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PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

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his paper attempts to define the relationship een the abnormal substances excreted in acute hyria and the clinical manifestations of this der of porphyrin metabolism. During an k of acute porphyria, patients usually excrete quantities of porphobilinogen either alone or certain porphyrins. The excretion of the hyrins and porphobilinogen is usually in direct ortion to the severity of the symptoms, suggest- causal relation, although Waldenström (1939) reported an authenticated case in which the nt did not pass uroporphyrin or porpho- ogen in the urine or bile during the attack, did so on other occasions. Several authors claimed that porphyrins may influence the ine or uterus (Supniewski, 1927; Gunther, Reitlinger and Klee, 1928; Vannotti, 1937; Simici, 1938). Critical appraisal of these ts has led us to repeat this work using por- ns of the kind known to be excreted in por- a, which were obtained by improved methods rification. The isolation of porphobilinogen stalline form (Westall, 1952) has for the first allowed pharmacological testing of the pure nce, although Waldenström and Wendt and Prunty (1945) had injected partially d porphobilinogen into rabbits.

METHODS

nal Experiments.—Observations were made on ood pressure (recorded with a cannula in the artery) and on the respiration of 13 cats and its (anaesthetized with chloralose (80 mg./kg.) duction with ether) and of 1 pithed cat, 1 pithed iscerate cat, and 1 decerebrate cat. Injections nade into the right femoral vein or the splenic

ulation of the distal end of the vagus, separated t in the neck, was with supramaximal 0.5 msec. at 10 c./s.

Isolated Organs.—Experiments were also made on isolated strips of guinea-pig ileum, non-pregnant rabbit uterus, rabbit jejunum and ileum, or cat ileum, set up in Tyrode's solution at 34° C. Contractions were recorded on smoked paper by a frontal writing lever. Experiments with light irradiation were done with an electric bulb of 300 w. at 25 cm. from the tissue in the organ bath.

Drugs.—The porphyrins, with the exception of haematoporphyrin, had been isolated from biological material as the methyl esters. Before use the esters were hydrolysed with 7 N HCl for 36 hours, at room temperature, the excess of HCl being then removed in a vacuum desiccator over KOH. Haematoporphyrin was prepared and used as the dihydrochloride. Pure crystalline porphobilinogen (Westall, 1952) was used; the porphobilin was obtained by Mr. R. G. Westall as a by-product in the preparation of porphobilinogen.

Porphobilinogen was dissolved in a minimum volume of 0.1 N $NH_4.OH$ and then made up to the required volume with 0.9% saline. For the porphyrins and porphobilin M/7 sodium bicarbonate was used as the solvent.

RESULTS

Anaesthetized Cats and Rabbits.—Recordings were made of the direct effect of porphobilinogen—and, in one experiment, of uroporphyrin I—on the blood pressure, respiration and vascular responses of the treated animals to acetylcholine, histamine, nicotine, adrenaline, noradrenaline, and vagal stimulation. The amount of porphobilinogen injected (up to 100 μg./kg.) was limited by the amount available; but from the known rate of excretion in patients (40–160 mg./day), and from the fact that tests for plasma porphobilinogen sensitive to 1 μg./ml. may be negative even at the height of an attack, it is likely that the blood levels obtained in our experiments were comparable to or even greater than those obtaining in acute porphyria. The only effect observed was an apparent potentiation by porphobilinogen of the response to adrenaline and noradrenaline in a few of the early

nber of Nuffield Unit for the investigation of Pyrrol Metabolism, Department of Chemical Pathology.

experiments (Fig. 1). This apparent potentiation of adrenaline and noradrenaline could not, however, be repeated and its interpretation is complicated by the fact that considerable spontaneous fluctuations in sensitivity to these drugs may occur.

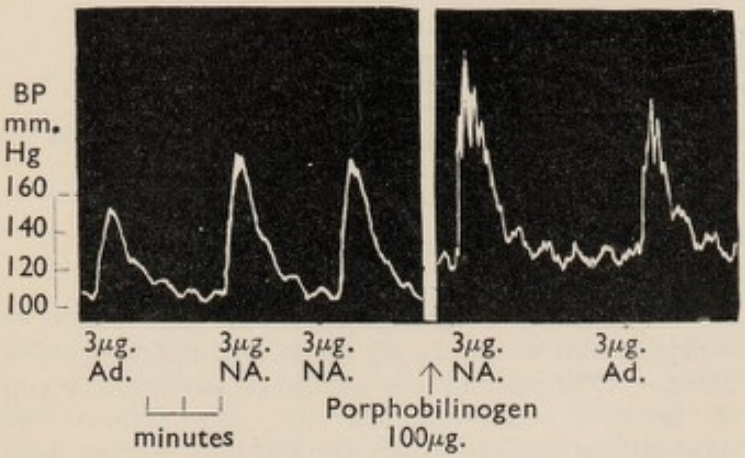


FIG. 1.—Cat.; chloralose; blood pressure recording; intravenous injections. Responses to 3 μg. adrenaline and noradrenaline before and after 100 μg. porphobilinogen.

Isolated Organs.—After obtaining records of spontaneous activity and tone, and of consistent responses to acetylcholine, histamine, adrenaline, and 5-hydroxytryptamine, the effect of adding porphyrins, porphobilinogen, and porphobilin to the preparation was investigated. With uroporphyrin I the effect of light irradiation was determined. A summary of these results is g in Table I.

The only significant responses were those haematoporphyrin (1/8,000) and to porphob The former produced a distinct waning contrac of guinea-pig ileum, followed by inactivity of intestine and a refractoriness—which became plete—to histamine and acetylcholine. R intestine was unaffected. Porphobilin produc histamine-like contraction, sensitive to mepyram but less so to atropine (Fig. 2); it was consid likely that the effect was due to contamination (c mg./g.) with histamine itself.

Test of Porphobilinogen on Unanaesthetized Ra —10 mg. porphobilinogen was injected intra ously into a rabbit (2.2 kg.) with an external bi fistula. The animal showed no abnormal sympt in the 3 days following the injection. There a slight rise in the level of bile protoporph during this period and a trace of uroporph was noted in the urine several hours after injection. No porphobilinogen was found in urine.

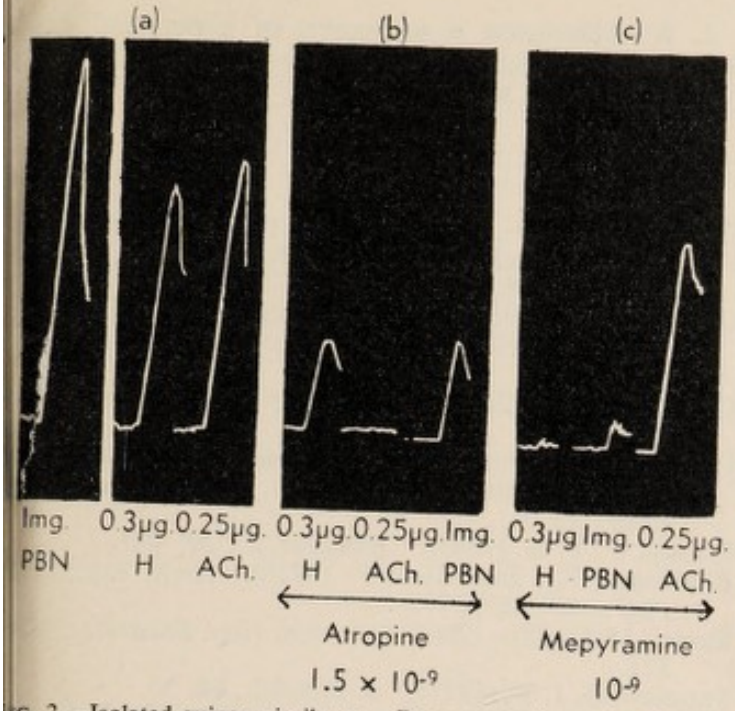
Test of Whole Urine from Patients with A Porphyria.—As a final test, to cover the possib that in porphyria some unidentified pharmac

TABLE I

TESTS OF PORPHOBILINOGEN AND PORPHYRINS ON ISOLATED TISSUES

		Porphobilinogen	Uroporphyrin I	Uroporphyrin III	Coproporphyrin I	Coproporphyrin III	Haematoporphyrin	Porphobil
Rabbit uterus	Drug concn.	1/40,000	1/10,000	1/40,000	1/40,000	1/40,000		
	Effect on spontaneous activity and tone	0	0	0	0	0		
	Effect on response to adrenaline	0	0	0	0	0		
Rabbit ileum or jejunum	Drug concn.	1/40,000	1/10,000				1/10,000	
	Effect on spontaneous activity and tone	0	0				0	
	Effect of combining with light irradiation		0					
	Effect on response to histamine, acetylcholine, adrenaline						0	
Guinea-pig ileum	Drug concn.	1/20,000	1/40–10,000	1/10,000	1/20,000	1/20,000	1/8,000	1/20,000
	Effect on spontaneous activity and tone	0	Slight + twice only	0	+ twice only	0	–	+ (histamine contaminant
	Effect on response to acetylcholine	0					–	
	Effect on response to 5-OH tryptamine	0		0	0	0		
	Effect on response to histamine		+ (50%) once only				–	

0 = No effect: + = increase; – = decrease.



IG. 2.—Isolated guinea-pig ileum. Response to 1 mg. porphobilin, 0.3 µg. histamine and 0.25 µg. acetylcholine (a) normally, (b) in presence of 1.5×10^{-9} atropine, (c) in presence of 10^{-9} mepyramine.

ically active substance is excreted, a sterile specimen f urine from a patient suffering a moderately evere attack of acute porphyria (with hypertension nd abdominal pain) was infused into an anaesetized cat at a rate of approximately 4 ml./min. r 15 min. This by itself produced no effect on lood pressure or respiration; and its effect on the sponses to adrenaline, noradrenaline, histamine, etylcholine, and nicotine was indistinguishable om that of a specimen of normal urine.

Discussion

Despite a great deal of research on acute porphyria the mechanism of the production of symptoms mains obscure. Recent work has tended to inimize the possible direct influence of the porphyrins (Waldenström, 1939) and to emphasize the portance of the pathological features of patchy elin change observed in the peripheral and tonomic nerves (Denny-Brown and Sciarra, 1945). ese authors considered that the changes might be used by an intermittent ischaemia, probably due a circulating vasoconstrictor substance. Follow- this, Wehrmacher (1952) reported clinical provement in acute porphyria with the use ganglion-blocking agents. A search for some h vasoconstrictor substance, which might be sent only in active cases of acute porphyria, uld be a reasonable approach to the problem. pharmacological testing of the known and ady purified excretion products was clearly essary. It would be unlikely that uroporphyrin and coproporphyrin could fulfil this role, since these are excreted in increased amounts in both congenital porphyria (as the series I isomers), and in porphyria cutanea tarda (as the series I and III isomers) where skin photosensitivity may be the only symptom. Porphobilinogen, however, is always excreted in the urine in attacks of acute porphyria and in those phases of porphyria cutanea tarda where acute symptoms are superimposed on the cutaneous syndrome. For this reason porphobilinogen or some closely related substance has been strongly suspected of being the *materia peccans* of acute porphyria (Lowry *et al.*, 1950).

Our experiments have failed to show that either the porphyrins or porphobilinogen have any significant pharmacological action. The initial animal experiments, in which porphobilinogen appeared to potentiate the blood pressure responses of the cat to adrenaline and noradrenaline, could not be repeated. These interesting results cannot be explained, although it is just possible that there may be an individual tissue and animal sensitivity to such drugs. Their inactivity in our hands is at variance with the results of some previous investigators. The difficulty of isolating porphyrins from biological materials, such as urine, in a state of purity that will guarantee freedom from possible histamine contamination, is very great. Our experience suggests that the contradictory results obtained by previous workers may possibly have been due to histamine contamination.

The results of intravenous injection of porphobilinogen into a rabbit confirm Prunty's (1945) findings, but contradict those of Waldenström and Wendt (1939), who found porphobilinogen in the urine of a rabbit into which they had previously injected the partially purified substance (amount used unknown).

Further work has tended to substantiate the absence of pharmacological activity of the porphyrins and porphobilinogen. An "experimental porphyria" or disturbance of porphyrin metabolism has been produced in rabbits by the non-hypnotic substance allyl *iso*propyl acetamide (Goldberg, 1953); very large quantities of uroporphyrin and porphobilinogen were excreted—in one animal for as long as three weeks—without obvious pharmacological effect. Apart from constipation, there is no evidence that the state of these rabbits compared with the clinical state of human acute porphyria, although the animals excreted proportionately greater quantities of the substances. Falk, Dresel, and Rimington (1953) have shown that porphobilinogen is a porphyrin precursor in a tissue system. This emphasizes that in porphobilinogen

we are probably dealing with a physiological substance. While our experiments therefore do not rule out the possibility that an unidentified vasoconstrictor substance may be produced in acute porphyria, or that the known excretion products may exert a pressor action by a mechanism at present unknown, they render these suggestions unlikely.

Summary

1. The porphobilinogen and the porphyrins usually excreted in acute porphyria, as well as haematoporphyrin and porphobilin, have been tested pharmacologically.

2. Apart from slight and variable action of uroporphyrin I, coproporphyrin I, and porphobilin, these substances show no pharmacological action.

3. The significance of the results in relation to the symptoms of acute porphyria is discussed.

We wish to thank Mr. R. G. Westall for generous supplies of porphobilinogen and Professor C. Rimington for specimens of porphyrins, and constant help through these experiments.

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