Experimental Study

Sensitization by Dihydrotachysterol (DHT) for Induction of Cardiac Lesions by Various Agents*

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IN A SERIES of previous publications we showed that, in rats, the oral administration of either sodium phosphate[1] or calcium acetate[2] qualitatively alters the cardiotoxic actions of certain steroid vitamin-D derivatives, such as dihydrotachysterol (DHT), so that an acute purulent myocarditis results. Furthermore, in rats simultaneously treated with comparatively small doses of DHT plus calcium acetate, the resulting cardiovascular lesions can be considerably aggravated by a variety of stressor agents—for example by cold baths, restraint, or noradrenalin.[2] On the other hand, pretreatment with similar doses of DHT alone did not suffice to render the cardiovascular system particularly stress-sensitive.[3] The production of an electrolyte-steroid-cardiopathy with necroses (ESCN) by combined treatment with certain corticoids and "sensitizing sodium salts" is likewise greatly enhanced by various stressors; indeed, corticoid conditioning alone suffices to so alter reactivity of the heart that it undergoes necrosis during exposure to stress.[3] All these observations show that suitable humoral conditioning with various steroids (hormones or vitamins) and electrolytes can selectively condition the cardiac muscle for the production of necroses by stress.

It is the object of this communication to report on experiments which indicate that intense acute overdosage with DHT alone, though not sufficient to produce cardiac necroses in itself, suffices to predispose the cardiac muscle to the production of necroses by cold or by papain, Plasmocid, noradrenalin, or vasopressin in doses themselves virtually ineffective.

MATERIAL AND METHODS

One hundred twenty female Sprague-Dawley rats, with an average initial body weight of 95 Gm (range 90–102 Gm), were subdivided into 12 equal groups, and treated as indicated in Table I.

Dihydrotachysterol (DHT) was administered by stomach tube in the form of a microcrystal suspension, at the dose of 150 mg in 0.5 ml of water, twice daily during the first two days and once on the third day.

TABLE I

Effect of Dihydrotachysterol upon the Potential Cardiotoxic Action of Various Agents

Group	Treatment	Cardiac necroses[a]	Mortality (%)
1	None	0	0
2	DHT	0.1 ± 0.10	0
3	Cold	0	0
4	Cold + DHT	1.2 ± 0.28	10
5	Papain	0.2 ± 0.15	0
6	Papain + DHT	2.3 ± 0.37	10
7	Plasmocid	0.1 ± 0.10	0
8	Plasmocid + DHT	2.6 ± 0.23	30
9	Noradrenalin	0.5 ± 0.17	0
10	Noradrenalin + DHT	1.6 ± 0.23	0
11	Vasopressin	0	0
12	Vasopressin + DHT	1.1 ± 0.38	0

[a] Intensity of cardiac lesions was assessed on a scale of 0 3; these figures are for the means of these determinations ± standard error.

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Exposure to *cold* consisted in the immersion of the rats in icy water, for a period of 3.5 minutes, twice during the second day.

Papain is a proteolytic enzyme contained in the dry latex of *Carica papaya*. Five grams of crude papain powder were added to 50 ml of distilled water; this turbid suspension was mixed in a Waring Blendor for ten minutes and then passed through a No. 40 Whatman filter paper; 0.2 ml of the resulting clear, amber-colored filtrate was injected into the jugular vein on the second day.

Plasmocid [8-(3-diethylaminopropylamino)-6-methoxyquinoline], 0.4 mg in 0.2 ml of water, was injected intraperitoneally three times during the second day.

Noradrenalin, 300 μg in 0.2 ml of oil, was administered subcutaneously twice on the second day.

Vasopressin, 10 i.u. in 1 ml of water, was injected subcutaneously twice on the second day.

Throughout the experiment all the animals were maintained on Purina Fox Chow. At the end of the third day, all surviving rats were killed with chloroform. The hearts were fixed in neutral Formalin for subsequent embedding in paraffin and histochemical determination of calcium with von Kóssa's technique. The intensity of the cardiac lesions was assessed in terms of an arbitrary scale of 0 to 3; the means of these determinations (with standard errors) are listed in Table I.

Results

As shown by the data in Table I, even at the high dose level at which it was given here, DHT did not produce any significant cardiac lesions during this acute three-day experiment (group 2). Among the other agents that we employed, papain and Plasmocid are known to produce widespread necroses in the myocardium, but only if they are given at higher dose levels. Cold, noradrenalin, and vasopressin rarely induce any extensive myocardial necroses, however, except in animals that are specially conditioned (e.g., by corticoids). In any event, in this series, the comparatively mild and brief treatment with cold (group 3), papain (group 5), Plasmocid (group 7), noradrenalin (group 9), or vasopressin (group 11) caused little or no histologically detectable cardiac damage. On the other hand, these same agents produced pronounced and often fatal cardiac necroses when they were administered concurrently with doses of DHT in themselves ineffective (groups 4, 6, 8, 10, and 12).

Histologic examination of the hearts revealed furthermore that, in the DHT-conditioned rat, the various additionally given agents produced histologic changes that simulated a much more severe DHT intoxication rather than the specific effects that would have been induced by higher doses of the eliciting agents themselves. For example, vasopressin, in itself, can produce minor cardiac lesions and, following corticoid conditioning, even extensive necroses, but it does not produce any lesions in the coronary arteries or calcification of the myocardium.[8] Yet, in group 12, in which vasopressin was administered conjointly with DHT, the latter in doses themselves ineffective, the resulting picture was typical of a severe DHT intoxication. Throughout the myocardium, the muscle fibers as well as the stroma between them became impregnated with von Kóssa-tingible calcium deposits (Fig. 1). The coronary arteries exhibited the Mönckeberg-sclerosis type of calcification, as well as an often very pronounced perivascular edema (Fig. 2).

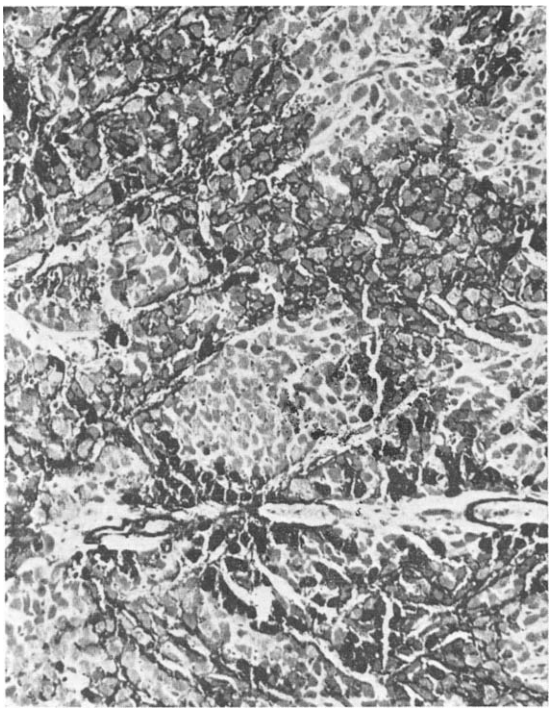

Fig. 1. Intense calcification of the coronary arteries and the myocardium in a rat of group 12 that was treated with vasopressin and DHT. In certain regions (*top*), calcification affects predominantly the stroma between the muscle fibers, while in other places (*bottom*), the entire muscle fibers are calcified. (von Kóssa; × 100)

Essentially similar changes were obtained in all the remaining groups in which DHT was administered in conjunction with another potentially cardiotoxic agent, yet the calcification was

FIG. 2. Another region from the myocardium shown in Figure 1. Here, in addition to the calcium deposition, there is marked perivascular edema, especially around the largest of the coronary arterioles. (von Kóssa; × 75)

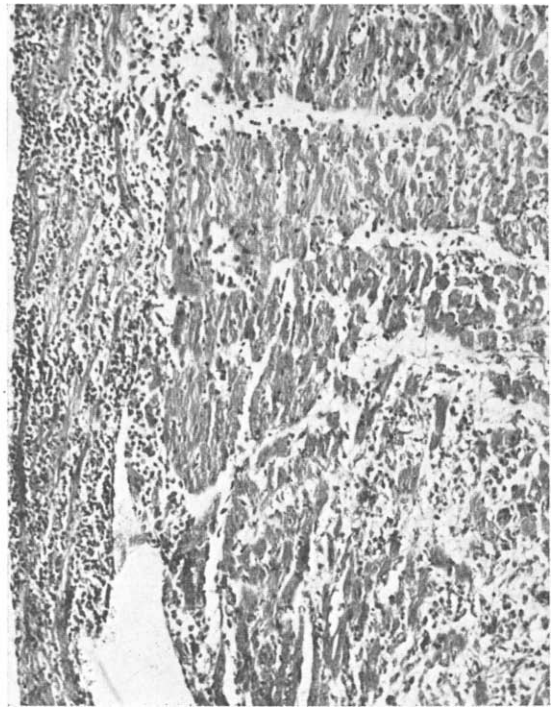

FIG. 3. Interstitial edema and inflammation without calcification, in the heart of a rat of group 4 treated with cold plus DHT. (von Kóssa; × 100)

not always equally pronounced. Often, in the same heart, certain regions were calcified; in others, the structural alterations consisted primarily of interstitial edema and inflammation, frequently accompanied by necrosis of muscle fibers (Fig. 3).

SUMMARY

Rats were treated, during three days, with doses of dihydrotachysterol (DHT) which, in themselves, caused no detectable cardiac lesions. In the hearts of these animals, necrosis, inflammation, and coronary calcification could be produced by various agents (cold, papain, Plasmocid, noradrenalin, vasopressin) which, in themselves, likewise produced no significant cardiac lesions under similar conditions of dosage.

After brief sensitization with small doses of DHT, even agents which normally have no tendency to produce coronary lesions or calcification of the myocardium (e.g., vasopressin) elicit these changes which are usually induced by more prolonged and intense treatment with DHT alone. Apparently, the potential cardiotoxic effect of mild DHT overdosage can be accentuated by a variety of nonspecific agents.

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