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MECHANISMS OF TENSION DEVELOPMENT INDUCED BY MONENSIN IN GUINEA-PIG AORTA : EFFECTS OF VERAPAMIL, OUABAIN, PRAZOSIN AND GLIBENCLAMIDE

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ABSTRACT :

The influence of monensin (10 µmol/L) alone and in the presence of verapamil (3 µmol/L) or ouabain (1 mmol) and prazosin (1 µmol/L) or glibenclamide (10 µmol/L) were studied on the muscle tension of guinea pig aorta. Changes in tissue sodium, potassium and calcium ion contents of the aortic muscle produced by monensin in the presence of prazosin were evaluated. Monensin in normal Tyrode's solution containing prazosin caused an increase in the resting tension followed by a decrease and returned to normal values. Verapamil reduced the muscle tension induced by monensin. After ouabain, monensin induced sustained increase in the resting tension. Glibenclamide partially reversed the relaxant phase of monensin in presence of norepinephrine. The findings of the present study indicate that the contraction induced by monensin in guinea pig aorta is due to the increased influx of calcium through voltage-dependent calcium channels as well as through Na^+ - Ca^{2+} exchange mechanisms. Monensin produced relaxation of guinea pig aorta in part through the increase in K^+ permeability via opening of K^+-ATP channels.

INTRODUCTION

Ionophores are useful biological tools for studying events associated with ion transport (1). In addition, some ionophores have been suggested as desirable drugs for treating low cardiac output syndrome. Among the ionophores, monensin is a member of the neutral subclass of ionophores (2). Monensin transports sodium ion mainly and to less extent potassium ion, down their concentration gradients across biological membranes (1).

It was reported that, monensin had a triphasic response on aortic

blood pressure. Arterial pressure falls bellow control value after monensin injection, followed by a gradual rise in blood pressure, and the aortic blood pressure then slowly returnes to control level (3). In addition, monensin induced a transient increase in tension of guinea pig aorta (4).

The aim of the present work is to study the mechanism (s) by which monensin induced its action on the smooth muscle represented by aorta of the guinea pig. The effects of verapamil Ca^{2+} free solution, ouabain, norepinephrine as well as prazosin and glibenclamide were studied on the muscle tension in presence of monensin, and tissue electrolytes were estimated to clarify the possible mechanism (s).

MATERIAL AND METHODS

1. Preparations :

The thoracic aorta was isolated from guinea-pigs (250-400 gm) killed by a below on the neck. for tension experiments, the aorta was cut into rings which were ligated at both ends with a fine silk suture. For the experiments of anion content, the aorta was cut into rings weighing 15-20mg (4).

2. Composition of solutions :

Normal Tyrode's solution contained mM : NaCl (136.9), KCl (5.4), $MgCl_2$ (0.5), NaH_2PO_4 (0.42), $NaHCO_3$ (11.9), $CaCl_2$ (1), glucose (5.6). In case of calcium free Tyrode's solution, it has the same composition as in normal Tyrode's solution (except calcium chloride) and contained in addition EGTA 5mM.

3. Measurement of tension :

Rings from guinea-pig aorta were suspended vertically in an organ

baths (5ml). One end was attached to an inductive force displacement transducer, the other end was recured to a glass rod support. The output of the inductive force displacement transducer was fed to a hellige carrier frequency preamplifier in conjunction with a low frequency pass filter. The muscle was then streteched by a 10 m/V force and relaxation to a new steady state was achieved within 60 min. in normal tyrode's solution. During this period, Tyrode's solution was replaced at least every 15 min. Bathing solutions were bubbled with carbogen (95% O_2 and 5% CO_2) and had pH 7.4 at 37°C (4).

4. Tissue electrolyte contents :

Tissue Na^+,K^+ and Ca^{2+} contents were estimated as follows: samples of aorta were weighed and dried at 105°C in hot oven for 36 hr. then ashed at 600°C in a muffle furnace (Heraeus KR 170) and the ash was then dissolved in distilled water and used for determination of Na^+, K^+ and Ca^{2+} content using specific kit for each ion.

5. Drugs :

The drugs used were ouabain (Serva, Heidelberg, Germany) verapamil (Knoll, Ludwigshafen, Germany), glibenclamide (Sigma, Deisenhafen (FRG), prazosin (Pfizer, Karls ruhe, Germany), monensin (Sigma, Deisenhafen, Germany) and norepinephrine (Serva, Heidelberg, Germany).

Statistical calculations :

The data were expressed as Mean ± SEM. Student's "t" test was used for statistical calculation.

RESULTS

1. Effect of monensin on resting tension of guinea-pig aorta :

In the first series of experiments, we examined the action of monensin (10 µmol/L) on contractility of guinea-pig aorta. As shown in figures (1), in normal Tyrode's solution containing prazosin (1 µmol/L), the addition of monensin (10 µmol/L) caused an increase in the resting tension 4.14±0.52 mN followed by a decrease and reached to normal values within 30 min. In the next series of experiments, the effect of monensin on the contraction was investigated in calcium free Tyrode's solution containing EGTA (5 mmol/l) and prazosin (1 µmol/L). Under these conditions, addition of monensin (10 µmol/L) had no effect on the resting tension of muscles. When the muscles were pretreated with verapamil (10 µmol/L) in normal Tyrode's solution containing prazosin (1 µmol/L), further addition of monensin (10 µmol/L) induced small increase in resting tension (1±0.17 mN) which returned to normal resting tension level within 15 min.

2. Effect of monensin on resting tension of guinea-pig aorta in the presence of ouabain :

We have shown so far that monensin (10 µmol/L) induced transient increase in tension of guinea-pig aorta in normal Tyrode's solution. It is not known why monensin induced a transient increase in tension of guinea-pig aorta. This effect of monensin may be due to stimulation of Na/K pump activity which may induce a hyperpolarization of the muscle. We have therefore investigated the effect of monensin on tension of guinea-pig aorta previously pretreated with ouabain. As shown in Figure (2), addition of ouabain (1 mmol/L) induced an increase in resting tension of guinea-pig aorta. Under these conditions, further addition of monensin

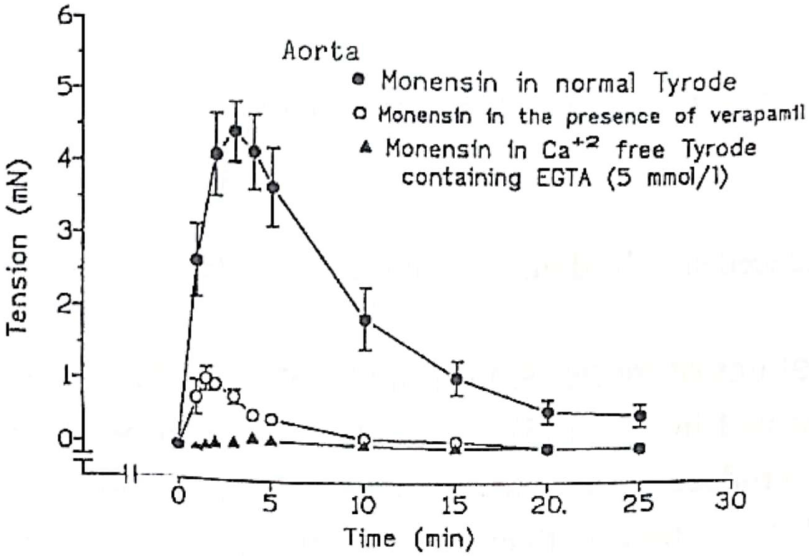

Figure (1). Influence of monensin (10 umol/1) on muscle tension in guinea-pig aort: a- in normal Tyrode's solution (closed circles), b- in normal Tyrode's solution containing verapamil (3 umol/1) (open circles), and c- in calcium free Tyrode's solution containing EGTA (5 mmol/1) (closed triangles). All experiments are carried out in the presence of prazosin (1 umol/1). Ordinate represents absolute values of tension in mN. Data are shown as mean ± S.E.M.

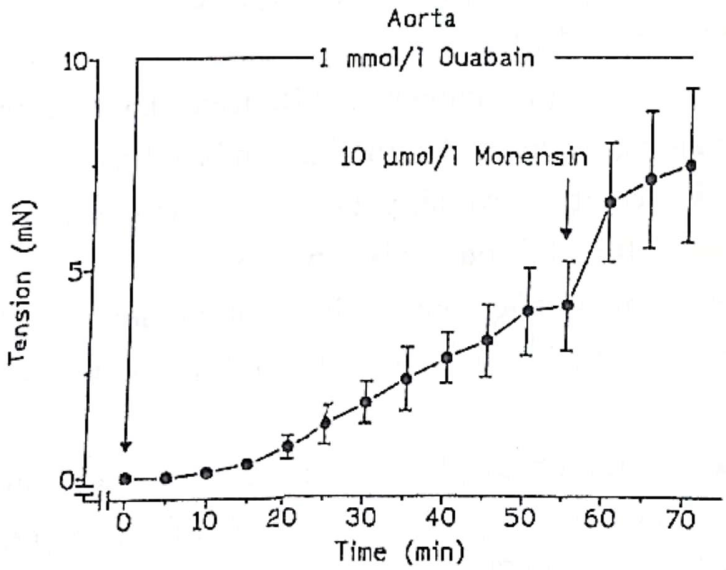

Figure (2). Effect of monensin (10 Umol/1) on muscle tension in guinea-pig aorta in the presence of ouabain (1 mmol/1) and prazosin (1 Umol/1). Ordinate represents absolute values of muscle tension in mN. Note that in the presence of ouabain, the increase in tension induced by monensin is not transient.

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(10 µmol/L) induced sustained increase in the resting tension.

3. Relaxant effect of monensin on guinea-pig aorta :

As represented in figure (3), EC50 of norepinephrine (1 µmol/L) was added to produce a sustained contraction of guinea-pig aorta in normal Tyrode's solution. Further addition of monensin (10 µmol/L) induce increase in tension (152±9%) of maximum response to norepinephrine) followed by a decrease in tension which reached to (55±10% of maximum response to norepinephrine). Under these conditions, further addition of glibenclamide (10µmol/L) partially reversed the relaxant effect of monensin from 55±10% to 80±9% of maximum norepinephrine ($P<0.05$).

4. Effect of monensin (10 µmol/L) on electrolyte changes in the tissues of guinea-pig aorta :

As demonstrated in Fig. (4), monensin (10 µmol/L) in normal Tyrode's solution containing prazosin (10 µmol/L), induced a significant increase of sodium tissue contents, while, potassium tissue contents was significantly decreased. In addition, calcium tissues contents was significantly increased 5 min. after the addition of monensin. Then, calcium tissue contents was gradually decrease toward the control values as shown in Fig. (5).

On the other hand, addition of glibenclamide, 100 min. after the application of monensin, induced a significant increase of potassium tissue contents from 66.2±3.7 mEq/kg to 79±3.0 mEq/kg.

DISCUSSION

As demonstrated in Figures (1 & 2), when monensin was added to isolated guinea-pig aorta in normal Tyrode's solution in the presence of

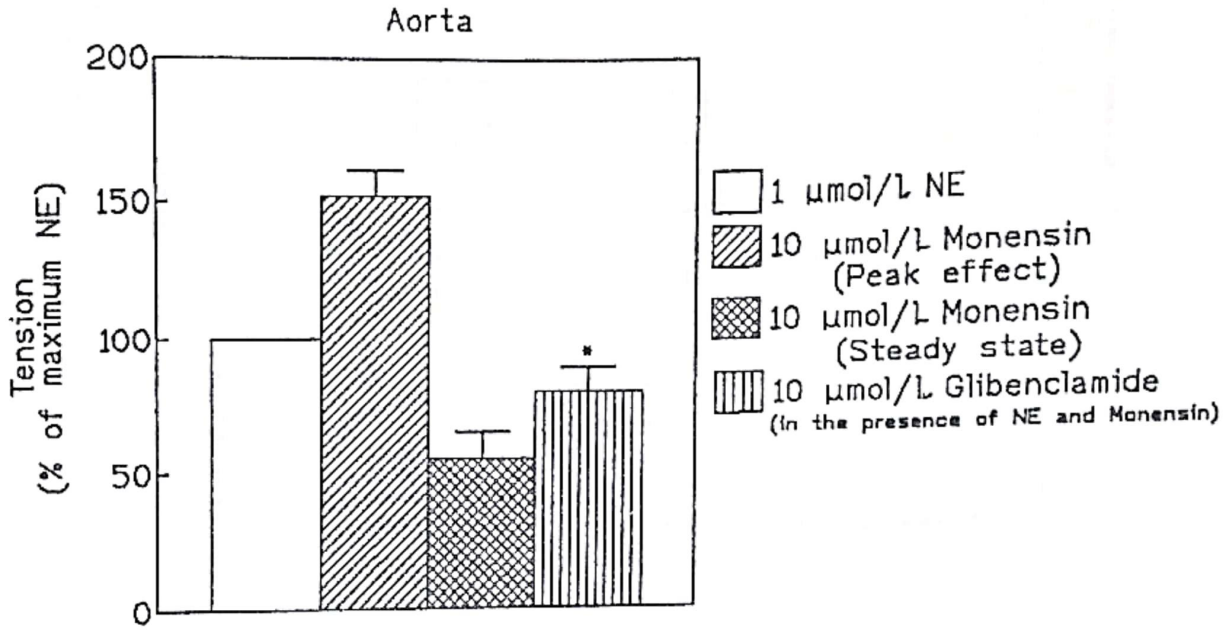

Figure (3). Relaxation in response to monensin. Helical strips from guinea-pig aorta were precontracted with nor-epinephrine (EC_{50}, 1 µmol/l) before addition of monensin. Relaxation to monensin is expressed as a percentage of contractile response to nor-epinephrine. Data are shown as mean ± S.E.M. of four experiments. * Significant difference from the relaxant effect induced by monensin.

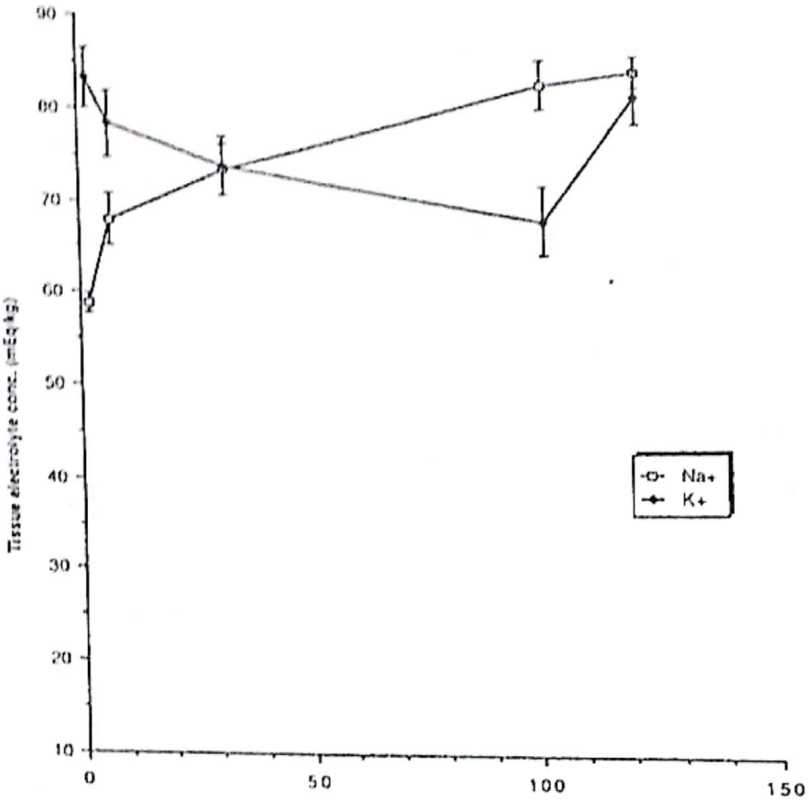

Fig. (4): Changes in tissue sodium and potassium content of guinea pig aorta produced by monensin(10 umol/1) in Tyrode's solution containing prazosin (1 umol/1)

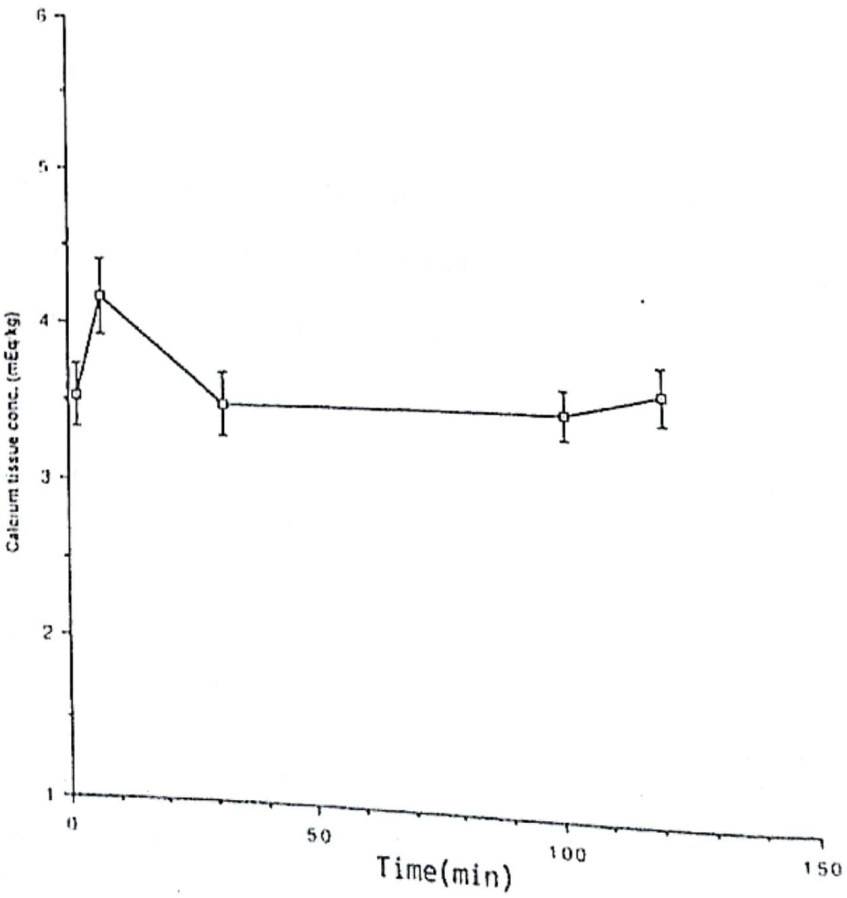

Fig. (5): Change in tissue Calcium conc. of guinea pig aorta produced by monensin (10 umol/1) in normal Tyrode's solution containing prazosing prazosin (1umol/1)

prazosin, there was a transient increase in tension during the observation period. Our results are in line with results of (4), who reported that monensin in normal K^+ solution induced rapid increase in tension followed by slow relaxation in guinea-pig aorta.

In the present study, monensin induced a significant increase in intracellular sodium content and at the same time decreased intracellular potassium content. This observation is in a good agreement with results of (4). These observations lead to suggestion that the contraction induced by monensin is determined by change in the sodium and potassium gradient across the cell membrane. There are two possible mechanisms :

1- An increase in calcium influx due to membrane depolarization and opening of voltage-sensitive calcium channels.
2- An increase in calcium influx and/or decrease in calcium efflux through Na^+ - Ca^{2+} exchange mechanism.

The first possibility is supported by the fact that verapamil reduced the contraction induced by monensin to about 25% of control Present study). In arterial smooth muscle, organic calcium antagonists like verapamil specifically inhibit the voltage-sensitive calcium channels (5) but not the calcium entry through Na^+ - Ca^{2+} exchange mechanism (6-7). In addition it was reported that the intracellular stores of Ca^{2+} in smooth muscle are relatively spare (8). Consequently, much of the Ca^{2+} necessary to activate the contractile mechanism enters the cell during the action potential.

Furthermore, in the presence of verapamil, the muscle strips still produced contraction. This verapamil-insensitive component of contractioay have been the result of increase in calcium influx and/or decrease in calcium efflux through a Na^+ - Ca^{2+} exchange mechanism following the decrease in the sodium gradient. The existance of a Na^+ -

Ca^{2+} exchange mechanism has been demonstrated in guineapig aorta (6-7) as well as in other smooth muscles (9-11).

On the basis of the present results, the contraction induced by monensin in guinea-pig aorta exhibits the following properties :

1- Monensin induced contraction in the presence of α-adrenoreceptor blocking agent.
2- Monensin had no effect on muscle tension in calcium free Tyrode's solution containing EGTA (5 mmol/L).
3- In the presence of organic calcium antagonist "verapamil" in normal Tyrode's solution, the effect of monensin on muscle tension of guinea-pig aorta was reduced to about 25% of control value.

These findings indicate that the contraction induced by monensin, in guinea-pig aorta, is due to the increased influx of calcium through voltage-dependent calcium channels as well as through Na^+ - Ca^{2+} exchange mechanism.

On the other hand, the decrease in tension observed after the contraction peak can be attributed to stimulation of Na-K pump. As the inhibition of Na-K pump by addition of ouabain and further addition of monensin induced sustained increase in tension (Present study). Na-K pump contributes in resting membrane potential in smooth muscle by about 20 mV (12). Stimulation of Na-K pump leads to an increase in resting membrane potential which may lead to a decrease of calcium influx through voltage-dependent calcium channels or Na^+ - Ca^{2+} exchange. furthermore, it has been reported that stimulation of the Na-K pump results in membrane hyperpolarization, muscle relaxation, and decreased cellular excitability, whereas inhibition of the Na-K pump results in membrane depolarization, muscle contraction, and increased cellular excitability (13).

Potassium channels that are inhibited by intracellular ATP are

present in vascular smooth muscle (14-16). These K^+ channels (K-ATP) are normally closed at physiological intracellular ATP concentration and open upon a diminuation of intracellular ATP concentration (17). Stimulation of ATP-dependent K^+ channel results in membrane hyperpolarization and relaxation of arterial smooth muscle, this hyperpolarization and relaxation can be reversed by glibenclamide (16).

The data in Fig. (3) show that monensin induced relaxation of guinea-pig aorta precontracted by norepinephrine. This relaxation was partially reversed by the addition of glibenclamide. glibenclamide is a potent blocker of K-ATP channels. A large number of pharmacological studies have demonstrated a competitive interaction of glibenclamide with the response to cromakalm (K-ATP channel opener) in vascular tissues (18-20).

In the present study, glibenclamide had partially reversed the relaxant effect induced by monensin in guinea-pig aorta. This leads to suggestion that monensin produced relaxation of guinea-pig aorta in part through the increase in K^+ permeability via opening of K-ATP channels.

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ميكانيكية الشد الناتج بالمونتسين في اورطى خنزير غينيا : تأثيرات الفيرابااميل والأوابين والبرازوسين والجليبنكلاميد

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فى هذا البحث تم دراسة تأثير عقار المونتسين منفردا وفى وجود الفيرابااميل او الاوابين مع البرازوسين والجليبنكلاميد على شد عضلات الاورطى فى خنزير غينيا. كما تم دراسة التغيرات فى محتوى أيونات الصوديوم والبوتاسيوم والكالسيوم فى عضلة الأورطى وذلك فى وجود المونتسين والبرازوسين.

وقد اظهرت نتائج البحث ان المونتسين فى محلول تيرود العادى المحتوى على البرازوسين يزيد الشد الطبيعى ويتبع ذلك تناقص فى مستوى الزيادة لتصل الى الحد الطبيعى ، وقد قلل الفيرابااميل الشد الحادث بالمونتسين ، وفى حالة الأوابين فإن المونتسين يحدث زيادة مستمرة فى مستوى الشد العضلى. ولكن عقار الجليبنكلاميد يعكس جزئيا مرحلة الإرتخاء الحادثه بالمونتسين فى وجود النورادرينالين.

من خلال نتائج البحث يمكن استنتاج ان الإنقباض الناتج بالمونتسين فى عضلة الأورطى يعزى زيادة دخول الكالسيوم من خلال قنواته الحساسة للفولت وايضا من خلال تبادله مع أيون الصوديوم ، بينما الإرتخاء الناتج فى المونتسين يمكن ان يكون على الأقل جزئيا نتيجة لزيادة مرور البوتاسيوم فى قنواته الحساسه للأدينوزين ثلاثى الفوسفات.