TWO PHASES OF THE INOTROPIC ACTION OF ADRENALIN AND ITS CALCIUM DEPENDENCE

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Two phases of the action of adrenalin on vertebrate heart muscle are known. The first phase of enhancement of the contractile properties of the myocardium is due to an increase in the calcium inflow into myocardial cells along slow sodium-potassium channels [12] and an increase in the quantity of calcium exchanged between the sarcoplasmic reticulum and myoplasm during contraction and relaxation of the muscle [10]. During prolonged profusion of heart muscle with adrenalin solution the first phase is gradually replaced by a second phase — inhibition of myocardial contractions, despite the presence of adrenalin in the perfusion solution [4]. The mechanism of this second phase of the action of adrenalin on the myocardium has hardly been studied at all. There is information that during prolonged perfusion of heart muscle with adrenalin, products of tissue metabolism are eliminated from the myocardium and modify the sensitivity of adrenoreceptors to adrenalin and the contractile properties of the myocardium [4].

The object of the present investigation was to study the biphasic action of adrenalin during prolonged (30 min) application and during a change in the intensity of inflow of Ca^{++} ions into the cell by changing the frequency of stimulation of the preparation, changing the external Ca^{++} concentration, and changing the adrenalin concentration.

EXPERIMENTAL METHOD

Experiments were carried out on a strip of the ventricle from *Rana temporaria* with perfusion with a continuous flow of Ringer's solution of the following composition (in mM): NaCl 110, KCl 2.5, NaH_2PO_4 0.08, $NaHCO_3$ 2.4, $CaCl_2$ 1.8, pH 7.64. The strips were stimulated by square pulses of current 5 msec in duration, of twice the threshold strength, and with a frequency of 0.1-1.0 Hz. Contractions under near-isometric conditions were recorded by means of a 6MKh1S mechanotron transducer on an N-306 xy recorder.

The stimulating effect of adrenalin on the heart was estimated as a percentage of the amplitude of the contractions before the beginning of perfusion with adrenalin. Inhibition of contractions during the second phase of action of adrenalin was estimated as a percentage of the maximal cardiostimulating effect. Each series consisted of 30 experiments (the curves in Figs. 1-3 represent mean results of 10 experiments).

EXPERIMENTAL RESULTS

In the experiments of series I the character of development of the cardiostimulating effect and the phase of inhibition of contractions in the presence of different concentrations of adrenalin were studied. It will be clear from Fig. 1 that at a frequency of stimulation of 0.25 Hz and in a Ca^{++} concentration of 1.8 mM, the maximal cardiostimulating effect of adrenalin (phase I) developed in the course of 3-5 min, and this was followed by a gradual fall in the strength of contractions (phase II). With an increase in the adrenalin concentration both the first and the second phases became more marked.

In the experiments of series II the effect of the frequency of stimulation on the inotropic effects of adrenalin was investigated. As Fig. 2A shows, the cardiostimulating action

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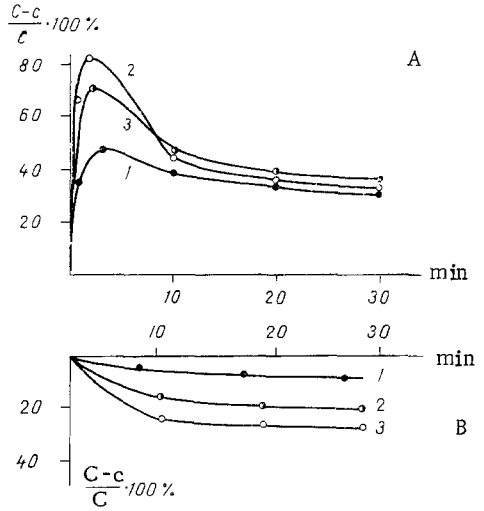

Fig. 1. Effect of different concentrations of adrenalin on frog myocardium. A—Development of inotropic effect in time. Ordinate: C) amplitude of contractions of strip during the action of adrenalin, c) amplitude of contractions before administration of adrenalin. B—Development of phase of decline of response to adrenalin. Ordinate: C) maximal amplitude of contractions under the influence of adrenalin, c) amplitude of contractions during phase of decline. Abscissa in A and B, time (in min). 1) Adrenalin 5×10^{-7} g/ml, 2) adrenalin 1×10^{-6} g/ml, 3) adrenalin 2×10^{-6} g/ml. Frequency of stimulation 0.25 Hz; Ca^{++} = 1.8 mM.

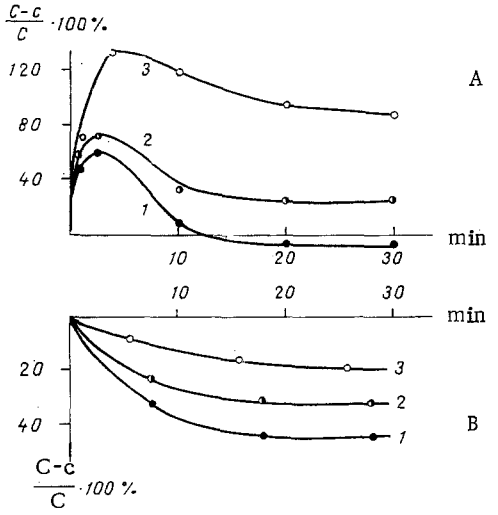

Fig. 2. Inotropic effect of adrenalin (2×10^{-2} g/ml) at different frequencies of stimulation of preparation. 1) 1 Hz, 2) 0.5 Hz, 3) 0.1 Hz. Ca^{++} = 1.8 mM. Remainder of legend as to Fig. 1.

of adrenalin was intensified with a decrease in the frequency of stimulation, and when the frequency reached 0.1 Hz it remained virtually unchanged throughout the period of subsequent perfusion with adrenalin, i.e., phase II was reduced or even disappeared completely. At high frequencies of stimulation (1.0 Hz) the cardiostimulating effect was quickly replaced by inhibition of the force of contractions, and under these circumstances the amplitude of contractions actually became less than before the beginning of perfusion with adrenalin.

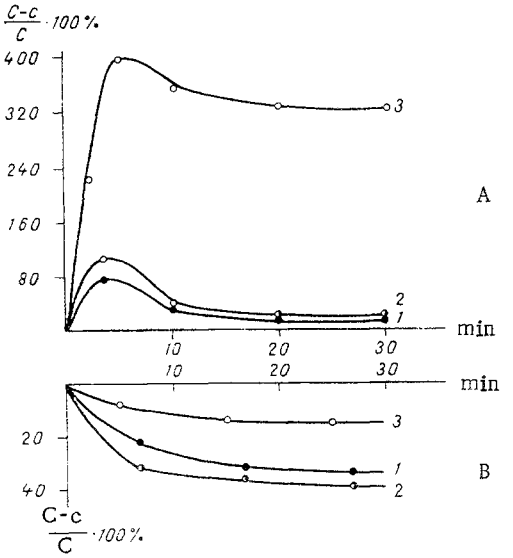

Fig. 3. Inotropic effect of adrenalin (2×10^{-6} g/ml) with different Ca^{++} concentrations in external medium. 1) Ca^{++} 1.8 mM, 2) 8.0 mM, 3) 0.5 mM. Frequency of stimulation 0.5 Hz. Remainder of legend as to Fig. 1.

In the experiments of series III the effect of a change in the external Ca^{++} concentration on development of the cardiostimulating action of adrenalin was investigated in the phase of inhibition of contractions (Fig. 3). The results showed that a decrease in the external Ca^{++} concentration caused a marked increase in the positive inotropic effect of adrenalin and the virtually complete disappearance of phase II. This last phase was significantly enhanced when the external Ca^{++} concentration was increased to 8.0 mM (Fig. 3A, B).

The magnitude of phase II thus depends directly on the quantity of Ca^{++} entering the myocardial cell, for with an increase in the intracellular Ca^{++} content produced by increasing the adrenalin concentration, the frequency of stimulation, or the external Ca^{++} concentration, the phase of inhibition of contractions became more marked.

On the basis of the data on calcium dependence of the biphasic effect of adrenalin three hypotheses can be put forward on mechanisms responsible for the development of phase II: 1) phase II is the result of desensitization of β-adrenoreceptors. The calcium dependence of desensitization has been demonstrated for acetylcholine receptors of the end-plate of skeletal muscles [3, 9] and has been suggested for the β-adrenoreceptors of the myocardium on the basis of an investigation of the role of calmodulin in regulation of the activity of the adenylate cyclase system [5]; 2) phase II is the result of activation of processes responsible for the removal of Ca^{++} from the myoplasm. It has been shown that adrenalin activates two processes at once: the inward flow of Ca^{++} along slow sodium-potassium channels and its outflow from the myoplasm [6-11]. It can be tentatively suggested that the first process predominates during the first 3-5 min of action of adrenalin, but later the second process predominates. A change in the rate of inflow of Ca^{++} is bound to change the relationship between these processes; 3) phase II is due to a decrease in the calcium conductance of the surface membrane on account of an increase in the intracellular Ca^{++} concentration. A similar mechanism of blocking of calcium conductance of the surface membrane with an increase in the intracellular Ca^{++} concentration has been demonstrated for skeletal muscle [8] and molluscan neurons [1, 2] and has been postulated in the mammalian myocardium [7].

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AN IMPROVED APPARATUS FOR VENOUS OCCLUSION PLETHYSMOGRAPHY

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KEY WORDS: venous occlusion plethysmography.

The method of venous occlusion plethysmography (VOP) is finding ever widening application in both physiological and clinical research [1, 2, 6]. The principle of the method is recording changes in the volume of a segment of the limb when the outflow of blood from the limb is blocked by compressing the veins with a pneumatic cuff. The cuff is inflated under a sufficient pressure to obstruct the veins completely but, at the same time, not to prevent the flow of arterial blood into the test segment. It has been shown [4, 5] that these demands are met by a pressure of about 50 mm Hg.

In the investigation described below the VOP method was used in the modification in [7]. Mercury–rubber transducers were mounted on the test limb. The electrical resistance of the transducer, proportional to changes in its length, was connected into one arm of a Wheatstone bridge. After amplification, the signal thus obtained was recorded by an automatic writer (N327-3).

It was considered useful to have not only a graph showing the change in volume of the limb segment, but also graphs of the rate of change of volume and of a logarithm of this value relative to time in order to study the time course of processes taking place during application (and, correspondingly, removal) of occlusion. For this purpose, the plethysmograph curve itself is recorded in the first channel of a three-channel automatic writer. The signal from the plethysmograph after passage through a signal differentiator (an operational amplifier was used), built to the circuit in [3], is led to the second channel of the automatic writer. The third channel records the signal from the plethysmograph of the passage through a logarithmic signal amplifier [3]. The circuit connecting the plethysmograph (from Loosco, The Netherlands), differentiator, and logarithmic signal amplifier to the three-channel automatic writer is illustrated in Fig. 1.

The apparatus described above, because it uses a differentiator (the first derivative of the change in volume of the limb segment in time is recorded), can determine more accurately and demonstratively the rates of change of limb volume (the rate of the arterial inflow when occlusion is applied and the rate of venous outflow when the occlusion is removed). The logarithmic device was introduced for the following reasons. The venous outflow curve is closely similar to a monoexponential curve or to a combination of two consecutive curves.

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