

Effect of aqueous extract of senna (*Cassia senna*) on K⁺-contracture of locust visceral muscles: A possible model to explain the laxative effect of senna.

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Abstract

The locust foregut and hindgut muscles K^+ -contracture were inhibited by aqueous extract of senna. This inhibitory action was stronger on foregut muscles and was dose-dependent on hindgut muscles. In single sucrose-gap method, isotonic KCl-saline depolarised the foregut muscle in the right chamber up to 6-8mV and 4-6mV in the left chamber followed with a slight contraction. Adding senna extract alone depolarised the muscle up to 0.3mV, followed by a hyper-polarisation up to 0.5mV, and was accompanied by a drop in the base line tension. Addition of 10^{-5} M 5-HT, depolarised the foregut muscle up to 1mV accompanied with a slight contraction.

In the double sucrose-gap method, 100mM K^+ induced an inward current of about 0.3 μ A without any increase in tension. Addition of senna extract alone induced an outward current of about 0.02 μ A accompanied with drop in base line tension. Pre-treatment with senna extract for 10 minutes before the addition of 100mM K^+ induced very small outward current while K^+ in presence of senna induced inward current of about 0.15 μ A but no clear tension was recorded. In contrast, 10^{-5} M 5-HT alone induced an outward current up to 0.07 μ A accompanied by a drop in the base line tension. Pre-treating the gut with senna extract for 10 minutes prior to 5-HT (10^{-5} M) induced an outward current of about 0.05 μ A accompanied by a drop in the base line tension. Upon returning to initial control after washing, 100mM K^+ induced an inward current of about 0.2 μ A but no muscle response was recorded.

The depolarisation induced by 100mM K^+ may release bound cellular Ca^{2+} needed for contraction, or it may even mobilise different cellular Ca^{2+} -stores. Senna inhibited the 5-HT activity in a reversible manner probably through binding with the serotonin receptors. Results reported in this

study suggest that senna may cause an inhibition of ionic exchange through cell membrane or alteration in electrolyte influx and efflux thus acting as a laxative.

Keywords: Senna; Locust; Visceral muscle; 5-HT

1. Introduction

The use of Senna as a laxative is an ancient example of the use of plant products in traditional medicine (Meelad 1983, 1987; Migahid 1978; Mutwally and Meelad 1997_a, 1997_b). In *Tibb* (Greco-Arabic) system of medicine the use of senna as a laxative first appeared around late 1000 AD and from there it was adapted into the western medicine. The botanical name of senna is derived from Hebrew language as *Cassia* which means 'to cut' and that is attributed to relieve the constipation. The laxative quality of senna is due to the presence of sennosides A and B in its leaves and pods, which were isolated in pure form by Stoll et al., (1950). The senna sennosides have been widely used but the information relating to their mode of action remains scant. In the last decades, various possible mode of action of sennosides as laxative has been explained including (a) stimulation of colon nerve plexuses thereby leading to defecation (Dobbs et al., 1975); (b) sennosides and their metabolites acting directly on large intestine motility (Garcia et al., 1980; Leng 1986_a); (c) changes in the colon motility and colonic fluid absorption (Leng 1986_b, 1989); and finally (d) involvement of prostaglandin E₂ in secretagogue action of the sennosides in small intestine has been suggested (Nijs et al., 1991).

To understand the mode of action of senna at the molecular level we sought a simpler model of the locust gut muscle. Firstly, using a single sucrose-gap technique its basic electrical, mechanical and ionic properties were established (Mutwally 1990). The K⁺-contraction and the membrane potential of this muscle were found to be uncoupled. Membrane depolarisation increased directly with increasing external K⁺-levels. Depolarisation persisted in Ca²⁺-free saline, a Na⁺-influx and not a Ca²⁺-influx probably mediated this depolarisation. Serotonin (5-HT) was seen to increase spontaneous activity and depolarised the locust foregut muscle. It also increased K⁺-induced contractures and depolarisation. A noticeable feature of 5-HT treatment was the induction of rapid spike-like membrane oscillations. The overall consistent actions of 5-HT suggest that this monoamine may act as a natural autonomic transmitter or modulator in the locust gut (Mutwally 1990).

The cellular electrical activity of muscle cells is generally monitored by the glass microelectrode technique. The glass electrodes have high resistance and poor penetration ability, and that sometime can damage very small size visceral smooth muscle (Tomita 1970; Mutwally 1990;

Mahmod and Huddart 1993). To overcome these problems, Stampfli (1954) introduced the sucrose-gap technique. Though, the single sucrose-gap method allows a continuous recording of electrical activity in visceral muscle and muscle exhibiting rhythmic contractile activity (Lennard and Huddart 1989; Mutwally 1990) but has some intrinsic limitations in its application. A double sucrose-gap method first introduced by Berger (1963) is advantageous in that it is possible to polarise a large region of cell membranes in an approximately uniform way. The usage of double sucrose-gap method allows a potential recording for long periods of time and measurement of membrane resistance and current.

In *Locusta*, Mutwally and Meelad (1997_{a, b}) showed existence of clear behavioural and pharmacological divergences between the foregut and the hindgut muscles. The present study is attempted to extend the investigation of senna aqueous extracts on 100mM K⁺-responses and 10⁻⁵M 5-HT responses of locust fore- and hindgut muscles. Moreover, this study used mechanical, single and double sucrose-gap techniques in the hope of casting some light on the possible role of *C. senna* and 5-HT on locust visceral muscles.

2. Materials and methods:

2.1 Preparation of senna extract

The dried leaves of *C. senna* were obtained from local herb shop in Makkah, Saudi Arabia. The leaves were pulverised into a fine powder in an electric grinder (Moulinex). Approximately 10g of powdered leaves was added to 200ml of distilled water then heated at 60°C for one hour on a water bath, filtered and used as senna extract. Such an extract may illustrate mainly water-soluble components from senna and may represent a synergistic effect of various bioactive components and ions contained there in on the muscle and nerve activity of the gut. It is noteworthy, that even today such an infusion is used by a large segment of population in the Arabian traditional medicine to relieve constipation.

2.2 Animals

Adult locusts of both sexes reared in laboratory culture were used throughout this study. The colony was maintained at 27°C and fed on green barley or grass daily with water supplied *ad libitum*.

2.3 Chemicals

All chemicals were of analytical grade and were obtained locally. 5-Hydroxytryptamine (5-HT) was obtained from Sigma Chemical Co, St Louis, Mo., USA. Isotonic KCl (172mM) saline and

isotonic sucrose (172mM) solutions were made in distilled water. The stock solution of 5-HT (10^{-5} M) was prepared in insect saline. All chemicals and 5-HT were freshly prepared from stock solutions and were added to the organ bath as indicated.

2.4 Muscle preparation and the experimental protocol

The details of insect saline, dissection and the preparation of the isolated fore- and hindgut muscle preparation were followed as described earlier (Mutwally and Jamel Al-Layl 1992). The single and double sucrose-gap techniques and experimental were set up according to Mutwally (1990), Huddart et al., (1992) and Nelson and Huddart (1992). For muscle tension recording, three different amounts of 0.25, 0.5 and 1 ml approximately 12.5, 25 and 50 mg of dried powder were chosen to evaluate the effect of senna extract. To test the effect of both senna extract (0.25 ml) and 5-HT (10^{-5} M) on 100mM K^{+} -responses they were added separately. The organ bath was maintained at 20°C with a Grant Closed Circuit Cooler and circulatory system and the bath contents were constantly aerated.

In this study, three different protocols were followed on single and double sucrose-gap preparations: (a) the effect of 100mM K^{+} -saline on foregut muscle electrical and mechanical responses; (b) the effect of test agent (senna extract or 5 HT) alone on the muscle electrical and mechanical responses and (c) the effect of 10 minutes pre-treatment with a test agent on subsequent 100mM K^{+} -induced responses. Each experiment was run for 30 minutes and was followed by 20 minutes washing period with normal saline.

3. Results

The K^{+} -contracture of locust foregut muscle was stronger than that of hindgut muscle and was inhibited with pre-treatment additions of senna aqueous extract (Fig 1 and 2). Inhibitory action of senna extract on fore- and hindgut muscles was significantly different at low doses, being about 28% in the foregut and about 3% in the hindgut. The inhibitory effect of senna extract on the hindgut was dose-dependent, and increased up to 20% at high concentration (1 ml) tested. In contrast, the inhibitory effect of senna on the foregut displayed a slight but not significant decrease at the higher concentration (Fig. 2). Post-treatment of senna aqueous extract on locust muscle divisions after K^{+} -responses did not inhibit the muscle responses. Whereas, addition of senna extract decreased the spontaneous activity of the hindgut induced by the addition of 100mM K^{+} (Fig. 3).

In single sucrose-gap experiment, the result in Figure 4a shows the depolarisation effect of the muscle in the right chamber with isotonic KCl. In eight separate preparations the compound

membrane potential was 6-8 mV accompanied with a slight contracture. Figure 4b shows the depolarisation effect of the tissue in the left chamber (test side) of the preparations with isotonic KCl. The compound membrane potential was between 4-6 mV and no tensions were seen. In all experiments, the depolarisation preceded the mechanical response but the mechanical responses declined despite the maintained K⁺-induced depolarisation. As shown in Figure 4c and Figure 4d electrical responses of the isometric preparations were highly variable (among preparations), after initial stable responses, irregular depolarisation occurred which later stabilised and force development became irregular. In addition, both 0.25 ml senna aqueous extract and 10⁻⁵M 5-HT induced relatively little depolarisation, weak isometric force accompanied by slow onset of contractions and the rhythmic nature of the responses recorded. Both 0.25 ml senna extract and 10⁻⁵M 5-HT induced fast spike-like membrane potential oscillations (Fig. 4c and Fig. 4d). Adding senna extract alone depolarised the preparation up to 0.3 mV followed by a hypo-polarisation up to 0.5 mV accompanied with a drop in base line tension of the foregut muscle up to 0.2 g (Fig. 4c). On the other hand, 10⁻⁵M 5-HT depolarised foregut muscle up to 1 mV accompanied with a small contracture up to 0.15 g (Fig. 4d). In all experiments the tension of the muscles and membrane potential returned to normal after washout with normal saline.

When foregut muscle was examined in the double sucrose-gap and voltage clamped at normal resting potential, 100mM K⁺-application was seen to induce an inward transmembrane current of about 0.3μA but no tension was seen (Fig. 5a). Addition of 0.25 ml senna extract alone on the foregut muscle in the double sucrose-gap and voltage clamped at normal resting potential induced an outward transmembrane current of about 0.02μA accompanied with drop in base line tension up to 0.06 g (Fig. 5b). The drug effect was reversible by washing the test chamber with normal saline, but the muscle tension was higher than normal after washing. When the preparation was treated with senna extract for 10 minutes prior to 100mM K⁺ application, senna induced very small outward current while K⁺-induced an inward current of about 0.15μA but no clear change in tension was observed (Fig. 5c). Addition of 10⁻⁵M 5-HT alone on the foregut muscle in the double sucrose-gap and voltage clamped at normal resting potential was seen to induce an outward current of about 0.07μA accompanied with drop in base line tension up to 0.06 g (Fig. 6a). When the preparation was treated with senna extract for 10 minutes prior to 10⁻⁵M 5-HT applications, senna induced an outward current of about 0.03μA and inhibited the 5-HT activity in a reversible manner. Both compounds induced an outward current of

about $0.05\mu\text{A}$, accompanied with a drop in the base line tension of about 0.02 g (Fig. 6b). The effect of both drugs was reversible upon wash out the test chamber with normal saline, but the activity of the muscle was higher than normal after washing. Upon returning to the control condition (addition of 100mM K^+) at the end of the experiment membrane potential returned to normal resting potential inducing an inward current of about $0.2\mu\text{A}$ but no muscle response was recorded (Fig. 6c).

4. DISCUSSION

C. senna aqueous extract inhibited 100mM K^+ -contracture of locust foregut up to 28% at low doses and 22% at high doses. The inhibition was of 3% at low doses and 20% at high doses on hindgut muscles. The differences in dose specificity could be related to both muscles structure and innervation differences between them. Post-treatment additions of *C. senna* aqueous extract on locust muscle divisions did not inhibit the K^+ -responses. This may suggest that pre-treatment with *C. senna* aqueous extract may block or compete with Ca^{2+} -channel or other channels such as Na^+ -channel and $\text{Na}^+ / \text{Ca}^{2+}$ -counter exchange since they were essential for muscle K^+ -contracture. Similarly, post-treatment with *C. senna* aqueous extract did not affect K^+ -contractures. This may be because of K^+ -depolarises both compartments fully and ion-channels are open. This suggests that *C. senna* aqueous extract has stronger effect on non-depolarised muscles than on the depolarised one.

Mutwally (1990) studied the electrical activity of the locust foregut muscle using single sucrose-gap technique applying different concentration of KCl and 5-HT. The K^+ -contraction and the membrane potential of this muscle were uncoupled. The mean compound membrane potential seen was $6\text{-}8\text{ mV}$ and the mean depolarisation with 100mM K^+ in the test chamber was $4\text{-}6\text{ mV}$. However, Dunbar (1980) measured the membrane potential of locust hindgut up to 38.6 mV with the glass microelectrode methods. This value was higher than those recorded in this study. This could be due to differences in muscle and innervation system and also to differences in the techniques being used. In the gill vein muscle of *Aplysia*, Dorsett and Evans (1989) reported that when membrane potential are measured by both techniques, the sucrose-gap recordings are invariably lower than those seen with glass micro electrodes. They also found that membrane depolarisation increased directly with increasing external K^+ -levels. In locust foregut the results of the present study similar were to those of (Mutwally 1990).

Single and double sucrose-gap voltage clamp studies show that control dose of 100mM K^+ used in this study induced a transmembrane inward current of the order of $0.3\mu\text{A}$, leading to a

membrane depolarisation of about 4 mV accompanied by a weak contracture. K^+ -induced depolarisation is thought to directly activate voltage operated Ca^{2+} -channel *via* voltage sensor sub-unit (Reuter 1983). This may suggest voltage-dependent events as described in mollusc (Huddart et al., 1992; Nelson and Huddart 1992). K^+ -induced depolarisation may release bound cellular Ca^{2+} needed for contraction by different membrane transduction routes, or it may even access different cellular Ca^{2+} -stores (Reilly and Peretz 1987). When depolarisation persisted in Ca^{2+} -free saline, a Na^+ - and not a Ca^{2+} -influx probably mediates this depolarisation. This may increase Na^+ -conductance, which is controlled by Ca^{2+} -bound to the membrane (Reilly and Peretz 1987; Huddart and Hill 1988; Mutwally 1990). In *Busycon* muscle, Hill and Licis (1981) suggested that there is a Na^+ / K^+ -pump involved during depolarisation. In addition, Dorsett and Evans (1989) concluded that in molluscan muscle, there is a Cl^- -transport involved in membrane potential development. These observation may reflect differences in the way $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ are mobilised to activate muscle contraction.

In conclusion, this study reports that senna extract alone induced a transient membrane potential change and lowered the base line tension, while 5-HT alone induced a depolarisation and caused small contraction. Senna extract also inhibited 5-HT activity in a reversible manner. This action may be through binding of active components of senna to the serotonin receptors and may confirm that senna is an antagonist of serotonin receptors. Mutwally (1990; 1993 and 1994) reported the appearance of 5-HT as the only consistent excitatory agent in the locust gut suggesting the presence of 5-HT-receptors. This observation allows the use of 5-HT as a tool to study the effect of *C. senna* aqueous extract on locust gut muscles. Moreover, 5-HT was seen to increase Spt.act and to depolarise locust foregut muscle. It also increased K^+ -induced contracture and depolarisation. A noticeable feature of 5-HT treatments was the induction of rapid spike-like membrane oscillations. The overall consistent actions of 5-HT suggest that it may act as a natural autonomic transmitter / modulator in locust foregut muscle. Sennosides and glycosides in senna extract may cause decrease in membrane potential, Na^+ / K^+ -ATPase activity due to fall in membrane potential which initially increases the excitability of muscle fibres, but as the membrane potential falls further, excitability begins to decrease. Such mechanism has also reported by Bowman and Rand (1984) in heart and intestine muscle.

If senna aqueous extract has comparable effects on mammalian intestine *i.e.* antagonistic effect of senna against 5-HT receptors as elicited in locust visceral muscle, that could help explain the

molecular mechanisms involved in the laxative effect of senna known to folkmedicine. The antagonism of senna sennosides towards 5-HT receptors in mammalian intestine remains to be evaluated.

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7. FIGURE LEGENDS

Fig. 1. Effect of pre-treatment with senna extract (250 μ l, 500 μ l and 1 ml, as indicated) on 100mM K⁺-contracture (as indicated) of locust (A) foregut and (B) hindgut muscles. Upper calibration apply to (A)traces and the lower apply to (B) traces and (W) wash out with normal saline.

Fig. 2. Effect of pre-treatment with senna extract on 100mM K⁺-contracture of locust foregut (o) and hindgut () muscles. The responses are plotted as a percent of the control response. Each point represents the mean and the \pm S.E of 8 replicates.

Fig. 3. Effect of post-treatment with senna extract (250 μ l, 500 μ l and 1 ml, as indicated) on 100mM K⁺-contracture (as indicated) of locust (A) foregut and (B) hindgut muscles. Upper calibration apply to (A) traces and the lower apply to (B) traces.

Fig. 4. First upper arrows represent the effect of isotonic K⁺-saline (A, in the right chamber) and (B, the control in the left chamber), (0.25ml) senna extract (C) and 10⁻⁵M 5-HT (D) on locust foregut muscles membrane potential in single sucrose-gap. Upper traces: membrane potential and lower traces: mechanical activities. Upper arrows represent the addition of normal saline for washing in all experiments. Membrane potential, force and time calibrations were standardized for each experiment and are as indicated on the right hand side of the Figures.

Fig. 5. Effect of 0.25ml senna extract, 10⁻⁵M 5-HT and 100mM K⁺ on locust foregut muscles in double sucrose-gap recording. Upper traces represent current membrane potential recordings while the lower traces indicate mechanical activities. (5 A) showing the inward current developed in response to 100mM K⁺-saline (first lower arrow), clamp potential was set at equivalent to natural resting potential, (5 B) recording of an outward current developed in response to addition of senna extract (first lower arrow), voltage clamp conditions were the same as above, (5 C) senna extract induced slight initial outward current (first lower arrow) followed by an inhibition of 100mM K⁺-induced current (second lower arrow) and (upper arrows) represent the addition of normal saline for washing in all experiments. The current, tension and time scales were identical in all the experiments above as indicated on the right hand side of the Figures.

Fig. 6. Effect of 0.25ml senna extract, 10^{-5} M 5-HT and 100mM K^+ on locust foregut muscles in double sucrose-gap recording. Upper traces represent current membrane potential recordings while the lower traces indicate mechanical activities. (6 A) showing the outward current developed in response to 5-HT (first lower arrow), clamp potential was equivalent to natural resting potential, (6 B) senna extract induced slight initial outward current (first lower arrow) followed by an inhibition of an outward current developed by 5-HT (second lower arrow), (6 C) at the end, after washing with normal saline the addition of 100mM K^+ -induced an inward current which stabilized to the control level under voltage clamp conditions were the clamp potential was set at natural resting potential. The current, tension and time scales were identical in all the experiments above as indicated on the right hand side of the Figures.

أثر المستخلص المائي لنبات السنّا مكّي كاسيا سنّا على الانقباضات العضلية الملساء للجراد الرحال المستحثة بالبوتاسيوم ؛ تجرّبه حديثه لإيضاح الأثر المدين للسنّا مكّي

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٢ معهد العلوم الأحيائية ، جامعة أودنسة ، كامسبوفج ٥٥ ، الدنمارك — ٥٢٣٠ أودنسة .

المُلخَص

لوحظ أن الانقباضات المستحثة بالبوتاسيوم للعضلات المعوية الأمامية والخلفية للجراد الرحال قد ثبتت عند إضافة المستخلص المائي للسنّا مكّي وكان هذا التثبيط قوياً على الأمعاء الأمامية وتدرجياً على الأمعاء الخلفية . وباستخدام تجربة الغشاء السكري المفرد العازل للكهرباء وجد أن البوتاسيوم قد أحدث لا إستقطاب لغشاء العضلات المحجوزة في المنطقه اليمنى بمقدار ٦ - ٨ مليفولت وفي الجهه اليسرى بمقدار ٤ - ٦ مليفولت متبوعه بانقباضه عضلية بسيطة وعند إضافة السنّا مكّي لوحده في الجهه اليسرى أحدثت لا إستقطاباً بمقدار ٣ . و مليفولت اتبعت بإستقطاب بمقدار ٥ . و مليفولت وكان مصاحباً بإنخفاض في مستوى الشد العضلي . وكذلك عند إضافة مركب السيروتونين عند تركيز 10^{-6} مولار أحدثت لا إستقطاب في غشاء العضلة المعوية الأمامية للجراد بمقدار ١ مليفولت مصاحبة بإنقباضه عضلية بسيطة .

وعند استخدام تجربة الغشاء السكري المزدوج العازل للكهرباء ، وجد أن إضافة السنّا مكّي على الأمعاء أحدثت مقاومة كهربائية خارجية بسيطة بينما عند إضافة البوتاسيوم مع السنّا مكّي وجد أنها أحدثت مقاومة كهربائية داخلية بمقدار ١٥ . و مايكروأم وبدون أي إنقباضة عضلية مصاحبه . وعند إضافة مركب السيروتونين عند تركيز 10^{-6} مولار وجد أنه قد أحدثت مقاومة كهربائية خارجية بمقدار ٧ . و ٥ - . و مايكروأم .

خلاصة البحث أوضحت أن الاستقطاب المستحدث من قبل إضافة ١٠٠ مليمول بوتاسيوم قد سبب في إطلاق أيونات الكالسيوم الخلوية المرتبطة والمهمة لحدوث الانقباضة العضلية أو أنها قد أثارت مخازن أخرى لأيونات الكالسيوم الخلوية . وكذلك وجد أن تثبيط السنّا مكّي لنشاط السيروتونين قد يكون عن طريق إرتباطه بمستقبلاته . والنتائج هنا تقترح بأن السنّا مكّي قد سبب تثبيطاً في التبادل الأيوني عبر الغشاء الخلوي أو أنها قد أحدثت تغيرات في نفاذية الأيونات عبر الأغشية الخلوية كما هي طبيعة الأدوية المئينة .