

**Action of chlorpromazine (CPZ) on  $K^+$ -contractures and field stimulation (FS) responses of locust foregut and hindgut muscles.**

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**ABSTRACT:** Addition of chlorpromazine (CPZ) at  $5 \times 10^{-6}$  M -  $5 \times 10^{-4}$  M severely inhibited  $100 \text{mM } K^+$ -contractures of locust both gut divisions in dose-dependent manner. Moreover, CPZ at  $5 \times 10^{-5}$  M induced spontaneous activity (Sp.Act.) of locust hindgut. However, the inhibitory effect of CPZ was stronger on hindgut muscle. These results suggested that CPZ may block  $Ca^{2+}$ -channels and may prevent  $Ca^{2+}$ -influx gradually, more likely via an inhibition of  $Ca^{2+}$ -movements into the intracellular compartments. Chlorpromazine ( $10^{-6}$  M -  $10^{-5}$  M) inhibited field stimulation (FS) responses of both gut divisions gradually. However, CPZ at  $10^{-6}$  M induced Sp.Act. of hindgut muscle. These results confirmed above suggestion of  $K^+$ -responses and previous conclusion of CPZ on locust mechanical activity and rat uterine muscles.

**GENERAL INTRODUCTION**

Cheung (1970) described calmodulin (CaM) as a heat stable  $Ca^{2+}$ -dependent protein activator. CaM and CaM-like proteins were found in a wide variety of mammalian, amphibian and invertebrate tissues (Waisman, et al, 1975; Sahaf, 1989) which suggests that many of calcium's physiological functions may be mediated by  $Ca^{2+}$ -receptor protein such as CaM (Lin, et al, 1974). Sahaf (1989) proposed CaM as a mediator in many  $Ca^{2+}$ -activator processes and in  $Ca^{2+}$ -stimulated release of neurotransmitter from frog nerve cells. In intact synaptosomes and isolated vesicle

preparations,  $\text{Ca}^{2+}$ -CaM has been shown to stimulate neurotransmitter release and parallel protein phosphorylation (DeLorenzo, 1982).

Recently, Mutwally and Sahaf (1994a) reported that both locust gut muscles are naturally active, but they do have some physiological differences. In addition, these myogenic activities are secondarily modulated by the stomatogastric nervous system, similar to the situation in the mammalian gut (Oldfield & Huddart, 1982; Mutwally, 1990 & 1993).

Mutwally and Sahaf (1994a & b) reported that cumulative additions of CPZ caused marked inhibition of both Sp.Act. and  $100\text{mMK}^+$ -contracture of rat uterine smooth muscle and natural activity of both locust gut preparations. They suggested that CPZ may block  $\text{Ca}^{2+}$ -channels and may slow down  $\text{Ca}^{2+}$ -influx mostly through inhibition of  $[\text{Ca}^{2+}]_i$ -movements into the intracellular environment of both guts. This action may occur through inhibition of CaM-activity, since CaM is found to mediate many  $\text{Ca}^{2+}$ -activated processes including muscle contractions. The aim of this study is to extend previous results and to explore views of CPZ, CaM-antagonists, and the possible involvement of CPZ / CaM in locust foregut and hindgut visceral muscle in term of excitation / contractions-coupling (EC-coupling) using  $100\text{mMK}^+$  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,  $\text{K}^+$ -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 & Sahaf, 1994a). 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 CPZ 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 CPZ on $K^+$ -contracture:

In Figure 1, both locust foregut (A) and hindgut (B) muscles were responded to  $100mMK^+$ -additions (control). The inhibitory effect of 3 minutes pretreatment CPZ ( $5 \times 10^{-6} M$ ,  $5 \times 10^{-5} M$  and  $5 \times 10^{-4} M$ ) on  $100mMK^+$ -responses were dose-dependent. However, the inhibitory effect was clearly stronger on hindgut muscle. In addition, CPZ -induced Sp.Act. on hindgut muscle. But at  $5 \times 10^{-4} M$ , CPZ demolished  $100mMK^+$ -responses. Moreover, both gut compartments did not resume their activities even after 2 washout. Figure 2, shows dose-response curves for CPZ additions and its percentage inhibition responses of both preparations.

### 2- The effect of CPZ on FS-responses:

Figure 3, shows that CPZ at  $10^{-6} M$  and  $10^{-5} M$  inhibited FS-responses of both gut divisions in dose-dependent manner. CPZ at  $10^{-6} M$  induced Sp.Act. of locust hindgut. However, the inhibitory effect of CPZ was stronger on hindgut muscles. The effect of CPZ on FS-responses of both preparations was plotted as a percentage of a control responses (Figure 4).

## DISCUSSION

Mutwally and Jamel Al-Layl (1992 & 1993) concluded that  $K^+$ -induced muscle depolarization and FS allows 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 established the role of CPZ in locust visceral muscles (Mutwally & Sahaf, 1994a). Huddart and Butler (1986) and Mutwally (1990) reported that FS-technique provide another  $Ca^{2+}$ -dependency of

both locust gut divisions. Moreover, locust foregut possesses  $\text{Ca}^{2+}$ -channels which are analogous to those of mammalian muscle in terms of their voltage inactivation/activation (Mutwally, 1990). However, locust hindgut electrophysiological studies indicated that action potential was generated by labial pacemaker areas (Dunbar, 1980). Natural activities,  $\text{K}^+$ -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 goes in harmony with previous studies and confirmed above conclusions.

Psychoactive drug like CPZ is very potent in inhibitory the alkaline phosphatase activity in rat brain which may lead to a change in neuronal permeability through glucocorticoid therapy affecting mode (Nag & Nandi, 1994). Argov and Yarri (1979) described CPZ as an antipsychotic and membrane stabilizing agent and is one of the CaM binding phenothiazines (Weiss, et al, 1980). In addition, CPZ was found to inhibit cholinergic transmission and adrenoceptors of animal and human nervous system (Argov & Yarri, 1979; Foster, 1990). Xiong and Li (1994) suggested that the inhibitory effect of CPZ on mice intestinal movement was produced by preventing the release of acetylcholine from the central nervous system. CaM was found to be a  $\text{Ca}^{2+}$ -dependent activator protein of  $\text{Ca}^{2+}$ -dependent cyclic nucleotide phosphodiesterase (Cheung, 1970). It was also found that some important enzymes in the brain, number of  $\text{Ca}^{2+}$ -dependent enzyme activities and muscles require the presence of CaM *in vivo* and *in vitro* (Cheung, 1970; Dabrowska & Hartshorne, 1978; Wolff & Brostrom, 1979). Moreover, Sahaf and Publicover (1987) concluded that CPZ have a stimulatory effect on transmitter release at frog neuromuscular junction, an effect which is suggested to be through an inhibition of CaM-activated  $\text{Ca}^{2+}$ -buffering processes. Mutwally and Sahaf (1994a & b) reported that CPZ induced Sp.Act. of locust hindgut, but caused severe inhibitions to the locust foregut and hindgut Sp.Act. and to the rat uterine natural activities and  $\text{K}^+$ -responses respectively. They suggested that CPZ may block  $\text{Ca}^{2+}$ -channels and prevent  $\text{Ca}^{2+}$ -influx gradually, more likely via an inhibition of  $\text{Ca}^{2+}$ -movements. Such an action may

involve interference of CaM activity in these muscle. Although, in this study, CPZ inhibited both responses of  $100\text{mMK}^+$  and FS, but it induced Sp.Act. of locust hindgut. This action could be due to structural and functional differences between these two guts. In addition, these results suggest that CPZ may have stimulatory effect on hindgut muscles, but its inhibitory effect could be through an inhibition of CaM-activated  $\text{Ca}^{2+}$ -buffering processes.

Barnette, et al (1983) suggested that several peptides were found in insect venom including melittin, apamin and mastoparan inhibited the activity of CaM-stimulated phosphodiesterase. Papazian, et al (1984) reported that  $\text{Ca}^{2+}$ -ATPases of synaptosomal plasma membrane are activated by CaM. It is therefore, anticipated that effective CaM inhibition would induce arise in interneuronal  $[\text{Ca}^{2+}]_i$  by inhibition of  $\text{Ca}^{2+}$ -pumping and such an action would bring about the stimulatory effect caused by CPZ on MEPP frequency in this study and other studies (Argov & Yaari, 1979; Publicover, 1983; Sahaf, 1989; Mutwally & Sahaf, 1994a & b). This stimulatory effect of CPZ on MEPP frequency at both normal saline and  $\text{Ca}^{2+}$ -free saline at a relatively high room temperature may be found as a result of elevation of  $[\text{Ca}^{2+}]_i$  which is possibly produced by inhibition of CaM-activated  $\text{Ca}^{2+}$ -buffering processes (Publicover & Duncan, 1979). It was reported previously, that the excitation-contraction coupling, FS- and electrical-responses of locust visceral muscles depend upon  $[\text{Ca}^{2+}]_o$  (Dunbar, 1980; Mutwally, 1990 & 1993; Mutwally & Jamel Al-Layl, 1993). In this study, CPZ inhibited both responses possibly in a similar way as described earlier.

Quastel, et al (1972) suggested that the increase in quantal content resulted from CPZ's ability to increase spontaneous transmitter release through an elevation of  $[\text{Ca}^{2+}]_i$ , possibly by inhibiting CaM-activated  $\text{Ca}^{2+}$ -buffering processes by the drug action (Sahaf, 1989; Mutwally & Sahaf, 1994a & b). Recently, Baines and Downer (1994) found that octopamine antagonist, CPZ partially blocked the octopamine-mediated increase in cockroach survival. Sepulveda, et al (1994) reported that the

effect of electrophysiological studies of CPZ on 5HT<sub>3</sub> was not voltage- or use-dependent and there was no blocking action when CPZ was applied from inside the mouse neuroblastoma cell. Tanabe, et al (1989) reported that parasites were more sensitive to calmidazolium and W-7, in addition, all Ca<sup>2+</sup>-blockers and CaM-inhibitors suppressed parasite development at later stages. They concluded that although all Ca<sup>2+</sup> and CaM-antagonists tested influence parasite development at later stages, they are multifunctional, having effects not directly associated with Ca<sup>2+</sup>-channels or CaM. In addition CPZ inhibited the binding of radiolabeled 5HT<sub>3</sub> receptor antagonist. These conclusions may also give support to CPZ inhibitory action in this present study, and would confirmed that CPZ block Ca<sup>2+</sup>-channel and inhibiting Ca<sup>2+</sup>-pumping or slow Ca<sup>2+</sup>-influx an action may suppressed CaM-activity which may caused EC-uncoupling. Moreover, the present results goes in harmony with previous studies of Argov and Yaari (1979) and Mutwally and Sahaf (1994a & b), and would take Tanabe, et al (1989) conclusion for concederation in explaining the action of CPZ on locust both gut divisions especially for hindgut muscles. More studies are needed for CPZ on insect electrophysiology and biochemistry before establishing a solid correlation between CPZ / CaM and EC-coupling.

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#### **REFERENCES**

**ARGOV**, Z and Yaari, Y. (1979) The action of chlorpromazine at an isolated chlonergic synapse. *Brain Res.*, Vol. 164: 227-236.

- BAINES, D.** and Downer, R.G.H. (1994) Octopamine enhances phagocytosis in cockroach hemocytes: Involvement of inositol trisphosphate. *Archives of Insect Biochem. and Physiol.*, Vol. 26 (4): 249-261.
- BARNETTE, M.S.;** Daly, R. and Weiss, B. (1983) Inhibition of calmodulin activity by insect venom peptides. *Biochem. Pharmacol.*, Vol. 32 (19): 2929-2933.
- CHEUNG, W.Y.** (1970) Cyclic 3', 5' -nucleotide phosphodiesterase. *Biochem. Biophys. Res. Commun.*, Vol. 38: 533-538.
- DABROWSKA, R.** and Hartshorne, D.J. (1978) A Ca<sup>2+</sup> and modulator dependent myosin light chain kinase from non-muscle cells. *Biochem. Biophys. Res. Commun.*, Vol. 85: 1352-1359.
- DeLORENZO, R.J.** (1982) Calmodulin in neurotransmitter release and synaptic function. *Fed. Pro.*, Vol. 41: 2265-2272.
- DUNBAR, S.J.** (1980) The morphology and neurophysiology of an insect visceral muscle. (Ph.D. Thesis), Lancaster University.
- FOSTER, R.W.** (1990) Basic pharmacology. Second Edition. Butter Worth, London.
- HUDDART, H.** Butler, D.J. (1986) Field stimulation responses of rat urinary bladder detrusor smooth-muscle. Dependence upon slow calcium channel activity determined by K<sup>+</sup>-depolarization and calcium antagonists. *Gen.Pharmacol.*, Vol. 17: 695-702.
- LIN, Y.M.,** Lin, Y.P. and Cheung, W.Y. (1974) Cyclic 3':5'-nucleotide phosphodiesterase. *J. Biol. Chem.*, Vol. 249: 4943-4954.
- MUTWALLY, H.M.A.** (1990) The structure, innervation and function of locust foregut visceral muscle. (Ph.D. Thesis). Biological Sciences. Lancaster University.
- MUTWALLY, H.M.A.** (1993) The effect of some biogenic amines on the spontaneous activity of locust *Locusta migratoria* foregut in the absence of extracellular calcium. *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol.11 (A): 47-56.

**MUTWALLY**, H.M.A. and Jamel Al-Layl, K.S. (1992) The effect of cyanobacterial neurotoxin on the locust *Locusta migratoria* foregut and hindgut visceral muscles. *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol.9 (A): 203-220.

**MUTWALLY**, H.M.A. and Jamel Al-Layl, K.S. (1993) The effect of cyanobacterial neurotoxin on field stimulation (FS) responses of locust *Locusta migratoria* foregut and hindgut visceral muscles. *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol.11 (A): 19-31.

**MUTWALLY**, H.M.A. and Sahaf, Z.Y. (1994a) The effect of cumulative dose of chlorpromazine (CPZ) on the spontaneous activity of the foregut and hindgut muscles of *Locusta migratoria*. *J. Fac. Edu. Ain Shams Univ. Cairo*, Vol. 19: 13-23

**MUTWALLY**, H.M.A. and Sahaf, Z.Y. (1994b) The effect of chlorpromazine (CPZ) on rat uterine smooth muscle. *Menoufiya Med. J. Cairo*, Vol. 6 (1): 13-27.

**NAG**, M. and Nandi, N. (1994) Chlorpromazine and other psychoactive drug induced alterations of a membrane bound enzyme in rat brain. *BioSci. Reports*, Vol. 14 (3): 139-144.

**OLDFIELD**, A.C. and Huddart, H. (1982) Spontaneous activity of foregut and hindgut visceral muscle of the locust *Locusta migratoria*. 1- Normal activity and the effect of KCL depolarization and glutamate. *Comp. Biochem. Physiol.*, Vol. 73 (C): 298-302.

**PAPAZIAN**, D.M.; Rahamimoff, H. and Goldin (1984) Partial purification and functional identification of a calmodulin activated, adenosine 5-triphosphate dependent calcium pump from synaptic plasma membrane. *J. Neurosci.*, Vol. 4: 1933-1943.

**PUBLICOVER**, S.J. (1983) Presynaptic action of trifluoperazine at the frog neuromuscular junction. *Naunyn-Schemiedeberge's Arch. Pharmacol.*, Vol. 322: 83-88.

- PUBLICOVER**, S.J. and Duncan, C.J. (1979) The action of verapamil on the rate of spontaneous release of transmitter at the frog neuromuscular junction. *Europ. J. Pharmacol.*, Vol. 54: 119-127.
- QUASTEL**, D.M.J., Hackett, T.J. and Okamoto (1972) Presynaptic action of central depressant drug: Inhibition of depolarization-secretion coupling. *Can. J. Physiol. Pharmacol.*, Vol. 50: 279-284.
- SAHAF**, Z.Y. (1989) Studies on transmitter release at the frog neuromuscular junction. (Ph.D. Thesis). Birmingham University.
- SAHAF**, Z.Y. and Publicover, S.J. (1987) Chlorpromazine but not chlorpromazine sulfoxide, stimulates transmitter release from motor nerve terminals. *Brain Res.*, Vol. 437: 397-401.
- SEPULVEDA**, M.I.; Baker, J. and Lummis, S.C.R. (1994) Chlorpromazine and QX222 block 5HT<sub>3</sub> receptors in N1E-115 neuroblastoma cells. *Neuropharm.*, Vol. 33 (3-4): 493-499.
- TANABE**, K.; Izumo,A.; Kato,M.; Miki,A. and Doi, S. (1989) Stage-dependent inhibition of plasmodium flaciparum by potent calcium and calmodulin modulators. *J. Protozool.*, Vol. 36 (2): 139-143.
- WAISMAN**, D.; Stevens, F.C. and Wang, J.H. (1975) The distribution of Ca<sup>2+</sup>-dependent protein activator of cyclic nucleotide phosphodiesterase in invertebrates. *Biochem. Biophys. Res. Commun.*, Vol. 65: 975-982.
- WEISS**, B., Prozialeck, W., Cimino, M., Barrett, M.S. and Wallace, T.L. (1980) Pharmacological regulation of calmodulin. *Ann. N. Y. Acad. Sci.*, Vol. 356: 319-345.
- WOLFF**, D.J. and Brostrom, C.O. (1979) Properties and function of the calcium-dependent regulator protein. *Adv. Cyclic Nucleotide Res.*, Vol. 11: 27-88.
- XIONG**, Y.Q. and Li, B.H. (1994) Inhibitory action of chlorpromazine on intestinal movement in mice and its antagonistic agents. *Acta Pharmacol. Sinica.*, Vol. 15 (3): 235-238.

## FIGURE LEGENDS

FIGURE 1: The effect of 3 minutes pretreatment with CPZ on locust foregut (A) and hindgut (B): In (A) and (B) Initial 100mMK<sup>+</sup>-responses (control). Then, pretreatment with CPZ ( $5 \times 10^{-6}$ M,  $5 \times 10^{-5}$ M and  $5 \times 10^{-4}$ M) (first points). Note, that CPZ-induced Sp.Act. of locust hindgut. In (A) and (B), the second points indicated the addition of 100mMK<sup>+</sup>. Lower points of all traces indicated washout. Calibration and time scales applies to all traces.

FIGURE 3: The effect of CPZ ( $10^{-6}$ M,  $10^{-5}$ M) (upper points) on FS-responses of intact isolated locust foregut (A) and hindgut (B) muscles. Lower points indicated washout. Calibration and time scales applies to all traces.

أثر الكلوربرومازين على الانقباضات المستحثة بالبوتاسيوم والمجال الكهربائي لعضلات

الجراد

الامامية والخلفية .

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الملخص:

اضافة مركب الكلوربرومازين بتركيز (١٠×٥<sup>-٦</sup> - ١٠×٥<sup>-٤</sup> مولار) قلل بشدة إنقباضات الامعاء الامامية والخلفية للجراد المستحثة بالبوتاسيوم (١٠٠ ملليمول) بشكل تصاعدي. بالإضافة إلى أنه عند تركيز ١٠×٥<sup>-٥</sup> مولار أحدث المركب إنقباضات طبيعية لمعي الجراد الخلفية إلا أن تأثيره التثبيطي كان أقوى على المعى الخلفي للجراد . نتائج هذه الدراسة تقترح أن الكلوربرومازين يحتمل أن يقلل قنوات الكالسيوم ويحتمل أن يمنع دخوله تدريجياً لداخل الخلية وذلك بتثبيط حركة الكالسيوم داخل مواقعها الداخلية .

الإنقباضات المستحثة بفعل المجال الكهربائي إنخفضت تدريجياً بإضافة مركب الكلوربرومازين بين التراكيز (١٠<sup>-٦</sup> مولار - ١٠<sup>-٥</sup> مولار) ولكن عند تركيز ١٠<sup>-٦</sup> مولار المركب أحدث إنقباضات طبيعية للمعى الخلفى للجراد . هذه النتائج أكدت الاقتراحات السابق ذكرها مع البوتاسيوم وكذلك النتائج السابقة على الانقباضات الطبيعية للجراد وعضلات رحم الفأر .