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EFFECTS OF QUINACRINE ON VASOPRESSIN-INDUCED CHANGES IN GLYCOGEN PHOSPHORYLASE ACTIVITY, Ca^{2+} TRANSPORT AND PHOSPHOINOSITIDE METABOLISM IN ISOLATED HEPATOCYTES

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Abstract—In isolated hepatocytes, quinacrine (150–250 μM) inhibited vasopressin-induced increases in glucose release, glycogen phosphorylase *a* activity and $^{45}Ca^{2+}$ efflux; and glucagon-induced increases in glucose release and cyclic AMP formation. These results indicate that a phospholipase A_2 enzyme sensitive to quinacrine is unlikely to be involved in the process by which vasopressin stimulates glycogen phosphorylase activity in the liver cell. In cells labelled with [^{3}H]inositol, much lower concentrations of quinacrine (20–50 μM) inhibited the stimulation by vasopressin of the accumulation of [^{3}H]inositol. The drug had little effect on vasopressin-induced accumulation of [^{3}H]inositol mono-, bis- and trisphosphates. In the absence of vasopressin, higher concentrations of quinacrine caused a small stimulation of glycogen phosphorylase activity, $^{45}Ca^{2+}$ release and the formation of [^{3}H]inositol polyphosphates. Quinacrine did not inhibit the degradation by liver homogenates of inositol 1-phosphate, inositol 4,5-bisphosphate or inositol 1,4,5-trisphosphate. It is concluded that concentrations of quinacrine comparable with those which inhibit phospholipase A_2 [G. J. Blackwell, W. G. Duncombe, R. J. Flower, M. F. Parsons and J. R. Vane, *Br. J. Pharmac.* **59**, 353–366 (1977)] inhibit the stimulation by vasopressin of inositol utilization without significantly affecting coupling between hormone receptors and adenyl cyclase or phosphoinositide-specific phosphodiesterase, the action of the phosphodiesterase, and the degradation of inositol triphosphate.

The physiological actions of vasopressin on hepatocytes are associated with stimulation of a phosphoinositide-specific phospholipase C [1, 2] and increases in the concentrations of inositol trisphosphates [3, 4], diacylglycerol [5, 6] and cytoplasmic free Ca^{2+} [7]. Considerable evidence which indicates that these agents act as intracellular messengers has now been obtained [3, 8–10]. In a number of cell types, the physiological actions of agonists which use these intracellular messengers are inhibited by quinacrine [11–19]. This ampiphilic compound binds tightly to phospholipids (reviewed in ref. 20) and inhibits a number of enzyme reactions (reviewed in refs 20–22), including phospholipases [12, 14, 23–26]. On the basis of the inhibition by quinacrine of the action of a number of agonists, including thrombin, on blood platelets and other cell types, it has been concluded that phospholipase A_2 is involved in the processes by which the agonists investigated induce cellular responses [11–19].

Quinacrine inhibits the actions of some agonists which bind to receptors coupled to phosphoinositol-specific phospholipase C [16, 18]. This suggests that quinacrine inhibits agonist-induced hydrolysis of phosphatidylinositol 4,5-bisphosphate, and hence might prove to be a useful tool in elucidation of the role of this reaction in the action of agonists on cells. The aim of the present experiments was to test whether quinacrine inhibits some of the early actions of vasopressin on hepatocytes, including the activation of phosphoinositol-specific phospholipase C. The results indicate that low concentrations of quinacrine inhibit vasopressin-stimulated inositol accumulation with little effect on the hydrolysis of phosphatidylinositol 4,5-bisphosphate or on the degradation of inositol 1,4,5-triphosphate. It is also shown that phospholipase A_2 is unlikely to be involved in the early actions of vasopressin on hepatocytes.

MATERIALS AND METHODS

Materials. Quinacrine hydrochloride, inositol trisphosphate and gelatin (swine skin, Type II) were obtained from the Sigma Chemical Co., St. Louis, MO; [^{3}H]*myo*-inositol, [^{14}C]inositol-1-phosphate, inositol-1-phosphate and $^{32}P_i$ from Amersham (Australia) Pty. Ltd., Surrey Hills, N.S.W., Australia; and Biorad AG 1-X8 (100–200 mesh) resin from Biorad Chemical Division, Richmond, CA. All other chemicals were of the highest grade available and were obtained from the sources described previously [5].

Methods. Hepatocytes were isolated from fed male rats as described by Hughes *et al.* [5]. Incubations were performed in cylindrical glass chambers in which the cells were maintained in suspension by a magnetic stirrer and continuously gassed with O_2/CO_2 (19:1) at 2 l/min [27] or in 20 ml sealed plastic pots, shaken at 90 oscillations per min (measurement of [^{3}H]inositol phosphates). The atmosphere in each pot was replenished with O_2/CO_2 (19:1) every

10 min. The incubation medium contained 117 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, 20 mM 2-([(2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino) ethansulphonic acid (TES)-NaOH, 1.3 mM $CaCl_2$ (except in experiments in which $^{45}Ca^{2+}$ release was measured) and hepatocytes (2×10^6 cells (30 mg wet wt.) per ml).

Rates of glucose release, cyclic AMP concentrations, $^{45}Ca^{2+}$ release at 0.1 mM extracellular Ca^{2+} [28], and initial rates of $^{45}Ca^{2+}$ exchange at 1.3 mM Ca^{2+} [27] were measured as described previously. The activity of glycogen phosphorylase *a* was measured as described by Hutson *et al.* [29]. One unit of activity is defined as one μmol [^{14}C]glucose-1-phosphate incorporated into glycogen per min per g wet wt. of cells [29].

Hepatocytes labelled with [^{3}H]inositol were prepared either by isolation of cells from the livers of rats which had received an intraperitoneal injection of [^{3}H]*myo*-inositol (100 μCi) [30] (*in vivo* labelling) or *in vitro* by the incubation of hepatocytes for 60 min in the presence of [^{3}H]*myo*-inositol (13 μCi/ml) [4] (*in vitro* labelling). For these incubations the medium also contained 15 mM glucose and 14 mg/ml gelatin. The concentration of cells was 54 mg wet wt/ml. The accumulation of trichloroacetic acid-soluble metabolites of [^{3}H]inositol in cells labelled with [^{3}H]inositol was measured as described by Prpic *et al.* [30]. The extraction and measurement of [^{3}H]inositol phosphates using anion exchange chromatography on Biorad AG 1-X8 resin [31, 32] to separate [^{3}H]-labelled inositol, inositol mono-, bis- and tris-phosphates were conducted as described by Thomas *et al.* [4]. Radioactivity was measured by liquid scintillation counting. At the concentrations employed, quinacrine did not cause significant quenching.

[4,5-^{32}P]Inositol 1,4,5-trisphosphate and [4-^{32}P]-inositol 1,4-bisphosphate were prepared from $^{32}P_i$-labelled human red blood cells [32] as described by Downes *et al.* [33]. The degradation of inositol phosphates by liver homogenates was measured as described by Storey *et al.* [34]. The hydrolysis of [4,5-^{32}P]inositol 1,4,5-trisphosphate (0.5 μM, 0.01 μCi/ml) was monitored by the decrease in [4,5-^{32}P]inositol 1,4,5-trisphosphate and by the formation of $^{32}P_i$; that of [4-^{32}P]inositol 1,4-bisphosphate (0.5 μM, 0.01 μCi/ml) by the decrease in [4-^{32}P]inositol 1,4-bisphosphate and by the formation of $^{32}P_i$; and that of [^{14}C]inositol 1-phosphate (25 μM, 0.04 μCi/ml) by the decrease in [^{14}C]inositol 1-phosphate and increase in [^{14}C]inositol. Except where indicated otherwise, the results are the means ± SE of the number of experiments indicate in parenthesis. Degrees of significance were determined using Student's *t*-test for paired samples. Values of $P < 0.05$ were considered to be not significant.

RESULTS

Glycogen metabolism and Ca^{2+} *transport*

Quinacrine inhibited the stimulation by vasopressin of glucose release and glycogen phosphorylase *a* activity (Fig. 1, Table 1). In the absence of vasopressin, quinacrine caused a small increase in phosphorylase activity (Fig. 1b). The time course for the activation of phosphorylase by 500 μM quinacrine is shown in Fig. 2. The effect of quinacrine was partially dependent on the presence of extracellular Ca^{2+} (Fig. 2). Quinacrine also inhibited glucose release induced by 8 μM adrenaline. The dose–response curve for quinacrine in the presence of adrenaline was similar to that obtained in the presence of vasopressin (results not shown). Half-maximal inhibition was observed at 40 μM quinacrine (Table 1). However, glucagon-stimulated glucose release was less sensitive to inhibition by quinacrine (Fig. 3a and Table 1). High concentrations of quinacrine were also required to inhibit the glucagon-stimulated increase in cyclic AMP (Fig. 3b and Table 1). *p*-Bromophenacylbromide, an inhibitor of phospholipase A_2 [35], had no effect on vasopressin-induced glucose release when tested in the range 0–100 μM (results not shown). In the absence of vasopressin this compound increased the rate of glucose release with 100 μM giving a 50% stimulation (results not shown).

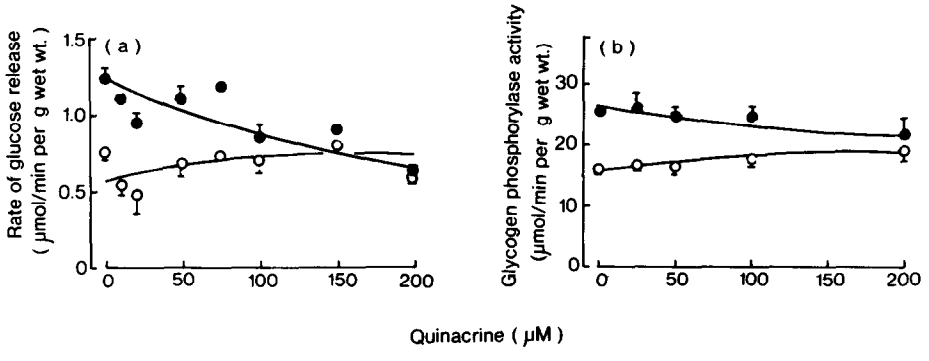

Fig. 1. Effects of quinacrine on glucose release (a) and glycogen phosphorylase *a* activity (b) in the absence and presence of vasopressin. Rates of glucose release and the activity of glycogen phosphorylase *a* were measured in the absence (○) or presence (●) of 13 nM vasopressin as described in Materials and Methods. Cells were added to the incubation medium and allowed to equilibrate for 10 min before the addition of quinacrine. After 5 min, vasopressin or vehicle was added and samples were removed for assay at 3 and 15 min (for calculation of the rates of glucose release) or 2 min (glycogen phosphorylase) after the addition of vasopressin. The results are the means ± SE of 3–6 experiments.

Table 1. Concentrations of quinacrine which give half-maximal inhibition of actions of vasopressin, adrenaline and glucagon on isolated hepatocytes*

Parameter measured	Hormone	Concentration of quinacrine which gives 50% inhibition (μM)
Glucose release	Vasopressin (13 nM)	120
	Adrenaline (8 μM)	90
	Glucagon (130 nM)	400
Glycogen phosphorylase activity	Vasopressin (13 nM)	200
$^{45}Ca^{2+}$ release	Vasopressin (13 nM)	250
	Adrenaline (8 μM)	40
Formation of [^{3}H]-labelled trichloroacetic acid-soluble metabolites of inositol	Vasopressin (10 nM)	20
Cyclic AMP concentration	Glucagon (130 nM)	250

* Values for the concentrations of quinacrine which gave 50% inhibition of the stimulation by hormone of the parameters tested were obtained from the following data: Figures 1a (glucose release), 1b (glycogen phosphorylase activity), 4 ($^{45}Ca^{2+}$ release) and 6 (formation of [^{3}H]-labelled acid-soluble metabolites of inositol) for the effects of vasopressin; and Figures 3a and 3b (glucose release and cyclic AMP, respectively) for the effects of glucagon. The values for the effects of adrenaline on glucose and $^{45}Ca^{2+}$ release were determined as described for vasopressin in the legends of Figs 1a and 4, respectively.

In cells incubated at 0.1 mM extracellular Ca^{2+}, addition of quinacrine alone induced the release of $^{45}Ca^{2+}$ (Fig. 4). The drug also inhibited the release of $^{45}Ca^{2+}$ induced by vasopressin (Fig. 4 and Table 1) or 8 μM adrenaline (results not shown). The plots obtained in the presence of adrenaline were similar to those obtained in the presence of vasopressin (results not shown). In the presence of adrenaline, half-maximal inhibition of $^{45}Ca^{2+}$ release was observed at 40 μM quinacrine (Table 1). The effects of quinacrine on $^{45}Ca^{2+}$ release in the presence and absence of vasopressin were near-maximal at 2 min exposure of the cells to quinacrine (results not shown). Quinacrine (150 μM) had no effect on the release of $^{45}Ca^{2+}$ induced by ionophore A23187 (results not shown). In cells incubated at 1.3 mM extracellular Ca^{2+}, concentrations of 50 and 100 μM quinacrine had no effect on the stimulation by vasopressin or adrenaline the initial rate of $^{45}Ca^{2+}$ exchange (results of three separate experiments, not shown).

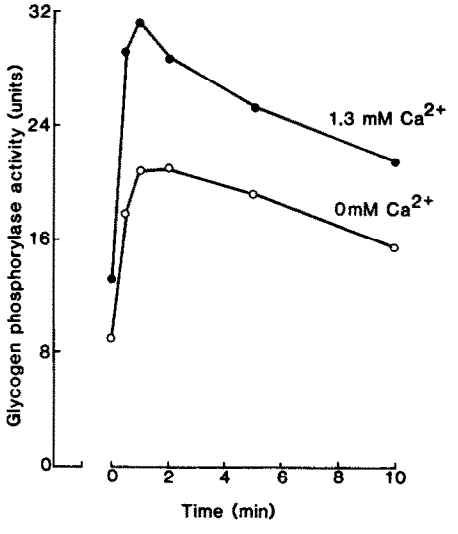

Fig. 2. Activation by a high concentration of quinacrine of glycogen phosphorylase *a*. Glycogen phosphorylase *a* activity was determined in cells incubated in the presence of 500 μM quinacrine (added at $t = 0$ min) in the absence (○) or presence (●) of 1.3 mM added extracellular Ca^{2+}, as described in Materials and Methods. The cells were equilibrated with incubation buffer for 15 min before addition of quinacrine. The results are the means of two experiments.

Phosphoinositide metabolism

As shown by others [30], vasopressin increases the formation of trichloroacetic acid-soluble metabolites in hepatocytes labelled with [^{3}H]inositol (Fig. 5a). This effect of vasopressin was almost completely inhibited by 100 μM quinacrine (Fig. 5b). The concentration of quinacrine which gave half-maximal inhibition was 20 μM (Fig. 6 and Table 1). The experiments described in Figs. 5 and 6 were conducted in the absence of Li^+. As reported previously [3, 4], in the presence of this cation, an inhibitor of the degradation of inositol monophosphate to inositol [36, 37], the stimulation by vasopressin of the rate of accumulation of acid-soluble metabolites of [^{3}H]inositol was much greater (Fig. 6, inset). Under these conditions, quinacrine also inhibited the action of vasopressin (inset (b) of Fig. 6). However, in the presence of Li^+ the fraction of the vasopressin-induced increase in acid-soluble metabolites of [^{3}H]inositol inhibited by quinacrine (inset (b) of Fig. 6) was considerably smaller than the fraction inhibited by quinacrine in the absence of Li^+ (inset (a) of Fig. 6).

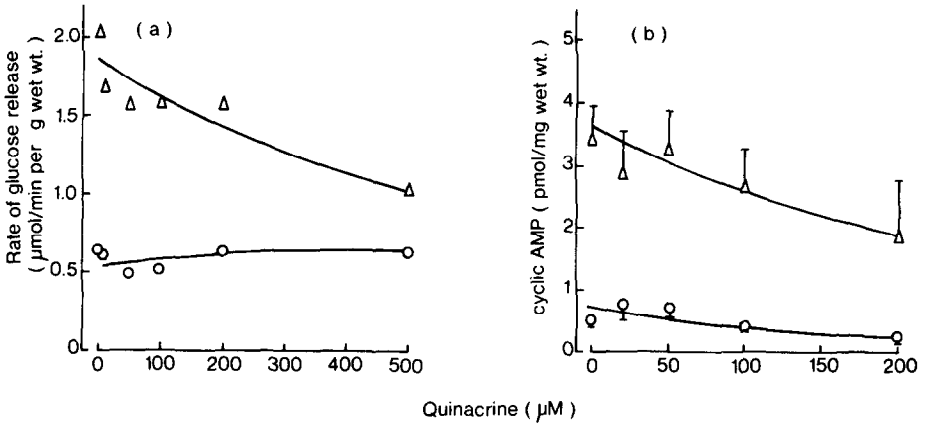

Fig. 3. Effects of quinacrine on glucose release (a) and cyclic AMP formation (b) in the presence and absence of glucagon. Rates of glucose release and cyclic AMP formation were measured in the absence (○) or presence (△) of 130 nM glucagon as described in Materials and Methods and in the legend of Fig. 1. For the measurement of cyclic AMP, cells were incubated in the presence of a given concentration of quinacrine for 5 min. Glucagon or vehicle was then added and samples removed for the assay of cyclic AMP after a further 2 min. The results are the means of two experiments (glucose release) and the means ± SE of three experiments (cyclic AMP).

Analysis of the inositol phosphates present in trichloroacetic acid extracts (Table 2) showed that, in the absence of Li^+, quinacrine inhibited the accumulation of [^{3}H]inositol induced by the treatment of cells with vasopressin for 30 min, but had no effect on vasopressin-induced increases in [^{3}H]InsP,* [^{3}H]InsP$_2$, and [^{3}H]InsP$_3$. In the absence of vasopressin, quinacrine caused small increases in [^{3}H]-labelled inositol polyphosphates (Table 2). Similar results were obtained for cells treated with vasopressin for 10 min (results not shown). When cell incubations were performed in the presence of 10 mM Li^+, it was also found that quinacrine did not inhibit the increase in [^{3}H]inositol trisphosphate induced by vasopressin. In the presence of the

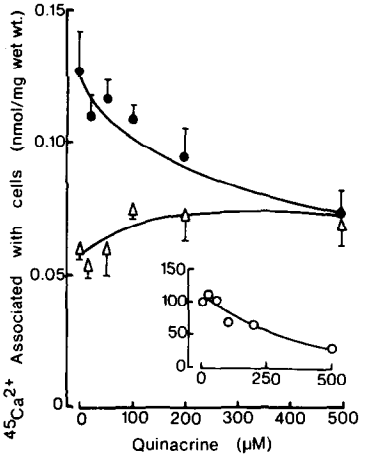

Fig. 4. Effects of quinacrine on the amount of $^{45}Ca^{2+}$ associated with hepatocytes incubated at 0.1 mM Ca^{2+} in the presence and absence of vasopressin. The amount of $^{45}Ca^{2+}$ associated with hepatocytes incubated in the absence (●) or presence (△) of 13 nM vasopressin was measured as described in Materials and Methods. Hepatocytes were incubated in the presence of 0.1 mM $^{45}Ca^{2+}$ for 28 min before the addition of quinacrine. After 2 min, vasopressin or vehicle was added, and after a further 4 min, samples were removed for measurement of the amount of $^{45}Ca^{2+}$ associated with the cells. The results are the means ± SE of 3–6 separate experiments. The inset shows the difference between the amount of $^{45}Ca^{2+}$ associated with cells in the absence and presence of vasopressin at a given concentration of quinacrine plotted as a function of the concentration of quinacrine. The difference is expressed as a percentage of the decrease induced by vasopressin in the absence of quinacrine.

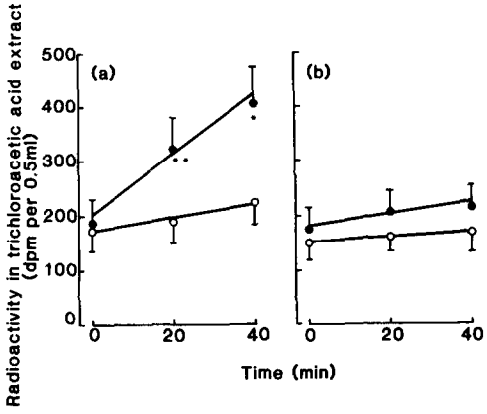

Fig. 5. Stimulation by vasopressin of the accumulation of metabolites of [^{3}H]inositol in trichloroacetic acid extracts of hepatocytes incubated in the absence (a) or presence (b) of quinacrine. The preparation of hepatocytes labelled with [^{3}H]inositol *in vivo*, incubation of labelled hepatocytes, and measurement of the amount of radioactivity in trichloroacetic acid extracts (0.5 ml) were performed as described in Materials and Methods. Cells labelled with [^{3}H]inositol were incubated for 5 min in the absence (a) and presence (b) of 100 μM quinacrine before the addition of vehicle (○) or 10 nM vasopressin (●) at 0 min. The values are the means ± SE for 4 separate experiments. The values of P, determined using Student's *t*-test for paired samples, are * $P < 0.01$; and ** $P < 0.005$.

* Abbreviations: InsP, inositol monophosphates; InsP$_2$, inositol bisphosphates; InsP$_3$, inositol trisphosphates.

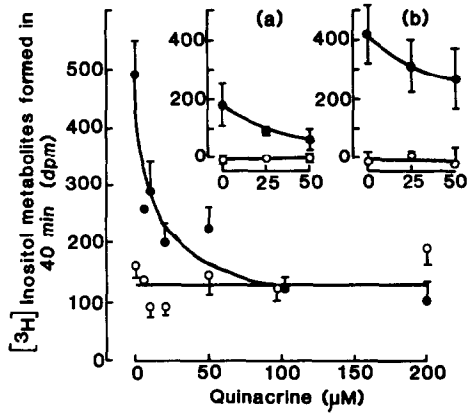

Fig. 6. Effect of increasing concentrations of quinacrine on the vasopressin-stimulated formation of acid-soluble metabolites of [^{3}H]inositol. The amount of radioactivity which accumulated in trichloracetic acid extracts (0.5 ml) over a period of 40 min was measured in cells incubated in the absence (○) and presence (●) of 10 nM vasopressin and in the presence of a given concentration of quinacrine as described in the legend of Fig. 5. For each incubation, the amount of radioactivity accumulated during the 40 min incubation period was calculated from linear regression analysis of the amount of radioactivity observed at 0, 20 and 40 min. The mean value for the amount of radioactivity present at 0 min was 490 ± 60 (N = 74) dpm per 0.5 ml trichloracetic acid. The results are the means ± SE of 3–7 separate experiments. The inset shows the effect of quinacrine in the absence (a) or presence (b) of 10 mM LiCl. Hepatocytes labelled with [^{3}H]inositol *in vitro* (Materials and Methods) were incubated for 25 min before the addition of quinacrine. After a further 4 min vehicle (○) or vasopressin (●) was added and samples removed at the times indicated. The mean value for the amount of radioactivity present at 0 min was 920 ± 70 (N = 46) dpm per 0.5 ml acid extract. The results are the means ± SE of 3 or 4 separate experiments.

hormone the amounts of [^{3}H]inositol trisphosphate were 166, 197, 208, and 218% (N = 2) of the controls at 0, 25, 50 and 100 μM quinacrine, respectively. Quinacrine (100 μM) had no effect on the rate of degradation of [^{14}C]InsP, [^{32}P]InsP$_2$ or [^{32}P]InsP$_3$ catalysed by liver homogenate (results not shown).

DISCUSSION

The concentrations of quinacrine which gave half-maximal inhibition of vasopressin-induced glucose release, glycogen phosphorylase activity and $^{45}Ca^{2+}$ release are comparable with those which inhibited glucagon-induced glucose release and cyclic AMP formation (Table 1). These results indicate that the inhibitory effect of quinacrine on these cellular responses is likely to be due to either inhibition of the binding of vasopressin or glucagon to the respective plasma membrane receptors, or to interference with the coupling between the receptors and phosphoinositide-specific phosphodiesterase or adenyl cyclase. The range of quinacrine concentrations which inhibited these processes is considerably higher than that which inhibits the action of phospholipase A$_2$ in intact cells [12, 14, 24, 25]. Moreover, the action of vasopressin on glucose release was not significantly inhibited by *p*-bromophenyacylbromide. These results indicate that a phospholipase A$_2$ enzyme sensitive to quinacrine is unlikely to be involved in the mechanism by which vasopressin alters Ca^{2+} distribution and activates glycogen phosphorylase in hepatocytes.

The concentrations of quinacrine which inhibited adrenaline-stimulated glucose and $^{45}Ca^{2+}$ release are lower than those which inhibited the action of vasopressin on these processes. This may reflect inhibition by quinacrine of the binding of adrenaline to α_1-adrenergic receptors since it has been shown that this process is inhibited by a variety of drugs [38–40]. The hyperbolic shape of the dose–response curves for inhibition by quinacrine of the parameters investigated suggests that the observed effects are caused by interaction of the drug at a single intracellular site. However, the results do not exclude the possibility that the effects of quinacrine are due to interaction at more than one site. In the case of adrenaline, one of these sites may be the α_1-adrenergic receptor.

At the concentrations of quinacrine which inhibited the vasopressin-stimulated accumulation of trichloroacetic acid-soluble metabolites of [^{3}H]inositol there was little effect on vasopressin-induced glucose release and Ca^{2+} transport. The latter effects of vasopressin involve the binding of the hormone to its receptor [41], activation of phosphoinositide-specific phosphodiesterase [1, 2], increased Ca^{2+} outflow from the endoplasmic reticulum [3, 4] and Ca^{2+} inflow across the plasma membrane [27], and activation by Ca^{2+} of calmodulin bound to glycogen phosphorylase kinase [42]. Therefore it may be concluded that in hepatocytes these processes are not significantly affected by quinacrine at the concentrations which inhibit the accumulation of acid-soluble metabolites of [^{3}H]inositol.

The results obtained with cells labelled with [^{3}H]inositol indicate that in cells treated with vasopressin and quinacrine the concentration of inositol is lower than that present in cells treated with vasopressin alone. The apparent resistance to complete inhibition by quinacrine of the accumulation of metabolites of [^{3}H]inositol measured in the presence of Li^+ (Fig. 6, inset b) may be due to the increased amounts of inositol mono- and poly-phosphates which accumulate in the presence of Li^+ [3, 4]. An effect of Li^+ on the accumulation of [^{3}H]inositol itself may also account for this resistance.

The observation that quinacrine did not affect the amounts of [^{3}H]-labelled inositol polyphosphates in cells treated with vasopressin indicates that at the concentrations employed the drug does not inhibit phosphoinositide-specific phospholipase C. Furthermore, the observation that, in the presence of vasopressin, quinacrine does not increase the amounts of [^{3}H]inositol polyphosphates in intact cells (cf. the increase induced by Li^+ [3, 4, 43, 44]); the failure of quinacrine (at concentrations which substantially decrease the amount of [^{3}H]inositol) to inhibit vasopressin-induced $^{45}Ca^{2+}$ release in intact cells; and the observation that quinacrine does not inhibit the degradation of InsP$_3$, InsP$_2$ or InsP *in vitro* indicate that quinacrine has little effect on the degradation

Table 2. Effect of quinacrine on the concentrations of [^{3}H]inositol and [^{3}H]inositol polyphosphates in hepatocytes labelled with [^{3}H]inositol and incubated in the absence of Li^+ in the absence and presence of vasopressin*

Metabolite	Amount of readioactivity (% of value in absence of quinacrine and vasopressin)		
	Quinacrine	Vasopressin	Quinacrine + vasopressin
Inositol	95 ± 1**	131 ± 4***	110 ± 1**
InsP	114 ± 9	131 ± 9*	129 ± 7*
$InsP_2$	115 ± 2*	157 ± 2**	157 ± 12**
$InsP_3$	122 ± 7***	520 ± 180***	510 ± 180****

* The preparation of hepatocytes labelled *in vitro* with [^{3}H]inositol, incubation of [^{3}H]inositol-labelled hepatocytes in the absence of Li^+, and the separation and measurement of [^{3}H]inositol and [^{3}H]inositol polyphosphates were performed as described in Materials and Methods. The incubation of hepatocytes labelled with [^{3}H]inositol was begun at 0 min, quinacrine (50 μM) or vehicle was added at 5 min, and vasopressin (13 nM) at 10 min. At 40 min samples were removed for the extraction and estimation of [^{3}H]inositol polyphosphates. For each experiment conducted in the presence of quinacrine, vasopressin, or quinacrine plus vasopressin, the amount of radioactivity present in a given inositol polyphosphate was expressed as a percentage of the value obtained in the absence of the agent. The mean values for the amounts of radioactivity present in the inositol, InsP, $InsP_2$ and $InsP_3$ fractions eluted from the anion exchange columns for samples incubated in the absence of both vasopressin and quinacrine were 62,000 ± 8000, 5300 ± 500, 2550 ± 490 and 114 ± 31 dpm per fraction (mean ± SE, N = 3), respectively. The values shown are the means ± SE of 3 experiments each conducted with a separate preparation of cells. The values of P for a comparison of the values obtained in the presence of quinacrine and/or vasopressin with the values obtained in the absence of these agents, are * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$ and **** $P < 0.005$. For the amount of radioactivity in [^{3}H]inositol, a value of $P < 0.005$ was obtained for comparison of the values obtained in the presence of vasopressin plus quinacrine with those obtained in the presence of vasopressin alone.

of inositol polyphosphates. It is concluded that the action of quinacrine is to stimulate one or more reactions which utilize inositol. These include the combination of inositol with CDP-diacylglycerol to form phosphatidylinositol. This conclusion is consistent with previous suggestions that quinacrine stimulates the synthesis of acidic phospholipids, including phosphoinositides [21, 22].

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REFERENCES

1. M. M. Billah and R. H. Michell, *Biochem. J.* **182**, 661 (1979).
2. J.A. Creba, C. P. Downes, P. T. Hawkins, G. Brewster, R. H. Michell and C. J. Kirk, *Biochem. J.* **212**, 733 (1983).
3. G. M. Burgess, P. P. Godfrey, J. S. McKinney, M. J. Berridge, R. F. Irvine and J. W. Putney Jr., *Nature, Lond.* **309**, 63 (1984).
4. A. P. Thomas, J. Alexander and J. R. Williamson, *J. biol. Chem.* **259**, 5574 (1984).
5. B. P. Hughes, K-A. Rye, L. B. Pickford, G. J. Barritt and A. H. Chalmers, *Biochem. J.* **222**, 535 (1984).
6. S. B. Bocckino, P. F. Blackmore and J. H. Exton, *J. biol. Chem.* **260**, 14201 (1985).
7. R. Charest, P. F. Blackmore, B. Berthon and J. H. Exton, *J. biol. Chem.* **258**, 8769 (1983).
8. F. D. Assimacopoulos-Jeannet, P. F. Blackmore and J. H. Exton, *J. biol. Chem.* **252**, 2662 (1977).
9. S. K. Joseph, A. P. Thomas, R. J. Williams, R. F. Irvine and J. R. Williamson, *J. biol. Chem.* **259**, 3077 (1984).
10. J. C. Garrison, D. E. Johnsen and C. P. Campanile, *J. biol. Chem.* **259**, 3283 (1984).
11. B. B. Vargaftig and N. Dao Hai, *J. Pharm. Pharmac.* **24**, 159 (1972).
12. G. J. Blackwell, W. G. Duncombe, R. J. Flower, M. F. Parsons and J. R. Vane, *Br. J. Pharmac.* **59**, 353 (1977).
13. Z. Naor and K. J. Catt, *J. biol. Chem.* **256**, 2226 (1981).
14. P. D. Winocour, R. L. Kinlough-Rathbone and J. F. Mustard, *Thrombos. Haemostas.* **45**, 257 (1981).
15. T. Takenawa, Y. Homma and Y. Nagai, *Biochem. J.* **208**, 549 (1982).
16. R. A. Frye and R. W. Holz, *Molec. Pharmac.* **23**, 547 (1983).
17. A. I. Tauber and E. R. Simons, *FEBS Lett.* **156**, 161 (1983).
18. A. Wada, S. Sakurai, H. Kobayashi, N. Yanagihara and F. Izumi, *Biochem. Pharmac.* **32**, 1175 (1983).
19. L. Best, A. Sener, P. C. F. Mathias and W. J. Malaisse, *Biochem. Pharmac.* **33**, 2657 (1984).
20. C. A. Dise, J. W. Burch and D. B. P. Goodman, *J. biol. Chem.* **257**, 4701 (1982).
21. A. A. Abdel-Latif, J. P. Smith and R. A. Akhtar, *Biochem. Pharmac.* **32**, 3815 (1983).
22. A. Erman, R. Azuri and A. Raz, *Biochem. Pharmac.* **32**, 2083 (1983).
23. R. J. Flower and G. J. Blackwell, *Biochem. Pharmac.* **25**, 285 (1976).
24. P. Mullorga, J. F. Tallman, R. C. Henneberry, F. Hirata, W. T. Strittmatter and J. Axelrod, *Proc. natn. Acad. Sci., U.S.A.*, **77**, 1341 (1980).
25. M. M. Billah, E. G. Lapetina and P. Cuatrecasas, *J. biol. Chem.* **256**, 5399 (1981).

26. A. C. Chan, E. T. Pritchard, J. M. Gerrard, R. Y. K. Man and P. C. Choy, *Biochim. biophys. Acta* **713**, 170 (1982).
27. G. J. Barritt, J. C. Parker and J. C. Wadsworth, *J. Physiol., Lond.* **312**, 29 (1981).
28. J. A. Whiting and G. J. Barritt, *Biochem. J.* **206**, 121 (1982).
29. N. J. Hutson, F. T. Brumley, F. D. Assimacopoulos, S. C. Harper and J. H. Exton, *J. biol. Chem.* **251**, 5200 (1976).
30. V. Prpic, P. F. Blackmore and J. H. Exton, *J. biol. Chem.* **257**, 11323 (1982).
31. M. J. Berridge, C. P. Downes and M. R. Hanley, *Biochem. J.* **206**, 587 (1982).
32. C. P. Downes and R. H. Michell, *Biochem. J.* **198**, 133 (1981).
33. C. P. Downes, M. C. Mussat and R. H. Michell, *Biochem. J.* **203**, 169 (1982).
34. D. J. Storey, S. B. Shears, C. J. Kirk and R. H. Michell, *Nature, Lond.* **312**, 374 (1984).
35. M. F. Roberts, R. A. Deems, T.C. Mincey and E.A. Dennis, *J. biol. Chem.* **252**, 2405 (1977).
36. W. F. Naccarato, R. E. Ray and W. W. Wells, *Archs Biochem. Biophys.* **164**, 194 (1974).
37. L. M. Hallcher and W. R. Sherman, *J. biol. Chem.* **255**, 10896 (1980).
38. P. F. Blackmore, M. F. El-Refai and J. H. Exton, *Molec. Pharmac.* **15**, 598 (1979).
39. P. H. Reinhart, W.M. Taylor and F. L. Bygrave, *Biochem. Internat.* **2**, 437 (1981).
40. H. J. Motulsky, A. S. Maisel, M. D. Snavely and P. A. Insel, *Circ. Res.* **55**, 376 (1984).
41. B. Cantau, S. Keppens, H. de Wulf and S. Jard, *J. Receptor Res.* **1**, 137 (1980).
42. T. D. Chrisman, J. E. Jordan and J. H. Exton, *J. biol. Chem.* **257**, 10798 (1982).
43. S. Palmer, P. T. Hawkins, R. H. Michell and C. J. Kirk, *Biochem. J.* **238**, 491 (1986).
44. J. S. Davis, L. L. Weakland, L. A. West and R. V. Farese, *Biochem. J.* **238**, 597 (1986).