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Investigation of the cellular mechanisms of actions of digoxin and monensin by using guinea-pig papillary muscles

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Abstract

This experiment was carried out to determine the possible mechanism of actions of monensin and digoxin by using guinea-pig papillary muscles. A total of 120 papillary muscles obtained from 60 guinea-pigs were used. Three treatment groups; control (%0,1 ethanol), monensin (10 µmol/l) and digoxin (3 µmol/l) were compared in normal, Na+-free, Ca^{2+}-free, and thapsigargin-added Krebs solutions. It was found that both digoxin and monensin caused a positive inotropic effect in normal and Ca^{2+}-free Krebs solutions. Although monensin produced a positive inotropic effect in thapsigargin-treated papillary muscles, digoxin did not show a positive inotropic effect. Because papillary muscles in Na^{+} -free Krebs solution showed spontaneous contractions, trusted results were not obtained in Na^{+} -free medium. It is concluded that monensin produces a positive inotropic effect by depending on both extracellular Ca^{2+} and Ca^{2+} released from the sarcoplasmic reticulum (SR). Digoxin, on the other hand, produces its effect by depending on the Ca^{2+} released from SR.

Keywords: Monensin, digoxin, guinea pig, papillary muscle

INTRODUCTION

It has been well demonstrated that the cardiac muscle contraction is initiated by increased intracellular Ca^{2+} concentration, $[Ca^{2+}]i$. Many positive inotropic agents, such as the digoxin, used for the treatment of congestive heart failure, and monensin, a carboxylic ionophore antibiotic, also act by increasing $[Ca^{2+}]i$ [16; 10; 7; 14;] By comparing the positive inotropic actions of monensin and digoxin in our previous experiments, it has been demonstrated that digoxin caused a positive inotropic effect in guinea-pig papillary muscles 1 h after the administration [8]. However, it lost its positive inotropic action with the passage of time and caused a negative rather than a positive inotropic action 3 and 4 h after administration. Monensin also caused a positive inotropic effect in guinea-pig papillary muscles 1 h after administration. However, it did not lose its positive inotropic action up to 4 h of experimental period [9] These results indicated that the effectiveness of digoxin with respect to its positive inotropic effect decreases in time whereas monensin remains effective. We designed another experiment to compare the possible mechanism of action of monensin to digoxin by using guinea-pig ventricular myocytes over a 2 h experimental period [9]. It was the first experiment that compared the monensin to digoxin with regard to their effects on $[Na^{+}]i$ and $[Ca^{2+}]$ i in guinea-pig ventricular myocytes. The results of the study led to the conclusion that the positive inotropic effect of digoxin depends on $[Na^{+}]o$. However, monensin increases $[Ca^{2+}]i$ in Na^{+}-dependent and -independent ways. Therefore, this experiment was carried out to determine the possible mechanism of actions of monensin and digoxin by using guinea-pig papillary muscles instead of isolated ventricular myocyte comparing the results obtained from both experiment. The present study is the first one that compares monensin to digoxin without electrically stimulating ventricular myocytes and monitors the effects of these drugs on $[Na^{+}]i$ and $[Ca^{2+}]i$ in guinea-pig ventricular myocytes for 2 h. Without using an electrical stimulus, the involvement of stimulating the voltage-dependent Ca^{2+} channels was avoided in the present experiment

MATERIALS AND METHODS

Preparation of papillary muscles

This experiment was performed in accordance with national and local ethical guidelines. Sixty male guinea-pigs, 400–600 g, were heparinized [1] 30 min before being decapitated. One animal was used per day. Hearts were removed rapidly and put in a beaker filled with ice-cold Krebs–Ringer bicarbonate buffer of the following composition (mmol/l): Na^{+}, 115.9; Ca^{2+}, 2.2; K^{+}, 4.0; Mg^{2+}, 1.3; Cl^{-}, 126.9; $H_2PO_4^{-}$, 2.1; HCO_3^{-}, 21.7; glucose, 10.9. The pericardium was removed, and the aorta and the pulmonary artery were excised. The hearts were transferred to a second beaker of the same solution, and then rapidly put in a glass pan filled with Krebs solution and bubbled with 100% O_2. The time lapse between the removal of the heart and its perfusion by oxygenated Krebs solution was less than a minute. Two papillary muscles with diameters of approximately 0.5–1.0 mm were dissected from the left ventricle of each guinea-pig and each muscle was mounted in an organ bath containing a Krebs solution with 2.2 mmol/l $CaCl_2$. The Krebs solution was perfused with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. The pH was maintained between 7.50 and 7.60. The tendon end of each muscle was attached to the force transducer by a

silk suture and the opposite end was held by a plastic clamp placed in the muscle chamber. The transducers to measure force were calibrated for each experiment using weights (starting force was calibrated to 700 mg).

Experiment 1: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in normal Krebs solution

A stock solution of 1.5 mmol/l digoxin (Sigma Chemical Co., St L ouis, MO) and a stock solution of 3.5 mmol/l monensin (Sigma Chemical Co.) prepared with alcohol (0.10%) were used. Chemicals were administered after an equilibration period of 35 min. 3 μmol/l digoxin, 10 μmol/l monensin or 0.10 % alcohol was administered to the chambers for evaluating the contraction force of papillary muscles. Each treatment was monitored for 2 h and ten papillary muscles from ten different guinea-pigs were used for each treatment. Two hours after the treatment 70 mmol/l phenylephrine was added to test whether the muscles would respond to this treatment.

Experiment 2: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in Na^+-free medium

To investigate whether an increase in contraction force induced by monensin or digoxin is due to Na^+ influx, a Na^+-free medium was used. The muscle preparation and treatment were the same as in experiment 1. However, treatment was made in a Na^+-free medium of following composition [mmol/l]; choline chloride, 116; $MgCl_2$, 1.3; K_2HPO_4, 2; glucose, 10.9; $CaCl_2$, 2.2; HEPES, 10.

Experiment 3: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in Ca^{2+}-free medium

To investigate whether an increase in contraction force induced by monensin or digoxin is due to Ca^{2+} influx, a Ca^{2+}-free medium was used. The muscle preparation and treatment were the same as in experiment 1. However, treatment was made in a Ca^{2+}-free medium of following composition [mmol/l]; Na^+, 115.9; EDTA, 0.01; K^+, 4.0; Mg^{2+}, 1.3; Cl^-, 126.9; $H_2PO_4^-$, 2.1; HCO_3^-, 21.7; glucose, 10.9. Two hours after the treatment 20 mmol/l caffeine was added to test whether the muscles would respond to this treatment.

Experiment 4: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle by depleting the SR with thapsigargin

Experiment was carried out in normal Krebs solution. Thapsigargin (200 nmol/l), which depletes the SR was administered after an equilibration period of 35 min. Two hours after the thapsigargin treatments, 3 μmol/l digoxin, 10 μmol/l monensin or 0.10 % alcohol was administered to the chambers for evaluating the contraction force of papillary muscles. Each treatment was monitored for 2 h and ten papillary muscles from ten different guinea-pigs were used for each treatment. Two hours after the treatment 70 mmol/l phenylephrine was added to test whether the muscles would respond to this treatment.

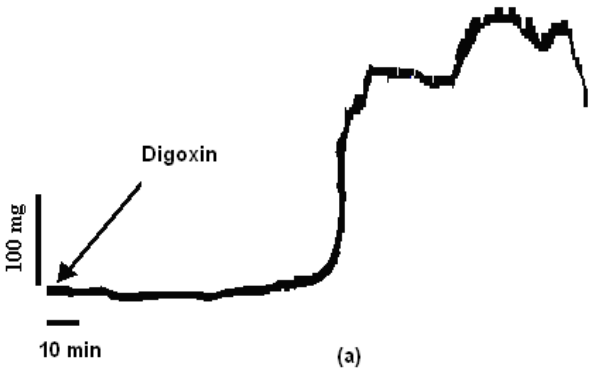
Treatment design, experimental design and statistical analysis

Three treatments (control, digoxin and monensin) were used in this study. Because drugs were prepared with alcohol, 0.10 %, alcohol was used as control to eliminate any secondary effect. Because of the large variability among animals, a balanced incomplete block design was selected as the experimental design. Each animal provided two muscles, individual muscles were the experimental unit for the experiment. The data were expressed as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA). Turkey's test was used to test for differences among means for which ANOVA indicated a significant ($P \leq 0.05$) F ratio (Snedecor and Cochran, 1989).

RESULTS

Experiment 1: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in normal Krebs solution

Effects of digoxin, monensin and alcohol on the contraction force of guinea-pig papillary muscle in normal Krebs solution are shown in Figures 1. Both digoxin and monensin produced a positive inotropic action 70 min after drug administration. However, alcohol administration did not produce any positive inotropic action. Phenylephrine administration produced a positive inotropic action in control muscles 2 h after alcohol administration indicating the viability of muscles.



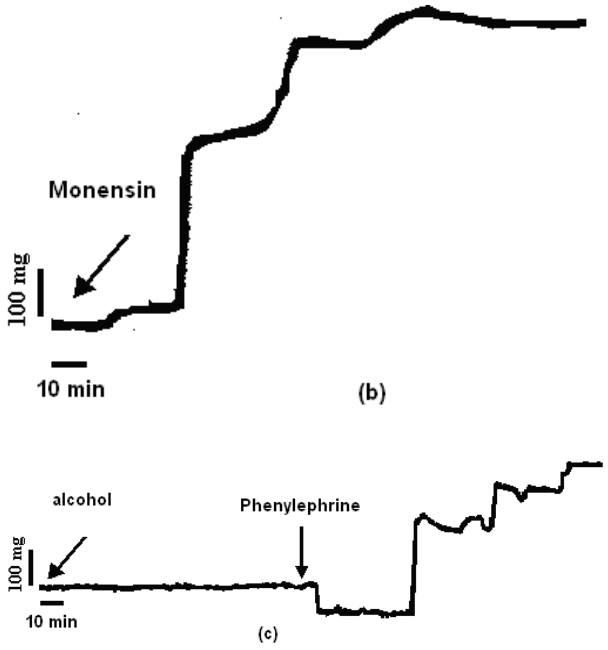


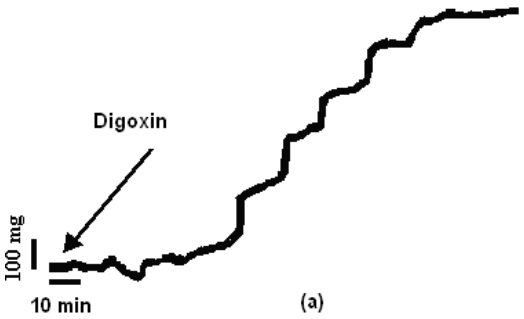
Figure 1. Effects of 3 µmol/l digoxin (a), 10 µmol/l monensin (b) and 0.10 % alcohol (c) on the contraction force of guinea-pig papillary muscle in normal Krebs solution.

Experiment 2: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in Na^+ free medium

This experiment could not be concluded due to early strong contractions occurred in muscles due to ionic imbalance.

Experiment 3: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle in Ca^{2+} free medium

Effects of digoxin, monensin and alcohol on the contraction force of guinea-pig papillary muscle in Ca^{2+} medium are shown in Figures 2. Both digoxin and monensin produced a positive inotropic action. Positive inotropic action of monensin was stronger and occurred earlier. However, alcohol administration did not produce any positive inotropic action. Caffeine administration produced a positive inotropic action in control muscles 2 h after alcohol administration indicating the viability of muscles.



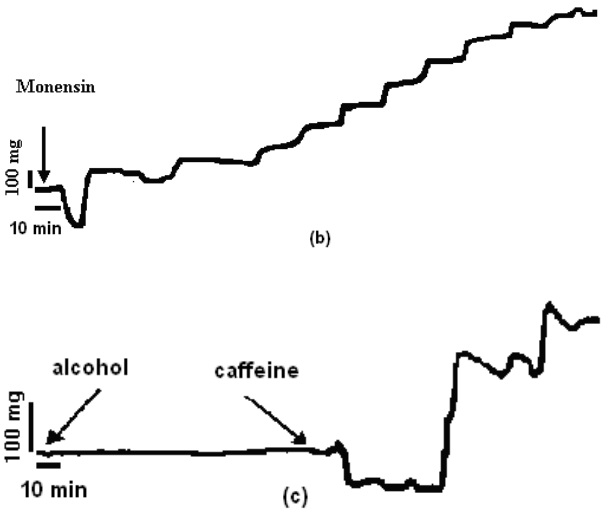


Figure 2. Effects of 3 µmol/l digoxin (a), 10 µmol/l monensin (b) and 0.10 % alcohol (c) on the contraction force of guinea-pig papillary muscle in Ca2+ free medium.

Experiment 4: Effects of digoxin and monensin on the contraction force of guinea-pig papillary muscle by depleting the SR with thapsigargin

Effects of digoxin, monensin and alcohol on the contraction force of guinea-pig papillary muscle after the SR depletion by thapsigargin are shown in Figures 3. Only monensin produced a positive inotropic action 90 min after drug administration. However, digoxin or alcohol administration did not produce any positive inotropic action. Phenylephrine administration produced a positive inotropic action in digoxin-treated or control muscles 2 h after alcohol administration indicating the viability of muscles.

DISCUSSION

This experiment was carried out to determine the possible mechanism of actions of monensin and digoxin by using isolated guinea-pig papillary muscles. Both digoxin and monensin produced a positive inotropic action 70 min after the drug administration. It has been suggested that the positive inotropic action of digoxin is caused by the inhibition of Na^+, K^+-ATPase. As a result, there is a gradual increase in $[Na^+]_i$ and a gradual small decrease in $[K^+]_i$ (Deitmer and Ellis, 1978; Balzan et al., 2000). It is the increase in $[Na^+]_i$ that can be judged to be crucially related to the positive inotropic effect of digoxin. An increase in $[Na^+]_i$ by digoxin raises the $[Ca^{2+}]_i$ due to the entry of Ca^{2+} into the cell by Na^+ (out)/Ca^{2+} (in) exchange (Barry and Bridge, 1993). Monensin has been characterized as a Na^+ ionophore by virtue of its ability to form a lipid-soluble cation complex that can rapidly traverse cell membranes. Monensin binds Na^+ outside the myocytes and carries it into the cell and increases $[Na^+]_i$

and thus $[Ca^{2+}]_i$ (Mollenhauer et al., 1990).

In this experiment it was possible to demonstrate that both digoxin and monensin increased the force of contraction of guinea-pig papillary muscles. The increase in the force of contraction by monensin and digoxin were gradual. It took about 70 min for them to reach a plateau. This result suggests that it takes time for digoxin and monensin to increase $[Ca^{2+}]_i$. This result is also consistent with the results of our previous experiment showing that both digoxin and monensin caused an increase in the $[Ca^{2+}]_i$ 70 min after the administration (Meral et al., 2002b).

It was also found that both digoxin and monensin caused a positive inotropic effect in Ca^{+2}-free Krebs solutions. Although monensin produced a positive inotropic effect in thapsigargin-treated papillary muscles, digoxin did not show a positive inotropic effect. This result indicated that monensin produces a positive inotropic effect by depending on both extracellular Ca^{+2} influx and Ca^{+2} released from the sarcoplasmic reticulum (SR). Digoxin, on the other hand, produces its effect by only depending on the Ca^{+2} released from SR. The action of these two drugs relies on an increase in $[Ca^{2+}]_i$ due to an increased $[Na^+]_i$ (Tsuchida and Otomo, 1990; Miura and Biedert, 1985; Barry and Bridge, 1993). It has been suggested that the positive inotropic effect of monensin in guinea-pig papillary muscles is nearly insensitive to inhibition by nifedipine (an L-type Ca^{2+} channel blocker) and is only partially blocked by ryanodine, a ryanodine channel blocker (Hugtenburg et al., 1989). They also suggested that the partial inhibitory effect of ryanodine suggests that a part of the monensin-induced $[Ca^{2+}]_i$ is taken up by the SR. These findings and the present results support the hypothesis that Ca^{2+} introduced into the cell by the Na^+/Ca^{2+} exchange, in addition to the influx through voltage dependent Ca^{2+} channels, might also contribute to the reloading of the SR (Fabiato, 1985). However, there may be an influx of Ca^{2+} through channels other than voltage dependent Ca^{2+} channels (Ehara et al., 1988). The positive inotropic action of digoxin is caused by the inhibition of Na^+, K^+-ATPase. As a result, there is a gradual increase in $[Na^+]_i$ and a gradual small decrease in $[K^+]_i$ (Deitmer and Ellis, 1978; Balzan et al., 2000). It is the increase in $[Na^+]_i$ that can be judged to be crucially related to the positive inotropic effect of digoxin. An increase in $[Na^+]_i$ by digoxin raises the $[Ca^{2+}]_i$ due to the entry of Ca^{2+} into the cell by Na^+ (out)/Ca^{2+} (in) exchange (Barry and Bridge, 1993; Nagai et al., 1996; Muller-Ehmsen et al., 1997).

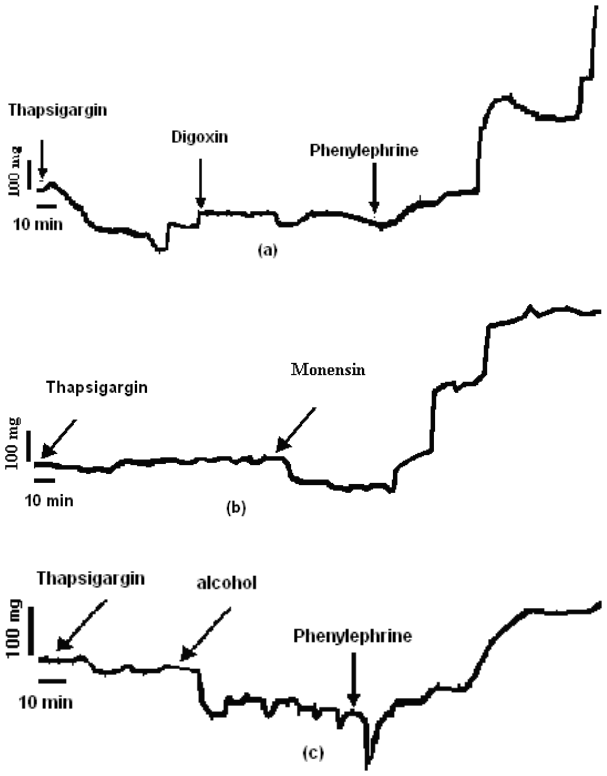


Figure 3. Effects of 3 μmol/l digoxin (a), 10 μmol/l monensin (b) and 0.10 % alcohol (c) on the contraction force of guinea-pig papillary muscle after the SR depletion by thapsigargin.

It is concluded that both digoxin and monensin produce a positive inotropic action in electrically non-stimulated papillary muscles. However, monensin produces a positive inotropic effect in papillary muscles by depending on both extracellular Ca^{+2} and Ca^{+2} released from the sarcoplasmic reticulum. Digoxin, on the other hand, produces its effect by depending on the Ca^{+2} released from SR.

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