Effect of Thyroxin on Heparin Inhibition of Cortisone A-Ring Reduction

Heparin inhibits hepatic reduction of the 4–5 double bond of Δ^4-3-ketosteroids both in vitro[1,2] and in vivo[3]. The mechanism is thought to involve competition between heparin and NADPH for attachment to the steroid reductases[4,5] because excess NADPH overcomes the inhibition but excess substrate does not. Lineweaver-Burk plots also indicate a competitive relationship between NADPH and heparin[5].

The rate of hepatic reduction of Δ^4-3-ketosteroids is controlled by the concentration of NADPH and the level of steroid reductase activity. Thyroxin treatment of the intact animal has an effect on both of these mechanisms[6]. Liver homogenates from rats treated with thyroxin for 3 days exhibited an increase in the rate of steroid reduction which could be explained by an increase in available NADPH. Treatment for 16 days resulted in a large increase in steroid 5α-reductase in liver microsomes. Thyroxin had no direct effect on steroid reductase when it was preincubated with rat liver microsomes at a concentration of $1.5 \times 10^{-3} M$.

If, as postulated, heparin competes with NADPH, increased availability of NADPH produced by acute thyroxin treatment might diminish heparin inhibition of cortisone reduction. This hypothesis was tested by treating 200 g female Holtzman rats with 2 μmoles of thyroxin daily for 3 days after which the livers were removed and assayed for cortisone reductase activity. According to McGuire and Tomkins[6] this treatment should markedly increase hepatic NADPH but it would not be sufficient time for induction of cortisone reductase.

Details of preparing whole liver homogenates and of the incubation and extraction procedures have been published[1]. No NADPH-generating system or exogenous NADPH was added to the incubates. Cortisone A-ring reduction was determined by UV-absorption at 240 nm.

The experiment is summarized in the Table. It is emphasized that no NADPH-generating system was added to the homogenates which were therefore completely dependent upon endogenous cofactors for activity. The rate of cortisone reduction by homogenates from control rats was 49 μg/10 min. This was much less metabolism than in similar experiments[1] which employed a NADPH-generating system (254 μg/10 min) and it again points up the fact that NADPH is a rate-determining factor in the reaction.

The thyroxin-treated animals had a significantly greater rate of reduction than the control rats (73 vs 49; $P = 0.02$) and an increase in available NADPH could account for the increased rate. Increasing concentrations of heparin produced progressive inhibition of cortisone reduction by homogenates from control rats. At the high concentration of 500 units in a final incubation volume of 7 ml, heparin almost completely (94%) inhibited the reaction. In contrast, homogenates from thyroxin-treated rats were not significantly inhibited even by the highest concentration of heparin. This is attributed to increased availability of NADPH produced by thyroxin.

Since NADPH is a rate limiting factor in cortisone A-ring reduction, the reaction rate under standard conditions is a reflection of the effective NADPH concentration. In view of this, it is interesting that conditions which produced cortisone reduction at the rate of 73 μg/10 min failed to exhibit heparin inhibition such as was seen in earlier experiments[1] in which exogenous NADPH produced a rate of 254 μg/10 min. Endogenous NADPH seems to be more effective in preventing heparin inhibition of cortisone reduction than is exogenous NADPH produced by a generating system. This might suggest that heparin is acting at the level of NADPH re-generation by the dehydrogenase systems in experiments which employed a generating system. However, it has been demonstrated that heparin does not inhibit the reduction of NADP by glucose-6-P dehydrogenase or isocitric dehydrogenase[5].

Intracellular localization of endogenous NADPH in thyroxin-treated rats could play a role in preventing heparin inhibition. The coenzyme may be intimately associated with microsomes and the steroid reductases in a manner that does not allow heparin to effectively compete with it for attachment to the reductase.

Finally, it is possible that thyroxin not only increases endogenous NADPH levels but also selectively increases the affinity between NADPH and its receptor site on the steroid reductase. If this is the case, the competition between heparin and NADPH for the attachment site

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Effect of heparin on reduction of the 4–5 double bond of cortisone by liver homogenates from control and thyroxin-treated rats

	Heparin added to homogenates-units							
	0	25	50	100	200	300	400	500
Controls								
Cortisone reduced	49 ± 4.6	41 ± 7.2	42 ± 5.9	34 ± 8.4	31 ± 8.1	25 ± 4.8	23 ± 3.9	3 ± 1.7
% inhibition	–	16	14	31	37	49	53	94
Thyroxin-treated								
Cortisone reduced	73 ± 7.5	76 ± 8.8	76 ± 8.1	72 ± 8.5	77 ± 8.1	75 ± 4.7	66 ± 5.6	70 ± 9.8
% inhibition	–	0	0	1	0	0	9	4

Cortisone reduction is expressed as μg/10 min/200 mg liver. Initial concentration of cortisone was 300 μg in final volume of 7 ml. Values are means of 5 determinations ± S.E.

might be altered in a manner that would favor binding of NADPH more than heparin. This situation would be analogous to that demonstrated by SOLOMON and SCHROGIE[7] in which thyroxin increased the affinity between warfarin and its receptor site and thereby potentiated the anticoagulant response to warfarin[8].

Zusammenfassung. Leberhomogenate aus mit Thyroxin injizierten Ratten reduzieren den Ring A von Kortison schneller als Homogenate von normalen Tieren. Die Reduktion von Kortison mit Kontrollpräparaten konnte mit Heparin leicht verhindert werden, nicht aber durch Einspritzen von Thyroxin.

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PRO EXPERIMENTIS

A Quantitative Method for Measuring the Uptake of Colloidal Carbon by Mouse Tissues

Out of several methods available for the study of the phagocytic function of the reticuloendothelial system in experimental animals, the one using colloidal carbon, introduced in its present form by HALPERN et al.[1,2], is among the most popular[3]. The main advantage of this method is that colloidal carbon can easily be determined in blood samples when the rate of clearance is studied and can also be readily seen in histological preparations. Difficulties can however arise when quantitative determinations of carbon uptake by organs or tissues are required.

The only method available for measuring carbon uptake by individual organs is the one originally described by HALPERN, BIOZZI et al.[1]. It is based on weighting the dry carbon residue after digestion of organs in strong alkali and repeated extractions and washings. This method is, however, limited by the amount of carbon injected into animals. Thus, with a dose of 16 mg/100 g body weight, which is generally used for measuring the reticuloendothelial phagocytic activity, the amount of carbon likely to be found in organs of individual laboratory animals smaller than rats is below that easily detectable on weight basis.

In the course of studies of the phagocytic activity of the reticuloendothelial system after irradiation it had become necessary to determine the amount of carbon taken up by organs of individual mice. A method, based on spectrophotometric measurement of carbon, has been developed which, being more sensitive, can be applied to small laboratory animals and limited amounts of carbon.

A suspension of colloidal carbon (C11/1431a) was obtained from Günther Wagner, Pelikan-Werke, Hanover (Germany). As the concentration of carbon is not specified for this preparation, amounts of carbon will be given as volumes of the original suspension. A series of dilutions of this suspension was prepared by diluting volumes of 20–100 µl (measured with microcaps-disposable micropipettes, Drummond Sci. Co.) up to 100–1000 ml distilled water. The optical density of these dilutions was measured on a Unicam SP 600 spectrophotometer at 800 nm with a 1 cm path length. From these readings the standard curve shown in Figure 1 was constructed. By making use of this curve and Table I any amount of original carbon suspension from as little as 0.25–400 µl or more can be easily determined.

Male CBA/H mice, aged 5–5½ months and weighing between 28 and 34 g were injected i.v. via a tail vein with 0.3 ml of carbon dilution containing 50 µl original carbon suspension (1 part of original carbon suspension plus 5 parts of 1% BDH gelatin in distilled water, pH 7). When the same volume of carbon dilution was diluted to 500 ml with distilled water it gave an OD of 0.300. This amount of carbon injected per mouse is close to 16 mg/100 g body weight for a 30 g mouse[4]. One group of the injected mice were bled 60 min later under slight ether anaesthesia from the orbital venous plexus and a 10 µl blood sample was lysed in 2 ml of 0.2% solution of Na_2CO_3 and used for estimating the amount of carbon in the blood. These mice were killed by cervical dislocation immediately after the blood sample had been taken

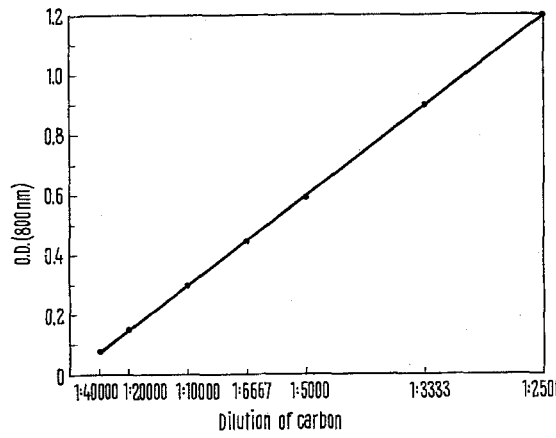

Fig. 1. Standard curve for dilutions of colloidal carbon C11/1431a.

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