Biochimica et Biophysica Acta, 640 (1981) 159—168

BBA 79045

ELEVATION OF APPARENT MEMBRANE VISCOSITY IN OVARIAN GRANULOSA CELLS BY FOLLICLE-STIMULATING HORMONE

B. STRULOVICI [a,**], H.R. LINDNER [a], M. SHINITZKY [b] and U. ZOR [a,*]

[a] *Department of Hormone Research and* [b] *Department of Membrane Research, The Weizmann Institute of Science, Rehovot (Israel)*

(Received June 16th, 1980)

Key words: Oleate; Cycloheximide; cyclic AMP; Fluorescence polarization; FSH; Membrane viscosity

Summary

Continued exposure of cultured granulosa cells to follicle-stimulating hormone (FSH) induced: (i) a rise in apparent membrane microviscosity, as reflected by an increase in fluorescence polarization of the lipid-soluble probe, 1,6-diphenyl-1,3,5,-hexatriene; and (ii) a progressive decline in the cyclic AMP response to renewed challenge with the same hormone. Both changes were reduced or prevented by pretreatment of the cells with oleic or linoleic acid, agents which reduce membrane viscosity, but not by elaidic or palmitic acid which increase the rigidity of membrane lipids. Other agents that inhibited FSH-induced changes in membrane fluidity (gonadotropin-releasing hormone, actinomycin D and cycloheximide) also prevented desensitization to FSH. Cyclic AMP and cyclic GMP derivatives did not mimic the effects of FSH on apparent membrane viscosity or desensitization.

Changes in membrane fluidity are unlikely to be the sole cause of desensitization since (i) pretreatment of the cells with fatty acids that increase lipid viscosity did not induce desensitization to FSH, and (ii) desensitization of granulosa cells to lutropin and prostaglandin E_2 by exposure to the homologous hormone was not attended by increased membrane viscosity.

The experiments described provide the first example of a hormonally induced increase in the target cell apparent membrane viscosity.

* To whom correspondence should be addressed.

** This work was carried out in partial fulfilment of the requirements for the Ph.D. degree of the Graduate School of the Weizmann Institute of Science.

Introduction

There is abundant evidence that receptors to hormones are freely mobile in the plasma membrane [1—4] and that they are physically separated from the membrane-bound adenylate cyclase [5—11]. Hormonal stimulation therefore depends on effective coupling of the hormone-receptor complex to the enzyme [12,13]. The degree of membrane fluidity significantly influences the rate of lateral mobility and coupling [14,15] of receptors. Thus, addition of certain unsaturated free fatty acids increases membrane fluidity and enhances the stimulatory effect of catecholamines on adenylate cyclase activity [15]. Conversely, addition of cholesterol increases membrane viscosity and abolishes prostaglandin E stimulation of platelet adenylate cyclase [16].

Prolonged exposure of cells to high concentrations of hormones leads to desensitization of the adenylate cyclase system to challenge with fresh hormones [12,17,18]. Although the mechanism underlying refractoriness differs from one hormone to another and from system to system, there is some indication that at least in some systems desensitization results from uncoupling of the hormone-receptor complex from the catalytic moiety of adenylate cyclase [12,19,21].

We now show that prolonged exposure of cultured granulosa cells to follicle-stimulating hormone (FSH) induces an increase in apparent membrane viscosity and that agents which prevent this change also inhibit the development of desensitization to FSH. The question is posed whether there is a causal relationship between the two phenomena.

Experimental procedure

Materials

Pregnant mare serum gonadotropin (PMSG) was purchased from N.V. Organon, Oss, Holland. Rat FSH (150 NIH U/mg), FSH-I-4 and ovine lutropin (NIAMDD-LH-S21) were kindly made available by Dr. A.F. Parlow, Harbor General Hospital, Torrance, CA. Prostaglandin E_2 was a generous gift from Dr. J. Pike of the Upjohn Co., Kalamazoo, MI. Gonadotropin-releasing hormone (GRH) was a gift of the Hoechst AG., Frankfurt, F.R.G.

Oleic, linoleic, linolenic, palmitic and elaidic acids of 99% purity were obtained from P-L Biochemicals, Inc., Milwaukee, WI. Stock solutions of fatty acids were prepared by dissolving 3 mg in 1 ml absolute ethanol. Arachidonic acid, cycloheximide, actinomycin D, 8-bromo-cyclic AMP, 8-bromo-cyclic GMP were purchased from Sigma, St. Louis, MO. 3-Isobutyl-1-methylxanthine was purchased from Aldrich Chemical Co., Milwaukee, WI. Diphenylhexatriene was obtained from Sigma and made up in tetrahydrofuran at 2 mM and stored in the dark at room temperature.

Preparation of granulosa cells

Immature (24-day-old) Wistar-derived rats from the departmental colony were injected with 20 I.U. PMSG and killed 48 h later by cervical dislocation. Granulosa cells were harvested at room temperature by puncturing the follicles with a blunt probe and applying gentle pressure to the follicles. Then the cells

were collected [22,23] and viability (about 70—80%) was examined by exclusion of trypan blue. Granulosa cells were cultured in McCoy's medium with addition of glutamine (2 mM), fetal calf serum (10%), penicillin (50 U/ml) and streptomycin (50 μg/ml) [23].

Cyclic AMP determinations

Cells were cultured in suspension or in monolayers for various times. After culture with the test substance, the cells were washed with phosphate-buffered saline, pH 7.2, and incubated with FSH for 30 min in a medium containing 3-isobutyl-1-methylxanthine (0.1 mg/ml). The cyclic AMP content of the cultured granulosa cells was then assayed by a modification [17] of the competitive protein binding assay of Gilman [24].

Determination of fluorescence polarization

Lipid fluidity was evaluated by fluorescence polarization of the lipid probe diphenylhexatriene (for a review, see Ref. 25).

Cells were cultured in suspension. At the end of the culture, the granulosa cells were washed with phosphate-buffered saline. 1 vol. of a $2 \cdot 10^{-6}$ M dispersion of diphenylhexatriene in phosphate-buffered saline was mixed with 1 vol. of $2 \cdot 10^{-6}$ granulosa cells/ml phosphate-buffered saline [26]. After 15 min incubation at 37°C the cells were washed and suspended in phosphate-buffered saline at 10^6 cells/ml. This suspension was used for measurement of fluorescence polarization. Since diphenylhexatriene can penetrate intracellular organelles, we have assessed the contribution of the cell plasma membranes to the recorded degree of fluorescence polarization using the method of Grunberger and Shinitzky (unpublished results). Diphenylhexatriene-labeled cells were exposed to 2,4,6-trinitrobenzenesulfonate (TNBS; 2 mg/ml) for 1 h at 4°C. Under these conditions, TNBS attaches only to the outer cell surface and almost totally quenches the diphenylhexatriene fluorescence in the plasma membrane. After three washes with phosphate-buffered saline, the fluorescence intensity and polarization were recorded and compared with an identical diphenylhexatriene-labeled cell sample which had not been treated with TNBS. The percentage of quenching exerted by TNBS labeling provided the percentage contribution of the plasma membrane to the recorded fluorescence polarization.

Results

Apparent membrane viscosity, as reflected by an increase in the degree of fluorescence polarization of diphenylhexatriene, increased with time at 6—18 h of incubation with FSH; at 3 h there was a slight, though not significant, increase in this parameter (Fig. 1). In contrast, prostaglandin E_2 or lutropin (LH) (1.0 μg/ml) did not change apparent membrane viscosity when tested at 6 or 18 h, respectively (data not presented). The 8-bromo derivatives of cyclic AMP and cyclic GMP also failed to mimic the effects of FSH on apparent membrane viscosity. Of the total diphenylhexatriene fluorescence associated with cells, 70—75% was quenched by TNBS and hence was assumed to be associated with the plasma membrane.

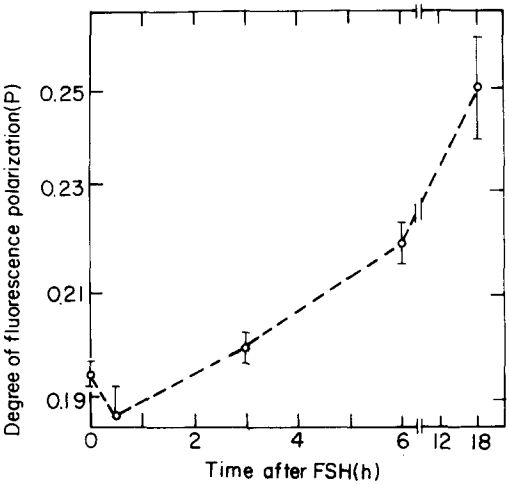

Fig. 1. Time course of effect of FSH on the degree of fluorescence polarization of diphenylhexatriene. Cells were cultured with FSH in suspension for the time indicated, washed and incubated with diphenylhexatriene for 15 min. Shown are mean values (n = 9) ± S.E. (vertical bars).

The response of granulosa cells cultured as monolayers to prolonged exposure to FSH, LH or prostaglandin E_2 in terms of stimulation of cyclic AMP formation is shown in Table I. Pretreatment with FSH for 12 h induced desensitization to challenge with fresh FSH, but left the response to LH partially unimpaired and to prostaglandin E_2 unaffected. Likewise, exposure in vitro to either LH or prostaglandin E_2 desensitized granulosa cells to the homologous hormone, but did not prevent the subsequent response to FSH. Analysis of the time-course of the effect of FSH on granulosa cell monolayers (Fig. 2) showed

TABLE I

SELECTIVE DESENSITIZATION OF GRANULOSA CELLS TO PROSTAGLANDIN E_2, LH or FSH

Granulosa cells were cultured for 24 h in a medium containing the hormone prostaglandin E_2 (1 μg/ml), LH (1 μg/ml) or FSH (0.25 μg/ml) as indicated, or without added hormone (control). The cells were thoroughly washed with phosphate-buffered saline, pH 7.2, incubated for 30 min in a medium containing 3-isobutyl-1-methylxanthine (100 μg/ml) with the hormone specified (challenge) and cyclic AMP accumulation was determined as described in Experimental Procedure. The results are expressed as mean values (n = 12) ± S.E. PGE_2, prostaglandin E_2.

Culture (24 h)	Challenge (30 min)	Cyclic AMP (pmol/5 · 10^5 granulosa cells)
Control	Control	2.0 ± 0.4
Control	FSH	60.0 ± 5.0
FSH	FSH	3.5 ± 0.4
FSH	LH	70.0 ± 3.5
FSH	PGE_2	82.5 ± 3.5
Control	LH	125.0 ± 10.0
LH	LH	6.2 ± 2.0
LH	FSH	32.0 ± 4.0
LH	PGE_2	56.0 ± 6.0
Control	PGE_2	60.0 ± 5.0
PGE_2	PGE_2	4.0 ± 1.0
PGE_2	LH	108.0 ± 5.0
PGE_2	FSH	60.0 ± 5.0

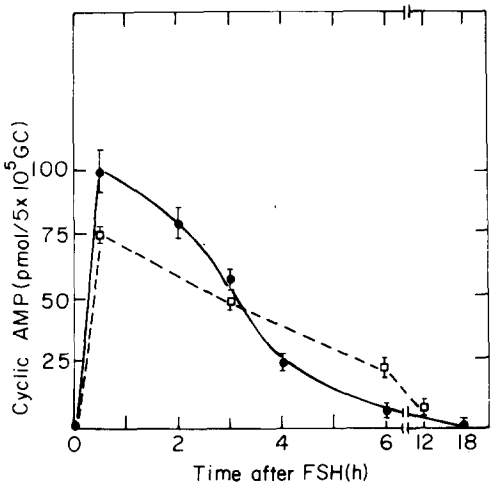

Fig. 2. Time-course of effect of FSH on cyclic AMP accumulation in granulosa cells (GC). Cells were cultured in monolayers (●) or suspensions (□) for the time indicated, washed and then challenged with FSH in a medium containing 3-isobutyl-1-methylxanthine. Cyclic AMP accumulation was determined as described under Experimental Procedure. Shown are mean values (n = 12) ± S.E. (vertical bars).

TABLE II

EFFECT OF VARIOUS AGENTS ON APPARENT MEMBRANE VISCOSITY AND FSH-INDUCED DESENSITIZATION IN GRANULOSA CELLS

Granulosa cells were cultured in suspension (apparent membrane viscosity studies) or as monolayers (cyclic AMP experiments). (A) After initial incubation with a fatty acid (10 μg/ml) or GRH (0.1 μg/ml) as indicated, FSH (0.25 μg/ml) was added to the medium and incubation was continued for a further 16 h. The suspension cultures were then used for measurement of fluorescence polarization after 15 min incubation with diphenylhexatriene. The monolayers were rinsed and challenged with FSH (0.25 μg/ml) in fresh medium containing 3-isobutyl-1-methylxanthine (100 μg/ml) for 30 min and cyclic AMP accumulation was assayed as described in Experimental Procedure. (B) Cell suspensions were cultured for 6 h with prostaglandin E_2 (PGE_2) or 20 h (all other treatments). Shown are mean values ± S.E. (n = 12). Basal level of cyclic AMP was 2 ± 0.4 pmol/5 · 10^5 cells.

Culture	Degree of fluorescence polarization (P) at 25°C	Cyclic AMP (pmol/5 · 10^5 granulosa cells)
(A)		
Control	0.190 ± 0.006	68 ± 4
FSH	0.250 ± 0.001	5 ± 0.1
Linoleic acid + FSH	0.176 ± 0.002	23 ± 2
Linolenic acid + FSH	0.180 ± 0.001	25 ± 3
Arachidonic acid + FSH	0.166 ± 0.003	26 ± 2
Palmitic acid + FSH	0.300 ± 0.005	1 ± 0.2
Elaidic acid + FSH	0.290 ± 0.003	2 ± 0.4
GRH + FSH	0.200 ± 0.004	24 ± 2
(B)		
Control	0.195 ± 0.003	
Cyclic AMP (1 mM)	0.200 ± 0.006	
Cyclic GMP (1 mM)	0.220 ± 0.001	
LH (1 μg/ml)	0.193 ± 0.002	
PGE_2 (1 μg/ml; 6 h only)	0.193 ± 0.003	

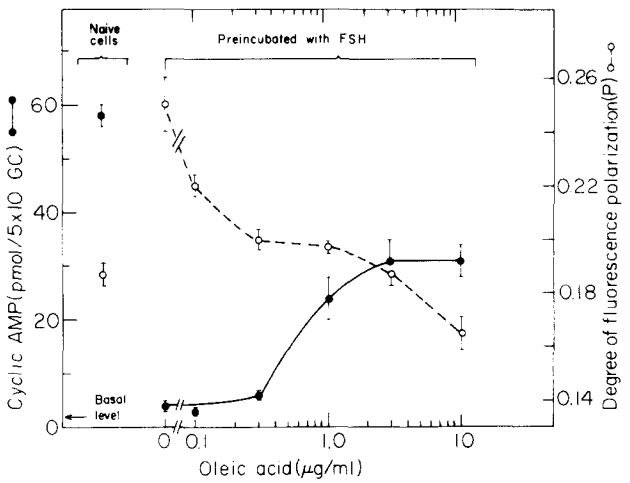

Fig. 3. Prevention by oleic acid of FSH-induced increase in fluorescence polarization and desensitization of cyclic AMP response in culture granulosa cells. Granulosa cells were preincubated with various doses of oleic acid for 4 h and then washed. FSH (0.25 μg/ml) was then added and incubation continued for another 18 h as suspension cultures. The degree of fluorescence polarization of diphenylhexatriene (o— — —o) or cyclic AMP accumulation (●———●) were then determined as described in the legends to Figs. 1 and 2. 'Naive cells' were cultured without added oleic acid or FSH under similar conditions and then challenged with FSH as above. Shown are mean values (n = 6—12) ± S.E.

that the cyclic AMP response to FSH was slightly, but not significantly, reduced at 2 h after the addition of FSH whereas desensitization was pronounced after 3 h and maximal (90%) after 6—18 h. Sustained treatment of granulosa cell suspensions with FSH give similar results, viz., progressive desensitization to the hormone was observed at 3 h (34% loss of response), which was maximal at more prolonged exposure to the hormone (60% at 6 h and 90% at 18 h).

Preincubation of granulosa cells in a medium supplemented with oleic acid

TABLE III

PREVENTION BY ACTINOMYCIN D AND CYCLOHEXIMIDE OF FSH-INDUCED INCREASE IN FLUORESCENCE POLARIZATION AND DESENSITIZATION OF CYCLIC AMP RESPONSE IN CULTURED GRANULOSA CELLS

Cells were cultured in control medium or with actinomycin D (AcD; 8 μg/ml) or cycloheximide (Ch; 5 μg/ml) together with FSH (0.25 μg/ml) for 24 h. Fluorescence polarization and cyclic AMP accumulation in the presence of 3-isobutyl-1-methylxanthine were then determined as described in the legends to Fig. 1 and Table I. Results are expressed as mean values (n = 6—12) ± S.E.

Culture (24 h)	Challenge (30 min)	Cyclic AMP (pmol/5 · 10^5 cells)	Degree of fluorescence polarization (P) at 25°C
Control	Control	0.5 ± 0.04	0.190 ± 0.006
Control	FSH	68.0 ± 4.0	0.188 ± 0.004
FSH	FSH	4.0 ± 0.2	0.250 ± 0.001
Ch + FSH	FSH	45.0 ± 3.0	0.190 ± 0.005
AcD + FSH	FSH	51.0 ± 3.0	0.120 ± 0.002
Ch	FSH	71.0 ± 5.0	0.192 ± 0.005
AcD	FSH	76.0 ± 6.0	0.170 ± 0.004
Ch	Control	0.8 ± 0.06	0.190 ± 0.006
AcD	Control	0.6 ± 0.02	0.142 ± 0.003

(1.0 μg/ml) prevented the FSH-induced increase in apparent membrane viscosity; at high concentrations (10 μg/ml), this fatty acid reduced apparent membrane viscosity to below the control level (Fig. 3). Exposure to oleic acid at 1.0—10 μg/ml reduced by about 50% the degree of desensitization of the cyclic AMP response of granulosa cells cultured as monolayers to a maximally effective dose of FSH (0.25 μg/ml; Fig. 3) and abolished the desensitization induced by a submaximal dose of FSH (0.1 μg/ml; data not shown). Three other unsaturated fatty acids, viz., linoleic (18 : 2), linolenic (18 : 3) and arachidonic acid (20 : 4), like oleic acid, prevented the FSH-induced increase in apparent membrane viscosity and reduced the extent of desensitization (Table II). However, supplementation of the medium with palmitic (16 : 0) or elaidic (*trans* 18 : 1) acids (10 μg/ml) caused a marked increase in fluorescence polarization from $P = 0.190$ (control) to $P = 0.260$ (palmitic) or to $P = 0.250$ (elaidic) without any accompanying change in the acute response to FSH (data not shown) or in the course of desensitization to the hormone (Table II).

Cycloheximide, actinomycin D (Table III) and GRH (Table II) prevented the FSH-induced changes in fluorescence polarization and partially prevented desensitization.

Discussion

The experiments described yielded three salient findings: (i) continued exposure of ovarian granulosa cells to FSH caused a progressive increase in fluorescence polarization of the lipid-soluble probe diphenylhexatriene; (ii) FSH also caused progressive desensitization of the cyclic AMP accumulation response of these cells; and (iii) diverse agents that prevented the FSH-induced changes in diphenylhexatriene fluorescence polarization, e.g., oleic acid, cycloheximide and GRH, reduced the degree of desensitization to the hormone. However, in a number of experimental situations (see below) these two parameters changed independently.

It has been proposed that measurements of fluorescence polarization, using embedded aromatic fluorophores such as diphenylhexatriene, reflect the microviscosity of the lipid bilayer and that an estimate of this microviscosity can be derived from such measurement by assuming that the Perrin relationship holds [25]. However, doubts have been expressed as to the validity of this assumption for steady-state polarization measurements which do not permit determination of rotational correlation times, and there is still some uncertainty as to the precise interpretation of the P values obtained in terms of biological membrane properties [27,28]. Moreover, there is evidence that the diphenylhexatriene lipid probe does not remain confined to the plasma membrane but penetrates internal membranous structures [29], though under the conditions of our experiment, viz., 15 min incubation, most of the fluorescence (75%) originated in the plasma membrane. Because of these uncertainties, we choose to present the untransformed polarization data rather than expressing these in terms of microviscosity units and prefer to use tentatively the term 'apparent microviscosity' in this discussion.

Desensitization of ovarian cells by FSH has been previously observed in organ cultures of Graafian follicles [18] and is here described for isolated

granulosa cells. In both instances, desensitization is specific for the homologous hormone. The precise mechanism underlying this desensitization is not clear, but work on LH and catecholamines suggests that it involves primarily an uncoupling of the hormone receptor from adenylate cyclase [12,20,21], followed in some cases by a reduction in the number of receptor sites [30,31].

Pari passu with the development of refractoriness to FSH, an increase in apparent microviscosity was observed. This raises the question as to whether a causal relationship exists between these two events. Several observations argue in favor of such a relationship: (i) the time course of the two changes was somewhat similar; (ii) the inclusion in the medium of fatty acids that reduce apparent membrane viscosity also reduced the degree of desensitization by FSH, whereas fatty acids that increase apparent membrane viscosity lack this effect; (iii) inhibitors of macromolecular synthesis such as cycloheximide and actinomycin D, which are known to prevent or delay desensitization [12], also mitigate the FSH-induced increase in apparent membrane viscosity; and (iv) GRH also inhibited both parameters.

However, a number of discrepant observations throw doubt on this interpretation: (i) after 3 h exposure of the cells to FSH, apparent membrane viscosity was not yet significantly higher than in untreated controls, whereas responsiveness of the adenylate cyclase-cyclic AMP system to the hormone was already reduced by about 40% (Figs. 1 and 2); (ii) the increase in apparent membrane viscosity induced by addition of palmitic or elaidic acid to the medium was not attended by a reduced responsiveness of adenylate cyclase to FSH; (iii) addition to the medium of oleic acid at a concentration (0.3 μg/ml) sufficient to reduce apparent membrane viscosity in FSH-desensitized cells to near the basal level (Fig. 3) failed to have a significant effect on the cyclic AMP response of the cells to FSH, while further increase in oleic acid concentration to 1.0 μg/ml reduced the degree of desensitization to FSH without a further fall in apparent membrane viscosity. It should also be noted that oleic acid supplementation at a dose (10 μg/ml) that reduced apparent membrane viscosity to below its basal level only partially prevented the desensitization (approx. 50%) caused by exposure to high doses of FSH (0.25 μg/ml), though complete protection against desensitization was afforded when a submaximal dose of the hormone (0.1 μg/ml) was used. Finally, continued exposure of the same cells to LH or prostaglandin E_2 resulted in desensitization to the homologous hormone, with approx. 90% reduction in responsiveness, without a concomitant change in apparent membrane viscosity. Thus, even if changes in apparent membrane viscosity do play a part in the development of desensitization to FSH, this cannot be a general mechanism applicable to all hormones. The three hormones studied also differ in the sensitivity of their action to agents that disrupt the structure and function of the microtubules and microfilaments [19,32].

It is of interest that insulin has also been reported to increase apparent membrane viscosity in liver plasma membranes, but the effect is a rapid one, manifest within 15 min at 4°C [33]. It appears, therefore, that this insulin effect does not depend on metabolic activity and thus may not be analogous to the FSH effect described here which requires macromolecular synthesis.

It would appear then that the data reported here do not fully support the

hypothesis that the membrane changes reflected by fluorescence polarization measurements, presumably related mainly to plasma membrane microviscosity, are solely responsible for the development of desensitization of granulosa cells to FSH. The deviations observed from the relationship between these two parameters, predicted by the above hypothesis, suggest at least that additional factors operate in bringing about the refractory state, such as newly formed protein [12], phosphorylation of a component involved in hormone receptor-cyclase coupling [34] and/or changes in cytoskeletal elements [19,32].

Five reports from other laboratories tend to support the view that the responsiveness of hormone-stimulable adenylate cyclase may be influenced by changes in membrane fluidity. Unsaturated fatty acids were shown to increase the response of turkey erythrocytes to catecholamines [14,15]. In the presence of methyl donors, catecholamines induced methylation of phospholipids resulting in increased microfluidity and responsivenss to these hormones [34,35]. Finally, increased microviscosity due to cholesterol enrichment of the cell membrane resulted in reduced responsiveness of platelets to prostaglandins [16].

The question remains by what mechanism could FSH influence membrane fluidity in its target cells. Mediation of the effect by cyclic nucleotides seems ruled out since (i) neither 8-bromo cyclic AMP nor 8-bromo cyclic GMP could mimic this FSH effect, and (ii) LH and prostaglandin E_2, which stimulate cyclic AMP production in granulosa cells, fail to influence apparent membrane viscosity (Table II). It is also possible that FSH, which causes differentiation of granulosa cells [37], induces phospholipase, triacylglyceride lipase or cholesterol esterase in granulosa cells, and as a result, influences the lipid composition of the membrane. Such effects were observed with catecholamines [36,38]. Exogenous phospholipases A and C (but not D) have not been shown to impede the response of adenylate cyclase in testicular homogenates to FSH [39,40]. Whilst the precise mechanism underlying the effect of FSH on fluorescence polarization of granulosa cells and its relation to the phenomenon of desensitization (if any) remain to be clarified, this appear to be the first report of a hormonally induced increase in apparent membrane microviscosity in the target cell.

References

1 Cuatrecasas, P., Hollenberg, M.D., Chang, K.-J. and Bennett, V. (1975) Rec. Prog. Horm. Res. 31, 37—94
2 Amsterdam, A., Nimrod, A., Lamprecht, S.A., Burstein, Y. and Lindner, H.R. (1979) Am. J. Physiol. 236, E129—138
3 Jacobs, S. and Cuatrecasas, P. (1976) Biochim. Biophys. Acta 433, 482—495
4 Schlessinger, J., Shechter, Y., Cuatrecasas, P., Willingham, M.C. and Pastan, I. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 5353—5357
5 Orly, J. and Schramm, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4410—4416
6 Ross, E.M. and Gilman, A.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3715—3718
7 Sahyoun, N., Hollenberg, M.D., Bennett, V. and Cuatrecasas, P. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2860—2864
8 Houslay, M.D., Ellory, J.C., Smith, G.A., Hesketh, T.R., Stein, J.M., Warren, G.B. and Metcalfe, J.C. (1977) Biochim. Biophys. Acta 467, 208—219
9 Schramm, M., Orly, J., Eimerl, S. and Korner, M. (1977) Nature 268, 310—313
10 Limbird, L.E. and Lefkowitz, R.J. (1977) J. Biol. Chem. 252, 799—802

11 Schramm, M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1174—1178
12 Lamprecht, S.A., Zor, U., Salomon, Y., Koch, Y., Ahrén, K. and Lindner, H.R. (1977) J. Cyclic Nucl. Res. 3, 69—83
13 Haga, T., Ross, E.M., Anderson, H.J. and Gilman, A.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2016—2020
14 Orly, J. and Schramm, M. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3433—3437
15 Rimon, G., Hanski, E., Braun, S. and Levitzki, A. (1978) Nature 276, 394—396
16 Sinha, A.K., Shattil, S.J. and Colman, R.W. (1977) J. Biol. Chem. 252, 3310—3314
17 Lamprecht, S.A., Zor, U., Tsafriri, A. and Lindner, H.R. (1973) J. Endocrinol. 57, 217—233
18 Zor, U., Lamprecht, S.A., Misulovin, Z., Koch, Y. and Lindner, H.R. (1976) Biochim. Biophys. Acta 428, 761—765
19 Zor, U., Strulovici, B., Lamprecht, S.A., Amsterdam, A., Oplatka, A. and Lindner, H.R. (1979) Prostaglanins 18, 869—882
20 Johnson, G.L., Woolfe, B.B., Harden, T.K., Molinoff, P.B. and Perkins, J.P. (1978) J. Biol. Chem. 253, 1472—1480
21 Su, Y.-F., Harden, T.K. and Perkins, J.P. (1979) J. Biol. Chem. 254, 38—41
22 Clark, M.R., Marsh, J.M. and LeMaire, W.J. (1978) J. Biol. Chem. 253, 7757—7761
23 Nimrod, A. and Lindner, H.R. (1976) Mol. Cell. Endocrinol. 5, 315—320
24 Gilman, A.G. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 305—312
25 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 1—42
26 Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133—149
27 Dale, R.E., Chen, L.A. and Brand, L. (1977) J. Biol. Chem. 252, 7500—7510
28 Sene, C., Genest, D., Obreinovitch, A., Wahl, P. and Monsigny, M. (1978) FEBS Lett. 88, 181—186
29 Johnson, S.M. and Nicolau, C. (1977) Biochem. Biophys. Res. Commun. 76, 869—874
30 Lefkowitz, R.J., Mullikin, D., Wood, C.L., Gore, T.B. and Mukherjee, C. (1977) J. Biol. Chem. 252, 5295—5303
31 Catt, K.J., Harwood, J.P., Aquilera, G. and Dufau, M.L. (1979) Nature 280, 109—116
32 Zor, U., Strulovici, B. and Lindner, H.R. (1978) Biochem. Biophys. Res. Commun. 80, 983—992
33 Luly, P. and Shinitzky, M. (1979) Biochemistry 18, 445—450
34 Ezra, E. and Salomon, Y. (1980) J. Biol. Chem. 255, 653—658
35 Hirata, F. and Axelrod, J. (1979) Nature 275, 219—220
36 Hirata, F., Strittmatter, W.T. and Axelrod, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 368—372
37 Richards, J.T. (1980) Physiol. Rev. 60, 51—89
38 Mallorga, P., Talman, J.F., Henneberry, R.C., Hirata, F., Strittmatter, W.T. and Axelrod, J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1341—1345
39 Abou-Issa, H. and Reichert, L.E., Jr. (1976) J. Biol. Chem. 251, 3326—3337
40 Abou-Issa, H. and Reichert, L.E. (1979) J. Endocrinol. 104, 189—193