Journal of Animal and Veterinary Advances 6 (4): 580-585, 2007

Pharmacology of Adrenergic Stimulation of Duodenal Smooth Muscle of Nigerian Muscovy Duck (*Cairina moschata*)

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Abstract: The response of the gastrointestinal smooth muscle to adrenergic stimulation was studied in Muscovy duck (*Cairina moschata*). Adrenergic stimulation was achieved by application of adrenaline on strips of isolated duodenum in organ bath. The potency, affinity and efficacy of the agonist alone and in the presence of antagonists were determined by EC_{50}, pA_2 and Emax, respectively. Adrenaline inhibited contractions of the smooth muscle of the gastrointestinal tract of *C. moschata* in a dose dependent fashion. Contractions of isolated duodenum recorded in the presence of adrenaline were predominantly of slow wave components. The cumulative concentration-response curve revealed that there were two phases of the response of duodenum to adrenaline. There was an initial concentration-dependent contraction of partially contracted segments from 1×10^{-9}-1×10^{-7}M of adrenaline, while concentrations higher than 1×10^{-7} M caused relaxation. Dibenamine and propranolol modified the response of duodenum to adrenaline and caused a rightward shift of adrenaline cumulative concentration-response curve in the isolated duodenum which indicate the presence of á- and β-receptors as mediators of adrenergic effects. Both antagonists significantly ($p<0.05$) reduced the potency (EC_{50}) of adrenaline. The pA_2 value was also reduced in the presence of dibenamine ($p>0.05$) and propranolol ($p<0.05$) which indicated a reduction in the affinity of adrenaline for the receptors. Dibenamine caused 85% reduction of maximal relaxant response of duodenum to adrenaline; this depression was statistically significant ($p< 0.001$). Pretreatment of the tissue with propranolol however caused a non-significant ($p>0.05$) depression of maximal response of duodenum to adrenaline. These all points to the fact that while dibenamine inhibits adrenaline non-competitively, propranolol acts as a competitive blocker. The study confirms that pharmacological responses to adrenergic stimulation in the gastrointestinal tract of *C. moschata* are mediated by á- or β- adrenergic receptors. This effect could be inhibitory or stimulatory depending on the dose of adrenaline administered. It was therefore concluded that the dose of catecholamines modulate what receptor is predominantly stimulated in a tissue at a particular time.

Key words: Pharmacology, adrenergic stimulation, duodenum, Nigerian Muscovy duck

INTRODUCTION

Generally, the motility of G.I tract is regulated by classical neurotransmitters, neuropeptides and humoral agents (Li *et al*., 2000). The gut smooth muscle in the intact conscious state exhibits three distinct types of contractions; rhythmic phasic contractions, tone and ultra propulsive contractions (Sarna, 1999). A dense network of extrinsic and intrinsic sensory nerves supply the gastrointestinal tract and the identity of the mediating excitatory and inhibitory neurotransmitters is well established (Holzer *et al*., 2001; Lecci *et al*., 2002). The excitatory motor neurons synthesize and release acetylcholine, tachykinins, serotonin and histamine which act through postjunctional muscarinic M_2 and M_3 or tachykinins NK1 NK2 or $5HT_{1-4}$ or histamine H_1, H_2 and H_3 respectively to induce smooth muscle contractions (Betaccini and Coruzzi, 1989., Ameh *et al*., 1994., Adolffson *et al*., 1999). Conversely, inhibitory motor neurons express Nitric Oxide (NO), Vasoactive Intestinal Peptide (VIP) to induce a coordinated muscle relaxation (Malone *et al*., 1999). Alongside these inhibitory inputs, the sympathetic division of autonomic nervous system also serve to inhibit intestinal smooth muscle through the adrenergic receptors (α and β) (Wood, 1999). Investigators overwhelmingly agreed in their findings that the adrenergic or sympathetic and enteric divisions of the autonomic nervous system are interactive in the determinations of the functional state of the digestive tract. Activation of the sympathetic input suppresses digestive function primarily through the release of nor-adrenaline at its synaptic interface with the enteric

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nervous system. Comparative study of autonomic regulation of gut motility of fish, amphibian, crocodiles, avian and mammals revealed that the control systems and signal transduction are amazingly similar between species and animal groups (Olsson and Holmgreen, 2001). Comparison among avian species by other contemporary workers confirms these findings that the distribution of neurotransmitters in neuronal structure is similar (Mensah-Brown and Lawrence, 2001). However, Kuenzel *et al.* (1999) established that a slight functional difference exist between wild and domestic avian species. They submitted that wild birds exhibit marked changes in body weight, a condition they related to shifts in balance between the sympathetic and parasympathetic nervous systems. They showed that domestic avian species known for growth rate display a dominance of the parasympathetic nervous system. These findings have been widely documented across many species and breeds, however not much work has done on adrenergic regulation of gastrointestinal tract of ducks. This study is one of the numerous efforts by this group to explore the biological reactivity of gastrointestinal tract of the Nigerian Muscovy duck (*Cairina moschata*).

MATERIALS AND METHODS

Experimental animals: Thirty Nigerian domestic adult ducks of both sexes were used for this study. The birds were kept under intensive system, while chicken finisher's mash and fresh water were provided *ad libitum* daily.

Drugs the agonist drug used was Adrenaline hydrochloride (British Drug House Chemicals Limited, Poole, England) and the antagonist drugs were Propranolol hydrochloride (ß-adrenergic receptor antagonist) (Imperial chemical Industries Limited, Wilmslow, Cheshire, U.K) and Dibenamine (α-adrenergic receptor antagonist) (Smithkline and French Laboratories Limited, Herts, England). Each of these drugs was dissolved in sterile distilled water and a stock solution of 10^{-2} M of each drug was prepared. Further dilutions were made from the stock as desired.

Tissue preparation: The birds were killed by stunning. Each duck was opened up and the gastrointestinal tract removed. The duodenal section of the gastrointestinal tract was identified, cut and taken out in a Petri dish containing Tyrode solution constantly being aerated by the air pump.

This section was cut into strips of about 2cm long, which was then cleared of ingesta and fat with Tyrode solution and placed in a 200 mL organ bath containing Tyrode solution, maintained at 37°C using a circotherm. The tissue was aerated with aeration pump. The lower end of the tissue was attached to the aerating tube inside the organ bath while the upper end was attached to a simple isotonic lever counterbalanced to provide a load of 2 g on the tissue. This frontal writing lever was aligned on a kymograph drum for recording.

Experimental procedure: Each strip of the tissue was allowed to equilibrate for at least 30 min before experimentation. Adrenaline was administered between the range of 1×10^{-9} and 3×10^{-4} M which is equivalent to the doses producing the threshold and maximal responses of G.I tract of *C. moschata* to adrenaline (Saba and Arowolo, 2006). The agonist drug was tested on the tissue in the absence and presence of 10^{-7} M dibenamine or 10^{-7} M propranolol as the case may be. The procedure was repeated five times for each agonist and agonist-antagonist interactions using new strip of tissue from different duck each time. Relaxant responses to adrenaline were taken from preparations that had been partially contracted (approximately 50 per cent maximum) to histamine (Chand and Eyre, 1977a). Relaxation was expressed as percentage of the maximum relaxation attainable to adrenaline.

The pooled data collected from dose-response curves were plotted, for the agonist alone and the agonist in the presence of the antagonist. These parameters include potency of the agonist as measured by $EC_{50,}$ the affinity of the Agonist (pA_2) and agonist's efficacy (Emax) for the agonist alone (Control) and the same parameters for the agonist in the presence of the antagonist (Test). The effectiveness of antagonism by 10^{-7} M dibenamine or 10^{-7} M propranolol was determined by the Concentration Ratio (C.R). The C.R is an indication of the relative effectiveness and specificity of antagonists. It is evaluated as the ratio of agonist concentrations giving equivalent responses in the presence and absence of antagonist.

It is expressed by the formula:
D.R =AB/AO

Where AB and AO represent agonist EC_{50} with and without antagonist respectively.

Statistical analysis: All the values were expressed as means with standard error. The test of significance of the difference of the means obtained for agonist alone (Control) and agonist-antagonist responses (Test) was done using student's t-test (Steel and Torrie, 1996).

RESULTS

Adrenaline produced relaxant effects on the smooth muscle of the G.I. tract of *C. moschata* in a dose

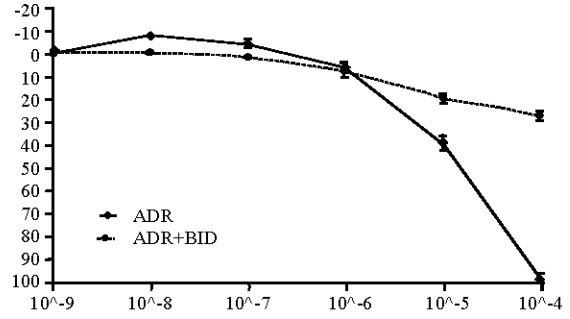

Fig. 1: The cumulative concentration-response curve for Adrenaline (ADR) and Adrenaline in the presence of 1×10^{-7}M Dibenamine (ADR + DIB) on the isolated duodenum of *Cairina moschata.* Numbers of observation = 5. Standard errors of means are indicated as verticalbars

dependent fashion. The contractions of the isolated duodenum recorded were predominantly of slow wave components. The cumulative concentration-response curve revealed that there were 2 phases of the response of duodenum to adrenaline. There was an initial concentration-dependent contraction of partially contracted segments from 1×10^{-9}-$1\times10^{-}$ M, while concentration higher that 1×10^{-7} M caused relaxation.

Inhibition of adrenaline-induced relaxation by dibenamine: Dibenamine inhibited the response of duodenum to adrenaline and caused a rightward shift of adrenaline cumulative concentration-response curve non-competitively in the isolated duodenum (Fig. 1).

EC_{50} of adrenaline in the presence and absence of dibenamine: The mean values of EC_{50} of adrenaline increased in the presence of dibenamine from $2.25\times10^{-5}\pm8.54\times10^{-7}$ to $4.73\times10^{-5}\pm6.25\times10^{-6}$. The difference of the means is statistically significant ($p<0.01$) (Table 1).

Adrenaline pA_2 in the presence and absence of dibenamine: Pretreatment of the tissues with dibenamine caused statistically non-significant ($p>0.05$) decrease in pA_2 value from 12.10±0.47 to 11.40±0.59 M.

Percentage maximal response (Emax) to adrenaline and the Concentration Ratio (C.R.): Dibenamine caused 85% reduction of maximal relaxant response of duodenum to adrenaline. This depression was also statistically significant ($p< 0.01$). The C.R. value was not determined because it is a non-competitive type of antagonism (Table 1).

Table 1: Pharmacodynamic values obtained for the effect of adrenaline on the isolated duodenum of C. moschata in the presence and absence of α- or β- adrenergic blockers

Parameters		Dibenamine	Propranolol
Potency			
(EC_{50})	Test	[b] $4.73\times10^{-5}\pm6.25\times10^{-6}$ (5)	[c] $8.50\times10^{-7}\pm3.31\times10^{-7}$ (5)
(Molar)	Control	[b] $2.25\times10^{-5}\pm8.54\times10^{-7}$ (5)	[c]$1.11\times10^{-10}\pm9.47\times10^{-11}$(5)
Affinity	Test	[d]11.40±0.59 (5)	
(pA_2)			[c]8.31±1.04(5)
(Molar)	Control	[c] 8.31±1.04(5)	[c]11.49±0.20(5)
Efficacy	Test	[a]8.10.15±4.32(5)	[d]89.26±2.27 (5)
(Ema×)			
(%)	Control	a80.01±5.13(5)	[d]96.22±2.34(5)
C.R		n.d. Fig.1	$1.50\times10^{5}\pm2.32\times10^{4}$

n.d = not determined

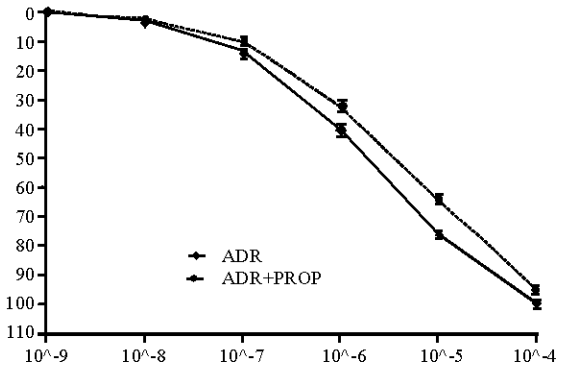

Fig. 2: The cumulative concentration-response curve for Adrenaline(ADR)and Adrenaline in the presence of 1×10^{-7}M Propranolol (ADR + PROP) on the isolated duodenum of *Cairina moschata.* Numbers of observation = 5. Standard errors of means are indicated as vertical bars.

Inhibition of adrenaline-induced relaxation by propranolol: The concentration-response curves obtained from the different strips of isolated duodenum of *C. moschata* revealed that propranolol competitively inhibited the responses of the tissue to adrenaline and caused a rightward shift of adrenaline cumulative concentration-response curve (Fig. 2).

EC_{50} of Adrenaline in the presence and absence of propranolol: The EC_{50} value of adrenaline in the presence of adrenaline ($8.50\times10^{-7}\pm3.31\times10^{-7}$M) is higher compared to the value obtained in the absence of adrenaline ($1.11\times10^{-10}\pm9.47\times10^{-11}$), the difference in the means is significant ($p<0.05$).

Adrenaline pA_2 in the presence and absence of Propranolol: Propranolol decreased the mean adrenaline pA_2 value from 11.49±0.20 to 8.31±1.04 M and this reduction is statistically significant ($p<0.001$).

Percentage maximal response (Emax) to adrenaline and the Concentration Ratio (C.R.): Pretreatment of the tissues with propranolol caused a non-significant ($p>0.05$) depression of maximal response of duodenum to adrenaline.

The C.R value was only determined for propranolol. The mean C.R value $1.50\times10^{5}\pm2.32\times10^{4}$ obtained is above unit which indicates effective antagonism by propranolol.

DISCUSSION

Response of gastrointestinal tract of *C. moschata* to adrenaline: Adrenaline inhibited contraction of the smooth muscle of the G.I. tract of *C. moschata* in a dose dependent fashion. The contractions of the isolated G.I. tissue recorded were predominantly of slow wave components. The cumulative concentration-response curve revealed that there were two phases of the response of duodenum to adrenaline. There was an initial concentration-dependent contraction of partially contracted segments from 1×10^{-9}-1×10^{-7}M, while concentration higher that 1×10^{-7} M caused relaxation (Fig. 1). The dual opposing effects of catecholamines on the smooth muscle in the body have also been reported in the intestine of mouse (Fontaine *et al*., 2002). A biphasic response comprising of contractions preceded by relaxation was reported in the bovine vasculature by Dina and Arowolo (1990).

Hoffman (2001) explained that presynaptic effects of catecholamines result in facilitation of the release of neurotransmitters such as acetylcholine. It is believed that the composite release of excitatory acetylcholine produces the contractile effect usually observed when catecholamines are administered on smooth muscle. These contractions either follow initial relaxations, as in this study or preceding it as reported by Dina and Arowolo (1990) on bovine vasculature. These foregoing reports corroborate the fact that pharmacological effects of catecholamines are generally diverse and complex depending on a number of factors. Hoffman (2001) reported that response of any cell or organ to sympathomimetic amines is proportional to the density and proportion of α - and β-adrenergic receptors present in the tissue. Furchgott (1972) reported that in the gastrointestinal tract the dominant effect of catecholamines is determined by the distribution of each type of receptors in the muscle layers, the neural elements of the intestinal wall, concentration of the catecholamine and on the experimental conditions. For example, it is generally accepted that both type of receptor (α or β) relaxes the smooth muscle of the intestine (Malcolm *et al*., 2000) but some workers believe that the preexisting condition of the tissue determines the way the event could go, if the muscle tone is already high, catecholamines causes relaxation but if the tone is low; contraction of the intestine is observed (Fontaine *et al*., 2002).

However, there is a general consensus that the dominant effect of adrenergic stimulation is relaxation of the gastrointestinal smooth muscle (Hoffman, 2001). The molecular mechanism underlying the inhibitory effect of adrenaline in smooth muscle is related to inhibition of adenylyl cyclase through the association of adrenergic receptors to the inhibitory Gi proteins. Adrenergic receptors are also postulated to activate G proteins gated K+ channels, resulting in membrane hyper polarization and relaxation (Mc Donald *et al*., 1994).

Dibenamine antagonism: Dibenamine inhibited adrenaline non-competitively in the duck's G.I. tract. Dibenamine significantly reduced the potency of adrenaline in the duodenum. These signify that adrenergic effect of adrenaline is mediated through α_1 and α_2 adrenergic receptors in the smooth muscle of G.I tract of *C. moschata*. Dibenamine does not discriminate between the two subtypes (Hoffman, 2001). While α_1 is a postsynaptic receptor and it causes relaxation of the gastrointestinal smooth muscle, the stimulation of α_2 receptors leads to suppression of the neuronal release of nor-adrenaline resulting in overall enhancement of smooth muscle tone and contractility (Hoffman, 2001).

Quite unlike the beta-blockers, which inhibit adrenaline-induced intestinal relaxation with concomitant enhancement of contraction of smooth muscle of G.I. tract (Malone *et al*, 1999). Dibenamine does not reverse adrenaline-induced relaxation; it rather causes further relaxation of the G.I. smooth muscle because of its ability to alkylate most autonomic receptors (adrenergic, serotonergic, cholinergic, histaminergic) (Hoffman,2001). The blockade of these receptors by alkylation account for the suppression of the non-sympathetic excitatory input of neurotransmitters like acetylcholine, serotonin or histamine to gut motility. The out come of non-specific blockade of these mentioned intestinal receptors accounts for the noncompetitive antagonism exhibited by dibenamine in this study. In addition, when presynaptic á$_2$ receptors are blocked it causes enhanced release of nor-adrenaline (Pozzoli *et al*., 2002) which leads to further relaxation of intestinal smooth muscle. Though the á receptors can be further classified into α_1A, α_1B, α_1D, α_2A, α_2B,α_2C but the distinction in their mechanism of action and tissue location have not been clearly defined (Hoffman and Taylor, 2001).

Propranolol antagonism: Propranolol elicited a rightward shift on the cumulative response curve obtained for the relaxant effect of adrenaline in the isolated duodenum of *C. moschata*. This shift to the right significantly indicates reduction of the potency of adrenaline by propranolol and the E_{max} was not significantly depressed. The C.R. values authenticate the effectiveness of competitive antagonism of propranolol. Propranolol is a non-subtype-selective, competitive β- adrenergic antagonist that is devoid of any agonist activity and it remains the prototype to which other β-adrenergic is compared (Pujet *et al*., 1992). Propranolol potently blocks β_1 and β_2 receptors (Lonnquist, 1993), but available reports shows that propranolol does not block the novel β_3 receptor which mediate responses to catecholamines at sites with atypical pharmacological characteristics e.g. adipose tissue (Summers *et al*., 1999). The findings in this study show that propranolol antagonized adrenaline-induced relaxations and also unmasked the contractions mediated by other non-adrenergic excitatory neurotransmitters like Ach and tachykinins etc. This is similar to what has been reported in mammalian G.I. tract (Malone *et al*., 1999), which confirms earlier report that stimulation of α- and β-adrenoceptors relaxes the G.I smooth muscle in avian and mammals alike. Hoffman (2001) however reported that newer evidences indicate that only the presynaptic α_2- and β_2 receptors mediates relaxation in the intestine and that the postsynaptic α_2- and β_2 receptors mediate contractions in the smooth muscle of intestines and in the blood vessels and heart in mammals. A further effort is therefore required to study in details the pharmacology of the different subclasses of α- and β-adrenergic receptors in the duck as well.

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