The Influence of Chorionic Gonadotrophin on the Reduced NADP-DCPIP Oxidoreductase System of Rat Testis

It has been demonstrated that specific hydroxylations carried out by testicular microsomes in vitro require NADPH and O_2[1]. In the rat testis the specific activities of 2 of the enzymes that reduce NADP, namely isocitric and glucose-6-phosphate dehydrogenases, can be increased by injecting chorionic gonadotrophin into normal rats[2].

The in vitro requirement for NADPH by synthetic processes carried out in the testes[1], and the capacity of testicular tissues to increase production of NADPH following the injection of chorionic gonadotrophin in normal rats[2], induced us to study the so-called NADPH-DCPIP oxidoreductase system, which is the first step in the oxidation of NADPH in rat testes, and the relationship of this system to the production of androgens.

Prepuberal rats, A × C inbred, weighing 18–20 g, and adult rats weighing 200 g at the onset of experiments were maintained in a constant temperature environment. Chorionic gonadotrophin was injected s.c. once daily for 5 days. The doses of hormones injected in each experiment are listed in the Tables. The animals were sacrificed by decapitation, and the capsule-free testicular tissue was immersed in chilled sucrose, 0.25 *M* (20 mg of tissue/ml sucrose).

The tissue was homogenized with a motor-driven Teflon pestle, and then submitted to differential centrifugation by the following procedure: A large particulate fraction was obtained at 800 g and discarded. The supernatant was then centrifuged at 10,000 *g*. The pellet obtained in this step was washed and resuspended in 0.25 *M* sucrose. This fraction was called fraction 1. The supernatant was called fraction 2. Fractions 1 and 2 were not extensively characterized, but fraction 1 corresponded to the mitochondrial fraction, and fraction 2 included microsomes and supernatant. In each fractionation, the tissue from 1 animal was used.

NADPH-DCPIP reductase was assayed by the method of CONNOVER et al.[3]. The assay medium contained: 0.3 μmole of NADPH, 0.24 μmole of DCPIP, 100 μmoles of 0.1 *M* phosphate buffer pH 7, and 0.2 ml of the enzymatic preparation (100 μg protein, approximately). As there is a non-specific reduction of DCPIP by the tissue, this reduction was allowed to come to completion before starting the reaction by the final addition of the reduced pyridine nucleotide. Fraction 2 was used in this assay because previous work done in our laboratory has shown that the activity of the enzyme is located entirely in this fraction in rat testis[4].

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Table I. Effects of Chorionic Gonadotrophin on the Activity of the testicular NADPH-DCPIP reductase of prepuberal animals

Experiment	No. of animals	Treatment	Testes weight mg tissue/10 g animal weight (± S.D.)	Seminal vesicles weight mg tissue/10 g animal weight (± S.D.)	Enzymatic activity μmoles DCPIP reduced per mg protein in 5 min (± S.D.)	% of increase	*t* test
1	4	controls	52.2 ± 3.5	2.1 ± 0.2	43 ± 2.1		
	4	0.5 IU	51.3 ± 2.9	5.8 ± 0.4	56 ± 5.8	30	0.01
2	4	controls	52.0 ± 4.1	2.6 ± 0.3	59 ± 2.7		
	4	2.5 IU	54.2 ± 4.0	7.9 ± 0.6	77 ± 3.8	30	0.001
3	4	controls	52.7 ± 3.6	2.4 ± 0.4	61.3 ± 4.1		
	4	10 IU	80.5 ± 5.2	11.2 ± 0.6	86.1 ± 8.8	40	0.001

Equal daily doses of chorionic gonadotrophin were injected s.c. for 5 days. The total injected doses expressed in international units (IU) are shown.

Table II. Effects of chorionic gonadotrophin on the activity of the testicular NADPH-DCPIP reductase of adult animals

Experiment	No. of animals	Treatment	Testes weight mg tissue/10 g animal weight (± S.D.)	Seminal vesicles weight mg tissue/ 10 g animal weight (± S.D.)	Enzymatic activity μmoles DCPIP reduced per mg protein in 5 min (± S.D.)	% of increase	*t* test
1	4	controls	1.270 ± 285	95 ± 8	53 ± 3.4		
	4	100 IU	1.293 ± 280	206 ± 15	65 ± 4.7	22	0.02–0.01
	4	250 IU	1.390 ± 182	237 ± 27	76 ± 7.9	43	0.01–0.001
2	4	controls	1.285 ± 230	91 ± 7	60 ± 3.8		
	4	500 IU	1.352 ± 250	258 ± 17	86 ± 8.9	43	0.1 –0.001
	4	1,000 IU	1.227 ± 172	272 ± 18	89 ± 7.3	48	0.001

Equal daily doses of chorionic gonadotrophin were injected s.c. for 5 days. The total injected doses expressed in international units (IU) are shown.

Results. NADPH-DCPIP reductase: 3 different experiments involving gonadotrophin treatment were performed on prepuberal rats, and the results are expressed in Table I. In Table II, the results obtained with the adult animals are listed.

These results show that the activity of the so-called NADPH' diaphorase of the postmitochondrial fraction of rat testis can be modified by in vivo administration of chorionic gonadotrophin. Dichlorophenolindophenol, the acceptor used in this study, can be reduced by the NADPH diaphorase as by the NADPH cytochrome C reductase[5]. Both enzymes are flavoproteins, and both are located in microsomes. As we have not purified our system, we must say that we have a NADPH-DCPIP oxidoreductase system which is under hormonal control localized in the postmitochondrial fraction. The subcellular localization of this system renders a relationship between it and the mitochondrial electron transport system unlikely. It is known that androgen-hydroxylating enzymes are located in microsomes in rat testis[1]; therefore we may presume that the increased activity observed is related to androgen synthesis. A flavoprotein component with the property of reducing DCPIP has been described in the electron transport system of adrenal microsomes by OMURA et al.[6]. This electron transport system acts on the hydroxylation of steroids in the adrenal. Such a system has not been described in testis, but we can presume that the activity we are concerned with is related to it.

Histochemical observations[7] are against the possibility of the activation of the diaphorase system by chorionic gonadotrophin in rat testis, but qualitative observations per se cannot give a measure of activity[8].

Resumen. La actividad de un sistema enzimatico que oxida el NADPH usando DCPIP como aceptor, en homogenados te sticulares de ratas prepuberales y adultas tratadas con gonadotrofina corionica ha sido estudiado. Los cambios encontrados se dis cuten en relación con la sintesis de androgenos.

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Renal and Pressor Actions of Angiotensin in Salt Loaded and Depleted Rabbits

Angiotensin is so named because it raises arterial pressure and its method of biological assay depends upon this action. In addition it has a marked effect on renal sodium excretion and may be important in the control of sodium homeostasis. The effect of infused angiotensin on renal sodium excretion is complex. Thus it may either increase or diminish sodium excretion depending on the amount infused[1-3]. Moreover, doses of angiotensin which are antinatriuretic when infused into normotensive subjects on a normal sodium intake may be natriuretic when given to salt depleted subjects[4] or to patients with cirrhosis and ascites[5]. In order to clarify its role in sodium homeostasis we have infused angiotensin in graded doses between 0.00005 and 0.5 μg/kg/min into conscious trained rabbits and determined the relative sensitivity of its pressor and renal actions and the dose range over which its natriuretic and antinatriuretic effects occur. In addition, we have infused it into the same animals when salt loaded and depleted to see the effect of changes in sodium status on the renal response to administered hormone.

The experiments were performed on 6 rabbits weighing between 2 and 2.5 kg, that had chronically implanted bladder, venous and arterial catheters. Over a period of 3–5 days the animals were either salt loaded by infusing 6 mEq sodium a day as $^1/_4$ strength Hartmann's solution, or depleted of 6–10 mEq of sodium by means of the diuretic frusemide and a salt free diet. Some animals were initially salt loaded and others depleted. During the experiment either $^1/_2$ strength Hartmann's solution in the case of salt loaded animals, or 2.5% dextrose in depleted ones was delivered at 0.75 ml/min by a constant infusion pump. When urine flow and sodium excretion were constant, synthetic angiotensin II (CIBA) was administered, dissolved in the appropriate infusate.

Infusion of angiotensin in small doses between 0.005 and 0.00005 μg/kg/min into salt loaded animals for periods ranging from 10–90 min consistently reduced urine flow and sodium excretion for the duration of the infusion. In contrast when given to salt depleted rabbits in doses of 0.0005–0.00005 μg/kg/min angiotensin had no effect on urine flow or sodium excretion, while doses of 0.005 μg/kg/min caused only an inconstant small reduction in these parameters. Table I compares the effects of identical 10 min infusions of angiotensin in 6 rabbits when salt loaded and depleted. It can be seen that sodium depletion greatly depresses both the antidiuretic and antinatriuretic response to small doses of angiotensin.

Larger doses of angiotensin caused a marked initial reduction in urine flow and sodium excretion in salt loaded animals. After an interval of 20 min however, particularly with very large doses, urine flow and especially sodium excretion were increased. In 3 animals the effects of doses of 0.05 μg/kg/min and above were com-

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