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Metabolism of isoprenaline in the intestine

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A method for the study of drug metabolism in isolated canine gut loops is described. This technique has been used to study the conjugation of isoprenaline with ethereal sulphate. After intraluminal injection of tritiated isoprenaline only 3·6% of the radioactivity in venous effluent appeared as free isoprenaline, and 68·0% as a sulphate conjugate. After pretreatment with salicylamide the proportion of free isoprenaline rose to 73·7% and only 3·3% was accounted for as sulphate conjugate. The significance of these findings is discussed in relation to a possible source of drug interaction.

The major clinical uses of isoprenaline are in the treatment of bronchial asthma and chronic heart block. It is recognized that the dosage requirements for isoprenaline vary widely according to the route of administration. When the drug is given intravenously, pharmacological effects are seen with only a few μg (Paterson, Conolly & others, 1968; Cleaveland, Rangno & Shand, 1972; George, Conolly & others, 1972), whereas tablets containing 180–360 mg may be required daily to control chronic heart block (Redwood, 1969). The explanation for these differences lies in the metabolism of isoprenaline which varies with the route of administration (Conolly, Davies & others, 1972). When given intravenously, isoprenaline is excreted mainly as free isoprenaline, whereas after oral dosing excretion is largely as an ethereal sulphate conjugate.

Pharmacokinetic analysis of these data indicates that isoprenaline undergoes extensive metabolism at the first pass after oral dosing, but the site of metabolism is not identified. We now describe a method for the study of conjugation in the small intestine, and its use in the investigation of isoprenaline metabolism by the gut.

MATERIALS AND METHODS

Six mongrel dogs of either sex, 16·9–27·3 kg, were anaesthetized with thiopentone sodium (25 mg kg^{-1}, i.v.) and anaesthesia was maintained with pentobarbitone (25 mg kg^{-1}). Each dog received phenoxybenzamine (100 mg i.v.) before further surgery to prevent sympathetic nervous inhibition of gastrointestinal motility which would thereby reduce absorption from the intestine (Groisser & Farrar, 1960, 1962).

The abdomen was opened through a mid-line incision and the duodenum identified and mobilized. A loop of terminal duodenum and proximal jejunum approximately 12 cm in length was isolated between two balloon catheters and venous blood from the loop was collected via a polyethylene cannula (Angiocath 1968) into heparinized tubes. After a previous irrigation with 0·9% w/v saline, [^{3}H]isoprenaline was injected into the lumen of the gut loop in a volume of 10 ml saline.

A check on the effectiveness of the isolation procedure was made by comparing the radioactivity in venous effluent with that in samples taken from a catheter situated in the abdominal aorta. Total radioactivity was determined on 1 ml aliquots of plasma dissolved in Instagel using a Packard Liquid Scintillation Spectrometer. Quantitative

measurements of isoprenaline, 3-*O*-methylisoprenaline, and conjugated isoprenaline were made according to Conolly & others (1972) and Ruthven & Sandler (1965).

In 3 of the 6 dogs, an attempt was made to deplete the supply of sulphate by pretreating the gut loops with salicylamide 1–2 g in 10 ml of water. Blood levels of salicylamide were measured in these dogs according to Levy & Matsuzawa (1967).

RESULTS

Adequate isolation of the gut loop was demonstrated in all instances by the high level of total radioactivity in venous blood from the loop and low levels of activity seen in systemic arterial blood (Fig. 1). Absorption of isoprenaline varied according to the length of the gut loop and ranged from 0·4–2·9 % of the administered dose in 0·5 h. In the experiments in which the gut loops were initially irrigated with saline, most of the radioactivity which appeared in venous blood was accounted for as conjugated isoprenaline (Table 1). Pretreatment of the gut loops with salicylamide led to a signi-

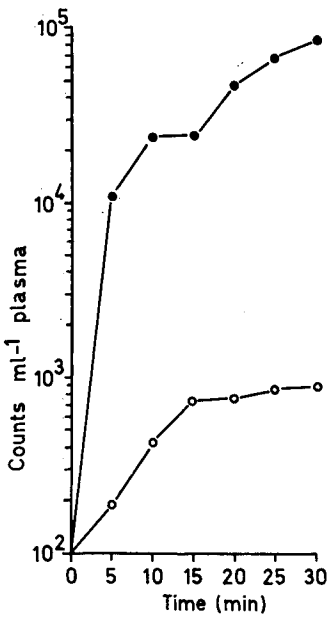

FIG. 1. Radioactivity in mesenteric venous (●——●) and systemic arterial plasma (○——○) after injection of [^{3}H]isoprenaline into the loop of small intestine. The difference in levels of radioactivity in the two circulations indicates effective isolation of the gut loop.

Table 1. *Pattern of metabolism of isoprenaline in isolated loops of dog gut.*

	Total radioactivity in venous effluent (%) found as:					
	Drug absorbed (%)	Free isoprenaline	Free 3-OMI	Sulphate conjugate of isoprenaline	Sulphate conjugate of 3-OMI	Total metabolites
(a) Saline washed	2·9	5·0	20·0	75·0	0	95·0
	1·3	1·6	19·5	60·9	7·2	87·6
	—	4·3	—	—	—	95·5
$\bar{x}$	2·10 ± 0·80	3·6 ± 1·0	19·8 ± 0·25	68·0 ± 7·0	3·6 ± 3·3	92·7 ± 2·6
b) Salicylamide pretreated	0·4	79·0	21·1	0	0	21·1
	1·0	74·2	26·5	0	0	26·5
	0·8	68·0	18·0	10·0	0	28·0
$\bar{x}$	0·73 ± 0·18	73·7 ± 3·2	21·9 ± 2·5	3·3 ± 3·3	0 ± 0	25·2 ± 2·1
Difference between groups		$P<0·001$	N.S.	$P<0·01$	N.S.	$P<0·001$

3-OMI = 3-*O*-methylisoprenaline.

ficant alteration in the amount of free isoprenaline present in the blood samples. Without salicylamide, only 3·6% of the total radioactivity was accounted for as free isoprenaline but after salicylamide 73·7% was free ($P<0·001$). The corresponding figures for conjugated isoprenaline were 68·0 and 3·3% respectively ($P<0·01$). In contrast, the percentage of radioactivity accounted for as 3-*O*-methyl isoprenaline was unaltered by pretreatment with salicylamide. The concentration of salicylamide in venous plasma varied from 62–180 μg ml^{-1}.

DISCUSSION

Although sulphokinases are known to exist throughout the intestine (Boström, Brömster & others, 1968) there are few previous studies indicating their physiological significance. Studies of the metabolism of isoprenaline administered by various routes (Conolly & others, 1972) showed that most of the drug administered orally was metabolized during absorption and that this metabolism probably occurred in the wall of the intestine. The present study confirms that the intestine is a major site of inactivation of isoprenaline absorbed following enteral administration. Almost 93% of the material absorbed and appearing in the venous effluent was metabolized at the "first pass" through the intestinal wall. Conjugation with sulphate was numerically the most important pathway of metabolism. Only 3·6% of the absorbed dose reached portal venous blood as pharmacologically active free isoprenaline and further metabolism in the liver is likely to reduce this proportion still more.

In contrast, after pretreatment with salicylamide the percentage of free isoprenaline appearing in the venous effluent increased twenty-fold to 73·7%. This interaction could have important pharmacological and therapeutic consequences in patients who are receiving large oral doses of isoprenaline for chronic heart block. Such an interaction could also be hazardous in patients who use pressurized aerosols containing isoprenaline for bronchial asthma, since it has been shown that more than 50% of an inhaled dose is subsequently swallowed and conjugated with ethereal sulphate (Blackwell, Conolly & others, 1970). Levy & Matsuzawa (1967) have shown that sulphate conjugation can be reduced by as little as 75 mg oral salicylamide, an amount which might be exceeded by patients medicating themselves.

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