J. Pharm. Pharmac., 1967, **19**, 509–519 Received February 15, 1967

Some pharmacodynamic effects of the babesicidal agents quinuronium and amicarbalide

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The intravenous injection of a therapeutic dose of quinuronium methylsulphate (1 mg/kg) causes a fall in blood pressure in sheep, which is partly prevented by mepyramine and abolished by atropine. Larger doses of quinuronium cause more marked hypotension and inhibition of respiratory movement, which are not affected by atropine. Quinuronium strongly increases the amplitude of contraction of the isolated rabbit heart. This effect is not antagonized by atropine or mepyramine. Contractions of plain muscle in the guinea-pig and sheep, and hypersecretion of gastric acid in the rat and of saliva in the sheep were all produced by quinuronium. The responses to acetylcholine were potentiated by quinuronium, an effect which was abolished by atropine. Amicarbalide isethionate by comparison was weakly active. The drug causes no change in blood pressure, smooth muscle contraction or salivary secretion, but stimulates gastric secretion and partially inhibits the actions of acetylcholine in these preparations.

QUINURONIUM methylsulphate was first synthesized in 1933 and is still one of the principal chemotherapeutic agents for piroplasmosis. Its main disadvantage is a very low therapeutic index. Therapeutic doses (1 mg/kg) cause salivation, defaecation and urination which may be accompanied by dyspnoea. Higher doses produce cyanosis, apnoea, collapse and death (Cernaianu, Schuldner & Magureanu, 1935).

Kronfeld (1959) suggested that the toxic signs were due to central respiratory inhibition. Rümmler & Laue (1961) showed that quinuronium reduced circulating cholinesterase activity in sheep and dogs and that partial protection could be produced by atropine and by pyridine 2-aldoxime methiodide—a cholinesterase reactivator (Wilson & Ginsburg, 1955).

The anticholinesterase action of quinuronium was confirmed *in vitro* and *in vivo* in a wide variety of species (Eyre, 1966a) and the release of histamine by quinuronium demonstrated in rats, mice and sheep (Eyre, 1966b).

Amicarbalide isethionate, another babesicidal agent with a better therapeutic index than quinuronium, became available in 1960. The toxic reactions of amicarbalide so far reported include local swelling at the injection site and mild ataxia in some animals (Ashley, Berg & Lucas, 1960; Beveridge, Thwaite & Shepherd, 1960).

Amicarbalide possesses weak anticholinesterase activity in many species (Eyre, 1966a) and releases histamine in rats (Eyre, 1966b).

The purpose of these investigations was to examine more precisely the pharmacodynamic actions of quinuronium and to make comparisons with amicarbalide.

Experimental

In vivo. CAROTID BLOOD PRESSURE AND RESPIRATION IN SHEEP

Adult "south country" Cheviot sheep of mixed sexes were used.

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Anaesthesia was induced with intravenous thiopentone sodium and maintained by closed-circuit cyclopropane and oxygen. Blood pressure was recorded kymographically by a mercury manometer, simultaneously with respiratory ventilation from a tracheal cannula. Drugs were injected into the tarsal vein.

RABBIT ISOLATED HEART

The method was a modification of Langendorff's (1895) technique, and the apparatus was described by Bartlet (1963).

RABBIT PERFUSED EAR

Ears from freshly killed rabbits were perfused in air through the central artery with Krebs solution (Krebs & Henseleit, 1932) preheated to 37°, and the perfusion rate measured kymographically by a photoelectric drop recorder and a Thorpe impulse counter. Drugs were injected into the perfusion fluid.

ISOLATED SMOOTH MUSCLE

Guinea-pig ileum. Short lengths of terminal guinea-pig ileum were set up in the usual way in aerated Tyrode solution at 35°, in a 5 ml organ bath.

Guinea-pig and sheep bladder. The mucous membrane was carefully removed from longitudinal strips of bladder wall, which were set up in an isolated organ bath in oxygenated Krebs solution at 35°.

EXOCRINE SECRETIONS

Parotid salivation in sheep. Anaesthesia was induced with intravenous thiopentone sodium and maintained with small doses of pentobarbitone sodium as necessary to maintain "surgical" anaesthesia. Salivary outflow was measured from the cannulated parotid duct by means of a photoelectric drop-counter recording kymographically.

Gastric acid secretion in rats. Gastric acid was measured by a modification of the method of Ghosh & Schild (1958). Saline heated to 38° was perfused through the stomach, collected over a given time and titrated immediately against 0·01N sodium hydroxide using methyl red as indicator.

Drugs were injected intravenously.

DRUGS

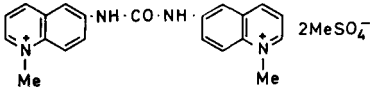
Quinuronium methylsulphate [*NN'*-diquinol-6-ylurea 1,1'-dimetho-(methylsulphate); I]; amicarbalide isethionate [*NN'*-di(3-amidinophenyl)-ureadi(2-hydroxyethanesulphonate); II]; acetylcholine chloride; histamine acid phosphate; adrenaline hydrogen tartrate; noradrenaline acid bitartrate; carbamoylcholine chloride (carbachol); atropine sulphate and mepyramine maleate were used.

Results

CAROTID BLOOD PRESSURE AND RESPIRATION IN SHEEP

Adrenaline increased, whereas acetylcholine and histamine decreased the blood pressure. Quinuronium had a variable effect in the

lower dose range (up to 1 mg/kg) whereas in therapeutic doses (1 to 2 mg/kg) there was always a fall in blood pressure, and a reduction in respiratory ventilation (Fig. 1).



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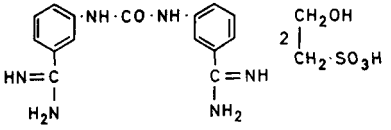

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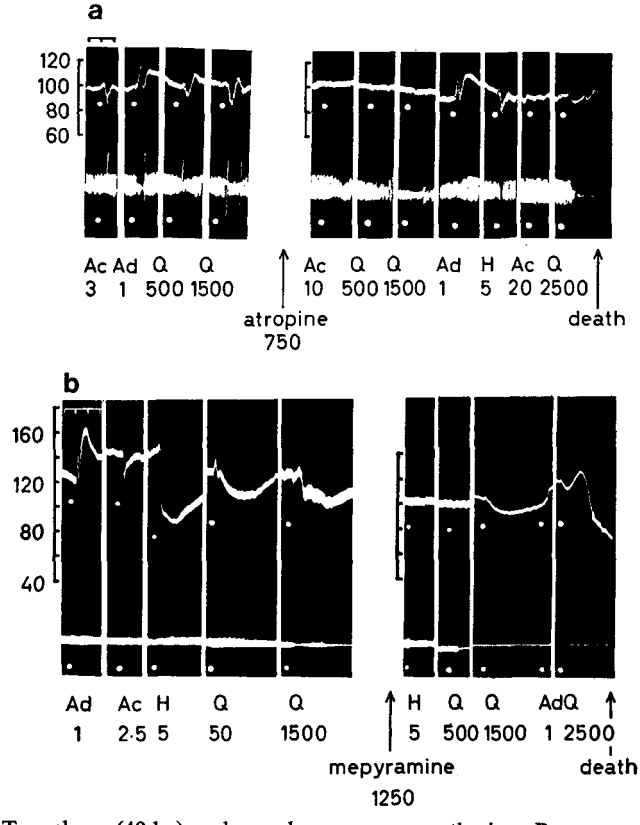

FIG. 1. Two sheep (40 kg) under cyclopropane anaesthesia. Responses of carotid blood pressure (mm Hg) (upper trace) and respiratory volume (lower trace) to intravenous injections of adrenaline (Ad), acetylcholine (Ac), histamine (H) and quinuronium (Q) before and after the administration of atropine (a) and mepyramine (b). Time scale in min. Drug doses, μg/kg i.v.

Atropine (0·75 mg/kg) antagonized the hypotensive action of quinuronium up to 1–2 mg/kg, but failed to protect above this dose.

Mepyramine (1·25 mg/kg) antagonized quinuronium up to 0·5 mg/kg. Neither atropine nor mepyramine showed any antagonism of the respiratory inhibition nor prevented death from overdosage with quinuronium.

Amicarbalide did not affect the blood pressure of sheep.

RABBIT ISOLATED HEART

Acetylcholine reduced the amplitude of contraction, whereas adrenaline, noradrenaline, histamine and quinuronium increased the contractility of the heart (Fig. 2). The doses of agonist drugs were adjusted to give

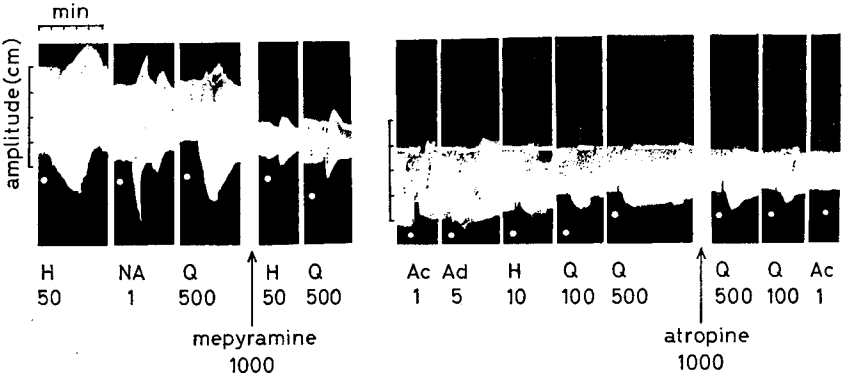

FIG. 2. The responses of isolated perfused rabbit heart to histamine (H), acetylcholine (Ac), adrenaline (Ad), noradrenaline (NA) quinuronium (Q), mepyramine and atropine, injected into the perfusion fluid. Drug doses in μg.

approximately equal responses. Atropine antagonized acetylcholine, but did not modify the action of quinuronium. Mepyramine depressed heart contractility by 60%, and did not inhibit the responses to quinuronium or histamine (Table 1). Amicarbalide had no demonstrable effect on the heart.

TABLE 1. THE RESPONSE OF ISOLATED PERFUSED RABBIT HEARTS TO ACETYLCHOLINE, ADRENALINE, NORADRENALINE, HISTAMINE, QUINURONIUM AND AMICARBALIDE AND THE INFLUENCE OF ATROPINE AND MEPYRAMINE.
The values are expressed as percentage *changes* in amplitude, and are means of four experiments. Standard errors are in parentheses.

Drug	Dose (μg)	Percentage change in amplitude of contraction		
		Drug alone	After atropine 1 mg	After mepyramine 1 mg
Acetylcholine ..	1·0	−29 (± 7)	0	
Adrenaline ..	5·0	+25 (± 6)		
Noradrenaline ..	1·0	+118 (±42)		
Histamine ..	50·0	+49 (±21)		+24 (± 8)
Quinuronium ..	100·0 500·0	+30 (±10) +78 (±28)	+55 (±16)	+76 (±23)

RABBIT PERFUSED EAR

Adrenaline, acetylcholine, histamine and quinuronium produced

vasoconstriction, but quinuronium had a much more prolonged action than the other drugs. Atropine inhibited acetylcholine and quinuronium, whereas mepyramine inhibited histamine but not quinuronium. Amicarbalide did not produce any effect on vascular resistance, but had some antagonism against acetylcholine and quinuronium (Fig. 3).

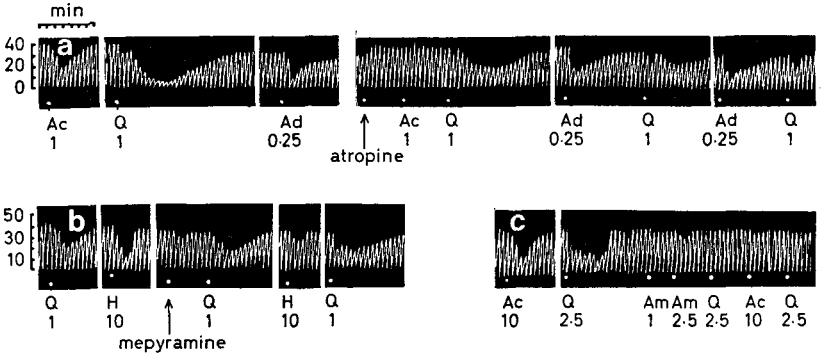

FIG. 3. Records of venous outflow from three perfused rabbit ears in response to intra-arterial injections of acetylcholine (Ac), adrenaline (Ad), histamine (H), quinuronium (Q), before and after atropine 1 mg (a) and mepyramine 2·0 mg (b); and of amicarbalide (Am) (c). Doses in μg for acetylcholine, adrenaline and histamine; in mg for quinuronium. Vertical scale: No. of drops.

ISOLATED SMOOTH MUSCLE

Guinea-pig ileum. A shortening of the ileum occurred in response to concentrations of quinuronium, $3{\cdot}5 \times 10^{-9}$ to $3{\cdot}5 \times 10^{-6}$ M. This increased tone was accompanied by increased spontaneous movement, illustrated in Fig. 4. The ileum is here contracting to alternate doses of 0·30 μg and 0·15 μg acetylcholine. Quinuronium, $3{\cdot}5 \times 10^{-9}$ to $3{\cdot}5 \times 10^{-7}$ M, produced in addition to increased tonus, a potentiation of the responses to acetylcholine, with a maximum in both phenomena at quinuronium, $3{\cdot}5 \times 10^{-8}$ M.

After a period of 5 to 10 min exposure to quinuronium, the muscle tone decreased to a level only slightly above the control. The spontaneous movement persisted however, but the responses to acetylcholine were inhibited. On washing out the quinuronium, the tone returned to normal and spontaneous movement disappeared almost immediately. The acetylcholine responses were restored to normal within 5 to 10 min with repeated washings.

In the presence of quinuronium in concentrations greater than 10^{-6} M there was little or no increase in muscle tone and the responses to acetylcholine and histamine (Fig. 4d, f) were depressed and finally abolished at a quinuronium concentration of $3{\cdot}5 \times 10^{-4}$ M. It became more difficult with increasing concentrations to "wash out" the quinuronium. After concentrations 10^{-5} and 10^{-4} the acetylcholine responses did not return to normal within an hour of repeated washing. Amicarbalide isethionate did not contract the ileum or potentiate acetylcholine at any concentration.

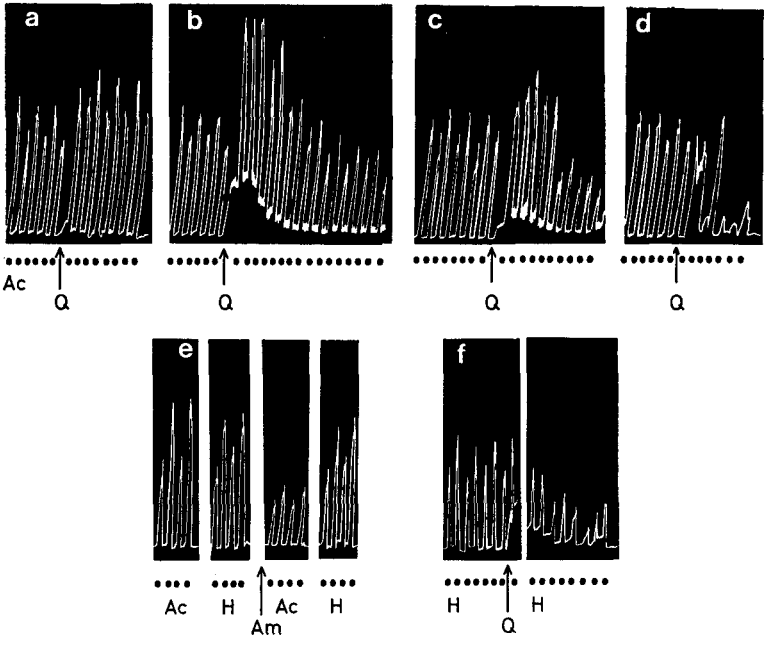

FIG. 4. (a), (b), (c) and (d). Isolated guinea-pig ileum in aerated Tyrode at 35°, contracting to alternate doses of 0·30 and 0·15 μg acetylcholine (Ac) added to the bath for 30 sec at 2 min intervals, showing the effects of increasing molar concentrations of quinuronium (Q) on muscle tone and the responses to acetylcholine. (a) $Q = 3{\cdot}5 \times 10^{-9}$M, (b) $Q = 3{\cdot}5 \times 10^{-8}$M, (c) $Q = 3{\cdot}5 \times 10^{-7}$M, (d) $Q = 3{\cdot}5 \times 10^{-6}$M. (e) Guinea-pig ileum contracting to alternate doses of 0·10 and 0·20 μg acetylcholine (Ac) and to alternate doses of 0·05 and 0·10 μg histamine (H), showing the effect of adding amicarbalide $2{\cdot}1 \times 10^{-4}$M (Am). (f) Guinea-pig ileum contracting to alternate doses of 0·04 and 0·08 μg histamine (H), showing the inhibitory action of $1{\cdot}0 \times 10^{-6}$M quinuronium (Q).

Large concentrations (above 10^{-4} M) inhibited responses to acetylcholine (Fig. 4e).

Guinea-pig and sheep bladder. Bladder strips from both species contracted strongly to histamine and acetylcholine. Quinuronium had a

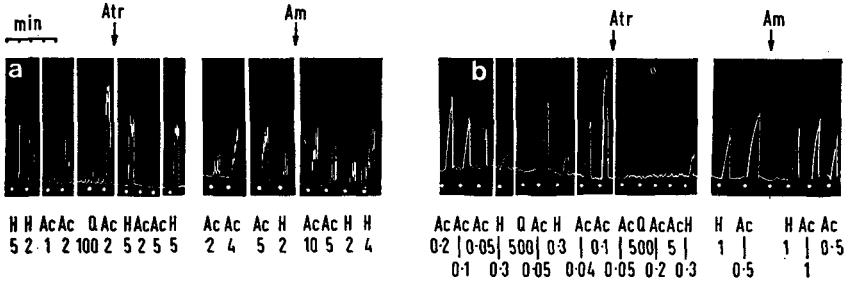

FIG. 5. Isolated longitudinal strips of (a) guinea-pig bladder and (b) sheep bladder in oxygenated Krebs, contracting to acetylcholine (Ac), histamine (H), quinuronium (Q), each added to the bath for 60 sec at 5 min intervals. Atropine (Atr) and amicarbalide (Am) added to the bath at the points indicated. Drug concentrations μg/ml.

variable and inconsistent effect. In all but one of six preparations from the guinea-pig there was muscle contraction and increased spontaneous movement, whereas in only one of five preparations from sheep was there a slight increase in tone. Repeated washings over a period of 5 to 15 min were required before the effects of quinuronium passed off, during which time the responses to acetylcholine were potentiated (Fig. 5). Atropine abolished the actions of acetylcholine and quinuronium.

Amicarbalide produced no contraction of the bladder muscle, but at high concentrations the compound partially inhibited the acetylcholine responses.

EXOCRINE SECRETIONS

Parotid salivation in sheep. A hypotensive dose of acetylcholine produced a brief increase in salivation, usually persisting for less than 1 min. A dose of histamine which was equally hypotensive produced little or no change in salivary flow. Doses of quinuronium less than 10 μg/kg produced no change, but above 100 μg/kg there was prolonged salivation which persisted for 10 to 40 min. Immediately after the effect of quinuronium had passed off, the responses to acetylcholine (both blood pressure and salivation) were potentiated. Atropine antagonized this effect (Table 2).

TABLE 2. THE ACTION OF ADRENALINE, HISTAMINE, QUINURONIUM AND ATROPINE ON PAROTID SALIVATION IN SHEEP.
Sheep, 30–40 kg, anaesthetized with thiopentone and pentobarbitone. Parotid salivary outflow in response to i.v. drug injections measured kymographically and expressed as mean *increases* (ml/min).

Drug	Dose (μg/kg)	Increase in mean salivary flow (ml/min)		
		Drug alone	After quinuronium	After atropine
Adrenaline	2	0·35	—	—
Histamine	4	0	—	0
	10	0	—	0
Acetylcholine	1	0	0·51	—
	2	0·20	0·60	0
	4	0·50	1·04	0
	15	—	—	0
Quinuronium	10	0	—	—
	100	0·32	—	—
	500	1·66	—	0
	1,000	2·10	—	0

Amicarbalide was without effect on this preparation.

Gastric acid secretion in rats. Histamine and acetylcholine stimulated the secretion of gastric acid for periods varying between 10 and 40 min. Doses of quinuronium less than 50 μg/kg induced no increase in secretion but potentiated the action of acetylcholine. Quinuronium at a rate greater than 100 μg/kg induced marked and prolonged acid secretion, which persisted for 1–2 hr. The response to acetylcholine was potentiated when the drug was given immediately after the effects of quinuronium had passed off (Table 3).

TABLE 3. THE ACTION ON GASTRIC SECRETION IN RATS OF ACETYLCHOLINE, HISTAMINE, QUINURONIUM, AMICARBALIDE, ATROPINE AND MEPYRAMINE
Rats, 150–250 g, anaesthetized with urethane. Gastric acid secretion in response to i.v. injection of drugs, expressed as percentage of resting secretion. Values are means with standard errors and numbers of experiments are in parentheses.

Drug	Dose μg/min for 10 min	Percentage increase in the mean gastric acid output per min				
		Drug alone	After atropine (1 mg/kg)	After mepyramine (2 mg/kg)	After quinuronium	After amicarbalide
Acetylcholine	10	485± 39 (20)	0 (4)	561± 65 (4)	826±24 (4)	273±33 (4)
Histamine ..	25	563± 37 (18)	553± 20 (4)	580± 28 (6)	550±19 (4)	545±41 (4)
Quinuronium	30	3128±201 (8)	320± 50 (4)	3606±350 (4)	—	—
Amicarbalide	200	433± 86 (8)	517±103 (4)	540±123 (4)	—	—
Mepyramine..	200	349±110 (18)	—	—	—	—

Atropine abolished the activity of acetylcholine and partially antagonized quinuronium. Mepyramine showed no antagonism against any of the drugs used.

Amicarbalide stimulated gastric secretion and was not affected by the presence of atropine. After amicarbalide, the response to acetylcholine was diminished.

Discussion

Therapeutic doses (1 mg/kg) of quinuronium caused hypotension in anaesthetized sheep, which was almost completely prevented by atropine and partially prevented by mepyramine. However, neither atropine nor mepyramine prevented or alleviated the respiratory inhibition, or the hypotensive action of larger doses of quinuronium (>1 mg/kg).

Quinuronium produced vasoconstriction of the rabbit ear which was completely inhibited by atropine and not at all by mepyramine, suggesting that the activity was "muscarinic" and that histamine effects were not involved in the action of quinuronium in this preparation. While amicarbalide itself showed no vascular action, the compound appeared to possess atropine-like activity, shown by its antagonism against acetylcholine and quinuronium.

In the isolated heart, quinuronium always produced an *increase* in amplitude of contraction which was unaffected by atropine. Both observations indicate that this action on the heart is not cholinergic, and is probably due to an action of quinuronium distinct from anticholinesterase activity. Mepyramine did not inhibit the stimulant effects of either quinuronium or histamine.

In 1910, Dale & Laidlaw showed that histamine stimulated the heart of cats and rabbits, and Went & Lissack (1935) found that histamine also stimulated the hearts of guinea-pigs.

Recently several authors have reported that the common antihistamine agents do not antagonize the effect of histamine on the heart of guinea-pigs (Lockett & Bartlet, 1956; Trendelenburg, 1960), and although Mannaioni (1960) reported that diphenhydramine antagonized the cardiac action of

histamine, this has not been confirmed (Bartlet, 1963). It is possible, therefore, that the cardiac action of quinuronium might involve some histamine-like activity, but in the absence of an antagonist this could neither be established nor denied.

The peripheral vasoconstriction and myocardial stimulation will not contribute to the production of hypotension by quinuronium. Other experiments designed to show the action of the drug on vascular resistance in the intestine, limb, and pulmonary bed of sheep have been negative (unpublished observations). Thus the results of experiments on the cardiovascular system have not as yet explained the fall in blood pressure which is such a prominent feature of quinuronium intoxication. A possibility is that cardiovascular changes occur as a consequence of the action of quinuronium on the central nervous system (Kronfeld, 1959) mediated for example through the vagus nerve. This possibly has not been investigated.

The actions of quinuronium on the ileum and urinary bladder were varied. In the ileum, small concentrations produced increased tonus and spontaneous movements invariably; whereas the effects on bladder muscle were less consistent.

In both muscles the responses to acetylcholine were potentiated by small concentrations of quinuronium (10^{-9} to 10^{-7} M). Larger concentrations ($>10^{-6}$ M) produced smaller increases in tone and the responses of the ileum to acetylcholine and histamine were inhibited.

Similar observations have been made by Heathcote (1932) and Shelley (1955) using eserine. It seems likely that the inhibitory action of quinuronium on the intestine, like that of eserine, is due to a factor not concerned with cholinesterase inhibition.

Amicarbalide is, comparatively, a very weak anticholinesterase (Eyre, 1966a). Shelley (1955) showed that increase in tonus of the intestine and acetylcholine potentiation occurred when less than 20% of the true cholinesterase activity of the tissue was inhibited. High concentrations of amicarbalide may inhibit cholinesterase to this extent (Eyre, 1966a), but in a series of experiments on the guinea-pig ileum, using a range of amicarbalide concentrations between 10^{-9} and 10^{-3} M, no increase in tone or acetylcholine potentiation has been observed before the onset of inhibition of the ileum. Amicarbalide thus inhibits the ileum in a manner which is almost certainly not related to anticholinesterase activity and which is different from the inhibition of the ileum by quinuronium, in that it is more specific for acetylcholine and is not preceded by contraction or potentiation of the responses to acetylcholine.

Comparable results were obtained when recording parotid salivation in sheep. Quinuronium itself induced prolonged salivation and potentiated the responses to acetylcholine.

Atropine effectively antagonized acetylcholine and quinuronium on plain muscle and on salivation.

Quinuronium increased gastric acid secretion in rats, an effect which could not be completely antagonized even by very large doses of atropine (approximately 5 mg/kg). Amicarbalide caused a mild transient hyper-

acidity which was atropine-resistant. It is known that atropine does not abolish gastric acid secretion (Gray & Ivy, 1937; Code, 1951); but in these experiments, since atropine completely antagonized the effect of acetylcholine, it may be that the atropine-resistant action of quinuronium on gastric secretion was non-cholinergic, although more evidence would be needed to establish the point. Both babesicides have been shown to release histamine in rats (Eyre, 1966b), but in the absence of an antagonist for the gastric secretory action of histamine it was not possible to show whether either compound stimulated gastric secretion by releasing histamine. [Many authors have reported that antihistamine compounds do not antagonize the gastric effects of histamine (Ashford, Heller & Smart, 1949; Loew, 1950; Paton & Schachter, 1951) and Mota & Da Silva (1960) showed that mepyramine released histamine *in vitro*.]

It was interesting that amicarbalide, although itself stimulating gastric acid secretion, appeared partly to antagonize the actions of acetylcholine but not of histamine. This observation is consistent with the activity of amicarbalide on smooth muscle.

The data presented confirm the many reports of the toxicity of therapeutic doses of quinuronium (Cernaianu & others, 1935; Egerov, 1951; Kronfeld, 1959; Rümmler & Laue, 1961; Eyre, 1966c), namely the signs of hypotension, dyspnoea, defaecation, micturition and salivation. Moreover they explain the rationale of using atropine during therapy with quinuronium sulphate.

The failure of atropine to alleviate the apnoea, collapse and death which characterizes more severe intoxication with quinuronium is not readily explained and will require further investigation. Kronfeld (1959) attributed the "respiratory-type death" to a failure of cellular respiration in the central nervous system (CNS) and (presumably) elsewhere. The observations may also be explained in part by the anticholinesterase activity of quinuronium (Rümmler & Laue, 1961; Eyre, 1966a) possibly causing bronchoconstriction initially, followed by CNS depression not antagonized by atropine (Modell & Krop, 1946). Atropine would prevent bronchoconstriction but at high doses of quinuronium the central effects would supervene.

In view of the quaternary nature of quinuronium sulphate it is unlikely to diffuse readily into the CNS and it may be a metabolite which causes the effects. This question has not been investigated, but if the respiratory effect of the drug were due principally to a metabolite there would probably be a time lag in the onset of respiratory inhibition. This is not so. In fact apnoea may be observed before the full hypotensive effect of the drug and the action is more likely due to quinuronium as such.

It has been observed through a large number of acute experiments in several species that after repeated dosage with quinuronium there is always a progressive deterioration in blood pressure and respiration which cannot be prevented or alleviated (Fig. 1). Although the metabolism of quinuronium has still to be described, the *in vivo* study of cholinesterase inhibition by quinuronium (Eyre, 1966a) showed that the enzyme activity remained depressed for up to 24 hr after a single injection of the drug,

which suggests either that the metabolism of quinuronium is comparatively slow or that a metabolite persists which also inhibits cholinesterase. The ultimate cardiovascular and respiratory failure is thus probably the manifestation of the persistence and/or accumulation of quinuronium and/or its metabolite(s) in the CNS and elsewhere, which would be consistent both with Kronfeld's (1959) explanation of cytotoxicity and with cholinesterase inhibition (Rümmler & Laue, 1961; Eyre, 1966a).

Quinuronium sulphate has thus been shown to possess a number of pharmacodynamic actions. The results support the conclusion that most of the toxic effects caused by therapeutic doses of quinuronium may be attributed to cholinesterase inhibition, the symptoms of which are largely prevented by atropine. Severe intoxication with quinuronium produces irreversible cardiovascular and respiratory depression, the precise mechanisms of which are not known.

Amicarbalide isethionate is, comparatively, much less active and produces no marked reactions even in high doses, the inhibition of cholinergic (muscarinic) responses being the only consistent finding.

Acknowledgements. I am grateful to Dr. F. Alexander for his encouragement; also to Evans Medical Limited, Liverpool for a gift of quinuronium (Pirevan) and to May & Baker Limited, Dagenham for a gift of amicarbalide (Diampron).

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