

Effect of Acetylcholine Agonist and Antagonist on the Buccal Mass Smooth Muscle of *Arion ater*

H.M.A. Mutwally

*Department of Biology, Faculty of Applied Sciences,
Umm Al-Qura University, Makkah Al-Mukkarama
P.O. Box 3711, Saudi Arabia*

Abstract: The present investigation aims to study the actions of acetylcholine (ACh) as well as ACh agonists [nicotine; carbamylcholine (Carbachol); propionylcholine (PCh); butyrylcholine (BCh) and valerylcholine (VCh) and ACh antagonists (atropine and gallamine) on the buccal mass smooth muscle of *Arion ater*. The results of this study showed that ACh is acting as the main up regulatory transmitter. Its action was dose-dependent and at low concentrations elicited phasic twitch activity. Moreover, results of nicotine, ACh analogue and ACh receptor (AChR) antagonist experiments point to the presence of an AChR which is a hybrid of the two mammalian types, nicotinic receptors (AChN) and muscarinic receptors (AChM).

Key Words: Acetylcholine (ACh), ACh agonists, ACh antagonists, ACh receptor, *Arion ater*.

Introduction

Arion ater, is a common temperate terrestrial slug. This slug is herbivorous, feeding on decaying vegetable matter and growing plants, often becoming a pest of agriculture and horticultural crops, or in the garden (Runham and Hunter, 1970; South, 1992).

Several neurotransmitter substances have been identified from the pulmonate nervous system. Acetylcholine (ACh) has been shown to exhibit both excitatory and inhibitory actions between neurones in the pulmonate central nervous system (Clark *et*

al., 1992; South, 1992). The classical neurotransmitters released at the terminals of neurones are ACh and noradrenaline (NA), which are indigenous to the parasympathetic and sympathetic nervous system respectively (Langton and Huddart, 1987 and 1988; Clark *et al.*, 1992).

The aim of the present study is to investigate the actions of ACh and the type of AChR present in the buccal mass muscle of *Arion ater* using receptor agonist and antagonist.

Materials and Methods

Experimental animals: A breeding

population of *Arion ater* was established. They were housed in plastic containers lined with moist compost, and fed on lettuce, carrot and wheat seeds. Only healthy and active slugs were selected for dissection.

Artificial saline preparation: The formula of the artificial saline was compiled by (Gibson and Logan, 1992) and was composed of (g/l): NaCl 5.2, KCl 0.3, CaCl₂ 2H₂O 1.03, Mg₂SO₄ (Anh) 0.476, Tris 0.6, Glucose 1.8. The pH was adjusted to 7.5 at room temperature using NaCl and HCl. The saline could be stored for up to one week at 0-5 °C.

Test drugs: The test drugs used in this study, were as follows: ACh, Nicotine, carbamylcholine (Carbachol), propionylcholine (PCh), butyrylcholine (BCh) and valerylcholine (VCh). These drugs were obtained from Sigma Chemical Company and were prepared freshly from stock solutions. They were added to the organ bath at known amount of stock solutions. After each test, the saline was drained and replaced with fresh saline from an overhead reservoir.

Dissection: The dissection and ligation of the buccal mass were done according to after (Bullough 1950 and Smith 1990) respectively. After selection the active slugs were anaesthetized with cold (in the fridge ~ 4 °C) for 3-5 minutes, the cut along the dorsal mid line and pinned open. The buccal mass was extracted by severing the associated nerve ganglion and oesophagus,

also by cutting around the mouth opening. During dissection and prior to mounting the preparation was kept moist with artificial saline.

Preparation mounting: Mounting the buccal mass in the organ bath was done as described by (Hill and Huddat 1995 and Wright *et al.*, 1996). Once removed, the buccal mass was ligated with cotton around the mouth opening from one end, and around the radula sac from the other end. Then was suspended in the 10 ml organ bath. This allowed the tissue to be held under tension in the organ bath but still gave scope for contraction. The ligature hook was attached to the plate of force displacement transducer, which detects changes in tension. Once in place the buccal mass was left to equilibrate in the bath for 30 minutes. This was essential for the tissue to become accustomed to the new environment prior to undertaking tests.

The apparatus and experimental routines: For muscle tension recording, the tissue is connected through an amplifier to four channel Grass Instrument model 79D polygraph and Grass FT 0.03 force-displacement transducers, which records contractile force as a curvilinear ink trace. An overflow system is present to finely adjust the bath volume to 10 ml. The saline is drained by gravity with the aid of a water pump. The organ bath was maintained at 10°C with a Grant closed circuit cooler/circulatory system and the organ bath

contents were gently constantly aerated. Each result was replicate of 8 experiments, run for 20 to 30 minutes and followed by 20 minutes washing period with normal saline.

Results

In this study, ACh appears to be the dominant neurotransmitter in this mollusc. Dose-dependent increases in tonic force were seen (Figure 1a). At a single doses of low concentrations of ACh agonist superimposed phasic twitch activity was also noticed (Figure 1b). The results also indicate that nicotine-induced response was not as high as ACh-induced response.

Further examinations into the type of ACh-receptor present were undertaken with the use of the muscarinic antagonistic atropine and the nicotinic receptor blocker, gallamine. Atropine and gallamine caused reduction in ACh-induced responses, However, gallamine-caused more reduction (Figures 2a and 2b).

Figure 3 shows the dose-dependent manner in which the ACh agonists increased contractile force. Variation is seen in the affinity of these agonist. Carbachol has the greatest affinity and VCh the least. Their affinity can be arranged in the following order: Carbachol > BCh > ACh > PCh > BCh.

Discussion

In this study, ACh elicited a dose-

dependent increase in contractile force with phasic twitch activity superimposed at lower concentrations. (Hill and McDonald-Ordzie, 1979 and Mutwally, 1998) concluded that ACh-induced responses were dependent on $[Na^+]_o$ and $[Ca^{21}]_o$, with a reduction in tension seen in Na^1 -free and Ca^{21} -free saline. This suggests a role for Na^1 in membrane depolarization and subsequent Ca^{21} -ion channel opening, supported by voltage clamping and sucrose-gap studies (Orkand and Orkand, 1975; Langton and Huddart, 1987 and 1988; Ram *et al.*, 1991). Moreover, sucrose-gap studies showed that the level of depolarization in Ca^{21} -free saline is not altered although the tension development nearly abolished. This reduction could be due to depolarization prevention or to the lack of available Ca^{21} to induce contraction (Nelson and Huddart, 1992; Huddart and Hill, 1996).

Molluscan muscle is known to have a very complex innervation. (Nelson, 1994) found that the radular retractor muscle of the whelk *Buccinum* had several types of nerve ending and four types of neurotransmitter vesicles, with evidence for co-transmission. (Hill and McDonald-Ordzie, 1979) suggested that ACh is the principle neurotransmitter in molluscs.

ACh was the first neurotransmitter substance isolated by (Dale, in 1914), and it has an important role in the neurotransmission of signals *via* the cholinergic neural pathway of the nervous system. The results

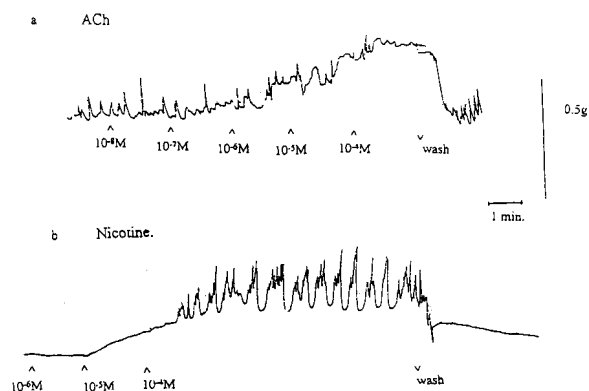


Figure 1. Show cumulative ACh-additions on buccal mass smooth muscles. (a) show dose-dependent responses of ACh (10^{-8} M- 10^{-4} M) (upper arrows), while (b) represent the effect of nicotine (10^{-6} M- 10^{-4} M) (upper arrows). Note that the muscle was more sensitive to ACh than to nicotine. Lower arrows indicated washout with normal saline. The force and time calibrations were standardized for each experiment reported in all experiments indicated on the right hand side of the Figures.

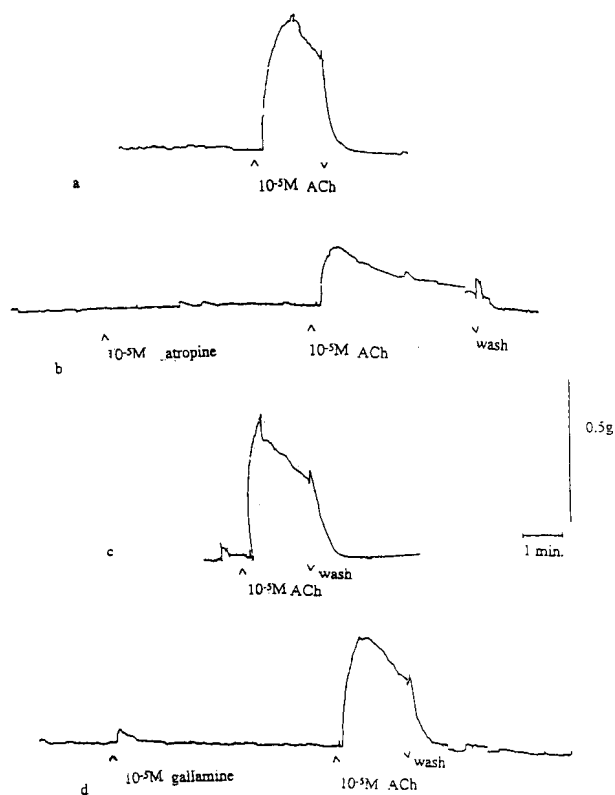


Figure 2. Effect of cholinergic antagonist. (a & c) show control effect of (10^{-5} M) ACh-induced responses (upper arrow). (b) represent the addition of (10^{-5} M) atropine (1^{st} upper arrow) caused inhibition in ACh-responses (2^{nd} upper arrow), while (d) show slight reduction in ACh-responses caused by (10^{-5} M) gallamine addition as indicated. Lower arrows indicated washout with normal saline. The force and time calibrations were standardized for each experiment reported in all experiments as indicated on the right hand side of the Figures.

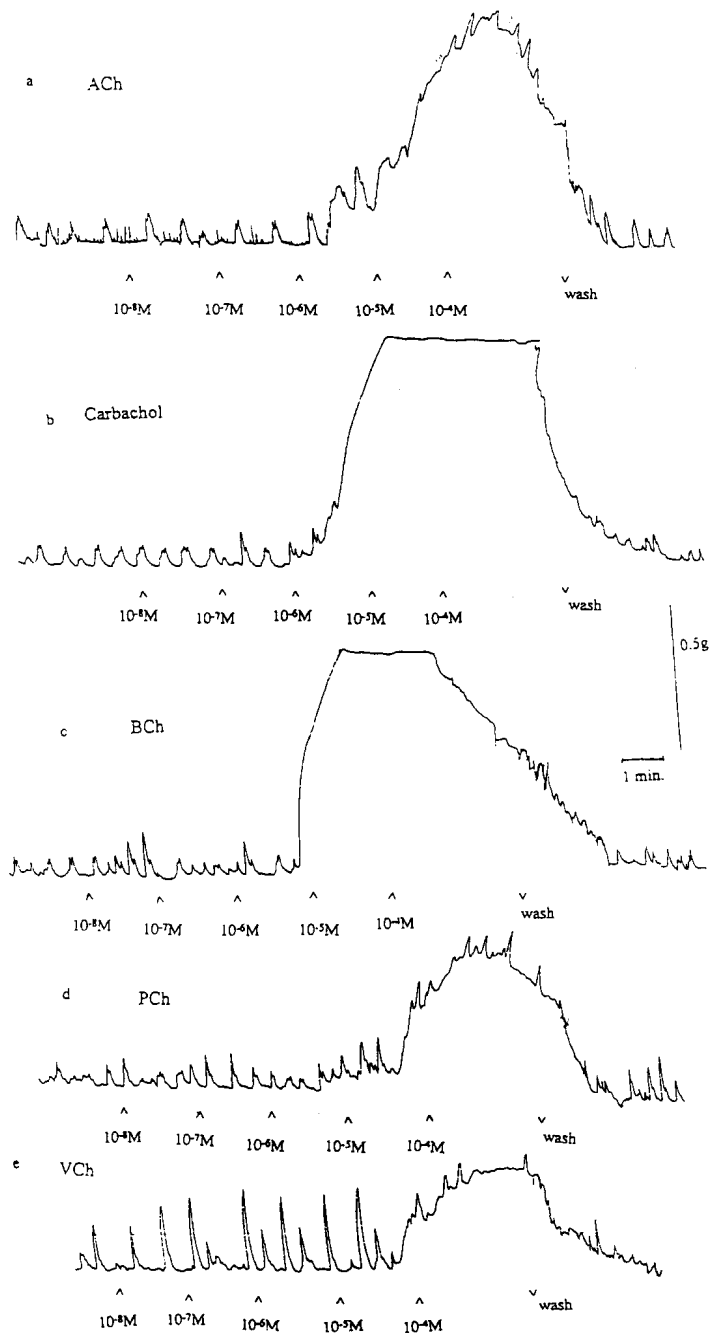


Figure 3. Represented cumulative addition of ACh and ACh-analogues (10^{-8}M - 10^{-4}M) (upper arrows). (a) Effect of control ACh-responses, (b) carbachol, (c) BCh, (d) PCh and (e) VCh as indicated respectively. In this figure, carbachol show the greatest affinity and VCh show the least. Lower arrow indicated washout with normal saline. The force and time calibrations were standardized for each experiment reported in all experiments as indicated on the right hand side of the Figures.

of this study show that the buccal mass of *Arion ater*, responded to the single and cumulative additions of ACh in a dose-dependent manner. This result suggested that the smooth muscles of the buccal mass have AChR. The present work also indicates that the buccal mass muscles did not respond to nicotine as ACh, but it is signifying the presence of mammalian AChN-like receptors. The findings of the present study are in agreement with those of (Burgen and Mitchell 1978; Huddart *et al.*, 1990 and Nelson 1992).

The present study, the phasic twitch activity seen at lower ACh-concentrations indicates the presence of two voltage dependent Ca^{21} -ion channels, one fast and one slow. (Langton and Huddart, 1987 and 1988) concluded that these twitch activities appear to occur from Ca^{21} -influx through the fast transmembrane channel, then as the cell becomes further depolarized the slow one takes over, the flow of Ca^{21} -ions through these channels is possible for increased tension.

Due to the fact nicotine evoked a response similar to that of ACh-induced response in *Arion ater*, the ACh shown to have properties of the nicotine like mammalian receptor. This is also supported by the present results of the ACh analogue tests, where carbachol and BCh showed a higher affinity for the AChR than ACh itself. This result is more inactive of the mammalian-type AChN, which binds the

carboxyl group of the molecule as opposed to the AChM, which binds the methyl group. Concerning the AChM receptor antagonists, atropine was more successful than gallamine in reducing ACh-elicited responses. There are two types of mammalian ACh receptors (AChR); the nicotine (AChN) and muscarinic (AChM) types (Wright *et al.*, 1996).

In this study, gallamine caused more reduction in ACh-induced responses than atropine. This result indicated the prevalence of muscarinic-like mammalian receptors in smooth muscles of the buccal mass. Moreover, this information indicates that the AChR in this mollusc is hybrid, having properties of both muscarinic and nicotine type mammalian receptors as were seen in molluscan muscle of *Buccinum undatum* (Nelson, 1992; Nelson and Huddart, 1992). In addition, this study also showed dose-dependent variations in the affinity of these ACh agonists affinity can be arranged in the following order: Carbachol > BCh > ACh > PCh > Vch. The extra CH_2 groups on VCh appear to hinder receptor binding (Burgen and Mitchell, 1978).

These results point to the presence of AChRs with properties of both mammalian-type receptors and are more characteristics of the mammalian-type nicotine receptor. It appears that the separate AChRs evolved later in higher animals. The results presented here, for the whole muscles not on individual muscles, give a good account of

possible neurotransmitters controlling the activation of muscles associated with feeding in *Arion ater*.

Acknowledgements

Author would like to thank Dr. H. Huddart, Department of Biology, Lancaster University, Lancaster, UK, for providing the facilities for experimental work carried out in his laboratory.

References

- Bullough, W.S. 1950. In: practical invertebrate Anatomy. Chapter 18. Macmillan & Co. Ltd.,
- Burgen, A.S.V. and Mitchell, J.F. 1978. Gaddum's pharmacology. 8th edition. Oxford University Press London.
- Dale, H.H. 1914. The action of certain esters and esters of choline and their relation to muscarine. *J. Pharmac. Exp. Ther.*, 6: 147-190.
- Gibson, I.C. and Logan, S.D. 1992. The actions of phorbol esters upon isolated calcium currents of *Helix aspersa* neurons. *Comp. Biochem. Physiol.* 102C, 297-303.
- Hill, R.B. and Huddart, 1995. Actions of GTP on molluscan buccal, cardiac, and visceral smooth muscle. *Comp. Biochem. Physiol.* 111C, 3: 389-396.
- Hill, R.B. and McDonald-Ordzie, P.E. 1979. Ionic dependency of the response to acetylcholine of a molluscan buccal muscle; the radular protractor of *Busycon canaliculatum*. *Comp. Biochem. Physiol.* 62C, 19-30.
- Huddart, H.; Brooks, D.D.; Lennard, R. and Hill, R.B. 1990. Diversity of mechanical responses and their possible underlying mechanisms in the proboscis muscles of *Busycon canaliculatum* *J. Comp. Physiol.* 159B, 717-725.
- Huddart, H. and Hill, R.B. 1996. Ionic dependency of membrane potential and autorhythmicity in the atrium of the whelk *Busycon canaliculatum*. *G. Pharmac.*, 27, 5: 819-825.
- Langon, P.D. and Huddart, 1987. The involvement of fast calcium channel activity in the selective activation of phasic contractions by partial depolarization in rat vas deferens smooth muscle. *Gen. Pharmac.*, 18: 47-55.
- Langton, P.D. and Huddart, H. 1988. Voltage, H. 1988. Voltage and time dependency of calcium mediated phasic and tonic responses in rat vas deferens smooth muscle. The effect of some calcium agonist and antagonist agents. *Gen. Pharmac.*, 19: 775-787.
- Mutwally, H.M.A. 1998. The pharmacology of buccal mass smooth muscle of *Arion ater*. *Umm Al-Qura Univ. J. in prepartaion*.
- Nelson, I.D. 1992. The physiology and pharmacology of muscles from the proboscis of two species of whelk: *Buccinum undatum* and *Busycon canaliculatum*. (Ph.D.). Lancaster University, UK.
- Nelson, I.D. 1994. The relation between excitation-contraction coupling and fine structure of a molluscan muscle, the radular retractor of the whelk, *Buccinum undatum*. *J. Comp.*

- Physiol. B.*, 164: 229-237.
- Nelson, I.D. and Huddart, H. 1992. The nature of the ACh receptor in *Buccinum* proboscis muscle examined by the sucrose-gap voltage-clamp technique. *G. Pharmac.*, 23, 3: 317-323.
- Orkand, P.M. and Orkand, R.K. 1975. Neuromuscular junctions in the buccal mass of *Aplysia*: fine structure and electrophysiology of excitatory transmission. *J. Neurobiol.*, 6, 531-548.
- Ram, J.L.; Zhang, F. and Liu, L. 1991. Contraction, serotonin-elicited modulation, and membrane currents of dissociated fibers of *Aplysia* buccal muscle. *Biol. Bull.* 180: 276-283.
- Runham, N.W. and Hunter, P.J. 1970. Terrestrial slugs. Chapter 1, and 3. Hutchinson and Co.,
- Smith, D.A. 1990. Comparative buccal anatomy in *Helisoma* (Molluscan, Pulmonata, Basommatophora). *J. Morphol.*, 203: 107-116.
- South, A. 1992. Terrestrial slugs, biology, ecology and control. Chapter 1,2,3 and 6. Chapman & Hall.
- Wright, T.J.; Huddart, H. and Edwards, J.P. 1996. A pharmacological study of the smooth muscle in the buccal mass of *Deroceras reticulatum*. *BCPC. Symps. Proc. (Slug & Snail Pests in Aric.)*, 66:341-346.

أثر منشطات ومثبطات مركب الأستاييل كولين على العضلات الملساء للتجويف الفمي للبزاق أريون أتر

حامد محمد عبد القادر متولي
قسم الأحياء ، كلية العلوم التطبيقية ، جامعة أم القرى ،
مكة المكرمة - ص.ب. ٣٧١١ المملكة العربية السعودية

الملخص: أظهرت هذه الدراسة أن مركب الأستاييل كولين (ACh) يعمل كموصل عصبي . ذلك لأن أثره الإستحاثي على عضلة التجويف الفمي للبزاق أريون أتر كان تصاعديا . وعند إضافة تراكيز متدنية من مركب الأستاييل كولين ، أظهرت هذه العضلة إنقباضات متسارعة من نوع الفيسيك (phasic twitches) . إضافة لذلك ، فإن النتائج المتعلقة بمركب النيكوتين (nicotine) ، والذي يعمل كمركب منشط مناظر أثر مركب الأستاييل كولين ، هذه المستقبلات هي ن النوع المماثل لكلا المستقبلين الموجودين في طائفة الثدييات والتي هي من نوع أسيتيل كولين نيكوتين (AChN) وأستاييل كولين مسكرين (AChM)