

# Tyramine And KCl-Action On The Foregut Muscle Of *Locusta Migratoria*

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**Abstract:** The preparation was first made to contract maximally with 100mM K<sup>+</sup>, and allowed to relax completely after washing in normal insect saline for 30-60 minutes before it was again recorded for spontaneous activity, stimulated with K<sup>+</sup>-contracture and finally field stimulation responses. Tyramine alone was un effective on the foregut muscle, suggesting a lack of either pre- or postsynaptic receptors responding exclusively to this monoamine. The fact that tyramine suppresses myogenic activity in the foregut muscle could imply that this amine may disrupt the electrogenic events that occur in the surface membrane of visceral muscle fibers. Until now, we know little of tyramine distribution in the visceral nervous system or of its release, so to implicate this catecholamine in inhibitory neurotransmission *in vivo* is premature.

**Key words:** Locust, foregut muscle, tyramine, spontaneous activity (Spt. Act), field stimulation (FS).

## Introduction

Inhibitory drugs inhibit the postsynaptic receptors and excitatory drugs excite the neurons, thus directly affecting the nerve-muscle synaptic-transmission. Excitation-contraction coupling describes the sequence of electrical and mechanical processes. In most muscles, the fibers only contract when the cell is first depolarized either by endogenous transmitter-induced action or by artificial stimulation such as that induced by elevated KCl levels or depolarizing square pulses (Huddart and Oldfield, 1982; Oldfield and Huddart, 1982; Mutwally, 1990).

The use of the field stimulation (FS) technique, which produces controllable consistent contractions of locust *Locusta migratoria* foregut, provides another method of examining

the Ca<sup>2+</sup>-dependency of contraction in this preparation. In conjunction with K<sup>+</sup>-induced depolarization, FS will allow the activity of any slow voltage-dependent Ca<sup>2+</sup>-channels to be analyzed (Mutwally, 1990; 1998a and 1998b; Mutwally and Jamel Al-Lyal, 1992 and 1993).

The present study has attempted to extend the previous investigations of the possible role of monoamines as visceral neuromuscular transmitters in locust *Locusta migratoria* foregut as well as carrying out recent techniques in insect physiology which measures the spontaneous activity (Spt. act) of locust foregut muscles as well as the K<sup>+</sup>-contracture and the effects of drugs on 100mM K<sup>+</sup>-contracture. The response induced by the same drugs under FS was also investigated in relation to the effects on the stimulation process and on the foregut visceral muscles.

## Materials and Methods

Adult locusts (*Locusta migratoria*) of both sexes were used throughout this study. The colony was maintained at 25°C-27°C and fed daily on fresh green barley and grass. Water was supplied *ad libitum*. According to Usherwood and Grundfest (1965) and Mutwally (1990) insect saline of the following compositions was used throughout this study (g/l): NaCl 8.12, KCl 0.74, CaCl<sub>2</sub> 0.294, MgCl<sub>2</sub> 0.406, NaH<sub>2</sub>PO<sub>4</sub> 0.936, NaHCO<sub>3</sub> 0.336 and Sucrose 34.2. All drugs were supplied from Sigma Company, dissolved in distilled water and adjusted to pH 6.8 with HCl and NaOH at room temperature. The formula was chosen due to its ability to maintain viable preparations for eight hours and more.

In both experimental techniques (mechanical responses and field stimulation) the whole locust foregut was used. Drugs used in both experiments were, potassium chloride (KCl) and tyramine. All drugs were added to the organ bath from standard laboratory concentrates, and were made up in distilled water as concentrated stock solutions. The drug was directly added to the perfusion baths, so that no more than 0.25ml (1% of bath volume) of the stock was required to yield the desired concentration. Dose-responses curves for tyramine were determined by a series of separate drug trials and not by serial additions (Mutwally 1990 & 1998b; Mutwally & Jamel Al-Lyl, 1993).

The whole foregut was isolated between the proventriculus and the gastric caeca from one side and from the buccal cavity, from the other. The innervation was left intact throughout this study. All preparations were equilibrated at room temperature for at least 30 minutes before records were taken.

To record tension the locust foregut was threaded from both ends by monofilament nylon thread. Two physiological experiments were involved in this study. The foregut was threaded and suspended between the glass hook of a 25ml jacketed organ bath and the cantilever arm of a Grass instruments FT 0.36 force-displacement transducer. The transducer was adjusted in the vertical plane to impose a passive load of approximately (0.2g) on the preparations. All subsequent changes in tension were recorded isometrically on a Grass Instruments 79D polygraph.

To record FS-responses, the foregut was threaded and passed through 2 silver/silver chloride ring electrodes, mounted 10mm apart on a Perspex rod. The electrode assemblies were then placed in a jacketed 25ml organ baths which were continuously aerated with O<sub>2</sub> and whose contents could be rapidly changed. The upper muscle ligatures were attached to a set of Grass FT 0.3 force displacement transducers held in adjustable rack work, and the racks were adjusted to put slight (Cal. 0.2g) tension on the preparations. After mounting in the perfusion baths the preparations were allowed 30-60 minutes to equilibrate, the bath saline being changed every 10-15 minutes. The force displacement transducer outputs were connected to a 4 Channel Grass model 79 Polygraph. The stimulation electrode terminals were attached to Grass SIU S isolation units connected to 2 Grass S48 stimulators driven in tandem. Stimulation train parameters varied in different preparations but common regime consisted of a 3000 ms (train durations) train of pulses at 80 HZ (PPS, stimulation rate), each pulse being 1ms (delay), at 60-120 Volts. Trains were automatically repeated usually at 60 sec intervals.

To prepare the preparation for the experiment, 100mM  $K^+$  was added in the organ bath to depolarize the preparation which was then left for about 30-60 minutes to relax. The Spt. act was recorded as well as the normal FS. In Spt. act and 100mM  $K^+$ -responses, tyramine was added in the organ bath then in 2-4 minutes later 100mM  $K^+$  was added. However, in FS experiments, preparations left to response to FS for about 2-4 minutes to create a control for each single experiment. Drugs then were added and left for about 8-12 minutes to record the effect of the drug on locust foregut whole preparation in FS-responses. Generally, after each experiment, saline was washed off and replaced automatically, and between each experiment there was a 10 minutes intervals. Numerical data presented are plotted as a percentage of a control of 100mM  $K^+$ -responses. Each point represents the mean positive and negative standard errors of 8 replicates (Mutwally, 1990; 1998a and 1998b; Mutwally and Jamel Al-Lyal, 1992 and 1993).

## Results

Locust foregut showed spontaneous rhythmic contractions even when it was denervated. Therefore, locust foregut visceral muscles can be classified as truly myogenic without regulation by an intrinsic neural network (Figure 1).

Raising the external  $K^+$ -concentration can bring about depolarization of locust foregut. The duration of  $K^+$ -induced contracture is variable. As KCl is increased the membrane progressively depolarize, accompanied by a graded mechanical contracture. Up to 100 mM  $K^+$ , where maximum tension was reached, it is clear that, tension is a linear function of membrane potential.

Generally, tyramine inhibited the response of 100mM  $K^+$  of locust foregut muscle (Figure 2 and 3).

Tyramine at  $10^{-7}M$  has no effect on 100mM  $K^+$ -response. But from  $5 \times 10^{-7}M$  -  $10^{-3}M$  the inhibition increased gradually from 12% to 49% (Figure 3). It is noticed in figure 2 that  $2.5 \times 10^{-4}M$  tyramine caused unexpected inhibitory result.

Preparations were field stimulated in normal saline for 3 hours without washing out. The responses became irregular as can be seen in figure 4. However, the Spt. act remains unchanged.

The effect of tyramine on FS-responses of foregut preparation was inhibitory (Figure 4 and 5). Tyramine from  $10^{-6}M$ - $2.5 \times 10^{-4}M$  decreased the responses of FS slightly. At high concentration of tyramine ( $5 \times 10^{-4}M$  -  $10^{-3}M$ ), the inhibitory effect increased of up to 18%. The drug shifted the base line tension at lower concentration, and lowered the base line tension at high concentration (Figure 4).

## Discussion

This study followed previous studies, which were adapted the single approach of drug response (Cook and Holman, 1978; 1980; Freeman, 1966; Huddart and Oldfield, 1982; Oldfield and Huddart, 1982; Mutwally, 1990; 1993 and 2002; Mutwally and Jamel Al-Lyal, 1992 and 1993).

The locust foregut is spontaneously active, and was continuously and rapidly separated by clear periods of quiescence as in the gut of locust and some insects (Huddart and Oldfield, 1982; Oldfield and Huddart, 1982; Dunbar, 1980 and Alohan, 1979; Mutwally, 1990; 2002).



Fig 1. The effect of tyramine (A)  $10^{-7}$  M, (B)  $10^{-5}$  M and (C)  $10^{-3}$  M, added at arrowed points, on locust foregut spontaneous activity. Lower arrows are washout.

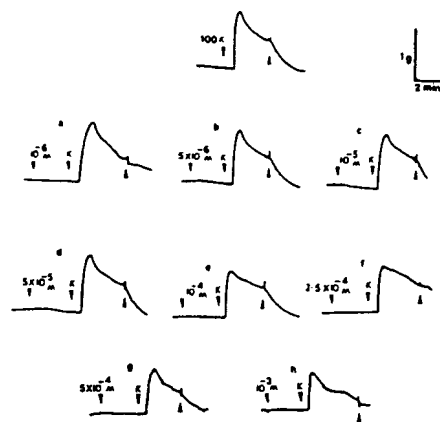


Fig 2. The effect of a 2 minute pretreatment with tyramine (first arrow) on 100mM  $K^+$ -contracture (second arrow), washout (lower arrow). Treatments were as follows: 1) control 100mM  $K^+$ -contracture. Tyramine concentrations: a)  $10^{-6}$  M Tyramine, b)  $5 \times 10^{-6}$  M Tyramine, c)  $10^{-5}$  M Tyramine, d)  $5 \times 10^{-5}$  M Tyramine, e)  $10^{-4}$  M Tyramine. f)  $2.5 \times 10^{-4}$  M Tyramine. g)  $5 \times 10^{-4}$  M Tyramine. h)  $10^{-3}$  M Tyramine. Calibrations: (1g and 2 min) apply to all traces.

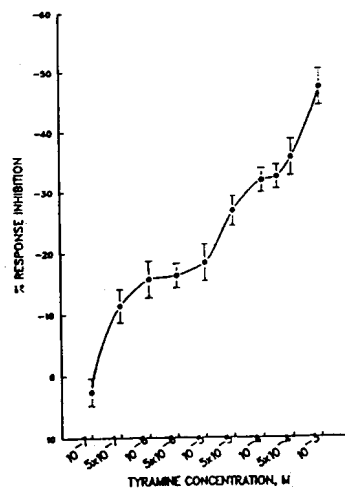


Fig 3. The effect of a 2 minute pretreatment with tyramine on 100 mM  $K^+$  responses of locust foregut muscle. The responses are plotted as a percentage of a control 100 mM  $K^+$  response. Each point represents the mean  $\pm$  S.E. n=8.

Mutwally (1990; 2002) reported that progressive elevation of the external  $K^+$  content of the bathing medium caused the development of contracture responses in the lowest foregut, with a threshold of about 10mM  $K^+$ . Tonic contracture of foregut muscles was obtained at  $K^+$ -concentrations of greater than 10mM  $K^+$ , for periods in excess of 10 minutes or until wash out. There is no obvious depolarization point at which  $Ca^{2+}$  is suddenly made available to the contractile apparatus. This event was unlike  $K^+$ -contracture of hindgut longitudinal muscle (Dunbar, 1980; Oldfield and Huddart, 1982; Mutwally, 1990 and 2002; Mutwally and Jamel Al-Lyal, 1992).

The present study, confirmed previous results of Huddart and Butler (1986) and Mutwally (1990; 1998b) that FS-responses of locust foregut visceral muscle and its natural Spt. act do indeed depend upon the activity of slow voltage-dependent  $Ca^{2+}$ -channels which provide the stimulus to maintain intracellular  $Ca^{2+}$ -mobilization.

Most insect muscle fibers are thus both multi-terminally and poly-neuronally innervated, conditions which are in complete contrast to that existing in vertebrates (Huddart, 1975).

Many visceral muscles are spontaneously active and their resting membrane potentials, are known to be far from being maintained at a stable level as in the case of skeletal muscles and multi-unit muscles (Huddart and Hunt, 1975).

A number of reports implicate tyramine in visceral muscle activity. Tyramine was shown to be the least effective stimulatory catecholamine on cockroach heart, and all other reports of tyramine action on visceral muscle implicate an inhibitory function. Cook and Holman

(1978) showed that tyramine ( $5 \times 10^{-6}M$ ) produced a marked suppression of *Leucophaea* foregut and hindgut Spt. act. In *Locusta*, tyramine had a similar depressant action on the foregut and hindgut in the concentration range from  $10^{-6}M$ - $10^{-7}M$  (Oldfield and Huddart, 1982). The present study found similar results.

The catecholamine tyramine, which suppressed Spt. act in both gut divisions, acted as a proctolin antagonist. It was found that tyramine at  $10^{-7}M$  inhibited the action of glutamate (Huddart, 1985).

This present study confirmed previous results, that the FS-responses as well as 100mM  $K^+$ -contracture were inhibited by tyramine, which confirmed that excitation-contraction coupling is achieved via  $Ca^{2+}$ -activated release of  $Ca^{2+}$ -ions bound to intracellular  $Ca^{2+}$ -stores (Dunbar, 1980; Dunbar and Huddart, 1982; Mutwally, 1990 and 2002).

It was found that  $K^+$ -contracture of the locust foregut is dependent upon the presence of  $Ca^{2+}$ -ions. In addition,  $Ca^{2+}$ -ions are considered to be necessary in reactions linking the electrical and mechanical events in muscle fibers (Mutwally, 1990 and 2002).

The dependence of FS-response of locust foregut visceral muscle upon extra-cellular  $Ca^{2+}$ -ions is clearly evident by their responses to  $Ca^{2+}$ -free media (Mutwally, 1990). This suggests that FS-response and Spt. act are mediated by similar trans-membrane  $Ca^{2+}$ -influx mechanism.

The present study describes the effect of tyramine on the isolated foregut of *Locusta migratoria* as inhibitory. Suggesting presence of either pre- or postsynaptic receptors responding exclusively to this monoamine tyramine. The inhibitory action of tyramine could be due to disruption in the electrogenic events that

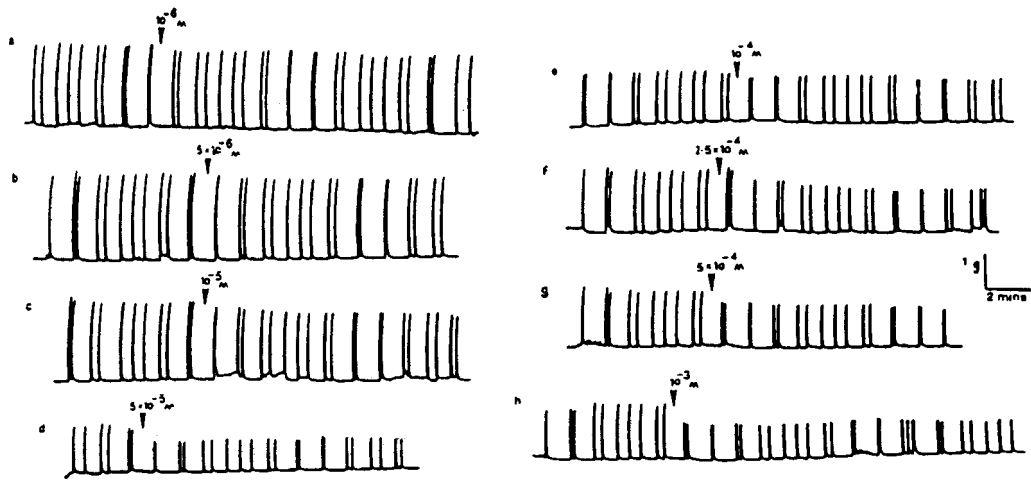


Fig 4. The effect of tyramine on FS responses of locust foregut muscle. Treatments were as follows: a)  $10^{-6}$ M tyramine, b)  $5 \times 10^{-6}$ M tyramine, c)  $10^{-5}$ M tyramine, d)  $5 \times 10^{-5}$ M tyramine, e)  $10^{-4}$ M tyramine, f)  $2.5 \times 10^{-4}$ M tyramine, g)  $5 \times 10^{-4}$ M tyramine, h)  $10^{-3}$ M tyramine. Calibrations: (1g and 2 min) apply to all traces.

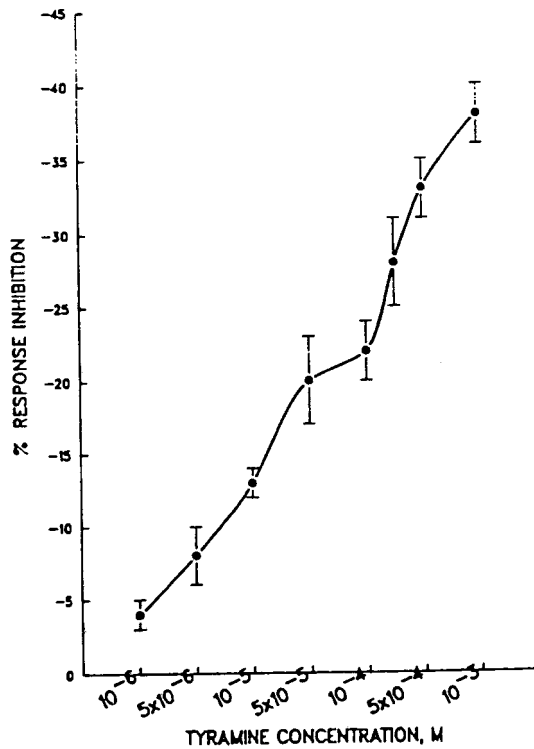


Fig 5. The effect of tyramine on field stimulation responses of locust foregut muscle. The responses are plotted as a percentage inhibition of a control response. Each point represents the mean  $\pm$  S.E. n=8.

occur in the cell membrane of locust foregut visceral muscles.

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## أثر التايرمين وكلوريد البوتاسيوم على عضلات الأمعاء الأمامية للجراد الرحال (لوكاستا ميقراتوريا)

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**ملخص البحث:** عند بداية التجربة عوملت عضلة الأمعاء الأمامية للجراد الرحال بجرعة ١٠٠ ملليمول بوتاسيوم بلغت الانقباضة العضلية أقصى مداها ، ثم تركت العضلة لترتخي كليا مدة تتراوح بين ٣٠ - ٦٠ دقيقة قبل تسجيل أي انقباضة طبيعية أو انقباضة مستحثة بالبوتاسيوم أو بالمجال الكهربائي . عند إضافة مركب التايرمين بمفرده على العضلة ، وجد أنها غير فاعلة بشكل قوي . يستنتج من ذلك غياب اتصال عصبي قبلي أو بعدي لهذا الحامض الأميني في هذه العضلة . ومع هذا فإن إضافة مركب التايرمين أحدث تشبيها جليا للانقباضات الطبيعية للأمعاء الأمامية للجراد الرحال والمستحثة بالبوتاسيوم وكذلك الناتجة عن استحثاثات المجال الكهربائي . هذه النتائج تقترح احتمال أن هذا المركب الأميني قد أحدث اختلافات في الإحداثيات الكهربائية العضلية الموجودة على غشاء الخلية العضلية الحشوية للأمعاء الأمامية للجراد الرحال . حتى هذا الوقت لم تتكون لدينا الصورة الكاملة عن أملكن تواجد وتوزيع وإفراز هذا المركب الأميني في الجهاز العصبي الحشوي . لذا فإنه من السابق لأوانه اعتماد مركب التايرمين كمثبط للموصلات العصبية بصورة قاطعة .