RELATIONSHIP BETWEEN THE EFFECTS OF GONIOPORA TOXIN ON ACTION POTENTIAL AND ON CONTRACTILE FORCE IN GUINEA-PIG PAPILLARY MUSCLE

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Abstract—Effects of Goniopora toxin (GPT) on cardiac action potential and on contractile force were investigated in isolated guinea-pig papillary muscle. GPT produced a positive inotropic effect by increasing contractile force and prolonging the relaxation time. The time-to-peak force was little affected. GPT prolonged the action potential duration but did not affect the resting membrane potential nor the amplitude of the action potential. Thus there was a correlation between the positive inotropic effect and prolongation of the action potential duration. Tetrodotoxin or a reduction in extracellular sodium concentration attenuated both the positive inotropic effect and the prolonged duration of the action potential induced by GPT. Lanthanum or a reduction in extracellular calcium concentration also inhibited the increased contraction but did not shorten the prolonged durations of contraction and action potential. Verapamil attenuated the positive inotropic effect by reducing both the contractile force and the duration of contraction, but did not shorten the action potential duration. These results show that the positive inotropic effect of GPT depends on the increase in both sodium and calcium influxes while the prolonging effect on the action potential probably depends only on an increase in sodium influx. Hence, it is concluded that the prolongation of the action potential due to the increased sodium permeability is an essential process for the appearance of the positive inotropic effect of GPT.

Some cardiotonic agents have β-receptor stimulating action while others have Na⁺,K⁺-ATPase inhibitory action. Goniopora toxin (GPT), a marine polypeptide isolated from a coral, has recently been found to produce a positive inotropic effect in rabbit atria at low concentrations (1). This toxin has neither β-receptor stimulating nor Na⁺,K⁺-ATPase inhibitory action. Thus, GPT appears to be a cardiotonic agent different from those mentioned above.

Previously, we found that GPT prolonged the duration of the action potential in rabbit atria (1). In the present study, we investigated the possible correlation between the prolonging effect on the duration of the action potential and the inotropic effect in isolated guinea-pig papillary muscle. The results obtained show that the positive inotropic effect of GPT is closely associated with a prolongation of the duration of the
action potential and that these electro-
mechanical responses are the results of a
common process, i.e., an increase in sodium
permeability through the sarcolemma.

MATERIALS AND METHODS

Right ventricular papillary muscles of less
than 1 mm in diameter were excised from the
hearts of guinea pigs (250–350 g body
weight) sacrificed by a sharp blow on the
skull. The preparations were horizontally
mounted on silicone rubber in a modified
two-chambered organ bath originally
described by Reiter (2). The bath capacity
was 45 ml. One end of the preparation was
connected with a silk thread to a force-
displacement transducer and the resting
length of the muscle was adjusted by applying
a stretching force of 400 mg. The bathing
medium was maintained at 30±0.5°C and
equilibrated with 95% O₂ and 5% CO₂
continuously. Electrical stimulation was
applied via the tips of two insulated platinum
wires located on the non-tendinous end of
the muscle. Stimulus parameters were:
duration 2 msec, frequency 0.5 Hz, strength
1.5× threshold voltage. An equilibration
period of at least 1 hr preceded each ex-
periment.

Transmembrane action potentials were
recorded with a 3 M KCl-filled glass micro-
electrode (10–30 megohms), connected to
a high input impedance preamplifier. Action
potentials and the developed contractile
force were displayed simultaneously on a
dual beam oscilloscope and recorded on
35 mm film. In some figures, action potential
and contraction curves were drawn from
experimental records and remounted for
greater clarity (Figs. 1A, 4A, 5A, C).

The following parameters of action
potential and isometric contraction were
investigated: resting membrane potential,
amplitude of action potential, duration of
action potential, peak of contractile force
developed, time-to-peak force (T₁), re-
laxation time (T₂). The duration of the
action potential was measured from the rapid
upstroke to the time of 90% repolarization of
the action potential. T₁ and T₂ were measured
at the 10% level of contractile force developed.

The bath was composed of Tyrode solution
of the following composition (mM): NaCl
140.0; KCl 3.0; MgCl₂ 1.0; CaCl₂ 1.8;
NaH₂PO₄ 0.4; NaHCO₃ 12.0; glucose 5.0;
pH 7.4. In order to depolarize the muscle,
the potassium concentration was raised to
27.0 mM by adding an appropriate amount of
a concentrated KCl solution. In low sodium
solution, the sodium concentration was
reduced to 106.7 mM by replacing with
choline chloride, and atropine in a concen-
tration of 1 μM was added to the solution
in order to block the activation of muscarinic
receptors by choline. In low calcium
solution, the calcium concentration was
reduced to 0.3 mM, without compensation.
For experiments with lanthanum, the Tyrode
solution was buffered by 10.0 mM tris
hydroxymethylaminoethane-HCl (TRIS) from
which carbonate and phosphate were omitted,
and was aerated with 100% O₂ (pH 7.4).

In all experiments, except in the case of
partially depolarized cardiac muscle by 27.0
mM potassium, propranolol (2 μM) was
added to the bath solution 20 min before the
beginning of each experiment to exclude the
effect of endogenous norepinephrine which
might be released by GPT. Statistical
analyses were performed with the Student’s
t-test. p-Values <0.05 were considered
statistically significant.

Goniopora toxin (GPT), a polypeptide
isolated from the coral Goniopora spp., has
a molecular weight of 12,000. GPT has the
following amino acid composition: Asp₂₉,
Thr₉, Ser₈, Gln₆, Pro₉, Gly₅, Ala₄, Cys₇, Val₇,
Met₁, Ile₁, Leu₁, Trp₂, Phe₁, Lys₁, His₁,
Arg₁, Trp₂. (3). The sequence of amino acids
is under investigation. The preparation of GPT
was as described in our previous paper (1).

Drugs used: verapamil hydrochloride (Knoll, N.J.), propranolol hydrochloride (Sumitomo, Osaka), tetrodotoxin (Sankyo, Tokyo), dl-isoproterenol hydrochloride (Nakarai, Kyoto).

RESULTS

Effects of GPT on contraction and action potential: In guinea-pig papillary muscle driven electrically at a frequency of 0.5 Hz, GPT in concentrations ranging from 3 to 100 nM produced an increase in contractile force and a prolongation of the duration of contraction. In parallel with these effects, the duration of the action potential was lengthened. A representative recording of the effect of 30 nM GPT is shown in Fig. 1A. The effects of GPT on the action potential and on contraction were characterized by prolongations of the repolarizing phase and the relaxing phase, respectively. The relaxation proceeded in two distinct phases at 90 min after addition of GPT: an initial rapid phase followed by a slow phase. The control values of resting membrane potential (-93±1.0, n=33), amplitude of the action potential (120±1.3, n=33), and resting tension were not changed significantly. Figure 1B shows the time course of the effects of GPT on the action potential and on the contraction of the same preparation as that in Fig. 1A. The duration of the action potential (AP), contractile force and relaxation time (T2) progressively increased after addition of GPT. The time course of changes of these parameters was in parallel. The time-to-peak force (T1), however, remained unchanged.

The effects of GPT were irreversible after exposure to the concentrations of whole range, as the effects persisted 120 min after washout at 20-min intervals. Small oscillations in the relaxing and repolarizing phase were often evident. At a high concentration such as 100 nM, GPT induced spontaneous contractions and eventually contractions. Figure 2 summarizes the effects of 10 and 30 nM GPT on the duration of the action potential and contractile force. There is a good correlation between the pro-
longation of the action potential and the positive inotropic effect. When the duration of the action potential was compared to the duration of contraction, there was also a good correlation (Fig. 3), as duration of contraction consists of $T_1$ and $T_2$, and $T_1$ always remained constant. It would thus appear that the positive inotropic effect of GPT consists of increase in contractile force and prolongation of contraction, and that such inotropic effect is closely associated with the prolongation of the action potential.

Effects of tetrodotoxin or reduction in concentration of extracellular sodium on GPT-induced positive inotropic effect: We have already reported that tetrodotoxin (TTX) effectively inhibited the inotropic effect of GPT (1). Thus we examined the effects of TTX on the configuration of the action potential and the contraction curve. When the muscle was treated with 10 nM GPT for 60 min (Fig. 4A), both the action potential and contraction were markedly prolonged. When 1 μM TTX was added to the solution containing GPT, the action potential and contraction were rapidly shortened and the contractile force was attenuated. Such effects of TTX were reversible by washing.

In another series of experiments using 10 and 30 nM GPT, similar results were obtained. In general, the change in the duration of the action potential occurred in parallel with the change in contraction. The spontaneous activity and contracture induced by a high concentration of GPT were also depressed by TTX (1 μM). TTX in concentrations lower than 0.1 μM was ineffective in reversing the effect of GPT.

The bathing medium was then replaced by low sodium (70% of the control) solution after a 30-min treatment with 30 nM GPT (Fig. 4B). Despite the continuous treatment with GPT, the increased contractile force was attenuated and the prolonged durations of both the action potential and contraction were shortened remarkably by the reduction in the concentration of extracellular sodium. From these experiments, it is apparent that the reduction in the concentration of extra-
cellular sodium produces the same effect as TTX on the GPT-induced electromechanical changes and that there is a good correlation between the duration of the action potential and the relaxing time or contractile force.

The effects of GPT never reverted to the control level after washing with toxin-free and normal sodium solution; rather the effects developed with time.

Effects of lanthanum, reduction in concentration of extracellular calcium or verapamil on GPT-induced positive inotropic effect: Addition of lanthanum (0.1 mM), after the application of 10 nM GPT for 60 min, attenuated the contractile force, especially the rapid upstroke phase which was enhanced by GPT (Fig. 5A). However, neither the duration of contraction nor the duration of the action potential was shortened; rather there was a progressive prolongation. Higher concentrations of lanthanum (above 0.3 mM) induced contracture in the GPT-treated muscles. In the muscles which had not been treated with GPT, 0.1 mM lanthanum abolished the contraction, shortened the

![Fig. 4A. Effect of tetrodotoxin (TTX) on the GPT-induced positive inotropic effect and prolonged duration of action potential. TTX was added 60 min after the application of GPT and the tissue was treated for 10 min.](image)

![Fig. 4B. Effect of reduction in the concentration of extracellular sodium on the GPT-induced positive inotropic effect and prolonged duration of action potential. The horizontal scales indicate 200 msec and the vertical scales 100 mg. Note that the time scales at 0 and 50 min differ from the others.](image)
duration of phase 2 of the action potential and slightly prolonged the total duration of the action potential.

In Fig. 5B, the bathing medium was replaced by low calcium (0.3 mM) and GPT-containing solution after the treatment with 30 nM GPT for 20 min. The contractile force was reduced despite the presence of GPT, but the durations of the action potential and contraction were prolonged considerably. Further addition of 1 μM TTX shortened the duration of the action potential and reduced the contractile force.

Verapamil (10 μM) attenuated the positive inotropic effect of GPT and shortened the duration of the contraction (Fig. 5C). The duration of the action potential, however, was progressively prolonged. In GPT-free solution this dose of verapamil abolished the contraction, shortened the duration of phase 2 of the action potential, and slightly prolonged the total duration of the action potential.

**Effect of GPT on partially depolarized muscle**: Guinea-pig papillary muscle was depolarized to about −40 mV by immersion into 27.0 mM potassium containing Tyrode solution. The muscle was then driven at a frequency of 0.1 Hz and treated with 1 μM
isoproterenol. Upstroke of the action potential was less steep and contraction developed slowly (Fig. 6). Under this condition the fast sodium channel is inactivated and the evoked action potential may primarily be dependent on calcium influx (4, 5). Application of GPT in concentrations ranging from 10 to 100 nM for 50 min had no effect on the action potential and contraction.

**DISCUSSION**

In the guinea-pig papillary muscle, GPT produced a potent positive inotropic response which was characterized not only by an increase in contractile force but also by a prolongation of the contraction period. Concomitant with such changes in the mechanical response, prolongation of the duration of the action potential was observed. Since there is a good correlation between the positive inotropic response (i.e., which consists of increase in contractile force and prolongation of contraction) and the duration of the action potential, it is considered that a certain common process participates in these effects of GPT.

In a previous paper, we suggested that the change in the duration of the action potential in rabbit atrial muscle is due to the altered sodium inward current, as the prolonged action potential in rabbit atria was effectively antagonized by TTX (1). In the present study using guinea-pig papillary muscle, the lengthened duration of the action potential was also shortened by TTX. The major effect of TTX in cardiac muscle is inhibition of the sodium current through the fast sodium channel (6), and in the normal state, the fast sodium channel of the cardiac muscle is rapidly activated and then rapidly inactivated upon depolarization (7, 8). And it is reported that TTX does not affect the duration of the action potential in atrial and/or ventricular muscle (9, 10) but only shortens that of the conducting system (10, 11). Thus the susceptibility of the prolonged action potential to TTX after treatment with GPT indicates that the prolongation is caused by a sustained activation of the fast sodium channel, in contrast to the normal state. Reduction in the concentration of extracellular sodium resulted in an effect similar to that seen after administration of TTX; thus supporting the possibility mentioned above.

Prolongation of the action potential duration after exposure to GPT could also be due to an increase in calcium permeability, in addition to the effect on the fast sodium channel. However, calcium antagonists such as verapamil (12–17) and lanthanum (18–20) or reduction in the concentration of extracellular calcium failed to shorten the duration of the action potential after the treatment with GPT, and slow action potential in depolarized muscle was not altered by GPT, even at high concentrations. As already suggested (1), prolongation of the action potential duration is probably the
The positive inotropic effect as well as the prolongation of the duration of the action potential following GPT was antagonized by TTX or by a reduction in the concentration of extracellular sodium. This indicates that the positive inotropic effect of GPT is also closely associated with the change in sodium permeability, through the sarcolemma. On the other hand, calcium antagonists such as verapamil and lanthanum or a reduction in the concentration of extracellular calcium attenuated the positive inotropic effect of GPT, while these antagonists had no effect on the duration of the action potential. These results indicate that the positive inotropic effect of GPT is associated with an increase in calcium influx as well. However, there was a dissociation between the electrical and the mechanical effects of GPT, under the conditions which attenuate calcium influx. Thus, it is likely that the primary action of GPT is prolongation of the duration of the action potential due to an increase in sodium permeability through the sarcolemma, and that the increase in sodium influx, in turn activates the sodium-calcium exchange mechanism (21) and produces the positive inotropic response (22, 23).

The effects of verapamil and lanthanum on the contractile force after exposure to GPT were similar; however, the effect on the contraction period differed between these two agents. Verapamil shortened the contraction period, while lanthanum prolonged the period, particularly the relaxing phase (T₂). Both lanthanum (20, 24–26) and verapamil (27–30) have cardiac actions other than the calcium antagonistic effect on the slow channel, and such actions may differ in extent between the two agents. Unlike verapamil, lanthanum has little effect on the sodium-calcium exchange mechanism (19, 31), and by itself produces cardiac contraction (25). On application, this difference between the effects of verapamil and lanthanum may be reflected to the duration of contraction prolonged by GPT. Thus, the prolonged duration of contraction which was antagonized by verapamil may be brought about by the sodium-calcium exchange mechanism.

The positive inotropic effect of GPT was characteristic in its marked prolongation of the relaxing phase, and this effect appeared to be associated closely with the increase in sodium permeability and the activation of the sodium-calcium exchange mechanism. In 1979, Roulet et al. (32) reported the relative importance of the sodium-calcium exchange mechanism, as compared with the internal calcium sequestration, in the relaxation of the twitch contraction of the frog atria. Compounds such as GPT, sea anemone toxin (33, 34) and anthopleurin-A (35), which increase sodium permeability should provide a useful tool for analyzing the relative contribution of the sodium-calcium exchange mechanism to cardiac contractility.

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