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The effect of nialamide, pargyline and tranylcypromine on the removal of amphetamine by the perfused liver

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Nialamide, pargyline and tranylcypromine, three monoamine oxidase inhibiting drugs, and the microsomal enzyme inhibitor SKF 525-A significantly decreased the amount of amphetamine removed from the portal circulation of the isolated perfused liver of the cat. The enhancement of the vascular actions and toxicity of amphetamine by monoamine oxidase inhibitors could be explained in terms of these findings.

The actions of amphetamine and its congeners are potentiated by monoamine oxidase inhibitors (Brownlee & Williams, 1963; Dally, 1962; Rand & Trinker, 1968; Sjöqvist, 1965; Trinker, Fearn & others, 1967; O'Dea & Rand, 1969; Morpurgo & Theobald, 1968). However, amphetamine is not a substrate for monoamine oxidase (Blaschko, Richter & Schlossman, 1937). The explanation of the paradox is that drugs termed "monoamine oxidase inhibitors" are not specific to that enzyme, but inhibit other enzymes including liver microsomal enzymes (Brodie, Gillette & La Du, 1958). Amphetamine is a substrate for liver microsomal enzymes (Axelrod, Reichenthal & Brodie, 1954; Axelrod, 1955), and the liver microsomal enzyme inhibitor proadifen (SKF 525-A) inhibits the metabolism of amphetamine by these enzymes *in vitro* (Cooper, Axelrod & Brodie, 1954).

The actions of amphetamine in the intact cat, as well as being potentiated by monoamine oxidase inhibitors, are also potentiated by proadifen or by excluding the liver from the circulation (Rand & Trinker, 1968). These findings suggest that metabolism of amphetamine by liver microsomal enzymes is important in limiting the pharmacological activity of amphetamine. This paper is concerned with the effects of nialamide, pargyline and tranylcypromine, representative monoamine oxidase inhibiting drugs, and of proadifen on the removal of amphetamine from a portal perfusate by the cat isolated liver.

Methods

Young cats of at least 3 months old and weighing 0·9 to 1·8 kg were used; in younger animals the liver microsomal enzyme systems are incompletely developed (Fouts & Adamson, 1959; Fouts, 1962; Conney, Schneidman & others, 1965). The cats were anaesthetized with halothane, one of the few anaesthetics that neither depress nor stimulate liver microsomal enzymes (Schimassek, Kunz & Gallwitz, 1966).

A midline abdominal incision was made from the xiphisternum to the pubic symphysis and the liver was exposed by displacing loops of the intestine to the left.

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The common bile duct and hepatic artery were tied and cut between ligatures. The portal vein was cannulated and perfusion was commenced with McEwen solution at 37° using a Watson-Marlow roller pump delivering 6 ml/min; this caused an immediate and uniform blanching of the liver parenchyma. The liver was freed from surrounding tissues and the hepatic veins and inferior vena cava were cut. The excised liver was weighed, suspended in a double jacket water bath maintained at 37° and perfused until the effluent was macroscopically free of blood.

A single dose of amphetamine was injected into the portal vein cannula in a dose equivalent to 12 μg/g of liver. A sample of effluent was collected before administration of amphetamine and thereafter serial samples were collected during 2 min periods. The amphetamine content of the effluent was determined spectrophotometrically by the method of Chapman, Shenoy & Campbell (1959) using the methyl orange reaction and adsorption at 515 μm. A standard curve was constructed for each experiment. Duplicate aliquots of each test sample were assayed.

Cats were pretreated as described by Rand & Trinker (1968), monoamine oxidase inhibitors being given intraperitoneally 16 to 18 h and proadifen 1 h before removing the liver.

The drugs used were (±)-amphetamine sulphate, nialamide hydrochloride, pargyline hydrochloride, proadifen hydrochloride (SKF 525-A) and tranylcypromine sulphate. The amounts referred to in the text are in terms of these salts.

RESULTS

No amphetamine was detected in the liver perfusate of both control and pretreated cats before administration of amphetamine. Our colleague Mr. H. J. Fearn also tested the samples for catecholamine activity by bioassay using the blood pressure of the pithed rat and the de Jalon preparation of the rat uterus, and obtained negative results.

In preliminary experiments it was established that after injection of amphetamine, the concentration in the effluent fell below detectable levels within 10 min. The mean total amount of amphetamine recovered in the effluent fluid from perfused livers of control untreated animals was 34·6% of that administered. Pretreatment with any of the three monoamine oxidase inhibitors resulted in substantial and highly significant increases in the amounts of amphetamine appearing in the effluent. The means, standard errors, and significances of difference from control values are summarized in Table 1. The monoamine oxidase inhibiting drugs included a hydrazine derivative (nialamide), two non-hydrazines (pargyline and tranylcypromine), and a drug having amphetamine-like sympathomimetic activity (tranylcypromine). Pretreatment with the microsomal enzyme inhibitor SKF 525-A had

Table 1. *Recovery of amphetamine from isolated perfused cat liver* %

Treatment	No. of animals	% recovery ± s.e.	*P* value
Control	9	34·6 ± 3·9	
Nialamide (50 mg/kg)	9	64·5 ± 4·7	<0·001
Pargyline (50 mg/kg)	5	68·8 ± 3·9	<0·001
Tranylcypromine (25 mg/kg)	3	60·0 ± 3·8	<0·001
SKF 525-A (20 mg/kg)	4	62·5 ± 6·4	<0·001

a similar effect to the monoamine oxidase inhibitors in increasing the amount of amphetamine appearing in the effluent from the liver (Table 1).

DISCUSSION

Only about one-third of the amphetamine injected into the perfusion fluid appeared in the effluent from the liver. This indicates that the liver takes up amphetamine avidly. The dose of amphetamine injected into the fluid perfusing the liver (12 μg/g of liver) is equivalent to the amount of 0·09 μmol/g which was found by Axelrod (1955) to be sufficient to saturate the liver microsomal enzymes *in vitro*. When the liver microsomal enzymes were inhibited by pretreatment of donor cats with monoamine oxidase inhibitors or proadifen, about two-thirds of the dose of amphetamine appeared in the effluent. How the metabolism of amphetamine by the liver is impaired is not clear; inhibition of the enzymes is probably not involved, but there may be a change in permeability of the microsomal membrane (Brodie, 1956; Netter, 1962), or in the endoplasmic reticulum affecting the access of drugs to the microsomes (Fouts, 1965). In any event, the microsomal enzyme system as a whole is efficient in removing amphetamine from the portal circulation. In intact animals the extent of potentiation of the action of amphetamine caused by excluding the liver from the circulation was about as great as that produced by pretreatment with nialamide or proadifen; no increase in effect was produced by either nialamide or proadifen together with exclusion of the liver (Rand & Trinker, 1968). These effects are explicable in terms of the increase in the amount of amphetamine appearing in the hepatic venous effluent after its administration into the portal vein of perfused isolated livers of cats that had been pretreated with the three drugs and proadifen.

These findings offer a rational basis for the greatly enhanced pressor responses observed after sympathomimetic amines, such as amphetamine, ephedrine and methylphenidate, that are not substrates for monoamine oxidase, in animals and in man receiving monoamine oxidase inhibiting drugs.

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