BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **233,** 200–202 (1997)
ARTICLE NO. RC976433

Prostaglandin $F_{2\alpha}$ Receptor (FP Receptor) Agonists Are Potent Adipose Differentiation Inhibitors for Primary Culture of Adipocyte Precursors in Defined Medium

Ginette Serrero*,[1] and Nancy M. Lepak[2]

**Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, and Program of Oncology, University of Maryland Cancer Center, 20 N. Pine Street, Baltimore, Maryland 21201-1180*

Received February 26, 1997

Prostaglandin $F_{2\alpha}$ inhibits adipose differentiation of primary culture of adipocyte precursors and of the adipogenic cell line 1246 in defined medium. In the present paper, we investigated the effect of FP receptor agonists cloprostenol and fluprostenol on the differentiation of newborn rat adipocyte precursors in primary culture. The results show that cloprostenol and fluprostenol are very potent inhibitors of adipose differentiation. Dose response studies indicate that both agonists are more potent than $PGF_{2\alpha}$ in inhibiting adipocyte precursors differentiation. 50% inhibition of adipose differentiation was observed at a concentration of 3×10^{-12} M for cloprostenol and 3 to 10×10^{-11} M for fluprostenol respectively whereas the $PGF_{2\alpha}$ concentration required to elicit the same effect was 10^{-8} M. In contrast compounds structurally related to PGE_2 such as 17-phenyl trinor PGE_2 had no effect on adipose differentiation except when added at a 10,000-fold higher concentration.

Cells cultivated in the presence of FP receptor agonists remained fibroblastic, typical of undifferentiated adipocytes. Triglyceride accumulation and increase in specific activity for glycerol-3-phosphate dehydrogenase activity were inhibited. In addition mRNA expression of adipocyte markers were such as lipoprotein lipase (LPL) and fatty acid binding protein (FAB) was decreased. The results provide new information on the biological activity of FP receptor agonists as differentiation inhibitors of adipocyte precursors freshly isolated from newborn rats.

Prostaglandin $F_{2\alpha}$ is a potent inhibitor of differentiation of adipocyte precursors in primary culture (1) and of the adipogenic cell line 1246 (2) 50% inhibition of differentiation was observed at a $PGF_{2\alpha}$ concentration of 10^{-8}M both for adipocyte precursors in primary culture and for the adipogenic cell line 1246 (1-2). Structure function studies were previously performed in order to examine the structural characteristics required by prostaglandin in order to exert a high adipose differentiation inhibitory activity. The results of these experiments showed that $9\alpha,11\beta PGF_{2\alpha}$ was equipotent to $PGF_{2\alpha}$ to inhibit adipose differentiation whereas $9\beta,11\alpha$ $PGF_{2\alpha}$ had no effect. Moreover PGD2 and PGE2 had no effect on differentiation except when added at 100-fold higher concentration where a 40% inhibition was observed (3). This order of potency of various prostaglandins suggested that inhibition of differentiation may be mediated via binding to the prostaglandin $F_{2\alpha}$ receptor classified as FP receptors (4).

Based on this possibility, we examined here the effect on adipose differentiation of two synthetic FP receptor agonists fluprostenol and cloprostenol. Fluprostenol and closprostenol which are stable analogues of $PGF_{2\alpha}$ are both potent FP receptor agonists and are also more selective than their parent compound (4-5). In particular, fluprostenol has no demonstrable agonist activity at any of the other four prostanoid receptor types (DP, EP, TP and IP). Because they are more selective, they are very useful to examine the effect of prostanoids on cells that have mixed prostanoid receptors populations such as the adipocytes (6). In the present paper, the effect of fluprostenol and cloprostenol on adipose differentiation was investigated using primary culture of rat newborn adipocyte precursors freshly isolated from inguinal fat pads of newborn rats since these cells can optimally proliferate and differentiate in defined medium (7). We have shown previously that in these defined culture conditions, 90% of adipocyte precursors from newborn rats differentiate within 8 days of plating (7) thus providing a useful cell culture system with which to investigate the effect of pharmacological agents on adipocyte differentiation in physiological conditions.

[1] Corresponding author. E-mail: gserrero@pharmacy.ab.umd.edu.
[2] Present address: Trudeau Institute, Saranac Lake, NY 12983.

0006-291X/97 $25.00

MATERIAL AND METHODS

$PGF_{2\alpha}$, cloprostenol, fluprostenol and 17-phenyl trinor PGE_2 were obtained from Cayman (Ann Harbor, MI) and were freshly prepared as ethanol solutions prior to being assayed. Tissue culture plasticware is from Beckton-Dickinson. Dulbecco's modified Eagle's medium (DME) and Ham's F12 nutrient media were from Gibco-BRL. Hormones and chemicals were from Sigma chemicals.

Culture of adipocyte precursors from inguinal fat pads of 2-day old Sprague-Dawley rats was carried out in defined medium as described previously (3-4). The defined medium consisted of DME-F12 nutrient mixture (1:1) supplemented with human fibronectin (0.2 μg/ml) bovine insulin (10 μg/ml) human transferrin (10 μg/ml) and bovine recombinant basic fibroblast growth factor 5 ng/ml. $PGF_{2\alpha}$ and FP receptor agonists were added at day 1 and maintained in the culture medium throughout the experiments. Medium was changed every three days and fresh agonist or prostaglandin added at that time. Each experiment was done with duplicate dishes for each condition and repeated at least three times. Differentiation was routinely followed by measuring glycerol-3-phosphate dehydrogenase specific activity (G3PDH). Total RNA was extracted with RNAzol (Cinnabiotech, Friendswood, TX). Northern blot analysis for measuring the mRNA expression of lipoprotein lipase (LPL), fatty acid binding protein (FAB) and ribosomal protein L32 (RPL32) were performed as described previously (8). Triglycerides were measured as described previously (9).

RESULTS AND DISCUSSION

We first examined the effect of increasing concentrations of cloprostenol and fluprostenol on the differentiation of adipocyte precursors by measuring glycerol-3-phosphate dehydrogenase (G3PDH) specific activity (figure 1). Fluprostenol and cloprostenol both inhibited G3PDH specific activity in a dose dependent fashion. 50% inhibition was observed at a concentration of 3×10^{-12}M for cloprostenol and a concentration of 3-10×10^{-11}M for fluprostenol. $PGF_{2\alpha}$ inhibited G3PDH specific activity by 50% when added at a concentration of 3×10^{-8} M. In contrast, a structural analogue of PGE2, 17-phenyl trinor PGE2 known to be an agonist of EP1 receptor did not inhibit differentiation until it was added at a concentration of 3×10^{-7} M which is a 1,000 to 10,000-fold higher than the respective concentration of fluprostenol and cloprostenol required to inhibit adipose differentiation (data not shown).

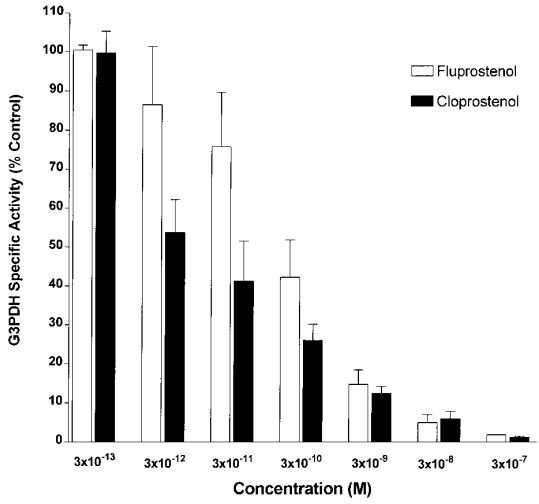

FIG. 1. Effect of increasing concentrations of fluprostenol and cloprostenol on adipose differentiation of adipocyte precursors in primary culture. Adipocyte precursors freshly isolated from inguinal fat pads of 2-day-old rats were cultivated in defined medium as described in the method section. Fluprostenol and cloprostenol were added at day 1 and maintained throughout the experiment (until day 8). At day 8, cells were harvested to measure Glycerol-3-phosphate (G3PDH) specific activity. Values for G3PDH specific activity are expressed as a percentage of the control corresponding to G3PDH specific activity in cells maintained in defined medium deprived of FP receptor agonist. 100% of control was 2087 ± 78 mU/mg protein.

TABLE 1

Culture conditions	Triglycerides (% control)
Control	100
Fluprostenol 3×10^{-10} M	24
Fluprostenol 3×10^{-8} M	5
Cloprostenol 3×10^{-10} M	11
Cloprostenol 3×10^{-8} M	5

Adipocyte precursors were cultivated in defined medium as described in the method section in the absence (control) or in the presence of either cloprostenol or fluprostenol. At day 8, the amount of triglycerides accumulated was determined as described previously (9). Results are expressed as % control. 100% value corresponded to the triglycerides accumulated in cells cultivated in defined medium only and was 860 $\mu g/10^6$ cells. SD was less than 10%.

In addition to having lower levels of G3PDH specific activity, adipocyte precursors treated with cloprostenol and fluprostenol remained fibroblastic and triglyceride accumulation was inhibited (table 1). In the presence of increasing concentrations of cloprostenol or fluprostenol, the cells accumulated from 80% to 95% less triglycerides than the control cells cultivated in defined medium in their absence.

Expression of mRNA for lipoprotein lipase (LPL) and of the adipose specific fatty acid binding protein (FAB) as early markers of adipose differentiation (10) was also examined in cells treated or not with FP receptor agonists (figure 2). When normalized to the level of ribosomal protein L_{32} (RPL_{32} mRNA) used as an internal standard, mRNA expression for adipocyte markers such as lipoprotein lipase and fatty acid binding protein was inhibited when the adipocyte precursors were cultivated in defined medium in the presence of cloprostenol or fluprostenol.

The results presented here demonstrate that FP receptors agonists cloprostenol and fluprostenol are potent inhibitors of adipose differentiation of primary culture of adipocyte precursors. Similar results were observed with the adipogenic cell line 1246 where

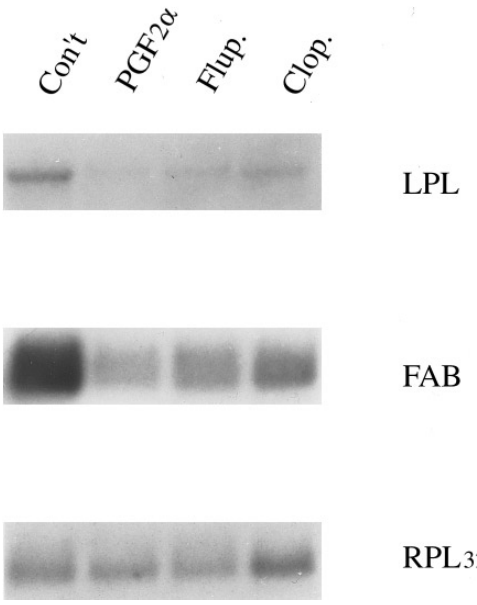

FIG. 2. Effect of fluprostenol, cloprostenol and $PGF_{2\alpha}$ on the mRNA expression of adipose differentiation markers in adipocyte precursors. Cells were cultivated in defined medium only (control) or in the presence of 3×10^{-10} M fluprostenol (Flup.), 3×10^{-10} M cloprostenol (Clop.) or 3×10^{-8}M $PGF_{2\alpha}$ ($PGF_{2\alpha}$). Cells were harvested at day 8 for extracting total RNA using RNAzol. Northern blot analysis was performed using 15 μg of total RNA from control and treated cells. Nitrocellulose filters were sequentially hybridized to ^{32}P-labeled cDNA probe corresponding to lipoprotein lipase (LPL), fatty acid binding protein (FAB). mRNA expression for ribosomal protein L_{32} (RPL_{32}) was used as internal standard to monitor RNA loading.

fluprostenol and cloprostenol inhibited adipose differentiation with similar ED_{50} as the ones determined for rat adipocyte precursors (data not shown). Interestingly for primary culture of adipocyte precursors and for the 1246 cells, both cloprostenol and fluprostenol were found at least 1,000-fold more potent that $PGF_{2\alpha}$ to inhibit differentiation.

FP receptor cDNAs have been cloned from human and rodent tissues and cells (11-14). The fact that fluprostenol is more potent than $PGF_{2\alpha}$ to inhibit adipose differentiation is consistent with the order of potency found for binding to expressed FP receptor cDNA cloned from rat corpus luteum cDNA libraries (13). One interesting point is the consistently higher potency of cloprostenol when compared to fluprostenol in its effectiveness to inhibit differentiation. Since freshly prepared preparations of both components were used in all our experiments, it is not clear whether the difference in potency could be only explained by a difference in stability of both compounds or rather a difference in the specificity for both agonists of FP receptors expressed on adipocyte precursors when compared to the ones expressed on other tissue types.

Since the experiments described here were carried out with adipocyte precursors freshly isolated from inguinal fat pads, these studies point out to the importance of FP receptor mediated pathways in the physiological regulation of adipose differentiation.

ACKNOWLEDGMENTS

This work was supported in part by grants PO1 DK 38639 from the National Institutes of Health, 3894 from the Council for Tobacco Research and 194174 from the Juvenile Diabetes Foundation International.

REFERENCES

1. Serrero, G., Lepak, N. M., and Goodrich, S. P. (1992) *Biochem. Biophys. Res. Commun.* **183,** 438–442.
2. Serrero, G., Lepak, N. M., and Goodrich, S. P. (1992) *Endocrinology* **131,** 2545–2551.
3. Lepak, N. M., and Serrero, G. (1993) *Prostaglandins* **46,** 511–517.
4. Dukes, M., Russell, W., and Walpole, A. L. (1974) *Nature* **250,** 330–331.
5. Coleman, R., Humphrey, P. P. A., and Kennedy, I. (1985) *in* Trends in Autonomic Pharmacology (Kalsner, S., Ed.), Vol. 3, p. 35, Taylor and Francis, London/Philadelphia.
6. Christ, E. J., and Nutgeren, D. H. (1970) *Biochim. Biophys. Acta* **218,** 296–307.
7. Serrero, G., and Mills, D. (1987) *In Vitro Cell. Devel. Biol.* **23,** 63–66.
8. Jiang, H. P., Harris, S. E., and Serrero, G. (1992) *Cell Growth & Differentiation* **3,** 21–30.
9. Serrero, G., and Mills, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3912–3916.
10. Bernlohr, D. A., Angus, W., Lane, M. D., Bolanowski, M., and Kelly, Jr. T. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5468–5472.
11. Abramovitz, M., Boie, Y., Nguyen, T., Rushmore, T. H., Bayne, M. A., Metters, K. M., Slipetz, D. M., and Grygorczyk, R. (1994) *J. Biol. Chem.* **269,** 2632–2636.
12. Sugimoto, Y., Hasumoto, K., Namba, T., Irie, A., Katsuyama, M., Negishi, M., Kakizuka, A., Narumiya, S., and Ichikawa, A. (1994) *J. Biol. Chem.* **269,** 1356–1360.
13. Lake, S., Gullberg, H., Wahlqvist, J., Sjogren, A. M., Lind, P., Hellstrom-Lindahl, E., and Stjernschantz, J. (1994) *FEBS Lett.* **355,** 317–325.
14. Graves, P. E., Pierce, K. L., Bailey, T. J., Rueda, B. R., Gil, D. W., Woodward, D. F., Yool, A. J., Hoyer, P. B., and Regan, J. W. (1995) *Endocrinology* **136,** 3430–3436.