Antagonism of Adrenocortical Extract and Cortisone to Desoxycorticosterone: Brain Excitability in Adrenalectomized Rats.* (18389)

DIXON M. WOODBURY, JOHN W. EMMETT, GEORGE V. HINCKLEY, NEWTON R. JACKSON, JOHN D. NEWTON, JOHN H. BATEMAN, LOUIS S. GOODMAN, AND GEORGE SAYERS.

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City

It has been demonstrated that adrenocortical extract (ACE)(1) and cortisone(2) will counteract the depressive effect of desoxycorticosterone acetate (DCA) on brain excitability in intact rats. The present report concerns the effects of ACE and cortisone on the brain excitability of adrenalectomized DCA-implanted rats.

Methods. Adult male rats from the Sprague-Dawley farm were employed. The technic for determining the electroshock threshold (EST) has been presented in detail by Davenport(3). Alterations in EST observed throughout the course of the experiments have been expressed as the percentage change from the control value determined at the conclusion of a period of stabilization(4). Two to 4 days elapsed between each EST measurement. At the end of the stabilization period the animals were divided into 6 groups of 6 rats each and given the following treatments. The rats in Groups 1 to 4 were bilaterally adrenalectomized and implanted subcutaneously with six 15-mg pellets of DCA.† In addition, the animals in these groups received the following treatment: Group 1, a 0.9% sodium chloride solution to drink; Group 2, a 0.9% sodium chloride solution to drink and 0.5 ml of ACE‡ injected subcutaneously twice daily from the 21st to the 27th day of the experiment; Group 3, a 0.9% sodium chloride solution to drink and 1 mg of cortisone acetate (suspended in 0.2 ml of 10% ethanol) injected subcutaneously twice daily from the 21st to the 27th day; Group 4, water to drink plus 1 mg of cortisone acetate injected subcutaneously twice daily from the 33rd to the 36th day of the experiment. Groups 5 and 6 were composed of intact rats given water to drink. The animals in Group 5 were implanted with DCA and received no further treatment. The rats in Group 6 served as untreated controls.

Results. The results of these experiments are illustrated graphically in Fig. 1 and 2. The lines connect the average EST values of the rats in each group. It can be observed in Fig. 1 that the intact untreated rats in Group 6 showed a slight elevation in EST starting on the 22nd day and reached a value of about plus 5% on the 37th day. This elevation of EST is associated with an increase in weight of the animals and has been noted previously by Davenport and Davenport(5). The intact DCA-implanted rats in Group 5 showed an elevation in EST which exceeded that of the controls; the elevation was first observed on the 17th day and appeared to have reached a maximum of about 13% by the 32nd day. The EST of the intact DCA-implanted rats increased at a relatively slow rate as compared to previously reported experiments(1). The animals in Group 4, which were adrenalectomized, implanted with DCA and given water to drink, showed a more rapid rate of increase of EST

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‡ Lipo-Adrenal Cortex, gift of Dr. Hailman of the Upjohn Co.

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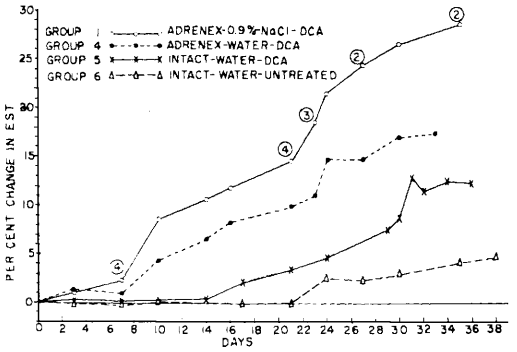

FIG. 1.

Effect of adrenalectomy, DCA and drinking fluid on EST. See text for explanation.

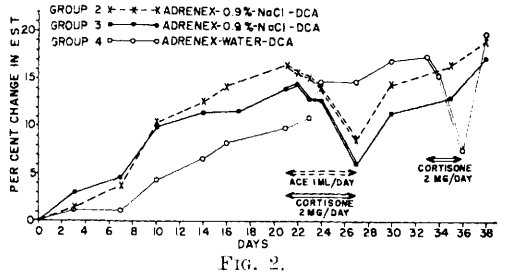

FIG. 2.

Effect of ACE and cortisone on the elevated EST of adrenalectomized DCA-implanted rats. See text for explanation.

than did those in Group 5. Furthermore, Group 4 reached a higher maximal elevation (17.5% after 33 days) than did Group 5. The EST of Group 1, the animals of which were adrenalectomized, implanted with DCA and given 0.9% sodium chloride solution to drink, rose rapidly and reached a value (plus 28.5% on the 35th day) much higher than those of the other groups. The figures in circles indicate the number of the original six animals which remained alive at the indicated times. No deaths occurred in the other groups.

Fig. 2 illustrates the data obtained on the effect of ACE and cortisone on the elevated EST of adrenalectomized DCA-implanted rats. Both ACE (Group 2) and cortisone (Group 3) decreased the elevated EST of DCA-implanted rats receiving 0.9% sodium chloride solution to drink; both substances, in the doses employed, induced about the same decrease (8.5%) in EST during the 6-day period of injection. It is of interest that these substances also induced loss in body weight; the decreases in weight during the period of administration were 9.5 and 9.1% for ACE and cortisone, respectively. Following withdrawal of the hormones the values of the EST slowly returned to levels slightly higher than those of the pre-injection period.

The adrenalectomized DCA-implanted rats, given water to drink (Group 4), showed a marked and rapid decrease in EST when treated with 2 mg of cortisone per day. The EST decreased 10% in 3 days as compared with 8.5% in 6 days for the animals on sodium chloride. The weight loss for this group during the injection period was 3.7% which is considerably less than that which occurred in the animals given sodium chloride solution to drink. It appears that the degree of reduction in EST is not related to the degree of loss of body weight. Following withdrawal of the cortisone the EST returned to a value greater than the preinjection threshold in 2 days as compared to 11 days for the animals on sodium chloride. Thus the EST of the DCA-treated rats on sodium chloride decreased less rapidly and recovered more slowly than did the EST of DCA-treated rats which received water to drink.

Discussion. The concept has been developed in this laboratory that DCA induces a state of hormone imbalance characterized by an excess of administered DCA and a deficiency of cortisone-like steroids of the adrenal cortex. Certain consequences of the deficiency can be overcome by stimulation of the adrenal cortex with ACTH or by the administration of ACE or cortisone. For example, in the intact rat it has been demonstrated (a) that DCA-induced insulin hypersensitivity can be counteracted by ACE administration(6), (b) that DCA-induced hypernatremia can be prevented by administration of ACTH or ACE (1), (c) that DCA-induced elevation in EST can be antagonized by ACTH, ACE or cortisone(1), and (d) that DCA-induced pathology (periarteritis nodosa, interstitial myocarditis and nephritis) can be markedly inhibited by ACTH or cortisone treatment(7). It has been suggested that the mechanism of

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action of DCA in producing a deficiency of cortisone-like steroids has two components: (1) inhibition of pituitary adrenocorticotrophic activity and (2) antagonism of DCA to cortisone-like steroids at their sites of action. Evidence in support of the first component is that ACTH counteracts certain effects of DCA and that DCA induces adrenocortical atrophy. [See review by Sayers(8)]. The data of the present report indicate that the second component is of major importance. Cortisone administration has been demonstrated to reduce EST in normal rats(4); in addition, the DCA-induced increase in EST in normal animals can be antagonized by cortisone(4). These facts alone could be interpreted to mean that cortisone affects brain excitability and that DCA exerts its effect on EST merely by inhibiting the pituitary release of ACTH, thus causing a cortisone deficit. However, it is herein shown that cortisone and ACE antagonize DCA in regard to brain excitability in the *adrenalectomized* animal in which the endogenous secretion of cortical steroids is nil; hence the antagonism is exerted in this instance not via the adenohypophysis but directly on brain excitability.

The nature of the antagonism between DCA and cortisone at their peripheral loci of action is unknown. The antagonism may be physiological, *i.e.*, the end result of two opposing metabolic actions which may or may not involve the same target tissue for each steroid. A more likely possibility is that DCA and cortisone, substances very similar in chemical structure, compete for the same strategic loci in the cell.

The fact that the EST of the adrenalectomized DCA-implanted animals given water to drink rose more rapidly than the EST of intact DCA-implanted rats given water to drink is additional evidence in support of the thesis that the secretion of the adrenal cortex opposes many of the actions of DCA. If DCA-implantation had completely inhibited ACTH release, one would not anticipate any difference between the response of adrenalectomized and intact rats to DCA treatment. However, such DCA treatment does not entirely suppress adrenocortical function. For example, adrenal atrophy is less marked in DCA-implanted animals than in hypophysectomized animals(1,9).

It is of interest that cortisone induced a more rapid decrease in EST in rats given water to drink than in rats given a solution of sodium chloride to drink and that the rate of increase of EST was greater in adrenalectomized rats given sodium chloride than in adrenalectomized rats given water to drink. It has been demonstrated that an important factor in determining brain excitability is the concentration of extracellular sodium; EST and the concentration of extracellular sodium parallel one another. One is tempted to interpret these results to mean that the rats loaded with sodium responded more slowly to cortisone because it took a longer period of time to reduce extracellular sodium in these animals. However, such an interpretation fails to explain the more rapid return of the EST to pre-injection levels in the rats given water to drink as compared to that in the rats given a solution of sodium chloride to drink. Electrolyte analyses may help to clarify this aspect of the problem.

The possibility that the DCA-cortisone antagonism on brain excitability may be used as a method of assay for cortisone-like compounds is suggested by the fact that cortisone and ACE gave similar responses in doses that have been found to be equivalent by other biological tests such as the Ingle-work test and the liver glycogen-deposition test(10). This type of assay is being explored by us.

Summary. The electroshock seizure thresholds (EST) of intact and adrenalectomized rats implanted with desoxycorticosterone acetate (DCA) and given water or a 0.9% sodium chloride solution to drink have been measured. The rate of elevation of EST was as follows, in increasing order: intact controls on water; intact DCA-implanted animals on water; adrenalectomized DCA-implanted rats

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on water; adrenalectomized DCA-implanted rats on 0.9% sodium chloride solution. These rates correspond to those predicted on the basis of the known effects of the secretion of the adrenal cortex and the concentration of extracellular sodium on brain excitability. Cortisone and adrenocortical extract (ACE) lowered the elevated EST of adrenalectomized DCA-implanted rats which were given a solution of sodium chloride to drink by 8.5% in 6 days. Following withdrawal of cortisone or ACE the EST returned to pretreatment levels in 11 days. Cortisone lowered the elevated EST of adrenalectomized DCA-implanted rats given water to drink by 10% in 3 days; following withdrawal of cortisone the EST returned to the pretreatment level in 2 days. It has been suggested that the results obtained provide evidence for the concept that cortisone and DCA compete for strategic loci in target cells.

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Hemagglutination by Col-MM-Virus. (18390)

SVEN GARD AND LEO HELLER. (Introduced by Albert B. Sabin.)

From the Department of Virus Research, Caroline Institute, Stockholm, Sweden.

Members of the EMC group of viruses(1,2) are capable of agglutinating sheep erythrocytes as first shown by Hallauer(3) and confirmed by Bremer and Mutsaars(4,5), Verlinde and de Baan(6) and Olitsky and Yager (7). Hemagglutinating capacity of the GD VII strain of Theiler's virus against human group O red blood cells was reported by Lahelle and Horsfall(8) and of Japanese B encephalitis virus for both sheep and chick erythrocytes by Sabin and Buescher(9). In all those cases, the hemagglutination could be inhibited by homologous immune sera.

Working with the Col-MM strain(10) of EMC virus, we have tried to establish a standard technic for assay of hemagglutination-inhibiting antibody for the purpose of studying the importance of this group of viruses as cause of disease in man(11-14). The difficulties encountered in virus hemagglutination (HA) experiments in general can be referred to the following complications: (a) the insensitivity of hemagglutination as a means of demonstrating the presence of virus, the hemagglutinating activity being inferior to infectivity by several powers of ten, (b) the appearance of false positives, caused by crude tissue extracts(7), bacterial contamination, so called spontaneous agglutination, etc. In order to increase the sensitivity of the HA reaction we have used very dilute cell suspensions [*cf.* Whitman(15)], the erythrocyte concentration ultimately adopted being 0.05%. Under such conditions the effects of salt agglutination and similar disturbances are more pronounced and the system has to be stabilized. This can be achieved by addi-

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