Inhibition of Human Testicular Glucose-6-Phosphate Dehydrogenase by Unsaturated C_{19}-Steroids

In previous publications[1,2], the inhibition of human red blood cell or placental glucose-6-phosphate dehydrogenase (G-6-PDH) by various C_{19}- and C_{21}-steroids has been reported. From these investigations a definite relationship could be established between the chemical structure of the particular steroid and its inhibitory activity. In continuation of such experiments the effect of 3β-substituted Δ^4- or Δ^5-C_{19}-steroids upon human testicular G-6-PDH was studied.

Testicular tissue was obtained from a 69-year-old male patient undergoing orchidectomy due to cancer of the prostate. After homogenization in 5 ml 0.9% sodium chloride/0.025% EDTA per g of wet tissue and a 20 min centrifugation at 18,000 *g*, the supernatant was submitted to an ammonium sulfate precipitation at 35% saturation. The precipitate was dissolved in 10 ml 0.05 *M* triethanolamine/0.005 *M* EDTA buffer of pH 7.6 and treated with 140 ml of a 13.1% suspension of hydroxyl apatite in 0.001 *M* phosphate buffer of pH 6–8 (SERVA Feinbiochemica, Heidelberg). Following a centrifugation the adsorbed enzyme activity was eluted from the precipitate by means of 200 ml 0.1 *M* phosphate buffer of pH 7.6. To the eluate sufficient ammonium sulfate was added to give a 50% saturation. Subsequently, the suspension was centrifuged for 20 min at 18,000 *g* and the precipitate dissolved in 6.0 ml triethanolamine/EDTA buffer. All assays of G-6-PDH activity were performed at 25 °C in 0.1 ml of the purified enzyme preparation, 3.0 ml triethanolamine/EDTA buffer, 0.1 ml 0.03 *M* NADP, 0.02 ml dioxan, eventually containing the steroid and 0.05 ml glucose-6-phosphate solution of varying concentration. The final concentration of steroid corresponded to a $10^{-5}M$ solution. From changes in the absorbance at 366 nm, registerd over 10 min, the enzyme activity was estimated and the inhibition constant K_i determined by the method of HUNTER and DOWNS[3].

Whereas the crude enzyme preparation exhibited a specific activity of 2.4 mU/mg protein, the purified enzyme possessed a specific activity of 126 mU/mg protein, indicating a 53-fold purification. The K_M-value of the purified enzyme was found to be $9.8 \times 10^{-5}M$ for glucose-6-phosphate as substrate. The inhibition of the enzyme by different steroids is demonstrated by their K_i-values in the Table. As can be seen 3β-hydroxy-4-androsten-17-one turned out to be the most effective inhibitor of the various steroids tested. This steroid with its equatorial hydroxy group and a planar half-chair configuration of rings A and B even surpassed 3β-hydroxy-5-androsten-17-one (dehydroepian-drosterone). The reduction of the 17-oxo group in the latter steroid, yielding 5-androstene-3β, 17β-diol (androstenediol), led to an almost complete loss of inhibitory activity. Additional functional groups, such as hydroxy groups at C-7, C-16 or C-19, reduced the biological activity of dehydroepiandrosterone as the parent compound to a considerable degree. Likewise, the replacement of the 3β-hydroxy group by a 3β-chloro group resulted in a remarkable decrease of activity. At the same time it can be stated that the 17-oxo group is required for inhibitory activity of C_{19}-streoids, as evidenced by the rather high K_i-value of 3β, 17β-dihydroxy-5-androsten-16-one. In every respect such findings agree with former results[1] and support the assumption that the presence of a 17-oxo-group, an equatorial hydroxy group at C-3 in the planar ring A/B configuration of 5α-, Δ^4- or Δ^5-C_{19}-steroids, as well as the lack of additional functional groups near C-5 provide the optimal structure for steroid inhibitors of G-6-PDH in the human organism. Since under physiological conditions, however, the levels of the more active steroids hardly reach a $10^{-6}M$ concentration – except perhaps in placental tissue – the participation of free C_{19}-steroids in the regulation of human G-6-PDH activity seems unlikely.

Inhibition of human testicular glucose-6-phosphate dehydrogenase by 3β-substituted Δ^4- or Δ^5-C_{19}-steroids

Steroid	K_i-value (for G-6-P)
3β-hydroxy-4-androsten-17-one	$6.6 \times 10^{-6}M$
3β-hydroxy-5-androsten-17-one	7.6
3β,16α-dihydroxy-5-androsten-17-one	$1.3 \times 10^{-5}M$
3β-hydroxy-5-androstene-16,17-dione	1.7
3β,7β-dihydroxy-5-androsten-17-one	2.9
3β,7α-dihydroxy-5-androsten-17-one	4.4
3β-chloro-5-androsten-17-one	4.6
3β-hydroxy-5-androstene-7,17-dione	6.9
3β,19-dihydroxy-5-androsten-17-one	8.6
3β,17β-dihydroxy-5-androsten-16-one	$1.4 \times 10^{-4}M$
5-androstene-3β,17β-diol	2.8
5-androstene-3β,16α, 17β-triol	4.5

Zusammenfassung. Die Bebrütung einer gereinigten G-6-PDH aus menschlichem Testisgewebe mit 3β-substituierten Δ^4- oder Δ^5-C_{19}-Steroiden zeigte, dass 3β-Hydroxy-Δ^4- oder Δ^5-androsten-17-on auch wirksame Inhibitoren der testikulären G-6-PDH darstellen. Zur gleichen Zeit konnten die früher gefundenen Zusammenhänge zwischen chemischer Struktur eines Steroids und seiner biologischen Aktivität im G-6-PDH-Hemmtest uneingeschränkt bestätigt werden.

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Determination of Methylguanidine in Serum and Urine from Normal and Uremic Subjects

The activated charcoal employed in the method of YATZIDIS et al.[1] for measuring monosubstituted guanidines in serum, catalyzes the oxidation of creatinine (CR) and creatine to methylguanidine (MG)[2]. Our previous observations on the MG contents in serum and urine obtained with a modification of this procedure[3,4], are falsely high and we retract them.

In the present paper a method is described to measure MG in body fluids, and the serum concentrations, as well as the daily urinary outputs found with it in normal subjects and in uremic patients, are reported.

Such procedure is based on the use of a strong cation-exchange resin to separate MG from creatine and CR, from the other Sakaguchi-reacting materials (arginine, guani-