Blood Pressure Reducing Effect of Actinomycin D in Rats.* (30297)

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Under a number of experimental conditions we find a positive correlation between blood pressure levels and concentration of potassium in arterial muscle cells(1). We consider that the aspect of blood pressure regulation most likely to be influenced by cellular potassium is arterial tone, rather than phasic contractility(2). The mechanism by which potassium regulates muscle tone is not clear but it may alter some biophysical characteristics of muscle protein such as viscoelasticity(1,3). The existence of a labile tonal protein component in arterial muscle would be implicated if impairment of protein synthesis were followed by a loss of arterial tone. Actinomycin D† was chosen as an agent to test this hypothesis.

Methods and results. Rats made hypertensive by renal ligation were treated with actinomycin D at 3 dosage levels administered intraperitoneally. Each rat received two injections of equal dosage 24 hours apart so that the total dosage was 24, 12 or 8 μg per 100 g body weight. The LD/50 is approximately 60 μg per 100 g body weight under such conditions. Rats receiving the dose of 24 μg per 100 g were listless for several days after the initial injection but recovered their vigor, while those receiving smaller amounts appeared to be in good health at all times. The other anti-metabolites, dinitrophenol and 5-fluorouracil were also administered to hypertensive rats at one-half their LD/50 dosages. Systolic blood pressures of all rats were obtained at regular intervals by microphonic manometer(4).

It can be observed (Fig. 1) that the average systolic blood pressures of all groups injected with actinomycin D promptly declined until the fourth day following the initial injection after which the blood pressure began to return to initial levels. It can be noted that both the degree of depression and rate of restoration of blood pressure levels are a function of the dosage of actinomycin D, but that maximum depression is reached on the fourth day for each dosage.

The relationship of dosage to changes in blood pressure suggests that this agent lowers blood pressure through a definite process rather than from the "non-specific" effect of a toxic substance. This deduction is supported by the failure of the 2 other anti-metabolites, dinitrophenol and 5-fluorouracil, to reduce the blood pressure.

A marked depression in blood pressure is also found following administration of actinomycin D to normal rats (Table I).

In view of the widespread effects of actinomycin D on the inhibition of DNA-dependent RNA synthesis, the hemodynamics of its depressor action cannot be accurately explained. Among several general factors responsible for this effect may be (1) disturbances in myocardial function, (2) reduction in circulating blood volume and (3) decreased peripheral resistance. There are few data available for evaluation of neurogenic, renal, or adrenal medullary mechanisms in the blood pressure depression due to actinomycin D. The following experiments explore the possibility that a state of adrenal cortical insufficiency was induced by this compound.

Actinomycin D (24 μg per 100 g body weight) was administered to hypertensive and normotensive rats. Two days after the initial injection, 2.5 mg prednisolone acetate‡ was injected daily. The systolic blood pressures of the rats continued to decline and returned to previous levels at the same rate and pattern as shown in Fig. 1. Apparently a lack of glucocorticoids was not responsible for the blood pressure changes induced by actinomycin D.

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‡ Meticortelone Acetate (prednisolone acetate) supplied by Schering Corp., Bloomfield, N. J., through the courtesy of Dr. Roger W. Cooper.

TABLE I. Blood Pressures of Normal Long-Evans Rats on Fourth Day Following Injection of Actinomycin D. Systolic blood pressures were measured by microphonic manometer and mean pressures by cannulation of abdominal aorta.

Group	No.	Avg systolic B. P. (mm Hg)	Avg mean B. P. (mm Hg)
Normotensive control	20	114 ± 9.1*	99 ± 8.2
Normotensive + actinomycin D, 24 μg/100 g	19	78 ± 11.6	69 ± 14.3
Statistical difference between groups		T = 7.3, P < .01	T = 6.6, P < .01

* S.D.

There is the other possibility that available adrenal steroids become ineffective in the peripheral tissues, an example being the failure of estrogenic steroids to induce normal uterine hypertrophy in the presence of actinomycin D(5). The following experiments indicate, however, that this anti-metabolite does not prevent glucocorticoids from exerting physiologic effects in the periphery. Adrenalectomized rats do not tolerate the administration of 24 μg actinomycin D per 100 g body weight, 13 of 15 rats dying by the fifth day after initial injection. When such rats are supplied with 2.5 mg prednisolone acetate daily only 2 of 15 died. This protective action was not obtained with desoxycorticosterone acetate administered in 5 mg doses daily, 12 of 15 adrenalectomized rats failing to survive.

It is of prime importance to determine whether actinomycin D lowers blood pressure by interference with the proteins of the arterial muscle. Therefore the functional capacity of the proteins involved in phasic contraction was tested in the following experiment: L-norepinephrine was administered

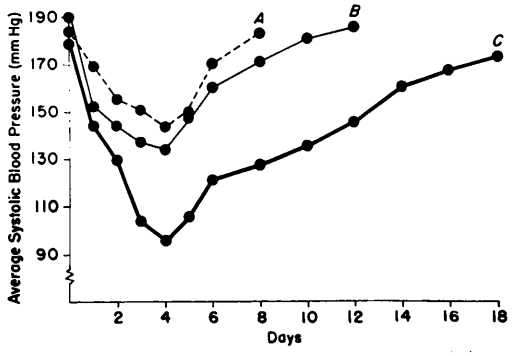

FIG. 1. Average systolic blood pressure of renal hypertensive rats in groups of 15 animals, following administration of actinomycin D. A—8 μg, B—12 μg, and C—24 μg/100 g body weight.

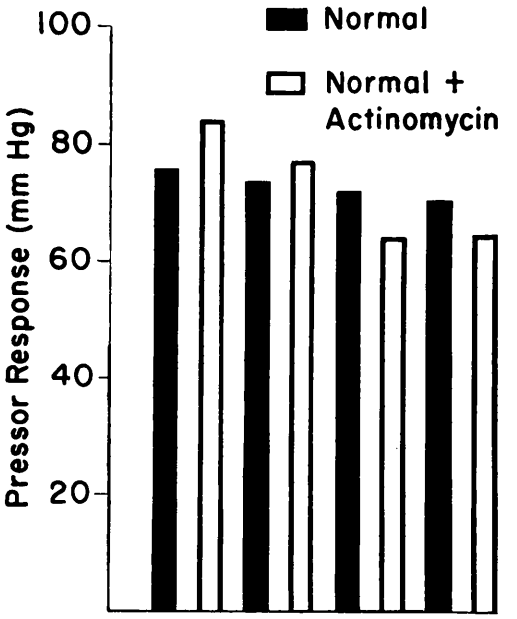

FIG. 2. Elevation of mean pressure in response to successive intravenous injections of 10 μg l-norepinephrine in 15 normal rats and in 15 treated with 24 μg actinomycin D/100 g body weight. Tests were repeated at 10-minute intervals.

to normal rats whose blood pressures were markedly depressed by actinomycin D and the pressor responses compared to those obtained in untreated normal rats. In this manner, 10 μg l-norepinephrine was injected intravenously and the rise in blood pressure measured by aortic cannulation. The injections of this drug were repeated 3 times at 10-minute intervals. It can be seen from Fig. 2 that there was little difference in pressor response between the two groups of rats.

The pressor response to l-norepinephrine involves myocardial as well as arterial muscle contraction. A response limited to the peripheral arteries was obtained by using the drug methoxamine in a smaller series of animals.

There was similarly no appreciable difference in response between normal and actinomycin D treated animals to the intravenous injection of 200 μg methoxamine.

Discussion. In view of the evidence that actinomycin D does not seriously interfere with the function of the phasic contractile elements, it appears that the blood pressure depression may be a reflection of a reduced arterial tone. Any attempt to explain the mechanism of this effect would be premature. To suggest that the cellular potassium-protein system participates in this response requires first of all proof that actinomycin D suppresses synthesis of muscle cell protein. Studies are now underway designed to resolve this question.

Summary. Actinomycin D causes a fall in blood pressure of normotensive and renal hypertensive rats; the reduction of pressure is proportional to the dosage. Giving prednisolone did not restore the pressure but hormonal activity is not blocked since prednisolone protects against a lethal dose of actinomycin D given to adrenalectomized rats. Vigorous arterial pressor response to norepinephrine and methoxamine limits any peripheral arterial effect to tonic rather than phasic contraction.

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Lipoprotein Lipase Activity (LLA) in Adipose, Muscle and Aortic Tissue from Rats of Different Age and in Human Subcutaneous Adipose Tissue.* (30298)

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The presence of lipoprotein lipase activity (LLA) in the adipose tissue of rats, rabbits and chickens is well established(1,2,3) but its presence in significant quantities in human adipose tissue has been questioned(4,5).

Angervall(4) found that lipoprotein lipase activity was considerably greater in chicken than in hen adipose tissue. Using a modification of the method described by Cherkes and Gordon(6), Chlouverakis(7) found that LLA in the adipose tissue of rats 4-5 weeks old was significantly greater than that of rats more than 5 months old. In interpreting this finding, however, it must be borne in mind that the protein/lipid ratio of young and old rat adipose tissue may differ(8).

This possibility was investigated by measuring the LLA in the adipose tissue of 2 groups of rats, one consisting of animals 4 to 6 weeks old and the other of much older animals (5 to 8 months), and expressing the results as LLA per unit of tissue wet weight and per unit of tissue protein. Other possible explanations for the apparent enzyme change associated with the ageing process, namely, a redistribution of the enzyme and its relationship to growth hormone, were also investigated.

Finally, in view of the conflicting reports regarding the enzyme activity in human adipose tissue, the LLA of subcutaneous fat was determined in material taken at biopsy.

Material and methods. Tissues. Male Wistar rats fed on a standard diet (Pillsbury's Thompson Diet Rat Cube, Birmingham, England), were used in all the experiments. "Young" rats were 4-6 weeks old and "old" rats were aged 5-8 months. Animals were killed by decapitation, adipose tissue was ob-

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