

# Histamine stimulated small intestine



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The longitudinal smooth muscle of the guinea-pig ileum small intestine contracts in response to acetylcholine. These contractions can be reduced by the application of adenosine and related compounds.

The guinea-pig ileum is innervated by the enteric, sympathetic and parasympathetic divisions of the autonomic nervous system. The parasympathetic and enteric fibres release acetylcholine which acts on muscarinic receptors.

The action of adenosine and its receptor antagonists can be assessed by comparing electrically induced contractions via electrical field stimulation and histamine induced contractions. Electrical field stimulation contractions cause presynaptic release of acetylcholine to produce contractions whereas the histamine induced contractions cause postsynaptic contractile responses.

Throughout this study adenosine and its receptor antagonist actions will be investigated and compared using electrical field stimulation and histamine.

The contraction of the gut

In gastrointestinal smooth muscles, researches show that there are two types of muscarinic receptors that are present as targets to the neurotransmitter acetylcholine (Okamoto et al., 2002). Acetylcholine and its derivatives produce contractions by activating muscarinic receptors. The muscarinic receptors types are known as M2 and M3. Binding Studies have portrayed that the number of M2 receptors is greater than that of the M3 receptors however functional studies have shown that M3 muscarinic

receptors play a fundamental role in mediating the contractile response (Eglen et al., 1996) and the functional role of M2 exists as unclear (Clague et al., 1985). The M3 receptor is coupled with G proteins, causing activation of phospholipase C and formation of inositol trisphosphate and diacylglycerol, which are expected to contribute in muscarinic receptor mediated smooth muscle contractions (Unno et al., 2005). They also mediate relaxation due to the release of nitric oxide from neighbouring endothelial cells. M3 receptors in visceral smooth muscle contribute to the smooth muscle stimulating effect of muscarinic agonists. However the muscarinic receptor most abundant in the ileum is the M2 which cause an indirect contraction of the guinea-pig ileum by preventing the relaxing effect of drugs (Ehlert and Thomas, 1995). Both muscarinic receptor subtypes are activated by acetylcholine and produce a contractile response; however they vary in their transduction mechanisms and signalling pathways.

## Adenosine

Adenosine has numerous diverse roles in normal physiology; such roles include promoting/maintaining sleep, regulating state of encouragement as well as local neuronal excitability and coupling cerebral blood flow to energy demand (Dunwiddie and Masino, 2001).

It exists free in the cytosol of all cells and is transported in and out of all cells mainly using a membrane transporter (Rang et al., 2007). Under normal conditions, adenosine is formed intracellularly as well as extracellularly (Fredholm et al., 2001). ATP is stored in vesicles and released by exocytosis. It is also available in the cytosol of cells and is taken up and released via a

specific membrane transporter. Released ATP and ADP are rapidly converted to adenosine by the action of tissue nucleotidases. Studies have shown that there are pathways that contribute to adenosine formation, a) by the action of adenylate kinase and cytosolic 5'-nucleotidase, b) formation from hydrolysis of adenosine 3, 5' phosphate and c) formation by the action of S-adenosylhomocysteine (SAH) hydrolase.

The pharmacological effects of adenosine include smooth muscle relaxation and inhibition of nerve activity, lipolysis and platelet aggregation (Daly et al., 1983). There is evidence that stimulation or inhibition of adenylate cyclase is involved in adenosine action and therefore it has been concluded that adenosine is mediated by cyclic AMP. Based on its ability to inhibit cell function and thus minimise the metabolic requirements of cells, one of its functions may be as a protective agent released when tissue integrity is threatened.

Adenosine exerts its physiological actions activation of a number of specific cell surface receptors. There are four different adenosine receptors known as A1, A2A, A2B, and A3. Some characteristics of these receptors are presented in Figure 1a. These subtypes have been distinguished on the basis of their agonist and antagonist selectivity. They belong to the G protein-coupled receptors.

#### Mechanism of Adenosine action

Adenosine A1 receptors are negatively coupled to the inhibition of adenylate cyclase, however they can act through other pathways such as stimulation of phospholipase C, activation of potassium channels and inhibition of N-type

calcium channels (Zizzo et al., 2009). A1 receptors are coupled to Gi and G0 proteins and lead to inhibition of adenylate cyclase and consequently cause a decrease in cAMP (Ranjit, 2008). Adenosine A2A and A2B are coupled for activation of adenylate cyclase whereas A3 receptors have been shown to stimulate phospholipase C and D, to inhibit adenylate cyclase and to activate ATP sensitive potassium channel (Ralevic and Burnstock, 1998). Activation of these receptors require comparatively high amounts of adenosine. A2A and A2B receptors have a high and a low affinity for adenosine respectively.

### Receptor Subtype

A1

A2A

A2B

A3

Transduction mechanism

Inhibits adenylyl cyclase

Activates adenylyl cyclase

Activates adenylyl cyclase

Inhibits adenylyl cyclase

Primary distribution

Brain (cortex, cerebellum, hippocampus). Dorsal horn of spinal cord.

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Eye, adrenal gland, atria

Spleen, thymus, leukocytes, blood platelets.

Straitopallidal GABAergic neurons, olfactory bulb

Caecum, colon, bladder

Testis, mast cells

Tissue functions

Antinociception,

Hypothermia.

Sedation,

Sleep,

Inhibition of lipolysis,

Cardio and neuroprotection

Reflex tachycardia, vasodilation, inhibition of platelet aggregation, sleep protection against ischemia

Relaxation of vascular and intestinal smooth muscle, cytokine production, inhibition of cell proliferation

Mast cell degranulation, coronary vasodilation and protection from reperfusion

## Selective antagonists

DPCPX

PSB 36

SCH 58261

PSB 1115 potassium salt

MRS 3777 hemioxalate

Figure 1a: Summary of adenosine receptors.

## Adenosine and the enteric functions of the Gut

The enteric nervous system (ENS) consists of a compilation of neurons in the gastrointestinal nervous system which is capable of functioning independently of the central nervous system. It moderates motility, secretion, microcirculation, inflammatory and immune responses of the gastrointestinal tract (Altaf and Sood, 2008). The ENS is composed of extrinsic, which consists of parasympathetic and sympathetic divisions and the intrinsic component which encloses neurons. Intestinal functions results from an interaction between the ENS, smooth muscle and the mucosal/immune system. The network is regulated by several mediators; however there is consolidating evidence that adenosine is a significant regulating agent (Bueno, 2000) (Wood, 2004). Studies show that in the small intestine adenosine and adenosine derivatives were found to inhibit cholinergic transmission in guinea-pigs via a prejunctional action on neurotransmitter release (Gustafsson et al., 1978). The action of the A1

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receptors allowed mediation of the inhibitory action of adenosine in the cholinergic transmission(Shinozuka et al., 1985) of motor neurones innervating circular and longitudinal smooth muscle however A2A receptors have been reported to reduce the cholinergic motor responses(Gustafsson et al., 1985a; Gustafsson et al., 1985b).

## Histamine

Histamine has a role as a primary transmitter or neuromodulator and it is widely distributed within mammalian tissues. (Izzo et al., 1998). Histamine is a vasoactive substance to be identified in the body which can rapidly metabolise and holds properties of being highly polar and not diffusing readily across cell membranes or the blood-brain barrier. It is stored in mast cells and basophils of blood and has two receptors known as H1 and H2. The release of histamine could cause changes in the cardiovascular system and induce anaphylactic shock.

Histamine has been shown to induce gastric acid secretion through the H2 receptors linked to cyclic AMP production in oxyntic cells. Researches show that gastric cells of the guinea-pig may have a class of binding sites for histamine which shows no relationship to adenylate cyclase and the H2 receptor. Histamine creates a spasmogenic effect on the intestine that results from H1 receptor stimulation(Guy A. and Settipane, 1988-1989).

There are three histamine subtypes known as H1, H2 AND H3 and all three have been identified to be present in the guinea pig small intestine. Studies show that H1 receptor subtypes mediate the contraction of the longitudinal muscle in the small intestine (Izzo et al., 1998). However researchers also

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state that the effect of histamine is predominantly due to the interaction with H1 receptors located on smooth muscle cells and moderately due to the interaction H2 receptors present on myenteric plexus interneurons (Bauer and Matusak, 1988).

## **AIMS**

The aim is to confirm the prejunctional action of adenosine and examine whether adenosine has the additional ability to relax the smooth muscle directly.

The project will use histamine to contract the smooth muscle and the objective is to find out whether adenosine can reduce these contractions and if so is the concentration range similar to that needed to inhibit the contractions to the electrical field-stimulation? It will also be investigated what adenosine receptor subtype is involved (A1, A2A, A2B, A3 identified using selective antagonists).

## **METHOD**

Animals and preparation of tissue

Dunkin Hartley guinea-pigs (250g +) of male sex that had previously been fed Harlan 2040, the guinea-pig diet and ad lib filtered tap water, were obtained from Harlan UK. They were group housed and provided with grade 6 woodchip and hay bedding. Their enrichment consisted of plastic and cardboard fun tunnels, plastic igloos and gnawing blocks. Furthermore they were kept at room temperatures of 19-23°C and at room humidity of 45-65%. They were provided with 12 hours light and 12 hours of dark lighting.

The guinea-pigs were stunned by a blow to the head and sacrificed by exsanguination. Two segments of 3cm length were removed from the distal part of the small intestine, for each tissue the ends were tied with cotton threads to the tissue holder and then suspended in 20ml organ baths containing Krebs solution (composition in mM: NaCl, 118; NaHCO<sub>3</sub>, 25; Glucose, 11; KCl, 4.7; CaCl<sub>2</sub>, 2.5; KH<sub>2</sub>PO<sub>4</sub>, 1.18; MgSO<sub>4</sub>, 1.18). This was aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37°C. The tissues were left for 30 minutes to equilibrate under a resting tension of 1g before starting the stimulation.

#### Experimental protocol

The organ baths were equipped with parallel electrodes which allowed electrical field stimulation to be transmitted at a frequency of 0.1 Hz, 40V, 0.5ms pulse duration. Contractions of the ileum were measured with isometric transducers (ADInstruments Force Transducers), amplified and recorded onto a data capture system (Lab Charts on the PC). The tissues were allowed to stabilise in the organ baths in order to reach steady contractions. Figure 1b represents the experimental set up.

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Figure 1b shows the laboratory designed set up for the experiment, with a set of two organ baths.

There were several parts (a-e) to the experiments to be carried out on the ileum. 'n' is the number of ileum used throughout the experiment n= 18. The number of experiments carried out on the ileum was 56.

Effect of Adenosine-the experiment consisted of stimulating the tissues continuously with electrical field stimulation and adding cumulative concentrations of adenosine ( $10^{-11}\text{M}$  -  $10^{-4}\text{M}$ ) to the organ baths and the responses were recorded.

Effect of Histamine- the tissues were stimulated with histamine, cumulative concentrations of histamine ( $10^{-11}\text{M}$  -  $10^{-4}\text{M}$ ) were added to the organ bath and responses were measured. The concentration  $1 \times 10^{-6}\text{M}$  gave the maximum response and a steady contraction, it was used to contract the tissue with adenosine.

Effect of Adenosine in the presence of Histamine- the concentration of histamine that gave the maximum and steady response was added to organ bath. The tissue was permitted to stabilise in order to reach steady contractions. Once reached, cumulative concentrations of adenosine were added ( $10^{-8}\text{M}$  -  $10^{-4}\text{M}$ ) in order to observe the responses of the ileum to adenosine in the presence of histamine.

Effect of PSB36( $10^{-8}\text{M}$ ) & SCH58261( $10^{-7}\text{M}$ ) - to confirm identity of receptors being investigated, cumulative dose-response curves for induced contractions by histamine were observed for adenosine in the presence of selective antagonists, PSB36 and SCH58261.

An experiment was also carried out, which involved electrical field stimulation to contract the tissue, adenosine was added in the presence of these antagonists.

Effect of Atropine - Atropine concentrations of  $10^{-6}M$  -  $10^{-7}M$  were added to establish the effects of atropine on the electrically field stimulated ileum.

For each drug that was being tested except atropine, the experiment was repeated at least six times. The tissue was washed out three times at least after an experiment was completed and was allowed to recover before another experiment was carried out. The electrical field stimulation was also switched off each time the tissue was washed out.

### Chemicals and drugs

The drugs that were used consisted of Adenosine hemisulphate salt, Histamine diphosphate salt and Atropine sulphate salt which were all purchased from Sigma-Aldrich, Poole, UK. PSB36 and SCH58261 and DMSO (Dimethyl sulfoxide) were purchased from Tocris -Cookson, Bristol, UK.

All drugs were dissolved in distilled water with exceptions of PSB36 AND SCH58261 which were diluted with DMSO. 10ml of stock solution were made up in each case.

### Statistical Analysis

All drug concentrations presented were final bath concentrations. The drug effects were expressed as twitch contraction (g). All data were given as means  $\pm$  S. E. M, where n represents the number of animals from which tissues were taken and on which observations were made. Inhibitory effects by adenosine in the field stimulated guinea pig ileum were measured and the responses of ileum twitch contraction were recorded for each concentration

applied. This was repeated when using adenosine receptor antagonists.

Adenosine responses were fitted onto concentration-response curve.

Effects of histamine stimulated guinea pig ileum were also recorded.

Adenosine responses and its receptor antagonist response were measured and plotted.

### **3. 0 RESULTS**

**a.**

**b.**

Figure 2. The effect of adenosine concentrations in the guinea-pig ileum.

Data are means  $\pm$  S. E. M and are expressed as an average of contractions

(g). a. Representative traces showing inhibitory responses induced by

adenosine. b. Concentration response curve for adenosine representing the

average twitch response (g) when cumulative adenosine concentrations

were added. Each point with bar represents the mean  $\pm$  S. E. M (n= 6).

Figure 3a: Original trace illustrating twitch response abolished by atropine

10-6M final bath concentration.

**b.**

**c.**

Figure 3. Influences of atropine and potent and selective A1 adenosine

receptor antagonist PSB36 10-7M AND 10-8M on guinea-pig ileum. b. Twitch

responses of the guinea-pig ileum preparation to electrical field stimulation

in the presence of PSB36 10-7M (n= 6) and PSB36 10-8M (n= 4), compared

to twitch responses to adenosine alone. The experiments carried out were

not paired. Each point with bar represents the mean  $\pm$  S. E. M. c.

Contractions of the guinea-pig ileum preparation stimulated by histamine in the presence of PSB36  $10^{-7}$ M (n= 6) and PSB36  $10^{-8}$ M (n= 4), compared to responses to adenosine when stimulated with histamine (n= 6). The experiments carried out were not paired. Each point with bar represents the mean  $\pm$  S. E. M.

**a.**

**b.**

Figure 4. Summarises the effect of potent and selective A<sub>2A</sub> adenosine receptor antagonist SCH58261  $10^{-7}$ M on guinea-pig ileum. a. Twitch responses of the guinea-pig ileum preparation to electrical field stimulation in the presence of SCH58261  $10^{-7}$ M (n= 6), compared to twitch responses to adenosine alone. The experiments carried out were not paired. Each point with bar represents the mean  $\pm$  S. E. M. b. Contractions of the guinea-pig ileum preparation stimulated by histamine in the presence of SCH58261  $10^{-7}$ M (n= 6), compared to responses to adenosine when stimulated with histamine (n= 6). The experiments carried out were not paired. Each point with bar represents the mean  $\pm$  S. E. M.

Figure 5a. A concentration-response curve illustrating the effects of adenosine on the guinea-pig ileum when the tissue is stimulated with histamine. . Each point with bar represents the mean  $\pm$  S. E. M (n= 6).

Figure 5b. Responses of the guinea-pig ileum to cumulative concentrations of histamine. Each point with bar represents the mean  $\pm$  S. E. M (n= 8).

Electrical field stimulated guinea-pig ileum produced reproducible twitch responses to adenosine. Adenosine concentrations ranging from  $10^{-8}$ M final bath concentration caused concentration dependent inhibitory effects. Administration of increasing adenosine concentrations decreased electrically evoked acetylcholine release from the cholinergic nerve endings of the ileum (Figure 2). Figure 2b clearly portrays that adenosine has no effect at low final bath concentrations of  $10^{-11}$ M.

Atropine  $10^{-6}$ M final bath concentration abolished the twitch responses to electrical field stimulation confirming they were produced by cholinergic nerve stimulation (Figure 3).

In the presence of selective A<sub>1</sub> adenosine receptor antagonist PSB36  $10^{-7}$ M and  $10^{-8}$ M concentration and electrical field stimulation, the twitch responses to adenosine decreased, when adenosine was applied at higher concentrations, the twitch responses became smaller as acetylcholine release was inhibited and completely prevented by PSB36.

Figure 3a shows the curve shifted to the right when PSB36  $10^{-8}$  was administered compared to the curve of adenosine. When PSB36  $10^{-7}$  was applied there is a slight shift of the curve to the right compared to the adenosine curve. The effects of the antagonist PSB36 are seen significantly at  $10^{-8}$ M final bath concentration as the shift of the curve is greater. The highest response the ileum produced was as at adenosine concentration of  $1 \times 10^{-8}$ M, the responses lowered slightly at  $1 \times 10^{-6}$ M and consequently decreased rapidly at  $1 \times 10^{-5}$ M final bath concentrations.

Histamine induces contractions in the ileum. Addition of cumulative concentrations of adenosine in the presence of PSB36  $10^{-7}\text{M}$  produced small responses compared to the contractions produced in the presence of PSB36  $10^{-8}\text{M}$ . The effect of PSB36  $10^{-8}\text{M}$  caused a rightward shift compared to the curve representing the effect of adenosine in the presence of histamine (Figure 3b).

Electrically field stimulated ileum in the presence of the selective adenosine receptor competitive antagonist SCH58261 produced smaller responses (Figure 4a). Following administration of cumulative concentrations the responses decreased however produced no right shift in the dose response curve.

Histamine excites the tissue causing it to contract and producing a high response, when adenosine is applied in the presence of SCH58261, the responses are inhibited and acetylcholine release is decreased and thus there is a decrease in the shape of the curve in Figure 4b.

The results expressed in Figure 5a illustrate the inhibitory effects of adenosine in the ileum. Figure 5b illustrates the effects of histamine concentrations on the ileum. The experiment was carried out to investigate the best concentration to use so that a maximum and strong, reproducible contraction would be produced; the figure confirmed  $1 \times 10^{-6}\text{M}$  final bath concentration to give the highest and steady contraction of the tissue. Moreover this permitted to obtain a dose-response curve for adenosine with histamine providing a suitable starting concentration for each drug at  $1 \times 10^{-8}\text{M}$ .



## **DISCUSSION**

The results of this present study show that adenosine plays an inhibitory role on muscular contractility in guinea-pig ileum.

Adenosine prevents the neuroeffector transmission in guinea-pig ileum. The action of adenosine appeared to be cholinergic prejunctional in nature, this is portrayed when adenosine is applied to electrically field stimulated guinea-pig ileum(Gustafsson et al., 1985b). The action of adenosine on histamine stimulated guinea-pig ileum in the absence and presence of adenosine antagonists also indicates reduction in neuroeffector transmission however due to postjunctional action.

The effect of adenosine on the guinea-pig ileum can be observed in Figure 2. Adenosine produced a dose-dependent depression on the response. It reduced the electrically evoked acetylcholine release from the ileum. A1 and A2 receptors have been reported to reduce acetylcholine release in the gastrointestinal tract(Tomaru et al., 1995). Adenosine released from neuronal endings is thought to have direct actions on smooth muscle as they illustrate relaxant neurotransmitters in the gastrointestinal tract(Storr et al., 2002).

Atropine is a competitive antagonist for the muscarinic acetylcholine receptor, consequently when applied to the ileum at  $10^{-6}$ M final bath concentration; there is rapid inhibition of response confirming that the twitch responses were produced by cholinergic nerve stimulation.

Adenosine inhibited the twitch response of the electrically stimulated guinea-pig ileum preparation, in the presence of PSB36  $10^{-8}$ M final bath

concentration there was a right shift in the adenosine curve thus interpreting that higher concentrations were required to lower the twitch response.

The general trends that Figure 3a displays is that the curves have the same form; the linear proportions of the curves are parallel. The traces help to show the changes in the response curve to adenosine and adenosine selective receptor antagonist PSB36. There is a slight fall in tension when adenosine  $3 \times 10^{-7} \text{M}$  was applied in the presence of PSB36  $10^{-8} \text{M}$ ; however there was a rapid decrease when adenosine  $1 \times 10^{-5} \text{M}$  was administered causing the right shift in the Figure. Upon cumulative additions of adenosine to field stimulated guinea pig ileum the concentration required to inhibit acetylcholine release was of  $3 \times 10^{-7} \text{M}$ , the effects diminish once concentration of adenosine  $1 \times 10^{-5} \text{M}$  was added providing evidence that adenosine has the ability to relax smooth muscle in the ileum.

Since PSB36 is a potent and selective A<sub>1</sub> adenosine receptor antagonist, the A<sub>2A</sub> receptors increase electrically induced twitch contractions in the guinea pig ileum, which contributes to assistance of acetylcholine release (Storr et al., 2002).

The effects of adenosine in the presence of PSB36  $10^{-7} \text{M}$  -  $10^{-8} \text{M}$  to histamine stimulated guinea pig ileum can be observed in Figure 3b. Histamine administration to the guinea pig ileum caused a tonic histamine contraction which was followed by after-relaxation response, and application of adenosine inhibited the acetylcholine release. The ileum responses were reduced significantly with lower concentrations of adenosine in the presence of PSB36  $10^{-7} \text{M}$  and  $10^{-8} \text{M}$  compared to the concentrations of adenosine

required in the electrically field stimulated ileum confirming that A1 receptors are the subtype present in the guinea pig ileum which cause the inhibition of acetylcholine release.

SCH58261 did not significantly affect the position of the rightward shift however additions of cumulative adenosine concentrations caused reduction in the tension produced by the ileum.

Large standard error bars can be observed in the figures, these may be due to human handling errors, i. e. micropipetting errors, administering less or more concentration of adenosine or antagonists. Protein build up causing contamination in organ baths can also contribute to acquiring inaccurate results. Particular cells of the tissue may have become inactive at that moment of time. Furthermore it could be that the piece of thread holding the tissue may have become loose i. e. equipment errors and consequently tension was not measured accurately. It could also be due to unknown errors.

Evidence that adenosine inhibits cholinergic neuroeffector transmission in the ileum by a prejunctional action on acetylcholine release can be of functional importance as adenine compounds are released during stimulation of intestinal nerves (Tomaru et al., 1995).

Antagonists selective for adenosine receptors are beneficial in the research treatment of numerous conditions including cardiovascular, neurodegenerative and inflammatory diseases.

In summary, the present study has confirmed the existence of presynaptic A1 receptors on the parasympathetic nerve terminals in the guinea pig ileum which upon activation causes inhibition of electrically induced neurogenic, cholinergic twitch contractions.