Vol. 131, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1985 Pages 449-455

DIFFERENTIAL SHORT-TERM EFFECTS OF GROWTH FACTORS ON FATTY ACID SYNTHESIS IN ISOLATED RAT-LIVER CELLS

W.J. Vaartjes, C.G.M. de Haas and S.G. van den Bergh

Laboratory of Veterinary Biochemistry, State University of Utrecht, P.O. Box 80.177, 3508 TD Utrecht, The Netherlands

Received July 19, 1985

SUMMARY: Hepatocytes in suspension, freshly isolated from meal-fed rats, were used to study the acute influence of growth factors on the rate of *de novo* fatty acid synthesis. Nerve growth factor (2.5 S) and epidermal growth factor caused a substantial increase in the rate of fatty acid synthesis, whereas fibroblast growth factor was inhibitory. Little effect was observed with nerve growth factor (7 S), bombesin or substance P. Transferrin did not affect hepatic fatty acid synthesis. The results are discussed in relation to the effects of insulin and tumor-promoting phorbol esters.

Lipogenesis in isolated rat hepatocytes offers a suitable experimental system to study two features of insulin. On the one hand, insulin has been implicated in the long-term control of hepatic lipogenesis *in vivo*. The coordinate induction of a set of lipogenic enzymes in liver was reported to start 3 hr after refeeding starved rats or after the provision of insulin to diabetic rats (1,2). Similarly, fatty acid synthesis was enhanced 3 hr after the addition of insulin to cultured hepatocytes isolated from starved rats (3). On the other hand, in short-term incubations of hepatocytes derived from fed rats insulin also caused an increase in the rate of fatty acid synthesis (4). This rapid stimulatory effect of insulin, presumably due to activation of acetyl-CoA carboxylase (4,5), was not mediated by cyclic AMP (6).

Neither the mechanism of these metabolic actions of insulin, nor the way in which the short-term and long-term effects of the hormone are linked, are yet clear. Regarding the latter problem, the simplest hypothesis is to assume that acute and long-term hormonal regulation of lipogenesis, though operating in a consecutive manner, are initiated simultaneously by the same early post-receptor event(s). In an attempt to further elucidate the cellular events induced by insulin, this hypothesis led us to consider the possi-

Abbreviations: NGF, nerve growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; PMA, phorbol 12-myristate 13-acetate.

0006-291X/85 $1.50

bility that other growth factors may also have rapid effects on hepatic lipogenesis. In this connection we recently demonstrated a short-term stimulation of fatty acid synthesis in liver cells by tumor-promoting phorbol esters (7). The present study deals with the effects of physiological growth factors like NGF, EGF and FGF. Our results suggest that the ability to rapidly enhance hepatic fatty acid synthesis is limited to those growth factors whose receptors are associated with ligand-induced tyrosine kinase activity. Whether or not these receptor kinases are involved in the stimulation of fatty acid synthesis and, if so, whether they serve to integrate short-term and long-term regulation of lipogenesis, remains to be established.

MATERIALS AND METHODS

Crystalline bovine insulin was kindly donated by Lilly Labs. (Indianapolis, IN, U.S.A.). Brain FGF was from Kor Biochemicals (Cambridge, MA, U.S.A.). Other growth factors and biochemicals were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ^3H_2O was supplied by Amersham Internat. (Amersham, U.K.). Repeated freezing and thawing of growth factor stock solutions, made up with 2%(w/v) bovine serum albumin, was avoided. Hepatocytes were isolated (4) from male Wistar rats (200-250 g), meal-fed a standard, pelleted diet. Fatty acid synthesis was measured with ^3H_2O (0.5 mCi/ml). Incubations were performed in triplicate. Other procedures were exactly as described previously (7). Statistical analysis was performed using paired *t*-testing.

RESULTS

Polypeptide growth factors represent a variety of regulatory substances that can be divided into subgroups characterized by structural similarities. Thus, the "insulin family" (8) includes NGF, insulin-like growth factors (IGFs) and relaxin. These factors share sequence homologies with insulin and exhibit insulin-like biological activities. Metabolic studies have been virtually limited to their effects on adipose tissue, heart, and skeletal muscle (9). Therefore, we decided to investigate the influence of NGF on fatty acid synthesis in isolated liver cells.

Native NGF is a multi-subunit complex, called 7 S NGF, which is formed from 2 α-subunits, a stable β-dimer and 2 γ-subunits (10). The β-dimer, known as 2.5 S NGF, is responsible for NGF-promoted neurite outgrowth (10), probably via a cyclic-AMP-independent mechanism (11,12). As shown in Figure 1, this 2.5 S NGF stimulated *de novo* fatty acid synthesis in isolated rat hepatocytes. Since glucagon or added cyclic AMP strongly inhibit lipogenesis (6,13), the NGF-induced stimulation (Fig. 1) is probably not mediated by cyclic AMP. Unlike classical hormones, NGF is proposed not to enter the circulation (10), thereby obviating reflections on the possible existence of hepatic NGF receptors. Rather, one may surmise cross-reaction of 2.5 S NGF, the subunit bearing the insulin-like amino acid sequences, with the insulin receptor. Such a competition for receptor binding is not uncommon within the "insulin family" (14) and its occurrence would further strengthen the hypo-

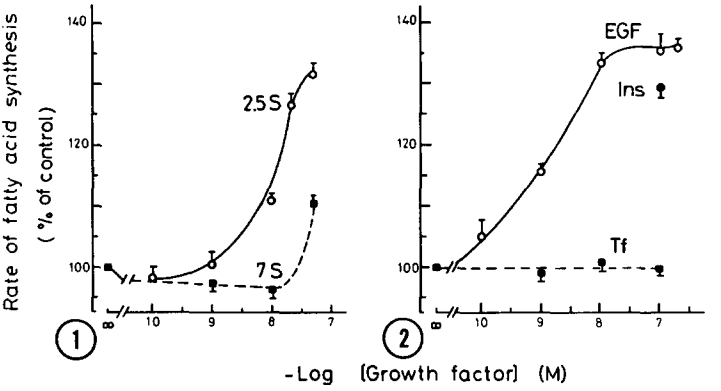

Fig.1. Effect of nerve growth factor (NGF) on the rate of fatty acid synthesis in isolated rat hepatocytes. Shown are means ± S.D. of 3 experiments (100%-control: 27.6 ± 2.5 nmol ^3H_2O incorporated/h per mg protein). O——O, 2.5 S NGF; ■— —■, 7 S NGF.

Fig.2. Effect of epidermal growth factor (EGF) and transferrin (Tf) on the rate of fatty acid synthesis in isolated rat hepatocytes. Shown are means ± S.D. of 4 (EGF) and 2 (Tf) experiments: O——O, EGF; ■— —■, transferrin. In experiments with EGF the effect of 100 nM insulin (●, Ins) was also determined. 100%-Control: 38.5 ± 13.2 (n=4) nmol ^3H_2O incorporated/h per mg protein.

thesis that the genes of insulin and 2.5 S NGF are related (cf. 15). When included in the native 7 S complex, the 2.5 S NGF subunit is much less effective in stimulating fatty acid synthesis (see Fig. 1). This may be due to steric hindrance of 2.5 S NGF - receptor interaction by the α- and γ-subunits.

The capacity to rapidly stimulate hepatic lipogenesis is not limited to members of the "insulin family", as EGF appeared to have the same effect. EGF, the main constituent of another polypeptide family, is a potent mitogen for a wide variety of cultured cells (16). Its biological effects are initiated by its binding to specific cell-surface receptors present in all EGF-sensitive cells, including hepatocytes (17). Figure 2 demonstrates that EGF is at least as effective as insulin in promoting fatty acid synthesis in isolated hepatocytes from meal-fed rats. Though being at variance with the reported inhibition by EGF of [^{14}C]acetate incorporation into the lipids of cultured hepatocytes (18), our results are in agreement with recent data of Holland and Hardie (19) obtained with hepatocytes from starved-refed rats.

Figure 2 also shows that stimulation of hepatic fatty acid synthesis is not common to all polypeptides. The well-known growth factor transferrin (20), structurally unrelated to insulin or EGF, did not affect the rate of fatty acid synthesis (Fig. 2) despite the presence of high-affinity binding sites for transferrin on the hepatocyte surface (21). As shown in Table 1, part A, other mitogens like bombesin (22), substance P (23) and FGF (24) appeared to inhibit rather than stimulate fatty acid synthesis. The weak effects observed with 10^{-7} M bombesin or substance P could be due to the lack of appropriate

Table 1. Influence of growth factors on fatty acid synthesis in isolated rat hepatocytes

	Additions	Growth factor concentration (M)	Rate of fatty acid synthesis (nmol ^3H_2O/h.mg)	(%)
(A)	None		23.9 ± 3.8	
	Insulin	10^{-7}	32.5 ± 1.8 [c]	(136.0)
	Bombesin	10^{-7}	20.9 ± 1.5 [b]	(87.4)
	Substance P	10^{-7}	21.9 ± 2.1 [a]	(91.6)
	FGF	10^{-8}	17.3 ± 0.8 [c]	(72.4)
(B)	None		17.7 ± 2.7	
	Insulin	10^{-7}	29.6 ± 3.4 [c]	(167.2)
	EGF	10^{-8}	27.3 ± 4.9 [c]	(154.2)
	Lactate		36.5 ± 0.7	
	+ insulin	10^{-7}	54.1 ± 6.4 [d]	(148.2)
	+ EGF	10^{-8}	58.9 ± 5.8 [d]	(161.4)
	2-Chloropropionate		33.2 ± 0.6	
	+ insulin	10^{-7}	48.1 ± 1.1 [d]	(144.9)
	+ EGF	10^{-8}	53.7 ± 1.4 [d]	(161.7)

Shown are means ± S.D. from two separate sets of 3 (A) and 2 (B) hepatocyte preparations. Growth factors, L-lactate (10 mM) and 2-chloropropionate (1 mM) were added as indicated. In parentheses, rate of fatty acid synthesis as percentage of the corresponding control value (without growth factors). Versus control with no additions: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; versus values with lactate or 2-chloropropionate alone: [d] $P < 0.001$.

receptor systems in liver cells. However, FGF at 10^{-8} M exerted a significant inhibitory effect.

The pronounced and reproducible stimulation of fatty acid synthesis by EGF made it worthwile to further locate its site of metabolic action by including L-lactate or 2-chloropropionate in the incubation medium. With exogenous lactate present, pyruvate derived from glycolysis plays only a minor role as carbon source for newly synthesized fatty acids. 2-Chloropropionate increases the availability of acetyl-CoA by activation of pyruvate dehydrogenase (25). As shown in part B of Table 1, the percentage stimulation of fatty acid synthesis by EGF or insulin was essentially unaltered in the presence of either lactate or 2-chloropropionate. This indicates that the observed stimulatory effects are not merely caused by an increased provision of carbon precursors, due to an increased glycolysis and/or pyruvate dehydrogenase activity, but that EGF and insulin affect the fatty-acid synthetic pathway itself, presumably at the level of the enzyme acetyl-CoA carboxylase (4,5,19).

DISCUSSION

The present study shows that, in addition to insulin, 2.5 S NGF (probably acting via the insulin receptor) and EGF are able to stimulate hepatic fatty acid synthesis. It is easily conceivable that these short-term effects may serve a long-term goal, viz., to provide acyl moieties for phospholipids in *de novo* synthesized biomembranes. However, full appreciation of the physiological significance is hampered by the fact that other mitogens like FGF (Table 1 A) and vasopressin (26) inhibit hepatic fatty acid synthesis.

EGF and insulin each bind to their own specific receptors (16,17,27). In addition, insulin at higher concentrations binds to IGF-1 receptors. King *et al.* (14) proposed earlier that the metabolic effects of insulin are mediated by the insulin receptor, whereas the mitogenic activity of insulin should be due to its cross-reaction with the IGF-1 receptor. However, in view of the fact that a well-known mitogen like EGF also affects cell metabolism (see Fig. 2) we suggest that binding of insulin to its own receptor may suffice to initiate both short-term and long-term effects.

The insulin and EGF receptors possess an intrinsic tyrosine kinase activity (27,28). The biological role of tyrosine-specific protein phosphorylation is not yet known, but its association with cellular growth control is generally assumed. It is tempting to speculate that these receptor kinases are also responsible for the short-term effects of EGF and insulin (29; cf. 30). If so, similarities between rapid effects of insulin and EGF may reside in their receptor kinase activities. This speculation is favored by recent reports indicating that insulin and EGF cause identical phosphorylation of hepatic acetyl-CoA carboxylase (19) and that their receptor kinases display overlapping substrate specificity (31,32). Incidentally, it may be noted that no such receptor kinase activity has been reported in case of growth factors like FGF, bombesin and vasopressin, which do not stimulate fatty acid synthesis.

Tumor-promoting phorbol esters also enhance hepatic fatty acid synthesis (7). This poses an intriguing problem as protein kinase C, the cellular target of phorbol esters (33), only phosphorylates serine and threonine residues. One may envisage that PMA, insulin and EGF all stimulate acetyl-CoA carboxylase via protein kinase C, with insulin and EGF acting indirectly on protein kinase C activity through their receptor kinases. Alternatively, receptor kinase activities in intact hepatocytes may cause phosphorylation of both serine and tyrosine residues (34,35). Moreover, PMA may activate another protein kinase activity (36,37) in addition to protein kinase C. A further possibility could be that growth factors, via receptor kinase activity, and PMA, via protein kinase C activity, separately set in motion a series of events, either independently leading to increased fatty

acid synthesis or having in common a particular protein kinase which exerts the final metabolic effect. A possible candidate in this last respect is the protease-activated kinase II, described by Traugh *et al.* (38,39). Our current research is aimed at eliminating some of these possibilities.

ACKNOWLEDGEMENTS

This research was supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1. Gibson, D.M., Lyons, R.T., Scott, D.F. and Muto, Y. (1972) Advan. Enzyme Regul. 10, 187-204.
2. Lakshmanan, M.R., Nepokroeff, C.M. and Porter, J.W. (1972) Proc. Natl. Acad. Sci. USA 69, 3516-3519.
3. Geelen, M.J.H. and Gibson, D.M. (1975) FEBS Lett. 58, 334-339.
4. Beynen, A.C., Vaartjes, W.J. and Geelen, M.J.H. (1979) Diabetes 28, 828-835.
5. Witters, L.A., Moriarity, D. and Martin, D.B. (1979) J. Biol. Chem. 254, 6644-6649.
6. Geelen, M.J.H., Vaartjes, W.J. and Gibson, D.M. (1977) Lipids 12, 577-580.
7. Vaartjes, W.J. and de Haas, C.G.M. (1985) Biochem. Biophys. Res. Commun., in press.
8. Carpenter, G. and Cohen, S. (1984) Trends Biochem. Sci. 9, 169-171.
9. Zapf, J., Froesch, E.R. and Humbel, R.E. (1981) Curr. Top. Cell. Regul. 19, 257-309.
10. Bradshaw, R.A. (1983) in Biochemical Actions of Hormones (Litwack, G., ed.), Vol. X, pp. 91-114, Academic Press, New York.
11. Connolly, J.L., Green, S.A. and Greene, L.A. (1984) J. Cell Biol. 98, 457-465.
12. Race, H.M. and Wagner, J.A. (1985) J. Neurochem. 44, 1588-1592.
13. Harris, R.A. (1975) Arch. Biochem. Biophys. 169, 168-180.
14. King, G.L., Kahn, C.R., Rechler, M.M. and Nissley, S.P. (1980) J. Clin. Invest. 66, 130-140.
15. Ullrich, A., Gray, A., Berman, C. and Dull, T.J. (1983) Nature 303, 821-825.
16. Fox, C.F., Linsley, P.S. and Wrann, M. (1982) Fed. Proc. 41, 2988-2995.
17. O'Keefe, E., Hollenberg, M.D. and Cuatrecasas, P. (1974) Arch. Biochem. Biophys. 164, 518-526.
18. Hayden, L.J. and Hollenberg, M.D. (1984) Fed. Proc. 43, 1863.
19. Holland, R. and Hardie, D.G. (1985) FEBS Lett. 181, 308-312.
20. Hayashi, I., Larner, J. and Sato, G. (1978) In Vitro 14, 23-30.
21. Young, S.P. and Aisen, P. (1980) Biochim. Biophys. Acta 633, 145-153.
22. Rozengurt, E. and Sinnet-Smith, J. (1983) Proc. Natl. Acad. Sci. USA 80, 2936-2940.
23. Nilsson, J., von Euler, A.M. and Dalsgaard, C.J. (1985) Nature 315, 61-63.
24. Gospodarowicz, D., Greenburg, G., Bialecki, H. and Zetter, B.R. (1978) In Vitro 14, 85-118.
25. Crabb, D.W. and Harris, R.A. (1979) Arch. Biochem. Biophys. 198, 145-152.
26. Palmer, T.N., Caldecourt, M.A., Watts, D.I. and Sugden, M.C. (1983) Biosci. Rep. 3, 1063-1070.
27. Kasuga, M., Zick, Y., Blithe, D.L., Crettaz, M. and Kahn, C.R. (1982) Nature 298, 667-669.
28. Ushiro, H. and Cohen, S. (1980) J. Biol. Chem. 255, 8363-8365.
29. Le Marchand-Brustel, Y., Grémeaux, T., Ballotti, R. and Van Obberghen, E. (1985) Nature 315, 676-679.
30. Zick, Y., Rees-Jones, R.W., Taylor, S.I., Gorden, P. and Roth, J. (1984) J. Biol. Chem. 259, 4396-4400.

31. Pike, L.J., Kuenzel, E.A., Casnellie, J.E. and Krebs, E.G. (1984) J. Biol. Chem. 259, 9913-9921.
32. Klein, H.H., Freidenberg, G.R., Cordera, R. and Olefsky, J.M. (1985) Biochem. Biophys. Res. Commun. 127, 254-263.
33. Nishizuka, Y. (1984) Nature 308, 693-698.
34. Kasuga, M., Zick, Y., Blith, D.L., Karlsson, F.A., Häring, H.U. and Kahn, C.R. (1982) J. Biol. Chem. 257, 9891-9894.
35. Gazzano, H., Kowalski, A., Fehlmann, M. and Van Obberghen, E. (1983) Biochem. J. 216, 575-582.
36. Bishop, R., Martinez, R., Nakamura, K.D. and Weber, M.J. (1983) Biochem. Biophys. Res. Commun. 115, 536-543.
37. Grunberger, G., Zick, Y., Taylor, S.I. and Gorden, P. (1984) Proc. Natl. Acad. Sci. USA 81, 2762-2766.
38. Trevillyan, J.M., Perisic, O., Traugh, J.A. and Byus, C.V. (1985) J. Biol. Chem. 260, 3041-3044.
39. Perisic, O. and Traugh, J.A. (1985) FEBS Lett. 183, 215-218.