ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Comparative study on the small intestinal motility of the juvenile and adult axolotl, *Ambystoma mexicanum*

Gamal Badawy✉,

Department of Zoology, Faculty of Science, Menoufiya University, Shebeen El-Koom, Egypt.

Manuscript Info

Manuscript History:

Received: 15 October 2014
Final Accepted: 22 November 2014
Published Online: December 2014

Key words:
Anatomy; Intestinal motility; Carbamylcholine; Noradrenalin; Peptides; neurotransmitters

Corresponding Author

✉**Gamal Badawy**,
Gamalbadawy940@yahoo.com

Abstract

The effects of four representative neuroendocrine substances, namely, carbamylcholine (CARB), noradrenalin (NAD), substance P (SP) and somatostatin (SS) on the in vitro small intestinal motility of juvenile and adult stages of the axolotl, *Ambystoma mexicanum*, were investigated. Both SP and CARB caused significant dose-dependent increases in contractility (affecting active and/or basal tone) which was more pronounced in case of SP. While NAD had significant dose-dependent inhibitory effects on the active tone, the weak effects of SS did not allow a firm conclusion regarding its possible involvement in control of the small intestinal motility. Split data analysis for each investigated neuroendocrine substance revealed that the juvenile small intestinal segments responded significantly different from their adult counterparts. Similarly, the significance of the data showed dependency on the investigated region of the small intestine. Interestingly, the proximal segments showed more significant responses to the excitatory effect provoked by SP and CARB than the distal segments. However, the latter showed more sensitivity to the inhibitory effects caused by NAD and SS. The overall effects exhibited a dose-dependent response with no significant influence for the lower concentrations on either active or basal tone.

Introduction

The transport of food materials within the intestine depends on the activity of the gastrointestinal smooth muscle which performs a co-ordinated pattern of motor behaviour called peristalsis (Olsson and Holmgren, 2011). The latter is in principle similar in different vertebrate groups and consists of a dual reflex resulting in contraction above and relaxation below the site of stimulation (Kunze and Furness, 1999). Ascending (orally directed) excitatory and descending (anally directed) inhibitory reflexes are usually trigged by mucosal contact or radial distension of the intestine by its contents and thus occur after feeding. The intestinal smooth muscle is largely regulated by the autonomic nervous system via hormones and other biologically active substances in order to perform these reflexes effectively (Shuttleworth and Keef, 1995; Olsson and Holmgren, 2001; Olsson and Holmgren, 2011). Both hormones and the biologically active substances are secreted into the blood from either the mucosal endocrine cells located in the intestinal mucosa or enteric nerve fibres in response to specific components of the chyme, and delivered to the intestinal smooth muscle via blood circulation (Furness and Costa, 1987).

Motor activity of the intestine is under the control of neuronal and hormonal mechanisms (Fujimiya and Inui, 2000). Nerves, hormones and smooth muscle act together in harmony to exert a co-ordinated control over intestinal function, which enables it to perform the different patterns of motility, accounted for at different stages of food processing. It is generally believed that the control of intestinal motility is regulated by the myenteric neurons and that the submucosal neurons are concerned mainly with the modulation of intestinal blood flow and ion transport (Surprenant, 1994). Isolated segments of the intestine can properly co-ordinate excitatory and inhibitory

responses of the smooth muscle despite the absence of connections to the brain and spinal cord (Hennig *et al.,* 1997). Therefore, it has been established that the enteric nervous system may act locally as an independent nervous system containing the necessary elements for maintaining normal gastrointestinal functions, including motility.

Since ACh and NAD were recognised as important neurotransmitters, a number of studies have investigated their effects on the in vitro intestinal motility. ACh is well known as the main excitatory neuromuscular transmitter in the intestine of not only mammals but also all vertebrate species examined so far and its action involves reduction of cyclic adenosine monophosphate (cAMP) levels in the smooth muscle cells (Furness and Costa, 1987; Naitoh *et al.,* 1990; Johnson *et al.,* 1994). Carbachol (CARB), a stable form of ACh, has been used in some studies and is known for its ability to exert the same excitatory effect displayed by ACh (Espanol and Sales, 2000). The catecholamines, NAD and adrenaline (AD) exert their actions through the mediation of adrenergic receptors which are subdivided into α and β types. α-adrenergic receptors are largely responsible for contraction while β -adrenergic receptors for relaxation of intestinal smooth muscle (Naitoh *et al.,* 1990). Both kinds of receptors can be involved in the effects produced by circulating catecholamines or by sympathetic nerve activity (Brookes *et al.,* 1991; Olsson and Holmgren, 2001). Beside ACh, NAD and AD as neurotransmitters, intestinal motility is under the influence of several neurohormonal peptides. Some of these peptides have direct excitatory and/or inhibitory effects on intestinal motility while others may have indirect effect via acting as neuromodulators.

SP has a wide anatomical distribution in the neuroendocrine control system of the vertebrate intestine and is consequently expected to exert many biological effects (Badawy, 2014 a). Its major effect on the intestinal motility is to stimulate powerful contractile activity of the circular muscle layer. SP or a related tachykinin is likely to be the major non-cholinergic excitatory neurotransmitter in the intestine of vertebrates and its release was found to increase only during ascending contraction (Olsson and Holmgren, 2001). Although generally excitatory, the precise patterns of SP activity vary with both the species and the investigated region of the intestine (Holzer and Lembeck, 1980). The variations in response to SP have been attributed to the fact that both direct and indirect effects are involved. The role of ACh and SP as the mediators of ascending contraction and the interplay between their pathways has been demonstrated in several species (Karila *et al.,* 1998).

Somatostatin (SS) is notoriously known for its inconsistent and weak effects upon the vertebrate intestinal motor activity. Despite the presence of SS in mammalian enteric neurons, no clearly defined role has been established for this peptide (Furness *et al.,* 1992). Its role has been regarded mainly as neuromodulator, e.g. SS has been shown to inhibit the release of SP from guinea pig ileum in response to NT (Monier and Kitabgi, 1981) and VIP (Katsoulis *et al.,* 1992). Moreover, the effects of SS can be contradictory, being either excitatory or inhibitory (Ormsbee *et al.,* 1978). In non-mammalian vertebrates, the effect of SS on intestinal motility is also weak and inconsistent (Holmgren *et al.,* 1985).

The parasympathetic and sympathetic nerves to the gut are known for their ability to modulate its activities and hence the importance of both ACh and noradrenalin (NAD) is well recognised. However, extrinsic denervation including vagotomy or sympathectomy has but little effect on motility (Furness and Costa, 1987). The intestine is therefore able to exhibit normal pattern of movements in the absence of extrinsic connections from the CNS (Wood, 1984). Consequently, it is possible to examine motility in vitro using isolated segments from the intestine. The effects of a given regulatory peptide or neurotransmitter can therefore be tested in vitro using isolated strip or ring intestinal preparations. Thus, the outstanding merit of the in vitro experiments is to allow study of a selected aspect of muscle function under conditions where the influence of external factors such as circulating hormones is removed, but the muscle itself performs in a manner analogous to its in vivo capacity (Percy, 1996). A considerable body of information regarding the role of different regulatory peptides and classical neurotransmitters has been accumulated through these in vitro experiments, using isolated rings and longitudinal or circular muscle strips of the intestine prepared in an organ bath (Olsson and Holmgren, 2001; Holmberg *et al.,* 2004). However, previous studies on the in vitro effects of different regulatory peptides and neurotransmitters upon the intestinal motility of vertebrates were performed mainly on mammals and considered only a few species (Hejazian *et al.,* 2007; Fu *et al.,* 2014). These studies indicated that despite variable responses between species a general line of neurotransmitter actions can be drawn. However, this generalization must be treated with caution as the exact function of a specific neurotransmitter may vary depending on both the receptors and its interactions with other neurotransmitters. By contrast, related studies utilising non-mammalian vertebrate species are evidently scarce. Therefore, the aim of this study was to investigate the possible effects, of representative gastrointestinal neuroendocrine substances namely CARB, NAD, SP and SS upon the in vitro small intestinal motility.

MATERIALS AND METHODS

ANIMALS AND DISSECTION

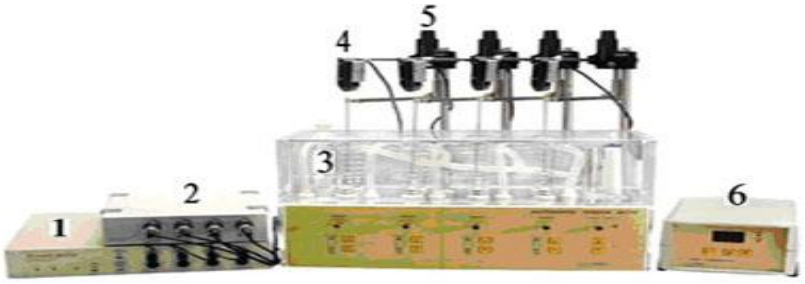
Juvenile and adult stages of the axolotl, *Ambystoma mexicanum*, were housed in a light-controlled room under alternate 12 h periods of light and darkness. The experimental animals were kept in tanks supplied with

aerated running dechlorinated tap water at a temperature of 19±1°C. Principles of animal care and use were followed according to the guidelines of the Biomedical services unit, School of Biosciences, Birmingham, UK.

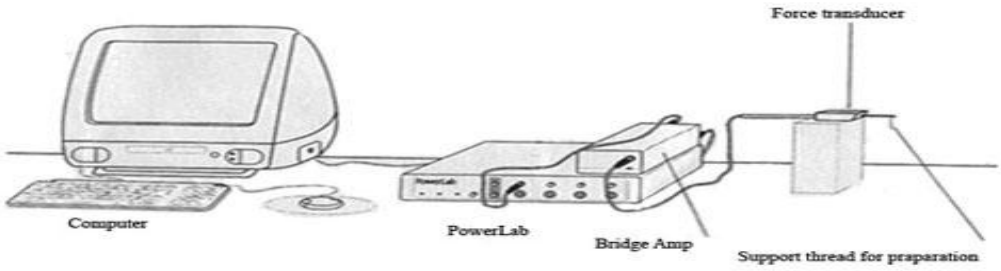
Data from a total of 9 juveniles and 6 adults, with a mean body weight of 11 and 82 g respectively, were included in the analysis. After deprivation from feeding for 12-24 h, the animals were transferred to the laboratory on the morning of the experiments and killed instantaneously by a sharp blow on the head. The small intestine was excised and immediately placed in ice-cold amphibian Ringer solution (composition in g /L-distilled water, 6.5 NaCl; 0.14 KCl; 0.16 $NaHCO_3$ and 0.16 $CaCl_2$). The pH was adjusted to 7.9 by adding Tris-HCl buffer. Care was taken not to stretch the intestine during excision and while surrounding tissues and mesenteries were dissected away. Four ring preparations (8-12 mm wide) were cut from each small intestine, two from the most proximal part and two from the most distal part. These were arranged according to their proximo-distal (PD) direction prior to their consecutive mounting in the organ baths.

EXPERIMENTAL SET-UP

Figure 1 A&B shows a four chamber organ bath system and a set-up of individual preparation respectively. Each experiment was conducted on four PD consecutive isolated small intestinal ring preparations from each animal. Individual preparation was carefully mounted on two opposite stainless steel hooks in an organ bath containing 30 ml of amphibian Ringer solution. One end of the preparation was attached to the hook at the bath bottom, and the other end to the short arm of a force displacement transducer (Model FTO3, Grass). The four consecutive preparations were therefore set-up in order in four separate organ baths aerated continuously via an air pump and maintained at 19F. All preparations were stretched smoothly by means of a micrometer adjustment in order to set the initial tension to a range of 200-500 mg. They were then allowed to equilibrate for not less than 30 min before starting the experiments.



A



B

FIG. 1: A- 4 chamber organ bath system: 1- Four channel PowerLab 2- Bridge amplifiers 3- Organ bath 4- Force transducer 5- Micrometer adjustment 6- Chart recorder. **B-** Schematic drawing showing a set-up of individual preparation with a single bridge amplifier connected to the first channel on a PowerLab.

Signals from the force transducer were fed into a four-channel chart recorder and the mean contractile tension of the circular smooth muscle recorded before adding drugs was considered as control or basal tone. Each drug was added to the organ baths cumulatively and after recording the effect of its strongest dose, the baths were drained, washed three times and refilled with fresh Ringer solution. Thus, cumulative concentrations response curves (CCRC) for CARB; NAD, SP and SS were generated. Because the inhibitory effects of NAD were irreversible, the viability of the tissues was, when necessarily, confirmed by the application of 10^{-6} M CARB after the third wash. At the end of each experiment, KCl (3M) was administrated until a steady level of maximum tone was obtained. Thereafter the rings were removed, blotted and weighed. The output of the force displacement transducer was processed through a PowerLab/8e analog-digital converter (AD Instrument, Inc.), recorded on a Macintosh computer and analyzed. Tension values were obtained by measuring the amplitude of all contractions recorded and the mean values were calculated accordingly.

CHEMICALS

Mammalian SP and SS (14) were purchased from Sigma Aldrich Co Ltd (Poole, Dorset). A stable form of ACh, carbamylcholine chloride (carbachol), and NAD were purchased from BDH Chemicals, UK. All compounds were freshly solved in distilled water at the day of the experiment except for SP and SS. For the latter, stock solutions with the concentration of 10^{-3} M were prepared in distilled water and kept at –20°C. After thawing they were diluted in the range of 10^{-3} to 10^{-7} M on the day of the experiment. However, CARB and NAD were diluted in the range of 10^{-1} to 10^{-5} M. Upon the injection of a quantity of 30 µl to the organ bath, these dilutions increased by 10^{-3} M.

DATA EVALUATION AND STATISTICAL ANALYSIS

All data were expressed as mean value ± standard error of the mean (SEM). Analysis of variance (ANOVA) was used to determine the significance between mean values. $P<0.05$ was used as the lowest criterion for statistical significance i.e. P values > 0.05 were considered insignificant. The significance of the obtained data had three categories i.e. *** $P<0.0001$, ** $P<0.03$ and * $P<0.05$. Data analysis was all computerized.

RESULTS

Carbachol

(A) Proximal segments

While low concentrations (10^{-8} and 10^{-7} M) exerted no significant effects, higher doses caused a significant dose-dependent contraction affecting both active and basal tones in case of the juvenile stage (Figs. 2A and 2B) and a gradual increase in the contractile frequency (Fig. 4A). The adult segments, on the other hand, showed a significant increase in the active tone which was accompanied with a gradual increase in the contractile frequency, while the basal tone exhibited no significant change (Figs. 2C and 4B).

(B) Distal segments

In contrast to the data obtained from the proximal segments, those from the distal segments displayed less significant values in terms of the contractile activity. The juvenile segments exhibited no significant change in either active or basal tone. The adult segments showed a significant increase in both active tone at concentrations of 10^{-5} and 10^{-4} M and basal tone at concentration of 10^{-4} M (Figs. 2D and 2E). However, this increase in both tones was significantly less than that displayed by the active tone of the proximal segment.

Noradrenalin

The preparations responded to NAD primarily by an inhibitory change in the active tone rather than any significant change in the basal tone. In some experiments, there was also insignificant excitatory change in the basal tone. All preparations included in the analysis showed a normal excitatory response to 10^{-6} M CARB indicating that the samples did not undergo significant fatigue during the duration of the experiments. Meanwhile, it was evident that the inhibitory effect caused by NAD is irreversible at least within the experimental duration.

(A) Proximal segments

Although the mean values of the active tone exhibited clear consecutive reduction the overall data were insignificant. Similarly, the basal tone displayed gradual insignificant increase. There was, however, one preparation which displayed a clear inhibitory change in the active tone (Fig. 4C). This original trace showed the clearest individual effect upon the juvenile proximal segment seen with NAD. The injection of CARB (10^{-6} M) into the organ bath resulted in a strong contraction after the irreversible relaxation caused by NAD. Adult segments, showed significant inhibitory changes in the active tone at concentrations of 10^{-5} and 10^{-4} M (Fig. 2F).

(B) Distal segments

Both juvenile (Fig. 2G) and adult (Fig. 2H) segments exhibited significant inhibitory changes in the active tone in a dose-dependent manner which was more prominent in case of the juvenile segments. This was accompanied with a gradual decrease in the contractile frequency with the basal tone showed notable but insignificant changes (Figs. 4D).

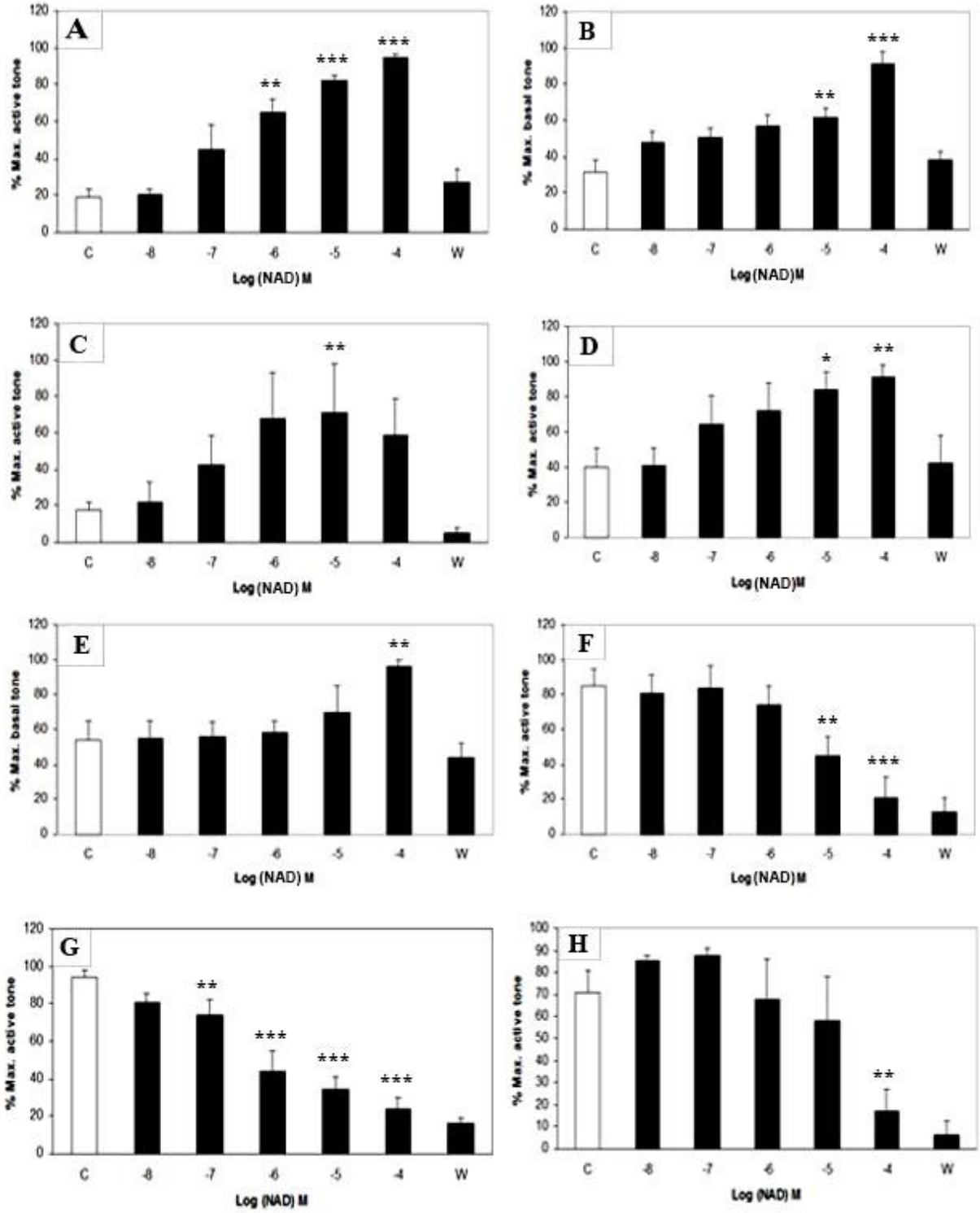


FIG. 2: Histograms showing the effect of cumulative concentrations of : **CARB** (A-E) on: **A-** the active tone of the proximal segments of the juvenile small intestine. **B-** the basal tone of the proximal segments of the juvenile small intestine. **C-** the active tone of the proximal segments of the adult small intestine. **D-** the active tone of the distal segments of the adult small intestine. **E-** the basal tone of the distal segments of the adult small intestine. **NAD** (F-H) on **F-** the active tone of the proximal segments of the adult small intestine. **G-** the active tone of the distal segments of the juvenile small intestine. **H-** the active tone of the distal segments of the adult small intestine.

Substance P

(A) Proximal segments

The juvenile proximal segments exhibited significant excitatory responses in both active and basal tones in a dose-dependent manner with a gradual increase in the contractile frequency. High concentrations (10^{-7} to 10^{-6} M) induced a stronger excitatory response (Figs. 3A, 3B and 4E). The adult proximal segments showed less significant excitatory effects on both active and basal tones only at high concentrations (Figs. 3C and 3D).

(B) Distal segments

The juvenile segments, showed a significant increase in the active tone with the high concentrations (10^{-8} to 10^{-6} M) without any significant change in the basal tone. The lower concentrations (10^{-10} to 10^{-9} M) had no significant effects on the same preparations (Fig. 4F). The adult segments, on the other hand, exhibited a slight but significant increase in the basal tone at the highest concentration (10^{-6} M) with no significant change in the active tone (Fig. 3E).

Somatostatin

The effects of SS upon the whole set of the small intestinal ring preparations were diverse and inconsistent. Because of the weakness in responses, only one significant difference was obtained with the highest concentration. This significant change in activity occurred in the juvenile distal segments where the basal tone exhibited small but significant inhibitory change at a concentration of 10^{-6} M (Fig. 3F). Despite the overall weak and insignificant effect of SS, there were some individual preparations which showed clear effects. Among the juvenile proximal segments, one exhibited an obvious increase in the active tone (Fig. 4G). Another clear response can also be seen with one of the adult proximal segments (Fig. 4H) where there was a moderate increase in the active tone. The contraction was dramatically augmented by CARB (10^{-6} M) post-treatment.

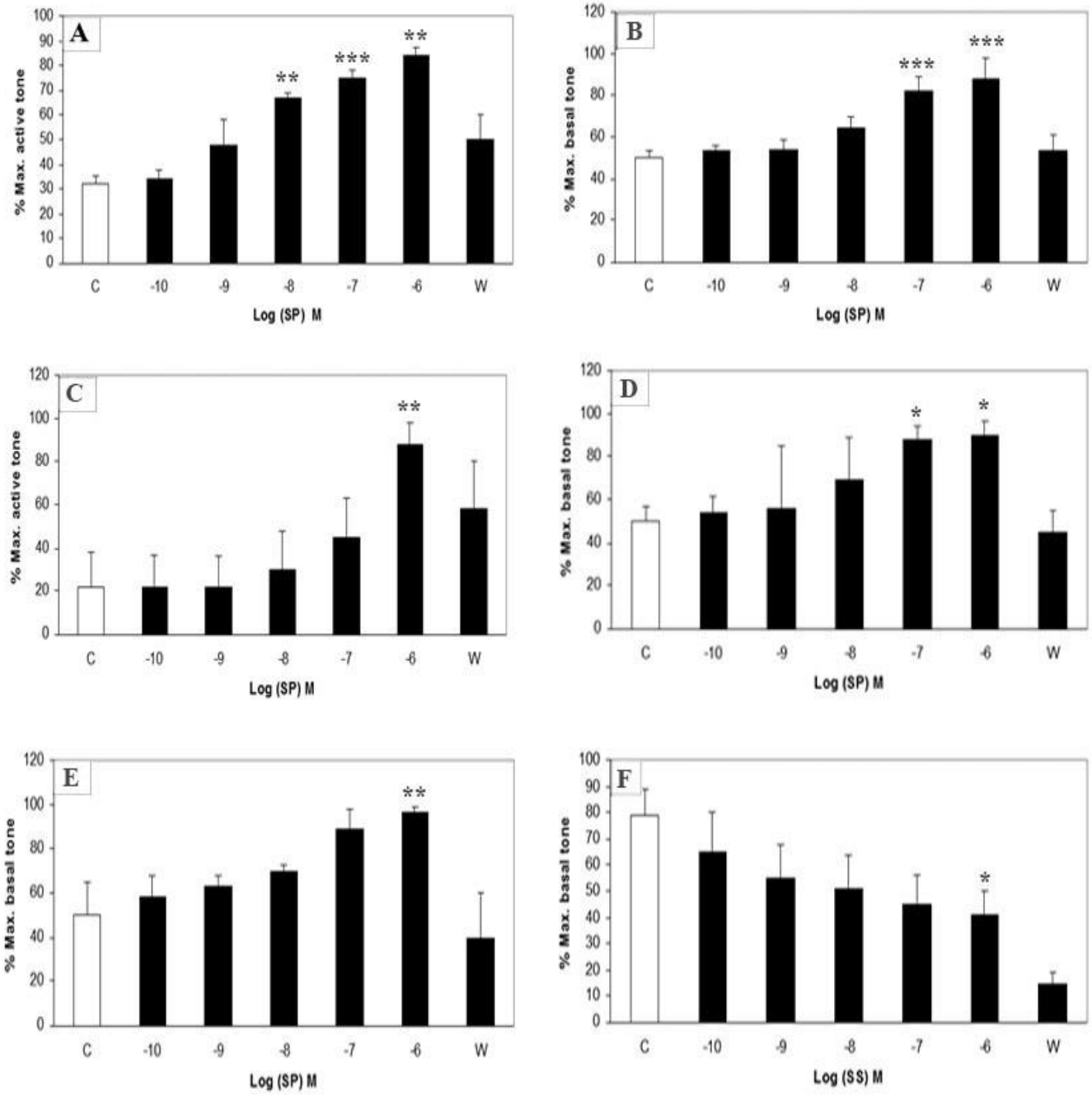


FIG. 3: Histograms showing the effect of cumulative concentrations of : **SP (A-E)** on: **A-** the active tone of the proximal segments of the juvenile small intestine. **B-** the basal tone of the proximal segments of the juvenile small intestine. **C-** the active tone of the proximal segments of the adult small intestine. **D-** the basal tone of the proximal segments of the adult small intestine. **E-** the basal tone of the distal segments of the adult small intestine. **SS (F)** the basal tone of the distal segments of the juvenile small intestine. The asterisks denote significant differences compared with the control value (C). *** P<0.0001, **P<0.03 and *P<0.05.

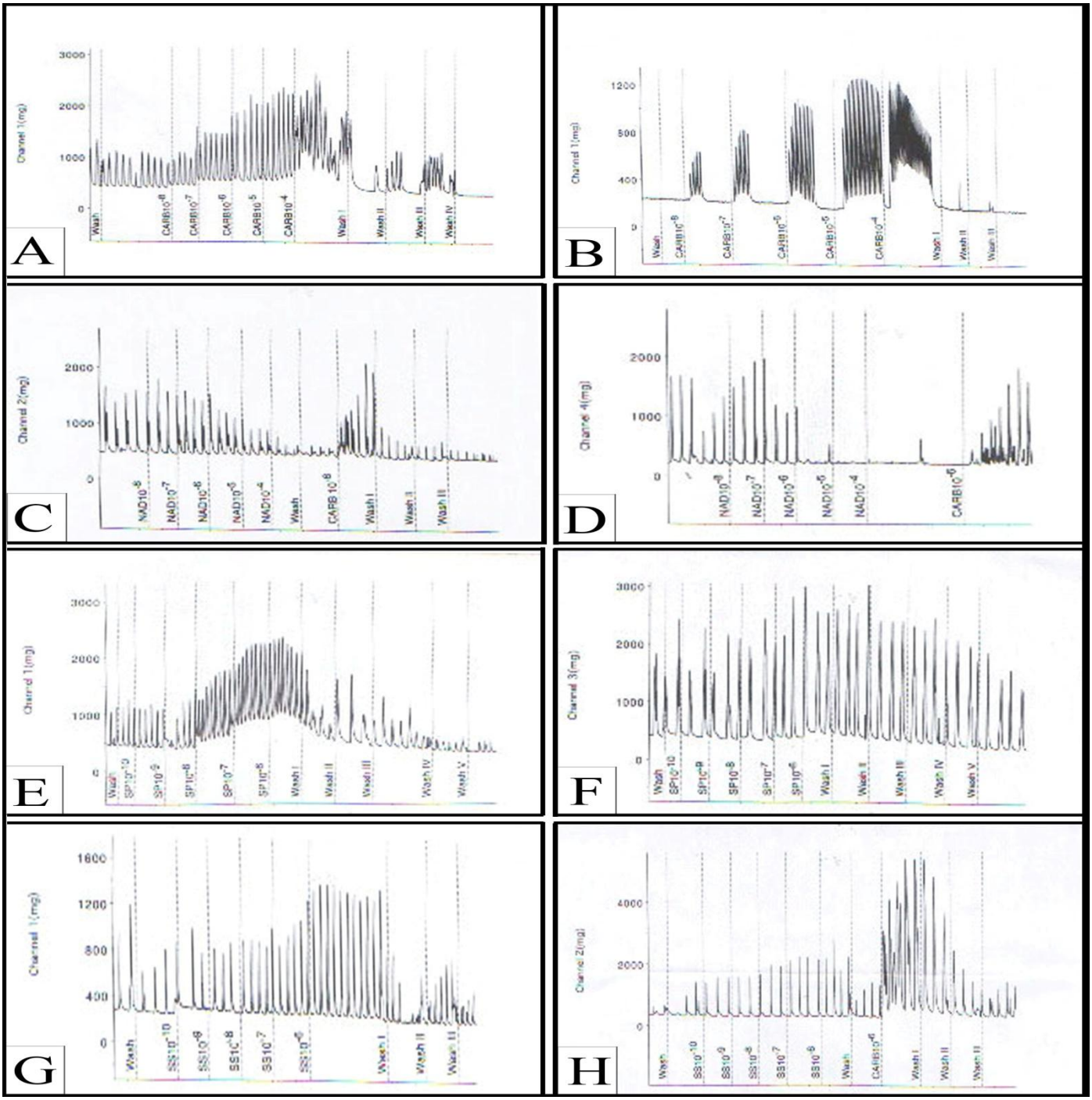


FIG. 4: Original traces showing the effect of cumulative concentration of: **CARB** (A-B) on: **A-** a proximal segment of the juvenile small intestine. **B-** a proximal segment of the adult small intestine. **NAD (**C-D) on: **C-** a proximal segment of the juvenile small intestine. D- a distal segment of the of the adult small intestine. **SP** (E-F) on **E-** a proximal segment of the juvenile small intestine. **F-** a distal segment of the juvenile small intestine. **SS** (F-H) on: F-G- a proximal segment of the juvenile small intestine. **H-** a proximal segment of the adult small intestine.

DISCUSSION

In order to explore the possible effects, if any, of some representatives' intestinal neuroendocrine substances upon the in vitro small intestinal motility of the axolotl, the present functional investigation was performed on isolated small intestinal ring preparations where the temperature and fluid environment were under control. ACh and NAD constituted the investigated neurotransmitters while SP and SS were the regulatory peptide representatives.

It is evident from the previous studies on the in vitro small intestinal motility that the actions of many regulatory peptides and neurotransmitters may be exerted in more than one manner as they can be excitatory in one way, but inhibitory in another (Olsson and Holmgren, 2001). As a result, occasional contrast may be noticed in the motility studies among different species, which is likely attributed to the presence of more than one kind of receptors combined with the fact that the physiology of intestinal hormones is complex because of their interaction with each other as well as with adrenergic and cholinergic nerves (Holmgren, 1989). Another minor, but possible, effect may result from the widespread experimental use of mammalian peptides in the motility studies of different vertebrates. The importance of using the native endogenous peptide instead of its mammalian counterpart in non-mammalian species studies has previously been highlighted by Shahbazi *et al.* (1998). Furthermore, the use of different methods and the possible effect of the tissue fatigue during the experiments cannot be excluded. However, general pattern can be drawn for the intestinal motility of vertebrates; the descending neurons often contain the relaxing agents nitric oxide (NO) and/or VIP, whereas the ascending motor neurons use ACh and/ or SP, or related peptides in the tachykinin group, as primary excitatory neurotransmitters (Holmberg *et al.,* 2006). Evidence of co-existing VIP and NO synthase, the enzyme responsible for the biosynthesis of NO, in the gastrointestinal tract of the axolotl has previously been revealed (Badawy and Reinecke, 2003). In the latter study, NOS-immunoreactivity was detected mainly in subpopulation of VIP-immunoreactive fibres that contacted submucosal arteries which indicate either a role in intestinal blood flow and/or motility.

The outcome of the present experiments as a whole highlighted the regional differences in terms of motility response to the drug. This in turn has led to the conclusion that for better results the area being investigated should always be specified and, if possible, all areas should be studied. Intestinal motility is supposed to differ from site to site for two main reasons. Firstly, the fluid loads presented to the intestine differ along its length. Secondly, different digestive and absorptive processes are occurring in different areas along its length. This speculation is also supported by the fact that the diameter of the small intestine is not constant as it decreases gradually in a PD direction. Moreover, although the overall structural pattern is similar throughout the intestinal tube, regional histological and immunohistochemical differences do exist (Badawy, 2014 b). In general, the responses obtained in the present experiments are in accordance with this hypothesis and reflect significant differences between the proximal and distal small intestinal segments. Furthermore, the present study revealed that, to a large degree, the proximal segments were more sensitive to the excitatory effect provoked by CARB and SP. However, the distal segments had the same character in response to the inhibitory effect caused by NAD and SS. This also reflects the peristaltic wave action of the small intestine in an ordinary PD direction, i.e. oral to anal pathway, and indicates a correlation between various neuroendocrine products and different regions of the intestine. Indeed, it has been reviewed by Kunze and Furness (1999) that all neurons which project orally to the intestinal smooth muscle are excitatory motor neurons and contain both ACh and SP, while those which project anally are inhibitory and contain VIP and NO. The importance of this general finding comes from the extreme scarcity of the comparative studies which utilised different intestinal regions. A clear discrepancy in responses between different parts of the urodele Necturus maculosus gut treated with bombazine and neurotensin has been reported by Holmgren *et al.* (1985).

ACh is well known for its exclusive excitatory effects upon the intestinal motility of vertebrates and acts via stimulating muscarinic ACh receptors (Espanol and Sales, 2000; Olsson and Holmgren, 2001). The present results demonstrate that CARB evokes strong excitatory responses in small intestinal ring preparations of the axolotl. Similar excitatory effects have previously been reported in the hagfish, Myxine glutinosa (Holmgren and Fange 1981), the Atlantic cod, Gadus morhua (Jensen and Holmgren, 1985) and Xenopus (Naitoh *et al.*, 1990). Excitatory effects have also been reported for Bufo marinus isolated gastric muscle cells which were attributed to stimulation of Ca^{2+} signals (Shonnard and Sanders, 1988). In a more recent study, Liu *et al.* (2002) revealed similar excitatory responses of gut preparations of the lungfish, Meoceratodus forsteri, to both ACh and SP.

Unlike the consistent excitatory effect which characterise ACh, NAD is known to have excitatory and/or inhibitory effects depending on the type of adrenergic receptors activated (Holmgren and Fange, 1981; Naitoh *et al.*, 1990). Furthermore, whether one or both of these receptor types are present on the smooth muscle differs among both the species and the investigated intestinal regions (Nilsson, 1983). The inhibitory effect of NAD on the spontaneous rhythmic activity is probably due to the abundance of β-adrenergic receptors in the small intestine of

the axolotl (Egginton, personal communication) coupled with the high affinity of NAD for β-adrenergic receptors. However, the insignificant increase in the basal tone suggests that α-adrenergic receptors might also be involved.

In a study on Xenopus, Naitoh *et al.* (1990) reported that both NAD and AD were not effective at a concentration of 10^{-7} M, produced slight increase in contraction at 10^{-4} to 10^{-6} M while induced a substantial decrease in contraction at 10^{-3} M. The authors consequently concluded that unlike the clear-cut excitatory effect of ACh, the effects of both NAD and AD are unclear and depend on their affinity for α- and/or β-adrenergic receptors. In accordance with the inhibitory effect of NAD, it has been reported that exogenous NAD reduces the amount of ACh released from intrinsic cholinergic neurons and thus causing motility inhibition (Furness *et al.,* 1992). Similar inhibitory effect for both NAD and AD has previously been reported by Fruhwald *et al.* (2002) using isolated segments of guinea pig small intestine. Thus, the responses of intestinal smooth muscle to NAD are probably mediated by interaction with specific α- and/or β-adrenergic receptors and both of them are often present in the same effector tissue. When α and β effects are antagonistic, one of the effects predominates. In non-sphincteric parts of the intestine, both α- and β-adrenergic receptors mediate inhibition, while in sphincteric parts α-excitatory adrenergic receptors predominate over β-inhibitory adrenergic receptors (Furness and Costa, 1987). The results of the present NAD experiments raise the possibility of species differences in the receptors themselves within amphibians. It is also possible that the observed insignificant excitatory effect after NAD treatment was due to the action of both α- and β-adrenergic receptors without significant dominancy for α-receptors.

SP, like ACh, is known for its excitatory action upon most intestinal preparations investigated with variable extent among different vertebrate species (Olsson and Holmgren, 2001; Liu *et al.,* 2002). The excitatory effect of SP on intestinal motility may depend either on a direct excitatory effect on the smooth muscle cells or indirectly mediated via the release of ACh from enteric neurons due to activation of cholinergic nerves (Holzer and Lembeck, 1980). The contraction of the intestinal smooth muscle caused by ACh has been shown to result via stimulating muscarinic Ach receptors (Nilsson, 1983). In dog ileum, the excitatory effect of SP has been found to be produced by direct action on the smooth muscle cells at high concentration and by a stimulation of cholinergic neurons at low concentration (Daniel *et al.,* 1982). However, in guinea pig ileum, the response to SP was divided into two phases, the first phase being a rapid contraction which was immediately followed by the second phase, a prolonged contraction of about half the amplitude of the initial response (Holzer and Lembeck, 1980). Two mechanisms of action were generally suggested for SP: an increase in the active tone which can be attributed to a direct action on the smooth muscle and an increase in the basal tone which can be explained by release of ACh (Jensen and Holmgren, 1991; Jensen *et al.,* 1993). As has been seen in the present experiments there was an increase in both active and basal tones indicating the involvement of the two mechanisms of action previously suggested for SP. Thus, it can be concluded that the major effect for SP upon the intestinal motility in both mammalian and non-mammalian vertebrates is excitatory. However, other investigations revealed that this is not always the case as the effects of tachykinins, including SP, is not only restricted to stimulation of intestinal motility but can also cause inhibition (Holzer *et al.,* 1995; Hökfelt *et al.,* 2001). This was attributed to the type of activated tachykinin receptors. Indeed, SP led to an increase in the active and/or basal tone of strip preparations from different gut regions of Necturus maculosus, but in some experiments an inhibition was observed with the longitudinal muscle strips of the pyloric stomach (Holmgren *et al.,* 1985). The potent excitatory effect provoked by SP in the present study is proportional to its high anatomical expression in both mucosal endocrine cells and enteric nerve fibres of the small intestine of the axolotl as has been recently revealed (Maake *et al.,* 2001; Badawy, 2014 a).

Despite its presence in neurons of myenteric and submucosal plexus as well as in the intestinal mucosal endocrine cells of mammals, SS seems to have little or even no direct effects on mammalian intestinal motor activity (Olsson and Holmgren, 2011). The finding that SS neurons do not project into the mammalian intestinal circular muscle layer makes it unlikely to influence smooth muscle cells directly (Keast *et al.,* 1984; Teitelbaum *et al.,* 1984). Consistent with that, SS had no contractile or relaxant effects on smooth muscle cells isolated from guinea pig or human intestine (McHenery *et al.,* 1991). However, it activates enteric inhibitory neurons and inhibits the release of ACh from cholinergic nerve terminals (Furness *et al.,* 1992). In rodents, two routes of SS inhibitory action have been proposed (Fujimiya and Inui, 2000), the first is via enhancing VIP release during descending relaxation and the second is through inhibiting ACh release from submucosal neurons. Gu *et al.* (1992) concluded that SS actions on motility are complex as it reversed VIP-induced relaxation of CARB stimulated contraction and had neither excitatory nor inhibitory effects on gastric smooth muscle cells. SS further inhibited the normal occurrence of cyclic interdigestive motor activity induced by motilin (Ormsbee *et al.,* 1978). The mechanism of SS effects on intestinal motility is probably related to the inhibition of basal release of ACh or to modulation of ACh release by other peptides (Yau and Youther, 1982). Indeed, SS inhibited ACh release induced by NT in guinea pig ileum but had no effect on ACh release induced by SP (Teitelbaum *et al.,* 1984). In the cod, Gadus morhua, SS caused relaxation followed by contraction (Jensen and Holmgren, 1985). In accordance with the effects reported for the

urodele amphibian, Necturus maculosus (Holmgren *et al.*, 1985), the data of the present study indicate a general weakness in response to SS. Therefore, no firm conclusion regarding the possible involvement of SS in the control of the intestinal motility of the axolotl could be drawn.

The phenomenon of the lack of significant effect in response to SS seen in most experiments draws attention to the crucial role of the enteric neurons upon the gut motility (Surprenant 1994). Meanwhile, the motility responses of SS may be explained in the light of the present results by one or more of the following possibilities: (1) SS is only expressed in mucosal endocrine cells but did not occur in the enteric neurons of the axolotl (Maake *et al.*, 2001; Badawy, 2014 a). This distribution pattern may be the basis for the weak effect obtained by SS especially that the regulatory peptides which are found in the mucosal endocrine cells may probably act as hormones rather than neurotransmitters (Holmgren and Jensen, 2001). Furthermore, the mucosal endocrine cells which showed immunoreactivity to SS were most numerous in the stomach, moderate in the small intestine and infrequent in the large intestine (Badawy, 2014 a); (2) SS may mainly control other intestinal functions rather than motility as the case with CGRP (Badawy, 2014 a and Umoh *et al.*, 2014); (3) SS may act as co-transmitter rather than transmitters and there might be a lack of interaction between SS and its co-agonist. The significant inhibitory effect of SS upon the basal tone of the juvenile distal segments and the somewhat clear effect obtained with some individual cases support the latter possibility and call for further investigation; or (4) there might be either low concentration of SS receptors or low affinity for the peptide itself.

In conclusion, the significant differences between juvenile and adult stages highlight the state of continuous change in the neuroendocrine system to meet the altered ontogenetic demands and indicate that the term neoteny can only be applied from the morphological aspect; however, it cannot be valid from the anatomical standpoint. This evidently contradicts the general belief that the relation between the neuroendocrine products and their target tissues in these late developmental stages i.e. juvenile and adult is well established. Moreover, the significant differences between Juvenile and adult small intestinal segments indicate possible differences in the expression of their neuroendocrine control system.

ACKNOWLEDGMENT

I am grateful to Professors Stuart Egginton (School of Biomedical Sciences, University of Leeds, UK), Ted Taylor (School of Biosciences, University of Birmingham, UK), Tobias Wang (Department of Zoophysiology, University of Aarhus, DENMARK).

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