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Chloroquine inhibits α_{1B}-adrenergic action in hepatocytes

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Abstract

Noradrenaline increased phosphorylase *a* activity through activation of α_{1B}-adrenoceptors in rat hepatocytes. Such effect was inhibited by chloroquine ($K_i \approx 55$ nM) and only slightly reduced by high concentrations of primaquine. Chloroquine did not inhibit the activation of phosphorylase *a* induced by vasopressin or angiotensin II. Binding competition experiments using [^{3}H]prazosin showed that both chloroquine and primaquine interact with α_{1B}-adrenoceptors, but only at very high concentrations. This indicates that the ability of chloroquine to block the α_{1B}-adrenergic action was not due to antagonism at the receptor level. Noradrenaline increased phosphatidylinositol resynthesis and inositol trisphosphate production; these effects were inhibited by chloroquine and phorbol 12-myristate 13-acetate. Staurosporine and Ro 31-8220 (3-[1-[3-(amidinothio)propyl-1H-indol-3-yl]-3-(1-methyl-1H-indol-3-yl)maleimide), reduced the inhibitions induced by the active phorbol ester and the antimalarial drug on adrenergic-stimulated phosphatidylinositol resynthesis. Similarly, staurosporine blocked the inhibitory actions of chloroquine and phorbol 12-myristate 13-acetate on noradrenaline-stimulated inositol trisphosphate production. These data suggest the possibility that protein kinases, such as protein kinase C, could be involved in the actions of chloroquine. © 1998 Elsevier Science B.V.

Keywords: α_{1B}-Adrenoceptor; Hepatocyte; Chloroquine; Protein kinase C

1. Introduction

The natural adrenergic amines, adrenaline and noradrenaline, are among the main modulators of liver metabolism (Hems and Whitton, 1980), both α_1 and β_2-adrenoceptors seem to mediate such modulation. The relative roles of these adrenoceptors and their densities in hepatocytes vary considerably among species (Sulakhe et al., 1988). In hepatocytes, from normal adult rats, α_1-adrenoceptors of the α_{1B} subtype (Han et al., 1987; García-Sáinz et al., 1992; García-Sáinz et al., 1994) are the main mediators of catecholamine actions. Activation of α_{1B}-adrenoceptors leads to phosphoinositide turnover (hydrolysis of phosphatidylinositol 4,5-bisphosphate, with production of the second messengers, diacylglycerol and IP_3 (inositol 1,4,5-trisphosphate), and subsequent resynthesis of phosphoinositides) and Ca^{2+} signaling (García-Sáinz, 1987; García-Sáinz, 1993; Minneman and Esbenshade, 1994; Graham et al., 1996).

Interestingly, activation of protein kinase C with active phorbol esters, such as phorbol 12-myristate 13-acetate (PMA) blocks immediately α_1-adrenergic actions, in cells such as hepatocytes (Corvera and García-Sáinz, 1984; Cooper et al., 1985; Lynch et al., 1985; García-Sáinz et al., 1985; Van de Werve et al., 1985; Corvera et al., 1986), smooth muscle cells (Leeb-Lundberg et al., 1985, 1987; Bouvier et al., 1987) or fibroblasts (Lattion et al., 1994; Diviani et al., 1996; Vázquez-Prado and García-Sáinz, 1996). This effect is associated to receptor phosphorylation (Leeb-Lundberg et al., 1985, 1987; Bouvier et al., 1987; Lattion et al., 1994; Diviani et al., 1996). This action of protein kinase C seems to be physiologically relevant in homologous and heterologous desensitization of the α_1-adrenergic action (García-Sáinz et al., 1986; Bouvier et al., 1987; Cowlen and Toews, 1988; Diviani et al., 1996). Although, other protein kinases also participate in these processes (Leeb-Lundberg et al., 1987; Lattion et al., 1994).

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Activation of protein kinase C not only induces the blockade/desensitization of α_1-adrenoceptors but also alters the subcellular distribution of these receptors in some cells (Lynch et al., 1985; Cowlen and Toews, 1988; Jagadeesh and Deth, 1988; Beeler and Cooper, 1995; Fonseca et al., 1995). Fonseca et al. (1995) have elegantly shown that PMA induces internalization to endosomes of α_{1b}-adrenoceptors stably expressed in embryonal kidney 293 cells. This effect is also induced by agonists and, interestingly, it is blocked by staurosporine, an inhibitor of protein kinase C, which suggests a physiological role of protein kinase C in agonist-induced receptor internalization (Fonseca et al., 1995).

Lysosomotropic agents, such as chloroquine and primaquine, are frequently used to study receptor endocytosis and recycling (Mellman et al., 1986). These weak bases seem to act by neutralizing the pH of endocytic vesicles which inhibits receptor recycling (Mellman et al., 1986). It has been shown that treatment of hepatocytes with primaquine induces a relatively rapid decrease ($\approx 30\%$) of plasma membrane α_1-adrenoceptors (Beeler and Cooper, 1995). Interestingly, the effects of primaquine and PMA on this parameter do not seem to be additive (Beeler and Cooper, 1995). Here, we show that chloroquine, inhibits the α_{1B}-adrenergic action in hepatocytes; our results suggest that protein kinase(s), such as protein kinase C, may be involved.

2. Materials and methods

L-Noradrenaline, DL-propranolol, chloroquine, primaquine, PMA, [Arg^8]vasopressin, angiotensin II, glucose-L-phosphate, glycogen, digitonin, caffeine, β-glycerophosphate and staurosporine, were obtained from Sigma Chemical. Collagenase was from Worthington and bovine serum albumin (fraction V) from Armour. Ro 31-8220 (3-[1-[3-(amidinothio)propyl-1H-indol-3-yl]-3-(1-methyl-1H-ndol-3-yl)maleimide) was obtained from Calbiochem. α-D-[^{14}C]Glucose-1-phosphate (313 mCi/mmol), [^{3}H]prazosin (71.8 Ci/mmol), [^{32}P]Pi (carrier free) and inositol-1,4,5-trisphosphate radioreceptor assay kits were from New England Nuclear. Phentolamine was a generous gift from Ciba–Geigy.

Hepatocytes were isolated, by the method of Berry and Friend (1969), from male Wistar rats (200–250 g) fed ad libitum. The cells (30–40 mg wet weight) were incubated in 1 ml of Krebs–Ringer bicarbonate buffer, under an atmosphere of 95% O_2/5% CO_2, pH 7.4 at 37°C in a water bath shaker as described (Corvera and García-Sáinz, 1984).

To quantify phosphorylase a activity, the cells were preincubated for 20 min in Krebs–Ringer bicarbonate buffer containing 10 mM glucose. Agents were added to the cells 1 min before stopping the reaction. Phosphorylase *a* activity was assayed as described by Stalmans and Hers (1975); activity is expressed in units, one unit is defined as the conversion of 1 μmol of substrate to product in 1 min per g of cells wet weight.

Phosphatidylinositol labeling was performed, as described (Corvera et al., 1986), by incubating the cells for 60 min in buffer supplemented with 10 μCi/ml of [^{32}P]Pi. At the end of the incubation lipids were extracted with chloroform/methanol (2:1) and phospholipids separated by one-dimensional thin-layer chromatography (García-Sáinz and Fain, 1980).

Production of IP_3 was quantified using a commercial radioreceptor kit following the manufacturer instructions with minor modifications. In brief, cell suspensions (1 ml containing 50 mg of cells wet weight) were incubated in presence of the agents to be tested. Reactions were stopped at 15 s or the indicated times by adding ice-cold perchloric acid (6% final concentration). Acidified samples were vortexed and kept on ice for 1 h before centrifugation at $2000 \times g$ for 15 min. Supernatants were removed and neutralized with 1.5 M KOH/75 mM HEPES; the neutralized supernatants were used for quantification, employing a commercial IP_3 radioreceptor assay kit.

Partially purified liver plasma membranes, were obtained by the method of Neville (1968). Membranes were washed and resuspended in 50 mM Tris, 10 mM $MgCl_2$, pH 7.5; aliquots of the membrane preparation were frozen and stored under liquid nitrogen until used (usually within two weeks), without any noticeable change in receptor binding. Radioligand binding studies were performed as described (García-Sáinz et al., 1994) by incubating membranes (75–100 μg protein) in 50 mM Tris, 10 mM $MgCl_2$, pH 7.5 with the radioactive ligand, alone or with the indicated agents. Incubations were in a total volume of 0.25 ml for 60 min in a water bath shaker at 25°C. At the end of the incubation, 2.5 ml of ice-cold buffer were added to the membrane suspension, the membranes were immediately filtered on GF/C filters and washed three-times (2.5 ml each time) with the same buffer. Saturation experiments were performed using concentrations between 0.05–12 nM [^{3}H]prazosin and binding competition studies with 1–1.5 nM of the radioactive ligand (75–85% receptor occupation). Non-specific binding was evaluated in the presence of 10 μM phentolamine; specific binding represented 80–90% of the total binding at the K_d. Binding saturation and competition data were analyzed using the EBDA and LIGAND (Munson and Rodbard, 1980) programs (Elsevier-Biosoft). K_i values were calculated according to Cheng and Prusoff (1973). Protein was quantified by the method of Lowry et al. (1951) using bovine serum albumin as standard.

3. Results

Noradrenaline (10 μM propranolol to block its β-adrenergic activity, was used in all the experiments in

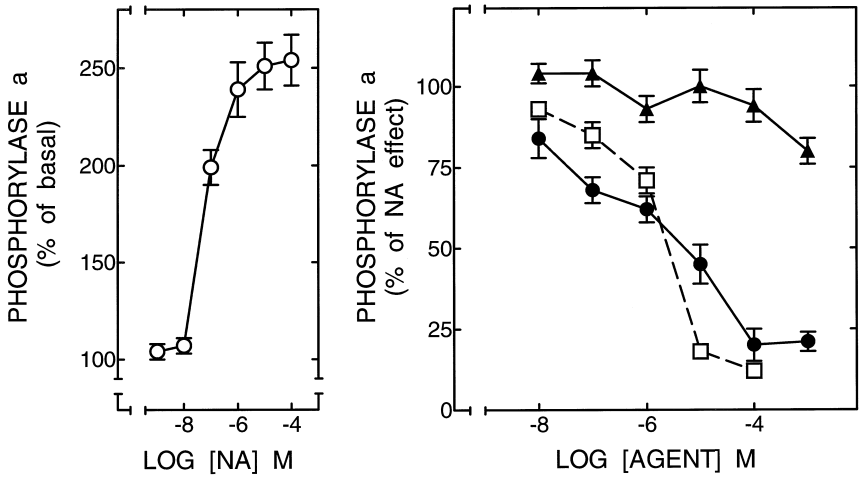

Fig. 1. Effect of noradrenaline, phentolamine, chloroquine and primaquine on phosphorylase a activity in isolated rat hepatocytes. Hepatocytes were incubated in the presence of 10 μM propranolol and different concentrations of noradrenaline (open circles) (left panel) or with 10 μM noradrenaline plus different concentrations of phentolamine (open squares), chloroquine (solid circles) or primaquine (solid triangles). Data are presented as percent of basal phosphorylase *a* activity (which was 20 ± 1 units, left panel) or as percent or noradrenaline effect (right panel). Plotted are the means and vertical lines represent the S.E.M. of 5–7 experiments using different cell preparations.

which this catecholamine was employed) induced a dose-dependent ($EC_{50} \approx 75$ nM) increase in phosphorylase *a* activity (Fig. 1, left panel). The increase in phosphorylase *a* activity induced by 10 μM noradrenaline was dose-dependently inhibited by phentolamine ($IC_{50} \approx 2$ μM, $K_i \approx$ 15 nM). Interestingly, the antimalarial drug, chloroquine, also inhibited in a dose-dependent fashion the adrenergic effect ($IC_{50} \approx 7.5$ μM, $K_i \approx 55$ nM) (Fig. 1, right panel). Primaquine, in contrast, induced a very modest decrease in the α_{1B}-adrenergic action and only at very high concentrations (Fig. 1, right panel). Neither the lysosomotropic agents, chloroquine and primaquine, nor the adrenergic

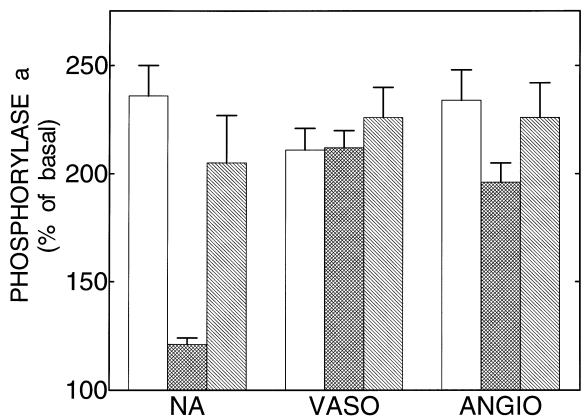

Fig. 2. Effects of chloroquine and primaquine on the stimulations of phosphorylase a activity induced by noradrenaline, vasopressin and angiotensin II. Hepatocytes were incubated with 10 μM noradrenaline plus 10 μM propranolol (NA), 10 nM vasopressin (VASO) or 100 nM angiotensin II (ANGIO) in the absence (open bars) or presence of 100 μM chloroquine (crisscrossed bars) or 100 μM primaquine (dashed bars). Plotted are the means and vertical lines represent the S.E.M. of 6–7 experiments using different cell preparations. Other indications as in Fig. 1.

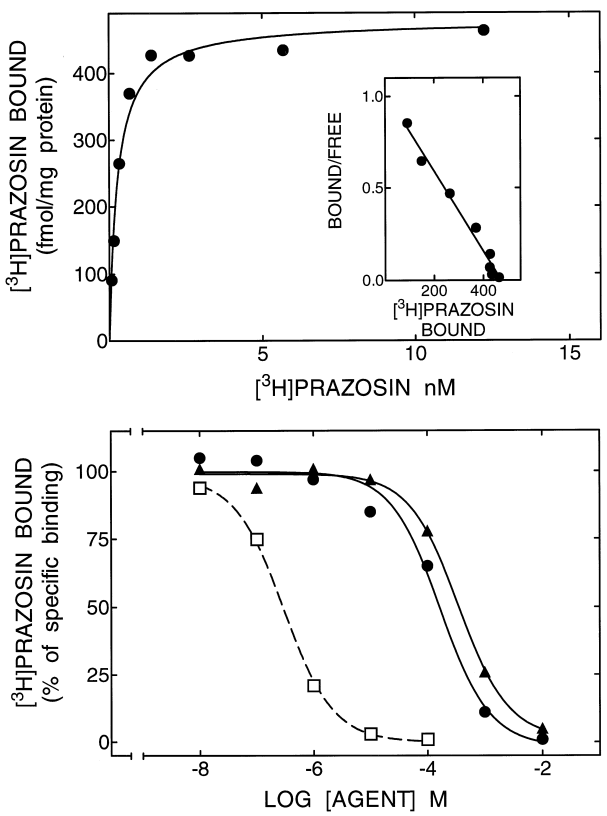

Fig. 3. [^{3}H]Prazosin saturation isotherm and binding competition studies. A saturation isotherm and the Rosenthal transformation of the data is presented in the upper panel. In the lower panel, binding competition studies using different concentrations of phentolamine (open squares), chloroquine (solid circles) and primaquine (solid triangles) are presented. The figures are representative of 4 (saturation) and 6–7 (competition) experiments using different membrane preparations.

antagonist, phentolamine, had any effect on basal phosphorylase activity at the concentrations tested (data not shown).

As shown in Fig. 2, vasopressin (10 nM) and angiotensin II (100 nM) were also able to induce marked increases in phosphorylase *a* activity. These effects of vasopressin and angiotensin II were not significantly affected by either chloroquine or primaquine, indicating that the effect of chloroquine was not general on hormone action but rather selective for the α_{1B}-adrenergic action.

The possibility that the effect of chloroquine could be mediated through its interaction with the α_{1B}-adrenoceptor was tested by performing binding competition experiments using partially purified liver plasma membranes. In agreement with previous findings (García-Sáinz et al., 1994), [^{3}H]prazosin bound rapidly, reversible and in a saturable fashion to liver plasma membranes. A representative saturation isotherm and the Rosenthal transformation of the data are presented in Fig. 3. The data indicate that the radioligand binds to a homogeneous type of sites with a B_{max} of 433 ± 35 fmol/ mg of membrane protein and a K_d of 0.18 ± 0.03 nM (means $\pm$ S.E.M. $n = 4$ in both cases). Binding competition experiments indicate that

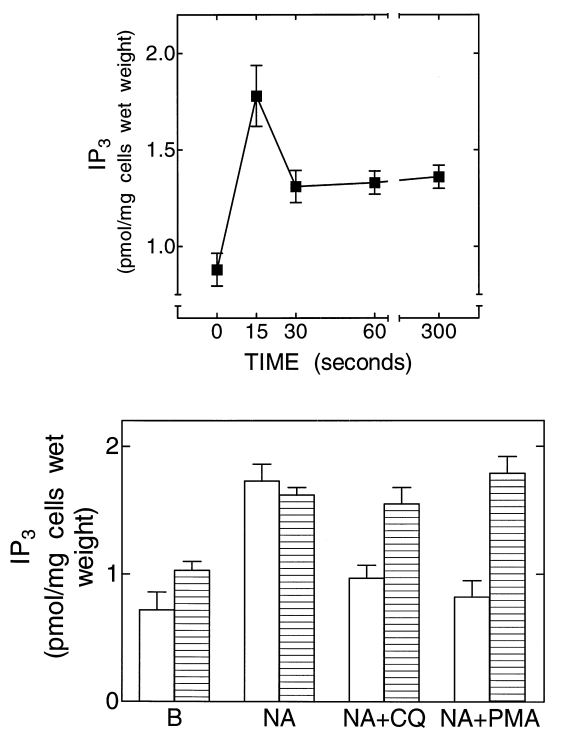

Fig. 4. Effect of noradrenaline, chloroquine and PMA on IP_3 production. Upper panel: Hepatocytes were incubated for the times indicated in the presence of 10 μM propranolol and 10 μM noradrenaline. Plotted are the means and vertical lines represent the S.E.M. of 3 experiments using different cell preparations. Lower panel: hepatocytes were preincubated for 20 min in the absence (open bars) or presence of 3 μM staurosporine (bars filled with horizontal lines) and further incubated for 15 s in the absence of any other agent (B) or presence of 10 μM propranolol plus 10 μM noradrenaline alone (NA) or with 100 μM chloroquine (NA + CQ) or 1 μM PMA (NA + PMA). Plotted are the means $\pm$ S.E.M. of 6 experiments.

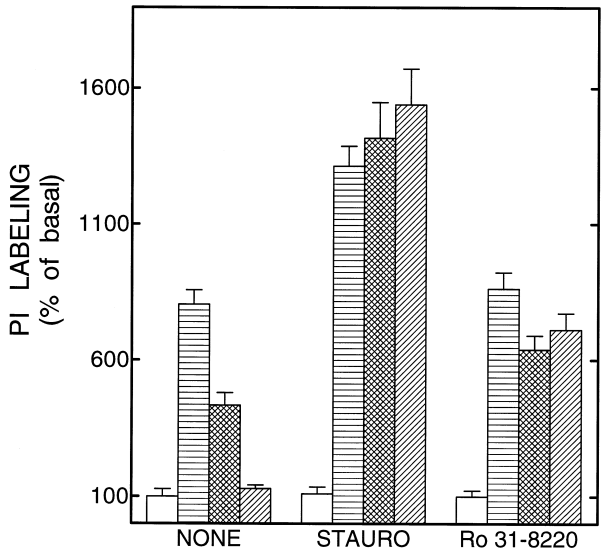

Fig. 5. Effect of staurosporine on the actions of chloroquine and PMA on the α_{1B}-adrenergic-mediated stimulation of phosphatidylinositol (PI) labeling. Hepatocytes were preincubated for 20 min in the absence (left group of bars, NONE) or presence of 3 μM staurosporine (middle group of bars, STAURO) or 10 μM RO 31-8220 (right group of bars, Ro 31-8220) and further incubated for 60 min in the absence of any other agent (open bars) or presence of 10 μM propranolol plus 10 μM noradrenaline alone (bars filled with horizontal lines); 10 μM propranolol plus 10 μM noradrenaline and 100 μM chloroquine (crisscrossed bars) or 10 μM propranolol plus 10 μM noradrenaline and 1 μM PMA (dashed bars). Basal phosphatidylinositol labelings were: 1487 ± 426 cpm/40 mg cells wet weight for cells preincubated in the absence of protein kinase inhibitors; 1549 ± 390 cpm/mg cells wet weight for cells preincubated in the presence of staurosporine, and 1560 ± 172 cpm/mg cells wet weight for cells preincubated in the presence of Ro 31-8220. Plotted are the means $\pm$ S.E.M. of 7–9 experiments.

phentolamine (K_i 53 ± 8 nM, slope 1.02 ± 0.06, $n = 7$), chloroquine (K_i 36 ± 4 μM, slope 1.05 ± 0.02, $n = 6$) and primaquine (K_i 74 ± 7 μM, slope 1.09 ± 0.03, $n = 6$) interacted with the α_{1B}-adrenoceptors, although the lysosomotropic agents only did it at very high concentrations.

Since chloroquine affinity for the α_{1B}-adrenoceptors was extremely low, other possibilities to explain its ability to block the adrenergic action were considered. The effect of chloroquine resembles that of PMA and therefore, the possibility that protein kinase C could be involved was tested through the use of inhibitors, such as staurosporine. However, staurosporine markedly interfered the hormonal activation of phosphorylase (data not shown) in agreement with the report of Gali et al. (1993).

Due to this, other parameters were tested. Noradrenaline induced a very rapid (Fig. 4, upper panel) increase in IP_3 production. This effect of noradrenaline was markedly decreased by chloroquine and PMA (Fig. 4, lower panel). Preincubation with 3 μM staurosporine increased slightly but consistently basal IP_3 (Fig. 4, lower panel). Nevertheless, under these conditions noradrenaline increased IP_3 production, in a magnitude similar to that observed in the absence of the protein kinase C inhibitor (Fig. 5). Interest-

ingly, when the cells were preincubated with staurosporine, the α_{1B}-adrenergic-mediated increase in IP_3 production was not inhibited by either chloroquine or PMA (Fig. 4, lower panel). Neither chloroquine nor PMA alter basal IP_3 production (data nor shown).

In order to confirm these results, we studied phosphatidylinositol resynthesis (phosphatidylinositol labeling). Noradrenaline induced a very big ($\approx$ 8-fold) increase in phosphatidylinositol labeling which was markedly decreased by chloroquine and essentially abolished by PMA (Fig. 5). Primaquine did not affect the α_{1B}-adrenergic effect (data not shown). In cells preincubated with staurosporine, there was no change in basal phosphatidylinositol labeling and the effect of noradrenaline was even bigger (14-fold). Consistent with the previous findings, in cells preincubated with staurosporine neither chloroquine nor PMA inhibited the effect of noradrenaline on phosphatidylinositol labeling (Fig. 5). Neither chloroquine nor PMA alter basal phosphatidylinositol labeling or IP_3 production (data nor shown). The effect of another protein kinase C inhibitor, Ro 31-8220, was also tested. This inhibitor affected neither basal phosphatidylinositol labeling nor the α_{1B}-adrenergic effect but markedly reduced the inhibitions induced by chloroquine and PMA (Fig. 5).

4. Discussion

The effect of the antimalarial lysosomotropic agents, chloroquine and primaquine, on the hormonal responsiveness of hepatocytes was examined. Our results clearly show that chloroquine, and to a much lesser extent primaquine, block the α_{1B}-adrenergic action in isolated rat hepatocytes. In contrast, the effect of the vasopressor peptides, vasopressin and angiotensin II was not significantly altered. These data indicate that the action of chloroquine was not due to general toxicity to the liver cells, perturbating their metabolism, but rather to a more selective action.

α_{1B}-Adrenergic agents, angiotensin II and vasopressin activate the phosphoinositide turnover/Ca^{2+} mobilization signal transduction pathway in a similar fashion (García-Sáinz, 1987). Their specific receptors seem to share the membrane molecular entities involved in such activation, i.e., G proteins and phospholipase C. Since the effect of chloroquine was selective, we considered the possibility that it could be taking place at the level of the α_{1B}-adrenoceptors. Binding competition experiments clearly indicated that chloroquine and primaquine could interact, although only at very high concentrations, with these receptors. Several arguments indicated that antagonism at the receptor level was not a good explanation for the effect of chloroquine on α_{1B}-adrenergic action. Firstly, there was a good relationship between the K_i values obtained with phentolamine in the binding competition (53 nM) and metabolic studies (15 nM); which was not the case with chloroquine, where a 650-fold difference was observed. Secondly, this enormous difference in K_i values suggested that chloroquine induced a 50% inhibition of the adrenergic effects at a concentration at which hardly any interaction with the receptor could have taken place. Thirdly, the marked difference in potency between chloroquine and primaquine, observed in the metabolic studies was not observed in the binding competition studies. Later, the studies using staurosporine and Ro 31-8220 also argue against the possibility that chloroquine exerted their effects by acting as an adrenergic antagonist.

The similarity of the action of chloroquine with that of PMA (Corvera and García-Sáinz, 1984) prompted us to test a possible role of protein kinase C. We used staurosporine, a protein kinase C inhibitor, for this purpose. Our initial experiments were frustrated by the inhibitory effect of staurosporine itself on the hormonal activation of phosphorylase (Gali et al., 1993). However, staurosporine clearly blocked the inhibition induced by chloroquine and PMA on the α_{1B}-adrenergic-mediated stimulation of phosphoinositide turnover (IP_3 production and phosphatidylinositol labeling). Ro 31-8220, a relatively more selective inhibitor of protein kinase C (Dieter and Fitzke, 1991) also attenuated the inhibitory action of chloroquine and PMA on the α_{1B}-adrenergic-mediated stimulation of phosphatidylinositol labeling. These data suggest a role of protein kinase C activation in the inhibitory actions of chloroquine. However, staurosporine and Ro 31-8220 are only relatively selective and also affect the activity of other protein kinases (Hofmann, 1997). Therefore, we can not rule out the possibility that other kinase(s) may participate in these effects. Further experimentation will be required to address these aspects.

Chloroquine and primaquine are frequently used to perturbate membrane trafficking by alkalinizing intracellular vesicles (Mellman et al., 1986). Our results indicate that caution should be exercised when these agents are used and its possible effect on protein kinases should be considered.

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References

Beeler, J.F., Cooper, R.H., 1995. Regulation of hepatocyte plasma membrane α1-adrenergic receptors by 4β-phorbol 12-myristate 13-acetate. Biochem. J. 305, 73–79.

Berry, M.N., Friend, D.S., 1969. High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. J. Cell. Biol. 43, 506–520.

Bouvier, M., Leeb-Lundberg, L.M.F., Benovic, J.L., Caron, M.G., Lefkowitz, R.J., 1987. Regulation of adrenergic receptor function by phosphorylation. II. Effects of agonist occupancy on phosphorylation of α1- and β2-adrenergic receptors by protein kinase C and the cyclic AMP-dependent protein kinase. J. Biol. Chem. 262, 3106–3113.

Cheng, Y.-C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Cooper, R.H., Coll, K.E., Williamson, J.R., 1985. Differential effects of phorbol ester on phenylephrine and vasopressin-induced Ca^{2+} mobilization in isolated hepatocytes. J. Biol. Chem. 260, 3281–3288.

Corvera, S., García-Sáinz, J.A., 1981. Phorbol esters inhibit α1-adrenergic regulation of hepatocyte metabolism. Biochem. Biophys. Res. Commun. 119, 1128–1133.

Corvera, S., Schwarz, K.R., Graham, R.M., García-Sáinz, J.A., 1986. Phorbol esters inhibit α1-adrenergic effects and decreases the affinity of liver α1-adrenergic receptors for epinephrine. J. Biol. Chem. 261, 520–526.

Cowlen, M.S., Toews, M.L., 1988. Evidence for alpha1-adrenergic receptor internalization in DDT1 MF-2 cells following exposure to agonists plus protein kinase C activators. Mol. Pharmacol. 34, 340–346.

Dieter, P., Fitzke, E., 1991. Ro 31-8220 and Ro 31-7549 show improved selectivity for protein kinase C over staurosporine in macrophages. Biochem. Biophys. Res. Commun. 181, 396–401.

Diviani, D., Lattion, A.L., Larbi, N., Kunapuli, P., Pronin, A., Benovic, J.L., Cotecchia, S., 1996. Effect of different G protein-coupled receptor kinases on phosphorylation and desensitization of the α_{1B}-adrenergic receptor. J. Biol. Chem. 271, 5049–5058.

Fonseca, M.I., Button, D.C., Brown, R.D., 1995. Agonist regulation of α_{1B}-adrenergic receptor subcellular distribution and function. J. Biol. Chem. 270, 8902–8909.

Gali, R.R., Pugazhenthi, S., Khandelwal, R., 1993. Reciprocal effects of the protein kinase C inhibitors Staurosporine and H-7 on the regulation of glycogen synthase and phosphorylase in the primary culture of hepatocytes. Metabolism 42, 1475–1480.

García-Sáinz, J.A., 1987. Pathways of the α1-adrenergic action. Comparison with V1-vasopressin and A1-angiotensin. Circ. Res. 61 (suppl. II), 1–5.

García-Sáinz, J.A., 1993. α1-Adrenergic action: Receptor subtypes, signal transduction and regulation. Cell. Sign. 5, 539–547.

García-Sáinz, J.A., Fain, J.N., 1980. Effect of insulin, catecholamines and calcium ions on phospholipid metabolism in isolated white fat cells. Biochem. J. 186, 781–789.

García-Sáinz, J.A., Mendlovic, F., Martínez-Olmedo, M.A., 1985. Effect of phorbol esters on α_1-adrenergic-mediated and glucagon-mediated acations in isolated hepatocytes. Biochem. J. 228, 277–280.

García-Sáinz, J.A., Hernández-Sotomayor, S.M.T., Tussie-Luna, M.I., 1986. Homologous and heterologous desensitization of one of the pathways of the α_1-adrenergic action. Effects of epinephrine,vasopressin, angiotensin II and phorbol 12-myristate 13-acetate. Biochim. Biophys. Acta 887, 73–79.

García-Sáinz, J.A., Romero-Avila, M.T., Alcántara-Hernández, R., Macías-Silva, M., Olivares-Reyes, J.A., González-Espinosa, C., 1992. Species heterogeneity of hepatic α_1-adrenoceptors: α_{1A}, α_{1B} and α_{1C} subtypes. Biochem. Biophys. Res. Commun. 186, 760–767.

García-Sáinz, J.A., Casas-González, P., Romero-Avila, M.T., González-Espinosa, C., 1994. Characterization of the hepatic α_{1B}-adrenoceptors of rats, mice and hamsters. Life Sci. 54, 1995–2003.

Graham, R.M., Perez, D.M., Hwa, J., Piasck, M.T., 1996. α_1-Adrenergic receptor subtypes. Molecular structure, function and signalling. Circ. Res. 78, 737–749.

Han, C., Abel, P.W., Minneman, K.P., 1987. Heterogeneity of α1-adrenergic receptors revealed by chlorethylclonidine. Mol. Pharmacol. 32, 505–510.

Hems, D.A., Whitton, P.D., 1980. Control of hepatic glycogenolysis. Physiol. Rev. 60, 1–50.

Hofmann, J., 1997. The potential for isoenzyme-selective modulation of protein kinase C. FASEB J. 11, 649–669.

Jagadeesh, G., Deth, R.C., 1988. Phorbol ester-induced modulation of agonist binding to alpha-1 adrenergic receptors in bovine aortic membranes. J. Pharmacol. Exp. Ther. 247, 196–202.

Lattion, A.-L., Diviani, D., Cotecchia, S., 1994. Truncation of the receptor caboxyl terminus impairs agonist-dependent phosphorylation and desensitization of the α_{1B}-adrenergic receptor. J. Biol. Chem. 269, 22887–22893.

Leeb-Lundberg, L.M.F., Cotecchia, S., DeBlasi, A., Caron, M.G., Lefkowitz, R.J., 1987. Regulation of adrenergic receptor function by phosphorylation. I. Agonist-promoted desensitization of α1-adrenoceptors coupled to inositol phospholipid metabolism in DDT1 MF-2 smooth muscle cells. J. Biol. Chem. 262, 3098–3105.

Leeb-Lundberg, L.M.F., Cotecchia, S., Lomasney, J.W., DeBernardis, J.F., Lefkowitz, R.J., Caron, M.G., 1985. Phorbol esters promote α1-adrenergic receptor phosphorylation and receptor uncoupling from inositol phospholipid metabolism. Proc. Natl. Acad. Sci. USA 82, 5651–5655.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lynch, C.J., Charest, R., Bocchino, S.B., Exton, J.H., Blackmore, P.F., 1985. Inhibition of hepatic α1-adrenergic effects and binding by phorbol myristate acetate. J. Biol. Chem. 260, 2844–2851.

Mellman, I., Fuchs, R., Helenius, A., 1986. Acidification of the endocytic and exocytic pathways. Ann. Rev. Biochem. 55, 663–700.

Minneman, K.P., Esbenshade, T.A., 1994. α1-Adrenergic receptors subtypes. Ann. Rev. Pharmacol. Toxicol. 34, 117–133.

Munson, P.J., Rodbard, D., 1980. LIGAND: A versatil computerized approach for the characterization of ligand binding systems. Anal. Biochem. 107, 220–239.

Neville, J.D.M., 1968. Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. Biochim. Biophys. Acta 154, 540–552.

Stalmans, W., Hers, H.-G., 1975. The stimulation of liver phosphorylase b by AMP, fluoride and sulfate. A technical note on the specific determination of the a and b forms of liver glycogen phosphorylase. Eur. J. Biochem. 54, 341–350.

Sulakhe, S.J., Pulga, V.B., Tran, S., 1988. Hepatic α1- and β-adrenergic receptors in various animal species. Mol. Cell. Biochem. 83, 81–88.

Van de Werve, G., Proietto, J., Jeanrenaud, B., 1985. Control of glycogen phosphorylase interconversion by phorbol esters, diacylglycerols, Ca^{2+} and hormones in isolated rat hepatocytes. Biochem. J. 231, 511–516.

Vázquez-Prado, J., García-Sáinz, J.A., 1996. Effect of phorbol myristate acetate on α1-adrenergic action in cells expressing recombinant α1-adrenoceptor subtypes. Mol. Pharmacol. 50, 17–22.