EFFECTS OF STEROIDS ON THE REGULATION OF THE LEVELS OF CYCLIC AMP IN HUMAN LYMPHOCYTES

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Summary

Glucorticosteroids, and estradiol increase the cyclic AMP response of lymphocytes to isoproterenol and PGE_1. This response, unlike the usual steroid responses initiated by specific cytoplasmic steroid receptors, does not require the biosynthesis of macromolecules for its activity. The effect may depend partially on the inhibitory effect of steroids on cyclic nucleotide phosphodiesterase. The potentiating effect of steroids however, is greater than that can be achieved with theophylline, a more potent inhibitor of phosphodiesterase prepared from lymphocytes.

Introduction

Glucocorticoids affect the metabolism of various tissues(1,2). The details of this effect have not been fully established, but glucocorticoids penetrate the cell membrane and bind to receptor proteins. The hormone-receptor complex in turn promotes messenger ribonucleic acid synthesis and ultimately cytoplasmic protein synthesis. Thus, macromolecular synthesis is required for many steroid actions(2).

Some of the effects of glucocorticoids may involve the formation(3,4) or the action of cyclic AMP(5,6). Mitogenesis of lymphocytes in response to phytohemagglutinin (PHA) and concanavalin-A (con A) is inhibited by cortisol and related glucocorticoid hormones(5,6). Agents such as prostaglandin E_1 (PGE_1) and isoproterenol elevate the level of cyclic AMP and also inhibit

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mitogenesis. This inhibitory effect of cyclic AMP and PGE_1 is potentiated by cortisol(5). Incubation of lymphocytes with PGE_1(5) or β-adrenergic agents(7) in the presence of cortisol result in a greater stimulation of the level of cyclic AMP in the cells than that observed with an agonist alone. This interaction between agonists (such as catecholamine and prostaglandins) and cortisol in regulating cyclic AMP might be responsible for cortisol's effect in the regulation of the metabolism of lymphocytes(5). Prednisolone(7) and dexamethasone(5) possess similar activity. For this reason, the potentiating effect of cortisol has been considered as a characteristic of glucocortioids. The purpose of the present study is to determine whether potentiation of the effect of PGE_1 and isoproterenol by a variety of steroids has any relation to their efficacy as a glucocorticoid and, whether the effect requires macromolecular synthesis.

Methods

Materials: Ficoll, cortisol, cortisone, corticosterone, deoxycorticosterone, aldosterone, progesterone, testosterone, estradiol, prednisolone, dexamethasone, triamcinolone, were purchased from the Sigma Chemical Company, St. Louis, Missouri. Stock solutions of steroids (10 mM) were prepared in ethanol and diluted with medium 199 (GIBCO, Grand Island, New York). Hypaque sodium (diatrizoate sodium) and l-isoproterenol-d-bitartrate were obtained from Winthrop Labs, Rensselear, New York. Prostaglandin E_1 was a gift of Dr. John Pike of the Upjohn Company, Kalamazoo, Michigan.

Isolation and Incubation of Lymphocytes: Procedures for the isolation of lymphocytes were modified from the method described by Boyum(8). The detail of the procedure for the isolation of the lymphocytes and the incubation has been described elsewhere(9). The levels of cyclic AMP were determined by the saturation assay(10).

Results and Discussion

Levels of cyclic AMP in lymphocytes respond maximally to isoproterenol and PGE_1 at the concentration of 10^{-5}M. The basal level of cyclic AMP varies among different individuals. Therefore the responses of all the drugs tested are expressed as the ratio of the cyclic AMP in the presence of added agonist(s) versus that in the absence of agonists (control). The potentiating effects of steroids were evaluated by the difference between values measured with the

Table 1

The Effect of Steroids on the Responses of Lymphocytes to Isoproterenol and PGE_1

(10^{-5}M) Steroid Added	Agonist Added: 0	Isop. (10^{-5}M)	PGE_1 (10^{-5}M)	Anti Inflamma Activi
	Ratio of Cyclic AMP (Agonist added/control)			
0	1.0	2.70±0.29	4.75±0.47	
I. Adrenosteroids				
1. Cortisol	1.24±0.07*	4.26±0.31**	7.28±0.67**	1.00
2. Cortisone	0.90±0.08	3.06±0.40	5.72±0.47**	0.65
3. Corticosterone	0.95±0.13	4.48±0.48**	7.00±1.02**	0.35
4. Deoxycorticorterone	1.04±0.18	3.92±0.25**	5.70±0.40**	0
5. Aldosterone	0.86±0.10	3.30±0.46	6.03±0.80**	0.35
II. Synthetic Glucocorticoids				
6. Prednisolone	1.38±0.28	5.82±0.80**	7.85±0.77**	4.0
7. Dexamethasone	1.00±0.17	3.46±0.40	6.37±0.74**	25.0
8. Triamcinolone	1.06±0.22	4.54±0.21**	6.50±0.50**	5.0
III. Sex Steroids				
9. Testosterone	1.08±0.10	2.81±0.53	4.98±0.20	-
10. Progesterone	1.01±0.14	2.82±0.30	4.69±0.21	-
11. 17β-estradiaol	1.12±0.09	4.17±0.34**	6.82±0.76**	-

* - Mean ± S.E.M. (Average of 9 subjects for each steroid)

** - Steroid vs no steroid $p < 0.05$

‡ - Values copied from Ref. 13

steroid and agonists (isoproterenol or PGE_1) together versus values measured with agonists alone. Most steroids had a marginal effect on the basal level of cyclic AMP at the concentration tested (10^{-5}M) (Table 1).

Among the adrenocorticoids tested, cortisol was the most active in po-

tentiating the effect of isoproterenol and PGE_1. Discrepancies between the glucocorticoid activity and the potentiating activity measured were evident.

A number of synthetic glucocosteroids, such as prednisolone, dexamethasone and triamcinolone are more potent anti-inflammatory agents than cortisol (11). In this class of steroids only prenisolone had a greater potentiating effect at the concentration of 10 μM than cortisol. The effects of dexamethasone and trimicinolone were moderate.

The potentiating effect was not limited to glucocorticoids. 11-deoxy corticosterone which is devoid of glucocorticosteroid activity in vivo(11,12) exhibited some potentiating effect on both isoproterenol and PGE_1. Of the sex steroids tested, progesterone and testosterone had no effect. Estradiol however, potentiated the effect of isoproterenol and PGE_1 almost as effectively as cortisol. There was no difference in the effect of the sex steroids on lymphocytes from men and women.

All steroids tested caused a small, dose dependent increase in the basal level of cyclic AMP (Table 2). The stimulatory effect of isoproterenol and PGE_1, also increase with the increasing concentration of steroids except progesterone and testosterone. Among different steroids there is no correlation between the stimulatory effect of a given steroid on basal level of cyclic AMP and its potentiating activity.

The effect of cortisol occures rapidly. Preincubation of the cells with cortisol is not needed. This effect lasts for at least 120 minutes. Preincubation of cells with actinomycin D (4 μM) or puromycin (500 μM) for 40 minutes, prior to the addition of cortisol, does not block the effect of cortisol (unpublished observation).

When the effect of steroids on the stimulatory activity of isoproterenol and PGE_1 is compared, there is a correlation between the potentiation of isoproterenol and PGE_1 (R=0.87). This may indicate that the potentiating effect of steroids is mediated through a common mechanism. It has been reported that cortisol inhibits cyclic nucleotide phosphodiesterase(13) At the concentration

Table 2

Effects of different dosage of steroids on the agonist regulated level of cyclic AMP in lymphocytes

Steroids Added	Agonist Added: 0	Isop (10^{-5})	PGE_1 (10^{-5})
	Ratio of cyclic AMP (Agonist added/control)		
1. Cortisol			
0	1.0	2.19 ± 0.2*	4.88±0.40
10 μM	1.21± 0.10	3.32 ± 0.35**	6.95±1.00**
100 μM	1.73±0.35	4.57 ± 0.7 **	10.19±1.50**
2. Estradial: 0	1.0		4.55±0.40
1 μM	1.08±0.20		4.65±0.29
10 μM	1.20±0.09		6.98±0.77**
100 μM	1.23±0.20		9.23±1.10**
3. Testosterone 0	1.0	3.62	
1 μM	1.0	3.28	
10 μM	1.0	3.70	
100 μM	1.28±0.10	3.30	
4. Dexamethasone 0	1.0		6.55 ± 0.50
1 μM	1.06±0.18		6.81 ± 0.89
10 μM	1.18±0.17		8.27 ± 0.68**
100 μM	1.44±0.23		12.66 ± 1.11**

* Mean ± S.E.M. (Average of 4 subjects for each steroid)
** Steroid vs No steroid $p<0.05$

used in the present experiments (10^{-5}M), it inhibited only about 10% of the activity of the phosphodiesterase in homogenate of lymphocytes. Theophylline (1 mM) inhibited more than 90% (unpublished data). The potentiating effect of cortisol on cyclic AMP levels is greater than that of the 1 mM theophylline (Table 3). These results would suggest that the inhibition of phosphodiesterase

Table 3

Effects of cortisol, theophylline, isoproterenol and PGE_1 in single or in combination on the level of cAMP in lymphocytes

Addition	Expt.1	Expt.2	Expt.3	Expt.4
	cyclic AMP (pmoles/10^6 cells)			
0 (control)	3.6*	2.71	2.76	4.09
cortisol, 10 μM	4.8	2.63	5.66	4.24
Theophylline, 1 mM	4.81	3.96	3.86	3.92
Isoproterenol, 10 μM	9.64	10.44		
Isop. + cortisol	17.3	15.29		
Isop. + theophylline	12.03	10.87		
PGE_1, 10 μM	21.34		14.6	8.97
PGE_1 + cortisol	47.0		38.1	19.27
PGE_1 + theophylline	27.5		32.6	15.2

* - Average of duplicates samples, the difference between duplicates is less than 10 %.

by cortisol only contributes partially to the potentiating effect.

In conclusion, it is felt that the potentiating effect of cortisol *in vitro* may not be a specific glucocorticoid action as has been suggested(5). High concentrations of steroids are needed and their effects do not correlate well with anti-inflammatory activity. However, as the regulation of cyclic AMP in lymphocytes is concerned, the mechanism of the potention deserves further investigation. In lymphocyte homogenates inhibition of cortisol cycli AMP phosphodiesterase(s) by cortisol does not fully account for the effect. In addition, cortisol does not potentiate membrane adenyl cyclase nor dihydroalprenolol binding. Therefore, further investigation is needed for elucidating the mechanism involved.

Bibliography

1. Baxter, J.D., and Forsham, P.H. (1972) Am. J. Med., 53, 573.

2. O'Malley, B.W., McGuire, W.L., Kohler, P.O., and Korenman, S.T. (1969) Recent Progress Hormone Res., 25, 105.

3. Friedmann, N., Exton, J.H., and Park, C.R. (1967) Biochem, Biophys. Res. Commun., 29, 113.

4. Schaeffer, L.D., Chenoweth, M., and Dunn, A. (1969) Biochem. Biophys. Acta., 192, 304.

5. Mendelsohn, J., Multer, M.M., and Boone, R.F. (1973) J. Clin. Invest., 52, 2129.

6. Nowell, P.C. (1961) Cancer Res., 21, 1518.

7. Parker, C.W., Huber, M.G., and Bauman, M.L. (1973) J. Clin. Invest., 52, 1342.

8. Boyum, A. (1967) Scand. J. Clin. Lab. Invest., 97 (Suppl.), 77.

9. Lee, T.P. (1977) J. Allergy Clin. Immun., 59, 79.

10. Brown, B.C., Alban, J.D.M., Ekins, R.P., Sgherzi, A.M., and Tampion, W. (1971) Biochem. J., 121, 561.

11. Sayers, G., and Travis, R.H. (1970) The Pharmacological basis of therapeutics, pg. 1604, McMillan Company, New York.

12. Thorn, G.W., Kerph, G.F., Lewis, R.A., and Olsen, E.F. (1940) J. Clin. Invest., 29, 813.

13. Schmidtke, J., Wienker, T., Filiigel, M., and Engel, W. (1976) Nature, 262, 593.