EFFECT OF LOW CONCENTRATION OF STEROIDS ON THE ACTIVITY OF GLYCYLGLYCINE DIPEPTIDASE IN VITRO

Enrique Piña, Alberto Hamabata, and José Laguna

Departamento de Bioquímica, Facultad de Medicina
Universidad Nacional Autónoma de México, México

Received October 25, 1962

Most of the information on the effect of steroidal hormones on enzyme activity has been derived from in vivo experiments; however, these results are difficult to interpret on account of the various physiological factors involved and the high doses utilized to produce such changes.

There are very few reports in the literature showing an effect of low concentrations of steroidal hormones on enzyme activity in vitro. The majority of observations have been made on NAD dependent enzymes: activation of hydroxysteroid dehydrogenases with estradiol (Hagerman and Villee, 1957), and with estrone and testosterone (Talalay and Williams-Ashman, 1958); the discussed activation of L-glutamic dehydrogenase with corticosterone or cortisone (Engel et al., 1960; Yielding et al., 1960), etc.

We have studied the effect of several steroids in vitro and we have been able to show that the glucocorticoids hydrocortisone and triamcinolone at a concentration of about 5×10^{-7} M produce a significant increase in the activity of muscle glycylglycine dipeptidase (CGD) an ubiquitous hydrolytic enzyme for which no coenzyme requirements are known. This enzyme was selected for several reasons: its relative abundance in the great mass of mammalian muscle; the fact that its activity may be enhanced in vivo by the administration of cortisone (Schwarz et al., 1956); furthermore, Rose

et al. (1959) have shown a clear parallelism between leucyl aminopeptidase (LAP) and protein catabolism, and evidence has been produced showing that LAP and GGD are the same protein enzyme, their only difference in apparent specificity being the type of cation (Mn^{++} or Co^{++}) present in the media (Vescia, 1956).

Hind leg muscles of adult male albino rats were homogenized in 0.1 M Tris (hydroxymethyl) aminomethane buffer, pH 7.6 (1:5), and centrifuged at 1,000 g at 0^0 C. for 10 minutes; the precipitate was discarded and the supernatant centrifuged at 19,000 g for 20 minutes; the supernatant was used as enzyme source. An aliquot of the enzyme preparation was incubated with the substrate and the hormone; another aliquot, without the hormone, was set up as control. Incubation of the enzyme was carried out according to Smith (1955) and the rate of hydrolysis was measured through the increase in alpha-amino nitrogen (Rosen's method, 1957); time interval used to measure enzyme activity was 2 hours. All determinations were carried out in duplicate.

As it is shown in table 1, where typical results are presented, a statistically significant increase in GGD activity was observed when either hydrocortisone or triamcinolone were added to the incubation media; the other steroidal hormones assayed showed a trend towards an enhancement of the enzyme activity, but in no case statistical significance could be found. This facts points to a biological specificity and not merely to a structural unspecific action, since only hydrocortisone and triamcinolone, having a similar physiological glucocorticoid action, showed an effect on the activity of GGD, a catabolic enzyme. The above mentioned effect, specially marked for hydrocortisone, may be of physiological importance inasmuch as it may operate throughout the whole muscular mass in the mammal. Fur-

thermore, in these experiments, effective hormonal concentration falls within the accepted physiological range for hydrocortisone.

Table 1

Effect of steroids on glycylglycine dipeptidase activity

Steroid	Concentration	Activity ± S.E.	Percentual increase	Significance "t" test
Hydrocortisone	0.0	9.83 ± 0.65 (16)*	**	
"	5.5×10^{-7} M	12.56 ± 0.55	26	p 0.005
Triamcinolone	0.0	8.08 ± 0.29 (7)		
"	4.2×10^{-7} M	9.12 ± 0.41	12.7	p 0.05
Estradiol	0.0	7.28 ± 0.83 (8)		
"	7.3×10^{-7} M	7.81 ± 0.92	7.2	no
"	7.3×10^{-6} M	8.29 ± 0.71	13.9	no
Testosterone	0.0	4.35 ± 0.51 (8)		
"	6.9×10^{-7} M	4.35 ± 0.63	0	no
"	6.9×10^{-6} M	4.83 ± 0.70	10.8	no
Desoxy-corticosterone	0.0	8.08 ± 0.29 (7)		
"	6.0×10^{-7} M	8.69 ± 0.64	7.5	no

The reaction mixture contained: 0.05 M Tris (hydroxymethyl) aminomethane buffer, pH 7.6; 0.05 M glycylglycine; 0.01 M $CoCl_2$; enzyme, 5.25 mg. of protein in final volume of 5.0 ml. Steroids were added in 0.01 ml. of ethyl alcohol; controls contained same amount of ethyl alcohol. Incubation time at 37^0 C., 2 hours. Activity expressed as micromoles of glycine liberated per gram of wet muscle per hour.

* Figures in parentheses indicate number of experiments with individual animals used for each steroid.

** Controls, with no steroid added, are considered as 100 % activity.

In summary, the observed increase in GGD activity in the presence of glucocorticoids might point to an enhancement of the well known catabolic effect of these steroids in the intact animal. This would be a particular case in which in vitro and physiologic effects of steroids could be related.

References

Engel, L. L., Scott, Y. F., and Colman, R. F., Fed. Proc. 19, 159 (1960).
Hagerman, D. D., and Villee, C. A., J. Biol. Chem. 229, 589 (1957).
Rose, H. G., Robertson, M. C., and Schwarz, T. B., Am. J. Physiol. 197, 1063 (1959).
Rosen, H., Arch. Biochem. Biophys. 67, 10 (1957).
Schwarz, T. B., Robertson, M. C., and Holmes, L. B., Endocrinology 58, 453 (1956).
Smith, E. L., in Methods in Enzymology, Colowick, S. P., and Kaplan, N. O. eds. New York, Academic Press Inc. II, p. 107 (1955).
Talalay, P., and Williams-Ashman, H. G., Proc. Nat. Acad. Sci. U.S. 44, 15 (1958).
Vescia, A., Biochim. Biophys. Acta 19, 174 (1956)
Yielding, K. L., Tomkins, G. M., Munday, J. S., and Curran, J. F., Biochem. Biophys. Res. Comm. 2, 303 (1960).