Reversible Inhibition of the Beat in Heart Fragment Tissue Cultures by Desoxycorticosterone. (18196)

IVOR CORNMAN. (Introduced by P. K. Smith)

From the Warwick Memorial for Cancer and Allied Diseases, and the Department of Anatomy, George Washington University School of Medicine, Washington, D.C.

In an effort to demonstrate an effect of adrenal cortical hormones on isolated living tissues, the embryonic heart was chosen because its action is readily observable and experimentally reversible. An embryonic heart can be divided into many fragments, 5 to 15 of which will pulsate and will quickly respond to changes in the culture medium. With potassium, for instance, the concentration can be gradually increased until any chosen percentage of the fragments stop beating or until a threshold is reached at which all fragments are blocked. A culture in this state of delicately suspended activity quickly responds to agents antagonistic to potassium. Thus it should provide a sensitive indicator of antagonism between desoxycorticosterone (DOC) and potassium if this antagonism operates at the cellular level. It is well known that potassium alters the activity of the heart *in situ,* whether the high potassium level results from injection(1,2) or from adrenal insufficiency(3,4). The accepted view assigns DOC effects to systemic decrease of potassium resulting from increased potassium excretion. Nevertheless, the possibility of direct effects of DOC on heart tissue and of DOC-potassium antagonism within tissues appears worth testing.

Materials and methods. Hearts of chick embryos 9-11 days old and of newborn mice or fetuses 9-14 mm in length, were cut into fragments, cemented in roller tubes with chicken plasma, and supplied with 1 ml of balanced salt solution (Gey's modification of Tyrode's). The pulsations were recorded during a 1-4 hour stabilization period before addition of isotonic KCl (1.08%) or the steroids suspended in balanced saline. Where serum was added, it was usually Difco lyophilized human placental serum. About 100 chick hearts and 150 mouse hearts were used in these experiments, providing a total of about 2500 pulsating fragments. Moreover, most fragments continued to beat for several days, so each could be tested for recovery and for uniformity of response even after long exposure. All results reported here have been obtained with both avian and mammalian heart fragments. Upjohn cortical extract, DOC (Ciba), DOC (Delta), DOCA (Delta), cortisone (Merck), cortisone acetate (Merck) and epinephrine (Parke, Davis) were tested on untreated hearts and on potassium-blocked hearts. The Ciba and Delta DOC behaved differently(5). Doses refer to the Delta product except where Ciba is specified. The steroids were dissolved in acetone and then suspended in saline. The concentrations reported here are all final levels, reached by gradual addition of the experimental agent over a period of hours. A culture was scored as blocked only when all the fragments had stopped beating, and as near-block when less than 20% continued beating. Stimulation could be detected as increase of speed and amplitude in beating fragments and as initiation of beat in fragments which had been quiet during the 1-4 hour preliminary observation.

Results. Action of cortical steroids or epinephrine on the potassium-blocked heart. Chick and newborn mouse hearts were blocked at final concentrations of 2%-7% of isotonic KCl, while fetal hearts required 7%-19%. The effect was readily reversible on return to balanced salt solution, even when the

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hearts had been immobilized for 12 hours. When hearts immobilized by KCl received a final concentration of 2%-5% cortical extract without diluting the KCl, some fragments began to pulsate within a few minutes. However, the extract is known to contain traces of epinephrine, and when ½ ppm. epinephrine was added alone, the fragments responded in a similar way. DOC at 0.005-0.067 mg/ml, its acetate at 0.015-0.077 mg/ml (in excess of saturation) or cortisone acetate at 0.025-0.166 mg/ml (in excess of saturation) administered immediately after or several hours before addition of potassium did not diminish the depression by potassium.

Suppression of heart beat by DOC. DOC by itself first stimulated and then depressed pulsation. Chick and newborn mouse hearts were blocked at 0.009-0.03 mg/ml in balanced saline, while 9-14 mm fetal mouse hearts required 0.046 mg/ml for a near block. In the absence of potassium (isotonic NaCl + $CaCl_2$) 0.005-0.01 mg/ml was sufficient to stop chick hearts, and 0.01 mg/ml blocked fetal mouse hearts. Blocked heart fragments slowly resumed pulsation when DOC was removed and replaced with balanced saline. In 7%-9% serum 0.1-0.2 mg/ml DOC was required to block hearts. Fragments which had been stopped by DOC recovered when serum was added up to 5%-10% without altering the concentration of DOC. Suppression and recovery could be repeated by adding more DOC and then more serum. This technic was employed to assay serum from different sources. There was no significant difference between lyophilized human placental serum, normal adult serum, or adult serum obtained 2 hours after adrenalin injection. Several influences could play a role in reversing the DOC effect: pH, general improvement in the tissue, adsorption or specific metabolic antagonism. Lyophilized serum shifts the cultures from the usual pH 7.4-7.6 to 8.2-8.6. However, DOC will still inhibit in cultures brought to pH 8.6 with $NaHCO_3$, and cultures in 8% serum acidified to pH 6.6 with CO_2 are resistant to DOC. Activity and survival of heart fragments is better in the presence of serum, but cultures transferred from serum to balanced saline, quickly scored and then poisoned with DOC fell well within the range of doses required to inactivate cultures maintained for 24 hours in balanced saline before dosage. To determine the site of the serum effect, cultures were dosed with enough DOC to quiet them in the presence of 8%-9% serum, but the serum was not added until most fragments had become inactive. When the serum was added, some of the fragments resumed their beating and then stopped again.

Effects of DOCA, cortisone, cortisone acetate, epinephrine or acetone. DOC acetate up to 0.038 mg/ml (persistent crystals visible within the culture tube) or cortisone alcohol or acetate up to 0.15 mg/ml were without effect on the beat. Cortisone up to 0.25 mg/ml did not decrease the inhibitory effect of DOC, and at the higher doses possibly had a slight synergistic influence. Epinephrine at 1:100,000 brought recovery lasting a few minutes to a few hours in 1 or 2 fragments per tube. Acetone at 1% did not affect the pulse. In most DOC-treated cultures the acetone carrier did not exceed 0.2%. The phenomena of blocking, recovery in serum, temporary recovery in DOC overdose, and non-antagonism between cortisone and DOC were verified with Ciba DOC. Chick and newborn mouse hearts were inactivated at 0.05 mg/ml, and recovered upon addition of 8%-9% serum, but responded poorly to 1:100,000 epinephrine.

Discussion. Resuscitation of potassium-blocked hearts by cortical extract so closely resembles the response to epinephrine at concentrations which can be present in the cortical extract, that it must for the present be regarded as an epinephrine effect(6). DOC exhibits neither a specific antagonism for potassium, nor antagonism of the sort exerted by aliphatic lipids(7). Nor is the DOC effect mediated by potassium, for DOC is more effective in the absence of potassium. Moreover, epinephrine acts upon most fragments subdued by potassium, but rarely—perhaps not significantly—on fragments in-

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activated by DOC. These effects of DOC on cultured heart fragments probably do not represent any normal function of the steroid within the body, although Cleghorn *et al.* have indicated the possibility of a specific beneficial influence of DOCA on the heart. Adequate DOCA therapy will abolish the cardiac arrhythmia of adrenal insufficiency(8) although maintenance doses sufficient to return plasma electrolyte values to normal may not improve the electrocardiogram(3). The DOC-induced inhibition of the heart fragments in culture may be related to certain systemic effects of DOCA overdosage. DOCA and other steroids act as depressants(9,10). Weakness of skeletal muscle and irregularity of heart beat induced by DOCA can be prevented by increased potassium intake(11). In cultures the heart was less inhibited by DOC in the presence of potassium. Difficulties are likewise encountered in trying to relate cortical steroid effects in the body and on cultures of kidney, where cortisone increases excretion by mesonephric tubules(12).

Serum most probably interferes with the DOC effect by adsorbing the steroid(13) although the temporary recovery of hearts in the presence of an overdose of DOC points to a possible site of counteraction within the cell. If there is an antagonist in serum, it would not appear to be cortisone inasmuch as cortisone alone will not interfere with DOC, and sera presumably high in cortisone activity (placental or post-epinephrine) proved no more antagonistic than normal adult serum. Nor could any synergist in serum be demonstrated by blocking hearts in serum and then adding cortisone. The inertness of DOC acetate may result merely from its low solubility, but could also be interpreted as evidence that only the unesterified steroid is physiologically active and that heart tissue is unable to hydrolyse it.

Summary. DOC reversibly inhibits the pulsation of mouse or chick heart fragments *in vitro*. The inhibition can be removed by serum but not by cortisone, and is more effective in the absence of potassium. DOC and cortisone have no influence upon potassium inhibition of the heart. Epinephrine will almost completely counteract potassium inhibition but revives only a few DOC-poisoned fragments.

I am indebted to Dr. Erwin P. Vollmer and Dr. Raymund L. Zwemer for their suggestions which guided these investigations. The Ciba DOC was contributed by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc. These studies were financed by an institutional grant from the American Cancer Society, Inc., to the Helen L. and Mary E. Warwick Memorial for Cancer and Allied Diseases.

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Received July 27, 1950. P.S.E.B.M., 1950, v75.

Factors Influencing Measurement of Hyaluronidase Activity by Dermal Spread of an Indicator.* (18197)

MARK A. HAYES AND THOMAS G. REED. (Introduced by B. L. Baker)

From the Departments of Surgery and Anatomy, University of Michigan Medical School.

The enzymatic hydrolysis of the hyaluronic acid component of connective tissue ground substance as measured by the dermal spread

* Supported in part by the Lawrence J. Montgomery Research Fund and by grants from the Division of Research Grants and Fellowships, The United States Public Health Service and from The Upjohn Company to Dr. B. L. Baker.