THE ANTIARRHYTHMIC EFFECT OF RYANODINE IN GUINEA PIGS WITH GLYCOSIDE POISONING

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Many forms of rhythm disturbances in pathological states such as ischemia, glycoside poisoning, and hypokaliemia, have been shown to arise through calcium overloading. An increase in the intracellular calcium ion concentration $[Ca^{++}]_i$ leads to diastolic waves of membrane potential (MP) which, on reaching the threshold level, induce extra-excitation of the heart [10]. The pharmacotherapy of these forms of arrhythmias is thus aimed at reducing the Ca^{++} inflow into the cells, by the use of blockers of the inward Ca and Na currents. At the same time, we know that diastolic fluctuations of MP and of myocardial tone in calcium overloading are based on oscillations of $[Ca^{++}]_i$, the source of which is the sarcoplasmic reticulum (SR) [2, 14]. It is therefore interesting to study the antiarrhythmic properties of preparations which modify SR function. The list of these preparations includes caffeine and ryanodine. We know that caffeine exhausts the calcium reserves in SR [4]. There are data in the literature on the blocking action of caffeine on oscillations of $[Ca^{++}]_i$, and on fluctuations of MP and myocardial tone in calcium overloading [3, 7]. However, it is impossible to study the action of caffeine in animals in vivo because its antiarrhythmic activity is exhibited only if present in high concentrations (over 3 mM). All the available physiological data indicate that the main site of action of ryanodine is also SR [2, 3, 8, 13]. Unlike caffeine, ryanodine exhibits its antiarrhythmic properties in nanomolar concentrations [13], as has been demonstrated in experiments in vivo [11]. It must be pointed out that so far an antiarrhythmic action of ryanodine in vivo has been obtained only in experiments on dogs [11], and in vitro, on guinea pigs and calves [13]. It was therefore decided to study the antiarrhythmic activity of ryanodine in vitro and in vivo on the same object.

In the investigation described below the action of ryanodine was studied on diastolic fluctuations on MP and tone of the papillary muscle of guinea pigs (in vitro) and on ventricular arrhythmias induced in guinea pigs by glucoside poisoning (in vivo).

EXPERIMENTAL METHOD

Action potentials (AP) and contractions were recorded in the papillary muscles isolated from the right ventricle of the guinea pig heart. The average length of the isolated papillary muscles was 3 mm and their diameter 0.6 mm. The isolated muscle was placed in a perfusion chamber and attached by means of a Kapron thread to a 6MKh1B mechanotron. The preliminary load on the muscle was 1 g/mm^2. The preparation was perfused with oxygenated (100% O_2) tyrode solution (35°C, pH 7.4) of the following composition (in mM): NaCl 150, KCl 4.0, $MgCl_2$ 0.5, Tris-HCl 25, $CaCl_2$ 2.5, glucose 10. Electrical activity was recorded by the standard microelectrode technique. Before the experiment began the muscle was adapted for 1.5-2 h to stimulation with a frequency of 1 Hz.

The standard bipolar ECG (leads I and II) was recorded from the body surface of anesthetized (pentobarbital, 40 µg/kg) guinea pigs weighing 300-400 g by means of 4 needle electrodes inserted into the limbs. The ECG was recorded on paper on a Mingograf-34 (Siemens). Glycoside poisoning of the animals was obtained by injecting ouabain from a microdosimeter into the jugular vein at the rate of 15-20 µg/kg body weight in 10 min until the appearance of

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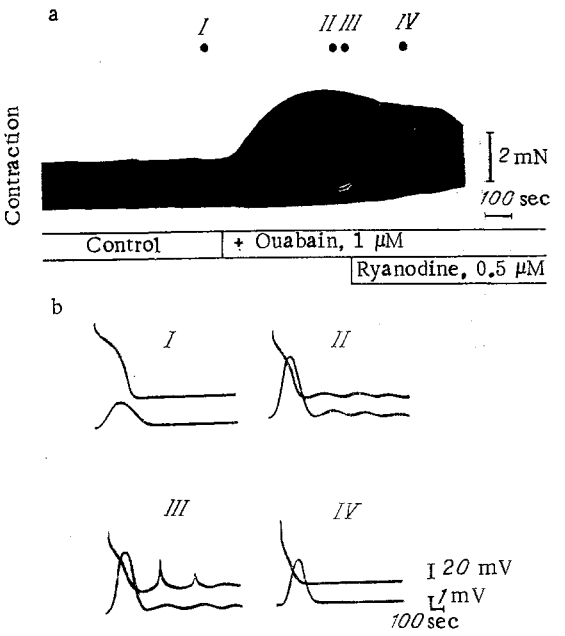

Fig. 1. Action of ryanodine on oscillations of MP and tone of guinea pig papillary muscle in ouabain poisoning. a) changes in force of contraction; b) AP and force of contraction recorded at times indicated in Fig. 1a by dots I, II, III, and IV.

stable ventricular arrhythmia. The final concentration of ouabain at which stable ventricular arrhythmia appeared varied in different experiments from 75 to 115 μg/kg. A preliminary tracheotomy was performed on the guinea pigs and they were connected to an artificial respiration apparatus.

EXPERIMENTAL RESULTS

The time course of the positive inotropic effect of ouabain is illustrated in Fig. 1a. The greatest increase in the force of contraction of the guinea pig papillary muscle under the influence of ouabain (1 μM) developed after 5-7 min and amounted on average to 133 ± 18% (n = 6). Later the force of contraction began to fall. Addition of ryanodine (0.5 μM) to the solution only delayed its fall initially, or actually increased the force of contraction (3 experiments), but later this continued to fall, against the background of increasing contracture. During the decrease in the force of contraction induced by ouabain, after-fluctuations of MP and tone appeared. AP and contractions at times indicated in Fig. 1a by the dots I, II, III, and IV are illustrated in Fig. 1b. The action of ouabain also caused a very small reduction in amplitude of the AP plateau and it shortened the duration of AP (Fig. 1b, II). Diastolic fluctuations of MP in different experiments varied from 5 to 10 mV and led to the development of autorhythmic activity, if ryanodine did not act on the preparation. On the addition of ryanodine to the solution after-fluctuations of MP and tone disappeared after 3-5 min and the duration of AP was shortened even more (Fig. 1b, III, IV). Under control conditions both AP and the force of contraction of the papillary muscle of the guinea pigs remained virtually unchanged under the influence of ryanodine (0.5 μM). Only some slight slowing of relaxation was observed.

Depression of the diastolic fluctuations of MP by ryanodine is evidence that this substance can abolish rhythm disturbances associated with calcium overloading. The action of ryanodine on ventricular arrhythmias was therefore studied in the next series of experiments (8 experiments) on guinea pigs with glycoside poisoning in vivo. In these experiments ryanodine was used in a concentration of 15 μg/kg, which corresponds to its concentration (0.5 μM) in the previous experiments on the guinea pig papillary muscle.

After intravenous injection of ouabain dispersion of the R-R intervals increased at first, but later, with ouabain in a concentration of 60-70 μg/kg, single ventricular extrasystoles appeared. As administration of ouabain continued, stable ventricular arrhythmia developed. The results of one typical experiment are given in Fig. 2. Ventricular arrhythmia appeared in the guinea pig in this case in response to ouabain in a concentration of 90 μg/kg. Injection

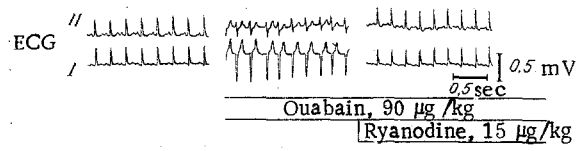

Fig. 2. Action of ryanodine on ventricular arrhythmia induced in a guinea pig by ouabain poisoning.

of ryanodine under these conditions abolished the ventricular arrhythmia afer 4-5 min, and restored the sinus rhythm after 8-10 min (Fig. 2c); the latter remained virtually unchanged during the next 40-50 min.

Similar results were obtained previously in experiments on anesthetized dogs in which ryanodine (2.4-18.6 μg/kg) abolished ventricular arrhythmias induced by digoxin poisoning [11].

The data described in this paper show that the antiarrhythmic effect of ryanodine in guinea pigs with glycoside poisoning is due to depression of diastolic fluctuations of MP arising as a result of calcium overloading. The blocking action of ryanodine in a concentration of 0.1-1 μM on after-fluctuations of MP and tone was observed previously [13] in experiments on the guinea pig papillary muscle under conditions of calcium overloading induced by a potassium-free solution. Under control conditions, however, just as in the present experiments, ryanodine did not change the parameters of AP. What is the mechanism which lies at the basis of the blocking action of ryanodine on MP fluctuations? As has already been stated, depression of diastolic fluctuations of MP by ryanodine was accompanied by an increase of contracture (Fig. 1). This may be evidence that ryanodine does not prevent overloading of the cells with Ca^{++}. It was in fact shown in [13] that ryanodine does not affect the slow inward current of calf Purkinje fibers. This substance probably has no direct action on ionic conductivity of guinea pig myocardial cells, for it virtually does not change the parameters of AP under control conditions. Shortening of the duration of AP induced by ouabain, and continuing on the addition of ryanodine to the solution (Fig. 1b), was evidently due to the outward current, activated by a raised $[Ca^{++}]_i$ level.

The main site of action of ryanodine, it is considered nowadays, is SR [6, 12]. The authors cited concluded from their physiological investigations that ryanodine, in nanomolar concentrations, blocks Ca^{++} release from SR. However, during the direct action of ryanodine on isolated vesicles of SR, the latter were not sensitive to such low concentrations of the drug. The blocking action of ryanodine on Ca^{++}-induced Ca^{++} release from vesicles of SR is exhibited in a concentration two orders of magnitude higher than that used in the physiological experiments [5]. The reason for these differences is that SR are most sensitive to ryanodine at the points of its contact with the T-system of the sarcolemma and it is important to take this into account when fractions of SR are isolated [12]. Investigations have shown that ryanodine abolishes oscillations of $[Ca^{++}]_i$ recorded with the aid of the Ca-sensitive protein aequorin [2, 14], and reduces the amplitude of mechanical noise reflecting SR activity [1].

Oscillations of $[Ca^{++}]_i$ have been shown to determine the inotropic state of the heart muscle, and an increase in their amplitude is the cause of the decrease in the force of contraction during calcium overloading [14]. Depression of oscillations of $[Ca^{++}]_i$ by ryanodine may therefore cause a brief positive inotropic effect, which was found in the present investigation.

Data in the literature point to an important role of SR in the mechanism of the autorhythmic activity of heart muscle [9]. In the opinion of the author cited, the spontaneous rhythm in partially depolarized human right atrial myocardium is determined by diastolic depolarization, which is largely controlled by SR, since the action of ryanodine (3 μM) depresses the spontaneous rhythm by 33%.

The data obtained in this investigation indicate that ryanodine can be used as a tool to analyze the mechanisms lying at the basis of cardiac arrhythmias in various pathological states accompanied by calcium overloading.

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EFFECT OF LITHIUM HYDROXYBUTYRATE ON THE PARAMETERS OF ACUTE INFLAMMATION

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Lithium hydroxybutyrate, which is used in the treatment of several pathological conditions (shock, diseases of the nervous and cardiovascular systems, allergic reactions), attracts increasing attention [2]. The aim of this investigation was to study some models of acute inflammation and the effect of lithium hydroxybutyrate on them.

EXPERIMENTAL METHOD

Experiments were carried out on 81 male Wistar rats weighing 120-150 g. Lithium hydroxybutyrate, dissolved in isotonic sodium chloride solution, was injected subcutaneously into the dorsal region of the animals in a dose of 200 mg/kg, 30 min before administration of the inflammation-inducing agents. As one model of inflammation peritonitis developing in the animals 3 h after intraperitoneal injection of 1 ml of a 0.1% aqueous solution of silver nitrate was used. The animals were then decapitated and the volume of fluid in the peritoneal cavity was determined. The mesentery was fixed with 96° alcohol, stained with 0.05% toluidine blue, and the number of degranulated mast cells was counted among 100 cells in the preparation. Acute edema of the rats' hind limbs after subplantar injection of 0.1 ml of an aqueous solution of histamine ($1 \cdot 10^{-7}$ g/ml), serotonin ($1 \cdot 10^{-7}$ g/ml), or prostaglandin E_2 (PGE_2; from Upjohn Ltd., England) — which, in a concentration of 10^{-6} g/ml, can also induce an acute inflammatory reaction [4] — was used as the other models of inflammation. Control animals were given an injection of 0.1 ml of distilled water. The volume of the limbs was measured 1 h after injection of the phlogogene in a measuring cylinder, the skin temperature of the limb was measured with an electrothermometer (TPEM-1), and sensitivity to pain was measured with an "Analgesymeter for the rat paw" (Italy). The results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

In the experiments of series I 3 h after injection of silver nitrate into the peritoneal cavity of the animals a reddish brown fluid appeared (diapedesis of erythrocytes) and total degranulation of the mast cells occurred in the mesentery (Table 1).

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