STUDIES ON THE ACTION OF CORTISONE ACETATE ON ISOLATED CARDIAC TISSUE

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Few definitive studies have been carried out on the direct effects of adrenocortical steroids upon isolated cardiac tissue. The first report intimating that the secretion from the adrenal cortex directly affected cardiac behavior was published by Rogoff and Stewart (1926) who noted a diminution in heart rate due to adrenal insufficiency. With the availability of cortisone and its use in therapeutics, reports were published by Hench et al. (1949), Percru et al. (1949), Somerville et al. (1950, 1951), and others, describing a return to normal in the electrocardiogram of patients being treated with cortisone for adrenal insufficiency.

In 1951 Abrams and Harris reported ECG changes in rabbits following the administration of cortisone, and concluded that this was the result of a direct action upon the heart. Several years later Hoffmann (1954) perfused isolated guinea pig and frog hearts with cortisone and observed that with low concentrations (1 to 10 µg/ml) a positive inotropic action resulted which was qualitatively confirmed by Emele and Bonnycastle (1956) using the hypodynamic cat papillary muscle preparation. In a series of reports by Booker's group (Booker et al., 1956, Shelton et al., 1956, Adeyemo et al., 1957 and Cannon et al., 1957) only a negative inotropic action was elicited by cortisone on the rabbit Langendorff preparation. Apparently, this can be explained by the high doses employed because Lacroix and Leusen (1958) reported a depression in myocardial oxygen consumption following large doses of cortisone.

The results reported herein were performed with the object of clarifying the action of cortisone upon in vitro cardiac preparations. Subsequent papers will be concerned with the cardiotonic action of other steroids.

METHODS. The cat papillary muscle preparation first described by Cattell and Gold (1938) was employed with certain modifications. The major improvement relates to electronic equipment designed and built so as to enable us to amplify and record papillary muscle contractions. This equipment makes use of an RCA mechanoelectronic transducer tube (5734), first announced by Olson (1947), and is similar in design to the amplifying instrument developed by Schilling (1958).

Papillary muscles were fixed so that their contractions displaced the shaft plate of the transducer tube. In this manner differences in electrical potential were developed and recorded directly on a Texas Instruments Rectriter.

Cats were anesthetized with ether and sacrificed by cardiectomy. Details of the procedures employed have been published elsewhere (Tanz, 1956, 1957). Briefly, 2 papillary muscles from the right ventricle were isolated without causing gross injury. A control and a treated muscle were stimulated in two identical muscle chambers at 60 times per minute by separate Grass (model S-4) stimulators using a monophasic square-wave stimulus of 10 msec duration. Contractile recordings and threshold voltages were obtained at 20-minute intervals. Threshold voltages ranged from 2 to 4½ volts. During the interval between 20-minute readings, stimulation voltages employed were approximately 2 volts higher than threshold and, unless otherwise stated, each experiment lasted 3 hours. Contractile force was expressed in terms of the per cent height of the initial contraction rather than grams for several reasons. The papillary muscles used varied in weight from 1 to 6 mg and it is obvious that the larger the muscle the greater is the force (grams) it can develop. As a consequence, in order to weigh equally the results from each experiment of a

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series, it is better to express contractile force in terms of the per cent height of a muscle's initial contraction. Therefore, the initial optimal tension for each muscle had to be individually determined which would upon stimulation yield the maximum force of contraction. Many investigators uniformly place papillary muscles under 1.5 to 2 g initial tension. However, numerous experiments in this laboratory correlating tension and force of contraction have shown that papillary muscle preparations yielding the most reliable and consistent results are those whose optimal initial tension is individually determined. Moreover, applying the same initial tension to all muscles regardless of their size, stretches some of them in an "unphysiological" manner. In this regard, we have been able to show that there is a range of optimal initial tension which can be correlated with the muscle's weight.

In the cortisone-treated muscles, cortisone acetate was added to the bath fluid within 1 minute after the initial reading, the final volume totalling 15 ml. The muscles were bathed in Krebs-Ringers bicarbonate solution enriched with dextrose, through which a mixture of 95% O2 and 5% CO2 was bubbled, and were maintained at a constant temperature of 37.5° C. The composition of the bathing fluid was: KCl 4.75 mM, KH2PO4 1.19 mM, MgSO4·7H2O 1.19 mM, CaCl2·2H2O 2.54 mM, NaHCO3 25 mM, NaCl 118 mM, and glucose 5.56 mM. The preferential use of a bicarbonate rather than a phosphate buffer lies in the fact that the former provides a more physiologic response (Sanyal and Saunders, 1957; Faust and Saunders, 1957).

Using two such systems, the individual muscles were attached to the electronic system in such a manner that both control and experimental observations could be recorded simultaneously.

Histologic sections were obtained from fresh papillary muscles and those stimulated at 60 times per minute for 6 hours in a concentration of 0 or 1 μg/ml of cortisone. Typical sections are compared in figure 1.

The whole isolated heart preparation as modified by Anderson and Craver (1948) was employed using cat hearts and enriched Krebs-Ringers bicarbonate solution as the bathing medium. This apparatus permits the original bathing fluid to be continuously recycled and in addition, failure can be induced by perfusing the preparation with fresh, unused perfusate. After failure was produced by this method the influence of cortisone acetate was investigated by adding it directly to the reservoir bottle in the concentrations indicated (see Results). In this manner a known concentration of cortisone acetate was assured of being in the bathing fluid before reaching the tissue.

RESULTS. Controls. Mean values of 11 control experiments are given in table 1. Under the conditions of the experimental arrangement, there is a rather pronounced diminution in the percent height of initial contraction during the first 20 minutes, followed by a more gradual decline thereafter, until the termination of the experiment at 3 hours. The resulting control curve is slightly higher than that reported by Lee (1953, 1954), but about equal to that reported by Loubatières (1948).

Cortisone acetate. Initially, a number of dose-response experiments were conducted employing concentrations of cortisone acetate4 of 0.01, 0.1, 1.0, 10, 20 and 40 μg/ml of perfusate. However, the minimum dosages yielding consistent results were only obtained using concentrations of 1 and 40 μg/ml.

The mean values of eight muscles treated with

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1 μg/ml and eight others treated with 40 μg/ml are presented in Table 1. In a concentration of 1 μg/ml a slight positive inotropic response occurred between 1 and 2 hours after administering the steroid. However, employing the *t* test of Fisher, all *P* values testing the statistical significance between the means obtained for the 1.0 μg/ml cortisone-treated muscles and those of the controls were greater than 0.05, indicating no significant difference (Fisher, 1948). (Using concentrations of cortisone between 5 and 35 μg/ml inconsistent results were obtained.) However, a consistent and significant depression developed within 40 minutes on eight other muscles when a concentration of 40 μg/ml was employed. It is also to be noted that there was a statistically significant difference between the 1 and 40 μg/ml cortisone-treated muscles from 40 to 100 minutes.

**Histology.** Typical histological sections of fresh, control, and cortisone-treated papillary muscles are illustrated in Figure 1. The fresh section shows good cellular integrity, lightly stained elongated nuclei and the typical cross-striations of cardiac tissue. The untreated control muscle, continuously stimulated for 6 hours, shows marked tissue degeneration. The section labelled cortisone was stimulated in a similar manner for 6 hours in a bath containing 1 μg of cortisone/ml, and again shows the typical cross-striations of cardiac tissue, good cellular integrity and lightly stained elongated nuclei. Indeed, the fresh and cortisone-treated tissues look very similar.

This apparently indicates that under the influence of a small amount of cortisone, cardiac tissue is histologically “protected” from degeneration. Presumably this occurs in the absence of some other “protecting” substance(s) which must undoubtedly be present in the normal intact animal. However, it should be remembered that results measuring the inotropic action on papillary muscles showed that there was no statistically significant difference between the 1 μg/ml cortisone-treated muscles and the controls.

**Toxic manifestations.** When a “toxic” concentration (40 μg/ml) of cortisone acetate was used, unexpected contraction patterns were often observed in the papillary muscle prepa-

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**Fig. 1.** Photomicrographs of papillary muscles. (H & E × 550)

Control and cortisone (1 μg/ml) preparations stimulated at 60 times per minute for 6 hours
ration about an hour and a half later. Figure 2 illustrates some of these abnormal patterns. In figure 2A pulsus alternans is shown; 2B shows the sudden onset of automaticity and a concomitant marked increase in diastolic tension; and 2C shows how, on occasion, increasing the current from threshold to suprathreshold voltages, a marked increase in the amplitude of contractions resulted with a reversal of this pattern when the voltage was returned to threshold levels.

Several reports have recently appeared indicating that the type of augmentation illustrated in figure 2C can be brought about solely by suprathreshold stimulation in cat papillary muscle, and that it probably is due to the endogenous release of adrenergic mediators (Whalen et al., 1958; Furchgott et al., 1959). However, the results reported by these authors could only be produced in this laboratory about an hour and a half following the addition of very high doses of cortisone acetate (40 µg/ml) or immediately following the addition of l-epinephrine bitartrate (1 to 5 µg/ml). The stepwise addition of acetylcholine (1 to 500 µg/ml) produced no changes in the contractile pattern at threshold or suprathreshold voltages.

In an attempt to ascertain the nature of the mediators responsible for the augmentation shown in figure 2C, phenolamine (Regitine) was added in very high concentrations (6.6 µg/ml) within 10 seconds. The results of one of these experiments is illustrated in figure 3A, and shows that under the influence of phenolamine a fall in the amplitude of contractions resulted in 2 minutes, but they never completely returned to control levels. If we assume that the augmentation described was due to the release of endogenous adrenergic mediators, then the results described are in agreement with those reported by Cotten et al. (1957), who showed a decline in cardiac contractile force to l-norepinephrine, l-epinephrine and isoproterenol following phenolamine (0.25 to 2.0 mg/kg) in vagotomized dogs. However, Moran (personal communication) now feels that the results reported employing phenolamine represent a nonspecific depression of the myocardium to both adrenergic and nonadrenergic cardiac stimulants.

In a similar manner a small amount of 1-(3,4-dichlorophenyl)-2-isopropylaminoethanol (DC1) (6.6 µg/ml)\(^8\) was added. Under the influence of DC1 the amplitude of contractions was returned to control levels within 3 minutes as illustrated in figure 3B. DC1 has previously been reported to inhibit this type of augmentation caused by epinephrine on cat papillary muscles (Dresel, 1958), as well as that caused solely by suprathreshold stimulation (Furchgott et al., 1959). It has also been shown to block the positive inotropic response of epinephrine, norepinephrine and isoproterenol in the Langendorff preparation using rabbit hearts (Moran and Perkins, 1958).

In order to determine whether DC1 was acting as a specific adrenergic blocking agent rather than as a cardiac depressant, several further experiments were carried out. The addition of DC1 alone in concentrations between 6.6 and 13.2 µg/ml resulted in no observable alterations in papillary muscle contractile force. Following the previously described augmentation due to cortisone acetate (40 µg/ml) and supramaximal stimulation, the addition of DC1 (6.6 µg/ml) prevented the onset of augmentation. Furthermore, the addition of DC1 1 minute prior to the administration of l-epinephrine bitartrate (1 µg/ml), with and without supramaximal stimulation, did not alter the contractile force.

Thus, it would appear that approximately an hour and a half following the addition of large

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\(^8\) The DC1 (Lilly 20522) was kindly supplied by Dr. I. H. Slater, Lilly Research Laboratories, Indianapolis.
amounts of cortisone acetate (40 µg/ml), cat papillary muscles make available (presumably) previously bound adrenergic mediators. Apparently it is the liberation and availability of endogenous adrenergic mediators which are responsible for (at least) some of the abnormal contractile patterns illustrated in figure 2.

**Isolated heart studies.** The whole isolated heart preparation was used, employing cat hearts. The results obtained from one of these experiments using cortisone acetate 1 and 10 µg/ml, are illustrated in figure 4. Following “failure,” brought about by substituting fresh, unused perfusate (see Methods), the administration of 1 µg of cortisone/ml resulted in a return to control levels in the amplitude of contraction and heart rate. The further administration of cortisone, bringing the total concentration up to 10 µg/ml, resulted in a marked decline in both amplitude and heart rate.

**Discussion.** A. These results are presented as evidence of the “protective” or “normalizing” action of small amounts of cortisone acetate on the whole isolated cat heart preparation and histological “protection” on the cat papillary muscle preparation. This action is not surprising in view of the fact that adrenal insufficiency is often characterized by cardiac impairment. Moreover, the protective action of certain glucocorticosteroids on skeletal muscle has been demonstrated (Bajusz and Selye, 1959), and Nasmyth (1957) has been able to show that a perfusion of 0.1 µg/ml of corticosterone through isolated rat hearts (taken from animals that had been adrenalectomized 72 hours previously) causes a slight increase in the amplitude of contractions. However, this response was often-times rather unpredictable. Nevertheless, Solomon et al. (1959) have demonstrated that the addition of corticosterone to the heart-lung preparation of adrenalectomized rats restored cardiac work capacity. Several studies on dogs have also demonstrated this “normalizing” ability of glucocorticoids on the heart. For instance, Nahas (1957) has shown that the administration of hydrocortisone will prevent the onset of acute acidic heart failure and Crossfield et al. (1958), using adrenalectomized dogs, have been able to demonstrate the disappearance of asystoles following the injection of certain glucocorticoids.

These results tend to support the belief that (depending on the species) certain naturally occurring glucocorticoids are necessary for normal cardiac function by virtue of a direct effect upon the myocardium.

B. It has been proposed (Hajdu, 1953) that the positive inotropic effect of cardiac glycosides is the result of a decrease in intracellular potassium. There is a great deal of information in the literature which shows that under the influence of nontoxic amounts of the cardiac glycosides, potassium is lost and sodium is taken up by the myocardium and that toxic amounts tend to reverse this process. Although contractile force and cationic flux are often correlated, it is this
The author's opinion that the mechanism of action whereby glucocorticoids may affect myocardial contractility is not primarily concerned with flux. This in no way implies that studies of this nature are not important, but rather that there is probably a more basic mechanism of action involved than solely membrane permeability and cationic flux. Admittedly, we still do not know at which level of cellular metabolism cortisone exerts its activity, although a great deal of work has been done along these lines. In general, our concepts take into account that steroids may act by (1) altering the permeability of target cells, (2) acting as carriers in ion transport or (3)

Fig. 4. Kymographic recording of isolated cat heart contractions using the Anderson-Craver heart perfusion apparatus.
First two sections show controls. At mark 1 (0 time), failure initiated by replacing the original with fresh perfusate. Mark 2 (40 min), failure having been established, 1 µg/ml of cortisone added. Mark 3 (120 min), recovery having been accomplished, more cortisone added making a total concentration of 10 µg/ml with failure resulting.
modifying specific tissue enzyme systems. Presumably, each mechanism, or a combination, may be responsible. There is little question, however, that the adrenocortical steroids regulate carbohydrate utilization in tissues (Winternitz et al., 1957) and there is a plethora of information that implicates cardiac glycosides acting in a similar manner in cardiac tissue.

C. About an hour and a half following the administration of cortisone acetate (40 μg/ml) in the papillary muscle preparation abnormal contractile patterns were often observed. In an attempt to ascertain the nature of the mediator(s) responsible for the augmentation pattern described, experiments were conducted employing phentolamine and DCI. The administration of phentolamine resulted in a diminution of the amplitude but did not completely return it to control levels. On the other hand, DCI returned the amplitude of contractions to control levels within 3 minutes.

On the basis of these experiments it is postulated that “toxic” doses of cortisone acetate may have liberated previously bound adrenergic mediators, i.e., epinephrine, norepinephrine, etc.

In any event, the main point should not be overlooked, namely, that in low doses cortisone apparently has the ability to histologically “protect” cat papillary muscle and bring back to control levels the amplitude of contractions in the whole isolated cat heart preparation, whereas in higher concentrations, it has the ability to initiate failure in both the cat papillary and whole isolated heart preparation. This biphasic action is typical of that caused by many pharmacologic agents, including the cardiac glycosides.

SUMMARY

The action of cortisone upon the isolated cat papillary muscle and isolated whole heart preparations was investigated. The results show that small doses (1 μg/ml) tend to restore the amplitude of contractions to control levels in the isolated heart preparation, and offer histological protection to papillary muscles. This effect is referred to as a “protective” or “normalizing” action.

The addition of “toxic” doses of cortisone (40 μg/ml), often resulted in abnormal papillary muscle contractile patterns about an hour and a half after administration. One of these patterns was the production of a marked augmentation following suprathreshold stimulation. Cursory experiments into the nature of this phenomena implicate the endogenous release of adrenergic mediators. A “toxic” dose of cortisone also brought about a negative inotropic response of both papillary muscle and intact heart preparations.

REFERENCES


