Volume 244, number 2, 465-468 FEB 06865 February 1989

Acridine orange, an inhibitor of protein kinase C, abolishes insulin and growth hormone stimulation of lipogenesis in rat adipocytes

Jean Smal, Satish Kathuria and Pierre De Meyts

Department of Diabetes, Endocrinology and Metabolism, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA

Received 13 Janury 1989

To determine whether protein kinase C plays a role in the actions of insulin and growth hormone in rat adipocytes, we tested the effect of acridine orange, a potent inhibitor of kinase C, on the lipogenic activity of both hormones. This compound completely inhibited the effects of insulin, growth hormone and phorbol ester 12-myristate 13-acetate, whereas 9-acridine carboxylic acid, an analog of acridine orange which does not inhibit kinase C, had no effect. Acridine orange did not act through inhibition of hormone binding. These data are consistent with the involvement of kinase C in the action of insulin and growth hormone on lipogenesis in rat fat cells.

Acridine orange; Protein kinase C; Enzyme inhibitor; Insulin action; Growth hormone action; (Rat adipocyte)

1. INTRODUCTION

Insulin and, under appropriate conditions [1], human growth hormone (hGH) stimulate lipogenesis in isolated rat adipocytes. The maximal amplitude of the hGH effect is one-fourth to one-sixth of that of insulin and their maximal activities are non-additive, suggesting that hGH activates a subset of the metabolic pathways stimulated by insulin [2]. Previous data, including the stimulation of lipogenesis by phorbol ester 12-myristate 13-acetate (PMA), suggested to us that protein kinase C may be involved in the putative common pathway shared by insulin and growth hormone. Accordingly, we showed that the effect of PMA on lipogenesis was non-additive to the effects of insulin or hGH and that down-regulation of protein kinase C markedly inhibited the effects of both hormones [3].

To evaluate further the role of kinase C in the action of insulin and growth hormone, we have investigated the effect of acridine orange on lipogenesis. We report here that this newly described inhibitor of kinase C [4] is also a potent inhibitor of insulin-, growth hormone- and PMA-stimulated lipogenesis in rat adipocytes.

Correspondence address: J. Small, Department of Diabetes, Endocrinology and Metabolism, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA

2. MATERIALS AND METHODS

2.1. *Materials*

Human growth hormone (hGH; NIDDK hGH bulk 2.4 IU/mg) was obtained from the National Hormone and Pituitary Program, NIDDK (Dr Raiti, University of Maryland School of Medicine). Porcine insulin, bovine serum albumin (BSA) fraction V (batch 17F-0149) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma (St. Louis, MO), D-[3-^{3}H]glucose from DuPont NEN (Boston, MA), collagenase (Cls II, batch 67157M) from Cooper Biomedical (Freehold, NJ), and acridine orange and 9-acridine carboxylic acid from Aldrich (Milwaukee, WI). Other chemicals were of reagent grade and were obtained from J.T. Baker Chemical (Phillisburg, NJ). ^{125}I-Tyr^{A14}-insulin and ^{125}I-hGH were prepared as described in [2].

2.2. *Animals*

Male Wistar rats (120–140 g) were obtained from Charles River (Wilmington, MA) and were maintained in light-dark cycles of 12 h and fed ad libitum.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

2.3. *Lipogenesis and binding experiments*

Preparation of adipocytes, lipogenesis assay and hormone binding were performed as in [2]. When used, PMA was dissolved (10 mg/ml) in dimethyl sulfoxide prior to dilution in lipogenesis buffer. The maximal dimethyl sulfoxide concentration used did not modify the cell response in control lipogenesis assays. Acridine orange and 9-acridine carboxylic acid were first dissolved (1 mM) in distilled water and then diluted in appropriate buffer. Each experiment was repeated at least three times, with comparable results.

3. RESULTS

3.1. *Effect of acridine orange on stimulated lipogenesis in rat adipocytes*

In order to restore the cell sensitivity to growth hormone [1,2], rat adipocytes were first preincubated for 4 h at 37°C without growth hormone in lipogenesis buffer prior to lipogenesis assay [2]. Fig.1 shows the effect of acridine orange on the incorporation of tritiated glucose into intracellular lipids. The maximal effect of insulin on lipogenesis was inhibited in a dose-dependent manner, with a half-maximal effective dose of 5–8 μM acridine orange. In parallel, increasing concentrations of acridine orange progressively inhibited both growth hormone- and PMA-stimulated lipogenesis as well as basal lipogenesis. Cell viability, as tested by the Trypan blue exclusion method, remained unchanged in the presence of 100 μM acridine orange.

The analog 9-acridine carboxylic acid, inactive on kinase C [4], had no inhibitory effect on basal or stimulated lipogenesis at concentrations up to 200 μM (fig.1).

Similar effects of acridine orange and lack of effect of 9-acridine carboxylic acid were observed on basal and insulin-stimulated lipogenesis in fresh, non-preincubated rat adipocytes (not shown).

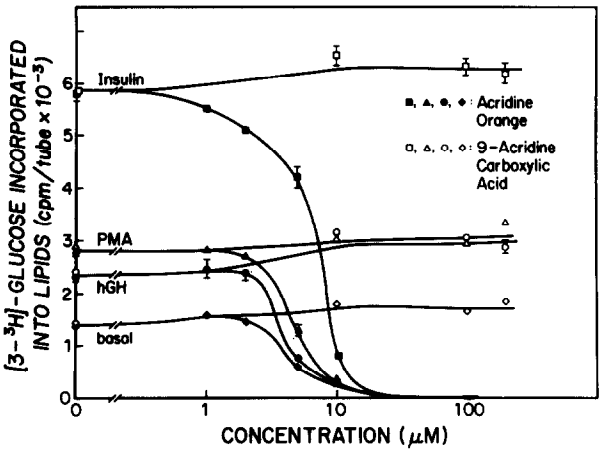

Fig.1. Effect of acridine orange and 9-acridine carboxylic acid on basal or stimulated lipogenesis in preincubated rat adipocytes. After 4 h of preincubation at 37°C without hormone, cells (4×10^4/tube) were incubated in triplicate for 2 h at 37°C without (basal) or with a maximally effective dose of insulin (10 ng/ml), hGH (1000 ng/ml) or PMA (100 ng/ml) and 9 nM D-[3-^{3}H]glucose in the presence of increasing concentrations of acridine orange or 9-acridine carboxylic acid. Results (means ± SD; expressed in cpm/tube) are plotted as a function of acridine derivative concentration.

3.2. *Effect of acridine orange on hormone binding to rat adipocytes*

We evaluated whether the inhibitory effect of acridine orange on growth hormone and insulin action was due to an inhibition of binding to their receptors. The binding experiments were conducted under conditions as close as possible to those used to measure lipogenesis [2]. Fig.2 shows that acridine orange had no significant effect on insulin and growth hormone binding at concentrations up to 10 μM which inhibited their effect on lipogenesis by more than 85% (fig.2). At 100 μM however, the binding of both hormones was decreased by 50%.

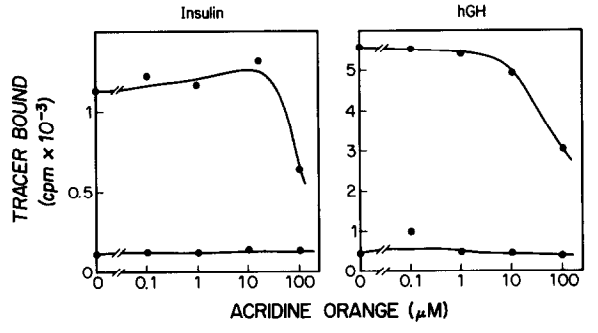

Fig.2. Effect of acridine orange on hormone binding to rat adipocytes. (Left) ^{125}I-Tyr^{A14}-insulin (30000 cpm/tube) was incubated in duplicate for 45 min at 37°C with isolated adipocytes (4×10^5 cells/tube) without (total binding) or with 10 μg/ml unlabeled insulin (nonspecific binding) in the presence of increasing concentrations of acridine orange. (Right) ^{125}I-hGH (50000 cpm/tube) was incubated in duplicate for 75 min at 37°C with isolated adipocytes (8×10^5 cells/tube) without (total binding) or with 10 μg/ml unlabeled hGH (nonspecific binding) in the presence of increasing concentration of acridine orange. ^{125}I-hormone binding, expressed in cpm/tube, is plotted as a function of acridine orange concentration.

4. DISCUSSION

The involvement of protein kinase C in insulin effects in rat adipocytes has been suggested by several investigators. Indeed, phorbol esters, which activate kinase C, mimick the action of insulin on phosphorylation of intracellular substrates, glucose transport, glycolysis and lipogenesis [3,5–11] while kinase C down-regulation significantly decreases insulin effects in these cells [3,11]. However, the interactions between phorbol esters and insulin are rather complex, since PMA also stimulates the phosphorylation of serine and threonine residues of the insulin receptor which results in inhibition of insulin's effects [12–15]. Moreover, unlike PMA, insulin does not induce translocation of protein kinase C from the cytosol to the membrane during enzyme activation [16] and its effectiveness on the breakdown of phosphatidylinositol phosphate generating inositol triphosphate and diacylglycerol, the intracellular activator of kinase C, remains controversial [17]. In an attempt to clarify and to evaluate further the potential role of kinase C in the action of insulin and growth hormone, we have used here a newly described inhibitor of kinase C, acridine orange. This compound inhibits kinase C by interacting with the regulatory domain of the enzyme in a more specific way than inhibitors such as H-7 which competes for the ATP-binding domain of the kinase [18]. The availability of close structural analogs of acridine orange which do not inhibit kinase C [4] allows unambiguous negative controls in intact cells. Our data showed that insulin- and growth hormone-stimulated lipogenesis is inhibited by acridine orange over a concentration range 10-times lower than that reported for the inhibition of kinase C in a micellar system in vitro [4]. In contrast, 9-acridine carboxylic acid, which does not inhibit kinase C [4], had no effect on the action of these hormones. The effect of acridine orange on insulin- and hGH-stimulated lipogenesis cannot be explained by an inhibition of the binding of hormones to their receptors, since it had no effect on hormone binding at a concentration (10 μM) which inhibits most of the lipogenic activity of the hormones. These results are in agreement with recent reports by our group and others that sphingosine, another inhibitor of kinase C, inhibits the actions of insulin [19–21] and growth hormone ([22]; Small and De Meyts, submitted).

Taken together, our results are consistent with the involvement of kinase C in insulin and growth hormone action on lipogenesis in fat cells. The fact that acridine orange also inhibited basal lipogenesis may suggest that rather than being a direct mediator in the mechanisms of action of both hormones, kinase C acts as a modulator with a permissive effect at a rate-limiting step in the pathways used by insulin to stimulate lipogenesis. The extracellular glucose concentration in our lipogenesis assay is low (0.27 mM) [2] and therefore glucose uptake represents an obvious limiting step in the incorporation of glucose into cellular lipids. Since phorbol esters have a maximal effect comparable to that of insulin on the translocation of glucose carriers in rat fat cells [23], it is tempting to speculate that kinase C exerts a permissive effect on glucose transporter translocation which is the first step in the stimulation of glucose transport.

Acknowledgements: J.S. is a postdoctoral fellow of the American Diabetes Association California Affiliate. This work was supported in part by Cancer Core Grant CA33572-09 to City of Hope. Wanda Mutter provided excellent secretarial assistance.

REFERENCES

[1] Goodman, H.M. (1968) Ann. NY Acad. Sci. 148, 419–439.
[2] Smal, J., Closset, J., Hennen, G. and De Meyts, P. (1987) J. Biol. Chem. 262, 11071–11079.
[3] Smal, J. and De Meyts, P. (1987) Biochem. Biophys. Res. Commun. 147, 1232–1240.
[4] Hannun, Y.A. and Bell, R.M. (1988) J. Biol. Chem. 263, 5124–5131.
[5] Graves, C.B. and McDonald, J.M. (1985) J. Biol. Chem. 260, 11286–11292.
[6] Kirsch, D., Obermaier, B. and Haring, H.U. (1985) Biochem. Biophys. Res. Commun. 128, 824–832.
[7] Cherqui, G., Caron, M., Wicek, D., Lascols, O., Capeau, J. and Picard, J. (1986) Endocrinology 118, 1759–1769.
[8] Van de Werve, G., Proietto, J. and Jeanrenaud, G. (1985) Biochem. J. 225, 523–527.
[9] Skoglund, G., Hansson, A. and Ingelman-Sundberg, M. (1985) Eur. J. Biochem. 148, 407–412.
[10] Avruch, J., Nemenoff, R.A., Pierce, M.W., Kwok, Y.C. and Blackshear, P.J. (1985) in: Molecular Basis of Insulin Action (Czech, M.P. ed.) pp.263–296, Plenum, New York.
[11] Cherqui, G., Caron, M., Wicek, D., Lascols, O., Capeau, J. and Picard, J. (1987) Endocrinology 120, 2192–2194.
[12] Takayama, S., White, M., Lauris, V. and Kahn, C.R. (1984) Proc. Natl. Acad. Sci. USA 81, 7797–7801.

[13] Haring, H.U., Kirsh, D., Obermaier, B., Ermel, B. and Machicao, F. (1986) J. Biol. Chem. 261, 3869–3875.
[14] Jacobs, S. and Cuatrecasas, P. (1986) J. Biol. Chem. 261, 934–939.
[15] Bollag, G.E., Roth, R.A., Beaudoin, J., Mochly-Rosen, D. and Koshland, D.E. (1986) Proc. Natl. Acad. Sci. USA 83, 5822–5824.
[16] Glynn, B.P., Colliton, J.W., McDermott, J.M. and Witters, L.A. (1986) Biochem. Biophys. Res. Commun. 135, 1119–1125.
[17] Augert, G. and Exton, J. (1988) J. Biol. Chem. 263, 3600–3609.
[18] Hidaka, H., Inagaki, M., Kawamoto, S. and Sasaki, Y. (1987) Biochemistry 23, 5036–5041.
[19] Robertson, D.G., Longo, N., Newby, F.D., Di Girolamo, M., Merrill, A.H. and Lambeth, J.D. (1987) The Endocrine Society, 69th Annual Meeting, p.39, abstr.71.
[20] Nelson, D.H. and Murray, D.K. (1986) Biochem. Biophys. Res. Commun. 138, 463–467.
[21] Civan, M.M., Peterson-Yantorno, K. and O'Brien, T.G. (1988) Proc. Natl. Acad. Sci. USA 85, 963–967.
[22] Smal, J., Fujita-Yamaguchi, Y. and De Meyts, P. (1988) The Endocrine Society, 70th Annual Meeting, p.34, abstr.253.
[23] Muhlbacher, C., Karnieli, E., Schaff, P., Obermaier, B., Mushack, J., Rattenhuber, E. and Haring, H.U. (1988) Biochem. J. 249, 865–870.