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Release of tissue histamine by the babesicidal agents quinuronium and amicarbalide

P. EYRE

Quinuronium sulphate liberated appreciable quantities of histamine from tissues of mice, rats and sheep. The signs of quinuronium poisoning in mice and rats were more severe than for compound 48/80. The animals acquired tolerance to the latter drug. Thus the toxicity of quinuronium probably depended on factors other than histamine release. Amicarbalide released amounts of histamine comparable with those liberated by quinuronium only in rat tissues.

THE two babesicidal agents investigated are both in common use in Britain for the treatment of bovine piroplasmosis.

Quinuronium sulphate [*NN'*-diquinol-6-ylurea 1,1'-dimetho(methyl sulphate)] has been in use since the early 1930's (Sergent, Donatein, Parrot & Lestoquard, 1933). Its marked toxicity was first recorded by Cernaianu, Schuldner & Magureanu (1935), who described profuse salivation, defaecation and micturition as invariable effects, usually accompanied by dyspnoea, muscular spasm and collapse. These were followed occasionally by death and were held to be cholinergic in nature. In 1935, however, Kikuth observed that the toxic symptoms bore a strong resemblance to "shock". Adrenaline was recommended as a suitable antidote to quinuronium poisoning, as it could partly antagonise certain cholinergic effects and also "shock" from histamine release. In 1959, Kronfeld published evidence of a "respiratory" type of death produced by quinuronium. He showed that quinuronium depressed cellular oxygen uptake in the brain and that sympathomimetic amines were useless in preventing death in these circumstances. In 1960, Rummler & Laue showed that quinuronium inhibited circulating cholinesterase, and atropine and pyridine 2-aldoxime methiodide together provided good clinical improvement in sheep "poisoned" by quinuroniun.

Recently a new babesicidal drug with a higher therapeutic index than quinuronium has been introduced, namely, amicarbalide [*NN'*-di(*m*-amidinophenyl) urea di-isethionate] (Ashley, Berg & Lucas, 1960; Beveridge, Thwaite & Shepherd, 1960). So far, the only untoward effect has been local swelling at the injection site and some inco-ordination.

The evidence for the cholinergic and central inhibitory aspects of quinuronium toxicity was clear, but in view of the shock-like nature of quinuronium poisoning, there was a possibility of histamine release. It therefore seemed important to investigate the role of histamine in the toxicity of quinuronium and to include amicarbalide in the investigations in view of its own toxic reactions.

From the Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall, Edinburgh, 9.

Experimental

METHODS

Male albino mice weighing 20–30 g, and male and female albino rats weighing 150–200 g obtained from the University animal breeding unit were used. Sheep diaphragm sections were obtained at slaughter.

In vitro *release of histamine from perfused rat hind-quarters.* The technique was described by Feldberg & Mongar (1954). The posterior part of the bisected body of the rat was perfused through the dorsal aorta with oxygenated Krebs solution and perfusate collected from the vena cava. Perfusion was with solutions containing quinuronium, 500 μg/ml; amicarbalide, 2 mg/ml and compound 48/80, 100 μg/ml. 100 ml each of control and drug perfusates were collected, treated with hydrochloric acid according to Feldberg & Talesnik (1953), reduced in volume by boiling, and assayed for histamine after neutralisation.

In vitro *release of histamine from sheep diaphragm strips.* Small areas of diaphragm were taken from freshly killed sheep, the area chosen being at the junction of the muscular and tendinous portions. From the peritoneal side of the muscle a thin sheet of tissue was prepared by splitting the musculature with a clean razor blade to leave peritoneum and a thin layer of attached muscle, about 3 mm thick. This sheet was then divided into a number of strips approximately 1 cm wide and 3 cm long, which were washed in warm (37°) saline for 1 min, carefully dried on cellulose tissue and weighed.

Sixteen such strips were prepared and each was placed in a tube containing 8 ml of Krebs solution. Four tubes remained as controls and the second, third and fourth groups of four contained, respectively: quinuronium, 500 μg/ml; amicarbalide, 2 mg/ml; and compound 48/80, 100 μg/ml (i.e. the same concentrations as those perfused through rat tissue). All tubes were incubated for 30 min at 37°, after which the tissues were removed and the solution assayed for histamine.

In vivo *histamine release in mice.* Experiments were designed according to the recommendations of Riley & West (1955), with a slight modification of the sub-acute experiment. Drugs were dissolved daily in fresh normal saline and injected on three successive days intraperitoneally into groups of six or eight mice. The doses measured in μg/g in the case of the babesicidal compounds approximated to between one and two times the therapeutic dose, and for 48/80 were within the doses recommended by Riley & West (1955), which were as follows: quinuronium (1) 1·0, (2) 1·5, (3) 2·0; amicarbalide (1) 20·0, (2) 25·0, (3) 30·0; Compound 48/80 (1) 2·0, (2) 2·5, (3) 3·0. A fourth group of mice received an equivalent volume of normal saline.

On the third day of injections, 3 hr after the final injection, four mice from each group were killed and skinned. A representative sample of skin was extracted and assayed for histamine.

In vivo *histamine release in rats.* A similar experiment was devised using white rats, which were injected intraperitoneally on three successive days, the daily dosage (μg/g) being as follows: quinuronium (1) 2·0

(2) 2:5, (3) 3·0; amicarbalide (1) 50·0, (2) 65·0, (3) 70·0; Compound 48/80 (1) 2·0, (2) 2·5, (3) 3·0. A fourth group received an equivalent volume of normal saline.

HISTAMINE EXTRACTION AND ASSAY

Perfusate and supernatant. The method has been described by Feldberg & Talesnik (1953).

To each sample, concentrated hydrochloric acid was added to produce an acid concentration of 20% v/v HCl. The samples were heated for 30 min in a boiling water-bath, then neutralised with concentrated sodium hydroxide solution using universal indicator paper. The volume was measured.

Histamine assays were performed on atropinised guinea-pig ileum (1×10^{-8} g atropine) in Tyrode solution. To exclude any non-histamine component, the solutions were re-assayed in Tyrode containing mepyramine 1×10^{-9} g.

Tissues. A portion of tissue was weighed, ground with silver sand and added to a small quantity of 20% v/v hydrochloric acid, which was boiled for 10 min, filtered through Whatman No. 1 filter paper and washed with three volumes of distilled water. The clear filtrate was neutralised with sodium hydroxide solution diluted with Tyrode as required and assayed as described above.

Results

In vitro *release of histamine from perfused rat hind-quarters.* Assays indicated that there was no detectable histamine released from the saline perfused tissues. Compound 48/80 released the largest amount of histamine, whereas quinuronium and amicarbalide released less than half this amount (Table 1).

In vitro *release of histamine from sheep diaphragm strips.* Table 1 shows that there was an appreciable quantity of histamine released from the control tissue. Compound 48/80 liberated a large quantity of histamine and quinuronium about half this amount, whereas the amount of histamine released by amicarbalide was not significantly different from that of the controls.

In vivo *histamine release in mice.* Within a few minutes of an intraperitoneal injection of quinuronium or compound 48/80, the mice became restless and showed signs of cyanosis. Mice which received quinuronium were very severely affected, showing also marked dyspnoea and collapse before finally recovering. Compound 48/80 was less severe in its effects than quinuronium and over a period of 3 days the mice acquired some tolerance to compound 48/80, but did not seem to show any tolerance to the action of quinuronium. Amicarbalide produced no marked toxic signs but the mice became slightly restless.

Table 1 shows that compound 48/80 and quinuronium reduced the skin histamine content by 50%, whereas amicarbalide did not show any measurable histamine release at the given dosage.

In vivo *histamine release in rats.* Within a few minutes of the injection of quinuronium, the rats began to show hyperactivity and face washing, followed by frenzied jumping movement and respiratory distress. A

TABLE 1. THE RELEASE OF HISTAMINE FROM THE TISSUES OF SHEEP, RAT AND MOUSE

	Tissue						
	In vitro			*In vivo*			
	Rat hind-quarters	Sheep diaphragm		Mouse		Rat	
Drug treatment	Mean histamine released in 100 ml perfusate (μg±s.e.)	Mean histamine released μg/g±s.e.	Hist-amine released %	Mean histamine content of skin μg/g±s.e.	Hista-amine released %	Mean Histamine content of skin μg/g±s.e.	Hist-amine released %
Physiological saline	0·00	1·63±0·60	13	26·1±2·27	0	33·21±2·80	0
Quinuronium Sulphate ..	101·0±40·0	5·62±0·66	45	14·1±1·85	46	16·80±3·44	49
Amicarbalide Isethionate ..	125·0±26·0	3·11±0·44	25	27·35±1·25	0	23·09±1·91	30
Compound 48/80	298·0±32·0	9·60±1·45	77	12·31±1·90	53	6·00±1·82	82

Mean histamine content of sheep diaphragm/peritoneum = 12·50 μg/g ± 1·50.
Number of estimations for all mean histamine values = 4.

period of quiescence preceded recovery. Amicarbalide produced some slight excitement in the rats, some of which showed face washing movements. Compound 48/80 produced similar signs to quinuronium with the addition of facial oedema. After three days of injections the rats became tolerant to the action of compound 48/80 but not to quinuronium. At the stated dosage levels, compound 48/80 released the most histamine; quinuronium released about half this quantity and amicarbalide about a third.

Discussion

Preliminary *in vitro* evidence from perfused rat hind-quarters indicated that quinuronium and amicarbalide were both capable of releasing significant quantities of histamine from rat tissues.

In sheep diaphragm, amicarbalide in the given concentration did not release histamine, whereas quinuronium and compound 48/80 did. A difficulty in the sheep experiments was a variable spontaneous release of histamine. Rocha e Silva & Schild (1949) reported this to occur in the rat diaphragm. These authors incubated rat diaphragms in oxygenated Tyrode at 37°, whereas in the sheep experiments described here, Krebs solution was not oxygenated. Factors which may have contributed to the variable release were (1) uneven thickness of tissue, (2) absolute thickness making oxygen and drug diffusion variable, and (3) trauma of tissue.

In vivo experiments in rats and mice were more informative. In mice, quinuronium and compound 48/80 were approximately equally active, but amicarbalide failed to liberate histamine at the doses used. In the rat,

quinuronium and amicarbalide released comparable quantities of histamine which were less than those produced by compound 48/80. The signs of toxicity in mice showed that although quinuronium and compound 48/80 released similar amounts of histamine, quinuronium was much more toxic. In addition mice acquired some tolerance to this dosage of compound 48/80 over a period of 3 days, but not to quinuronium.

In rats where quinuronium and amicarbalide released similar amounts of histamine, quinuronium was distinctly more toxic,. This suggested that although quinuronium was a potent histamine liberator, the drug possessed toxic properties other than histamine release, which agreed with the observations of central respiratory inhibition by Kronfeld (1959) and the anticholinesterase activity observed by Rummler & Laue (1960).

In the experiments described, it was clear that histamine release was a significant part of the overall toxicity of quinuronium in mice, rats and sheep. Amicarbalide at these doses only appeared to release comparable quantities of histamine in rats.

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