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# PREVENTION OF ADRENALIN-INDUCED ARRHYTHMIAS BY THE CALMODULIN BLOCKER TRIFLUOPERAZINE

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Opening of $Ca^{++}$ channels and increased entry of $Ca^{++}$ into the cardiomyocytes are an essential stage in the cardiotonic effect of catecholamines [8], and at the same time they play an important role in the development of adrenergic and, in particular, of stress-induced heart damage [2]. Accordingly blockers of slow $Ca^{++}$ channels, mainly verapamil, diltiazem, etc., have proved to be effective cardioprotective and, in particular, antiarrhythmic drugs [8]. However, this protective effect of $Ca^{++}$-channel blockers is limited by the fact that after the excess of $Ca^{++}$ has entered the cell or has arisen in the sarcoplasm due to release from the sarcoplasmic reticulum or other depots, damage induced by the excess of $Ca^{++}$ cannot be abolished by blockers of $Ca^{++}$ channels. Accordingly the possibility of preventing the damaging and, in particular, the arrhythmogenic effect of catecholamines by means of blockers of the main $Ca^{++}$ receptor (calmodulin), in the form of a complex with which $Ca^{++}$ can activate phospholipolysis [4], lipolysis [2], proteolysis [6, 12], and peroxidation [1, 10], i.e., processes playing a principal role in the development of adrenergic damage [2], is very interesting.

The aim of this investigation was to study the possibility of preventing depression of the contractile function of the heart and arrhythmias which regularly arise in response to the action of toxic doses of catecholamines, by means of the calmodulin blocker trifluoperazine, and to compare the effect witb the cardioprotective action of the $Ca^{++}$ blocker, verapamil.

## EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats. The rats were heparinized (200 U/100 g body weight, intraperitoneally) and, under pentobarbital anesthesia (50 mg/100 g, intraperitoneally) the heart was removed and placed in a Langendorff perfusion system. Standard Krebs–Henseleit solution (glucose 11 mM) was used for perfusion. The solution was aerated with a mixture of 95% $O_2$ and 5% $CO_2$ at 37°C and the pH maintained between 7.3 and 7.4. The perfusion pressure was 9.5 kPa (97 cm water). Mechanical activity of the isolated heart was recorded by Straube's method, using a TD-112S isotonic transducer, and the ECG and mechanical activity of the heart were recorded with the aid of the specialized modules of the RM-6000 polygraph and VC-9 oscilloscope (Nihon Kohden, Japan). One electrode for recording the ECG was placed

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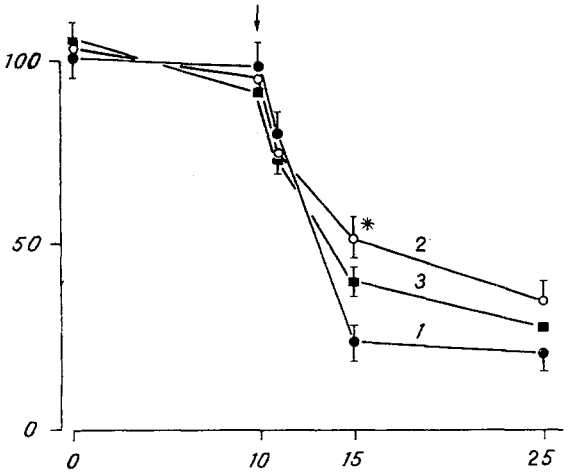


Fig. 1. Effect of trifluoperazine ($10^{-6}$ M) and verapamil ($2.5 \times 10^{-8}$ M) on the amplitude of contraction of the isolated heart in response to cardiotoxic doses of adrenalin ($5 \times 10^{-5}$ M). Abscissa, time after beginning of experiment (in min); ordinate, amplitude of contraction (in % of initial value). 1) Control; 2) trifluoperazine; 3) verapamil; arrow indicates injection of adrenalin.

on the aorta, the other on the left ventricle. To reproduce adrenergic heart damage cardiotoxic doses of adrenalin ($5 \times 10^{-5}$ M) were added to the perfusion fluid. Injection of the calmodulin blocker trifluoperazine, in a dose of $10^{-6}$ M, began 10 min before perfusion with adrenalin and continued throughout the experiment. The calcium channel blocker verapamil was injected in accordance with the same scheme in a dose of $2.5 \times 10^{-8}$ M.

## EXPERIMENTAL RESULTS

The data given in Fig. 1 and Table 1 show that as a result of perfusion of the isolated heart with cardiotoxic doses of adrenalin, depression of the contractile function developed during the first 15 min, and was reflected in a fivefold decrease in amplitude of the contractions and by the development of marked contracture, in which the degree of permanent shortening of the left ventricle was close to the amplitude of its contraction before the action of adrenalin began. Meanwhile, against the background of depression of the contractile function, disturbances of the cardiac rhythm developed, in the form of extrasystoles in all the hearts studied, ventricular tachycardia (in four of 11 hearts), bradyarrhythmia (in three of 11 hearts), and fibrillation (in one heart) in fewer than half of the cases. These arrhythmias occurred soon (27 sec) after the beginning of adrenalin infusion. Thus cardiotoxic damage to the isolated heart is characterized by the fact that marked depression of contraction and partial contracture of the heart muscle take place against the background of sinus tachycardia, and after a short latent period ventricular arrhythmias develop, mainly in the form of extrasystoles, and also of ventricular tachycardia.

The calmodulin inhibitor trifluoperazine did not affect the basic sinus rhythm but, at the same time, it significantly limited the disturbances of contraction and the arrhythmias arising in response to the cardiotoxic action of adrenalin. At the 15th minute after the beginning of adrenalin infusion the isolated hearts, into whose perfusion fluid trifluoperazine had started to be injected 10 min previously, possessed an amplitude almost twice greater, and were characterized by less marked contracture. The latent period of onset of the arrhythmias was correspondingly increased more than tenfold compared with that of the unprotected hearts, and extrasystoles were observed in fewer than half of the hearts (five of 11), their average duration being reduced by 17 times. Manifestations of ventricular tachycardia and fibrillation of the heart were not observed in any single case, and trifluoperazine in this concentration was ineffective against sinus bradyarrhythmias. Judging by the parameters of the contractile function and cardiac rhythm, the protective action of verapamil was significantly weaker (Fig. 1). The antiarrhythmic effect of verapamil also was much weaker. For example,

TABLE 1. Effect of Trifluoperazine on Cardiac Arrhythmias Induced by Adrenalin

| Parameter | | Adrenalin | |
|---|---|---|---|
| | Control (n = 11) | Trifluoperazine (n = 11) | Verapamil (n = 11) |
| Arrhythmia: number of hearts | 11 | 6 | 11 |
| Extrasystoles: | | | |
| number of hearts | 11 | 5 | 10 |
| total number of extrasystoles | 171 | 10 | 42 |
| mean number of extrasystoles calculated per heart (M ± m) | 16.5 ± 3.5 | 0.9 ± 0.4† | 3.8 ± 0.9* |
| Ventricular tachycardia: | | | |
| number of hearts | 4 | 0 | 2 |
| total duration, sec | 12 | 0 | 4 |
| average duration calculated per heart, sec (M ± m) | 3.0 ± 1.3 | 0 | 0.4 ± 0.1* |
| Bradyarrhythmia: | | | |
| number of hearts | 3 | 2 | 1 |
| total duration, sec | 311 | 300 | 26 |
| average duration calculated per heart, sec (M ± m) | 28.3 ± 8.2 | 27.2 ± 5.9 | 2.3 ± 2.3 |
| Ventricular fibrillation: number of hearts | 1 | 0 | 0 |
| Latent period of arrhythmias, sec | 27 ± 4 | 294 ± 94* | 29 ± 6 |

Legend. Significance of differences compared with control: *) $p < 0.05$, †$p < 0.01$.

the total number of extrasystoles for the groups of animals compared was reduced by verapamil by 4 times, not by 17 times as during the action of trifluoperazine; the latent period of onset of arrhythmias was not significantly altered by verapamil. Verapamil prevented the increase of ventricular tachycardias and reduced more than tenfold the total duration of sinus bradyarrhythmias.

The calmodulin blocker trifluoperazine thus prevents depression of cardiac contractility more effectively than the slow calcium channel blocker and reduces the duration and frequency of ventricular arrhythmias in response to the action of cardiotoxic doses of catecholamines. The antiarrhythmic effect of calcium antagonists under conditions of ischemia is frequently explained on the grounds that, by depressing cardiac contractility, they limit the deficiency of high-energy phosphates and reduce disturbances of function of the cationic pumps [7]. It will be evident that the antiarrhythmic effect of the calmodulin inhibitor in catecholamine-induced cardiac damage has a different mechanism, for the drug enhances the contractile function. In order to understand this phenomenon the following considerations must be examined.

1. Because of the great difference in the affinity of calmodulin for enzymes of different metabolic pathways, it is possible that the calmodulin inhibitor limits effects of cAMP by a greater degree in the cells than the calmodulin-dependent activity of the contractile system, as a result of which adrenalin-induced arrhythmias are suppressed against a background of comparatively high contractile function, as was observed in the present experiments.

2. Trifluoperazine is an antioxidant [9, 11] and, inserted directly into the membrane it produces a membrane-stabilizing effect. In this way it can depress activation of LPO, membrane damage, and cell depolarization, which is usually observed during the action of catecholamines [3]. As a result, arrhythmias also are prevented.

3. Drugs of the phenothiazine series are known to interact directly with α-adrenoreceptors, blocking them [5]. This mechanism probably also may lie at the basis of the cardioprotective and, in particular, the antiarrhythmic effect of trifluoperazine during the action of toxic doses of catecholamines.

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# EFFECT OF SODIUM HYDROXYBUTYRATE ON CATECHOLAMINE AND SEROTONIN LEVELS AND MONOAMINE OXIDASE ACTIVITY IN ALCOHOLICS

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Modulation of activity of the GABA-benzodiazepine receptor complex is not the only mechanism involved in realization of the anxiolytic effect. Drugs affecting the noradrenergic, dopaminergic, and serotoninergic systems of the brain also have an anxiolytic action [5, 10, 12]. Sodium hydroxybutyrate, the sodium salt of γ-hydroxybutyric acid (GHBA), is a GABA metabolite in brain tissue and gives GABA-positive effects [8]. Meanwhile GHBA causes accumulation of noradrenalin, blocks the conduction of impulses along dopaminergic nerve fibers, accelerates serotonin metabolism, and inhibits monoamine oxidase (MAO) activity in the CNS [7, 13-15]. The close similarity of the chemical structures of sodium hydroxybutyrate and GHBA suggests that the mechanism of action of this compound is based on the effects of GHBA. Sodium hydroxybutyrate has been used in the treatment of drug addiction as a substance to abolish addiction to alcohol and to treat the alcohol withdrawal syndrome [1]. The aim of this investigation was to study the psychotropic effect of sodium hydroxybutyrate and to compare it with its effect on plasma adrenalin (A), dopamine (DA), noradrenalin (NA), and serotonin (5-HT) levels and on platelet MAO activity in alcoholics.

## EXPERIMENTAL METHOD

The subjects were 36 patients with chronic alcoholism in stage II (average age 35.1 ± 6.1 years) 2 weeks after abolition of the alcohol withdrawal syndrome. During the 10 days before the investigation the patients received no medication. A mean single dose of 2 g (40 ml of a 5% sugar syrup solution) was used as the test dose of sodium hydroxybutyrate. The psychotropic effect of the drug was evaluated by a clinical-descriptive method and recorded on a 4-point psychometric scale: 0) no effect, 1) weak effect, 2) moderate effect, 3) strong effect. Plasma levels of A, NA, DA, and 5-HT and platelet MAO type B activity (MAO-B) were

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