CHAPTER TWO

EXPERIMENTAL
INTRODUCTION

In recent years chloroquine and amodiaquin, the 4-aminoquinoline antimalarial agents, have been investigated for their therapeutic utility in a number of other unrelated conditions. Thus chloroquine has been found to be useful in amoebic hepatitis, cardiac arrhythmias, lupus erythematosus, certain light sensitive eruptions, pemphigus, Sjögren's syndrome, rheumatoid arthritis, infectious mononucleosis, taeniasis and giardiasis, bronchial asthma and as a desludging agent in vascular disease process (20,151,170,213). Comparatively, amodiaquin has received lesser attention in therapeutics but appears to share the utility of chloroquine in cardiac arrhythmias, rheumatoid arthritis, lupus erythematosus, giardiasis and in dermatological practice including conditions like the light sensitive eruptions (21,77,113,215,261).

As for the acute pharmacological effects of these compounds chloroquine has been shown to possess potent antiarrhythmic action (9,40), and antiveratrinic (6), anticholinergic and vagolytic (1), antihistaminic and antianaphylactic (2), anticoagulant and local anaesthetic (149,181), and antiinflammatory (241) action. Intravenous administration of chloroquine produces hypotension in animals (9,24,69) and in man (169,255) to which the cardiac inhibitory action of this compound probably contributes to
some extent (1, 24, 128). After the transient fall in blood pressure responses to administered catecholamines are found to be augmented (146). Amodiaquin again appears to exhibit many of the pharmacological actions of chloroquine, though this agent has not been thoroughly investigated for these actions. The reported pharmacological actions of amodiaquin include a potentiating effect on the pressor action of injected catecholamines (148), and antiveratrinic (6), uterine stimulant (150), local anaesthetic (149), hypotensive (69, 148) and neuromuscular blocking (149) effect. Though amodiaquin has an antiarrhythmic effect on heart (8) and has been clinically investigated in this connection it can activate ectopic foci in ventricles and increase sensitivity of the ventricles towards adrenaline induced arrhythmias (7). Recently both chloroquine and amodiaquin were found to prolong significantly the barbiturate sleeping time in mice (71) and to exert an adrenergic neuronal blocking effect (147).

Though chloroquine and amodiaquin were reported to augment the pressor responses to injected catecholamines, the mechanism of this potentiation has not been clarified so far. There is also a dearth of information on the interaction between the 4-aminoquinolines and various vasoactive agents other than the catecholamines in vivo. The present work was primarily undertaken to confirm the potentiating effect of chloroquine and amodiaquin on the
pressor responses to injected catecholamines and to characterize the mechanism through which this potentiation occurs. The interaction between the 4-aminoquinolines and other vasoactive agents was also studied in intact animal preparations. The details of these experiments are presented in Part I.

One of the possible explanations for the potentiating effect of the 4-aminoquinolines seen in the intact animal experiments could have been the sensitizing effect exerted by these compounds on the peripheral tissues. Moreover it was observed that somewhat larger doses of amodiaquin sometimes did not augment but inhibited the pressor responses to injected catecholamines, probably by blocking the \( \alpha \)-adrenergic receptors. For these reasons it was thought worthwhile to study the effect of the 4-aminoquinolines on responses of isolated tissue preparations mediated through the \( \alpha \)-or \( \beta \)-adrenergic receptors. The details of this study are presented as Part II.

During the experiments designed to characterize the potentiating effect of the 4-aminoquinolines on the catecholamine pressor responses, the compounds were also found to induce a mild supersensitivity to injected tyramine. It was also seen that the 4-aminoquinolines which on intravenous administration always produce a fall in blood pressure, produce a somewhat sustained pressor
response after the initial hypotension if the animals are previously treated with either dexamphetamine or methylamphetamine half an hour earlier. This pressor effect and the potentiation of injected catecholamines and of tyramine which follows these agents in untreated animals were considered to be of pharmacological interest being reminiscent of similar effects following acute intravenous administration of reserpine (see, 31, for references).

Yelnosky et al (880) reported that in amphetamine pretreated animals reserpine given intravenously produced brisk pressor responses which are adrenergic in origin. The experiments designed to characterize the pressor responses of the 4-aminoquinolines occurring in methylamphetamine pretreated animals showed that these responses are mediated through a brisk release of catecholamines from the tissue stores. These experiments are described in Part III as they demonstrate the capacity of chloroquine and amodiaquine to interact with tissue catecholamine stores and therefore tend to help in the final analysis of the mechanism by which the 4-aminoquinolines augment the pressor responses to injected catecholamines.
PART I : EXPERIMENTS WITH INTACT ANIMAL PREPARATIONS.

METHODS

GENERAL

Cats and dogs. Male or female cats (with weights between 2.5 and 3.5 kg) and mongrel dogs (with weights between 9 and 13 kg) were used for experiments. Prior to use the animals were observed for 10 to 12 days to be healthy with respect to their food intake, bowel function and behaviour; in this period their sodium intake was from 70 to 130 mEq/day. Spinal preparations were obtained under ether anaesthesia by dividing the spinal cord at the level of second cervical vertebra as described by Burn (34); bilateral vagotomy was performed routinely. In some experiments the dogs were anaesthetized with pentobarbitone (35 mg/kg, intraperitoneally) or with phenobarbitone sodium (150 mg/kg, intraperitoneally). Blood pressure was recorded with a mercury manometer connected to left common carotid artery; the heart rate was measured from Lead II of electrocardiogram. Drugs were injected through a cannulated femoral vein. Contractions of the right gravidating membrane were recorded by a frontal writing lever (ratio 1:10) putting the membrane under a stretch of 3.5 g in case of cats and 1.5 g in case of dogs; splenic volume
was monitored as described by Burn (34); contractile force of the exposed heart was recorded by a Cushney myocardioograph. Spinal and open chest preparations were artificially ventilated. Rectal temperature of the animals was maintained at 37 - 38 C with aid of heating lamps. All preparations were stabilised for 1 hr.

Rabbits. Albino rabbits (with weights between 1.7 to 2.3 kg) were anaesthetised with urethane (1.5 g/kg, subcutaneously). Blood pressure was measured with a Condon's mercury manometer connected to left common carotid artery; drugs were injected through a cannulated jugular vein.

**EXPERIMENTAL PROCEDURE.**

At the beginning of experiment responses to increasing doses of adrenaline and noradrenaline were determined by rapid intravenous administration of the amines (in a completely randomized fashion) in a fixed volume of 1 ml of normal saline which was washed in with 1.5 ml of the saline. Doses of amines used ranged between 0.06 to 4.0 µg/kg and produced pressor responses from 25 to 75 % of the maximal; enough time was allowed between the injections for return of various parameters to the resting values. In some experiments a single dose (about ED50 -blood pressure) of the catecholamines was used. Other vasoactive agents tested were acetylcholine (1-3 µg/kg), histamine (0.5-2 µg/kg), isoprenaline (1-3 µg/kg),
posterior pituitary extract (0.4 I.U./kg), angiotensin (0.25-1.5 mg/kg) and tyramine (0.2-0.5 mg/kg). Responses to these agents were determined in duplicate with an interval of 25 to 35 min in between the injections. When responses to the agonists were reproducible, chloroquine (10 mg/kg for cats and dogs and 3 mg/kg for rabbits) was slowly infused intravenously over a period of 8-10 min; the drug was dissolved in a fixed quantity of saline (8 ml for cats, 16 ml for dogs and 3 ml for rabbits) for the infusion. In studies on potentiation due to amodiaquin, the agent (150 μg/kg, unless stated otherwise) was slowly infused intravenously over a period of 5-7 min; the drug was dissolved in a fixed quantity of saline (6 ml for the dogs and 3 ml for the rabbits) for infusion. Responses to the same doses of the agonists were determined again after 60 min in experiments with chloroquine and after 90 min in experiments with amodiaquin; in a few experiments responses to catecholamines were determined every 10 min after the infusion of either of the 4-aminoquinolines.

The following groups of spinal cats were used for studying the potentiating effect of chloroquine.

Group 1 (20 animals) : untreated (control);
Group 2 (5 animals) : pretreated with cocaine (5 mg/kg, intravenously, and the same dose intramuscularly every 30 min thereafter; testing of drugs began 30 min after the intravenous injection);
Group 3 (3 animals): pretreated with hexamethonium (10 mg/kg, intravenously, followed by an intravenous infusion at 10 mg/kg/hr; testing of drugs began 30 min after starting the infusion);

Group 4 (3 animals): pretreated with reserpine (0.5 mg/kg, intramuscularly, 24 hr before the experiment);

Group 5 (3 animals): pretreated with nialamide (15 mg/kg, intraperitoneally, 4 hr before the experiment).

The following groups of phenobarbitone anaesthetised dogs were used for studying the potentiating effect of amodiaquin.

Group 1 (16 animals): untreated (control);

Group 2 (5 animals): pretreated with cocaine;

Group 3 (3 animals): pretreated with hexamethonium;

Group 4 (3 animals): pretreated with reserpine;

Group 5 (3 animals): pretreated with nialamide and

Group 6 (3 animals): injected with amodiaquin (0.5 mg/kg, intramuscularly) every day for 10 days and used 24 hr after the last injection for determination of their sensitivity to injected catecholamines. The details of pretreatment were as in groups of cats used for studies on chloroquine.
Chronic treatment with chloroquine. In 4 cats, chloroquine (5 mg/kg) was injected intramuscularly every day for 10 days; the animals were used 24 hr after the last injection.

**ESTIMATION OF 'POTENTIATION'.**

**Experiments with chloroquine.**

Adrenaline and noradrenaline: The augmentation in the height (in mm of Hg.) and in the 'half life' (that is, the time to 50% recovery, in seconds) of pressor responses was estimated from 'potentiation factor'. These were determined as the mean ratio (±s.e.) of the response after and before administration of chloroquine. The change in the chronotropic effect of the amines on the heart was estimated by comparing the % change in heart rate (over the resting value) induced by the amines before and after chloroquine. These estimates were determined at two dose levels of the amines, the ED₂₅ and ED₇₀ (blood pressure). As chloroquine variably affected the magnitude and the duration of the amine pressor responses, it was desirable to estimate the overall change in the amine pressor action; for this purpose, the mean area (mm²) under the amine pressor response curves (called as 'total effect' hereafter) was studied before and after chloroquine.

Other agents: Effect of chloroquine on blood pressure responses to other drugs was estimated from % change in responses after chloroquine.
Experiments with amodiaquin.

As amodiaquin did not materially alter the duration of pressor responses to adrenaline and noradrenaline, its augmenting effect on these responses was estimated by comparison of dose/pressor response (mm of Hg) curves obtained before and after the administration of amodiaquin. Effect of amodiaquin on positive chronotropic effect of the amines on heart and on the blood pressure responses to agents other than adrenaline and noradrenaline were estimated as described above (see, 'Experiments with chloroquine').

DRUGS.

Chloroquine sulphate, amodiaquin hydrochloride, hexamethonium chloride, cocaine hydrochloride, (±)-noradrenaline hydrochloride, (±)-isoprenaline hydrochloride, tyramine hydrochloride, histamine acid phosphate, acetylcholine chloride and pronethalol hydrochloride, were used throughout the experiments. The doses refer to the salts. 2.5 mg/ml solution of reserpine (Serpasil, Ciba) was used after diluting it in normal saline. Adrenaline base, angiotensin (Hypertensin, Ciba), nialamide, and posterior pituitary extract (10 I.U./ml solution) were made up fresh in normal saline immediately prior to use.
RESULTS

A. CHLOROQUINE.

SPINAL VAGOTOMISED CATS.

Effect of chloroquine on the action of catecholamines on cardiovascular system, splenic volume and nictitating membrane. Untreated cats (Group 1).

The mean resting blood pressure of this group was 83 ± 6 mm of Hg; the heart rate was 144 ± 9 / min. Infusion of chloroquine raised the blood pressure for a brief period of time (see below); 15 min later, however, the blood pressure had attained the resting value. Pressor responses to adrenaline and noradrenaline were first found to be augmented 15 to 30 min after the chloroquine infusion. The augmentation was maximal by 45 to 60 min after the infusion and remained so for 3 to 4 hr, that is, during the period of study. When the amine responses were thus augmented (7 experiments), the positive inotropic effect of amines on the heart was either unchanged (2 experiments) or was insignificantly augmented (2 experiments); the contractile effect of the amines on the spleen (3 experiments) or on the nictitating membrane (5 experiments) was unaltered.

Augmenting effect of chloroquine on the amine pressor responses was analysed in 5 other experiments (Table I; Fig.1). The effect of chloroquine on chronotropic effect of the amines on heart is shown in Table II. Height of pressor responses to both the amines was enhanced after
Augmentation of pressor responses to adrenaline and noradrenaline by chloroquine in spinal cats and its modification by cocaine, hexamethonium, reserpine and nialamide.

\[ a \text{ and } b \] : The 'Potentiation Factors' signifying the augmentation in the height and the 'half life' (i.e. the time to 50% recovery) of pressor responses, respectively. These were computed from data obtained before and 60 min after intravenous administration of chloroquine (10 mg/kg). * Values significantly \((p < 0.05)\) different from those in control group.

**TABLE I**

Augmentation of pressor responses to adrenaline and noradrenaline by chloroquine in spinal cats and its modification by cocaine, hexamethonium, reserpine and nialamide.

\[ a \text{ and } b \] : The 'Potentiation Factors' signifying the augmentation in the height and the 'half life' (i.e. the time to 50% recovery) of pressor responses, respectively. These were computed from data obtained before and 60 min after intravenous administration of chloroquine (10 mg/kg). * Values significantly \((p < 0.05)\) different from those in control group.

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<th>Noradrenaline</th>
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<td>(ED_{70})</td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
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<td>±.06</td>
</tr>
<tr>
<td></td>
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<td>±.12</td>
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<td>Reserpine (3)</td>
<td>*1.08</td>
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<td></td>
<td>*1.04</td>
<td>±.09</td>
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<td></td>
<td>1.48</td>
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<td>Nialamide (3)</td>
<td>*1.46</td>
<td>±.15</td>
</tr>
<tr>
<td></td>
<td>*1.2</td>
<td>±.11</td>
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<tr>
<td></td>
<td>1.5</td>
<td>±.13</td>
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</tbody>
</table>

1 See, Methods, for details.
FIGURE 1.

Dose/response curves of the pressor effects of adrenaline (ADR) and noradrenaline (NADR) in spinal cats before (______) and 60 min after slow, intravenous administration of 10 mg/kg of chloroquine (______). Abscissa, log dose (μg/kg) of the amines; ordinate, the area (mm²) under the pressor response curves (recorded by a mercury manometer on kymograph paper moving at 6.5 mm/min). Each point shows the mean response, vertical bars showing S.E. of the mean.

A, untreated (control) animals; B, animals pretreated with cocaine; C, animals pretreated with hexamethonium; D, animals pretreated with reserpine; E, animals pretreated with nialamide; (see, Methods, for the details of pretreatment).
FIG. 1
Augmentation of positive chronotropic effect of adrenaline and noradrenaline in spinal cats following chloroquine and its modification by cocaine, hexamethonium, reserpine and nialamide.

The rate stimulant action of the amines on the heart (expressed as % change in heart rate above the resting value) (a) before and (b) 60 min after administration of chloroquine (10 mg/kg).

Data from the same experiments as detailed in Table I. * Values significantly (p<0.05) different from those in control experiments.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>No. of experiments</th>
<th>Adrenaline</th>
<th></th>
<th>Noradrenaline</th>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>a  b</td>
<td>a  b</td>
<td></td>
<td>a  b</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>33 ± 5 51.8 ± 8</td>
<td>81 ± 9 108 ± 10</td>
<td>25 ± 4 38 ± 5</td>
<td>52 ± 7 68 ± 6</td>
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<tr>
<td>Cocaine</td>
<td>5</td>
<td>30 ± 4 21.8 ± 5</td>
<td>89 ± 10 47 ± 6</td>
<td>30 ± 6 32 ± 7</td>
<td>57 ± 10 49 ± 8</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>3</td>
<td>29 ± 6 48 ± 7</td>
<td>76 ± 6 96 ± 12</td>
<td>22 ± 6 33 ± 8</td>
<td>50 ± 11 570 ± 12</td>
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<tr>
<td>Reserpine</td>
<td>3</td>
<td>29 ± 4 34 ± 5</td>
<td>77 ± 5 56 ± 10</td>
<td>22 ± 6 27 ± 5</td>
<td>55 ± 7 56 ± 8</td>
</tr>
<tr>
<td>Nialamide</td>
<td>3</td>
<td>36 ± 4 54 ± 8</td>
<td>90 ± 6 114 ± 9</td>
<td>29 ± 5 42 ± 5</td>
<td>56 ± 7 77 ± 9</td>
</tr>
</tbody>
</table>

Values under the columns are means (±s.e.).
chloroquine; half life of responses to noradrenaline was also enhanced. Positive chronotropic effect of the amines as well as their 'total pressor effect' was augmented at all dose levels.

To test whether these changes were due to spontaneous change in sensitivity of the preparations 8 ml of normal saline (vehicle used for chloroquine) was infused alone in 3 other spinal cats in place of chloroquine. Though not shown in Fig.1 there was no significant change in the effect of catecholamines in subsequent 3 to 4 hr.

Effect of pretreatment with various agents on chloroquine induced augmentation of the amine responses. (Table I & II)

Cocaine (Group 2). Pretreatment of animals with cocaine increased their sensitivity to catecholamines, particularly to noradrenaline. Resting blood pressure and heart rate was as in Group 1 both before and 60 min after chloroquine infusion. In comparision to Group 1 chloroquine had a lesser augmenting effect on (a) heights of pressor responses to both amines; (b) half life of responses to noradrenaline; (c) positive chronotropic effect of both the amines and (d) the 'total effect' of both amines.

Hexamethonium (Group 3). Hexamethonium increased the sensitivity of the animals to catecholamines to some extent. Resting blood pressure and heart rate was as in Group 1
throughout the experiment. Chloroquine induced augmentation of (a) height of pressor responses to both amines; of (b) half life of noradrenaline responses and of (c) 'total effect' of both the amines was less in this group in comparison to Group 1.

Reserpine (Group 4). The resting blood pressure and the sensitivity of reserpinized cats to catecholamines was as in Group 1. The animals had some bradycardia which was not statistically significant. In this group, chloroquine had very little augmenting effect on the heights of pressor responses to catecholamines. There was considerable enhancement of half life of pressor responses to adrenaline, an effect not seen in Group 1. Augmentation of positive chronotropic effect and of 'total effect' of both the amines were severely reduced.

Nialamide (Group 5). Pretreatment of the animals with nialamide did not significantly alter the sensitivity of the animals to catecholamines. The resting blood pressure and the heart rate was as in Group 1. In this group, chloroquine had very little augmenting action on the height of the pressor responses to adrenaline and noradrenaline. However there was a marked enhancement in half life of pressor responses to adrenaline, as in Group 4 animals.
Effect of intravenous infusion of chloroquine on blood pressure.

Either during or immediately after the slow, intravenous infusion of chloroquine there was a slow, moderate but somewhat sustained rise in blood pressure which lasted for 6 to 12 min (Fig. 2). This was seen in 9 out of 12 experiments using untreated animals; the mean maximal rise in blood pressure was $28 \pm 6$ mm of Hg. In cocaine and hexamethonium pretreated animals the corresponding rise in blood pressure was by $20 \pm 8$ and $22 \pm 6$ mm of Hg, respectively. The rise was less remarkable in reserpine pretreated and nialamide pretreated animals where the mean rise in blood pressure was by $13 \pm 4$ and $10 \pm 6$ mm of Hg, respectively. Infusion of equivalent volume of normal saline (5 experiments) did not give rise to any significant or persistent pressor effect.

Effect of chloroquine on blood pressure responses to other vasoactive agents.

In a total of 8 experiments in which chloroquine augmented the pressor responses to catecholamines, the responses to other vasoactive agents were variably altered. Acetylcholine. Chloroquine did not significantly alter the depressor responses to acetylcholine (3 experiments).

Histamine. Depressor responses to histamine were reduced by 15 to 40% after chloroquine (3 experiments); the half life of the responses was not significantly changed.
FIGURE 2.

Record of carotid arterial blood pressure (B.P.) of a spinal cat. Responses to adrenaline (0.5 µg/kg) in 1; to posterior pituitary extract (0.4 I.U./kg) in 2; to angiotensin (0.75 µg/kg) in 3 and to tyramine (0.3 mg/kg) in 4.

Panel A; control responses. Panel B; response to slow, intravenous infusion of chloroquine (10 mg/kg); the infusion was delivered over a period of 8 min, the arrow marking the end of infusion. Panel C; responses 60 min after B. All injections were intravenous. Time, 2 min.
Isoprenaline. There was no consistent effect of chloroquine on depressor responses to isoprenaline. The responses were unchanged in 3 experiments and were augmented in 2 experiments by about 20%. In 4 out of 5 experiments, the positive chronotropic effect of the amine was enhanced by 15 to 35%.

Posterior pituitary extract. The magnitude and duration of pressor responses to this agent was reduced by about 20% in 2 experiments; in one, the response was not changed.

Angiotensin. Pressor response to angiotensin was reduced by chloroquine in all 4 experiments, giving a mean block of 30%. Half life of the response was not significantly changed.

Tyramine. Chloroquine augmented the pressor responses to tyramine (5 out of 7 experiments); the augmentation was by 15 to 50%; the half life of the responses was enhanced by about 20%.

Fig. 2 illustrates the effect of chloroquine on blood pressure responses to adrenaline, angiotensin, posterior pituitary extract and tyramine.

Effect of chronic administration of chloroquine.

The general activity of 4 cats treated daily with chloroquine was considerably reduced after 4 to 6 days. In two of the animals there was also an inhibition of reactivity to painful stimuli like pin prick and gross pressure. All cats required considerably less quantity of ether as an
anaesthetic prior to spinalization.

The mean resting blood pressure of these animals after spinalization was 26 ± 7 mm of Hg; the mean heart rate was 174 ± 8/min. The hypotension and tachycardia was significant (p < 0.05) in comparison to control animals. The preparations were also significantly (p < 0.05) subsensitive to catecholamines both in respect to the pressor and positive chronotropic effect of the amines (Fig. 3). Isoprenaline (upto 100 μg/kg) also had very little hypotensive or positive chronotropic effect. An approximate comparison of BD_{50} (blood pressure) in this group of cats and in control group indicated that the animals chronically treated with chloroquine were 2 to 4 times less sensitive to tyramine and angiotensin, revealing a generalized cardiovascular subsensitivity.

OTHER PREPARATIONS.

The effect of chloroquine on pressor responses to adrenaline, noradrenaline and tyramine was studied in spinal vagotomised dogs (5 experiments), in anaesthetized dogs (5 experiments) and in anaesthetized rabbits (3 experiments). Infusion of chloroquine elicited some pressor response only in 2 out of 5 spinal dogs; in anaesthetized dogs and rabbits chloroquine infusion had no major effect. 60 min after chloroquine infusion the height of pressor responses to sympathomimetic amines was found to be considerably augmented. The half life of pressor responses to noradrenaline was also enhanced in spinal dogs (Table III).
FIGURE 3.

Dose/response curves of the pressor effects of adrenaline (ADR) and noradrenaline (NAAD) in spinal cats, untreated (control) animals ( ) and animals treated with chloroquine (5 mg/kg, intramuscularly) every day for 10 days and used 24 hr after the last injection ( ). Abscissa, log dose of catecholamines (mg/kg); ordinate, pressor response (mm of Hg). Each point shows mean response, the vertical bars indicating the S.E. of the mean.
FIG. 3
Augmentation of pressor responses to adrenaline, noradrenaline and tyramine by chloroquine in dogs and rabbits.

a and b : The 'Potentiation Factors' signifying the augmentation in the height and the half life (i.e. the time to 50% recovery) of pressor responses, respectively. These were computed from the data obtained before and 60 min after the intravenous administration of chloroquine. The dose of each amine used was approximately $BD_{50}$ (blood pressure).

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<td>a</td>
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<tr>
<td>Spinal, Vagotomised dogs</td>
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<td>1.01 ± .09</td>
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<td>Anaesthetised rabbits</td>
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<td>2.0 ± .15</td>
<td>1.05 ± .08</td>
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* The dose (10 mg/kg for the dogs, and 3 mg/kg for rabbits) was infused over a period of 8-10 min in a fixed quantity of saline (15 ml for dogs, 3 ml for rabbits).
Effect of amodiaquin on the action of catecholamines on cardiovascular system, nictitating membrane and splenic volume. Untreated dogs (Group 1).

The mean resting blood pressure of 10 dogs in this group was 151± 7 mm of Hg; the mean resting heart rate was 154± 8 / min. Slow, intravenous administration of amodiaquin (150 mg/kg) had no appreciable effect on blood pressure or heart rate. Pressor responses to adrenaline or noradrenaline were first found to be augmented 20 to 40 min after amodiaquin infusion. The augmentation was maximal by 60 to 90 min after the infusion and remained so for next 2 to 3½ hr, that is, during the entire period of study. When the amine responses were thus augmented (9 out of 10 experiments) the positive inotropic effect of the amines on heart was either unchanged (3 experiments) or was reduced by about 15 % (2 experiments); the contractile effect of the amines on nictitating membrane or on spleen was not significantly altered (5 experiments). Fig.4 shows the finding in a typical experiment.

Effect of amodiaquin on the pressor and the chronotropic effect of the amines on heart was analysed further in 5 other experiments (Table IV, Fig.5). There
**FIGURE 4.**

Dog, phenobarbitone anaesthesia.

Records of carotid arterial blood pressure (B.P.), cardiac contractions (C.C.) and contractions of nictitating membrane (N.M.).

Responses (at dots) to adrenaline (1.5 μg/kg, intravenously) in 1 and 3 and to noradrenaline (1.5 μg/kg, intravenously) in 2 and 4. A; control responses. B; responses 60 min after slow, intravenous administration of amodiaquin (150 μg/kg). Time, 2 min.
Effect of amodiaquin on blood pressure, heart rate and the chronicotropic effect of catecholamines on heart in anaesthetized dogs.

<table>
<thead>
<tr>
<th>Pretreatment (no of experiments in parenthesis)</th>
<th>Mean resting blood pressure (mm of Hg) ± S.E.</th>
<th>Mean resting heart rate (/min) ± S.E.</th>
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<tr>
<td>B</td>
<td>147±7</td>
<td>158±10</td>
<td>34</td>
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</table>

B & A : Values before and 30 min after amodiaquin (150 μg/kg), respectively. I & II : Effect produced by ED$_{30}$ & ED$_{60}$ (blood pressure) of catecholamines, respectively.

See methods, for details. Calculated as % change in heart rate over the resting value: 'A' under this column denotes the difference produced by amodiaquin in the value 'B' which is the control effect (↑, increment; ↓, reduction).
FIGURE 5.

Dose/response curves of the pressor effects of adrenaline (ADR) and noradrenaline (NADR) in anaesthetised dogs before and 90 min after slow, intravenous administration of 150 µg/kg of amodiaquin. Abscissa, log dose of catecholamines (µg/kg); ordinate, pressor response (mm of Hg). Each point shows the mean response, vertical bars indicating the S.E. of the mean.

A, control (untreated) animals; B, animals pretreated with cocaine; C, animals pretreated with reserpine and D, animals pretreated with nialamide (see Methods, for the details of pretreatment).
was a significant (p < 0.05) augmentation in pressor responses to all dose levels of adrenaline and noradrenaline. Duration of responses, however, was not significantly changed. Tachycardia induced by adrenaline was reduced; the reduction was significant (p < 0.05) for higher doses. Bradycardia induced by noradrenaline was further enhanced; again the change was significant (p < 0.05) for higher dose levels of the amine.

To test whether these changes were due to a fortuitous change in sensitivity of the preparations, 5 ml of normal saline was infused alone in place of amodiaquin solution in 3 other experiments. Though not shown in Fig. 5 there was no significant change in the effects of catecholamines in subsequent 3 to 4 hr in these experiments.

Effect of pretreatment of the animals with various agents on amodiaquin induced augmentation of the catecholamine responses (Table IV, Fig. 5).

Effect of cocaine (Group 2). Cocaine pretreatment of the animals did not significantly alter the resting blood pressure or the heart rate. However, the animals were distinctly more sensitive to administered catecholamines, particularly to noradrenaline. 90 min after amodiaquin infusion all animals showed some fall in blood pressure and in heart rate; the change, however, was not significant. Pressor responses to catecholamines were significantly (p < 0.05) enhanced at all
dose levels. The augmentation was apparently even more than that in Group 1. Chronotropic effect of the amines on the heart was not significantly changed. This feature contrasts with the findings in Group 1.

**Effect of hexamethonium (Group 3).** Pretreatment of animals with hexamethonium did not significantly alter the resting blood pressure or heart rate, though there was some tachycardia. These animals were somewhat more sensitive to administered catecholamines; noradrenaline always caused some tachycardia. 90 min after amodiaquine infusion pressor responses to catecholamines were augmented as in Group 1. Tachycardia due to higher doses of adrenaline was reduced, but the reduction was significantly lesser than that in Group 1 (p < 0.05).

**Effect of reserpine (Group 4).** Reserpinized animals had significant (p < 0.05) bradycardia in comparison to Group 1. 90 min after amodiaquine infusion, the heart rate was still lower than in Group 1. In this group, there was very little augmentation of the pressor effect of the catecholamines. The antagonism of the positive chronotropic effect on heart of adrenaline by amodiaquine was significantly less (p < 0.05) than that in Group 1.

**Effect of nialamide (Group 5).** Pretreatment of the animals with nialamide had no significant effect on resting blood
pressure or heart rate. Sensitivity of these animals to injected catecholamines was practically as in Group 1, but the responses to tyramine were markedly augmented. 90 min after amodiaquin infusion, the pressor responses to the catecholamines were found to be reduced; the reduction was significant \((p < 0.05)\) for higher dose levels of the amines. The antagonism of tachycardia due to adrenaline by amodiaquin was significantly \((p < 0.05)\) less than that in Group 1.

**Effect of chronic administration of amodiaquin on cardiovascular sensitivity to catecholamines (Group 6).** (Table IV; Fig.6).

Dogs chronically injected with amodiaquin had significant \((p < 0.05)\) bradycardia. The pressor responses to all dose levels of catecholamines were significantly \((p < 0.01)\) higher than those in untreated dogs. In this group the positive chronotropic effect of adrenaline on heart was significantly \((p < 0.05)\) less.

Comparative study of pressor activity of tyramine and angiotensin in this group and the control group was not made using different dose levels. However, from comparison of approximate ED\(_{50}\) (blood pressure) of these agents, animals in this group appeared to be two to four times more sensitive to tyramine and to angiotensin.
FIGURE 6.

Dose/response curves of the pressor effects of adrenaline (ADR) and noradrenaline (NADE) in anaesthetized dogs, untreated (control) animals (● ● ●) and animals treated with amodiaquin (0.5 mg/kg, intramuscularly) every day for 10 days and used 24 hr after the last injection (●●●●●●). Abscissa, log dose of catecholamines (μg/kg); ordinate, pressor response (mm of Hg). Each point shows the mean response, the vertical bars indicating the S.E. of the mean.
FIG. 6
Modification of blood pressure responses to other vasoactive agents by amodiaquin (Fig. 7).

The augmenting effect of amodiaquin on pressor responses to catecholamines was not due to a generalised rise in cardiovascular sensitivity; this was evident in 8 experiments in which amodiaquin augmented pressor responses to catecholamines but variably altered responses to other vasoactive agents.

Acetylcholine and histamine. Depressor responses to acetylcholine or histamine were not significantly affected (4 experiments) by amodiaquin.

Isoprenaline. Depressor responses to isoprenaline were either augmented by 15 - 20 % (2 experiments) or were reduced to some extent (2 experiments) by amodiaquin.

Angiotensin. Amodiaquin reduced the pressor responses to angiotensin (4 experiments) giving a mean reduction of 21 %.

Posterior pituitary extract. Magnitude of pressor responses to posterior pituitary extract was not appreciably affected by amodiaquin, though duration of these responses was reduced by 25 to 35 % (3 experiments).

Tyramine. Amodiaquin augmented pressor response to tyramine by 20 to 35 % in 5 experiments; in one experiment the response was unaltered.
FIGURE 7.

Dog, phenobarbitone anaesthesia.
Record of carotid arterial blood pressure (B.P.). Responses to adrenaline (1.0 mg/kg, in 1); to angiotensin (1.5 mg/kg, in 2); to posterior pituitary extract (0.4 I.U./kg, in 3) and to tyramine (0.3 mg/kg, in 4) before (in A) and 90 min after slow, intravenous administration of 150 µg/kg of amodiaquin (in B). All injections were intravenous. Time, 2 min.
FIG. 7
Effect of larger doses of amodiaquin on pressor responses to catecholamines.

In dogs infused with 10 mg/kg (5 experiments) or 5 mg/kg (6 experiments) of amodiaquin, the resting blood pressure declined gradually during the course of experiment; the fall was from 20 to 36 mm of Hg in 2 to 3½ hr in different experiments. One animal in each of these groups died after injecting adrenaline; these findings agree with an earlier report (7). In 4 of the remaining experiments, pressor responses to catecholamines were augmented by 25 to 40 %; in one experiment they were unaffected, while in 4 experiments, there was a clear inhibition.

It has been reported that administration of pronethelol, a specific -adrenergic blocking agent (25), reduces the blocking effect of several adrenergic blocking agents (119); to test whether the aforesaid inhibition of catecholamine pressor responses was due to blockade of adrenergic receptors, pronethelol (4 mg/kg) was injected intravenously. 10 min later pressor responses to adrenaline and noradrenaline were practically restored in all 4 experiments (Fig. 8).
FIGURE 8.

Dog, phenobarbitone anaesthesia.
Record of carotid arterial blood pressure (B.P.). Responses (at white dots) to adrenaline (2.0 µg/kg) in 1 and to noradrenaline (2.0 µg/kg) in 2. Panel A shows the control responses. The responses were inhibited 90 min after slow intravenous administration of 5.0 mg/kg of amodiaquin (Panel B). 10 min after the administration of pronethalol (4.0 mg/kg) the responses were restored to normal (Panel C). All injections were intravenous. Time, 2 min.
FIG. 8
SPINAL VAGOTOMISED DOGS.

The mean resting blood pressure (60±5 mm of Hg) and heart rate (124±8 /min) of 4 spinal vagotomised dogs was not significantly changed 90 min after a slow intravenous infusion of amodiaquin. At this time the pressor effect of all dose levels of adrenaline and noradrenaline was significantly enhanced (p<0.05); tachycardia induced by adrenaline was also significantly enhanced (p<0.05); that induced by noradrenaline was also enhanced but the change was not significant (Fig. 9). Pressor response to tyramine (ED$_{50}$) was augmented in all experiments; the augmentation was by 20 - 38% of the control responses. Duration of responses to the amines was not significantly changed. In these preparations the time course of development of supersensitivity to catecholamines was as in anaesthetized dogs.

ANAESTHETIZED RABBITS.

The mean resting blood pressure of anaesthetized rabbits was 130±12 mm of Hg (3 experiments). 90 min after the infusion of amodiaquin, it was reduced to 120±7 mm of Hg. Pressor responses to adrenaline, noradrenaline and tyramine were augmented by 60 - 85%, 55 - 70% and 40 - 65% respectively; duration of responses was not significantly altered.
Figure 9.

Dose/response curves of the pressor effects of adrenaline (ADR) and noradrenaline (NADE) in spinal dogs, before ( ._______.) and 90 min after slow intravenous administration of 150 µg/kg of amediaquin ( .-----.). Abcissa, log dose of catecholamines (µg/kg); ordinate, pressor response (mm of Hg). Each point represents the mean response, vertical bars indicating the S.E. of the mean. The figures near each of the points indicate the mean % rise in heart rate (over the resting value) due to the corresponding dose of the amines.
FIG. 9
PART II: EXPERIMENTS WITH ISOLATED TISSUE PREPARATIONS.

METHODS

The rabbit aortic strip preparation. Aortic strips were obtained from thoracic aortae of young rabbits and prepared in the manner described by Furushot and Bhadrakom (109). Spirally cut strips, about 3.5 cm in length, were mounted in a 30 ml organ bath and bathed in Kreb's solution (see, Appendix A, for composition). The solution was maintained at 37° to 38°C and 5% carbon dioxide in oxygen was bubbled through the solution in bath and the reservoir; pH of the solution was now 7.4. Contractions were recorded with an isotonic frontal writing lever (ratio 1:10) and the load on the tissue was 3 g. The preparations were stabilized for 2 hr in vitro before starting the experiments.

Cumulative administration of increasing concentrations of adrenaline or noradrenaline (1 x 10^{-9} to 8 x 10^{-9}) elicited dose related contractile responses from the aortic strips. Cumulative dose response curves for the amines (usually three and with responses between 20 to 65% of the maximal) were determined at 30 min intervals till the responses were reproducible. In some experiments the contractile effect of noradrenaline (1 - to 2 x 10^{-9}), histamine (1.5 x 10^{-7}), angiotensin (0.5 - to 2 x 10^{-9}) and acetylcholine (3 - to 5 x 10^{-5})
was determined in duplicate. The doses used in individual experiments produced responses which were 50 to 75% of the maximal and were so spaced that an individual agonist was administered at an interval of at least 35 to 45 min to avoid tachyphylaxis. Chloroquine or amodiaquin was placed in the bath at different time intervals before the next administration of the agonists and remained in the bath thereafter.

The rat seminal vesicle preparation. Seminal vesicles of albino rats were prepared in the manner described by Leitch, Liebig and Haley (174). The preparations were suspended in a continuous flow (at 12 ml/min) of De Jalon's fluid (see Appendix A, for composition). Contractions were recorded with a frontal writing lever (ratio 1:10) placing the tissue under a tension of 300 mg. The bathing solution was continuously gassed with 5% carbon dioxide in oxygen. Flow was interrupted when the stimulant drugs were added to the bath (12 ml capacity).

Adrenaline (0.5 - to 2.5 x 10^{-6}) or noradrenaline (1 - to 3 x 10^{-6}) elicited dose related contractile responses from the preparations. Though sensitivity of the preparations to the amines varied in different experiments, a linear relationship was always obtained when responses (expressed as % of the maximal contraction) were plotted
against the log dose of the amines. At least 4 doses of the amines were used in constructing the dose/response curves. After obtaining reproducible responses to the amines the preparations were perfused with fluid containing amodiaquin or chloroquine for 15 min and the responses to the amines determined again.

The rabbit tracheal chain preparation. The rabbit tracheal chains were set up as described by Castillo and de Beer(60). The chains were suspended in a bath (30 ml capacity) filled with Kreb's solution (see, Appendix A, for composition) maintained at 37° - 38°C and gassed with 5% carbon dioxide in oxygen. The tone of the preparations was recorded with a frontal writing lever (ratio 1 : 10) placing the chains under a stretch of 1.5 to 2 g. Pilocarpine nitrate (0.01 mg/100 ml) was added to the bathing fluid to raise the tone. Inhibition of the tone produced by adrenaline ($5 \times 10^{-5}$ to $3.3 \times 10^{-6}$) or by isoprenaline ($3 \times 10^{-5}$ to $9 \times 10^{-5}$) was recorded at the intervals of 12 min, when the pilocarpine induced spasm reached a steady plateau. Amodiaquin or chloroquine was placed in the bath 5 min before the next administration of the amines and remained in the bath thereafter.
Isolated rabbit atria. Isolated rabbit atria were set up as described by Burn (34). The preparations were mounted in a 80 ml organ bath in well oxygenated Locke solution (see, Appendix A, for composition). Temperature of the bath was maintained at 28°C. The amplitude of contractions was recorded with a spring-loaded lever. The preparations were stabilized for 45 min before the experiments started.

The positive chronotropic and inotropic effects of adrenaline (4 - to 5 x 10^-8) and isoprenaline (2.5 - to 3 x 10^-8) were recorded at 15 min intervals. When the responses were reproducible, amodiaquin or chloroquine was added to the bath 5 min before the next administration of the amines and it remained in the bath thereafter.

Perfused heart of frog. Frog hearts were perfused through the posterior venae cavae at room temperature (28 ± 1°C) with well oxygenated from Ringer solution (see, Appendix A, for composition). Perfusion pressure head was maintained constant throughout. The hearts operated against an artificial resistance of constant value. Contractions were recorded with a light spring loaded lever with a diastolic loading of 0.75 g. When responses to adrenaline (0.005 to 0.01 µg) or isoprenaline (0.001 to 0.01 µg), injected close to the perfusion cannula, were constant,
amodiaquin or chloroquine was added to the perfusion fluid. The responses to the amines were determined again at variable intervals of time thereafter.

The isolated rabbit/guinea-pig ileum preparation. Pieces of ileum, 3 to 4 cm in length, were obtained from freshly killed animals and were set up in a 30 ml organ bath in well oxygenated Tyrode solution (see, Appendix A, for composition). The temperature of the bath was maintained constant at 32°C. The longitudinal movements were recorded by a frontal writing lever. When the inhibition of the tone and of the longitudinal movements produced by adrenaline (0.5 - to 1 x 10^{-6}) or noradrenaline (1 - to 2 x 10^{-6}) was reproducible, amodiaquin or chloroquine was placed in the bath 3 min before the next administration of the amines and remained in the bath thereafter.

**DRUGS:** Amodiaquin hydrochloride, chloroquine sulphate, (\(\pm\))-noradrenaline hydrochloride, (\(\pm\))-isoprenaline hydrochloride, histamine acid phosphate, acetylcholine chloride and pilocarpine nitrate were used throughout the experiments. The doses refer to the salts. Adrenaline base and angiotensin (Hypertensin, Ciba) were made up fresh in normal saline immediately prior to use.
RESULTS

The rabbit aortic strip preparation.

Effect of chloroquine and amodiaquin on contraction induced by adrenaline and noradrenaline.

Chloroquine ($5 \times 10^{-9}$ to $1 \times 10^{-8}$, 4 experiments) and amodiaquin (0.5 - to $1 \times 10^{-7}$, 4 experiments) had no effect on contractile effect of adrenaline or noradrenaline on the rabbit aortic strips, when the preparations were exposed to the 4-aminquinolines for 5 to 15 min. Chloroquine (1 - to $5 \times 10^{-8}$) augmented the contractile effect of lower doses of the amines in 3 out of 6 experiments; the augmentation was by 15 to 23% of the control responses. Effect of higher doses of the amines was however, not altered.

Chloroquine ($1.5 \times 10^{-7}$ to $7.5 \times 10^{-7}$, 7 experiments) and amodiaquin ($1 \times 10^{-6}$ to $2.5 \times 10^{-7}$, 8 experiments) inhibited the responses of the strips to adrenaline and noradrenaline, shifting the dose/response curves for the amines to the right. In the concentration range of the aminquinolines which was studied, the shift was parallel, without an appreciable change in the slope of the dose/response curves. The inhibitory effect of the same concentration of the aminquinolines was variable in different experiments; however, in the same experiment
the inhibition was related to the dose. Findings in a typical experiment with chloroquine are shown in Fig.10. The inhibition was completely reversible after washing the preparations repeatedly for 40 to 90 min.

Effect of chloroquine and amodiaquin on contractions induced by other vasoactive drugs.

Furchgott (107) demonstrated that the rabbit aortic strip contains separate and specific receptors for noradrenaline, histamine, acetylcholine and 5-HT. Angiotensin, on the other hand, produces a contraction of the strip probably by a direct action on the smooth muscle. In order to determine whether the inhibitory effect of the aminoquinolines was restricted to noradrenaline their effect was also investigated on the effect of other agents which produce a contraction of the strip.

Chloroquine.

Chloroquine (7 x 10^{-8}) reduced the contraction of the aortic strip produced by histamine by 50 to 80%; this concentration of chloroquine had no effect on the contractions produced by noradrenaline, angiotensin and acetylcholine (3 experiments). Chloroquine (3 - to 5 x 10^{-7}) reduced the effect both of noradrenaline and of angiotensin by 30 to 50%; the effect of acetylcholine was unaltered.
FIGURE 10.

Rabbit aortic strip preparation:

dose/response curves for noradrenaline alone (●), in presence of $3 \times 10^{-7}$ of chloroquine (▲) and $6 \times 10^{-7}$ of chloroquine (○). Contact time for chloroquine, 5 min. Chloroquine caused a parallel shift in dose/response curves to the right. The magnitude of the shift was related to the dose of chloroquine.
FIG. 10
Still higher concentrations of chloroquine (0.6 - to 1 x 10^{-6}) severely reduced the effect of noradrenaline, histamine and angiotensin; the effect of acetylcholine was reduced by 30 to 60%. Findings in one such experiment are shown in Fig. 11.

Amodiaquin.

Exposure of the strips to amodiaquin (0.5 - to 1 x 10^{-6}) for 5 min inhibited the contraction of the strip due to histamine and noradrenaline by 40 to 80% and by 30 to 50% respectively; contraction due to angiotensin was practically unchanged while that due to acetylcholine was either unaltered (2 experiments) or slightly augmented (2 experiments). Amodiaquin (2.5 - to 5 x 10^{-6}) practically abolished the contractile effect of acetylcholine; that of angiotensin was reduced by 20 to 50% (2 experiments).
Rabbit aortic strip preparation.

Responses (at white dots) to angiotensin
\((1 \times 10^{-9})\) in 1, to acetylcholine \((4 \times 10^{-5})\) in 2, to noradrenaline \((1.5 \times 10^{-9})\) in 3 and
to histamine \((1.5 \times 10^{-7})\) in 4. A; control
responses. B; responses in presence of
chloroquine \((7 \times 10^{-8})\). C; responses in
presence of chloroquine \((5 \times 10^{-7})\). Chloroquine
was added 5 min before the addition of the
agonists.
Chloroquine (2 x 10^{-7} to 1 x 10^{-6}) and amodiaquin (7.5 x 10^{-7} to 5 x 10^{-6}) inhibited the contractile responses of the seminal vesicles to adrenaline and noradrenaline. This was seen in a total of 22 experiments. The inhibition was evident in a shift of the dose/response curves for the amines to the right. In the range of concentration of the aminoquinolines studied, the shift was more or less parallel, without a change in the slope of the curves. Inhibition due to a fixed concentration of the aminoquinolines developed fully after 15 min and was completely reversible when the preparations were perfused with normal fluid for 30 to 60 min. Inhibition was related to the dose of the aminoquinoline in individual experiments, though that due to the same concentration of the agents, varied in different experiments.

Findings in an experiment with chloroquine are shown in Fig. 12.
FIGURE 12.

Rat seminal vesicle preparation:

Log dose/response curves for adrenaline alone ( ), in presence of chloroquine $4 \times 10^{-7}$ ( ) and chloroquine $8 \times 10^{-7}$ ( ). Chloroquine causes a parallel shift in dose/response curves to the right; the magnitude of the shift is related to the dose of chloroquine.
FIG. 12

RESPONSE (% OF MAXIMAL) vs. LOG DOSE ADRENALINE (µg/ml)
The rabbit tracheal chain preparation.

Ordinarily the responses of the rabbit tracheal chain to adrenaline or isoprenalinine were variable and small in magnitude as this preparation exhibits little residual tone. Addition of pilocarpine to the bathing medium, however, raised the tone and enabled uniform and considerably larger responses to be obtained over a period of 3 to 4 hr.

Chloroquine (1 - to 3 x 10^{-7}) and amodiaquin (1 - to 5 x 10^{-7}) had no effect on pilocarpine induced spasm of the preparations or on the relaxant action of adrenaline or isoprenalinine (8 experiments). Chloroquine (5 - to 7 x 10^{-7}) reduced the pilocarpine spasm to a small but variable extent; the relaxant effect of adrenaline (Fig.13) and isoprenalinine was augmented in 5 out of 7 experiments; the augmentation was by 15 to 20 % of the control responses and was short lasting, when the preparations were washed out. Concentrations of chloroquine (0.7 - to 1.5 x 10^{-6}, 4 experiments) and of amodiaquin (1 - to 7 x 10^{-6}, 3 experiments) reduced the pilocarpine induced spasm to a greater extent; at this time the relaxant effect of the amines was reduced by about 50 % and 30 % respectively. In view of a gross alteration in the spasm the effect of higher concentrations of the 4-aminoquinolines on the relaxant effect of the amines could not be studied satisfactorily.
FIGURE 13.

Response of the rabbit tracheal chain to adrenaline (3.3 x 10^-6) at dots, alone (A, D & F) and in presence of chloroquine, 2 x 10^-7 (B), chloroquine, 6 x 10^-7 (C), and chloroquine, 1.2 x 10^-5 (E). Contact time for adrenaline, 3 min. Chloroquine was placed in the bath 5 min before the addition of adrenaline.
Isolated rabbit atria.

Exposure of isolated rabbit atria to chloroquine (1 - to 3 x 10^{-6}) or to amodiaquin (1.6 to 5 x 10^{-6}) for 5 min resulted in some reduction in the beating amplitude, and a marked reduction in the positive chronotropic and positive inotropic effect of adrenaline and isoprenaline (Table V). Concentrations lesser than this had no major effect on atria or on the effect of catecholamines on the hearts. Higher concentrations of chloroquine or amodiaquin caused a severe reduction in the beating amplitude and gross alterations in regularity of beating, and they were not studied further.

Perfused heart of frog (Fig. 14).

Perfusion of the frog hearts with fluid containing chloroquine (2 - to 8 x 10^{-7}) or amodiaquin (1 - to 3 x 10^{-6}) for a period of 10 to 15 min resulted in a marked reduction of the beating amplitude and some reduction in the rate of beating. The positive chronotropic and positive inotropic effect of adrenaline and isoprenaline was also markedly reduced at this time (Table V). The inhibition of heart produced by the 4-aminquinolines was partially reversible on a continued perfusion with normal fluid for 30 to 40 min.
### TABLE V

**Isolated rabbit atria and perfused heart of frog**

**Effect of 4-aminoquinolines on spontaneous beating and on positive chronotropic and inotropic effect of adrenaline.**

<table>
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<th>Agent and Concentration</th>
<th>Time of contact (min)</th>
<th>Spontaneous rate of beating (/min)</th>
<th>% change in amplitude of beating.</th>
<th>% change in rate in the ton. (aln) beating</th>
<th>% change in the amplitude of beating.</th>
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<td>* -34</td>
<td>* +18</td>
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</table>

**Isolated rabbit atria (5 experiments)**

| Dose of adrenaline was 5 x 10^-8 for rabbit atria and 8 x 10^-9 for perfused frog heart. |
| Values significantly (P < 0.05) from those during the control period. |
FIGURE 1A.

Record of contractions of a perfused heart of frog. Responses (at white dots) to adrenaline (8 x 10^{-9}). A, control response; response after 10 min of perfusion with fluid containing 1 x 10^{-7} of amodiaquin (B) and 1 x 10^{-6} of amodiaquin (C). The responses to adrenaline were partially recovered 30 min after perfusion with normal fluid (D). Time, 2 min.
FIG. 14
Isolated rabbit ileum/guinea-pig ileum preparation.

Exposure of rabbit ileum to chloroquine (1 x 10⁻⁸ to 5 x 10⁻⁷, 5 experiments) or to amodiaquin (1 to 8 x 10⁻⁷, 5 experiments) for 3 min had no effect on the tone or spontaneous contractions of the ileum or on the relaxant effect of adrenaline or noradrenaline. In 3 out of 5 experiments amodiaquin (1 to 2 x 10⁻⁶) enhanced the relaxant effect of the amines by 10 to 20 %.

Chloroquine (0.5 to 1.5 x 10⁻⁵) produced a marked inhibition of the tone and motility of the ilea (4 experiments) during the 3 min contact period. Due to this relaxation the effect of the amines could not be satisfactorily studied in presence of these concentrations of chloroquine. On the other hand, amodiaquin (5 x 10⁻⁶ to 1 x 10⁻⁵) markedly increased the tone of the preparations during the 3 min contact period. At this time the amplitude of contractions was much reduced; the relaxant effect of adrenaline was reduced by 30 to 60 %, while that of noradrenaline was reduced by a greater extent (4 experiments, Fig. 15).

Amodiaquin and chloroquine were found to produce qualitatively similar effects when guinea-pig ilea were used as test objects (7 experiments).
Isolated rabbit ileum. Record of longitudinal contractions. Responses (at dots) to adrenaline (1 x 10^-6) in 1, 3 & 5 and to noradrenaline (1.5 x 10^-6) in 2, 4 & 6 alone (in A), in presence of amodiaquin (1.5 x 10^-6, in B) and amodiaquin (9.5 x 10^-6, in C). Amodiaquin was allowed to act for 3 min before addition of the amines. Time, 1 min.
PART III: MECHANISM OF THE PRESSOR EFFECT OF AMODIAQUIN
AND CHLOROQUINE IN METHYLAMPHETAMINE PRETREATED
ANAESTHETIZED DOGS.

METHODS

Mongrel dogs of either sex weighing between 8
and 14 kg were used and were anaesthetized with phenobarbi­
tone sodium (150 mg/kg, intraperitoneally). Blood pressure
was recorded with a mercury manometer connected to a carotid
artery. Intravenous injections were made through a polyethy­
lene cannula inserted into a femoral vein.

Amphetamine pretreatment. Preliminary experiments performed
to evolve the amphetamine treatment schedule which was optimal
in eliciting satisfactory pressor responses to amodiaquin
and chloroquine administered later demonstrated that the
pressor response to a fixed dose of either of the 4-amino­
quinolines was always maximal between 20 and 45 min after
an injection of 0.25 mg/kg of methylamphetamine; the
response was smaller if a smaller dose of methylamphetamine
was used or if the 4-aminoquinolines were injected earlier
or later. Dexamphetamine was equally satisfactory for the
pretreatment of the animals but was not used. Hereafter
the administration of the above dose of methylamphetamine
is referred to as 'methylamphetamine pretreatment'.
Rat isolated stomach strip bathed in blood. Strips of stomach obtained from the fundus of the reserpine pretreated rats were prepared in the manner described by Vane (242). The strips were suspended in a continuous stream of oxygenated blood (243) pumped from a carotid artery of a dog (anaesthetized with phenobarbitone) at a constant rate of 10 ml/min into a jacketed bath of 10 ml capacity. From the bath the blood was drained back into a jugular vein. The temperature of the external circuit was maintained at 37°C. Heparin (1000 units/kg) was given intravenously 10 min before the experiment to prevent coagulation of blood.

Treatment with reserpine. Dogs were injected with reserpine (0.5 mg/kg) intraperitoneally 24 hr before the experiment. Rats were given a subcutaneous injection of reserpine (3 mg/kg) on two consecutive days and were used 24 hr after the second injection.

DRUGS. Amodiaquin hydrochloride, chloroquine sulphate, methylamphetamine sulphate, tolazoline hydrochloride, 1,4-(bis-1,4-benzodioxan-2-yl-methyl) piperazine (Dibozane), pronethalol hydrochloride, hexamethonium chloride, tetraethylammonium bromide, mecamylamine hydrochloride, atropine sulphate, N-diethylaminoethyl-N-isopentyl-N,N'-diisopropy lurea (P-286), (±)-noradrenaline hydrochloride, dimethyl-
phenylpiperazinium iodide, tyramine hydrochloride, and 4-(m-chlorophenyl carbamoyloxy)-2-butynyltrimethyl ammonium chloride (MeN-A-343) were used throughout the experiments; doses refer to the salts. A 1 mg/ml solution of reserpine in 20% ascorbic acid was used as such or diluted with 0.9% saline. Adrenaline base was dissolved in 0.9% saline immediately before use.
RESULTS

The following observations were made in elementary experiments regarding the pattern and reproducibility of pressor responses to amodiaquin (2 mg/kg) and to chloroquine (3 mg/kg). (1) The injection of amodiaquin or chloroquine in anaesthetized dogs produced a brisk but transient fall in blood pressure. After methylamphetamine pretreatment (see Methods) however, the agents produced the initial hypotension which was followed by a somewhat sustained rise in blood pressure (Fig. 16). The pressor responses to amodiaquin (4 experiments) or to chloroquine (4 experiments) obtained by injecting the agents at 25, 35 and 45 min after methylamphetamine pretreatment were remarkably constant in magnitude. (2) In six other experiments the responses to one of the two compounds obtained at 25 and 45 min after methylamphetamine pretreatment were also remarkably similar when the other compound was administered in between at 35 min. The results obtained in such an experiment are shown in Fig. 16 which also shows the typical responses to the 4-aminooquinolines. (3) In 6 experiments, equipressor responses to amodiaquin or to chloroquine were obtained when methylamphetamine pretreatment was repeatedly given for four times every 40 min and followed by the
FIGURE 16.

Dog (13.5 kg), phenobarbitone anaesthesia. Record of carotid arterial blood pressure (B.P.). Responses to intravenous injections (at dots) of amodiaquin (2 mg/kg at Amo) and of chloroquine (2 mg/kg at Chlr). A: responses during the control period elicited at the interval of 10 min. B: responses elicited at 25, 35 and 45 min after the administration of methylamphetamines (0.25 mg/kg at Meth). Time, 2 min.
administration of amodiaquin or chloroquine at 30 min each time. (4) Pressor responses to chloroquine and amodiaquin were found to be unaltered (4 experiments) when tachyphylaxis was experimentally induced to methylamphetamine (Fig. 17).

These experiments showed that tachyphylaxis to amodiaquin or chloroquine does not develop very readily, that the pressor effect of one compound was not affected by the other, and that pressor responses to the 4-aminoquinolines are unaffected even when there is tachyphylaxis to pressor action to methylamphetamine. One of the above experimental designs was, hence, always adopted in further work while studying the pressor action of the 4-aminoquinolines.

**Pressor action of amodiaquin and of chloroquine in methylamphetamine pretreated dogs.**

Effects produced by increasing doses of amodiaquin (1 to 3 mg/kg) and chloroquine (2 to 4 mg/kg) administered at 25, 35 and 45 min after methylamphetamine pretreatment were studied in a total of 22 experiments. The results obtained are summarized in Table VI. The initial hypotension due to the 4-aminoquinolines was variable in magnitude and duration and was not further analysed. The pressor responses to the same dose of either of these agents
FIGURE 17.

Dog (9.5 kg), phenobarbitone anaesthesia. Record of carotid arterial blood pressure (B.P.). Responses (at dots) to methylamphetamine (0.25 mg/kg) in A and to amodiaquin (2 mg/kg) in B (obtained 30 min after A). Administration of methylamphetamine was repeated every 8 min after B; responses, at dots, to the 3rd and 4th administration in C and D respectively showing tachyphylaxis to this agent. Amodiaquin (2 mg/kg, at dot) produced the usual pressor response (E) when injected 10 min after D. All injections were intravenous. Time, 2 min.
**TABLE VI**

Pressor action of amodiaquin and of chloroquine in methylamphetamine pretreated, anaesthetized dogs and its modification by reserpine pretreatment of the animals.

The responses to amodiaquin and to chloroquine were elicited between 25 and 35 min after administration of methylamphetamine (0.25 mg/kg, intravenously).

a) No. of experiments; b) mean of maximal responses, ± s.e. of mean (P for difference between response and control, in parenthesis), in mm of Hg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Amodiaquin</th>
<th>Chloroquine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>15 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>36 ± 10</td>
<td>5 10 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>42 ± 12</td>
<td>13 32 ± 10</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>4 40 ± 9</td>
</tr>
<tr>
<td>Reserpine (0.5 mg/kg 24 hr before)</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 ± 4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>Reserpine (0.5 mg/kg 24 hr before) + noradrenaline (infusion, at 15 ug/kg/min for 15 min)</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 ± 6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 ± 3</td>
<td>3 5 ± 4</td>
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<tr>
<td></td>
<td></td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
</tr>
</tbody>
</table>
varied considerably in different experiments; however, they were related to the dose in individual experiments. A comparison made at the level of about 50% of the maximal pressor responses to amodiaquine and chloroquine (six experiments) revealed that the pressor activity of amodiaquine base was about 1.4 times that of chloroquine base.

**Effect of prior treatment with adrenergic blocking agents.**

In four experiments control pressor responses to amodiaquine (2 mg/kg) and chloroquine (2 mg/kg) were determined 30 min after methylamphetamine pretreatment. The responses were redetermined 30 min after methylamphetamine treatment and 10 min after tolazoline (10 mg/kg). In four similar experiments dibozane (3 mg/kg) was used as the blocking agent. In these doses tolazoline and dibozane always abolished the pressor effect of noradrenaline (8 µg/kg); the pressor responses to amodiaquine and chloroquine were much reduced but not abolished (Fig. 18-b).

Persistence of some pressor effect of the 4-aminoquinolines after adequate doses of the α-adrenergic blocking drugs could have been due to persistent cardiac action of the 4-aminoquinolines. To test this possibility prone-thalol (4 mg/kg) was administered; 10 min after its administration the pressor effect of amodiaquine and chloroquine was totally abolished (Fig. 18-c).
Records of carotid arterial blood pressure (B.P.) of two dogs (A & B) anaesthetized with phenobarbitone. Responses (at dots) to amodiaquin (2 mg/kg) in A and to chloroquine (2 mg/kg) in B. (a) Control responses 30 min after methyramphetamine (0.25 mg/kg). (b) Responses 30 min after methyramphetamine (in the same dose) and 10 min after tolasoline (10 mg/kg) given at TOL. (c) Responses 10 min after subsequent administration of pronethalol (4 mg/kg) given at PRO. Time, 2 min. All injections were intravenous.
Modification of pressor action of amodiaquin and chloroquine by treatment with various other drugs.

**Effect of ganglion blocking agents.**

In anaesthetized rats physostigmine (246, 247) and galantamine (53) are known to produce pressor effects due to central adrenergic excitation. Dimethylphenylpiperazinium and nicotine raise the blood pressure by augmenting sympathoadrenal discharge; McN-A-343 and certain other agents also induce pressor responses by an 'unusual' ganglionic stimulant action (217,176). To test whether the 4-aminoquinolines raise the blood pressure through similar mechanisms the effect of various ganglion blocking agents was investigated.

In 4 dogs control responses to amodiaquin (2 mg/kg), to chloroquine (2 mg/kg) and to dimethylphenylpiperazinium (30 mg/kg) were determined 25 min after methylamphetamine pretreatment, and hexamethonium (10 mg/kg) was administered. This agent produced a moderate fall in blood pressure. 10 min later the pressor effect of dimethylphenylpiperazinium was greatly reduced, while the pressor responses to amodiaquin and to chloroquine were augmented. Similar results were obtained when tetraethylammonium (3 mg/kg) or mecamylamine (3.5 mg/kg) was used as a ganglion blocking agent (4 experiments).
In five similar experiments where McN-A-343 was used as a ganglionic stimulant, atropine (100 μg/kg) completely abolished the pressor effect of McN-A-343 (50 μg/kg), while it did not significantly alter the pressor action of amodiaquin or chloroquine.

Fig. 19 shows the effect of hexamethonium and of atropine on the pressor responses to chloroquine.

**Effect of reserpine pretreatment.**

It is now generally held that the sympathomimetic effect of tyramine and related amines is caused by a release of noradrenaline from nerve ending (36, 102), the action of the released amine being probably augmented by tyramine itself (178, 230). To test whether the pressor responses to amodiaquin and chloroquine are mediated through a similar release of catecholamines, their modification by reserpine pretreatment of the animals was investigated.

In three dogs pretreated with reserpine methylamphetamine given as pretreatment produced only a small pressor response (Fig. 20-B). 30 min later, amodiaquin and chloroquine also produced very small pressor responses in comparison with those produced in control animals (Table VI, Fig. 20-B). In three other reserpine pretreated dogs noradrenaline (15 μg/kg/min) was infused for 15 min
FIGURE 19.

Records of carotid arterial blood pressure (B.P.) of two dogs, A and B, anesthetized with phenobarbitone. Responses (at dots) to chloroquine (2 mg/kg) in 1 & 4 (in both A & B); to dimethylphenylpiperazinium (30 μg/kg) in 2 & 3 (in dog A) and to McH MA-343 (50 mg/kg) in 2 & 3 (in dog B). Methylamphetamine (0.25 mg/kg) was given 25 min before 1; 10 min before 3, hexamethonium (10 mg/kg) and atropine (100 μg/kg) was administered in dog A (at C=6) and in dog B (at Atr) respectively. Drugs were given intravenously. Time, 2 min.
FIG. 19
FIGURE 20.

Records of carotid arterial
blood pressure (B.P.) of three dogs, 
A, B and C, anaesthetized with pheno-
barbitone. Dog A-untreated (control); 
dog B—given reserpine (0.5 mg/kg, 
intraperitoneally) 24 hr before; dog C—
given reserpine as in dog B and infused 
with noradrenaline (15 μg/kg/hr) for 
15 min, 10 min before the experiment. 
Responses (at dots) to methylamphetamine 
(0.25 mg/kg) in 1; to amodiaquin (2 mg/kg) 
in 2 and to chloroquine (2 mg/kg in 3; 
records 2 and 3 obtained 30 and 40 min 
after 1, respectively. Injections were 
intravenous. Time, 2 min.
at the beginning of experiments. 10 min after the infusion pressor action of methylamphetamine given as pretreatment was found to be remarkably greater than that in reserpine-treated animals not infused with noradrenaline. However, pressor responses to amodiaquin and chloroquine given 30 min later were still very small in magnitude (Fig. 20-C).

Effect of P-286.

P-286, an active aminoalkylurea studied by Gardier, Abreu, Richards and Herrlich (110), when given in small doses, appears to block specifically the release of catecholamines from adrenal medulla occurring in response to various drugs (68) without producing a concomitant blockade of sympathetic ganglia. Effect of such small dose of P-286 on the pressor action of the 4-aminoquinolines was therefore investigated with the hope of evaluating the role of adrenal medulla in this action.

In four experiments the pressor effect of amodiaquin (2 mg/kg) or of chloroquine (2 mg/kg) and of dimethylphenylpiperazinium (10 μg/kg) was determined 30 min after methylamphetamine pretreatment. The pressor effect of such small dose of dimethylphenylpiperazinium is predominantly due to a release of catecholamines from adrenal medulla. The responses due to the above drugs were determined again 30 min after methylamphetamine and
15 min after P-286 (3.5 mg/kg). It was observed that while P-286 considerably reduced the pressor response of dimethyldiphenylpiperazinium; the pressor responses to amodiaquin and chloroquine were reduced by 10 to 20% only. Finding in a typical experiment are shown in Fig. 21.

**The rat isolated stomach strip bathed in blood.**

A stomach strip obtained from a reserpine pretreated rat and continuously bathed in blood of an anaesthetized animal (243) is a convenient and sensitive test preparation for monitoring the changes in circulating catecholamine levels. This preparation was used for obtaining information on the nature of the possible release of catecholamines during the rise of blood pressure induced by the 4-aminoquinolines.

Adrenaline (0.5 μg/kg) injected intravenously into the dog produced a small pressor response which was accompanied by a relaxation of the stomach strip (Fig. 22). Administration of amodiaquin (0.6 mg) or of chloroquine (0.7 mg) directly in the external circuit produced some relaxation of the stomach strip but had no effect on blood pressure; 10 min later a second similar administration, did not, however, produce any effect (Fig. 22, 5 experiments). In four other experiments amodiaquin (2 mg/kg) or chloroquine (3 mg/kg) injected intravenously produced the usual brief fall in blood pressure along with relaxation of the stomach.
FIGURE 21.

Dog (12 kg), phenobarbitone anaesthesia. Record of carotid arterial blood pressure (B.P.). Responses (at dots) to amodiaquin (2 mg/kg) in 1 and 5; to chloroquine (2 mg/kg) in 2 and 6 and to dimethylphenylpipеразин (10 µg/kg) in 3 and 4.

A; control responses obtained 30 min after methyhamphetamine (0.25 mg/kg).

B; responses obtained 30 min after the same dose of methyhamphetamine and 15 min after P-286 (3.5 mg/kg). All injections were intravenous. Time, 2 min.
FIGURE 22.

Dog (10 kg), phenobarbitone anaesthesia. Records of femoral arterial blood pressure (B.P.) and the tone of the blood bathed stomach strip prepared from a reserpine pretreated rat (R.S.S.). Responses (at dots) to adrenaline (5 mg, intravenously) in A, D and F; to amodiaquin (20 mg, intravenously) in E; to dimethylphenylpiperazinium (175 mg, intravenously) in G and to injection of amodiaquin (0.6 mg) directly into the external circuit, in B and after 10 min again, in C. 30 min before E, methylamphetamine (0.25 mg/kg) was administered intravenously at Mth.

Time, 2 min.
strip; again a second similar administration did not produce any effect on the stomach strip, though it produced a fall in blood pressure.

In these experiment when the stomach strips had thus become insensitive due to repeated administration of the drugs either in the external circuit or in the animal, their sensitivity to adrenaline was unchanged (Fig.22). Administration of methylenedamine at this time had very little effect on the strips (2 experiments) or there was a rise in tone of the strips (7 experiments) which occurred a little later after administration of methylenedamine and then persisted during the rise in blood pressure. 30 min after methylenedamine intravenous administration of amodiaquin (2 mg/kg, 4 experiments) or of chloroquine (3 mg/kg, 3 experiments) produced a pressor response but did not produce any relaxation of the stomach strip. At this time sensitivity of the strip to injected adrenaline was unchanged; also dimethoxyphenylpiperazinium (17.5 μg/kg) given intravenously produced a pressor response associated with a marked relaxation of the stomach strip (Fig.22).