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Effects of Different Concentrations of Monensin on the Contractility Changes of Guinea-pig Papillary Muscle

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Abstract: Effects of different concentrations of monensin on contraction amplitude (CA) which is the contraction force (CF), initial contraction velocity (ICV), average contraction velocity (ACV) and initial relaxation velocity (IRV) of guinea-pig papillary muscle were studied by using male guinea-pigs. Hearts were removed and papillary muscles were isolated from the right ventricles. The muscles were then attached to a capacitance transducer, electrically connected to a Beckman recorder and the isometric contractions of guinea-pig papillary muscles were recorded. The five hours of measurement in this experiment were divided into five periods (T0 was equilibration, T1, T2, T3 and T4 were respectively one, two, three and four hours after drug administration). Two µmol/l monensin did not change any parameters at any time period. Although 12 µmol/l monensin did not change the CA, ACV or IRV at any time period, it increased the ICV at T2. Seven µmol/l monensin, on the other hand, increased the CA and IRV at T2, T3 and T4 and the ICV and ACV at T1, T2, T3 and T4. It is concluded that the effect of monensin in guinea-pig papillary muscles is dose dependent. It is also concluded that although the therapeutic range of monensin is very narrow, it should be considered as an alternative drug for the treatment of congestive heart failure (CHF). However, more studies are needed to domenstrate the effectiveness of monensin on the tratment of congestive heart failure.

Key Words: Monensin, papillary muscle, calcium, positive inotrope, guinea-pig

Monensinin Farklı Dozlarının Kobay Papillar Kas Kontraktilitesi Üzerindeki Etkileri

Özet : Monensinin farklı dozlarının popillar kas kasılma gücü (KG), başlangıç ve ortalama kasılma hızı (BKH ve OKH) ve başlangıç gevşeme hızı (BGH) üzerindeki etkileri erkek kobaylar kullanılarak çalışıldı. Kalp vucuttan uzaklaştırıldıktan sonra papillar kaslar sağ ventrikulusdan izole edildi. İzole edilen kaslar elektriksel olarak bir poligrafa bağımlı transdüsere bağlandıktan sonra izometrik kasılmalar ölçüldü. Ölçümler beş değişik zaman diliminde yapıldı (T0 denge ve T1, T2, T3, T4 ise sırasıyla ilaç uygulamasından 1, 2, 3 ve 4 saat sonrasını ifade etti). İki µmol/l monensin herhangi bir parametrede, hiçbir zaman diliminde istatistiksel olarak önemli bir değişiklik oluşturmazken, 12 µmol/l monensin sadece BKH'ı T2'de artırdı. Buna karşılık 7 µmol/l monensin KG ve BGH'ı T2, T3 ve T4'de ve BKH ve OKH'ı T1, T2, T3 ve T4'de artırdı. Bu sonuçlar monensinin kobay papillar kaslar üzerindeki etkisinin doza bağımlı olduğunu ortaya koydu. Monensinin terepatik oranı oldukça dar sınırlar içerisinde olmasına rağmen, kalp yetmezliğinin tedavisinde kullanılmak üzere alternatif bir ilaç olarak üzerinde çalışılması gerektiği sonucuna varıldı. Ancak monensinin kalp yetmezliğinin tedavisinde etkili bir şekilde kullanılabileceğini kanıtlamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Sözcükler: Monensin, papillar kas, kalsiyum, pozitif inotrop, kobay

Introduction

Ionophores carry ions across lipid barriers as complexes soluble in the lipid phase of membranes. The potential use of ionophores as probes of biological function, or as potential therapeutic agents, was recognized very early, but major economic importance was not forthcoming until the discovery of monensin in 1967 (1).

Initially, Shumard and Callender, 1969 (2) recognized the effectiveness of monensin against experimental infections of coccidoia in chickens and subsequently it was used widely in birds for control of coccidiosis. Monensin is also efficacious in the control of coccidiosis in lambs and calves (3, 4). In beef cattle, it improves the feed efficiency by 10-15% due to an increase in the production of more propionic acid which yields more energy than acetic or butyric acids following ruminant fermentation (5).

Of the more than one hundred ionophores that have been reported, three, monensin, lasalocid, and salinomycin, have wide spread commercial use and of these, licenced monensin is probably used most widely (1). Among the domestic animals, horses are most sensitive to monensin toxicity (6). If monensin is used

as a coccidiostat or growth promoter in other species, the drug-treated feed would be dangerous if accidentally consumed.

Monensin has also a strong positive inotropic effect in cardiac muscle (7, 8, 9). This effect occurs because, as an ionophore, monensin has strong affinity for $[Na^+]_o$. The affinity of monensin for Na^+ is ten times that for K^+, its nearest competitor in biological systems (1). The monensin-induced increase in $[Na^+]_i$ facilitates the entry of Ca^{2+} into the cell by a Na^+ (out) / Ca^{2+} (in) exchange mechanism (10). This Ca^{2+} shift is the primary factor mediating the cellular response. Another factor modifying the cellular response includes the alteration of the pH of intracellular components (pH_i) since monensin increases the pH_i by transferring H^+ out of the cell (1).

In the present study, effects of different concentrations of monensin on contraction amplitude (CA) which is the contraction force (CF), inital contraction velocity (ICV), average contraction velocity (ACV) and initial relaxation velocity (IRV) of guinea-pig papillary muscle were investigated over a four-hour experimental period. Many pharmacological experiments have compared different drugs according to their initial effects. Treatment of papillary muscles with monensin for four hours gave us a better understanding of the effect of monensin with time.

The purpose of the present study was to evaluate the inotropic effect of monensin in guinea-pig papillary muscle by using different concentrations of monensin and to investigate whether this effect of monensin would change in time. The objective of this experiment was to establish the need for further investigation of monensin for the possibility of using it in the management of certain clinical syndromes such as congstive heart failure (CHF).

Materials and Methods

Preparation of papillary muscles:

Male guinea-pigs, 500-600 g, were heparinized (1000 IU per animal, IP injection) 30 minutes before being decapitated. One animal was used per day. Hearts were removed rapidly and put in a beaker filled with ice-cold KRB of the following composition [mmol/1]: Na^+, 115.9: Ca^{2+}, 2.2: K^++, 4.0: Mg^{2+}, 1.3: Cl^-, 126.9: H_2PO_4-, 2.1: HCO_3-, 2.17: 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 bubled with 100% O_2. Two papillary muscles with diameters of approximately 1.0-1.5 mm were dissected from the right 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 Kreb solution was perfused with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. The pH was maintained between 7.50-7.60. The muscles were attached to a capacitance transducer (Harvard isometric capacitance transducer, Harvard apparatus, inc., South Notick, MA) electrically connected to a Beckman recorder (R611). The tendon end of each muscle was attached to the transducer by a silk suture and the opposite end was held by a plastic clamp placed in the muscle chamber. Muscles were stimulated at a frequency of 0.2 PPS by using a pair of platinum field effect electrodes. The transducers to measure force were calibrated for each experiment by using weights (1 g equaled a displacement of 40 mm). Frequency was calibrated by an oscilloscope.

Stock solutions:

Stock solutions of 1, 3.5 and 6 mmol/l monensin (Sigma Chemical Co., St. Louis, MO) prepared with alcohol were used. Four treatments were used; control (0.18% alcohol), and monensin at 2, 7 and 12 µmol/l. Each treatment was replicated four times. Treatments were administered after an equilibration period of 35 min.

Contractility Experiment:

Sixteen papillary muscles were obtained from 8 guinea-pigs as described in the preceding section. Two different treatments were used on the same day on two different papillary muscles obtained from one guinea pig. Data were obtained from each muscle five times (before treatment application and 1, 2, 3 and 4 hours after continuos treatment). Variables measured at each period included contraction amplitude, contraction angle, relaxation angle and stimulus to response time. Contraction force (CF), initial contraction velocity (ICV), average contraction velocity (ACV) and initial relaxation velocity (IRV) were calculated as functions of the measured variables.

Treatment design, experimental design and statistical analysis:

Four treatments were used in this study. 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. Blocks were size of two; with each block containing two muscles from the same animal. Experimental units (a single muscle) were randomly allocated to treatments. Analyses of variance were obtained using SAS (SAS intitute, Inc., 1988, release 6.03, Cary, NC). Unbiased estimates of treatment means and their errors were obtained using the LSMEANs statement. The ANOVA indicated a significance when P≤0.05. Contrasts (11) were used to test differences among means.

Results

Effects of different concentrations of monensin on the CA, ICV, ACV and IRV of guinea-pig papillary muscle are presented in table 1, 2, 3 and 4, respectively. Two μmol/l monensin did not change any parameters at any time period (Table 1, 2, 3 and 4). Although 12 μmol/l monensin did not change the CA, ACV or IRV at any time period (Table 1, 3 and 4), it increased the ICV at T2 (Table 2). Seven μmol/l monensin, on the other hand, increased the CA and IRV at T2, T3 and T4 (Table 1 and 4) and the ICV and ACV at T1, T2, T3 and T4 (Table 2 and 3).

Table 1. Effects of different concentrations of monensin on the contraction amplitude (mm) of guinea-pig papillary muscle. Mean ± standard error is shown (n=4). M. monensin.

Time (hour)	Control	M-2	M-7	M-12
0	12.6±2.7	13.5±1.4	1.28±1.6	14.2±1.9
1	14.5±3.9	15.8±2.2	23.5±5.6	21.1±4.8
2	12.1±4.1	14.3±2.9	27.4±4.4*	19.2±6.8
3	11.2±3.9	12.8±2.7	25.6±3.2*	13.8±4.4
4	10.8±3.5	12.6±3.2	21.3±2.8*	11.6±4.9

* denotes a significant difference between treatment and control group (P≤0.05).

Table 2. Effects of different concentrations of monensin on the initial contraction velocity (g/sec) of guinea-pig papillary muscle. Mean ± standard error is shown (n=4). M. monensin.

Time (hour)	Control	M-2	M-7	M-12
0	1.14±0.2	13.4±0.3	1.34±0.1	1.60±0.5
1	1.34±0.3	1.45±0.2	3.07±0.4*	2.62±0.6*
2	1.31±0.3	1.56±0.2	4.63±0.7*	2.82±1.0
3	1.61±0.4	1.56±0.2	4.17±0.5*	3.40±1.5
4	1.49±0.3	1.55±0.2	3.79±0.4*	2.38±0.6

* denotes a significant difference between treatment and control group (P≤0.05).

Table 3. Effects of different concentrations of monensin on the average contraction velocity (g/sec) of guinea-pig papillary muscle. Mean ± standard error is shown (n=4). M. monensin.

Time (hour)	Control	M-2	M-7	M-12
0	0.88±0.2	0.89±0.2	0.98±0.1	1.17±0.4
1	1.18±0.3	1.13±0.1	2.66±0.6*	2.79±1.3
2	1.09±0.3	1.15±0.2	3.67±0.4*	2.57±1.0
3	1.45±0.3	1.06±0.2	4.10±0.8*	2.51±0.9
4	1.38±0.3	1.03±0.2	3.87±0.5*	2.00±0.6

* denotes a significant difference between treatment and control group (P≤0.05).

Table 4. Effects of different concentrations of monensin on the initial relaxation velocity (g/sec) of guinea-pig papillary muscle. Mean ± standard error is shown (n=4). M. monensin.

Time (hour)	Control	M-2	M-7	M-12
0	0.63±0.1	0.61±0.2	0.66±0.1	0.68±0.2
1	0.91±0.2	0.75±0.2	1.78±0.5	1.59±0.6
2	0.86±0.3	0.97±0.2	3.30±0.5*	1.43±0.4
3	0.96±0.4	0.94±0.2	2.54±0.5*	1.08±0.4
4	1.10±0.2	0.93±0.2	2.63±0.3*	0.87±0.4

* denotes a significant difference between treatment and control group (P≤0.05).

Discussion

This study was undertaken to evaluate the inotropic and toxic effects of the different concentrations of monensin in guinea-pig papillary muscle and to investigate whether these effects of monensin would change in time. Contractility is the performance of a heart at a given preload and afterload (18). An increase in contractility (positive inotropic effect) is not only reflected by increments in developed force but also reflected by increments in velocity (18).

In this experiment it was found that 2 μmol/l monensin did not change any parameter at any time period. Although 12 μmol/l monensin did not change the CA, ACV or IRV at any time period, it increased the ICV at T2 (Table 2). Seven μmol/l monensin, on the other hand, increased the CA and IRV at T2, T3 and T4 (Table 1 and 4) and the ICV and ACV at T1, T2, T3 and T4 (Table 2 and 3). These results indi-

cated that the effect of monensin on the contractility of guinea-pig papillary muscles is dose dependent.

The 7 μmol/l monensin administration increased the CA of guinea-pig papillary muscles at T2(120 min after the treatment) and did not lose its positive inotropic effect with the elapse of time (Table 1). The action of monensin rely on an increase in $[Ca^{2+}]_i$ due to an increased $[Na^+]_i$. Monensin binds Na^+ outside of the cell and carries it into the cell and increases $[Na^+]_i$ and thus $[Ca^{2+}]_i$ (1). Although a Ca^{2+} shift is the primary factor mediating the cellular response of monensin, another factor modifying the cellular response is the pH_i change. Classically an acidosis reduces contractions by inhibiting the Na^+-Ca^{2+} exchange (12, 13), whereas an alkalosis increases contractions by stimulating Na^+-Ca^{2+} exchange and the release of Ca^{2+} from the sarcoplasmic reticulum (SR) (14). Monensin increases the pH_i due to the increased exit of a H^+ when monensin is transported out of the cell causing an increase in the CA (1).

To be able to demonstrate whether monensin-induced increase in $[Ca^{2+}]_i$ is due to an influx or a release from the SR, we measured the ICV and the ACV. There are two components, early (P1) and the late (P2) components, of guinea-pig papillary muscle contraction wave (15). The P1 is suggested to be generated by Ca^{+2} released from SR whereas P2 is thought to be result from Ca^{+2} influx (15). The ICV would be affected by the change in P1 and the ACV would be affected by those factors that produce both P1 nd P2 of the contraction wave (16). An increase in the rate of Ca^{2+} release from the SR would increase the ICV. The positive inotropic effect of monensin is thought to be mediated by an increase in activator Ca^{2+} which enters the cell and causes the release of Ca^{2+} from the SR. Seven μmol/l monensin increased the ICV and the ACV of guinea pig papillary muscles at T1 and did not lose its effect with the elapse of time (Table 2 and 3). This result indicated that monensin might increase the rate of Ca^{2+} release from the SR in guinea pig papillary muscles. However, more studies are needed.

The IRV is affected by two mechanisms. First, a SR Ca^{2+} -ATPase that pumps Ca^{2+} into the SR thus decreases $[Ca^{2+}]_i$. Second, a Na^+(in)/Ca^{2+} (out) exchange (17). Under steady-state conditions, the amount of Ca^{2+} entering the cell equals that which exits the cell, and the amount released by the SR equals that sequestered by the SR (17). If an abrupt change in this balance of fluxes takes place, then the Ca^{2+} content of the SR will be affected. Seven μmol/l monensin increased the IRV of guinea-pig papillary muscles at T2 (Table 4). This increase in IRV was due to the SR Ca^{2+}-ATPase and the Na^+(in)/Ca^{2+} (out) exchange which would be stimulated when $[Ca^{2+}]_i$ increased in order to lower the increased $[Ca^{2+}]_i$.

It is concluded that although the therapeutic range of monensin is very narrow, it should be considered as an alternative drug for the treatment of congestive heart failure (CHF). It is also concluded that increased contraction velocity, as compard the control, is important; as an increased contraction velocity acts as an decreased afterload to a functional heart and can be an important contributor to heart failure. However, more studies are needed to demonstrate the effectiveness of monensin on the treatment of congestive heart failure.

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