Title: Responses of adrenergic drugs (noradrenaline and guanethidine) on the sympathetic nervous system of rabbit jejunum.

Abstract (296)

The autonomic nervous system is responsible for regulating involuntary physiological processes and consists of 3 sections: sympathetic, parasympathetic, and enteric. Rabbit jejunum is part of the enteric nervous system and is also modulated by a sympathetic nerve supply from the mesentery.

Noradrenaline is a neurotransmitter of the sympathetic nervous system. It works by binding to adrenergic receptors on target cells promoting relaxation of muscle. Guanethidine is a sympatholytic drug which blocks noradrenaline from fulfilling its action.

In this study adrenergic drugs, noradrenaline and guanethidine, were added to a sample of rabbit jejunum with stock concentrations of 5×10^{-7} and 2×10^{-3} respectively. The jejunum was suspended in a bath of Krebs solution with the mesentery attached. Contraction responses were analysed using a trace generated by LabChart reader. Alongside the application of drugs, electrical stimuli (15Hz, 15V) were applied to the jejunum, pre and post addition of guanethidine. The procedure was conducted on the bases of the Finkleman preparation, with the objective of exploring the effect of these stimuli on the sympathetic nervous system of the rabbit jejunum.

Results indicate that both electrical stimulation and addition of noradrenaline inhibit spontaneous contractions. Guanethidine shows a slight decrease in contraction amplitude, however, not a big enough change to demonstrate a full inhibition. Post guanethidine, electrical stimulation shows negligible change in contraction amplitude however, noradrenaline continues to inhibit contractions.

To conclude a discussion of why this inhibition occurred was explored. A decrease in spontaneous contraction amplitude by electrical stimulation was deducted to be due to the endogenous release of noradrenaline from the mesentery. Noradrenaline directly inhibits contractions by binding to beta-adrenergic receptors. Guanethidine is proved to be a noradrenergic neurone-blocking drug as it acts presynaptically, blocking transmission from noradrenergic nerve terminal and thus, depleting postganglionic sympathetic nerve endings of noradrenaline.

Introduction (390)

The autonomic nervous system, part of the peripheral nervous system, is responsible for regulating involuntary physiological processes and consists of three divisions: sympathetic, parasympathetic, and enteric. Sympathetic is responsible for the fight or flight responses, including pupil dilation, increasing heart rate, and inhibiting peristalsis [1]. The parasympathetic nervous system controls the body's rest and digest responses.

Rabbit jejunum is part of the intrinsic enteric nervous system. Attached to the jejunum is a mesentery which contains a sympathetic nerve supply. These nerve fibres can inhibit the

gastrointestinal movement by influencing the smooth muscle activity on the jejunum [2]. The jejunum is in the middle of the small intestine, between the duodenum and ileum. Its function is to absorb nutrients, water and food passing through the stomach as well as facilitate peristalsis.

Noradrenaline and Guanethidine are adrenergic drugs. Noradrenaline is released by sympathetic nerve endings and binds to adrenergic receptors on the jejunum muscle. Its function is to regulate the motility of the smooth muscle in the gastrointestinal system. It does this by binding to alpha and beta-adrenergic receptors on smooth muscle cells bringing about a relaxation, slowing down peristalsis and decreasing motility [2]. Guanethidine's role is to interfere with the release of noradrenaline. It is classed as a sympatholytic drug and, more specifically, an adrenergic antagonist as it depletes noradrenaline by reducing the release of it from adrenergic postganglionic terminals.

This experiment was conducted following the bases of the Finkelman preparation. This preparation is widely used to show the response of sympathetic nerve stimulation and the effects of various drugs blocking these nerves [3]. The jejunum is a common model used to demonstrate results due to its power of regular rhythmic contractions. These spontaneous contractions are influenced by cardiac tissue pacemaker cells which conduct a myogenic rhythm [4]. They have an unstable resting membrane potential that continuously depolarise due to the leaky membrane nature thus, generating a membrane current. Every time an action potential is reached, the muscle contracts.

In this study, a sample rabbit jejunum with attached mesentery is electrically stimulated and exogenous adrenergic drugs: noradrenaline and guanethidine are added. The effects these stimuli have on smooth muscle contraction are analysed and recorded using LabChart reader. As well as this, the responses presented when these stimuli are used in conjunction are noted, generating a profile on how these drugs work.

Methods (247)

This experiment was conducted following the methods outlined in the practical handbook.

To deduct the responses to sympathetic nerve stimulation on rabbit jejunum, three stimuli were used: electrical stimulation, noradrenaline and guanethidine.

Krebs solution was used as the diluent to make up stock concentrations. For noradrenaline, 5ml Krebs and 5ml noradrenaline ($1x\ 10^{-2}$) were combined followed by two 1 in 10 dilutions to create a $5x10^{-5}$ stock solution. For Guanethidine, 8ml Krebs and 2ml Guanethidine ($1x10^{-2}$) were mixed to make a $2x10^{-3}$ stock solution.

A sample of rabbit jejunum was suspended in Krebs solution. A pulse baseline was distinguished and consequently an electrical impulse of parameters 15V, pulse width 1ms and frequency 15Hz was added for 15 seconds. Between each electrical stimulus applied a 1-minute recovery time was allocated. Results were recorded.

Following the electrical stimulus, a stock concentration of $5x10^{-5}$M noradrenaline was added to the bath (final concentration of 5×10^{-7}). The result was recorded. Once applied, rabbit jejunum was washed out 6 times to ensure complete removal of the drug.

A new baseline was recorded. Guanethidine was added to the bath (final concentration of 2×10^{-5}). Response was observed for 2 minutes. Further electrical stimulus was applied using the same original parameters (15V, 15Hz) without washing out guanethidine. This was repeated 7 times, in 3-minute intervals, followed by the addition of 5×10^{-7}M noradrenaline. Bath was washed out thoroughly.

All results were recorded using LabChart Reader, producing a trace, and later analysed.

Results (526)

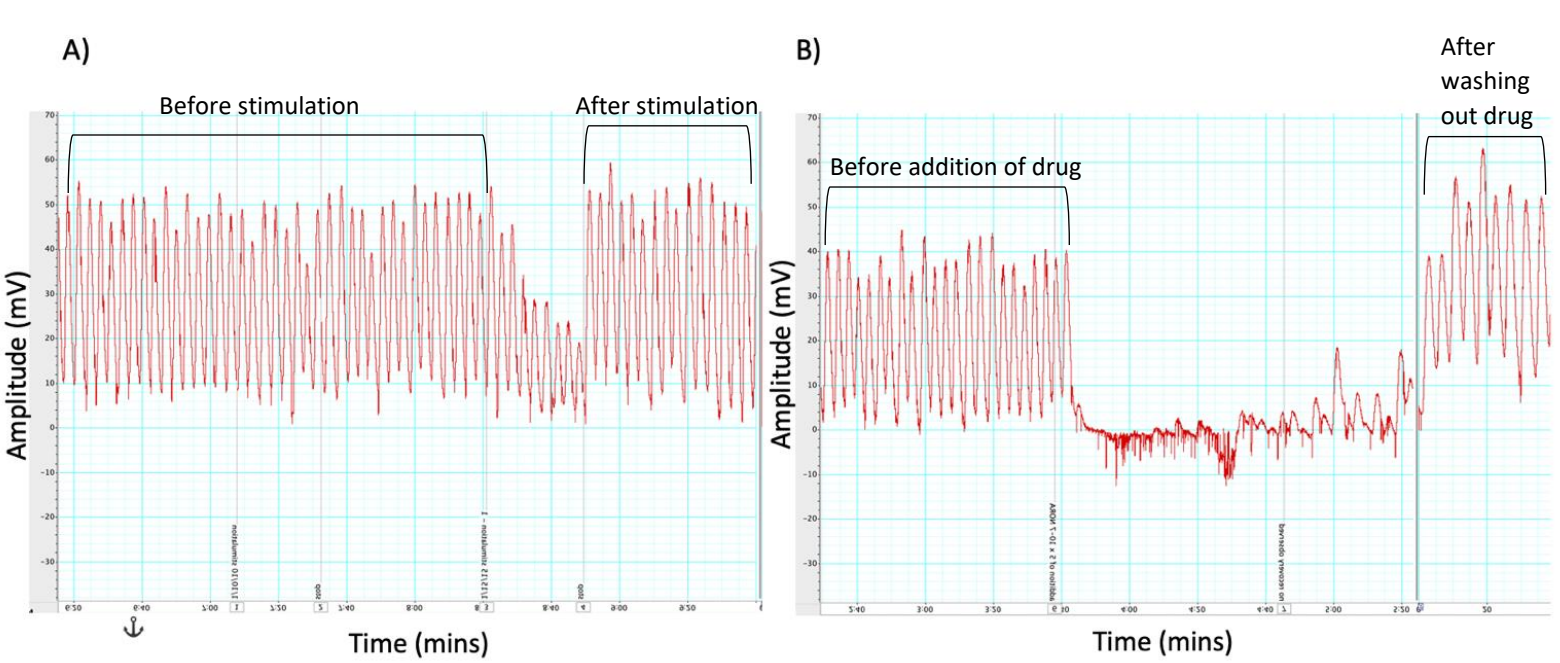

Figure 1; Responses to electrical stimulation and addition of exogenous noradrenaline displayed by rabbit jejunum. Representative traces recorded on LabChart reader to show contraction cycles and show the time passed (minutes) on the x-axis and the amplitude (mV) on the Y-axis. A) Trace of spontaneous baseline contractions prior to application of electrical stimulation (15 Hz, 15V) and the response post-stimulation. B) Trace of spontaneous baseline contractions prior to application of $5x10^{-7}$ M noradrenaline and response after washing the drug out.

Rabbit jejunum shows spontaneous rhythmic contractions before any stimulation was applied, see fig1A. Electrical stimulation (15Hz, 15V) reduces and/or inhibits smooth muscle contraction seen by a decrease in amplitudes during the 15 seconds the stimulus was applied (fig1A). There amplitude decreases by 35mV when electrical stimulation is applied. Once stimulus stopped, original baseline returned. Similar pattern exhibited by addition of exogenous noradrenaline; fig1B. Contractions are inhibited during exposure to the drug shown by a decrease of amplitude from 40mV to 2mV, however, after washing out noradrenaline from the bath, the previous baseline returns.

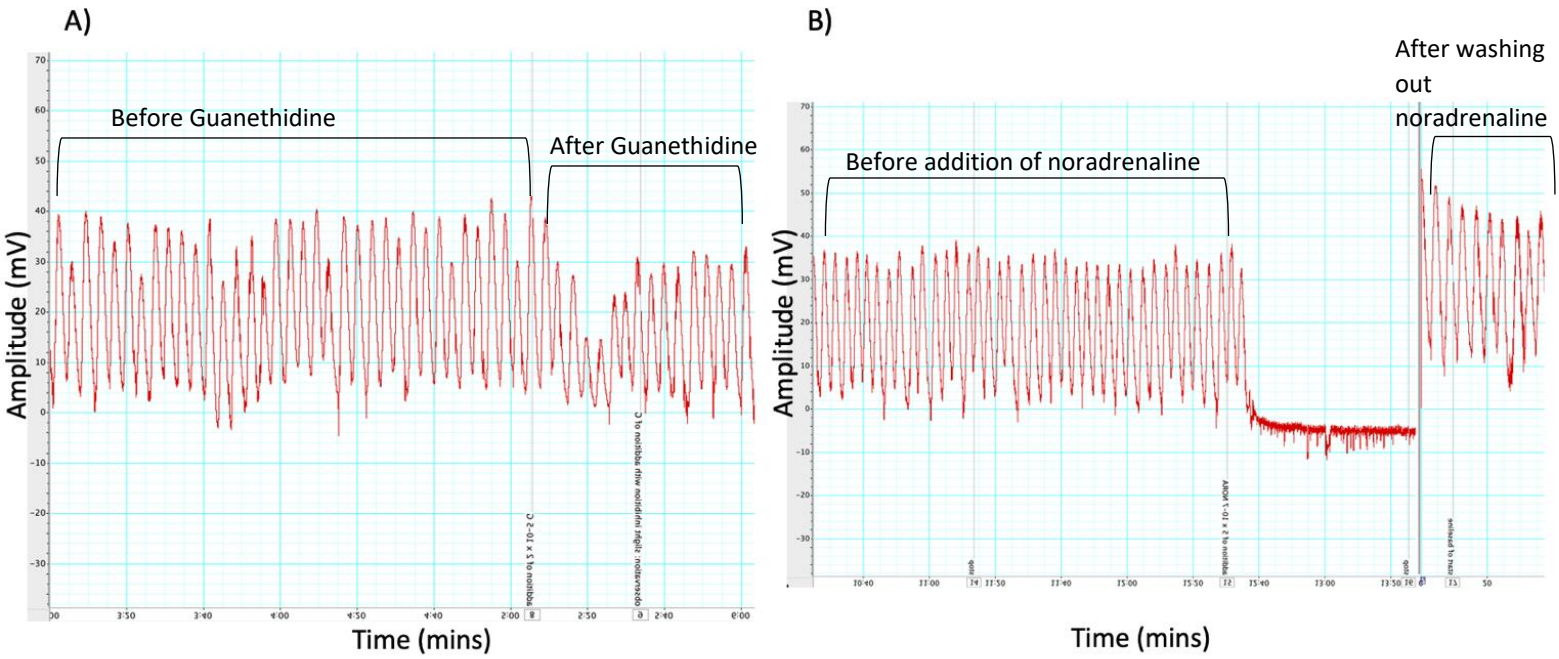

Figure 2; Responses to addition of exogenous guanethidine and noradrenaline displayed by rabbit jejunum. Representative traces recorded on LabChart reader to show contraction cycles and show the time passed (minutes) on the x-axis and the amplitude (mV) on the Y-axis. Stock concentrations of $5x10^{-7}$ M noradrenaline and $2x10^{-5}$ M guanethidine used. A) Trace of spontaneous baseline contractions before and after application of guanethidine. B) Trace of spontaneous baseline contractions prior to application of noradrenaline, during drug stimulation and after the washing out of drug (post addition of guanethidine).

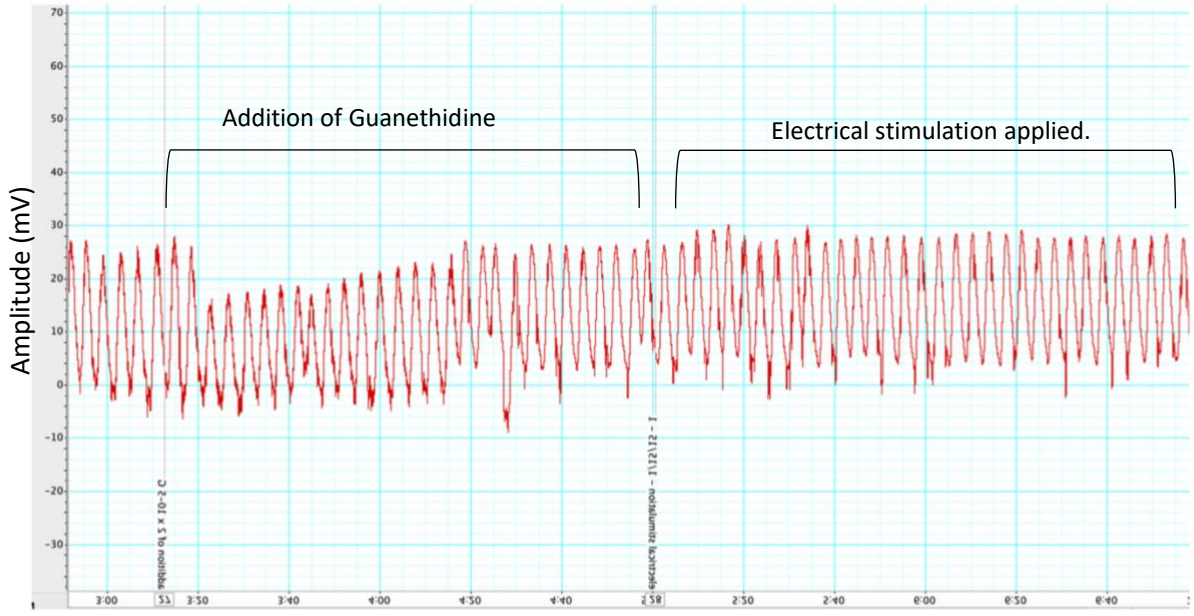

Figure 3; Response of electrical stimulation on rabbit jejunum following addition of guanethidine. Representative traces recorded on LabChart reader to show contraction cycles. Time passed (minutes) is indicated on the x-axis and the amplitude (mV) is on the Y-axis. Trace shows the change in amplitude directly after adding guanethidine, followed by applying an electrical stimulation (15 Hz, 15V) without washing guanethidine out.

Addition of guanethidine results in a small decrease of contraction amplitude, shown in fig2A and fig3. Noradrenaline continues to inhibit the contractions of jejunum smooth muscle following guanethidine. However, the electrical stimulation loses the ability of inhibition as there is no change in amplitude; see fig4 for further details.

Figure 4; Table showing the percentage inhibition of contraction from each stimulus before and after addition of guanethidine. E1-E7 represents the 7 consecutive electrical stimuli applied post addition of guanethidine, each stimulation was conducted 1 minute apart for a duration of 15 seconds. % inhibition measured by the difference in contraction amplitudes.

Stimulation	% inhibition of contraction
Electrical pre guanethidine	69.23
Noradrenaline	89.47
Guanethidine	31.58
E1 post guanethidine	0.00
E2 post guanethidine	9.09
E3 post guanethidine	9.09
E4 post guanethidine	18.18
E5 post guanethidine	27.27
E6 post guanethidine	36.36
E7 post guanethidine	36.36
Noradrenaline post guanethidine	94.73

Noradrenaline shows a large % inhibition of contraction before and after the addition of guanethidine (see fig1B&2B). However, as seen in fig4, noradrenaline displays a bigger % inhibition of contraction following the addition of guanethidine in comparison to before. Electrical stimulation has a significant decrease of 69.23% pre guanethidine (fig4) which contrasts between post guanethidine where % inhibition is low directly after and there is negligible effect of electrical stimulation directly after guanethidine exposure. Contraction recovery is shown as % inhibition increases from E1-E7 (fig4).

Discussion (500)

Results demonstrate that noradrenaline inhibits myogenic spontaneous contractions of the rabbit jejunum, fig1B&2B. This happens because noradrenaline binds to β-adrenergic receptors in smooth muscle which inhibit pacemaker currents by activating G-proteins and adenylate cyclase, increasing production of cAMP, which in turn regulates muscle contraction [4].

Indicated in fig4, Noradrenaline shows an increase in % inhibition of contraction after the addition of guanethidine (89.47% to 94.73%), showing that it is more potent at inhibiting the activity of smooth muscle contraction in the presence of guanethidine. Noradrenaline is inactivated by its reuptake into the presynaptic terminal by a high affinity transporter system NET (norepinephrine transporter). Guanethidine enters the nerve terminal by the same NET mechanism, thereby blocking the reuptake of noradrenaline into its storage vesicles and displacing it [5][6]. As a result, the exogenously added noradrenaline can remain in the synaptic cleft, without being inactivated, and can continue to bind to the β-adrenergic receptors, eliciting a response.

Electrical stimulation provokes secretion of endogenous noradrenaline from presynaptic nerve terminals, inhibiting the spontaneous smooth muscle contraction. `shown by the decrease in contraction amplitude by 35mV in fig1A, electrical stimulation depolarises the sympathetic nerve endings of the jejunum which depolarises the membrane and causes the voltage-gated calcium channels to open [7]. This influx of calcium ions into the nerve terminal leads to the exocytosis of noradrenaline from its synaptic vesicles.

However, once guanethidine was applied to the bath, electrical stimulation caused no inhibition at first, fig4, and a much smaller inhibition of contraction as time passed. This promotes the idea that guanethidine acts within the postganglionic sympathetic nerve fibres. Guanethidine is taken up by the vesicles used for exocytosis of noradrenaline, therefore leaving noradrenaline to be metabolised by a monoamine oxidase enzyme inside the neuron [5]. This leads to a reduction in available endogenous noradrenaline for release when stimulated. Furthermore, guanethidine demonstrates long lasting effects (shown in fig4) which infers that it has slow clearance and is not rapidly metabolised[8].

The reduced inhibition by electrical stimuli compared to the greater inhibition by exogenous noradrenaline shows that guanethidine is a noradrenergic neurone-blocking drug and acts presynaptically. It blocks transmissions from noradrenergic nerve terminal, thus, depleting postganglionic sympathetic nerve endings of noradrenaline and inhibiting the release of noradrenaline from the sympathetic nerve terminals [9]. This confirms guanethidine's role as an adrenergic antagonist drug.

When guanethidine was added, a slight inhibition of spontaneous contraction was exhibited, fig2A,3&4. This does not follow the expected trend and may be due to experimental error. However, it may also be due to a side effect of displacing noradrenaline from its vesicles directly into the synaptic cleft where it may bind to the adrenergic receptors. The effect doesn't last long as guanethidine quickly blocks the exocytosis.

To conclude, results obtained indicate that noradrenaline depletes the smooth muscle contraction of a rabbit jejunum (when released endogenously and added exogenously). However, guanethidine blocks noradrenaline's endogenous pathway of release in the presynaptic neurone. An inhibition can only be seen when noradrenaline is added exogenously with guanethidine.

References (10 maximum**)**.

1. Hussain LS, Reddy V, Maani CV. Physiology, noradrenergic synapse.
2. Mcgregor DD. The Effect of Sympathetic Nerve Stimulation of Vasoconstrictor Responses in Perfused Mesentric Blood Vessels of The Rat. J Physiol. 1965 Mar;177(1):21-30.
3. Finkleman B. On the nature of inhibition in the intestine. The Journal of Physiology. 1930 Sep 9;70(2):145.
4. Jun JY, Choi S, Yeum CH, Chang IY, Park CK, Kim MY, et al. Noradrenaline inhibits pacemaker currents through stimulation of beta 1-adrenoceptors in cultured interstitial cells of Cajal from murine small intestine. Br J Pharmacol. 2004 Feb;141(4):670-7.
5. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. *Rang and Dale's Pharmacology*. 9th ed. Elsevier; 2019.

6. Chang CC, Chang JC, Su CY. Studies on the interactions of guanethidine and bretylium with noradrenaline stores. British Journal of Pharmacology and Chemotherapy. 1967 Jun;30(2):213.
7. Perrino BA. Calcium sensitization mechanisms in gastrointestinal smooth muscles. Journal of Neurogastroenterology and Motility. 2016 Apr;22(2):213.
8. Paton DM. THE CLINICAL PHARMACOLOGY OF ADRENERGIC NEURON BLOCKING AGENTS. InThe Release of Catecholamines from Adrenergic Neurons 1979 Jan 1 (pp. 373-379). Pergamon.
9. Abercrombie GF, Davies BN. The action of guanethidine with particular reference to the sympathetic nervous system. British Journal of Pharmacology and Chemotherapy. 1963 Feb;20(1):171-7.