EARLY ACTIONS OF CADMIUM IN THE RAT AND DOMESTIC FOWL TESTIS

V. INHIBITION OF CARBONIC ANHYDRASE*

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Summary. Carbonic anhydrase (CA) activity in the testis of the rat and fowl was inhibited *in vivo* and *in vitro* by cadmium chloride (CdCl_2). Testes were removed from control animals and from animals which had previously received injections of 0.03 mmol CdCl_2/kg . In both species, there was an initial inhibition followed by an increase in enzyme activity 10 min after injection. Exposure of testis tissue *in vitro* to 0.0001, 0.001, 0.01 or 0.1 M- CdCl_2 revealed that the CA activity in the testis tissue of both species was equally inhibited. The data verify that cadmium causes biochemical changes in the testis soon after injection. The fact that testicular CA activity in both the rat and fowl is inhibited by Cd, suggests either that inhibition of this enzyme is not the mode of action resulting in testicular damage or that the fowl testis is tolerant to CA inhibition.

INTRODUCTION

The deleterious effects of cadmium (Cd) on the scrotal testis, involving oedema, haemorrhage and destruction of the germinal epithelium, have been reviewed by Pařízek (1960) and Gunn & Gould (1970). In contrast to the effects of Cd on the normal scrotal testis of most mammals, the abdominal testis of the domestic fowl is not altered or damaged by subcutaneous (s.c.) or intraperitoneal (i.p.) injections of Cd (Guthrie, 1964; Erickson & Pincus, 1964; Chiquoine, 1964).

Early changes in the scrotal testis caused by Cd are histologically evident as early as a $\frac{1}{2}$ to 1 hr (Hodgen, Butler & Gomes, 1969) and certainly by 2 to 6 hr after injection (Pařízek, 1960; Gunn, Gould & Anderson, 1963; Mason, Brown, Young & Nesbit, 1964; Chiquoine, 1964). Other early testicular changes which have been reported are: decreased blood flow at 3 hr (Waites & Setchell, 1966); increased temperature by 5 min in the rat testis, but not the domestic fowl (Johnson, Gomes & VanDemark, 1970); altered vasculature (Niemi & Kormano, 1965); and inhibition of carbonic anhydrase (CA) activity by 30 min

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in the rat testis (Hodgen *et al.*, 1969). Carbonic anhydrase was studied because it is a metallo-enzyme containing Zn, a molecule which might be replaced by Cd.

This work has suggested that biochemical changes in the testis occur extremely early after injection of Cd. Other work had indicated that ^{109}Cd reaches a high level in the testis of both the rat and domestic fowl at $2\frac{1}{2}$ min after i.p. injection (Johnson & Miller, 1970). With this information, the present study was undertaken to determine the very early changes brought about in testicular CA after Cd injection in the rat and domestic fowl.

MATERIALS AND METHODS

Studies in vivo of the action of cadmium on the activity of carbonic anhydrase

For a study of Cd inhibition of CA activity *in vivo*, eighteen male rats of the Wistar strain (0.24 to 0.36 kg) and eighteen male domestic fowl of the Rhode Island Red breed (2.5 to 3.5 kg) were used. These animals were injected s.c. with CdCl_2 (0.03 mmol/ml) in the interscapular region at the level of 0.03 mmol/kg of body weight. At 0 (control), $2\frac{1}{2}$, 5, 10, 20 and 40 min following injection, both testes were removed under sodium pentobarbital anaesthesia, and immersed immediately in ice water. Four samples of each testis, free of tunica albuginea, weighing 90 to 110 mg each, were used for the determination of CA activity.

Testis samples were placed in 6 ml of 0.02 M-veronal buffer (pH 8.3) and homogenized in a stainless steel homogenizer (Omnimixer). A pH probe (Combination electrode Corning No. 476051), attached to a pH meter (Orion, Model 801), was inserted into the homogenized sample, 4 ml of CO_2 -saturated water at 2°C was added and the time required for the pH to drop from 8.3 to 6.3 was recorded. The units of enzyme activity were calculated from a standard curve which was plotted using purified CA (Schwarz BioResearch Inc., Orangeburg, N.Y.) and using the method designed for the assay by Wilbur & Anderson (1948).

Inhibition study in vitro of the action of cadmium on carbonic anhydrase activity

The testes of four animals from each species were removed and CdCl_2 solution was added to tissue samples (in triplicate), weighing 90 to 100 mg each, to bring the final Cd concentration to 0, 0.0001, 0.001, 0.01 or 0.1 M. An equal volume at varying concentrations was added to reach the final concentrations. The samples were then assayed as for the work *in vivo* outlined in the previous section.

RESULTS

The action of cadmium in vivo

The changes brought about in CA activity in the testes of the rat and fowl on the basis of enzyme units/g of wet weight are shown in Text-fig. 1. The levels of enzyme activity in the controls for the two species were not significantly different, and similar trends, following injection of Cd, were evident in both

TEXT-FIG. 1. Carbonic anhydrase activity/g of wet tissue, in the testis of the rat and domestic fowl at various times following s.c. injection of 0.03 mmol CdCl₂/kg.

TEXT-FIG. 2. The changes in the testicular carbonic anhydrase activity/g of dry tissue, in the rat and domestic fowl following s.c. injection of CdCl₂ (0.03 mmol/kg).

species. The decrease immediately after injection was significantly lower ($P < 0.05$) than the control values at $2\frac{1}{2}$ min and, in the rat testis, the activity was still significantly lower ($P < 0.05$) than in the controls at 5 min after injection. In both species, this inhibition was followed by an apparent stimulation of CA activity at 10 min. This was followed by return to a normal level in the rat and a significant decrease ($P < 0.05$) in the domestic fowl.

On the basis of CA/g of dry weight as shown in Text-fig. 2, the same general trends as on a wet weight basis were apparent. Again, control values for the two species were similar. At $2\frac{1}{2}$ min, the CA activity had decreased ($P < 0.01$) to 64 and 81 % of the control values for the rat and domestic fowl, respectively. At 10 min, there was an increase ($P < 0.05$) in CA activity above control values in both species. Values returned to normal in the rat but the levels at 20 and 40 min after injection in the domestic fowl were decreased ($P < 0.05$) below control values.

The action of cadmium in vitro

Text-figure 3 shows the percentage inhibition by various concentrations of CdCl_2 of the CA activity of testis tissue *in vitro*. It is evident that Cd inhibits

TEXT-FIG. 3. The percentage inhibition of the testicular carbonic anhydrase of the rat (—) and domestic fowl (---) at different concentrations of CdCl_2 *in vitro*.

CA activity about equally in the testis tissue of both the rat and the domestic fowl. Responses were nearly linear in both species and CA inhibition increased with each increase in the concentration of Cd.

DISCUSSION

The primary rôle of CA in the animal body is that of buffering which involves conversion of the bicarbonate ion to CO_2 , as well as the reverse of this reaction (Maren, 1967b). In interstitial fluid and urine, this involves the conservation of Na^+ ions and water at the expense of H^+ ions. The presence of CA has been reported in several reproductive tissues of various mammals (Lutwak-Mann,

1955; Pincus & Bialy, 1964; McIntosh & Lutwak-Mann, 1967) and fowl (Benesch, Barron & Mawson, 1944; Bernard & Genest, 1945; Maren 1967a).

Initially, CA was examined in connection with Cd inhibition by Hodgen *et al.* (1969) following the suggestion of Pařízek (1957, 1960) that Cd may play a rôle in the inhibition of metallo-enzymes by displacing Zn. Cotzias, Borg & Selleck (1961) established that in many tissues Zn was not distinguished from Cd. If CA was inhibited in a tissue, the exchange of ions would be impaired and adverse osmotic pressures would result that might cause oedema and even cellular rupture. Oedema and rupture of blood vessels are evident in the mammalian testis following Cd injection.

It is apparent from the present study that Cd acts very rapidly on the testis after injection. This is not surprising since high levels of ^{109}Cd have been found in the testis $2\frac{1}{2}$ min after injection (Johnson & Miller, 1970). In the rat testis, increases in temperature occurred 5 min after injection (Johnson *et al.*, 1970). The only marked difference in the CA inhibition in the testis of the rat and domestic fowl at this early stage is a slightly greater inhibition in the rat at $2\frac{1}{2}$ min which persisted through the 5-min post-injection period. Whether this difference is great enough to result in testicular damage in the testis of the rat and not in the domestic fowl is highly questionable. The reason for the increase of CA above normal at 10 min may be due to an increased number of CA-rich erythrocytes resulting from greater blood flow, which has been suggested during this period (Johnson *et al.*, 1970). The decrease in CA in the domestic fowl at 20 and 40 min may be the result of an eventual decrease in such erythrocytes in the domestic fowl but not in the rat. At 30 and 60 min in the rat, Hodgen *et al.* (1969) found an inhibition of CA activity, but at 20 and 40 min in this study it was not evident. The prolonged decrease in CA activity in the domestic fowl during the period from 10 to 40 min would suggest that if CA inhibition is the mode of action by which Cd inflicts testicular damage, the testis of the domestic fowl and not that of the rat should be damaged.

There was no difference in the inhibition rate of CA in the testis tissue of the rat and of the fowl *in vitro*. This equal inhibition substantiates the work *in vivo* in that the CA of the testes of both species was inhibited. If CA inhibition in the testis is the way in which Cd causes testicular damage, it might be expected that the testis of the domestic fowl as well as that of the rat would be affected. It is possible, however, that inhibition of CA activity can be tolerated without detriment to the testicular tissue in the fowl, but not in the rat.

It is evident that there is little difference in the effect of Cd on the CA activity in the testes of the domestic fowl and the rat, though the testes of the two species are strikingly different. Inhibition of CA activity in the vast pampiniform plexus present in the rat (Harrison, 1949) might result in the breakdown of the vessel wall. This, in turn, would alter blood flow and interfere with counter-current heat exchange, resulting in detrimental temperature changes. In the domestic fowl, which lacks a pampiniform plexus, there may be no requirement for counter-current heat exchange for normal testis function so that with no amassing of blood in one single area, there would be no site at which CA inhibition would be so critical. Thus, there would be no increase in testicular temperature (Johnson *et al.*, 1970) in the domestic fowl following Cd injection

and no damage, even though there was as much Cd in the testis of the domestic fowl as in the rat (Johnson & Miller, 1970). The inhibition of CA appears not to be the direct mechanism by which Cd damages the testis of the rat. This inhibition of CA might, however, interfere with the functions of the pampini-form plexus resulting in altered blood flow and increased temperature in the testis of the rat.

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