Action of Adrenal Cortical Steroids and Nor-Epinephrine on Vascular Responses of Stress in Adrenalectomized Rats

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In recently published work from this laboratory (1), it was demonstrated that muscular fatigue in the adrenalectomized animal was not a resultant of intrinsic metabolic disturbances in muscle or nerve, since isolated muscles from adrenalectomized rats, stimulated in vitro, were capable of the same work performance as comparable muscles from normal rats. It seemed possible, therefore, that the well-known fatigability exhibited by the muscles of adrenalectomized animals, when stimulated in vivo, may be due to inadequate circulation to the working part. In another series of experiments (2) using adrenalectomized dogs, it was shown that a continuous fall in blood pressure always preceded the muscular fatigue. Conversely, it was shown that normal work performance could be regained by restoring the blood pressure to normal values. In the course of these experiments, it was found that the adrenalectomized dog required abnormally high amounts of nor-epinephrine, even for the temporary maintenance of desired blood pressure levels. It was also noted that the venous pressure and hematocrit values remained within normal limits during the period of hypotension in the adrenalectomized dog.

The stress (work) hypotension of these animals was, therefore, interpreted as being due largely to a failure of peripheral resistance.

In an attempt to evaluate more intimately the possible factors responsible for the circulatory failure exhibited by adrenalectomized animals subjected to stress, it was felt desirable to visualize the circulation in the small blood vessels of the splanchnic area, by employing the Chambers-Zweifach rat mesoappendix preparation (3). The method affords an opportunity to determine directly the sensitivity of the vessels to various agents applied topically and parenterally in both normal and adrenalectomized animals, under varying experimental conditions.

METHODS

Sprague-Dawley male rats, ranging between 100 and 200 gm., were adrenalectomized and maintained on a 1 per cent salt solution and food ad libitum for at least 6 days prior to microscopic observation of the mesoappendix. The mesoappendix was isolated and examined according to the methods of Chambers and Zweifach (3, 4), using a modified gelatin (one-half %)-Ringer's solution for irrigation (5).

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Light sodium pentobarbital (Nembutal, i.m.) anesthesia was used. Nor-epinephrine\(^3\) (ascorbic acid added for stabilization) in various concentrations was applied topically to test for vasoconstrictor responsiveness; histamine hydrochloride and acetylcholine were applied topically to test for vasodilator responsiveness; and the adrenergic blocking agent, Dibenamine hydrochloride, was injected intramuscularly to observe the differences of response to the various agents listed above. To prevent undesirable side actions of Dibenamine in the adrenalectomized rats, it was necessary to divide the total dose (10 mg/kg.) into 3 equal amounts, injected 30 minutes apart.

A subcutaneous injection of ‘neutral’ formalin (\(\delta\)) (0.3 cc. of a 4\% solution) was used to provide a standard stress to normal and adrenalectomized rats (6-8). Some of the animals in each group were given Dibenamine prior to the injection of formalin.

**RESULTS**

**Comparison of Vasomotor Responses of Normal and Adrenalectomized Rats.** In 25 normal rats, the blood vessels of the mesoappendix constricted in response to dilutions of nor-epinephrine varying from 1:600,000 to 1:1,000,000 when .05 cc. of such solutions was topically applied. Once the original sensitivity was determined, it was observed that the vascular bed continued to be responsive to the same concentration of the drug without signs of exhaustion. The maximum length of time such a preparation was observed was 4 hours, at the end of which time it was still responsive. Vasomotion, which is an alternate constriction and dilatation of precapillaries and meta-arterioles (3), occurred spontaneously in the untreated normal rat for the duration of observations. Dilatation of vessels followed application of 0.05 cc. of histamine hydrochloride (1:1000); immediate relaxation of vessels constricted by nor-epinephrine followed the topical application of .05 cc. of acetylcholine (1:300,000).

The behavior of the vessels of the adrenalectomized rat mesoappendix offered sharp contrasts to that of the normal controls. There was no spontaneous vasomotion, and the vessels had less tone than did those of normal rats. Histamine responsiveness was the same in both normal and adrenalectomized rats. Initial concentrations of nor-epinephrine required to induce vasoconstriction varied within the same range as in the normals. The most striking differences were noted when repeated applications of nor-epinephrine were made. In the majority of adrenalectomized rats tested, there developed a gradual unresponsiveness to a concentration of nor-epinephrine which had initially induced vasoconstriction. With greater concentrations, there was again vasoconstriction; but in the majority of rats tested, there developed unresponsiveness even to the more concentrated solutions. This phenomenon never occurred in normal rats. There was no spontaneous recovery of initial responsiveness in those adrenalectomized animals which showed vasomotor exhaustion (fig. 1).

In 6 adrenalectomized animals, the concentrations of nor-epinephrine necessary to induce vasoconstriction rose from 1:600,000 or less to 1:100,000. In each of these rats, 0.2 cc. of an aqueous adrenal cortical extract (Wilson Laboratories) was placed topically on the mesoappendix. Within 20 to 30 minutes, there was invariably a return of responsiveness to the initial effective concentration of nor-epinephrine. In

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\(^3\) Supplied by the Winthrop-Stern Laboratories, through the courtesy of Dr. Scriver.
2 other animals which showed vasomotor exhaustion, there was no recovery of sensitivity to nor-epinephrine if comparable amounts of ACE were placed in the peritoneal cavity, but not directly on the mesoappendix. Neither was there recovery of sensitivity when desoxycorticosterone glucoside (0.5 mg.) or the preservative present in the cortical extract was applied topically (fig. 1).

Blood vessels of the normal rat mesoappendix invariably recovered from the effects of nor-epinephrine in 1 to 3 minutes. On the other hand, blood vessels of adrenalectomized preparations frequently failed to recover, with a resultant stasis occasionally accompanied by escape of red blood cells into interstitial spaces. This occurred most often in the salt-treated, adrenalectomized rat when more than the minimal effective concentration of nor-epinephrine was applied topically.

In order to examine more thoroughly the differences between adrenalectomized and normal rats, in so far as blood vessel sensitivity to nor-epinephrine is concerned, a constant drip of nor-epinephrine was used in the irrigating gelatin-Ringer's solution for one set of experiments. In addition, the blood vessels of the mesoappendix were observed as nor-epinephrine was injected subcutaneously. In both sets of experiments, marked differences between normal and adrenalectomized animals manifested themselves. The blood vessels of normal rats responded to a 1:250,000 drip of nor-epinephrine by alternate vasoconstriction and vasodilatation, with the vessels remaining predominantly in the constricted phase for the duration of the experiment (about 2 hours). There was no red blood cell escape, no permanent stasis, nor any varicosities of the vascular walls. In short, the normal preparation at the end of 2 hours looked virtually identical to its initial appearance.

On the other hand, blood vessels of the adrenalectomized rat under the same conditions displayed the following important differences: 1) Although initially there was alternate vasoconstriction and vasodilatation with vessels remaining predomi-
nantly in the constricted phase, the blood vessels soon exhibited 'escape' from the irrigating nor-epinephrine solution; and with time, the vessels remained predominantly in the vasodilator phase. This is, in effect, an atonia of the small vessels and a refractoriness to nor-epinephrine. 2) At the end of an hour, approximately, a moderate leakage of red blood cells into interstitial spaces was evident. This happened neither in normal controls nor in adrenalectomized rats whose mesoappendix was irrigated with simple gelatin-Ringer's solution for comparable lengths of time. 3) Frequently, nodular-like bulging of the walls of blood vessels (varicosities) of adrenalectomized animals appeared in response to a constant nor-epinephrine drip. Also, flow in the veins frequently became more sluggish with time. This did not occur in normal controls.

Analogous types of reactions were noted when nor-epinephrine was injected subcutaneously into adrenalectomized rats. Fifty γ of nor-epinephrine was injected at 15-minute intervals while the mesoappendix was being examined microscopically. After the third injection (total of 150 γ), an irreversible stasis appeared in the majority of small vessels examined, and red blood cells subsequently leaked into interstitial spaces more extensively than when a constant drip of nor-epinephrine was supplied. However, in normal rats, equal amounts of nor-epinephrine (1 mg/kg.) produced no visible effects except an enhancement of vasomotion. Effects microscopically comparable to those occurring in the blood vessels of adrenalectomized animals resulted when 5 mg/kg. nor-epinephrine was injected subcutaneously. Even then, the stasis frequently was reversible, if enough time were allowed for recovery. It is, therefore, evident that the parenteral dose of nor-epinephrine, required to produce severe circulatory changes in the mesoappendix of normal rats is at least 5 times as great as that required in the adrenalectomized preparation.

Two cc. of aqueous ACE injected intramuscularly in adrenalectomized animals prevented the adverse circulatory phenomena described above from appearing in response to nor-epinephrine administration. Comparable amounts given to normal rats did not appreciably alter the response of blood vessels to nor-epinephrine administration at any dose level.

The results given above demonstrate that the stress of the technical procedure involved in exposure of the mesoappendix was sufficient to elicit in the adrenalectomized animal insensitivity of its blood vessels to nor-epinephrine. While larger than normally effective amounts of nor-epinephrine did lead to vasoconstriction in the adrenalectomized preparation, recovery was frequently poor and incomplete. ACE corrected both the insensitivity and the occurrence of irreversible stasis.

In order to study further the role of physiologically occurring vasoconstrictor substances in the vascular phenomena of stress, formalin was used to impose an acute standard stress situation.

**Differences in Reactivity to Formalin.** Normal rats tolerated subcutaneous injections of formalin (0.3 cc. of a 4% solution) without showing any adverse circulatory changes in the mesoappendix. Vasomotion was enhanced. On the other hand, within 15 minutes after the formalin was injected, adrenalectomized rats displayed a sluggish blood flow, with stasis and multiple microscopic hemorrhages. The blood vessels were maximally dilated, and did not respond to topical applications of his-
tamine. The rate of flow was slow and irregular, and ultimately ceased in all vessels discernible in the mesoappendix. Death followed within an hour. When a larger amount of formalin was injected (0.5 cc. of a 4% solution) the majority of normal rats remained unaffected, but all the adrenalectomized animals died in from 20 to 45 minutes after injection. The sequence of circulatory reactions in the adrenalectomized rat which followed administration of a larger dose of formalin was the same as for the smaller dose, but the time interval for this sequence to occur was shortened. Invariably, there was complete cessation of flow in the mesoappendix blood vessels prior to death.

**Effects of Dibenamine.** In both normal and adrenalectomized rats, pre-treatment with Dibenamine resulted in a complete non-responsiveness to nor-epinephrine, but the responsiveness to histamine was retained. In the normal, as in the adrenalectomized, there was no spontaneous vasomotion. Injection of formalin (0.3 cc. of a 4% solution) had no apparent effects on the circulation in the mesoappendix of either the normal or the adrenalectomized rat. This is in marked contrast to the effect this dose of formalin in the non-dibenaminized, adrenalectomized rat, which developed severe circulatory symptoms culminating in a cessation of flow in the blood vessels of the mesoappendix and death.

Because of these results, the effects of Dibenamine on non-anesthetized, adrenalectomized rats, under stress, were tested. Pre-treatment with Dibenamine significantly prolonged the survival time of adrenalectomized animals injected subcutaneously with a lethal dose of formalin (fig. 2). This suggests that formalin stress acts, at least in part, through the release of sympathetic neurohumoral substances.

When non-anesthetized normal rats were injected with a dose of formalin that was lethal to adrenalectomized animals, no fatalities resulted.

The question arose whether Dibenamine exhibits protective action against formalin only, or whether it serves to protect the adrenalectomized animal generally against stress situations. Since it was known that 1 mg/kg. nor-epinephrine injected subcutaneously produced typical circulatory changes in the adrenalectomized rat, it was decided to employ this agent in large amounts to see the extent of pro-
tection that Dibenamine offered. As much as 13 mg/kg. had no effect upon the meso-appendix circulation of the dibenaminized, adrenalectomized rat. This, of course, was not interpreted as being protection against a general stress, since it is well known that Dibenamine pharmacologically is an adrenergic blocking agent (9). The more generalized stress by which the action of Dibenamine could be tested was thought to be survival time in hours of animals exposed to cold. Subsequently, adrenalectomized animals were placed in an environment maintained at 8° to 10° C. until death, after first being adjusted to a temperature of 15° to 20° C. for 3 hours. Pretreatment with Dibenamine resulted in an increased ability to withstand cold stress. Work is now in progress to determine if Dibenamine protects adrenalectomized animals against other stresses such as histamine.

![Graph](Fig. 3. COMPARISON OF DIBENAMINE reversal, as measured by blood vessel sensitivity to nor-adrenaline, in normal and adrenalectomized rats. Reversal agents (formalin, ACE or cortisone) injected at time indicated by arrow. Note logarithmic ordinates. Graph represents a composite of experiments on 4 rats from each series (24 rats in toto).)

**Antagonism Between Dibenamine and Adrenal Cortical Hormones.** It was observed in the course of examining normal rats which had been Dibenaminized, that a formalin injection given within 3 hours after initiation of dibenaminization restored the sensitivity of blood vessels to nor-epinephrine applied topically. If instead of formalin (0.3 cc. of a 4% solution), either cortisone (2 mg.) in propylene glycol or suspended in human plasma, or an aqueous adrenal cortical extract (Wilson, 3 cc.) was injected intramuscularly, there was also restoration of sensitivity within 30 to 40 minutes. On the contrary, formalin injection into the dibenaminized, adrenalectomized rat did not elicit a return of sensitivity to nor-epinephrine. But responsiveness did return if either cortisone or aqueous cortical extract was administered intramuscularly. A latent period of 30 to 40 minutes between the injection of adrenal cortical hormones and the return of sensitivity was observed in the adrenalectomized as well as in the normal dibenaminized rats. In neither group was there spontaneous recovery of sensitivity to any concentration of nor-epinephrine as long as 8 hours after injection of Dibenamine; and even at the end of 22 hours after Dibenamine injection,
only high concentrations of nor-epinephrine could elicit vasoconstriction \((1:100,000)\) (fig. 3).

**DISCUSSION**

Since the present data have shown that under certain stress situations (namely exposure of the mesoappendix while topically applying various agents), the blood vessels of the adrenalectomized animal become increasingly less sensitive to nor-epinephrine, it is justifiable to conclude that the C-11 oxysteroids in some way are necessary for blood vessels to respond optimally to sympathin E (nor-epinephrine) \((10)\). Cleghorn et al. \((11)\) demonstrated that adrenalectomized dogs show a smaller than normal pressor response to epinephrine, barium chloride, Pitressin, and nicotine. Their criterion for vasconstriction was elevation of blood pressure, and they did not distinguish between increased peripheral resistance, cardiac output, etc. Zweifach et al. \((12)\) demonstrated in a preliminary report that blood vessels of adrenalectomized rats became refractory to epinephrine. Thus, from both the direct observations here reported, and from data on intact animals, it can be generally concluded that blood vessels constrict repeatedly in response to various agents only if they are somehow 'primed' by 11-oxygenated steroids. It is likely that the 11-oxygenated steroids exert their action at the blood vessel wall itself, since the amount of cortical steroids necessary to restore responsiveness is very much smaller when applied topically than when given intramuscularly. Further substantiation comes from the fact that topical application of small amounts of cortical steroids to areas other than those being examined microscopically will not aid in restoring responsiveness to the area under observation.

Vasomotor exhaustion probably did not result from electrolyte imbalance, since serum chloride levels of both the normal and adrenalectomized rats tested were within the normal range.

It is apparent that failure of the blood vessels of adrenalectomized animals to respond optimally to nor-epinephrine cannot alone account for the hypersensitivity to all types of stress. The other type of abnormality peculiar to the blood vessels of adrenalectomized animals—namely the damaging effects of large amounts of nor-epinephrine—deserves special attention as a possible contributing factor.

At first glance, it seems peculiar that Dibenamine protects adrenalectomized animals from formalin and from cold stress; yet, it must be recalled that the initial vascular effects of formalin injection resemble those produced by subcutaneous injection of nor-epinephrine, varying only in degree of severity. For purposes of constructing a working hypothesis, let it be assumed that a neuro-humoral circuit exists consisting of afferents from peripheral or splanchnic regions; a vasomotor center; efferents; and effector organs (blood vessels). Let it be assumed further that normal functioning of this circuit is necessary for cardiovascular homeostasis, and that derangement of this circuit (as may happen in adrenal insufficiency in stress situations) leads to exhaustion of the effector organs a resultant mass dilatation and atonia of the vascular tree, accompanied by stasis. Finally, let it be assumed that interruption of this circuit at any point will prevent the circulatory failure which otherwise would have resulted from derangement of it.
If a circuit with such properties existed, then one would expect sympathectomy —by cutting the efferent fibers from the postulated vasomotor center—to protect the adrenalectomized animal from stress situations. Dibenaminization, which may be regarded as functional sympathectomy, was shown to be able to protect adrenalectomized rats against the damaging effects of formalin and cold. This shows that the stresses tested act, at least in part, through sympathomimetic substances. A possible interpretation of the protective action of elimination of bombardment by adrenergic nerve fibers is the maintenance of tonus in blood vessel walls at an intermediate stage still compatible with blood pressure maintenance, while intense bombardment results in exhaustion and atonia of blood vessel walls with resultant circulatory failure. Such an interpretation must be modified, in view of the more recent findings of Ramey, Goldstein and Levine (13). Nor-epinephrine infusion into the working adrenalectomized dog actually helped to maintain blood pressure levels. Thus, it appears that under certain circumstances, an excess of nor-epinephrine helps to prolong life, while in other circumstances, a deficiency of nor-epinephrine is protective against stress. Resolution of this paradox must await further work.

Strong additional support for the neuro-humoral circuit hypothesis is derived from the work of Swingle et al. (14, 15). They (14, 15) demonstrated that adrenalectomized dogs subjected to intestinal stripping did not go into circulatory failure if spinal anesthesia were used, or if the spinal cord were cut. When none of these precautions was taken, the animal showed a rapid and fatal drop in blood pressure, even if DCA were administered (16). If II-oxygenated steroids were supplied, or if only the adrenal medullae were removed (17), the animal recovered satisfactorily from such stress situations. It can be seen that cutting the afferent portion of the circuit prevented acute circulatory failure which always follows intestinal stripping in adrenalectomized dogs; and that the presence of II-oxygenated steroids somehow ‘corrected’ the deranged circuit.

These facts, plus present observations, point strongly to overactivity of neuro-humoral substances as a major factor in the production of circulatory failure in adrenalectomized animals exposed to stress.

The early effects of Dibenamine can be reversed by II-oxygenated steroids in amounts comparable to that secreted in an alarm reaction. It is interesting that Nickerson (9) comments on the ‘completeness and ‘non-equilibrium’ character of the adrenergic blockage’ produced by Dibenamine in its long-range action, but that an equilibrium between epinephrine and Dibenamine does exist in the first 1½ hours of action. From this, and other data, he concludes that Dibenamine blockage develops in two stages, and that the first and shorter one probably involves an ‘approximation of Dibenamine to its site of action;’ while the second, less reversible, stage involves a possible ‘covalent chemical bonding of blocking agent to some grouping near its site of action’ (9). If this is actually so, then it may be that II-oxygenated steroids permit the early stage of dibenaminization to be competitively overcome by lesser quantities of nor-epinephrine than is otherwise necessary to throw the equilibrium toward the nor-epinephrine responsive side.

These crucial differences in blood flow between normal and adrenalectomized animals provide additional insight into the possible causes of circulatory failure in
the adrenalectomized animal subjected to stress. The inability of blood vessel walls to respond repetitively to minute amounts of nor-epinephrine, in addition to the observed effects that a standard stress has upon the blood vessels of the mesoappendix in the adrenalectomized animal, suggests the following provisional working hypothesis:

Both in the adrenalectomized and normal animals, various mechanisms induce an increased blood supply to essential working areas (i.e. muscle in the case of exercise and brain in the case of anoxia). Compensations to this vasodilatation are mediated through a vasomotor center which sends out vasoconstrictor impulses to blood vessels in relatively non-active areas. As working parts remain active, and more vasoconstrictor impulses arrive, the blood vessels of the adrenalectomized animal become refractory to the action of sympathin E. These blood vessel walls not only fail to constrict; they become completely atonic and stasis results. Adequate shunting is prevented, and when the circulating blood no longer fills the diluted vascular tree, profound circulatory failure occurs. The II-oxygenated steroids permit blood vessel walls to respond to minute amounts of nor-epinephrine without vasomotor exhaustion, thereby allowing normal function of the homeostatic mechanisms which govern shunting of blood. The II-oxygenated steroids also serve to nullify or neutralize the toxic action of certain neurohumeral substances released in stress situations.

SUMMARY AND CONCLUSIONS

The adrenalectomized animal dies in circulatory failure in response to stresses to which the normal can adjust. In an attempt to evaluate the ability of the adrenalectomized animal to maintain its peripheral resistance, the Chambers-Zweifach rat mesoappendix preparation was utilized to observe blood flow under various experimental conditions. From these direct microscopic observations, the following was demonstrated:

Blood vessels of the mesoappendix of the adrenalectomized rat became refractory to repeated topical applications of nor-epinephrine, while those of normal controls retained their sensitivity. Responsiveness to nor-epinephrine was restored by the topical application of aqueous adrenal cortical extracts. Formalin injection did not impair the circulation in the mesoappendix of normal rats, but induced a sluggishness of flow, terminating in stasis and a complete cessation of flow prior to death, in the mesoappendix of the adrenalectomized rat. These changes were prevented by Dibenamine. Dibenamine also prolonged the survival time of adrenalectomized rats which were given lethal amounts of formalin. The early stage of Dibenamine blockade of adrenergic agents was overcome by adequate amounts of adrenal cortical extracts in both normal and adrenalectomized rats.

Interpretation of these data and a provisional working hypothesis were presented. In essence, cortical steroids seem to be necessary to allow blood vessels to respond regularly and repeatedly to minute amounts of sympathin E, and at the same time, to prevent the toxic effects upon blood vessels of large amounts of sympathomimetics, specifically nor-epinephrine.

REFERENCES