The Effects of an Extra-stimulation on Post-extra-systolic Potentiation in Papillary Muscle of Rats

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SUMMARY

The mechanism of post-extrasystolic potentiation (PESP) is unclear. It has previously been suggested that changes in both the calcium release from the sarcoplasmic reticulum (SR) and the transsarcolemmal calcium influx are factors in the development of PESP in guinea-pigs. This experiment investigated the effects of a resting interval and a coupling interval on PESP in rat papillary muscles, which have a well-developed SR. An extra-stimulation was induced at various coupling intervals and a variety of post-extrasystolic intervals were set. The PESP was not dependent on the coupling interval. The post-extrasystolic interval at which the maximal % PESP was obtained was about 90 sec, and post-extrasystolic interval-% potentiation of the PESP relationship curve consisted of an ascending limb and a descending limb. Caffeine eliminated the PESP in a concentration-dependent manner. These findings suggest that SR calcium release plays an important role in the mechanism of PESP in rats. This is consistent with results from guinea-pigs, and implies that the calcium capacity and/or retention of the SR may characterize the post-extrasystolic interval-% PESP relationship in muscle from different species.

Additional Indexing Words:
Post-extrasystolic potentiation Calcium Sarcoplasmic reticulum Caffeine

It is well known that extra-stimulation induces a transient augmentation of subsequent twitch tension (post-extrasystolic potentiation; PESP).\textsuperscript{1,2} Recently, it was reported that theophylline and caffeine, which suppress the sarcoplasmic reticulum (SR), removed the dependence of PESP both on the coupling interval of an extra-stimulation and on the post-extrasystolic inter-

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Received for publication June 28, 1989.
Accepted November 8, 1989.
val in papillary muscles of guinea-pigs. These data suggest that SR calcium release is very important in the development of PESP in guinea-pigs. However, Bers reported that there is a species difference in the relative contribution of the SR to excitation-contraction coupling, such that the SR may be more important in the development of contraction in the rat ventricle than in the guinea-pig ventricle. The object of this study was to use caffeine as a tool to investigate the role of the SR in the generation of PESP in rat papillary muscles, and to compare the findings in rats with our previous findings in guinea-pigs.

**METHODS**

Male and female Wistar rats weighing about 200 g were used. The heart was quickly removed under urethane anesthesia (100 mg/100 g, body weight), and placed in normal physiologic saline containing 115.3 mM NaCl, 2.5 mM KCl, 1.55 mM Na₂HPO₄, 1.55 mM NaH₂PO₄, 1.8 mM CaCl₂, and 0.013 mM tubocurarine chloride. After the papillary muscles (1–2 mm in diameter) were removed from the right ventricles under a microscope, they were mounted in a 2-ml chamber with one end fixed and the other end connected to an isometric tension transducer (MEC, Isometric amplifier, model ME-4021) with surgical silk. Muscles were stretched to 1.2 times their resting lengths and isometric tension (T) was recorded (National pen recorder). The control perfusate was a modified Tyrode's solution containing 136.9 mM NaCl, 4.0 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.33 mM NaH₂PO₄, 2.44 mM Na₂HPO₄, and 5.5 mM glucose. This solution had been prewarmed to 30°C and bubbled with 100% O₂ before entering the recorder chamber. Muscles were stimulated by passing a voltage of 5V amplitude and 2 msec duration; they were driven at a basic stimulating interval of 1500 msec for 30 min to obtain steady-state twitch tensions.

After equilibration of the twitch tension for 30 min, an extra-stimulation was imposed after the train of 40 regular stimuli. The post Extrasystolic interval was set at either 10 sec, 30 sec, 60 sec, 90 sec, 3 min, 5 min, or 10 min. To examine the influence of an extra-stimulation on PESP, two coupling ratios (coupling interval/to basic stimulating interval) of 0.67 and 0.99 were chosen to represent early and late prematurity of extra-stimulation. Furthermore, the coupling interval was increased from 500 to 1499 msec every 100 msec to examine whether the prematurity of extra-stimulation depends on PESP. The influence of the stimulation frequency on PESP was compared between the basic stimulating intervals of 1500 and 3000 msec. In addition 1 mM and 5 mM caffeine was applied to investigate the effect of caffeine on PESP.
Caffeine was allowed to act for at least 30 min prior to an experiment to assess the effects on PESP. All data were analyzed statistically using Student’s t-test.

RESULTS

The magnitude of PESP with coupling ratios of 0.67 and 0.99 are shown in Fig. 1. The magnitude of PESP varied with the prolongation of a post-extrasystolic interval. The post-extrasystolic interval-% PESP relationship curve consisted of an ascending limb and a descending limb (Fig. 2). The maximal % potentiation was obtained at a 90 sec post-extrasystolic interval. When the post-extrasystolic interval was equal, the magnitude of PSEP did not vary with the coupling ratio. The influence of the prematurity of extra-stimulation on PESP is shown in Fig. 3. The basic stimulating interval was 1500 msec and the post-extrasystolic interval was 90 sec. The magnitude of PESP was invariant across coupling intervals.

The influence of the basic stimulating interval on the post-extrasystolic interval-% PESP relationship curve is shown in Fig. 4. The magnitude of PESP was smaller at a basic stimulating interval of 3000 msec than at 1500 msec for an identical post-extrasystolic interval. Thus, the post-extrasystolic interval-% PESP relationship tended to be shifted downward by the prolongation of a basic stimulating interval, but this shift was not significant.

The effects of caffeine on regular contractions and PESP are shown in Fig. 5. After 1 mM caffeine was applied, the PESP decreased gradually, but the magnitude of regular contractions did not change. The influence

Fig. 1. Influences of post-extrasystolic intervals of 10 sec, 30 sec, 60 sec, and 90 sec and coupling ratios of 0.67 and 0.99 on PESP. Recordings are shown from one papillary muscle driven at a basic stimulus interval of 1500 msec. Although the magnitude of PESP varied with the post-extrasystolic interval, the magnitude of PESP did not vary with the coupling ratios when the post-extrasystolic intervals were identical.
Fig. 2. The post-extrasystolic interval-% PESP curves with coupling ratios of 0.67(○) and 0.99(●) at a basic stimulating interval of 1500 msec. These curves consist of an ascending limb and a descending limb. The maximal % PESP developed at a post-extrasystolic interval of 90 sec. All points represent the mean of 6 experiments; the vertical bars indicate SE. The difference between the curves with a coupling ratio of 0.67 and that of 0.99 was not statistically significant.

Fig. 3. Influence of coupling intervals on PESP. The coupling interval was increased from 500 msec to 1499 msec in 100 msec increments. The basic stimulation interval was 1500 msec. All points represent the mean of 5 experiments; the vertical bars indicate SE. There was no significant difference in the magnitude of PESP between coupling ratios.
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Fig. 4. Influence of basic stimulation intervals on PESP. The post-extrasystolic interval-% PESP curves at basic stimulating intervals of 1500 msec (○) and 3000 msec (□) are shown. All points represent the mean of 5 experiments; the vertical bars indicate SE. The curves for the two stimulation intervals did not differ significantly.

Fig. 5. Effects of 1 mM caffeine on steady-state twitch tension and PESP. After the administration of caffeine, the PESP decreased gradually to less than the steady-state twitch tension. The muscle was driven at a basic stimulation interval of 1500 msec and an extra-stimulation was imposed with a coupling ratio of 0.67.

Of 1 mM and 5 mM caffeine on the post-extrasystolic interval-% PESP relationship is shown in Fig. 6. Both doses of caffeine blocked PESP in a concentration-dependent manner. In the case of 5 mM caffeine application, the maximum PESP was seen at the shortest post-extrasystolic interval and % potentiation decreased gradually as a post-extrasystolic interval was prolonged. In the case of 1 mM caffeine application, the maximal % potentiation was obtained at a 30 sec post-extrasystolic interval.
Fig. 6. Effects of 1 mM (∆) and 5 mM (▲) caffeine on the post-extrasystolic interval-% PESP relationship. The basic stimulation interval was 1500 msec and an extra-stimulation was imposed at a coupling ratio of 0.67. Caffeine significantly eliminated the PESP in a concentration-dependent manner. All points represent the mean of 5 experiments; the vertical bars indicate SE.

**DISCUSSION**

In this study, the post-extrasystolic interval-% PESP relationship consisted of an ascending limb and a descending limb. Although the general shape of these curves is consistent with our previous results at 30°C in guinea-pigs,²,³ the rat data differ quantitatively from the results in guinea-pigs.³ In guinea-pigs, the maximum % potentiation appeared at a post-extrasystolic interval of 3 to 4 sec, while the maximum % potentiation was obtained at 90 sec in rats. Furthermore, the slope of the descending limb is much steeper in guinea-pigs.

It is also of great interest that PESP is independent of the coupling interval in rats. This contrasts with the dependence of PESP on the coupling interval in guinea-pigs.³ Since PESP at an extra-stimulation of coupling ratio of 0.99 is regarded as the absence of extra-stimulation, it is clear that the magnitude of PESP does not depend on extra-stimulation in rats.

The post-extrasystolic interval at which the maximal potentiation develops is called the optimal interval.⁶ The optimal interval appears to vary with species and experimental conditions. In the present study, the optimal interval for rat papillary muscle was 90 sec. It was reported at 35 sec for ferret ventricular muscle paced at 1 Hz at 37°C⁷ and 120 sec for canine ventricular muscle paced 0.5 Hz at 37°C.⁸ On the other hand, a 20 sec optimal in-
interval was reported for cat ventricular papillary muscle paced at 1.3 Hz at 30°C. For guinea-pig ventricular muscle, the 2 sec optimal interval appeared at a basic stimulating interval of 1500 msec and increased to 10 sec at a basic stimulating interval of 3000 msec at 30°C. Thus, the optimal intervals may differ among the species, stimulating frequency, and temperature.

Winegrad described conflicting opinions on the period which is required for the calcium movement in the SR, with estimates ranging from less than 1 sec to several seconds. Calcium fluxes in the SR would be one of the determining factors of the optimal interval. One possibility, then, is that calcium movement in the SR of rats is slow compared with that of guinea-pigs.

It has been reported that the magnitude of PESP depends on the prematurity of an extrasystole in guinea-pigs and human beings; the shorter the prematurity, the larger the potentiation. We reported that the magnitude of PESP depends on the coupling ratio of extra-stimulation in guinea-pigs. However, in rats, there was no significant difference in the magnitude of PESP among any coupling ratio. The basis for this species difference is unclear.

When a basic stimulating interval was shortened, the maximal % PESP increased and the optimal interval shortened in canine ventricular muscles at 37°C. We also reported a similar result using guinea-pig muscle at 30°C. In this experiment, though, the post-extrasystolic interval-% PESP curve for rat muscle was not affected significantly by prolongation of a basic stimulating interval. The present study was not performed at a physiological temperature (37°C). However, we suspect in these findings that there is no fundamental difference in the post-extrasystolic interval-% PESP relationship between 30°C and 37°C since these phenomena are based on the recycling time of calcium in the SR and intracellular calcium kinetics.

It is known that caffeine inhibits SR function by decreasing calcium uptake by the SR and increasing calcium release from the SR. Caffeine abolished the dependence of PESP on the post-extrasystolic interval in a concentration-dependent manner. The magnitude of PESP in the absence of caffeine is supported by the combination of calcium influx and calcium release from the SR. It is reasonable to conclude that the difference in the magnitude of PESP after caffeine application represents an estimate of the relative contribution of calcium release from the SR. If this is the case, the contribution of calcium release from the SR to PESP must be greater than the contribution of the calcium influx in rats. The relative contributions of calcium release from the SR and calcium influx to the maximal PESP was estimated as a ratio of two to one from Fig. 6. This is consistent with
reports that the relative contribution of calcium influx to tension development is as follows: frog>toad>fetal>rabbit>guinea-pig>cat>dog>ferret>rat.51,17)-19)

Although the magnitude of PESP disappeared at a post-extrasystolic interval of 3 min in the guinea-pigs,3) the decrease in PESP with the prolongation of post-extrasystolic interval was small in rats. This decrease in PESP with the prolongation of a post-extrasystolic interval is believed to be due to the transmembrane calcium extrusion20) from the SR and cytosol. Since the slope of the descending limbs of post-extrasystolic interval-% PESP relationships are different in rats and in guinea-pigs, calcium capacity and/or retention of the SR may be better developed in rats.

Fabiato21) reported that the activation and inactivation of calcium-induced calcium release from SR are dependent on both time and calcium concentration. The optimal interval is the post-extrasystolic interval at which the calcium release from the terminal cisternae of SR is maximal. This implies that relative contributions of calcium release from the SR (which is probably influenced by the calcium movement in the SR10),11),22)) the activation-inactivation status of the SR calcium releasing mechanism,21) the transsarcolemmal calcium influx and calcium extrusion are determinants of the post-extrasystolic interval-% PESP curve, and that the magnitude of PESP was mainly based on calcium release from the SR. We suspect that the characteristics of SR in rat papillary muscle may produce the typical post-extrasystolic interval-% potentiation curves and the independence of PESP from the coupling interval in this species.

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