The present investigations show that the injection of either hormone into the hind-limb has an immediate effect on blood flow but that angiotensin I is much less effective than angiotensin II. We have found in other investigations that both hormones used in the present studies are removed equally by the hind-limb of the sheep. A possible explanation for the present findings is that much of the angiotensin I is inhibited or destroyed rather than converted to angiotensin II when it is removed from the circulation. Our findings in the sheep differ from those of Ng & Vane (1968) in the dog and they are in agreement with those reported in several species by the earlier workers. These studies were made in the Dr. Leonard West Research Laboratory of Sully Hospital, Sully, Glamorgan, and we gratefully acknowledge the expert technical assistance of Mr. J. Wilson, Mr. O. F. Mason and Mr. P. Stock.

*Departments of Medicine and Metabolic Medicine,**
Welsh National School of Medicine,
Cardiff Royal Infirmary,
Cardiff.
Sully Hospital,
Sully,
Glamorgan, U.K.

E. C. OSBORN
G. TILDESLEY
K. G. LEACH
R. F. MAHLER

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* Correspondence to Dr. E. C. Osborn at this address.

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Is 5-hydroxytryptamine involved in the mechanism of action of fenfluramine?

Jespersen & Scheel-Krüger (1970) recently reported that methysergide blocked the hypothermic effect of fenfluramine in dogs and concluded that 5-hydroxytryptamine (5-HT) played an important role in the mechanism of action of fenfluramine, an anorectic drug that does not produce central stimulation in most animals (Le Douarec, Schmitt & Laubie, 1966). On the other hand, Opitz (1967) found that fenfluramine inhibited the appetite of rats in which brain 5-HT had been depleted by *p*-chlorophenylalanine, an experiment that strongly suggested that 5-HT was not required for the action of fenfluramine.

We have confirmed the results obtained by Opitz (1967) in rats trained to eat their day's food in 2 h (Hollifield & Parson, 1962). A single intraperitoneal injection of 300 mg/kg of *p*-chlorophenylalanine lowered food intake by 30% in a group of 15 rats on the third day; an effect which may arise from irritation of the gut since, in other experiments we have found marked intestinal damage after intraperitoneal administration of the drug. On the fourth day, food consumption was inhibited about 80% after the administration of 8 mg/kg of (±)-fenfluramine hydrochloride. This is about the same inhibition as that previously found after the use of fenfluramine alone (Fig. 1). Chemical analyses of the brains of 15 other rats given 300 mg/kg of *p*-chlorophenylalanine showed that the 5-HT content was markedly decreased on the fourth day.

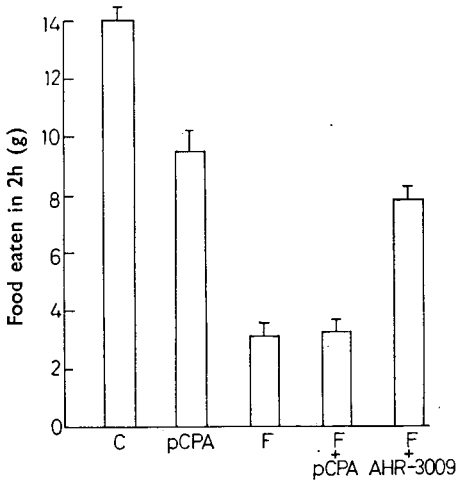

FIG. 1. Effect of drugs on food consumption of rats (n = 15) trained to eat their daily diet in 2 h. Control animals (C) received 1 ml of saline intraperitoneally. *p*-Chlorophenylalanine (pCPA), at 300 mg/kg, lowered food consumption by 30% after 3 days (thought to be due to gastrointestinal irritation; see text). Fenfluramine (F) markedly inhibited food intake when administered alone and to rats pretreated with pCPA 4 days earlier. 8β-Carbobenzyloxy-aminomethyl-1-methyl-10α-ergoline (AHR-3009) antagonized the action of fenfluramine when these drugs were given together.

In a further 15 trained rats, appetite depression usually produced by 8 mg/kg of fenfluramine intraperitoneally was partially blocked by 1 mg/kg by the same route of AHR-3009 (8β-carbobenzyloxy-aminomethyl-1-methyl-10α-ergoline), a potent inhibitor of 5-HT (Beretta, Glässer & others 1965). This is consistent with the finding of Jespersen & Scheel-Krüger (1970) who also found evidence that a 5-HT antagonist blocked the appetite depressant action of fenfluramine. Attempts to antagonize the anorectic action of (+)-amphetamine or chlorphentermine with AHR-3009 have been unsuccessful.

Since the appetite-depressant action of fenfluramine in rats can be blocked by 5-HT antagonist at least in part and it continues to act at a time when the brain content is much decreased, fenfluramine may act by stimulating tryptaminergic neurons directly.

Department of Pharmacology,
A. H. Robins Company, Inc.,
1211 *Sherwood Avenue,*
Richmond, Virginia, 23220, *U.S.A.*

WILLIAM H. FUNDERBURK
JEAN C. HAZELWOOD
ROBERT T. RUCKART
JOHN W. WARD

January 28, 1971

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Acetylcholine and "auto-inhibition"

The evidence that the release of noradrenaline from sympathetic fibres is due to the acetylcholine released by the nerve impulse, has now been strengthened by the work of Eränkö, Rechardt & others (1970), and by that of Malik (1970). The former have stained the pineal gland of the rat by the thiocholine method, and have shown that adrenergic terminals seen in electron micrographs are closely invested with acetylcholinesterase. The pineal gland is innervated entirely by fibres from the superior cervical ganglion, and when the ganglia of both sides are removed, both the acetylcholinesterase and the small granular vesicles containing noradrenaline disappear.

Malik perfused the superior mesenteric artery and its branches in the rat, and recorded the constrictor response in the arteries to postganglionic stimulation. He found that the response to stimulation of frequencies from 1 to 6 s was increased when anticholinesterases were added to the perfusion fluid, and in about 100 experiments showed that the increase was greatest at the lowest frequency, diminishing as the frequency rose until at 6 s the increase was imperceptible. Since the investigations of Eränkö & others (1970) and of Malik (1970) provide very clear evidence, the recent work of Löffelholz (1971) requires consideration.

Löffelholz has carried out experiments on the isolated heart of the rabbit, in which the sympathetic postganglionic nerves were stimulated, and the noradrenaline appearing in the effluent was measured. In the course of these experiments either acetylcholine (plus atropine) was added to the perfusion fluid for a short period, or nicotine, or DMPP, was added, and the noradrenaline released by these substances was measured.

The concentration of acetylcholine infused was large, $2{\cdot}1 \times 10^{-4}$M, and this caused a release of a large amount of noradrenaline. However this release was very brief, continuing for 5 to 10 s only, although the infusion of acetylcholine was maintained for 9 min.

The author considered that the cessation of noradrenaline release after 5 to 10 s was due to "auto-inhibition", the receptors for acetylcholine being blocked by the infusion. The important point was that he found that during this "auto-inhibition" the response to sympathetic stimulation was unchanged. He said "when the nicotinic block was established, the noradrenaline released by electrical stimulation was not inhibited", and his implication was that the receptors on which acetylcholine acted to release noradrenaline were not involved in the release of noradrenaline by sympathetic stimulation.

Since the evidence from anticholinesterases shows that sympathetic stimulation involves receptors for acetylcholine, it follows that the "auto-inhibition" must occur at some other point. The same problem was raised by the experiments of Daly & Scott (1961) who found that acetylcholine, injected into the splenic artery, released noradrenaline, but that this release was blocked by hexamethonium, whereas the response to stimulation of the splenic nerves was not. It seemed possible that the