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RESPONSE OF ISOLATED WHITE FAT CELLS TO ANTI-MALARIAL DRUGS*

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Abstract—The anti-malarial drugs, chloroquine, quinacrine and quinidine, were examined for their effects on isolated rat white fat cells and on isolated rat white fat cell ghosts. Quinidine, 0·1 to 1·0 mM, antagonized the lipolytic activity of theophylline but either enhanced, antagonized or had no effect on the lipolytic activity due to dibutyryl cyclic AMP depending upon the experimental conditions. Quinidine (0·5 mM) inhibited glucose oxidation to CO_2 by more than 60 per cent, and similar effects were seen with quinacrine. Chloroquine and quinacrine at all concentrations tested inhibited lipolysis induced by dibutyryl cyclic AMP, norepinephrine and theophylline. Quinacrine and quinidine (0·1–1·0 mM) inhibited the activation of adenylate cyclase activity in fat cell ghosts due to glucagon or epinephrine. These drugs had a lesser effect on the increase in adenylate cyclase activity due to fluoride. Quinidine (0·2–1·0 mM) decreased cyclic AMP accumulation in intact fat cells.

THESE STUDIES were designed to investigate the mechanisms by which anti-malarial drugs inhibit lipolysis in fat cells. Markus and Ball[1] suggested that these drugs preferentially antagonized triglyceride lipase but Benoit[2] proposed that they inhibited a basic energy system. Recently, it has been suggested[3,4] that active transport of FFA is involved in fatty acid release. Those studies were done in the presence of millimolar concentrations of anti-malarials so that release could be studied in the absence of lipolysis. Because of the potential significance of such findings, further experiments were carried out to characterize other possible effects of anti-malarials on fat cell metabolism.

EXPERIMENTAL PROCEDURE

Isolated white fat cells were obtained from the parametrial adipose tissue of 120–160 g female Sprague–Dawley rats (Charles River CD strain) fed laboratory chow and water *ad lib.* For each experiment, three to four rats were killed by decapitation, the fat pads excised and the cells isolated from the pooled tissue. The fat pads were incubated for 60 min at 37° in 1-oz plastic bottles containing 0·5 mg/ml of crude bacterial collagenase (Worthington) in Krebs–Ringer phosphate buffer containing 4% Armour lot no. 38311 bovine serum albumin. The composition of the phosphate buffer was as follows: NaCl, 128 mM; $CaCl_2$, 1·4 mM; $MgSO_4$, 1·4 mM; KCl, 5·2 mM; and Na_2HPO_4, 10 mM. The buffer was made fresh daily and adjusted to pH 7·4 with NaOH after the addition of the albumin. All incubations were carried out at 37° in a shaking water bath.

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Adenylate cyclase activity was determined by a modification[5] of the method of Krishna *et al.*[6] using fat cell ghosts prepared by hypotonic lysis.[7] Protein content was assayed following the procedure of Lowry *et al.*[8]

FFA release was determined using a modification[9] of the procedure of Dole and Meinertz.[10] Due to the interference with the enzymatic assay for glycerol release,[11] the anti-malarials had to be removed prior to analysis. One hundred μl of 20 mM phosphate buffer (pH 6·0) containing 6% (w/v) charcoal (Norit A) was added to 100 μl of medium. The suspension was mixed and then centrifuged for 10 min.

An aliquot of the supernatant was taken for glycerol analysis. The standards were also subjected to charcoal treatment. Comparative studies showed that the charcoal treatment removed the anti-malarials but not the glycerol. Cyclic AMP accumulation was assayed according to a modification of the method of Gilman[12] as described elsewhere.[5] Labeled glucose oxidation to labeled CO_2 was measured by collecting $^{14}CO_2$ in phenethylamine-treated $3 \times 10 \times 0{\cdot}16$ mm paper strips (Whatman no. 1) situated in hanging wells.[13]

The data for fat cells when expressed as per g of fat cells are calculated from the amount of triglyceride present per tube.[13] Quinacrine hydrochloride, chloroquine diphosphate, primaquine diphosphate and quinidine hydrochloride were obtained from Sigma Chemical Co., St. Louis, Mo.

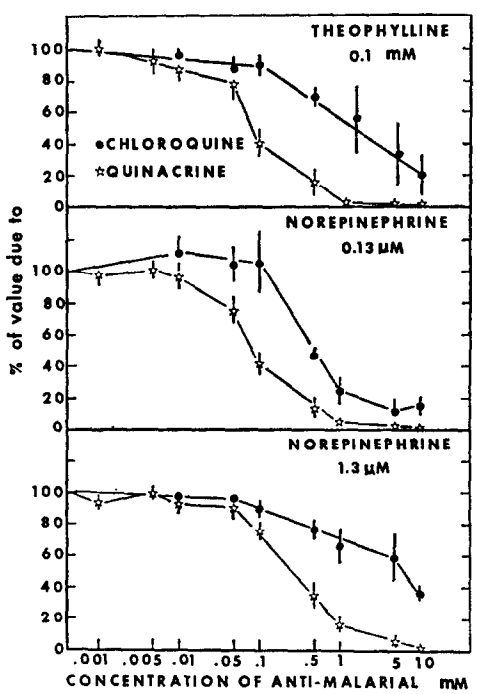

FIG. 1. Effects of varying the concentration of quinacrine and chloroquine on FFA release induced by norepinephrine and theophylline in fat cells. Fat cells (18 mg/tube) were incubated for 1 hr in 1·2 ml of buffer containing 4% albumin. The effects of the anti-malarial drugs are shown as the per cent ± standard error for four paired experiments of the values seen with the lipolytic agents alone. The FFA release in the presence of 0·13 μM and 1·3 μM norepinephrine and 0·1 mM theophylline was 55, 87 and 54 μmole/g respectively. FFA release in the absence of added agents was 1·5 μmole/g.

RESULTS

Figure 1 illustrates the effects of chloroquine and quinacrine on FFA release induced by theophylline and norepinephrine. The lipolytic response to 0·1 mM theophylline or 0·13 μM norepinephrine was antagonized at quinacrine concentrations in the range of 0·05–0·5 mM. Increasing the concentration of norepinephrine 10-fold only slightly shifted the dose–response curve. Chloroquine was considerably less active as an inhibitor of lipolysis than quinacrine.

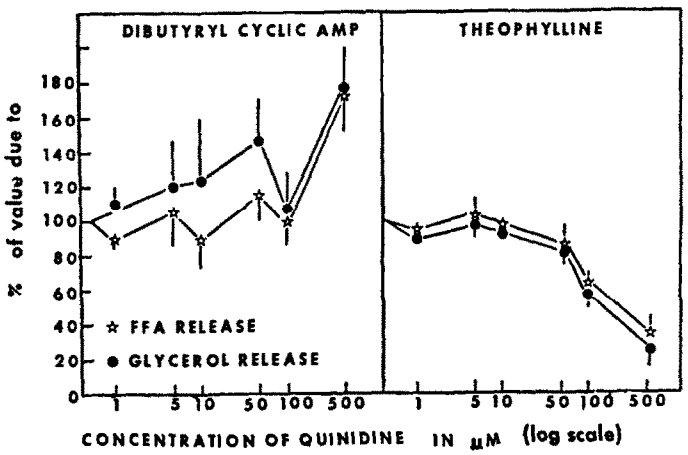

FIG. 2. Effects of quinidine on lipolysis due to dibutyryl cyclic AMP and theophylline in fat cells. Fat cells (27 mg/tube) were incubated for 1 hr in 1·2 ml of buffer containing 4% albumin. The effects of quinidine are shown as the per cent ± standard error of the value seen with the lipolytic agents for four paired experiments. The FFA release and glycerol release in the absence of lipolytic agents were 1·0 and 0·4 μmole/g respectively. The FFA release for dibutyryl cyclic AMP (0·5 mM) and theophylline (0·1 mM) was 14 and 19 μmole/g and for glycerol release was 4·2 and 7·2 μmole/g respectively.

Figure 2 shows the effects of quinidine on FFA and glycerol release due to 0·1 mM theophylline and 0·5 mM dibutyryl cyclic AMP. Quinidine (0·5 mM) potentiated by nearly 80 per cent the dibutyryl cyclic AMP-induced response while inhibiting by nearly 80 per cent the theophylline-induced response.

Quinidine, chloroquine and quinacrine at 5 mM inhibited lipolysis due to dibutyryl cyclic AMP and other lipolytic agents (Table 1). Again chloroquine was less active as an inhibitor of lipolysis. Quinidine (0·5 mM) in this series of experiments only enhanced lipolysis by 28 per cent as compared with 80 per cent as shown in Fig. 2.

Because of the variability in the potentiation of lipolysis by 0·5 mM quinidine, further experiments were performed to more clearly define this effect. Figure 3 shows that the magnitude of the quinidine enhancement of lipolysis due to dibutyryl cyclic AMP varied from one experiment to the next. The lipolytic response induced by 0·5 mM dibutyryl cyclic AMP was enhanced by quinidine at 0·7 mM in one experiment and 0·2 mM in another. When the concentration of dibutyryl cyclic AMP was lowered to 0·25 mM, the enhancement by quinidine occurred at 0·1 and 0·7 mM in the respective experiments, the point being that there is only a small margin between inhibition and potentiation.

TABLE 1. EFFECT OF QUINIDINE, CHLOROQUINE AND QUINACRINE ON LIPOLYSIS DUE TO NOREPINEPHRINE, DIBUTYRYL CYCLIC AMP AND THEOPHYLLINE IN FAT CELLS*

Lipolytic agent	(μmole/g)	% Change from value seen with the lipolytic agent alone					
		Quinidine		Chloroquine		Quinacrine	
		(0·5 mM)	(5·0 mM)	(0·5 mM)	(5·0 mM)	(0·5 mM)	(5·0 MM)
	Fatty acid release						
Norepinephrine 0·13 μM	25·8	−66 ± 14	−86 ± 14	−59 ± 8	−79 ± 8	−89 ± 6	−98 ± 1
Theophylline, 0·1 mM	30·3	−38 ± 9	−97 ± 2	−28 ± 13	−85 ± 7	−78 ± 12	−91 ± 4
Dibutyryl cyclic AMP, 0·5 mM	20·7	+28 ± 12	−82 ± 11	−46 ± 17	−68 ± 16	−38 ± 14	−97 ± 2
Theophylline, 0·1 mM + Dibutyryl cyclic AMP, 0·5 mM	36·2	−11 ± 6	−79 ± 14	−25 ± 4	−45 ± 5	−57 ± 7	−94 ± 3
	Glycerol release						
Norepinephrine, 0·13 μM	10·5	−59 ± 21	−98 ± 1	−42 ± 3	−75 ± 10	−82 ± 7	−83 ± 4
Theophylline, 0·1 mM	16·5	−51 ± 14	−91 ± 1	−51 ± 15	−87 ± 5	−86 ± 4	−88 ± 5
Dibutyryl cyclic AMP, 0·5 mM	12·0	+10 ± 17	−82 ± 8	−38 ± 7	−50 ± 10	−28 ± 20	−83 ± 7
Theophylline, 0·1 mM + Dibutyryl cyclic AMP, 0·5 mM	17·9	−17 ± 7	−76 ± 5	−5 ± 19	−41 ± 10	−40 ± 6	−88 ± 8

* Fat cells (31 mg/tube) were incubated for 1 hr in 1·2 ml of buffer containing 4% albumin. The effects of the anti-malarials are shown as the per cent change from the value seen with the lipolytic agents and are the means ± standard errors for four paired experiments. Minus values refer to percentage decreases and plus values to potentiations of lipolysis due to the drugs. The FFA release in the absence of lipolytic agents was 3·9 μmole/g and glycerol release was 1·3 μmole/g and were unaffected by all concentrations of the drugs tested.

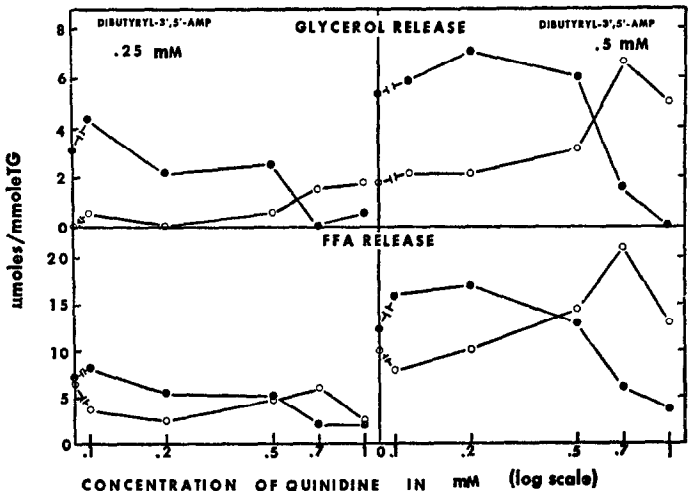

FIG. 3. Effects of quinidine on FFA and glycerol release due to dibutyryl cyclic AMP. Fat cells (36 mg/tube) were incubated for 1 hr in 1·2 ml of buffer containing 4% albumin. The open and closed circles each represent two separate experiments.

It was of interest to investigate the effects of anti-malarials on membrane bound adenylate cyclase in fat cells. The data in Fig. 4 show that primaquine and quinacrine antagonized both norepinephrine and fluoride stimulation of adenylate cyclase activity by fat cell ghosts. Quinacrine at 1·0 mM abolished the increase in adenylate cyclase activity due to norepinephrine while the increase due to fluoride was inhibited to a lesser extent (Table 2 and Fig. 4). Table 2 shows that quinacrine and quinidine antagonized adenylate cyclase activity due to epinephrine as well as that due to glucagon. Similar effects were seen on cyclic AMP accumulation. The data summarized in Table 3 show that cyclic AMP accumulation induced by norepinephrine and theophylline decreased as the concentration of quinidine increased.

TABLE 2. INHIBITION OF ADENYLATE CYCLASE BY ANTI-MALARIALS*

		Additions		
	Basal	+Glucagon (2 μg/ml)	+Epinephrine (0·2 mM)	+Sodium fluoride (10 mM)
		(nmole/mg protein)		
	< 0·1	7·0	10·0	18·8
		% Change ± S. E. due to added agents		
Quinacrine, 0·1 mM			−7 ± 11	+11 ± 10
1·0 mM		−74 ± 5	−58 ± 11	−35 ± 20
Quinidine, 0·1 mM			−44 ± 13	+2 ± 6
1·0 mM		−66 ± 10	−70 ± 5	−32 ± 14

* Fat cell ghosts (107 μg of protein/tube) were incubated for 20 min. The values are for four paired experiments. Plus values refer to per cent stimulation and negative to per cent inhibition.

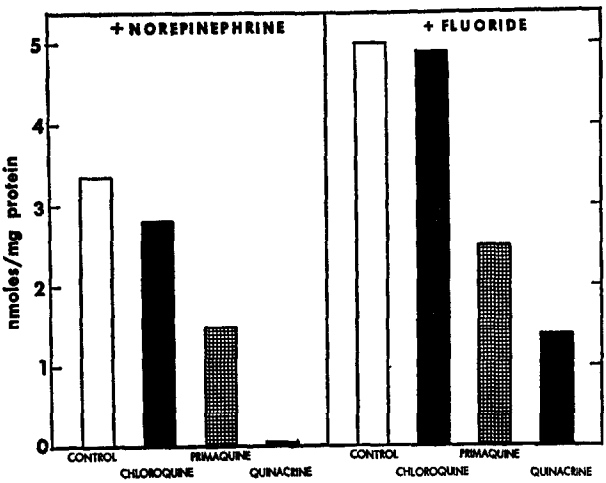

FIG. 4. Effect of anti-malarial agents on adenylate cyclase. Fat cell ghosts (85 μg of protein/tube) were incubated for 20 min. The values are the means of two paired experiments and basal adenylate cyclase activity was less than 0·2 nmole/mg of protein. The anti-malarials were added *in vitro* at a concentration of 1 mM. The concentrations of norepinephrine and fluoride were 0·2 mM and 10 mM respectively.

Since quinidine in the range of 0·1 to 1·0 mM potentiated lipolysis due to dibutyryl cyclic AMP, it was of interest to determine if quinidine had similar effects on glucose oxidation. Figure 5 shows that quinidine and quinacrine inhibited basal glucose oxidation to CO_2 at all concentrations tested.

TABLE 3. INHIBITION OF CYCLIC AMP ACCUMULATION DUE TO QUINIDINE*

Quinidine (mM)	Cyclic AMP accumulation (nmole/g)	Fatty acid release (μmole/g)
0	57	42
	Per cent inhibition ± standard error due to quinidine	
0·2	21 ± 15	44 ± 9
0·5	72 ± 15	66 ± 7
1·0	83 ± 6	100 ± 10

* Fat cells (30 mg/tube) were incubated with quinidine for 10 min prior to the addition of norepinephrine (1·5 μM) plus theophylline (0·2 mM). Samples for cyclic AMP accumulation were taken after 2 min and for fatty acid accumulation after 30-min incubation. The values are from three paired replications.

DISCUSSION

Chloroquine generally had a lesser inhibitory effect on lipolysis induced by catecholamines than did quinidine and quinacrine (Fig. 1). Quinacrine appeared to be the more potent antagonist of lipolysis in the present investigation whereas quinidine was more powerful in adipose tissue slices.[1] Our studies and those of others[1–4,14]

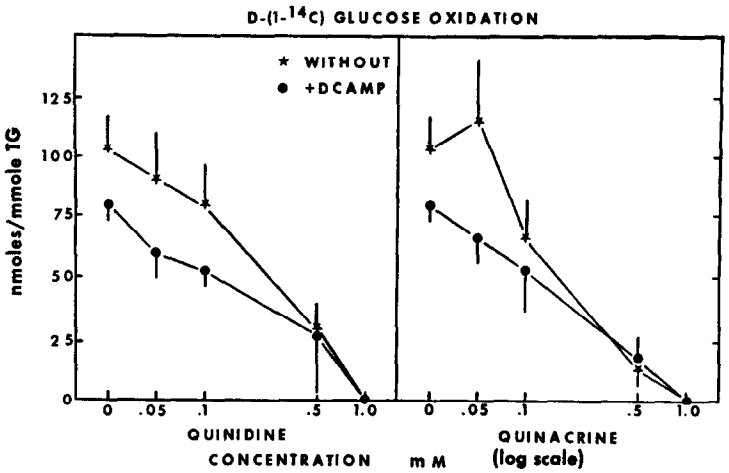

FIG. 5. Effects of quinidine and quinacrine on D-(1-^{14}C) glucose oxidation to $^{14}CO_2$ in fat cells. Fat cells (25 mg/tube) were incubated for 1 hr in 1·2 ml of buffer containing 4% albumin. The values are the mean ± standard error of three paired experiments for cells incubated in the absence (★) or presence of dibutyryl cyclic AMP at 0·5 mM (●).

suggest that millimolar concentrations of anti-malarials inhibit lipolysis due to all lipolytic agents tested by 50–100 per cent.

Schimmel and Goodman[3] measured the release of FFA from adipose tissue in the presence of 3 mM quinacrine as a preferential inhibitor of triglyceride lipase. These investigators concluded that the fatty acid efflux seen was an active energy-requiring process, since this efflux was not seen in the presence of metabolic inhibitors such as dinitrophenol and iodoacetate. However, our studies and those of Benoit[2] suggest that the energy requirement might be related to overcoming the toxic effects of anti-malarials on fat cell metabolism. Quinidine at concentrations in the range 0·1–0·7 mM can enhance lipolysis due to dibutyryl cyclic AMP in fat cells but no similar effect was seen with other lipolytic agents or anti-malarials. It may be that quinidine also stimulates the uptake or conversion of dibutyryl cyclic AMP to an active form and this offsets any inhibition of cyclic AMP action at low concentrations of quinidine.

Quinacrine and quinidine (0·1–1·0 mM) inhibited basal glucose oxidation to CO_2 in intact fat cells. Others[15] have reported that 0·5 mM quinidine depresses glucose uptake in rat heart slices and homogenates, while 0·3 mM quinidine depressed basal respiration and anaerobic glycolysis in rat heart slices.[16] The mechanisms by which the preceding effects are seen may be related to the action of quinidine on membrane ATPase. Quinidine (0·005 mM to 0·5 mM) inhibited ATPase in rat skeletal muscle[17] and in toad cardiac muscle.[18] On the other hand, quinacrine at 0·75 mM stimulates rat liver mitochondrial ATPase and inhibits it at 3·0 mM.[19] It is possible that the availability of ATP or membrane permeability might explain the effects seen. However, the high concentrations of the drugs used may result in toxic metabolic effects.

The present studies do not support the hypothesis that anti-malarials preferentially antagonize the tryglyceride lipase as suggested by others.[1] Rather these drugs appear to inhibit lipolysis by poisoning all metabolic processes. Considering the large dosages required to produce the effects of anti-malarials on lipolysis in adipose cells and tissue, it is not surprising that a variety of non-specific effects are seen.

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