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The inhibition of gluconeogenesis by chloroquine contributes to its hypoglycaemic action

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Abstract

The effect of chloroquine on gluconeogenesis in isolated hepatocytes and kidney-cortex tubules of rabbit has been studied. The inhibitory action of 200 μM chloroquine was the highest in hepatocytes and renal tubules incubated with glutamine and glutamate + glycerol + octanoate, respectively, while in the presence of other substrates the drug action was less pronounced. With amino acids as substrates, the inhibition of gluconeogenesis was accompanied by a decreased glutamine production, resulting from a decline of glutamate dehydrogenase activity. A decrease in the urea production by hepatocytes incubated with chloroquine in the presence of glutamine but not NH_4Cl as the source of ammonium is in agreement with this suggestion. The degree of inhibition by chloroquine of the rate of gluconeogenesis in renal tubules isolated from control rabbits was similar to that determined in diabetic animals. Chloroquine-induced changes in levels of intracellular gluconeogenic intermediates indicate a decrease in phosphoenolpyruvate carboxykinase and glucose-6-phosphatase activities probably due to increased concentration of 2-oxoglutarate, an inhibitor of these two enzymes. In view of the data, it is likely that inhibition by chloroquine of glucose formation in liver and kidney may contribute to the hypoglycaemic action of this drug. The importance of the inhibitory effect of chloroquine on glutamate dehydrogenase activity in the antihyperglycaemic action of the drug is discussed.

Keywords: Hypoglycaemia; Chloroquine; Gluconeogenesis; Hepatocyte; Renal tubule

1. Introduction

Chloroquine is a drug with over 60 years of safe clinical use in the treatment of malaria and inflammatory disorders. However, despite various actions of this readily penetrable lipid bilayer compound, the mechanism by which the drug mediates its effects has not been fully characterised (cf. O'Neill et al., 1998, for review).

Case reports and clinical studies suggest that chloroquine induces hypoglycaemia in situations including overdose (Bamber and Redpath, 1987; Seltzer, 1989) and in some patients with rheumatologic disorders (Abu-Shakra and Lee, 1994; Petri, 1996) while it does not affect overall glucose tolerance in the healthy volunteers (Smith et al., 1987). There is, however, a statistically significant reduction in fasting plasma glucose after aminoquinolines treatment in the non-diabetic subjects (Davis et al., 1996). Chloroquine improves glucose tolerance in patients with non-insulin-dependent diabetes mellitus (Smith et al., 1987), increases peripheral glucose disposal and decreases metabolic clearance rate of insulin (Powrie et al., 1991). Moreover, in insulin-dependent diabetes mellitus the drug has been shown to reduce insulin resistance (Blazar et al., 1984). The hypoglycaemic action of chloroquine is attributed to a decreased degradation of insulin in the presence of the drug (Bevan et al., 1997) due to augmented binding of insulin to its receptor (Bevan et al., 1995).

Although the maintenance of appropriate plasma glucose concentration is largely a function of the hormone action, the direct effect of chloroquine on glucose metabolism cannot be excluded. It has been reported that chloroquine inhibits the rate of glucose formation in rat hepatocytes incubated with lactate (Crabb et al., 1980). According to an observation from our laboratory (Jarzyna et al., 1997), chloroquine is a potent inhibitor of glutamate dehydrogenase, a key enzyme in amino acid metabolism of both liver and kidney-cortex. Since (i) amino acids are considered to be main precursors of glucose formation, (ii) kidney in addition to liver makes significant contribution to glucose whole body metabolism (Stumvoll et al., 1997; Meyer et al., 1999; Cersosimo et al., 2000), (iii) glutamine

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is predominantly a renal gluconeogenic substrate (Stumvoll et al., 1999), (iv) in diabetic animals renal gluconeogenesis is also increased together with that in liver (Kida et al., 1978), and (v) chloroquine is known to accumulate in liver, kidney, spleen and lung (McChesney et al., 1967; Grundmann et al., 1971), we were prompted to investigate in detail the potential inhibitory effect of chloroquine on gluconeogenesis in both hepatocytes and kidney-cortex tubules of rabbit, which exhibit similar to human intracellular localisation of gluconeogenic enzymes (Usatenko, 1970).

2. Material and methods

2.1. Animals and isolation of kidney-cortex tubules, hepatocytes and mitochondria

Male white Termond rabbits were used throughout. Animals were maintained on the standard rabbit chow with free access to water and food.

Alloxan diabetes was induced by the single injection of alloxan (150 mg kg^{-1} body weight) dissolved in 10 mM citrate buffer (pH 4.5) into the marginal vein of the ear of a rabbit starved for 48 h. After injection, the animals were allowed a standard diet and water ad libitum. To avoid hypoglycaemic shock, animals were given 1% glucose solution to drink during 24 h after the alloxan treatment. Only those alloxan-treated animals that exhibited decreased or stabilised body weight and blood glucose concentrations in excess of 300 mg/100 ml 3 days after treatment were considered diabetic and used for experiments.

Rabbit kidney-cortex tubules were obtained from the left kidneys as described by Zabłocki et al. (1983). Briefly, rabbits were anaesthetised by intravenous injection of pentobarbital (about 50 mg kg^{-1}). The left kidney was perfused for 10 min through the left renal artery with Ca^{2+}-free Krebs-Ringer bicarbonate buffer. Then, perfusion was continued for 10–15 min in a recirculating manner with Krebs-Ringer buffer containing 2.5 mM Ca^{2+} and 0.02% collagenase. The perfusion medium was kept in equilibrium with O_2:CO_2 (95%:5%) at 37 °C. In the next step, the kidney was removed from the animal and the cortex tissue was suspended in the same collagenase medium and shaken in the polyethylene bottle at 37 °C continuously gassed with O_2:CO_2. After 45 min of collagenase treatment, the twofold volume of cold Krebs-Ringer buffer was added to stop tissue digestion. After sedimentation of unbroken particles, the supernatant was centrifuged at low speed ($10 \times g$ for 30 s). The tubule pellet was resuspended and washed twice on the centrifuge. Finally, the tubules were suspended in about 10 vol. of the buffer and immediately used for incubation.

Hepatocytes were obtained as described by Zaleski and Bryła (1978). Briefly, After 48 h of fasting, the rabbits were anaesthetised by intravenous injection of pentobarbital (about 50 mg per kg). Liver was perfused for 10 min with Ca^{2+}-free Krebs-Ringer bicarbonate buffer. Then, perfusion was continued for 10–15 min in a recirculating manner with Krebs-Ringer buffer containing 2.5 mM Ca^{2+} and 0.02% collagenase. The perfusion medium was kept in equilibrium with O_2:CO_2 (95%:5%) at 37 °C. Dispersed liver was removed from the animal and shaken for 15 min at 37 °C in the same collagenase medium and continuously gassed with O_2:CO_2. The suspension was filtered through the nylon cell strainer and centrifuged at $50 \times g$ for 2 min. The cells were resuspended in 10 vol. of Krebs-Ringer bicarbonate buffer and centrifuged as above. Finally, the cells were suspended in 8 vol. of medium and immediately used for incubation.

Mitochondria were isolated from both the kidney-cortex and the liver using a solution containing 225 mM mannitol, 75 mM sucrose, 5 mM 3-[*N*-Morpholino]propanesulfonic acid (MOPS), 0.1 mM EDTA and 1% Bovine serum albumin (pH adjusted to 7.2 with Tris) as reported by Harris et al. (1971), but the final wash was made with 0.3 M mannitol.

2.2. Incubation of renal tubules and hepatocytes

Both the isolated kidney-cortex tubules and the hepatocytes were incubated at 37 °C in 25 ml Erlenmeyer flask sealed with rubber stoppers under atmosphere of O_2:CO_2 (95%:5%) in Krebs-Ringer bicarbonate buffer, pH 7.4, in the presence of substrates indicated in legends to figures and tables. Reactions were stopped following 60 min of incubation by the addition of 1 ml sample to 0.1 ml of 35% perchloric acid.

2.3. Analytical methods

Glucose-6-phosphatase activity was measured according to Gierow and Jergil (1982) with modifications. Incubation medium (2 ml) consisted of 12.5 mM Tris adjusted with maleic acid to pH 6.5, 10 mM phenol with 0.4 mM aminoantipyrine, 75 U of glucose oxidase, 8 U of peroxidase and a 0.6–1.2 mg of kidney cortex cytosolic protein. The incubation mixture was preincubated for 5 min at 30 °C and then the reaction was started by the addition of 5 mM glucose-6-phosphate. The formation of quinoneimine was followed for 3 min at 510 nm. The result was corrected for changes of absorption in the absence of the substrate.

Glucose-6-phosphate dehydrogenase activity was assayed by the method of Lohr and Wahler (1974) measuring the generation of NADPH spectrophotometrically at 355 nm (Wagner et al., 1998).

Pyruvate carboxylase activity was measured as described by Michalik and Bryła (1987) with some modifications. The incubation medium (3 ml) contained 15 mM KCl, 5 mM $MgCl_2$, 10 mM phosphate buffer pH 7.4, 50

mM Tris–HCl pH 7.4, kidney-cortex mitochondria about 10 mg protein and 5 mM pyruvate or 5 mM alanine + 5 mM oxoglutarate. The mixture was continuously stirred and gassed with O_2:CO_2 (95%:5%), maintained in thermostatised glass chambers at 30 °C and preincubated with or without 200 μM chloroquine for 5 min. Reaction was started by the addition of 30 mM $KHCO_3$ with trace amount of [^{14}C] sodium bicarbonate (1 μCi/mmol). At 0 and 10 min of incubations, 1 ml samples were withdrawn from suspension, deproteinized with 0.1 vol. of 35% $HCLO_4$ (v/v) and centrifuged. Supernatants were collected and shaken for 30 min. The resulting acid-stable non-volatile residue was used for the determination of radioactivity by liquid scintillation counting.

The phosphoenolpyruvate carboxykinase activity in mitochondria was measured according to Bryła and Dzik (1977), while that in cytosol was measured in agreement with Bentle and Lardy (1976) and MacDonald et al. (1978) using GTP instead of ITP.

The intracellular content of metabolites in isolated kidney tubules was measured in samples taken at 60 min of incubation following centrifugation through silicone oil into a perchloric acid solution as described previously (Zaleski et al., 1982). Glucose, lactate, pyruvate, 2-oxoglutarate, malate, phosphoenolpyruvate, triose phosphates, 3 phosphoglycerate + 1,3 bisphosphoglycerate, fructose-1,6-bisphosphate, fructose-6-phosphate and glucose-6-phosphate were estimated either spectrophotometrically or fluorimetrically according to Bergmeyer (1965). Urea was measured colorimetrically as described by Bryła and Harris (1976). Amino acids were determined by high performance liquid chromatography (HPLC) (Beckman Instruments) as their DABS-derivatives according to Chang et al. (1983).

2.4. Chemicals

Enzymes, coenzymes and nucleotides for metabolite determination were from Boehringer, (Mannheim, Germany), Aqua Scynt for C^{14} labelled CO_2 incorporation was from Biocare (Warsaw, Poland). All others chemicals were obtained from Sigma (St. Louis, MO).

2.5. Expression of results

Data shown are means ± S.D. for three separate experiments. The statistical significance of differences was calculated by Student's *t* test.

3. Results

3.1. Glucose formation and oxygen consumption

In contrast to hepatocytes, rabbit kidney-cortex tubules produce efficiently glucose from amino acids only in the presence of glycerol and either fatty acids (Lietz et al., 1999) or ketone bodies (Lietz et al., 1997). Therefore, in all experiments rabbit renal tubules were incubated with glutamate, alanine or aspartate at 2 mM concentrations in the presence of 2 mM glycerol and 0.5 mM octanoate, while other substrates were added at 5 mM concentrations.

Increasing concentrations of chloroquine resulted in an augmented decrease of glucose formation in kidney-cortex tubules (Fig. 1). In subsequent experiments, 200 μM chloroquine was applied to avoid the complete inhibition of gluconeogenesis. As shown in Table 1, the inhibitory action of the drug at 200 μM concentration was the highest (about 50%) in hepatocytes and renal tubules incubated with glutamine or glutamate + glycerol + octanoate, respectively, while in the presence of other substrates a decline of the rate of glucose production by chloroquine was less pronounced (about 20–30%). Moreover, other aminoquinolines (quinacrine, quinidine and quinine) were more potent than chloroquine in the inhibition of glucose synthesis (Table 2).

In agreement with previous reports (Kida et al., 1978), gluconeogenesis in diabetic rabbit renal tubules was increased for about 20–30% (Table 3). However, the degree of inhibitory effect of chloroquine on this process from alanine, pyruvate or dihydroxyacetone was similar to that measured in renal tubules isolated from control animals. Although chloroquine did not affect the intracellular ATP levels (data not shown), it decreased significantly the oxygen uptake in renal tubules incubated with pyruvate, glutamate or aspartate. On the contrary, the drug did not alter the oxygen consumption in the presence of alanine or malate as substrates (Table 4), suggesting the substrate-dependent mechanism of the inhibitory action of chloroquine.

3.2. Cellular intermediary metabolites

In order to identify the gluconeogenic steps responsible for the inhibition by chloroquine of glucose formation, we

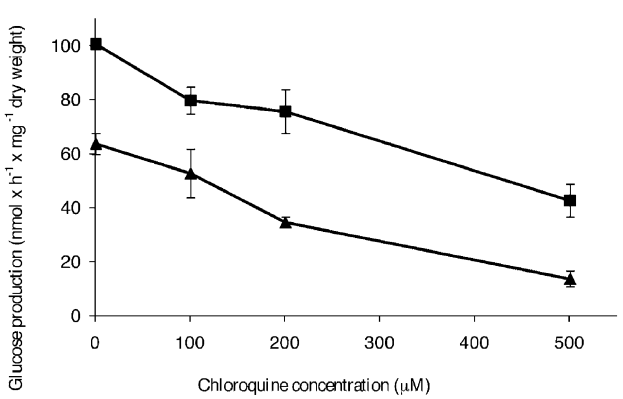

Fig. 1. The effect of increasing chloroquine concentrations on glucose formation in rabbit renal tubules incubated with pyruvate (squares) or glutamate + glycerol + octanoate (triangles). Renal tubules were incubated with either 5 mM pyruvate or 2 mM glutamate + 2 mM glycerol + 0.5 mM octanoate, without or with chloroquine, as indicated. Points with vertical lines represent means ± S.D. for three separate experiments.

Table 1
The effect of chloroquine on glucose synthesis in isolated rabbit kidney-cortex tubules and hepatocytes incubated in the presence of various substrates

Substrates	Glucose production (nmol h^{-1} mg^{-1} dry weight)			
	Kidney-cortex tubules		Hepatocytes	
	−CQ	+CQ	−CQ	+CQ
Alanine	78±3	59±6[a]	46±2	36±0[a]
Glutamine	n.d.	n.d.	17±1	9±2[b]
Glutamate	66±6	35±3[b]	n.d.	n.d.
Aspartate	65±6	44±7[a]	n.d.	n.d.
Malate	142±7	105±9[a]	n.d.	n.d.
Pyruvate	98±4	71±7[a]	68±6	55±5[a]
Lactate	n.d.	n.d.	101±19	83±15[a]

Hepatocytes were incubated in the presence of 5 mM substrates. In experiments with kidney-cortex tubules, the reaction medium containing 2 mM amino acid as substrate was supplemented with 2 mM glycerol and 0.5 mM octanoate while other substrates were added at 5 mM concentrations. Chloroquine (CQ) was added at 200 μM concentration.
[a] $P < 0.05$.
[b] $P < 0.02$.

have measured the chloroquine-induced changes of gluconeogenic intermediates in renal tubules incubated with alanine + glycerol + octanoate or glutamate + glycerol + octanoate. As shown in Table 5, with no chloroquine added to the reaction medium the intracellular content of the intermediary metabolites was dependent on the glucose precursor. In the presence of glutamate, the intracellular malate was sixfold higher than that with alanine while phosphoenolpyruvate and triose phosphate concentrations were almost twice lower than those with alanine. However, chloroquine resulted in a marked accumulation of malate with the two substrate studied accompanied by a significant decline of phosphoenolpyruvate content, particularly in the presence of glutamate, indicating a decrease in phosphoenolpyruvate carboxykinase activity (Fig. 2). With alanine as a substrate, the chloroquine-induced elevation of intracellular malate content was not due to an enhanced

Table 2
Effects of aminoquinoline drugs on glucose synthesis in isolated rabbit renal tubules and hepatocytes

Drug	Glucose production (nmol h^{-1} mg^{-1} dry weight)	
	Kidney-cortex tubules	Hepatocytes
None	80±11	96±2
Chloroquine	58±9[a]	73±2[b]
Quinacrine	23±9[b]	50±1[b]
Quinidine	41±10[c]	58+5[b]
Quinine	48±2[a]	59±7[c]

Kidney-cortex tubules and hepatocytes were incubated in the presence of 5 mM pyruvate and 5 mM lactate, respectively. Drugs were added at 200 μM concentration.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.02$.

Table 3
The effect of chloroquine on glucose formation in kidney-cortex tubules isolated from alloxan-diabetic and control rabbits

Substrate	Glucose production (nmol h^{-1} mg^{-1} dry weight)			
	Control rabbits		Diabetic rabbits	
	−CQ	+CQ	−CQ	+CQ
Alanine	62±2	47±4	76±13	63±9
Pyruvate	90±7	66±6[a]	108±9	83±7[a]
Dihydroxyacetone	56±6	35±5[b]	67±1	43±5[b]

Kidney-cortex tubules were incubated with 2 mM alanine in the presence of 2 mM glycerol and 0.5 mM octanoate, while pyruvate, dihydroxyacetone and chloroquine (CQ) were added at 5, 5, and 0.2 mM concentrations, respectively, where indicated.
[a] $P < 0.05$.
[b] $P < 0.02$.

pyruvate carboxylation as in the presence of alanine and 2-oxoglutarate chloroquine did not affect the incorporation of $[^{14}C]CO_2$ into isolated kidney-cortex mitochondria (49 ± 1 and 51 ± 1 nmol 10 min^{-1} mg^{-1} of protein without and with 200 μM chloroquine, respectively). The same concentration of chloroquine affected neither malate dehydrogenase nor alanine and aspartate aminotransferases (data not shown). Another possibility to elevate the intracellular malate content is an inhibition by chloroquine of malic enzyme activity. However, if this were the case, a drop between malate and phosphoenolpyruvate would not appear. Thus, it seems very likely that such a drop is due to a chloroquine-induced decline of the phosphoenolpyruvate carboxykinase activity.

The levels of intermediates from 3-phosphoglycerate to glucose-6-phosphate were slightly increased while intracellular glucose concentration decreased following the chloroquine addition into the renal tubule suspension, suggesting a diminished glucose-6-phosphatase activity. The glucose-6-phosphate content could also have been elevated due to an inhibition by chloroquine of glucose-6-phosphate

Table 4
The effect of chloroquine on oxygen consumption by kidney-cortex tubules incubated with various substrates

Substrate	Oxygen consumption (nmol min^{-1} mg^{-1} dry weight)	
	−Chloroquine	+Chloroquine
Malate	10.0±0.3	9.0±1.4
Aspartate	11.6±2.0	9.8±1.4[a]
Glutamate	6.7±0.5	4.5±1.2[a]
Alanine	10.2±0.7	10.2±0.9
Pyruvate	6.0±0.6	5.2±0.4[a]

Renal tubules were incubated without or with 200 μM chloroquine. Amino acids were added at 2 mM concentrations in the presence of 2 mM glycerol and 0.5 mM octanoate while other substrates were included at 5 mM concentrations, where indicated. Oxygen consumption was measured with Clark electrode, following 1 h of incubation.
[a] $P < 0.05$.

Table 5
Intracellular content of gluconeogenic intermediates in renal tubules incubated in the presence of amino acids + glycerol + octanoate

Metabolite	Substrate	
	Alanine	Glutamate
Pyruvate	0.58 ± 0.05	n.d.
2-Oxoglutarate	0.27 ± 0.02	0.83 ± 0.16
Malate	0.34 ± 0.01	1.90 ± 0.45
Phosphoeneolpyruvate	0.41 ± 0.04	0.22 ± 0.06
3-Phosphoglycerate + bisphosphoglycerate	0.39 ± 0.01	0.38 ± 0.05
Triosephosphate	0.52 ± 0.13	0.30 ± 0.04
Fructose-1-6-bisphosphate	0.13 ± 0.02	0.10 ± 0.01
Fructose-6-phosphate	0.07 ± 0.00	0.07 ± 0.01
Glucose-6-phosphate	0.25 ± 0.07	0.26 ± 0.06
Glucose	6.00 ± 0.30	5.82 ± 0.62

Kidney-cortex tubules were incubated with either 2 mM alanine or 2 mM glutamate in the presence of 2 mM glycerol and 0.5 mM octanoate. Metabolite content (nmol mg^{-1} dry weight) was determined following 1 h of incubation and centrifugation through a silicon oil layer, as described under Materials and methods.

dehydrogenase. However, the activity of this enzyme measured in the cytosolic fraction of kidney cortex was stimulated on the addition of 100 μM chloroquine to the incubation medium (from 74 ± 6 to 145 ± 9 nmol min^{-1} mg^{-1} of protein with and without chloroquine, respectively), in agreement with data reported for rat liver (Abdel-Gayoum et al., 1992). Despite such a significant activation of glucose-6-phosphate dehydrogenase followed by an increased utilisation of glucose-6-phoshate, the intracellular level of glucose-6-phosphate remained increased while the content of glucose was decreased indicating a decline of glucose formation via glucose-6-phosphatase.

Surprisingly, chloroquine at 500 μM concentration affected neither glucose-6-phosphatase activity in the cytosolic fraction of rabbit kidney-cortex (10.0 ± 1.2 and 10.1 ± 0.8 without and with chloroquine, respectively) nor phosphoenolpyruvate carboxykinase activity in both cytosol (32.0 ± 2.1 and 31.7 ± 1.9 nmol min^{-1} mg^{-1} of protein without and with chloroquine, respectively) and mitochondria (19.1 ± 1.4 and 18.5 ± 2.1 nmol min^{-1} mg^{-1} of protein without and with chloroquine, respectively). However, the inhibitory action of chloroquine on both phosphoenolpyruvate carboxykinase and glucose-6-phosphatase activities in renal tubules might result from an elevation of the intracellular content of 2-oxoglutarate, an inhibitor of these two enzymes (Mithieux et al., 1990; Titheradge et al., 1970). As determined in the presence of glutamate + glycerol + octanoate following the addition of chloroquine, the intracellular 2-oxoglutarate level in renal tubules increases from 0.8 ± 0.1 to 2.0 ± 0.2 nmol mg^{-1} of dry weight. According to Titheradge et al. (1970), phosphoenolpyruvate carboxykinase activity is inhibited by 2-oxoglutarate competitively (K_i with respect to oxaloacetate 0.32 ± 0.04 mM). Taking into account the intracellular water (5.1 ± 0.3 mg mg^{-1} dry weight) in the kidney-cortex tubules incubated in the presence of glutamate + glycerol + octanoate without and with chloroquine, 2-oxoglutarate concentration is equal to 0.16 ± 0.02 mM and 0.40 ± 0.04 mM, respectively. Thus, the intracellular 2-oxoglutarate content in chloroquine-treated renal tubules seems to be high enough to inhibit phoshoenolpyruvate carboxykinase activity. Similarly, Mithieux et al. (1990) have suggested that the inhibition of glucose-6-phosphate activity might be related to the intracellular synthesis of 2-oxoglutarate from glutamine. These observations support our suggestion with respect to the inhibitory effect of increased 2-oxoglutarate levels on both phoshoenolpyruvate carboxykinase and glucose-6-phosphatase activities.

In agreement with our previous observations (Jarzyna et al., 1997), an increased intracellular accumulation of 2-oxoglutarate might be due to the inhibition by chloroquine of glutamate dehydrogenase activity, as indicated by a decline of glutamine production with both glutamate + glycerol + octanoate (110 ± 3 and 66 ± 12 nmol h^{-1} mg^{-1} of dry weight, without and with chloroquine, respectively)

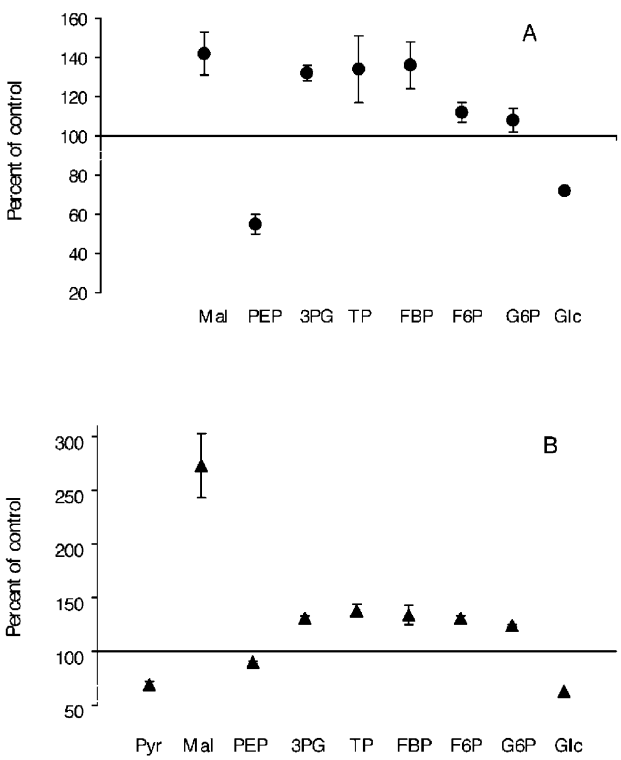

Fig. 2. The effect of chloroquine on changes of intracellular levels of gluconeogenic intermediates in renal tubules incubated with glycerol and octanoate in the presence of either glutamate (A) or alanine (B). Experimental conditions as well as control values of metabolites are presented in Table 5. The concentrations of intracellular metabolites in the presence of 200 μM chloroquine are expressed as percentage of control values measured without chloroquine. Points with vertical lines represent the means ± S.D. for three separate experiments. Metabolite listed from left to right are: pyruvate, Pyr; malate, Mal; phosphoenolpyruvate, PEP; 3-phosphoglycerate plus 1,3-diphosphoglycerate, 3PG; glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate, TP; fructose-1,6-bisphosphate, FBP; fructose-6-phosphate, F6P; glucose-6-phosphate, G6P; glucose, Glc.

and alanine + glycerol + octanoate (51 ± 2 and 32 ± 1 nmol h^{-1} mg^{-1} of dry weight without and with chloroquine, respectively). The inhibition by chloroquine of urea production from glutamine in rabbit hepatocytes (104 ± 14 and 73 ± 13 nmol h^{-1} mg^{-1} of dry weight without and with chloroquine, respectively), as well as the lack of the chloroquine inhibition of this process in the presence of NH_4Cl as ammonium source (87 ± 3 and 93 ± 1 nmol h^{-1} mg^{-1} of dry weight without and with chloroquine, respectively) are also in accordance with this hypothesis. Thus, the inhibitory effect of chloroquine on glutamate dehydrogenase activity might contribute to the mechanism of drug-induced inhibition of gluconeogenesis in the presence of amino acids.

4. Discussion

The hypoglycaemic action of chloroquine is believed to be mediated by insulin. Bevan et al. (1995, 1997) showed that the drug augmented binding hormone to its receptor which extends the lifetime of activated insulin receptor complex. As the presented data indicate that chloroquine (cf. Fig. 1, Table 1) and other antimalaric drugs (cf. Table 2) inhibit gluconeogenesis in both rabbit kidney cortex tubules and hepatocytes, it seems likely that the inhibition of glucose production by chloroquine may also contribute to its hypoglycaemic effect.

Although chloroquine increases peripheral glucose disposal in patients with non-insulin dependent diabetes mellitus (NIDDM) (Powrie et al., 1991), it has little effect on the blood glucose levels in healthy volunteers (Smith et al., 1987). Therefore, one may consider chloroquine rather as an antihyperglycaemic than a hypoglycaemic agent. The metabolism of patient with NIDDM is altered in comparison with that of a healthy person. Renal glutamine gluconeogenesis is increased in NIDDM (Stumvoll et al., 1999). Moreover, in diabetic animals there is a rise in renal gluconeogenic enzymes activity (Lemieux et al., 1984) and in renal gluconeogenesis (Kida et al., 1978; Kang et al., 1982), likewise in patients with insulin-dependent diabetes mellitus (IDDM) taken off regular insulin (Mitrakou et al., 1996). Because in human almost 80% of the systemic glutamine gluconeogenesis takes place in the kidney (Stumvoll et al., 1998), all these observations indicate that in diabetes mellitus (despite the type) there is an activation of renal glutamine gluconeogenesis. Our studies indicate that chloroquine is a more potent inhibitor of gluconeogenesis from glutamine (cf. Table 1) and glutamate (cf. Table 1 and Fig. 1) than from any other substrate, probably due to a strong inhibition of glutamate dehydrogenase activity (Jarzyna et al., 1997). Both the decline in the urea production in hepatocytes incubated with glutamine and chloroquine, and the decrease in glutamine formation in kidney tubules incubated with chloroquine and amino acids are in agreement with this suggestion. Thus, the inhibition of renal gluconeogenesis from glutamine and glutamate may contribute to chloroquine mediated antihyperglycaemic action pronounced particularly in diabetic patients.

In addition to being a gluconeogenic substrate, glutamine may also act as a regulator of this process. In isolated renal tubules, glutamine stimulates the incorporation of fructose, dihydroxyacetone and lactate into glucose (Guder and Wirthensohn, 1979). An infusion of glutamine that increases plasma glutamine concentration threefold leads to a sevenfold increase of glucose formation from glutamine in the absence of changes in plasma insulin levels. Glutamine also stimulates glucose formation from alanine in the absence of changes in plasma alanine content (Perriello et al., 1997). As increases in the concentrations of other gluconeogenic precursors such as lactate (Jenssen et al., 1993), alanine (Aikawa et al., 1972) and glycerol (Nurjhan et al., 1992) produce only a proportional increase in their incorporation into glucose, these observations provide evidence that glutamine exerts a stimulatory effect on gluconeogenesis. Increased glutamine utilisation could stimulate the aspartate-malate shuttle (Friedrichs, 1975) generating the increased reducing equivalents and ATP required for increased gluconeogenesis (Cornell et al., 1974). And finally, glutamine has been reported to increase the activity of phosphoenolpyruvate carboxykinase in rat kidney cortex homogenates (Peters et al., 1976). In view of these observations, chloroquine-induced decrease in both the glutamine content and glutamate utilisation may contribute to its antihyperglycaemic action in addition to extended lifetime of insulin receptor complex.

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