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ANTIARRHYTHMIC EFFECT OF ANTIBODIES TO DIGOXIN IN EXPERIMENTAL MYOCARDIAL INFARCTION (THE ARRHYTHMOGENIC ACTION OF ENDOGENOUS DIGOXINLIKE FACTOR)

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The blood and several tissues of man and animals contain a substance (or substances) possessing immunoreactivity and biological properties similar to those of digitalis glycosides, and which has been called endogenous digoxinlike factor (EDLF) [6, 10]. The chemical structure of EDLF has not yet been discovered but it has been shown that, since it possesses natriuretic activity and inhibits active sodium transport through the plasma membrane in different kinds of tissues, this substance participates in the regulation of water and mineral metabolism and is involved in the pathogenesis of arterial hypertension [14]. One proof of the latter hypothesis has been obtained in experiments during which administration of antidigoxin serum (ADS), which binds EDLF, caused the blood pressure (BP) to fall in rats with certain forms of experimental hypertension [15]. Digitalis glycosides are known to give rise to serious disturbances of the cardiac rhythm such as

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TABLE 1. Effect of ADS on Frequency of Appearance of Ventricular Arrhythmias in Rats with MI and in Mice with Hypoxia Induced by Inhalation of Chloroform Vapor

Compound, dose, mg/kg intravenously	Etiology of arrhythmias	Number of animals in group	Disturbances of rhythm (number of animals)		
			extrasystoles	VT	VF
Control	MI	6 rats	5	6	2
ADS, 5.0	MI	6 rats	1	1*	1
Control	Chloroform	10 mice	10	—	10
ADS, 2.5	Chloroform	10 mice	10	—	8
ADS, 20.0	Chloroform	10 mice	7	—	5*
Lidocaine , 2.5	Chloroform	10 mice	9	—	8
Lidocaine , 10.0	Chloroform	10 mice	5*	—	5*

Legend. Asterisk indicates groups of animals in which frequency of appearance of arrhythmias differed significantly ($p < 0.05$) from control (t test). Lignocaine was injected for comparison in experiments on mice.

ventricular fibrillation and ventricular tachycardia (VF and VT), and in myocardial infarction (MI) the ability of digitalis preparations to cause arrhythmias is considerably increased [13]. VF and VT, in turn, are the main causes of sudden death from MI and acute coronary insufficiency [13]. The basis for the present investigation was the view that EDLP has a possible role in the pathogenesis of ventricular arrhythmias accompanying acute myocardial ischemia and hypoxia, for as long ago as in the late 1940s, the formation of a hypothetical endogenous "strophanthinlike substance" was linked with the development of hypoxia [9]. Isolation of EDLF possessing a positive inotropic action could be the response to a sudden decrease in cardiac ejection in acute myocardial ischemia. The antiarrhythmic action of ADS was one factor confirming our hypothesis. The aim of the present investigation was to study the effect of ADS on the electrical threshold of ventricular defibrillation (TVF) in cats with experimental MI and on arrhythmias induced by MI in rats and by hypoxia in mice.

EXPERIMENTAL METHOD

ADS was obtained by immunizing rabbits with a conjugate of digoxin and bovine serum albumin [15]. TVF was determined in mature male cats weighing 3-5 kg. Under pentobarbital anesthesia (8 mg/kg, intramuscularly) and with artificial ventilation of the lungs, left-sided thoracotomy was performed on the animals and the descending branch of the left coronary artery (CA) was ligated in its lower third. TVF was determined 5 min after ligation, by applying single increasing square pulses with a duration of 5 msec to the left ventricular myocardium every 30 sec, in the so-called "vulnerable phase" (the beginning of the descending limb of the T wave). The minimal amplitude of the pulse inducing VF was taken to be TVF. In three series of experiments TVF was determined in intact animals and in cats with MI, receiving 5 mg/kg of ADS or 2 ml of physiological saline intravenously 5 min before ligation of CA. The ECG and BP were recorded continuously during the experiments. High ligation of the left CA in anesthetized (pentobarbital 40 mg/kg, intramuscularly) and artificially ventilated male Wistar rats (230-300 g) was carried out by the method described previously [11]. The animals were given an intravenous injection of ADS or 0.2 ml of physiological saline 5 min before ligation, and the ECG was recorded for 20 min after ligation. In experiments on albino mice (19-24 g) of both sexes the effect of ADS was studied on the development of arrhythmias caused by inhalation of chloroform vapor; the pathogenesis of chloroform arrhythmias has been linked with myocardial hypoxia [7]. To study the possible role of EDLF in adrenergic cotransmission in the heart the effect of ADS was studied on the positive inotropic effect of indirect transmural electrical stimulation (TES) of adrenergic nerve endings of the rat atria [5]. Isolated atria of male Wistar rats (150-200 g) were placed in a thermostated bath containing Krebs' solution at a temperature of 32.5°C, through which was passed a mixture of 95% O_2 and 5% CO_2. Contractions evoked under an initial load of about 1 g were recorded by means of a strain gauge transducer. TES was applied in "bursts" of square pulses 2 msec in duration and with a frequency of 25 Hz; the potential difference was chosen so that the amplitude of atrial contractions increased by 25-100%. To abolish the cholinergic component of TES, atropine (1 μM) was added to the solution.

EXPERIMENTAL RESULTS

TVF in intact cats (n = 11) was 25.5 ± 3.3 V. In untreated animals with MI, TVF was significantly lower, namely 11.3 ± 1.6 V (n = 11). Meanwhile, in animals with MI and receiving ADS, not only did TVF not fall, but it was actually higher than in the control group, at 53.3 ± 8.1 V (n = 9). Injection of ADS caused no appreciable changes in heart rate, BP, or parameters of the ECG in the animals.

In experiments on rats and mice ADS also had an antiarrhythmic action. As Table 1 shows, preliminary injection of ADS in both series of experiments led to a decrease in the frequency of appearance of ventricular arrhythmias compared with the control. In experiments on mice the effective dose of ADS with respect to its antifibrillatory activity corresponded to a comparatively high dose of lignocaine.

Our results indicate that EDLF plays a definite role in the pathogenesis of ventricular arrhythmias in acute myocardial ischemia. It is noteworthy that the doses of ADS which we used were of the same order as those which lowered BP in rats with experimental hypertension [15], and the possible mechanisms of the arrhythmogenic action of EDLF are therefore of great interest. Receptors of cardiac glycosides in the heart are known to be distributed not only on the cardiomyocyte membrane, but also on adrenergic nerve endings [10]. Preparations of digitalis interact with Na,K-ATPase of adrenergic terminals in much lower concentrations than those in which they act on cardiomyocytes [10]. It has been shown that a local increase in noradrenalin release in the heart plays a role in the pathogenesis of arrhythmias in MI [2]. Taking these data into consideration, it could be postulated that EDLF promotes the appearance of arrhythmias in MI by acting like an adrenergic cotransmitter. To test this hypothesis we studied the effect of ADS on the positive inotropic effect of TES of the rat atria; as was shown previously, TES causes release not only of noradrenalin, but also of its cotransmitters [5]. In our experiments ADS (1-100 μg) did not affect the magnitude or duration of the positive inotropic response of the atria to TES. Thus EDLF is not an adrenergic cotransmitter in the heart, and other mechanisms lie at the basis of its arrhythmogenic action. The following possibilities seem to be most likely to be correct. First, it may be a question of the action of circulating EDLF, discovered for the first time in patients with arterial hypertension. Second, the possibility of formation of EDLF as a component of the stress-protein system of the cell cannot be ruled out; the formation of "heat-shock" proteins in myocardial ischemia was proved in [3]; it has also been shown that they can regulate Na,K-ATPase activity [1]. Finally, the possibility of a central action of EDLF cannot be ruled out. Stimulation of certain brain structures containing glycosidelike substances, the area postrema and area AV3V [14] can induce disturbances of the cardiac rhythm [4].

The discovery of the antifibrillatory action of ADS may therefore widen our opportunities for antiarrhythmic therapy in MI, more especially because antibodies to digoxin have already been successfully used in the clinical treatment of cardiac glycoside poisoning [12].

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