

Effect of ACh agonist and antagonist on the buccal mass smooth muscle of

Arion ater

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ABSTRACT The result 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 from nicotine, ACh analogue and AChR antagonist experiments point to the presence of an AChR which is a hybrid of the two mammalian types, AChN and AChM.

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 agricultural and horticultural crops, or in the garden (Runham & Hunter, 1970; South, 1992).

Several neurotransmitter substances have been identified from the pulmonate nervous system. Acetylcholine (ACh) has been shown to be both excitatory and inhibitory actions between neurones in the pulmonate central nervous system (NS) (Clark *et al.*, 1992; South, 1992). The classical neurotransmitters released at the terminals of neurones are acetylcholine (ACh) and noradrenaline (NA) which are indigenous to the parasympathetic and sympathetic nervous system (NS) respectively (Clark *et al.*, 1992; Langton & Huddart, 1987 & 1988).

The aim of this study is to extend previous investigation of Mutwally (1998, In Preparation) and to focus on the action 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 ater* was established and 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 as follows (g / l): NaCl 5.2, KCl 0.3, CaCl₂·2H₂O 1.03, Mg₂SO₄ (Anh) 0.476, Tris 0.6, Glucose 1.8, adjusted to pH 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: Nicotine, ACh, 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 to the organ bath. After each test, the saline was drained and replaced with fresh saline from an overhead reservoir.

Dissection: The dissection and ligature the buccal mass was 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, then 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 after Hill and Huddart (1995) and Wright *et al.* (1996). Once removed, the buccal mass was ligature 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 a 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 a 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 the agonist superimposed phasic twitch activity was also visible. Figure 1b compares the development of ACh- and nicotine-induced tension. Although, nicotine-induced response but it 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 receptor antagonist, atropine and the nicotinic receptor blocker, gallamine. Atropine and gallamine caused reduction in ACh-induced responses. However, gallamine-caused more reduction (Figure 2a & 2b).

Figure 3a to 3e shows the dose-dependent manner in which these compounds increased contractile force. Variation is seen in the affinity of these agonist have for the AChR. Carbachol has the greatest affinity and VCh the least as follows: Carbachol > BCh > ACh > PCh > VCh.

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, In Preparation) concluded that ACh-induced responses were dependent on $[Na^+]_o$ and $[Ca^{2+}]_o$, with a reduction in tension seen in Na^+ -free and Ca^{2+} -free saline. This suggests a role for Na^+ in membrane depolarization and subsequent Ca^{2+} -ion channel opening, supported by voltage clamping and sucrose-gap studies (Orkand & Orkand, 1975; Langton & Huddart, 1987 & 1988; Ram *et al.*, 1991). Moreover, sucrose-gap studies showed that the level of depolarization in Ca^{2+} -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^{2+} to induce contraction (Nelson & Huddart, 1992; Huddart & Hill, 1996).

Molluscan muscle is known to have a very complex innervation. A study of the radular retractor muscle of the whelk *Buccinum* (Nelson, 1994) found several types of nerve ending and four types of neurotransmitter vesicles, with evidence for co-transmission.

ACh was the first neurotransmitter substance isolated by Dale in (1914). Its function is in the neurotransmission of signals *via* the cholinergic neural pathway of the nervous system. The result of this study shows that the buccal mass of *Arion ater*, responded to the single and cumulative additions of ACh in dose-dependent manner. This result suggested that this muscle have AChR. Although, the buccal mass muscles did not response to nicotine as ACh, but it is signifying the presence of mammalian AChN-like receptors. This study also goes in agreement with Burgen and Mitchell (1978), Huddart *et al.* (1990) and Nelson (1992).

The phasic twitch activity seen at lower ACh-concentrations indicates the presence of two voltage dependent Ca^{2+} -ion channels, one fast and one slow. Langton and Huddart (1987 & 1988) concluded that these twitch activities appears to occur from Ca^{2+} -influx through the fast transmembrane channel, then as the cell becomes further depolarized the slow one takes over, the flow of Ca^{2+} -ions through which is possible for increased tension.

Due to the fact that nicotine evoked a response same as that of ACh-induced response in *Arion ater*, the ACh is shown to have properties of the nicotinic 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 indicative of the mammalian-type AChN, which binds the carboxyl group of the molecule as opposed to the AChM, which binds the methyl group. However, the AChM receptor antagonist, atropine was more successful than gallamine in reducing ACh-elicited responses. Hill and McDonald-Ordzie (1979) suggested that ACh is the principle neurotransmitter in molluscs, and there are two types of mammalian ACh receptor (AChR) can be found; the nicotinic (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. Moreover, this information indicates that the AChR in this mollusc is hybrid, having properties of both muscarinic and nicotinic type mammalian receptors as were seen in molluscan tissues (Nelson, 1992; Nelson & Huddart, 1992). In addition, this study also showed dose-dependent variations in the affinity of these agonist have for the AChR, Carbachol > BCh > ACh > PCh > VCh. The extra CH₂ groups on VCh appear to hinder receptor binding (Burgen & Mitchell, 1978).

These results point to the presence of an AChR with properties of both mammalian-type receptors and are more characteristic of the mammalian-type nicotinic receptor. It appears that the separate AChRs evolved later in higher animals. The result 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*.

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FIGURE LEGENDS

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 as 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 (1st upper arrow) caused inhibition in ACh-responses (2nd 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.

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

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السعودية

الملخص:

أظهرت هذه الدراسة أن مركب الأستاييل كولين (ACh) يعمل كموصل عصبي . ذلك لأن أثره الإستحثاثي على

عضلة التجويف الفمي للبزاق أريون آثر 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 لأثر مركب الأستاييل كولين، وكذلك من نتائج

المركبات المثبطة للقنوات المستقبلية لأثر مركب الأستاييل كولين فإنها جميعا تدلل على وجود مستقبلات لمركب الأستاييل

كولين. هذه المستقبلات هي من النوع المماثل لكلا المستقبلين الموجودين في طائفة الثدييات والتي هي من نوع أسيتيل كولين

نيكوتين (AChN) وأستاييل كولين مسكرين (AChM) .