Vol. 103, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1981 Pages 638-646

PREVENTION OF PROSTAGLANDIN E_2-INDUCED DESENSITIZATION OF RAT OVARIAN CYCLIC AMP RESPONSE BY CONCANAVALIN A

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Received October 9,1981

SUMMARY

Prolonged exposure (> 6 h) of cultured granulosa cells to Prostaglandin E_2 (PGE_2; 1 µg/ml) led to a near-total loss of the cyclic AMP response to subsequent addition of fresh hormone. Pre-treatment of the cells with concanavalin A (ConA; 2.0 µg/ml) for 1 h blocked the desensitizing action of PGE_2, so that the decline in the response was reduced by 60% with the hormone at high concentration (1.0 µg/ml); a full response was preserved at submaximal concentration of PGE_2 (0.1 - 0.3µg/ml). Other lectins (succinyl Con A, peanut agglutinin and, to a lesser extent, phytohemagglutinin and wheat germ agglutinin) had a stabilizing effect similar to that of Con A. Addition of alpha-methyl-mannoside either with Con A or various times following the addition of Con A to the cells prevented the protective effect of Con A. Concomitant treatment with colchicine or cytochalasin B abolished the ability of Con A to prevent PGE_2-induced desensitization.

INTRODUCTION

Prolonged exposure of various cell types to PGE initiates induction of desensitization of the adenylate cyclase-cyclic AMP system to rechallenge with fresh hormone (1-7). Recently, it has been suggested that "down regulation" of PGE receptors is responsible for the desensitization (5-6). We have recently shown that the cytoskeletal system may be involved in the

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* In partial fulfilment of the requirements for the Ph.D. degree of the Graduate School of the Weizmann Institute of Science.

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Supported by grants to Dr. H.R. Lindner from the Ford Foundation and the Rockefeller Foundation.

0006-291X/81/220638-09$01.00/0

process of desensitization to PGE_2 since agents that stabilized the microtubules-microfilament systems prevented desensitization (4, 8).

Receptors to concanavalin A are widely distributed among various cells (9-10), including ovarian follicles (11) and granulosa cells (12-14). Con A binding to the cell surface modulates the mobility of various membrane receptors (9, 15) and inhibits the formation of patches and caps (16), a process which may involve the cytoskeleton (9, 14 and 16). In addition, Con A significantly reduced the rate of internalization and degradation of receptors to acetylcholine in muscle cells (17) and prevented desensitization induced by acetylcholine (18) or glutamate (19).

In the present study we examined whether Con A and other lectins could prevent PGE_2-induced desensitization and if the cytoskeletal system is involved in this process.

MATERIALS AND METHODS

Materials

PGE_2 was a generous gift from Dr. J. Pike of the Upjohn Co., Kalamazoo, Michigan. Concanavalin A (Con A, 2 x crystallized), peanut agglutinin (PNA), phytohemmaglutinin (PHA) and wheat-germ agglutinin (WGA) were purchased from Miles-Yeda Ltd., Israel; succinyl concanavalin A from Polysciences, Inc., Warrington, Pennsylvania, D-galactose and alpha-methyl D-mannoside from Sigma Chemical Co., St. Louis, Missouri, and pregnant mare serum gonadotropin (PMSG) from Organon-Oss, Holland. Cytochalasin B and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Aldrich, England. The cytochalasin B was dissolved in dimethylsulfoxide (DMSO, Fluka AG, Buch SG, Switzerland) as a stock solution of 5 mg/ml and the final concen-

tration in the medium did not exceed 0.05% (v/v). DMSO at the same concentration was added to control follicles. Colchicine was purchased from Merck, W. Germany.

Methods

24-day old Wistar rats from our departmental colony were injected with PMSG (15 I.U./rat) and sacrificed 48 h later. Large ovarian preovulatory follicles were punctured, and the granulosa cells were expressed gently and cultured in McCoy's medium containing 10% fetal calf serum, and 20 mM glutamine, 100 U/ml penicillin and 50 μg/ml streptomycin. Multiwell tissue culture plates (Costar No. 3524) were used with each well containing 0.5×10^6 cells in 0.5 ml medium. After the initial 24 h, medium was aspirated from the monolayers and replaced with fresh serum-free medium.

Induction of desensitization to PGE_2

The protocol consists of three steps: I) preincubation of the cells with or without lectin for 1 h; II) addition of PGE_2 for various periods of time; and III) rechallenging the cells with fresh PGE_2 and 3-isobutyl-1-methyl-xanthine (IBMX; 0.1 mg/ml), a phosphodiesterase inhibitor, for an additional 30 min. The cells were washed between steps I and II and between steps II and III. In the experiment described in Figure 4, cells were not washed between steps I and II.

cAMP Measurement

The above reaction was terminated by the addition of 1 M of Na acetate (pH 4). The cAMP content of the total culture system (media + cells) was determined by a modification (20) of the protein binding assay described by Gilman (21).

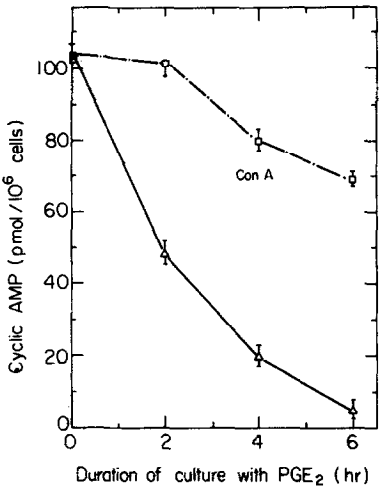


Fig. 1. Protective effect of Con A against PGE_2-induced desensitization. Granulosa cells were preincubated with (—□—) or without (—△—) Con A (2 μg/ml) for 1 h, washed and further incubated with PGE_2 (1 μg/ml) for the time indicated. At the end of this period, cells were rechallenged with PGE_2 in the presence of IBMX for 30 min and their cAMP content was determined. The data shown represents mean values (n = 6-12) ± S.E.M.

RESULTS

Cultured granulosa cells responded to addition of the maximally effective dose of PGE_2 with an immediate rise in cAMP production (Fig. 1-4). Prolonged exposure of granulosa cells to PGE_2 induced a progressive decline (50%) in the cAMP accumulation after 2 h, and a 95% reduction after 6 h (Fig. 1). However, exposure of granulosa cells to Con A 1 h prior to addition of PGE_2, preserved the cells' ability to respond to the hormone and the degree of decline was significantly attenuated (Fig. 1). The partial desensitization which occurs at 2 h of incubation with PGE_2 was completely prevented by the lectin, while at 6 h 60% of the response to PGE_2 was preserved (Fig. 1-2). Moreover, full protection by Con A was achieved at 6 h if submaximal doses of PGE_2 (0.1 - 0.3 μ/ml) were used in order to induce desensitization (Fig. 2). Other lectins such as

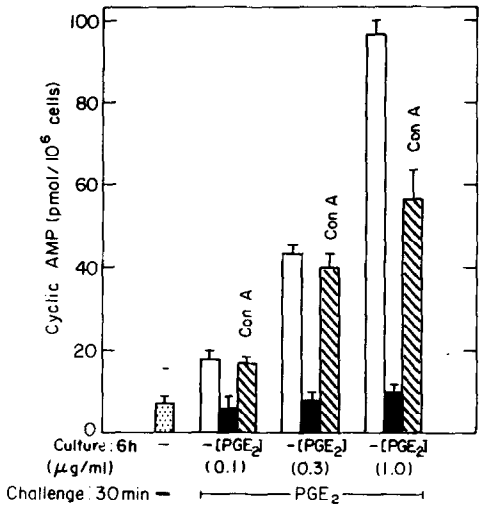
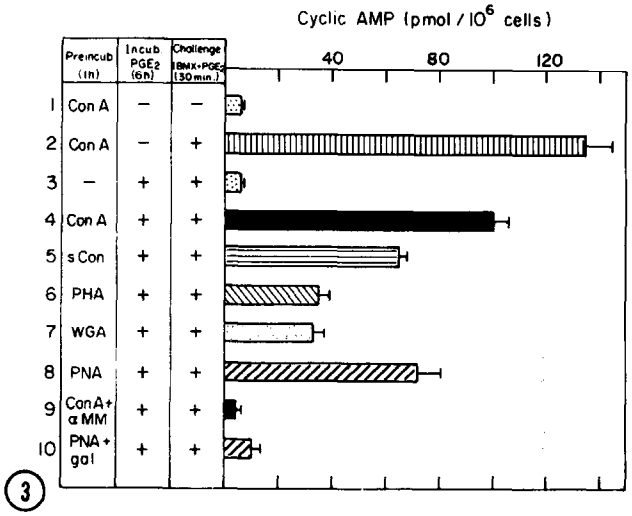


Fig. 2. Effect of Con A on the induction of desensitization by submaximal doses of PGE_2. The cells were preincubated with (▨) or without (□ ; ■) Con A (2 μg/ml) for 1 h, washed and then cultured for 6 h longer with (■ ; ▨) or without (□) addition of various doses of PGE_2 as indicated in the figure. Subsequently, the cells were challenged with PGE_2 at the same doses as in the culture period. The data shown represents means of three experiments ± S.E.M..

succinyl Con A, PNA, PHA and WGA prevented the development of refractoriness to PGE_2 similar to Con A (Fig. 3).

The question of whether continuous binding of Con A to plasma membranes is essential for its protective effect against development of refractoriness was examinded. To dissociate the Con A from the membrane, alpha MM (150 mM) was used. Addition of alpha MM to ConA-treated cells either 30 min before addition of PGE_2 or together with PGE_2 prevented the effect of Con A. In similar experiments, D-galactose (200 mM) inhibited the protective effect of PNA (Fig. 3). In order to achieve protection against PGE_2-induced desensitization, the cells must be incubated with Con A prior to the addition of PGE_2. Simultaneous addition of Con A with PGE_2 to the cells did not prevent desensitization. Moreover, in order to protect cells against development of refractoriness, Con A should be present on the cell sur-



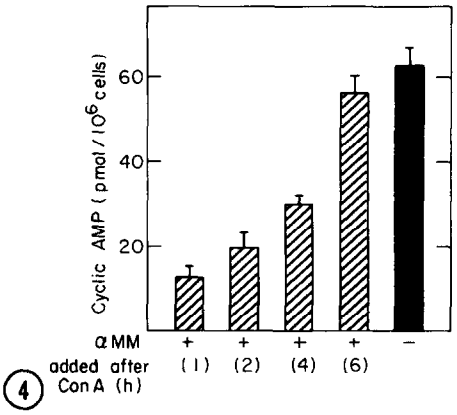


Fig. 3. Effect of various lectins on PGE_2-induced desensitization. Granulosa cells were preinbuated for 1 h with either Con A (2 μg/ml), sCon A (succinyl Con A, 20 μg/ml), PNA (peanut agglutinin), PHA (phytohemmagglutinin) or WGA (wheat germ agglutinin) each at 2 μg/ml as indicated. To group nine alpha MM (alpha-methyl-D-manoside; 150 mM) was added with Con A while to group ten, gal (D-galactose; 200 mM) was added with PNA. PGE_2 (1 μg/ml) was added to group 3-10 for another 6 h. After challenge with IBMX (group 1) or IBMX + PGE_2 (groups 2-10), cAMP levels were measured. The results represent the mean of 4 determinations of 3 separate experiments ± S.E.M.

Fig. 4. Prevention by alpha-methyl-D-mannoside (alpha MM) of the protective effect of Con A on PGE_2-induced desensitization. Granulosa cells were incubated with Con A for 1 h, then cultured with PGE_2 for additional 6 h. Alpha MM (150 mM) was added 1, 2, 4 or 6 h after ConA: ■ no alpha MM added; cAMP was measured after rechallenge with PGE_2 and IBMX for 30 min. The response of desensitized cells in the absence of Con A was 7 pmol/10^6 Cells. Vertical brackets, ± S.E.M.(n = 8).

face throughout the incubation period since stripping of Con A by alpha MM even after 4 hr of exposure resulted in less protection (Fig. 4).

The role of cytoskeletal elements in the protective effect of Con A was investigated by using the microtubule inhibitor colchicine and the microfilament inhibitor cytochalasin B (Fig. 5). Each of these drugs, when added with Con A to the cells 1 h prior to prolonged exposure to PGE_2, inhibited the protective effect of Con A. In the absence of Con A, the acute stimulation

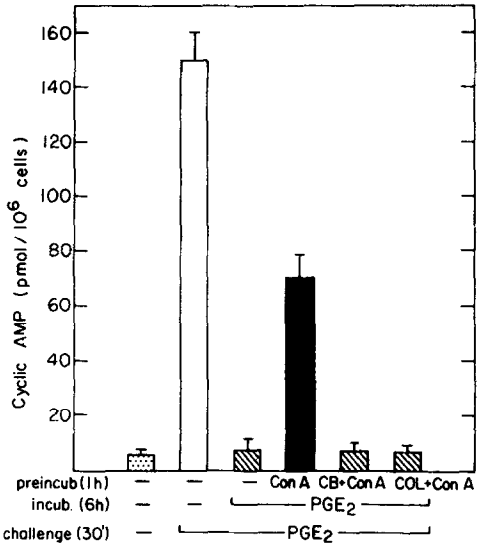


Fig. 5. Abolishment of the protective effect of Con A by cytochalasine B or colchicine. Granulosa cells were preincubated for 1 h with Con A alone or with Con A + cytochalasine B (CB; 3 μg/ml), or with Con A + colchicine (COL; 1 μg/ml), washed and then PGE_2 was added for an additional 6 h as indicated in the figure. Challenge to PGE_2 was done as described in Fig. 4. In control experiments, cells were preincubated with CB or COL for 1 h, washed, and further cultured in drug-free medium for 6 h. Cyclic AMP levels when challenged with PGE_2 + IBMX were 138 ± 12 pmol/10^6 cells for the CB group or 125 ± 6 pmol/ 10^6 cells for the COL group. Shown are means ± S.E.M. of 3 experiments.

of cAMP formation by PGE_2 as well as PGE_2-induced desensitization were unaffected by these drugs (Legend to Fig. 5).

DISCUSSION

The present study shows that Con A and other lectins prevent desensitization induced by PGE_2. Con A must be added prior to the hormone and must remain bound to the cell surface throughout the culture period. The integrity of the microtubule--microfilament system seems to be essential for expressing the protective effect of ConA.

Although we have no conclusive evidence to point to the mechanism of Con A action, our data strongly suggests that Con A may inhibit and retard the mobility and internalization of recep-

tors to PGE, probably via the rearrangement of the cytoskeleton. This hypothesis is in agreement with the effect of Con A on mobility (9,15) and half-life (17) of membrane receptors and with the study showing PGE-induced receptor "down regulation" during desensitization (5-6).

The mobility of membrane receptors is controlled inter alia by the microtubule-microfilament sytems (15,22). Binding of Con A to various cells results in realignment of the cytoskeleton components beneath the plasma membrane (14, 23-24) which may lead to the rearrangement of PGE receptors, increasing their half-life and, as a consequence, prevent desensitization. This is in accord with our previous results showing that stabilizers of the cytoskeleton prevent desensitization to PGE_2 (4,8). Moreover, this hypothesis is also in agreement with the results of the present study as well as other studies demonstrating that Con A loses its activity when agents that disrupt the cytoskeleton are added with the lectins (9, 15).

It was suggested that in the process of hormone-induced desensitization the hormone-receptor complex is uncoupled from the GTP regulatory binding protein and from the adenylate cyclase moiety (7, 25-27). The mechanism of Con A action may be related to its ability to prevent the hormone-receptor from being uncoupled from the regulatory protein following prolonged exposure to PGE_2.

REFERENCES

1. Newcombe, D.S., Ciosek, C.P., Jr., Tshikava, Y, and Fahey, J.V. (1975) Proc. Nat. Acad. Sci. U.S.A., 72, 3124-3128.
2. Su, Y.-F., Johnson, G.L., Cubeddu, X.L., Leichtling, B.H., Ortmann, R., and Perkins, J.P. (1976) J. Cycl. Nucl. Res. 2, 271-285.

3. Ayad, S.R. and Burns, G.R.J. (1979) Exper. Cell Res. 118, 229-235.
4. Zor, U., Strulovici, B., Lamprecht, S.A., Oplatka, A., and Lindner, H.R. (1979) Prostaglandins 18, 869-882.
5. Lefkowitz, R.J., Mullikin, D., Wood, C.L., Gore, T.B., and Mukherjee, C. (1979) J. Biol. Chem. 252, 5295-5303.
6. Davies, P.J.A., Chabay, R., Zech, L., Berman, M., and Pastan, I., (1980) Biochim. Biophys. Acta 629, 282-291.
7 Lamprecht, S.A., Zor, U., Salomon, Y., Koch, Y., Ahren, K., and Lindner, H.R. (1977) J. Cycl. Nucl. Res. 3, 69-83.
8. Zor, U., Strulovici,B., Lamprecht, S.A., Oplatka, A., and Lindner, H.R. (1979) Ovarian Follicular and Corpus Luteum Function, Eds. C.P.Channing, J.M.Marsh and W.A. Sadler. pp. 679-686, Plenum Publishing Corporation.
9. Edelman, G.M. (1976) Science 192, 218-226.
10. Goldstein, I.J. and Hayes, C.E. (1978) Adv. in Carbohydrate Chemistry and Biochemistry, Vol. 35, pp. 128-154, Academic Press, New York.
11. Lee, C.Y. and Ryan, R.J. (1979) Biol. of Reprod. 21, 973-977.
12. Albertini, D.F. and Clark, J.I. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 4976-4980.
13. Clark, J.I. and Albertini, D.F. (1976) Cell Motility, Cold Spring Harbor Laboratory, pp. 323.
14. Albertini, D.F. and Anderson, E. (1977) J. Cell Biol. 73, 111-127.
15. Henis, Y.I. and Elson, E.L. (1981) Proc. Nat. Acad. Sci. U.S.A. 78, 1072-1076.
16. Yahara, I. and Edelman, G.M. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 1583-1590.
17. Prives, J., Hoffman, L., Tarrab-Hazdai, R., Fuchs, S., and Amsterdam, A. (1979) Life Sci. 24, 1713-1718.
18. Kirpekar, S.M. and Prat, J.C. (1978) Br. J. Pharmac. 62, 549-552.
19. Mathers, D.A. and Usherwood, P.N.R. (1976) Nature 259, 409-411.
20. Lamprecht, S.A., Zor, U., Tsafriri, A., and Lindner, H.R. (1973) J. Endocrinol. 57, 217-233.
21. Gilman, A.G. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 305-312.
22. Cherksey, D.B., Zadunaisky, A.J., and Murphy, B.R. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6401-6405.
23. Ash, J.F. and Singer, S.J. (1976) Proc. Natl. Acad. Sci. U.S.A., 73, 4575-4579.
24. Geiger, B. and Singer, S.J. (1979) Cell 16, 213-222.
25. Su, Y.-F., Harden, T.K., and Perkins, J.P. (1980) J.Biol. Chem. 255, 7410-7419.
26. Iyengar, R. and Birnbaumer, L. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 3189-3193.
27. Ezra, E. and Solomon, Y. (1980) J. Biol. Chem. 255, 653-658.