SUPPRESSING EFFECTS OF CAFFEINE ON POSTEXTRASYSTOLIC
POTENTIATION IN PAPILLARY MUSCLES OF GUINEA PIGS

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It is well known that the strength of postextrasystolic potentiation (PESP) is
dependent on the prematurity of the ectopic beat, though the fundamental
mechanism of the potentiation is still obscure. In this study, the effect of a
resting interval on the strength of PESP was investigated in isolated papillary
muscles of guinea pigs in the presence or absence of caffeine, which inhibits
the functions of sarcoplasmic reticulum (SR). PESP of a fixed coupling
interval increased and then decreased as the resting interval was prolonged.
The maximum of PESP was obtained at a resting interval of 3 to 4 sec. The
dependency of PESP on a coupling interval was decreased considerably by
$5 \times 10^{-4}$M caffeine and removed completely by $10^{-2}$M caffeine. Although
$5 \times 10^{-4}$M caffeine decreased the degree of contraction of postextrasystole,
the maximum contraction of postextrasystole was still obtained at a resting
interval of 3 to 4 sec. After the application of $10^{-2}$M caffeine, the post-
extrasystolic contraction gradually declined as the resting interval was pro-
longed. We conclude that SR Ca release contributes largely to a mechanism
of PESP and increases in contribution as the coupling interval of an extra-
stimulation shortens, and that the optimal resting interval is determined by
a balance between the activity of SR function and the activity of the
sarcolemmal Ca extrusion mechanism.

POSTEXTRASYSTOLIC potentiation1 (PESP) is
a fundamental characteristic of the heart
and its strength is dependent on the prematurity
of the ectopic beat.2 PESP following sponta-
neous or induced premature beats has been
studied as a possible means of defining myo-
cardial contractile reserve,3 but the precise
mechanism of potentiation is still obscure. It
is generally accepted that both transsarcolemmal
Ca influx and sarcoplasmic reticulum (SR) Ca
release contribute to a contraction of cardiac
muscle though there is substantial variation in
the SR Ca release-to-Ca influx ratio in different
cardiac preparations.4,5 This study was designed
to examine the relation between SR Ca release
and PESP in isolated papillary muscles of guinea
pigs with the aid of caffeine, which suppresses
SR function via a mechanism of caffeine-induced
Ca release.6

METHODS

Now living guinea pigs weighing 200–300g
were used in this experiment. The right ven-
tricular papillary muscle with a diameter of
less than 1 mm and a length of more than 4 mm
was removed and mounted in the muscle bath
through which an oxygenated normal Tyrode

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solution containing (mM) NaCl 136.8, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaHCO₃ 11.9, glucose 6 at 30°C was superfused. The muscle was stretched to near 120% of rest length. One end was fixed and the other end was attached to a tension transducer (ME-4021; MEC). Muscles were field-stimulated with a 2-msec square wave of 2 times threshold through a bipolar electrode. Isometric force development was recorded. Steady-state tension development of papillary muscles, stimulated at a basic cycle length of 1500 msec, was reached after 30–60 min. Two pacing protocols were designed with a program stimulator (Cardiac stimulator BC-02A, Fukuda Denshi) as shown in Fig. 1. An extra-stimulation was added after trains of 40 regular pulses.

Protocol A consists of a fixed coupling interval followed by varying resting intervals. Protocol B, on the other hand, consists of varying coupling intervals followed by a fixed resting interval. After obtaining a baseline measurement, caffeine (Sigma) was added as a solid to the perfusate. Caffeine was allowed to act for more than 30 min before measurement of PESP in the presence of caffeine. First, the effects of either 5 × 10⁻⁴ M caffeine or 10⁻² M caffeine on PESP were measured precisely in 3 muscles respectively, using protocols A and B. Second, to make a statistical comparison between the effects of 5 × 10⁻⁴ M caffeine and 10⁻² M caffeine on potentiation, measurements were obtained sequentially at 4 resting intervals of 2, 3.5, 5, and 20 sec for each of the 2 coupling ratios of 500/1500 and 1300/1500 in each of the 7 muscle preparations. Coupling ratios of 500/1500 and 1300/1500 were chosen as representatives in early and late prematurity of an extra-stimulation.

Data were analysed statistically using a paired Student’s t-test. A p value of less than 0.05 was chosen to indicate statistical significance.

RESULTS

Figure 2 shows a representative record of tension after superfusion of the papillary muscle with solution containing either 5 × 10⁻⁴ M caffeine or 10⁻² M caffeine. Application of 5 × 10⁻⁴ M caffeine decreased PESP gradually while regular contractions almost maintained that of baseline strength (the mean ± SD: 88 ± 18%, N = 10). On the other hand 10⁻² M caffeine removed PESP quickly and completely, and increased the strength of regular contractions to about 5 times that of baseline strength (the mean ± SD: 496 ± 140%, N = 8).

Effects of a resting interval on PESP at baseline condition are shown in Fig. 3. At both coupling ratios of 500/1500 and 1300/1500 in the absence of caffeine, PESP of the fixed coupling ratio increased and then decreased as the resting interval was prolonged. PESP reached a maximum after a rest of 3 to 4 sec. The resting interval-potentiation curves shifted downward at the late prematurity of an extra-stimulation. While 5 × 10⁻⁴ M caffeine shifted the curves downward considerably under both 2 conditions of the prematurity, the maximum of PESP was still obtained at a resting interval of 3 to 4 sec.
Effects of Caffeine on PESP

Fig. 3. Representative examples of relationship between % potentiation and resting interval of protocol A in two muscles.
Open symbols: baseline, closed symbols: caffeine application (0.5 mM in Muscle 1 and 10 mM in Muscle 2), square: a coupling ratio of 500/1500, triangle: a coupling ratio of 1300/1500.

Fig. 4. Representative results of caffeine on coupling interval - % potentiation relation.
Open symbols: baseline, closed symbols: caffeine (0.5 mM in Muscle 3 and 10 mM in Muscle 4), square: at a coupling interval of 4 sec, triangle: at a resting interval of 15 sec.

On the other hand, $10^{-2}$ M caffeine removed the difference in potentiation between the 2 conditions of prematurity, and the maximum tension of postextrasystole was obtained at the shortest resting interval as can be seen in Fig. 3.

Effects of a coupling ratio on PESP at baseline condition and after caffeine application are shown in Fig. 4. At both resting intervals of 4 sec and 15 sec, the dependency of PESP on a coupling interval was obtained at baseline condition, in agreement with a previous observation. The coupling interval-potentiation curves shifted downward at the longer resting interval. Even after application of $5 \times 10^{-4}$ M caffeine, the

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dependency of PESP on a coupling interval was maintained while the maximum % potentiation was decreased considerably at the 2 different resting intervals, that is, the dependency of PESP on a coupling interval was decreased markedly by $5 \times 10^{-4}$ M caffeine. On the other hand, $10^{-2}$ M caffeine removed the dependency almost completely, that is, % potentiation was constant at any coupling interval in both resting intervals of 4 sec and of 15 sec. Of course, the % potentiation at the longer resting interval is less than that at the shorter one. Essentially, the same results were obtained in other muscle preparations at both concentrations of caffeine, respectively.

Figure 5 shows the statistical comparison between the effects of coupling ratios of 500/1500 and 1300/1500 on potentiation at 4 resting intervals of 2, 3.5, 5, 20 sec during 3 sequential treatments consisting of baseline condition, $5 \times 10^{-4}$ M caffeine, and $10^{-2}$ M caffeine application for each of the 7 muscle preparations. At treatments of baseline condition and at $5 \times 10^{-4}$ M caffeine application in 7 muscle preparations, the % potentiation at a postextrasystolic interval of 3.5 sec was bigger than those at the other 3 coupling intervals, and the % potentiation at a shorter coupling ratio was bigger than at a longer one. After application of $10^{-2}$ M caffeine, the % potentiation at the shorter resting interval was the biggest among the selected 4 resting intervals, and the % potentiation decreased as the resting interval prolonged. The significant difference of % potentiation between coupling ratios of 500/1500 and 1300/1500 disappeared.

**DISCUSSION**

Sleator et al. proposed that in isolated cardiac muscle preparations, several separate...
Fig. 6. Our hypothetical schema that SR calcium release contributes largely to a mechanism of PESP.
(B: increased Ca sensitivity)

"Ca pools" may contribute to a Ca transient or Ca that activates muscle contraction. Postrest contraction\textsuperscript{9,10} is the first contraction after a pause longer than the usual interval between beats and can be regarded as PESP of a coupling ratio of near 1.0\textsuperscript{11} In 1981 Temma et al\textsuperscript{12} reported that postrest contraction was apparently dependent on the rest interval and related ryanodine-sensitive, verapamil-insensitive Ca pool. In 1986 Malecot et al\textsuperscript{13} reported that milrinone split the twitch tension of ferret cardiac muscles into 2 components (P\textsubscript{1} and P\textsubscript{2}), and ryanodine and caffeine suppressed P\textsubscript{1} of postrest contraction while P\textsubscript{2} of postrest contraction was increased by Ca and decreased by cobalt. From these evidence P\textsubscript{1} is considered to be attributable to ryanodine-sensitive Ca pool, that is, SR Ca release, and P\textsubscript{2} is considered to be caused by verapamil-sensitive Ca pool, that is, transsarcolemmal Ca influx. In this study we investigated the relation between SR Ca release and PESP.

Although it is well known that caffeine affects the Ca transport in the SR,\textsuperscript{14} 10\textsuperscript{-4}M caffeine had no effects on both PESP and on regular contraction (Data were not shown.). After application of 5 \times 10\textsuperscript{-4}M caffeine, a transient increase in strength of regular contractions, thought to be due to the increase in intracellular Ca owing to an impairment of Ca reuptake by the SR, was observed. During the next 15 to 60 min, the increased strength returned to near baseline level, and was thought to be due to Ca loss into the extracellular space\textsuperscript{15,16} Immediately after application of 10\textsuperscript{-2}M caffeine, there was a rapid and marked increase in the strength of regular contractions. In the next 10 min, the regular contractions decreased and reached a steady-state of about 5 times that of baseline contractions. This decrease was thought to be due to a complete loss of SR Ca into the extracellular space and a sustained inhibitory activity of phosphodiesterase\textsuperscript{17} Then after 30 min of caffeine application, SR function is negligible. The dependency of PESP on either a coupling interval or a resting interval was decreased considerably or removed completely depending on the concentration of caffeine. These findings support the idea that PESP is dependent on Ca release from the SR and not on an increase in intracellular Ca owing to transsarcolemmal Ca influx, and that the SR Ca release increases in contribution as the coupling interval of extra-stimulation shortens.

As already reported\textsuperscript{11,18} we have found that at a fixed coupling interval, PESP increased and then decreased as the resting interval lengthened regardless of the prematurity of an extra-stimulation, that is, the curve of postextrasystolic interval-potentiation relationship of PESP consisted of an ascending limb and a descending limb. According to previous observations\textsuperscript{15,16} the decay of postrest contraction with longer rest intervals may reflect a fall in intracellular Ca owing to extrusion into the extracellular space. Thus, the decay of PESP under baseline condition and caffeine application was considered to be due to the extrusion of intracellular Ca. In 1985 Kerker et al\textsuperscript{19} proposed that PESP de-
pended on an enhanced Ca shift within the SR, and in 1986 Capogrossi et al.\textsuperscript{20} reported that the reloading of releasable SR Ca pool was time-dependent. Furthermore, Fabiato\textsuperscript{21} reported on the time and calcium dependency of activation and inactivation of calcium-induced release of calcium from the SR. It is then reasonable to postulate that the time required for obtaining the maximal augmentation of PESP is dependent on a balance of the speed of the Ca shift within the SR, the activation-inactivation of SR releasing mechanism, and the activity of the sarcolemmal Ca extrusion. We concluded that the curve of resting interval-potentiation relationship of PESP consisted of an ascending limb and a descending limb. Caffeine suppresses SR function including Ca uptake, Ca shift, and Ca release, and then it removes PESP, as shown in Fig. 6.

It is now recognized that PESP can augment contractile function in segments unresponsive to catecholamines\textsuperscript{22,23} and should therefore be a good intervention for eliciting maximal contractile reserve.\textsuperscript{4} So far, aequorin is the only intracellular Ca indicator used successfully to detect the Ca transients of cardiac muscle in conjunction with mechanical measurements.\textsuperscript{24} In this study, however, SR Ca release is not measured directly. Hagane et al.\textsuperscript{25} regarded postrest contraction as a SR function and concluded that the cardio-depressant action of doxorubicin is due to a drug-induced dysfunction of the SR in guinea pig. If it is true that SR Ca release contributes to one of the mechanism of PESP, as shown in Fig. 6, it will be possible to evaluate roughly SR function and sarcolemma function in vivo.

In conclusion, SR Ca release contributes largely to a mechanism of PESP and increases in contribution as a coupling ratio of an extra-stimulation shortens. In addition, the optimal postextrasystolic interval is determined by a balance between the activity of SR function and the activity of the sarcolemmal Ca extrusion mechanism. Drugs such as caffeine suppressing SR function remove PESP. We suspect that PESP can be clinically used as the rough representative of SR function.

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