Polyunsaturated fatty acids inhibit mouse hepatic glucocorticoid receptor activation in vitro

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Received 15 December 2003; revised 26 August 2004

The effect of saturated fatty acids (SFAs) stearic and palmitic acids and polyunsaturated fatty acids (PUFAs) oleic, linoleic and arachidonic acids was studied on in vitro heat activation of mouse hepatic glucocorticoid receptor (GR) complex, as assessed by binding to DNA-cellulose and purified nuclei. Significant dose-dependent inhibition of heat activation of hormone-receptor complex by the PUFAs was observed. Linoleic and arachidonic acids were found to be more potent (caused ~70% inhibition maximally at 160 μM) inhibitors of GR heat activation, compared to oleic acid (~38% inhibition at 40 μM). However, stearic and palmitic acids were unable to modulate GR heat activation, suggesting that the unsaturated moieties in PUFAs are possibly the important determinants of receptor activation. Thus, our study shows an inhibitory effect of PUFAs on in vitro hepatic GR activation.

Keywords: Fatty acids, mice, liver, activation, glucocorticoid receptor

Glucocorticoid receptors (GRs) mediate the action of glucocorticoids, the key regulators of different physiological activities of target tissues in human and animals. They are versatile regulators of gene transcription and cell signaling. The GR has a characteristic domain structure, with the C- and N-terminal domains possessing hormone-binding and modulatory role, respectively. The central domain, called the DNA-binding domain has two zinc finger motifs which are involved in DNA binding. The GR exists as a large hetero-oligomeric complex with several chaperone proteins, such as hsp90, hsp70, p23 acidic protein, and heat-shock organizing protein, from which it dissociates upon ligand binding by a process called activation. Activated GR then translocates into the nucleus, where it binds to conserved glucocorticoid responsive elements (GREs) within promoters of target genes, thus modulating gene expression. This represents the classical genomic action mechanism of glucocorticoids. Modulation of GR function by various factors is one of the key elements that determines responsiveness of target cells towards glucocorticoids. One of the key steps associated with modulation is activation, which involves the dissociation of bound chaperones from GR complex that keeps the receptor in an inactive (unactivated) state. Hence, agents that modulate receptor activation presumably regulate the dissociation of bound chaperones from the receptor complex.

Glucocorticoids acting through GRs facilitate lipolysis in adipocytes and the released fatty acids are metabolized in many tissues, including liver. Fatty acids and glucocorticoids have important roles in liver and adipocytes, involving energy metabolism and other functions that control growth and development. Hence, they may interact with each other in modulating hepatic functions. Earlier, polyunsaturated fatty acids (PUFAs) were shown to inhibit hormone binding to GR in different tissues of animals. Our previous study demonstrated the inhibitory effect of linoleic and arachidonic acids on hepatic and renal GR heat activation in immature and aged mice. In the present paper, we studied the effect of PUFAs (oleic, linoleic and arachidonic acids) and saturated fatty acids (SFAs), palmitic and stearic acids on in vitro heat activation of mouse hepatic GR complex, as assessed by binding to DNA-cellulose and purified nuclei.

Materials and Methods

[1, 2, 4, 6, 7-3H] Dexamethasone (specific activity, 91 Ci/mmol) was purchased from Amersham Biotech, England. Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and arachidonic (C20:4) acids, DNA-cellulose, dextran T-70 and dimethylsulfoxide (Me2SO) were obtained from Sigma Chemical Co., St. Louis, USA. Buffers used were: (A) 0.25 M sucrose/10 mM Tris-HCl, pH 7.6; and (B) 0.25 M sucrose/10 mM Tris-HCl/0.5% (v/v) Triton X-100, pH 7.6.

Notes
Male Swiss albino (balb/c strain) mice aged 120 days, maintained under normal laboratory conditions at 25±2°C were fed with standard food pellets (Amrut, Pune) and tap water ad libitum. The photoperiod was of 12 hr light/12 hr dark cycle. The fatty acids were dissolved in Me2SO and made to 1 mM stock. All assays were conducted at 0-2°C, unless otherwise indicated. Radioactive counts (CPM) were obtained using Wallac 1409 liquid scintillation counter, having 68% efficiency for tritium.

Preparation and activation of glucocorticoid-receptor complexes

Mice were sacrificed by cervical dislocation at a fixed time of the day (11:00 hr) and the livers were quickly washed twice in chilled normal saline (0.9% NaCl) and blotted dry. Pooled livers from 4-5 separate mice were homogenized (20% homogenate) in buffer A and centrifuged at 2000 g for 10 min at 2°C to sediment nuclei and other cellular debris. Supernatant, thus obtained was further centrifuged at 27, 500 g for 1 hr at 2°C to yield cytosol, which was made fat-free using Pasteur pipette and incubated with 40 nM [3H] dexamethasone (final concentration) for 4 hr at 0°C, during which maximal saturation binding occurred. Bound [3H] dex-receptor complexes were separated using dextran-coated charcoal (4% activated charcoal plus 0.4% dextran T-70, prepared in buffer A)8.

Bound [3H] dex-receptor complexes were subjected to activation at 0°C (control) and 25°C for 45 min, in order to determine the heat activation of the complex. For fatty acids modulation of GR activation, [3H] dex-receptor complexes were heat activated at 25°C for 45 min in the absence or presence of varying final concentrations (0-200 μM) of PUFAs and SFAs separately. Control tubes received equal volume of Me2SO, without the fatty acids. The extent of heat activation and activation-modulation by fatty acids was determined using DNA-cellulose and nuclear binding assays8.

DNA-cellulose binding assay

Preformed heat-activated [3H]dex-receptor complexes (~30,000 CPM) were incubated with pre-washed DNA-cellulose pellet containing 100-150 μg DNA for 1 hr at 0°C and agitated gently at regular intervals to keep the cellulose particles in suspension for better [3H]dex-receptor complex interaction7,8. DNA-cellulose bound [3H] dex-receptor complexes were then obtained by washing the pellets twice with buffer A. The final pellet was suspended in 4 ml scintillation cocktail-T and bound radioactivity (CPM) counted in a liquid scintillation counter.

Nuclear binding assay

Crude nuclear pellet obtained above was gently homogenized using chilled buffer B, followed by centrifugation at 2000 g for 10 min at 4°C and washed thrice with buffer A to get purified nuclei, which were finally suspended in buffer A. Nuclear pellets containing 100-150 μg DNA were then incubated with [3H]dex-receptor complexes (~30,000 CPM) and processed in the same manner as described for DNA-cellulose binding assay. DNA content of purified nuclear suspension was determined as described15. Results obtained were expressed as [3H] dexamethasone-receptor complex bound to DNA-cellulose/purified nuclei (CPM/100 μg DNA) and finally, expressed in terms of per cent (%) inhibition. Control samples (without fatty acids) were attributed zero per cent inhibition and the per cent inhibition in the presence of fatty acids was calculated by subtracting the actual values (Y-CPM/100 μg DNA) from control values (X-CPM/100 μg DNA), divided by the control (X-CPM/100 μg DNA) value and multiplied with 100.

Statistical analysis

Data obtained from different sets of experiments were analyzed statistically. The level of significance (P-value) between two sets of data was evaluated according to Student’s t-test, with P<0.05 indicating significance.

Results and Discussion

Activation of GR-complex at 25°C, as ascertained by both DNA-cellulose and nuclear binding assays showed approximately 3.2-fold increase in acceptor binding, compared to the same at 0°C, indicating that heat significantly induces GR-complex activation (Fig. 1). The present study was aimed to understand a dose-dependent modulatory effect of different SFAs and PUFAs on hepatic GR activation. To assess in vitro receptor activation-modulation by fatty acids, DNA-cellulose binding assay was employed and the results obtained were unexpected. Palmitic and stearic acids, when used at increasing concentrations (0-160 μM) were unable to inhibit the heat activation
of GR-complexes (data not shown). In contrast, the three PUFAs showed a dose-dependent inhibition of GR-complexes at 0-200 μM concentrations. Oleic acid showed a maximal inhibition of heat activation of ~38% at 40 μM, whereas linoleic and arachidonic acids exhibited maximal inhibition (~70%) at 160 μM (Fig. 2).

Since DNA-cellulose is a non-specific assay system to assess activation, it could not unequivocally implicate the inhibitory effects of PUFAs on acceptor binding by activated hormone-receptor complexes. Hence we employed purified nuclei from hepatic cells to provide a more relevant physiological assay system. Nuclear binding assay results also showed the inability of palmitic and stearic acids in inhibiting GR heat activation (data not shown), thereby corroborating with the DNA-cellulose binding data. The magnitude of GR heat activation-inhibition by PUFAs, as ascertained by nuclear binding assay matched closely (Fig. 3), with the DNA-cellulose binding data. Hence, both assays revealed a similar pattern of GR heat activation-inhibition by the three PUFAs.

Earlier, free fatty acids have been shown to modulate receptor functions of many steroidal hormones, including glucocorticoids. In the present study, we propose that SFAs and PUFAs might be the modulators of hepatic GR activation. We showed that all the three PUFAs inhibited GR heat activation, whereas SFAs were unable to inhibit the activation, possibly due to the absence of unsaturation. Amongst the PUFAs, the higher per cent inhibition by linoleic and arachidonic acids, compared to oleic acid suggests increase in inhibitory potency concomitant to increase in carbon chain length and unsaturation to a certain extent. However, the exact mechanism of the inhibitory effect by PUFAs on GR activation is unknown. The particular domain in the GR, responsible for the inhibition of activation has not been characterized and the group(s) in the PUFAs that interact with such domain is not known. PUFAs, probably induce some conformational change in the receptor, through the involvement of their unsaturated...
moieties, thereby limiting or inhibiting the dissociation of bound heat shock proteins. The inhibitory effects of PUFAs in the binding of [\( ^3 \)H] dexamethasone to hepatic GR in fishes\(^\text{18}\) suggest the unsaturated moiety(ies) as the likely candidate; however, this remains to be determined. From our data, we hypothesize that unsaturated moieties in PUFAs are important determinants of GR activation. The role of nutrients in the regulation of intracellular signaling via members of nuclear receptor superfamily has become an exciting area of research\(^\text{19,20}\). Fatty acids may be one of the simple nutritionally-derived molecules that exert a modulatory effect on glucocorticoid signaling. Our in vitro findings reveal the role of fatty acids in GR function modulation and suggest that GR function could be regulated directly and/or indirectly by the fatty acids, wherein, the degree of unsaturation may act as suitable binder to the receptor. The level of PUFAs may, thus govern the functionality of GR in controlling the metabolic status of the tissues.

Acknowledgement

We express gratitude towards the Department of Biochemistry, North Eastern Hill University, Shillong, for providing research facilities. The grant-in-aid from the Department of Science and Technology (Govt. of India), New Delhi is also gratefully acknowledged.

References