seen more clearly (Fig. 2). Fractionation of plasma radioactivity revealed that unchanged isoprenaline was a major component, accounting for about 75% of the total, while 3-*O*-methyl isoprenaline made up the remainder. Table 2 summarizes the results for three dogs. Acid hydrolysis of representative samples produced little change in the distribution of radioactivity, confirming the absence of significant levels of conjugated isoprenaline or 3-*O*-methyl isoprenaline in plasma.

Both urine and bile were collected for the period of the experiments (2–7 h). During this time an average of 46·1% of the radioactive dose was excreted in urine while less than 0·5% was excreted in bile.

Quantitative examination of the urine (Table 2) revealed that unchanged iso-

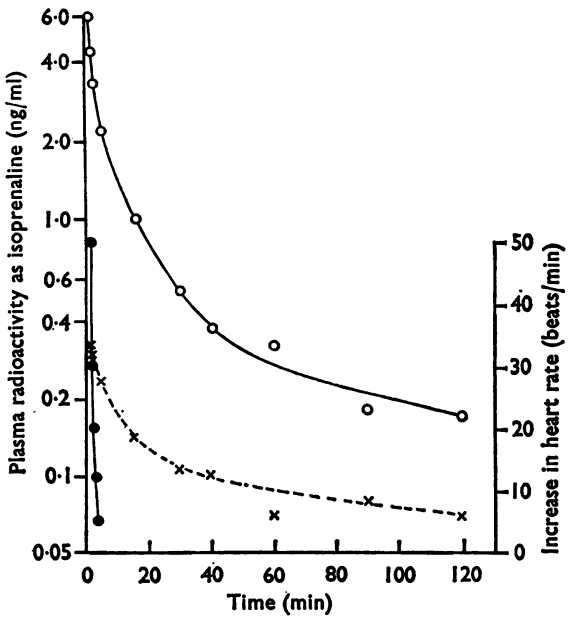

FIG. 2. Increase in heart rate (●——●) and plasma levels of free isoprenaline (○——○) and 3-*O*-methyl isoprenaline (×----×) in Dog 1 following an intravenous dose of 0·64 μg/kg.

prenaline was the major radioactive component, accounting for 60–80% of the total urinary activity. Free 3-*O*-methyl isoprenaline accounted for 20–40% of the total activity recovered.

Oral administration to man

Three patients received a small dose (from 0·04 to 0·09 mg/kg, 50 μCi) of isoprenaline dissolved in water; a fourth patient received 0·2 mg/kg (50 μCi). At the lower dose level no effect on heart rate was detected but in the patient receiving

TABLE 2. *Isoprenaline and metabolites in plasma and urine after intravenous dosing in dogs*

		Plasma			Urine (% dose)		
Dog no.	Dose (μg/kg)	Time of sample (min)	Isoprenaline* (ng/ml)	3-*O*-methyl isoprenaline* (ng/ml)	Time of sample (h)	Isoprenaline*	3-*O*-methyl isoprenaline*
1	0·64	1·0	6·00	—	2	42·9	12·1
		2·5	4·38 (100)	0·34 (94)			
		5·0	2·18 (100)	0·24 (96)			
		60	0·31 (100)	0·08 (88)			
		120	0·16 (100)	0·07			
2	0·50	0·5	5·65 (100)	0·49 (100)	7	38·7	9·4
		20	0·87 (100)	0·13 (100)			
		90	0·24 (100)	0·07 (100)			
		210	0·19 (100)	0·04 (100)			
3	0·27	1	1·71 (100)	0·27 (100)	3	21·1 (97·2)	14·2 (100)
		3	0·92 (100)	0·13 (100)			
		10	0·53 (100)	—			
		60	0·17 (100)	—			
		180	0·05 (100)	—			

* Figures in parentheses indicate the percentage of each radioactive component appearing in the unconjugated form.

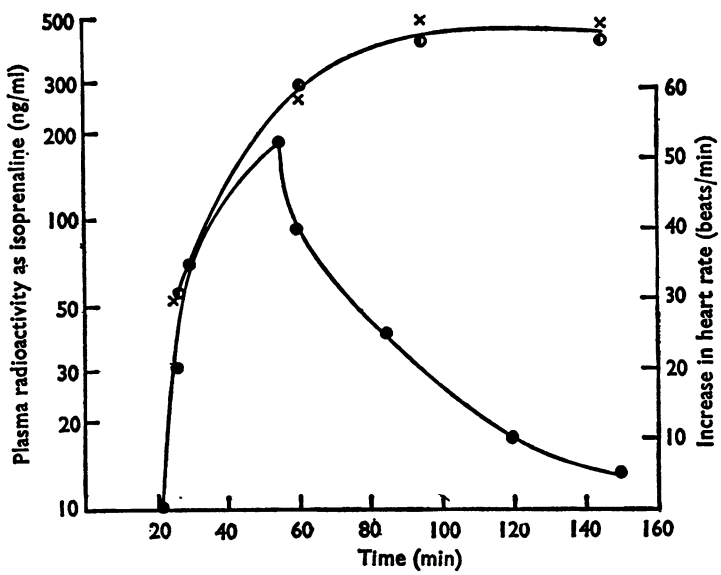

FIG. 3. Time course of changes in heart rate (●——●) and plasma levels of radioactivity (◐——◐) and conjugated isoprenaline (×----×) in man following an oral dose of 0·2 mg/kg (50 μCi).

the higher dose, the heart rate increased sharply 20 min after administration, rising to a peak of 55 beats/min above resting levels after about 55 min (Fig. 3). It then regressed to normal, with a half-life of approximately 40 minutes. In this subject, plasma radioactivity rose sharply between 20 and 60 min after ingestion and reached a plateau level at approximately 80 min where it remained during the period the blood samples were taken. Total radioactivity was still rising between 60 and 80 min after dosage, at which time the heart rate had begun to fall. When plasma radioactivity was fractionated no free isoprenaline or 3-*O*-methyl isoprenaline was detected. However, following acid hydrolysis, high concentrations of isoprenaline were recovered; no other radioactive compounds were found.

In three patients 48 h faecal collections were made and the total radioactivity was determined. Faecal recovery ranged from 12·2 to 27·0% of the administered radioactivity but the identity of the material was not determined. Between 59·1 and 106·8% of the dose of radioactivity was excreted in urine over a period of 30–48 hours. Chromatographic examination of the urine revealed the presence of a broad radioactive band with an Rf of 0·22. After acid hydrolysis, a narrower band with an Rf value of 0·53 was observed. As the Rf value for authentic isoprenaline was 0·50, conjugated isoprenaline was presumably present in unhydrolyzed urine.

Quantitative examination revealed that conjugated isoprenaline was the major metabolite in urine from all patients (Table 3). A small percentage of free isoprenaline was also detected (6·5 to 16·2%); 2·6–11·4% of the dose was excreted as 3-*O*-methyl isoprenaline mostly in conjugated form and this accounted for the remaining radioactivity. No acidic metabolites of isoprenaline were found in these studies.

TABLE 3. *Analysis of urinary radioactivity following oral administration of isoprenaline to man*

Subject no.	Dose (mg/kg)	Time of sample (h)	Urine (% dose) Isoprenaline*	3-*O*-methyl isoprenaline*
1	0·21	0–3	36·2 (11)	4·1 (17)
		3–6	45·1 (5)	5·8 (11)
		6–20	13·4 (3)	1·5 (9)
		20–30	0·7 (3)	
		Total	95·4 (7·0)	11·4 (13)
2	0·044	0–1	—	—
		1–3	6·1 (0)	0·2 —
		3–6	14·8 (42)	0·4 (64)
		6–24	31·8 (8·9)	1·4 (36)
		24–48	8·3 (9·8)	0·6 (46)
		Total	61·0 (16·2)	2·6
3	0·049	0–9·5	30·5 (8·5)	4·5 (25)
		9·5–36	20·3 (3·5)	3·8 (15)
		Total	50·8 (6·5)	8·3 (20)
4	0·087	0–0·5	0·4 (53·0)	0·03
		0·5–2	15·3	0·80 (64)
		2–4	17·7	1·4
		4–24	37·8 (4·1)	8·1
		24–48	6·3 (5·8)	0·7 (25)
		Total	77·5	11·03

* Figures in parentheses indicate the percentage of each radioactive component appearing in the unconjugated form. In the 'Total' lines these percentages are calculated, not measured, values.

The nature of the conjugates of isoprenaline and 3-*O*-methyl isoprenaline in urine was investigated by incubating a sample of urine with glusulase or β-glucuronidase. The results (Table 4) show that hydrolysis with acid or glusulase greatly enhanced the recovery of free isoprenaline and 3-*O*-methyl isoprenaline. Treatment with β-glucuronidase did not change the recovery of free isoprenaline but did slightly increase the level of 3-*O*-methyl isoprenaline.

TABLE 4. *Analysis of urine radioactivity before and after hydrolysis with hydrochloric acid, β-glucuronidase or glusulase after oral administration of isoprenaline to man*

Total activity (dpm/ml)	Free isoprenaline (dpm/ml)	Free isoprenaline recoverable after acid hydrolysis (dpm/ml)	Free isoprenaline recoverable after hydrolysis with glucuronidase (dpm/ml)	Free isoprenaline recoverable after hydrolysis with glusulase (dpm/ml)	Free 3-*O*MI (dpm/ml)	Free 3-*O*MI recoverable after acid hydrolysis (dpm/ml)	Free 3-*O*MI recoverable after hydrolysis with glucuronidase (dpm/ml)	Free 3-*O*MI recoverable after hydrolysis with glusulase (dpm/ml)
8,569	259	7,677	224	8,313	75	646	247	400

Intraduodenal administration in the dog

Doses of approximately 1 mg/kg administered intraduodenally to three dogs produced an increase in heart rate within 2 minutes. A peak of 45–70 beats/min above resting rates was seen at 2–12 minutes. Thereafter, values fell to base-line with a half-life of 10–25 minutes. Plasma radioactivity rose sharply to reach a plateau at 80 minutes, where it remained for the rest of the experiment (Fig. 4).

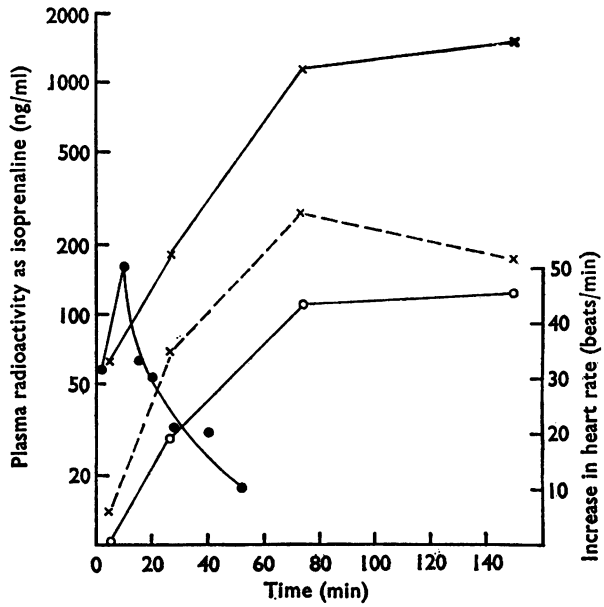

FIG. 4. Time course of changes in heart rate (●——●) and plasma levels of free isoprenaline (○——○), conjugated isoprenaline (×——×) and 3-*O*-methyl isoprenaline (×----×) in dog following an intraduodenal dose of 1 mg/kg (131 μCi).

Fractionation of the radioactivity on alumina and cation exchange columns before and after acid hydrolysis of the plasma samples showed that more than 55% of the radioactivity in plasma at all times studied was a conjugate of isoprenaline. Approximately 10% of the activity was in the form of free 3-*O*-methyl isoprenaline which rose to a level of approximately 200 ng/ml at 80 minutes. Free isoprenaline was the only other radioactive compound detected in plasma, accounting for approximately 10% of the activity. However, although free isoprenaline was a minor component following this route of administration (Fig. 4), the plasma levels of the drug were considerably higher than following small intravenous doses. After intraduodenal administration, plasma levels of unchanged isoprenaline rose to 100 ng/ml and this rise occurred at a time when the heart rate was falling back to normal.

Table 5 contains a summary of the plasma concentrations of isoprenaline and metabolites in the three dogs after intraduodenal dosage. Conjugated isoprenaline is the major metabolite in plasma at all times studied. However, significant amounts of 3-*O*-methyl isoprenaline and free isoprenaline were detected in all dogs studied, the plasma levels of these two components being of the order of 100 ng/ml.

In two dogs 42·1 and 5·0% of the administered radioactivity was recovered in urine, largely as free or conjugated isoprenaline. Quantitative examination showed that from 76 to 92% of the isoprenaline in urine was conjugated. Free 3-*O*-methyl isoprenaline made up the remainder of the activity.

Intraportal infusion in dog

A large dose of isoprenaline was infused into the portal vein of one dog at approximately the same rate as that at which it was absorbed following intraduodenal administration. Heart rate rose to a maximum of 70 beats above resting level after approximately 2 min and remained at this level during the remainder

TABLE 5. *Isoprenaline and metabolites in plasma and urine after intraduodenal dosing in dogs*

		Plasma			Urine (% dose)		
Dog No.	Dose (mg/kg)	Time of sample (min)	Isoprenaline* (ng/ml)	3-*O*-methyl isoprenaline*	Time of sample (h)	Isoprenaline*	3-*O*-methyl isoprenaline*
1	0·91 (131 μCi)	5	65 (15)	14 (100)	—	—	—
		27	212 (14)	70 (100)			
		74	1,279 (8·7)	277 (100)			
		150	1,620 (7·6)	170 (100)			
2	1·05 (239 μCi)	4	—	30 (100)	4	36·9 (8·1)	5·2 (97)
		60	2,190	110 (100)			
		120	2,360 (8·9)	140 (100)			
		240	—	80 (100)			
3	0·63 (259 μCi)	4	60 (32)	14 (100)	3	3·5 (24)	1·5 (94)
		32	106	34 (100)			
		62	292	50 (100)			
		124	352 (7·3)	41 (100)			
		179	273 (3·7)	50 (100)			

* Figures in parentheses indicate the percentage of each radioactive component appearing in the unconjugated form.

of the infusion period of 80 minutes. When the infusion was stopped, heart rate fell rapidly with a half-life of approximately 5 min (Fig. 5).

Plasma radioactivity rose steadily during the infusion and then fell off slowly. Fractionation of plasma radioactivity revealed that free isoprenaline was the major component in plasma during and for some time after the infusion. The concentration of isoprenaline had risen to 300 ng/ml at the end of the infusion and then fell slowly and was greater than 200 ng/ml when heart rate had returned to normal. Plasma 3-*O*-methyl isoprenaline concentration rose to 20 ng/ml during the infusion and fell slowly with a half-life of approximately 3 hours. No conjugated isoprenaline could be detected in plasma during the infusion but levels rose steadily after the infusion and at 4 h it was the major metabolite accounting for more than 70% of plasma radioactivity.

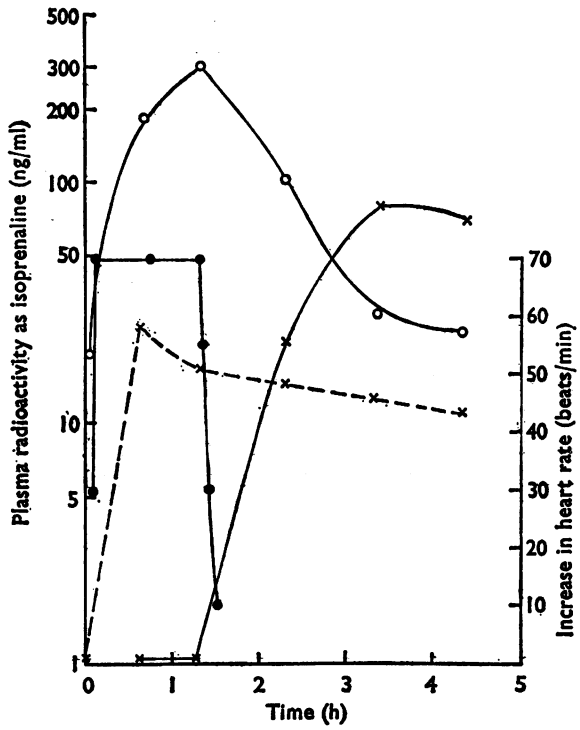

FIG. 5. Time course of changes in heart rate (●——●) and plasma levels of free isoprenaline (○——○), conjugated isoprenaline (×——×) and 3-*O*-methyl isoprenaline (×----×) following an intraportal infusion of 0·2 mg/kg of ^{3}H-isoprenaline over 80 minutes.

TABLE 6. *Analysis of urinary radioactivity following intraportal infusion of isoprenaline* (0·2 *mg/kg*) *in dog*

Urine sample	Time of sample (h)	Urine (% dose) Isoprenaline*	3-*O*-methyl isoprenaline*
1	0–1·3	—	—
2	1·3–2·3	23·0 (67·0)	13·6 (95·0)
3	2·3–4·3	13·6 (39·0)	6·4 (81·0)
Total	4·3	36·6 (56·5)	20·0 (90·5)

* Figures in parentheses indicate the percentage of each radioactive component appearing in the unconjugated form. In the 'Total' line these percentages are calculated, not measured, values.

In 4·3 h, 56·6% of the administered radioactivity was excreted in urine (Table 6). A total of 36·6% of the dose was excreted as isoprenaline, 67% of which was unconjugated in the early samples (0–2·3 h) falling to 39% in the latter stages. A total of 20% of the dose was excreted as 3-*O*-methyl isoprenaline which was largely in the free form.

Discussion

These studies show that in man and dog isoprenaline is extensively metabolized by a relatively small number of reactions. The drug is either conjugated with sulphate or *O*-methylated by COMT ; the *O*-methyl metabolite may also be conjugated. We were unable to confirm that 4-hydroxy-3-methoxymandelic acid (VMA) is a metabolite of isoprenaline in the dog (Conway *et al.*, 1968) and thus support the view that isoprenaline is not a substrate for MAO (Hertting, 1964; Morgan, 1969 ; Leeper, Weissbach & Udenfriend, 1958). This negative finding also indicates that isoprenaline is not N-dealkylated to a significant extent in man or dog (Hertting, 1964 ; Morgan, 1969).

In both man and dog the extent of *O*-methylation or conjugation of isoprenaline depended largely on the route of administration. Following small intravenous doses of the drug in man, about half of the administered amount was excreted as unchanged drug and no conjugated amine was detected although 24 to 35% was conjugated after *O*-methylation. Small doses given intravenously to dogs resulted in a similar pattern of metabolism. These data are not in agreement with those of Conway *et al.* (1968) who studied the pattern of urinary metabolites in one dog given an intravenous dose of isoprenaline. They found that 80% of the dose was excreted over 6 h of which only 5% was isoprenaline, almost entirely conjugated. In their study, 3-*O*-methyl isoprenaline was the major metabolite and over 80% of it was unconjugated. We have no explanation for the lesser role played by COMT in our investigation but our findings were consistent in the three dogs studied.

We do not agree with the conclusions of Hertting (1964) and Conway *et al.* (1968) that the rapid inactivation of intravenously administered isoprenaline is due mainly to metabolism by COMT. In their single experiment, Conway and his colleagues found that 3-*O*-methyl isoprenaline accounted for more than 65% of the plasma radioactivity 1 min and 3 min after an intravenous dose of isoprenaline, while unchanged amine could not be detected. In our experiments on dogs ten samples, obtained over a period of 2 h, all showed unchanged isoprenaline to be the major radioactive compound in plasma. After 1 min free isoprenaline accounted for 90% of plasma radioactivity falling to 70% at 2 hours. Free isoprenaline in plasma declined biphasically, the rapid phase having a half-life of 1–2 minutes. This value approximates to the half-life for the fall in heart rate and suggests that the initial inactivation of isoprenaline may be due to rapid removal from the circulation.

Isoprenaline is a substrate for the $Uptake_2$ mechanism described by Iversen (1967) which transports amines into tissues such as smooth muscle. A recent study (Lightman & Iversen, 1969) has shown that, contrary to previous opinion, there is probably not a threshold concentration below which $Uptake_2$ does not function. Thus it may be reasonable to assume that at the concentrations of isoprenaline observed in these studies, such a mechanism would be effective. Rapid

removal of isoprenaline from the circulation of the dog has been described by Gryglewski & Vane (1970). Isoprenaline removed by $Uptake_2$ might be *O*-methylated at some of the sites of uptake since it has been shown that COMT is present in smooth muscles (Axelrod, Whitby, Hertting & Kopin, 1961). Many drugs are known to be potent inhibitors of $Uptake_2$ (Iversen, 1967) and it might thus be possible to increase and prolong the effects of isoprenaline by their prior administration.

The agreement between the fall of free isoprenaline and the decline of heart rate after small rapid intravenous doses of the drug in man and dog was not seen after intraduodenal and intraportal infusions of large doses in the dog. In these studies, free isoprenaline in plasma rose to high concentrations and remained elevated at a time when the heart rate had fallen to normal. The dogs thus seem to exhibit tachyphylaxis to the cardiac effects of isoprenaline. A similar phenomenon was seen after infusion of isoprenaline over a prolonged period, when the response to rapid intravenous shots of isoprenaline was diminished 3–4 fold (Conolly, Davies, Dollery & George, 1971).

Oral administration of isoprenaline in man and intraduodenal administration in the dog produce a pattern of metabolism which differs markedly from that seen after intravenous dosage. In these studies, the major metabolite in plasma and urine at all times in both man and dog was a sulphate conjugate of isoprenaline. These results explain why orally administered isoprenaline, which is well absorbed, is some 1,000 times less active than intravenously administered isoprenaline on a weight for weight basis.

The site of conjugation of orally administered isoprenaline has not been determined finally but it seems likely that it occurs largely in the intestinal wall and perhaps to a lesser extent in liver, two known sites of sulphate conjugation (Nose & Lipmann, 1958). Evidence for the lesser role for the liver during sulphate conjugation of isoprenaline was obtained when a large dose was infused into the hepatic portal vein of a dog over a long period. In contrast to the intraduodenal study, large amounts of free isoprenaline appeared in urine, indicating a much lower sulphate conjugating ability. However, some conjugated isoprenaline was detected in the urine and also started to accumulate in plasma an hour after the infusion. The delayed appearance of conjugate in plasma suggests that during prolonged infusion, isoprenaline, a basic drug, might diffuse from plasma into the acid medium of the stomach. It could then be conjugated with sulphate during re-absorption lower down the gastro-intestinal tract. This would be consistent with our finding that after a rapid intravenous dose, when equilibrium with stomach contents is less likely to occur, no conjugated isoprenaline was found in plasma or urine. Further studies are needed to confirm that sulphate conjugation of isoprenaline takes place in the gut wall and to investigate any possible role of gut flora.

This type of conjugation is limited in the body by the supply of inorganic sulphate and this can be depleted by ingestion of drugs such as salicylamide which are themselves extensively conjugated with sulphate (Levy, 1968). In the absence of sulphate conjugation, isoprenaline might be absorbed unchanged from the gastro-intestinal tract, and its pharmacological effects might then be increased by an order of magnitude.

Since it is thought that a large proportion of aerosol doses of isoprenaline is swallowed (Blackwell, Conolly, Davies & Dollery, 1970), any defect in the inactivation of isoprenaline by sulphate conjugation may be of importance in a consideration of the possible involvement of aerosols in asthma deaths. Such a conjugation defect has recently been identified in patients with tyramine sensitive migraine (Youdim, Bonham-Carter, Sandler, Hanington & Wilkinson, 1971 ; Smith, Kellow, Mullen & Hanington, 1971). Perhaps intending isoprenaline users should in the future be investigated for the integrity of their sulphate conjugation mechanism.

The metabolic results in man do not suggest an obvious explanation for the correlation between increased asthma deaths and the use of pressurized aerosols of isoprenaline. It was thought that accumulation of 3-*O*-methyl isoprenaline, a weak β-adrenoceptor blocking agent might be of importance but the extent of *O*-methylation is not large and the short half-life of the metabolite would make excessive accumulation unlikely. The tolerance which develops to isoprenaline during a prolonged period of exposure to the drug may be of more importance in explaining the asthma deaths (Conolly *et al.*, 1971).

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