

**Action of Mg²⁺-ions on K⁺-contractures and field stimulation (FS)
responses of locust foregut and hindgut visceral muscles.**

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ABSTRACT: In $2mM Ca^{2+} + Mg^{2+}$ -saline the responses of $100mM K^+$ of locust foregut and hindgut muscles were decreased, whereas in $2mM Mg^{2+}$ -media (Ca^{2+} -free), the K^+ -contractures were demolished and the inhibition was stronger on hindgut muscle. This indicates that K^+ -contractures of both guts depend on $[Ca^{2+}]_o$. In field stimulation (FS), the responses of both gut compartments were inhibited in $2mM Mg^{2+}$ -saline. In addition, these responses of locust hindgut were decreased and disturbed in $2mM Ca^{2+} + Mg^{2+}$ -saline. However, the foregut muscle base line tension was lowered and FS-responses were increased. Addition of $10^{-4} M$ 5-HT in $2mM Mg^{2+}$ -saline (Ca^{2+} -free), lowered the base line tension but had no effect on FS-responses of the foregut muscle, while it significantly increased the hindgut muscle responses. On the other hand, the FS-responses of both gut divisions in $2mM Ca^{2+} + Mg^{2+}$ -saline increased at $10^{-4} M$ 5-HT slightly. These results suggested that both divalent ions compete with each other on the cellular membrane, therefore, Mg^{2+} -ions can not replace Ca^{2+} -ions in these muscles. These results confirm previous study of Mutwally (1994) that both gut responses are dependent upon $[Ca^{2+}]_o$ and also Ca^{2+} / Mg^{2+} -pump of both guts may be disturbed. Moreover, the present study indicates that the responses of both muscles in Ca^{2+} -free saline and in mixed media may be due to $[Ca^{2+}]_i$ and to the reactive sites themselves and their use in an inhibition of Mg^{2+} -ions in plasma membrane and in cellular Ca^{2+} -binding loci. Such action may cause complete excitation/contraction EC-uncoupling. Serotonin (5-HT) failed to induce any response in $2mM Mg^{2+}$ -media (Ca^{2+} -free) of

the foregut which indicate that this amine depends on $[Ca^{2+}]_o$ and the competitive action of Mg^{2+} -ions to Ca^{2+} -ions on the cellular membrane, whereas in locust hindgut muscles, 5-HT induced FS-responses which could be via $[Ca^{2+}]_i$. It can also be concluded that 5-HT possibly acted as normal autonomic transmitter/modulator especially on the foregut. After washout with normal saline ($2mM Ca^{2+}$), both muscle preparations quickly resumed their activities. This might be due to fast sequestration of Ca^{2+} on cellular membrane and intracellular organoids. These results revealed clear differences in function between these two gut compartments.

GENERAL INTRODUCTION

It is well known that all muscle cells, be they from skeletal; cardiac and visceral muscles, have a basically similar organization, when considered in the most simple manner. Contractile activity is mediated by metabolically active cells. The specific membrane conductance or permeability to an ion is highest at its equilibrium potential, when the electrochemical driving force is zero (Huddart, 1975; Huddart & Hunt, 1975).

The membrane capacitance of visceral muscle is rather low compared to that of the skeletal muscle. The difference in capacitance is almost certainly related to the T-system, which is well developed in skeletal muscle and usually completely absent in visceral muscle; with the exception of arthropod gut muscles (Huddart & Hunt, 1975). Intracellular microelectrode techniques confirmed that contraction of a muscle fibre would occur only after the cell was first activated by a depolarizing electrical disturbance of the plasma membrane- whether this be caused by a naturally-occurring action potential or graded response, by artificially-applied stimulation, or by increased KCl-level (Huddart, 1975; Huddart & Hunt, 1975).

As in skeletal muscles, visceral muscles are activated by a rise in myoplasmic free Ca^{2+} , and it seems probable that the great bulk of this activating Ca^{2+} has to be translocated from surface sites (either from the extracellular fluid through the plasma membrane or from storage sites immediately below the surface) (Syson, 1974; Huddart, 1975 & 1985). This procedure is called CIRC (Huddart, 1985; Mutwally, 1990). It was known that muscle contractures due to KCl-depolarization are

mediated via an increase in myoplasmic free Ca^{2+} (Syson, 1974). The depolarization of the SR induces a rapid release of its bound Ca^{2+} in both amphibian (Endo & Nakajima, 1973), mammalian (Miyamoto & Kasai, 1973) and insect (Huddart & Oates, 1970; Huddart, 1975; Dunbar, 1980; Mutwally, 1990).

Carvalho (1966 & 1968) showed that the reticulum possessed powerful Ca^{2+} -pumping activity, exchangeably binding Ca^{2+} for Mg^{2+} at reticular $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase binding sites under various conditions. He concluded that cations also have a major effect at a later stage of coupling, at the level of the SR. Plasma membrane fraction possesses considerable power of Ca^{2+} -sequestration compared with the mitochondria fraction. Mitochondria play some role in Ca^{2+} -translocation during EC-coupling in visceral muscles, but this role may be only a general 'mopping up' during relaxation. The greater binding tenacity of the plasma membrane vesicular fraction indicates that it is this fraction which may be responsible for the ultimate pumping of sarcoplasmic Ca^{2+} down to the levels low enough to permit relaxation (Huddart & Hunt, 1975).

In insect neuromuscular system, both Ca^{2+} - and Mg^{2+} -ions have pre- and postsynaptic effect (Hoyle, 1955). In addition, traces of Ca^{2+} and Mg^{2+} -ions were bound to amino acids in locust haemolymph (Duchateau, et al, 1953; Clements & May, 1974). Kitada (1994) reported that, in frog glossopharyngeal nerve, the enhanced Mg^{2+} -response and the enhanced Na^{+} -response were greatly inhibited by the addition of Ca^{2+} -ions, and the enhanced Ca^{2+} -response was inhibited by the addition of Mg^{2+} - or Na^{+} -ions, suggesting that competitive antagonism occurs between Ca^{2+} and Mg^{2+} -ions and between Ca^{2+} and Na^{+} -ions in the presence of Ni^{2+} -ions. Mutwally (1994) showed clear functional differences between locust foregut and hindgut muscles; due to structural and innervation variations. He also concluded that both gut responses are dependent upon $[\text{Ca}^{2+}]_o$ and also $\text{Ca}^{2+}/$

Mg²⁺-pump of both guts may be disturbed. Moreover, he found that 5-HT has slight effect on both gut divisions in mixed saline, but has no effect in Mg²⁺-media (Ca²⁺-free). The aim of this study is to extend previous results and to explore our view of Mg²⁺, and the possible involvement of Mg²⁺/Ca²⁺-ions counterexchange in locust foregut and hindgut visceral muscle in terms of excitation/contractions-coupling (EC-coupling) using 100mMK⁺ and field stimulation techniques.

MATERIALS AND METHODS

Adult locust *Locusta migratoria* of both sexes, reared in laboratory culture were used throughout this study. The details of the dissection of the isolated foregut and hindgut muscles, the salines used, the methods to record tension, K⁺-responses and field stimulation (FS) have all been described in the preceding papers (Oldfield & Huddart, 1982; Mutwally, 1990; Mutwally & Jamel Al-Layl, 1992 & 1993; Mutwally, 1994). All the drugs and chemicals used in this study were obtained from Sigma Chemical Co., and were added to the organ baths from standard laboratory concentrates and freshly made up in distilled water just before use. Dose-response curves for K⁺-contractures, 5-HT and FS-responses in Mg²⁺- and Mg²⁺ + Ca²⁺-saline, were determined by a series of separated drug trails and not by serial addition. Data presented in this study show the mean, the standard error and number of replicates.

RESULTS

1- The effect of K⁺-contractures in Mg²⁺- and Mg²⁺ + Ca²⁺-saline:

In Figures 1 and 2, both locust foregut (A & B) and hindgut (C & D) visceral muscles responded to initial 100mMK⁺-additions (control) in normal saline (2mM Ca²⁺). However, in a combination of salines (2mM Ca²⁺/Mg²⁺), 100mMK⁺-contractures of both muscles were decreased gradually. This treatment affected both the phasic- and the tonic-responses. On the contrary, Ca²⁺-free media severely inhibited 100mMK⁺-responses. The inhibitory effect was stronger on the hindgut muscles. Both muscles resumed their activities after washout with normal saline.

2- The effect of Mg^{2+} - and $Mg^{2+} + Ca^{2+}$ -saline on FS-responses:

Figures 3 and 4 show that FS-responses of both gut compartments were abolished gradually in $2mM Mg^{2+}$ -saline. Both guts resumed their activities after washout with normal saline. However, these responses were increased and the base line tension of foregut muscle was lowered in $Ca^{2+} + Mg^{2+}$ -media. FS-responses were increased compared with control, after washing with normal saline, but FS-responses of hindgut muscles were inhibited and disturbed.

3- The effect of 5-HT on FS-responses in Mg^{2+} - and $Mg^{2+} + Ca^{2+}$ -saline:

Figures 5 and 6 show that in $2mM Ca^{2+} + Mg^{2+}$ -media, 5-HT (10^{-4} M) lowered the base line tension of locust foregut muscle and slightly increased FS-responses of both locust muscles. However, in $2mM Mg^{2+}$ -media (Ca^{2+} -free), 5-HT (10^{-4} M) had no effect on the foregut FS-responses, whereas it increased FS-responses of the hindgut muscle. The FS-responses of both gut divisions quickly resumed their activities after washing with normal saline and the responses were stronger and higher than the control, the base line tension also returned to normal.

All data presented here (Figures 2, 4 & 6) were plotted as a percentage of control responses. Each point is a mean of eight replicates (\pm S.E. of mean of both gut muscles).

DISCUSSION

The locust foregut and hindgut muscles share with vertebrate heart and bladder the property of being a distensible hollow organ capable of functioning as a pump. Almost all work on EC-coupling has been carried out on skeletal muscle, and a little on cardiac muscles and visceral muscle was far behind (Huddart, 1975; Huddart & Hunt, 1975). Dunbar (1980) and Mutwally (1990) have studied in detail structural, innervation and function of locust hindgut and foregut muscles respectively. Mutwally and Jamel Al-Layl (1992 & 1993) concluded that K^{+} -induced muscle depolarization and FS allow to study the activity of any slow voltage-dependent Ca^{2+} -channels along with their modulation by drug additions. This technique was applied to extend previous studies and to establish the role of Mg^{2+} -ions in locust

visceral muscles (Mutwally, 1994). Huddart and Butler (1986) and Mutwally (1990) reported that FS-techniques provide another Ca^{2+} -dependency of both locust gut divisions. Moreover, locust foregut possesses Ca^{2+} -channels which are analogous to those of mammalian muscle in terms of their voltage inactivation/activation (Mutwally, 1990). However, electrophysiological studies of locust hindgut indicated that action potential was generated by labial pacemaker areas (Dunbar, 1980). Natural activities, K^+ -contractures and FS-responses of locust both gut muscles are dependent upon $[\text{Ca}^{2+}]_o$ (Dunbar, 1980; Mutwally, 1990; Mutwally & Jamel Al-Layl, 1993). The results of this present study also go in harmony with the previous studies and confirm the above conclusions.

Different visceral muscles differ in the relative balance of mitochondria/surface vesicles, and in some muscles the mitochondria may play a more significant role than that found in rat ileal muscle (Somlyo, 1972). It may well be that Ca^{2+} -storage within visceral muscle cells is low, and that the binding activity of 'reticulum' or plasma membrane vesicles or mitochondria, or all of these agencies, may be adequate to reduce myoplasmic Ca^{2+} (Huddart & Hunt, 1975). The effective concentrations of cations needed in insect muscle are somewhat higher (about 0.5mM) (Huddart, 1968), but this may merely reflect the rather poor penetration of externally applied agents into insect muscle (Huddart, 1975).

Since EC-coupling is a circuitous process involving a series of separate but interlinked stages, a variety of physical and chemical treatments can disrupt coupling by modifying or suppressing one or more of the stages in the cycle (Huddart, 1975). To cause relaxation *in vivo*, the reticular Ca^{2+} -pump must be able to reduce sarcoplasmic Ca^{2+} -level to below this 10^{-7} M level from at least 10^{-6} M level; in normal state, the pump becomes reactivated when the membrane potential has returned to normal negative polarity either by an electrotonic flow change in SR or via pH change of the sarcoplasm as suggested by Nakamaru and Schwartz (1970). Aidley (1965) concluded that, in locust leg muscle, Ca^{2+} is probably uniquely

important in EC-coupling. He showed that divalent-ions and other substitute cations act by displacing Ca^{2+} from some site at which it is bound in the muscle.

Fuch, et al (1968) showed that cardiac SR accumulates Ca^{2+} in the presence of ATP and Mg^{2+} -ions, and this accumulating ability is reduced in the presence of Na^+ -ions. Francois, et al (1993) concluded that there was a competitive behaviour between Ca^{2+} and Mg^{2+} -ions in eel skeletal muscles. It appears that cations are able to selectively block the rapid K^+ -efflux from muscle, the action responsible for normal repolarization (Huddart, 1968 & 1975). In this study, K^+ -responses were severely inhibited in Mg^{2+} -saline, and were decreased in $\text{Ca}^{2+} + \text{Mg}^{2+}$ -saline. This indicates that K^+ -contractures are dependent on $[\text{Ca}^{2+}]_o$. Sando and Isaacson (1966) concluded that the primary action of cations in EC-coupling was at some readily accessible membrane sites such as plasma membrane or the T-tubules or SR (Carvalho, 1968). Carvalho and Leo (1967) reported that the enormous Ca^{2+} -binding shown by the reticulum in the presence of ATP takes place by a displacement of cations such as Mg^{2+} and K^+ . They suggested that the total cations binding capacity of the reticulum is fixed and not transiently variable. Although the binding sites will bind Ca^{2+} , Mg^{2+} , K^+ and H^+ -ions, the nature of what is actually bound or replaced varies with pH.

Both locust foregut and hindgut muscles *in vitro* induced a rapid, frequency-dependent contraction with maximum responses at $2\text{mM}\text{Ca}^{2+}$ (normal saline), and also they responded to FS. This contraction was partly decreased by a combination of $2\text{mM}\text{Ca}^{2+} + \text{Mg}^{2+}$ -saline and was totally blocked by $2\text{mM}\text{Mg}^{2+}$ -saline (Ca^{2+} -free). This was due to stimulation of the excitable membrane, and probably due to a direct activation of the EC-coupling mechanisms. Pretreated muscles with 5-HT exhibited multiphasic response to FS. After washout with normal saline, FS-responses of both treatments of both preparations returned to normal, but over quite different time scales. Potassium-induced depolarization, which inactivate fast Ca^{2+} -

channels. Field stimulation-responses persisted and additively superimposed upon the K^+ -induced tension, suggesting that FS-induced depolarization had activated slow voltage-dependent Ca^{2+} -channels (Huddart & Butler, 1986). In this study, Mg^{2+} -saline eliminated FS-responses, but a combination media of $Ca^{2+}+Mg^{2+}$ partly blocked FS-responses of both preparations. These results confirm that both K^+ -contractions and FS-responses are dependent strongly upon a sustained Ca^{2+} -influx through these channels for the sustained release of $[Ca^{2+}]_i$ and the maintenance of contractile force.

Serotonin (5-HT) is well known as an excitatory agent in various animal tissues (for more references see Mutwally, 1990). This agent is active in pharmacological concentrations, in most cases 10^{-7} M being needed to induce the state effect; and at 10^{-3} M, 5-HT reversed the action (Miller, 1975; Mutwally, 1990). A role for 5-HT as a neurotransmitter is suggested by these responses, and by the fact that 5-HT has been found in many insect nervous tissues (Klemm & Axelsson, 1973; Hiripi & S-Rozsa, 1974; Huddart & Oldfield, 1982; Mutwally, 1990 & 1993).

Banner, et al (1986 & 1987) and Mutwally (1990) found that 5-HT caused dose-dependent relaxation of the locust foregut. The foregut of cockroach and locust were consistently more sensitive to 5-HT than the hindgut (Cock & Holman, 1978; Mutwally, 1993 & 1994). Serotonin (5-HT) caused >150% stimulation of Ca^{2+} -efflux from locust foregut and caused slight stimulation effect from locust hindgut (Mutwally, 1990 & 1994). Similar results were found in this present study. In addition, 5-HT had no effect on FS-responses in Mg^{2+} -saline, whereas in $Ca^{2+}+Mg^{2+}$ -saline, FS-responses were increased slightly with 5-HT treatment. The agonist action and the stimulatory effects on Ca^{2+} -efflux and the related contractile responses were abolished in Mg^{2+} -saline and in $Ca^{2+}+Mg^{2+}$ -media. Both locust muscles showed a clear neurochemical dichotomy in response to this agonist (5-HT), both in terms of contractile responses and cellular Ca^{2+} -translocation (Huddart,

1986). Thus, the effect of 5-HT on both K^+ -contractions and FS-responses resulted from a rise in cellular free Ca^{2+} . The mechanism for elevating activator Ca^{2+} during EC-coupling appears to be an agonist-induced influx of Ca^{2+} from the external medium via voltage-dependent slow Ca^{2+} -channels. This inward Ca^{2+} -signal is secondarily amplified by the triggering of Ca^{2+} -release from cellular storage sites (Huddart, 1986; Mutwally, 1990 & 1994). This conclusion goes in harmony with this present study.

The result of this study suggested that the combination saline slightly stimulated Ca^{2+} -binding by microsomes and mitochondria, while Mg^{2+} -saline inhibited Ca^{2+} -binding by both sub-cellular fractions in both gut divisions. Moreover, the inhibitory action of Mg^{2+} on these muscles appears to be due to inhibition of Ca^{2+} -influx coupled with some stimulation of intracellular binding. In addition, 5-HT excitatory action appears to be related to stimulation of Ca^{2+} -influx and inhibition of cellular Ca^{2+} -binding. At 10^{-4} M, 5-HT increased the frequency and amplitude of contraction and general tonus of isolated locust foregut muscle; while hindgut muscles were less sensitive to 5-HT. The 5-HT-receptor populations in these two gut compartments were essentially homogenous. Both gut divisions have remarkably different pharmacological properties, reflecting differences in innervation and extrajunctional monoamine receptor affinities (Huddart & Oldfield, 1982; Mutwally, 1990 & 1994).

The plasma membrane may constitute a major Ca^{2+} -pool for the activation of contraction in an annelid visceral muscle (Alohan & Huddart, 1981a & b) and in locust foregut and hindgut muscle (Dunbar, 1980; Mutwally, 1990). In rat ileal smooth muscle, Mg^{2+} -free saline stimulated ^{45}Ca -efflux in the presence of $[Ca^{2+}]_o$, but in Ca^{2+} -free conditions, Mg^{2+} -free media inhibited ^{45}Ca -efflux, indicating the presence of a Mg^{2+} -dependent Ca^{2+} -efflux. Evidently, Ca^{2+} -

regulation in rat muscle depends upon counterexchanges with Na^+ and Mg^{2+} as well as cytoplasmic buffering (Huddart & Latham, 1981). This would explain the effect of Mg^{2+} -ions in this study. Tension development, in fish intestinal muscle, appears strongly dependent on an influx of $[\text{Ca}^{2+}]_o$ and $[\text{Ca}^{2+}]_i$, and $[\text{Ca}^{2+}]_i$ -fluxes may play only a minor role in the overall Ca^{2+} -utilization during contraction (Barratt & Huddart, 1979). In rat ileal muscle, Mg^{2+} -free media increased tension and enhanced spontaneous contractions, whereas high Mg^{2+} -saline inhibited contractility. This suggests that a linked counterexchange system of Ca^{2+} with Mg^{2+} and Na^+ would seem more likely in rat ileal muscles (Huddart & Saad, 1978). In locust gut muscles, Mutwally (1994) reported similar conclusions which may apply to this present study.

Hubbard, et al (1968) showed that Ca^{2+} and Mg^{2+} competed for the same sites in mammalian neuromuscular junction. Washio and Inouye (1978) suggested that Ca^{2+} and Mg^{2+} -ions acted at the same surface sites in the presynaptic membrane of cockroach leg muscles. These results would imply that Mg^{2+} has at least some ability to trigger spontaneous release of transmitter in a Ca^{2+} -free medium at insect neuromuscular junctions as it was found in frog (Blioch, et al, 1968) and mammalian (Hubbard, et al, 1968) neuromuscular junctions. Fukuda, et al (1977) concluded that in the larval muscle of beetles, the muscle membrane remained non-excitable even in the presence of high $[\text{Mg}^{2+}]_o$ (40mM) unless a certain level of Ca^{2+} -ions was present in the external space. This conclusion goes in harmony with the results of this present study and also indicates that the penetration of Mg^{2+} -ions into the cell is very small or none.

In conclusions, the results of this study show the competitive action of Mg^{2+} -ions to Ca^{2+} -ions. These results suggest that Mg^{2+} -ions can not replace Ca^{2+} -ions in these muscles. In addition, it may give support to Mg^{2+} -ions inhibitory action and

would confirm that Mg^{2+} blocks the Ca^{2+} -channel inhibiting Ca^{2+} -pumping or slows Ca^{2+} -influx, an action that may cause EC-uncoupling. The responses of locust foregut and hindgut muscles are strongly dependent on $[Ca^{2+}]_o$ and probably on Ca^{2+}/Na^+ -counterexchange rather than Ca^{2+}/Mg^{2+} -counterexchange. Moreover, the present results go in harmony with previous studies and more recently studies of Mutwally (1994).

ACKNOWLEDGEMENT

This work was carried out under a kind invitation from Dr. H. Huddart at University of Lancaster, Biology Department, U.K. The author would like to thank him for allowing him using the laboratory; and giving him some of his valuable time for discussion. Also, I thank my colleagues in Computer centres at U.Q.U. in Makkah and K. A. U. in Jeddah. Moreover, I thank Dr.A. Yassin for reading the manuscript and to Mr. W. Amody and Mr. F. Badry for their help.

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FIGURE LEGENDS

Figure 1: Initial K^+ -contractures (control) were made to all traces. The K^+ -contractures (second lowered points) of locust foregut (A & B) and hindgut (C & D) muscles were abolished gradually in $2mMMg^{2+}$ -media (first lowered points) (B & D). Whereas, in $2mMCa^{2+}+Mg^{2+}$ -saline (A & D), the K^+ -contractures were reduced. The muscle resumed their activities after washout (W) with normal saline ($2mMCa^{2+}$). Tension 0.5g, time 2 minutes apply to all traces.

Figure 3: Both locust preparations responded to FS (A & B). In $2mMCa^{2+}+Mg^{2+}$ -saline (lowered points), FS-responses of foregut muscles were increased and the base line tension was lowered, while the responses of hindgut muscles were decreased and disturbed. However, in $2mMMg^{2+}$ -media (lowered points), both gut muscles were severely inhibited. On returning to normal saline (W), both gut compartments resumed their normal FS-responses. Tention 0.5g, time 2 minutes apply to all traces.

Figure 5: Shows the effect of 5-HT (10^{-4} M) (second lowered points) of locust foregut (A & B) (first lowered points) and hindgut (C & D) (first lowered points). In $2mMCa^{2+}+Mg^{2+}$ -media, 5-HT (10^{-4} M) dropped foregut base line tention and slightly increased FS-responses, but was without effect in $2mMMg^{2+}$ -media. However, 5-HT (10^{-4} M), in $2mMCa^{2+}+Mg^{2+}$ -media, slightly increased FS-responses of hindgut muscles. Whereas, in $2mMMg^{2+}$ -media, 5-HT (10^{-4} M) clearly increased FS-responses. Both gut divisions returned to normal after washout (W). Tension 0.5g, time 2 minutes apply to all traces.

Figure 2: The effect of $2mMMg^{2+}$ -media (Ca^{2+} -free) and $2mMCa^{2+}+Mg^{2+}$ -media on $100mMK^+$ -contractures of both locust foregut (O) and hindgut () visceral muscles. The responses were plotted as a percentage of $100mMK^+$ -responses. Each point is the mean of 8 replicates (\pm SE of mean of both gut preparations).

Figure 4: FS-responses of both locust foregut (O) and hindgut () visceral muscles in $2mMMg^{2+}$ -media (Ca^{2+} -free) and $2mMCa^{2+}+Mg^{2+}$ -media. The responses were plotted as a percentage of a control responses. Each point is the mean of 8 replicates (\pm SE of mean of both gut compartments).

Figure 6: The effect of 5-HT (10^{-4} M) on FS-responses of both locust foregut (O) and hindgut () visceral muscles in $2mMMg^{2+}$ -media (Ca^{2+} -free) and $2mMCa^{2+}+Mg^{2+}$ -media. The responses were plotted as a percentage of a control responses. Each point is the mean of 8 replicates (\pm SE of mean of both gut muscle fibr).

أثر المغنسيوم على الانقباضات المستحثة بالبوتاسيوم والمجال الكهربائي للعضلات الملساء لمعي الجراد الأمامية والخلفية .

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الملخص :

أثر الإنقباضات المستحثة بإضافة ١٠٠ مليمول بوتاسيوم لكلا معي الجراد الامامية والخلفية إنخفضت في المحلول المحتوي على ٢ مليمول كالسيوم/مغنسيوم . في حين أنها اختفت في المحلول الخالي من الكالسيوم والمحتوي على المغنسيوم فقط . التأثير كان واضحاً وقوياً على الامعاء الخلفية للجراد . هذا يوضح أن هذه الانقباضات المستحثة لكلا معي الجراد تعتمد على وجود الكالسيوم في المحيط الخارجي .

تحت تأثير المجال الكهربائي ، كلا الانقباضات المستحثة كهربائياً لكلا معي الجراد إنخفضت بشده في وجود المغنسيوم وغياب الكالسيوم من المحلول الفسيولوجي. ولكنها زادت من انقباضات الامعاء الامامية مع إنخفاض في مستوى الشد وذلك في وجود ٢مليمول مغنسيوم / كالسيوم ، إلا أنها سببت اضطراباً وإنخفاضاً في انقباضات الامعاء الخلفية . قد يكون سبب ذلك المنافسة الشديده بين ايونات المغنسيوم و الكالسيوم على الغشاء الخلوي وكذلك على مراكز تجمع الكالسيوم داخل الخلية. نتيجة هذا التثبيط قد يسبب في انفصال الارتباط الوثيق بين عمليتي التهيج و الانقباض العضلي. بالرغم من ذلك فإن الانقباضات العضليه المستحثة بالبوتاسيوم والمجال الكهربائي، التي شوهدت في وجود ايونات المغنسيوم فإن سببها قد يكون عن طريق ايونات الكالسيوم الموجوده داخل الخلية. كلا معي الجراد استعادا نشاطهما بعد غسلهما بالمحلول الفسيولوجي الطبيعي سريعاً . قد يكون سبب ذلك هو سرعه استعادته الغشاء الخلوي والمخازن الداخليه للكالسيوم بعد غسل العضلات من ايونات المغنسيوم المنافسه. نتيجة هذه الدراسه تقترح بأن المغنسيوم لايمكن أن يحل أو ينوب عن الكالسيوم في استحثاث أو استمرار الانقباضات العضليه لمعي الجراد، وذلك للمنافسه الشديده بينهما.

إضافة مركب السيروتونين ($5-HT$) بتركيز 10^{-4} مولار ، أدى إلى إنخفاض في مستوى الشد العضلي إلا انها لم تؤثر على الامعاء الامامية في غياب الكالسيوم ووجود المغنسيوم . ولكنها زادت في انقباضات الامعاء الخلفية، قد يكون سبب ذلك هو ايونات الكالسيوم الموجوده داخل الخلية. وفي وجود المغنسيوم والكالسيوم كان لمركب السيروتونين ($5-HT$) أثر ضعيف على كلا معي الجراد. هذه النتائج تقترح احتمال اضطراب مضخة الكالسيوم/المغنسيوم لكلا معي الجراد ، وكذلك دلت على أن مركب السيروتونين ($5-HT$) يعتمد على الكالسيوم الموجود في المحيط الخارجي ، ويحتمل أن يؤدي وظيفه موصل عصبي ومنظم في الجهاز السمثاوي خصوصاً في الامعاء الامامية للجراد . هذه الدراسه تؤكد النتائج السابقه لمتولي (١٩٩٤)، وكذلك تؤكد على وجود الاختلافات الطبيعيه بين كلا معي الجراد.