Br. J. Pharmacol. (1988), **93**, 684–692

Development of non-cholinergic, non-adrenergic excitatory and inhibitory responses to intramural nerve stimulation in rat stomach

Shigeo Ito, Atsuko Kimura & Akira Ohga

Department of Pharmacology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

1 The onset and development of functional innervation of intramural neurones were examined by transmural nerve stimulation in circular muscle strips isolated from the rat stomach during the period from embryonic day (ED) 15 to 7-days postnatal.

2 At ED 15, transmural stimulation elicited an atropine-sensitive contraction in about half of the preparations. From ED 16, it caused a frequency-dependent contraction in all preparations. Physostigmine significantly potentiated the amplitude of the nerve-mediated contraction until ED 18.

3 Atropine inhibited but failed to abolish the contractile response to nerve stimulation in all preparations from ED 16.

4 During the contraction induced by carbamylcholine (CCh), transmural stimulation caused a biphasic response consisting of a contraction followed by a relaxation at ED 18 and ED 19, but caused a triphasic response consisting of a rapid relaxation followed by the biphasic response after birth.

5 CCh and substance P (SP) elicited contractions at ED 15 and vasoactive intestinal polypeptide (VIP) caused a relaxation at ED 16. The sensitivity to CCh and VIP increased with development but that to SP did not change.

6 The results suggest that functional intramural cholinergic and non-cholinergic excitatory innervations in the rat stomach are established almost simultaneously by ED 16, and the onset of functional intramural non-adrenergic inhibitory innervation lags about 2 days behind that of functional excitatory innervations.

Introduction

It is well-known that neurotransmitters involved in neural regulation of the gastrointestinal tract are acetylcholine and noradrenaline. The ontogenesis of cholinergic neurones has been studied by eliciting the mechanical response of gastrointestinal smooth muscles to transmural nerve stimulation. Results from this approach suggest that functional cholinergic innervation is established by embryonic day (ED) 17 in the rabbit intestine (Gershon & Thompson, 1973) and by ED 16 in the rat intestine (Miyazaki *et al.*, 1982). The adrenergic innervation of the intestine has been reported to start functioning a few days after birth in the rabbit (Burn, 1968; Gershon & Thompson, 1973; Gulati & Panchal, 1978). These results indicate that functional intrinsic cholinergic neurones develop earlier than the extrinsic adrenergic neurones in the gastrointestinal tract.

In addition to classical cholinergic and adrenergic neurones, there is evidence for the existence of intramural nerves such as non-adrenergic inhibitory (Bennett *et al.*, 1966) and non-cholinergic excitatory neurones (Furness & Costa, 1980) which influence the motility of gastrointestinal smooth muscles. The non-adrenergic inhibitory innervation was found to start functioning simultaneously with cholinergic innervation in the rabbit and mouse intestine (Gershon & Thompson, 1973). However, in the early foetal stages, the relaxant response of the intestine to transmural nerve stimulation appeared to result from movement of the longitudinal muscle of the intestine caused by contraction of the circular smooth muscle layer (Miyazaki *et al.*, 1982). Therefore, the onset and development of function in these intramural neurones is not fully understood.

Recently, many biologically active peptides have been isolated. In particular, vasoactive intestinal polypeptide (VIP) and substance P (SP) may be neurotransmitters of intramural non-adrenergic

inhibitory (Fahrenkrug *et al.*, 1978) and non-cholinergic excitatory neurones (Barthó, & Holtzer, 1985), respectively. SP and VIP immunoreactivities in the myenteric plexus of the rat stomach develop respectively at the perinatal period and after birth (Larsson, 1977; Sikoro *et al.*, 1984). If this is the case, it would be of interest to examine the onset and development of functional innervation of intramural non-cholinergic, non-adrenergic inhibitory and excitatory neurones in the rat stomach. For this purpose, we examined the onset and development of the mechanical response to transmural nerve stimulation using the circular muscle strip of the stomach isolated from foetal and newborn rats. In the course of this study, we have also investigated responses of the smooth muscle to carbamylcholine (CCh), SP and VIP at each developmental stage.

Methods

Animals

Foetuses and neonates of Wistar rats at various stages from ED 15 to 7-days postnatal (7-DP) were used. Four females were placed together with a male in a cage over night. When sperm was found in the vaginal smear the next morning, the age of the foetus was designated as ED 0. The age of the neonate which was usually born on ED 21 was designated as 0-DP on that day, so that the preparations of ED 21 included both foetus and neonate.

Recording of mechanical activity

Foetuses were removed, by Caesarean section, from female rats, which had been stunned and bled to death. Foetuses and neonates were killed by decapitation and the stomach isolated. A circular smooth muscle segment of the stomach (2–9 mm, length; 0.5–1.5 mm, width) was prepared from the region of the corpus with the aid of a binocular stereomicroscope. The mucosal layer of the isolated preparation was carefully removed from ED 20 specimens. In earlier foetal stages, the preparation was so fragile that the mucosal layer could not be removed. The strip was then suspended in a 0.8 ml bath containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 (pH 7.3–7.4), bubbled with 95% O_2 and 5% CO_2, and maintained at 34–36°C. The solution flowed continuously at a rate of 1 ml min^{-1} into the bath from a reservoir through polyethylene tubing connected to the bottom of the bath and the overflowing fluid was removed by suction. Drugs were applied to the bath through the perfusing solution to give the final concentration required. The mechanical activity of the smooth muscle segment was recorded by means of an isotonic transducer (Nihon Kohoden, TD-112S) on a thermal pen recording oscillograph (San.ei, recti-Horiz-8K). The tension loaded to the smooth muscle preparation was 5–10 mg at ED 15 and ED 16, 10–30 mg at ED 17 and ED 18, 50–100 mg at ED 19 to ED 21 and 100–300 mg at 1-DP to 7-DP, respectively.

Transmural nerve stimulation was applied to the preparation through two parallel Ag-AgCl electrodes (2 × 8 mm, separated by 4 mm) placed at either side along the muscle segment. An electronic stimulator (Nihon Kohoden. SEN-3201) was used to deliver the rectangular pulses with supramaximal voltage, 0.1–30 Hz and 1 ms duration.

Statistical analysis

Values are expressed as mean ± s.e. mean. Results were analysed by Student's unpaired t test. A P value less than 0.05 was considered significant.

Results

Development of contractile activity

To examine the development of the contractile activity of the circular muscle layer of the rat stomach, contractions induced by CCh (0.1 mM), SP (10 μM) and transmural nerve stimulation (10 Hz, 1 ms, supramaximal voltage) were observed at various stages of development. The amplitude of the contractile response to CCh, SP and transmural stimulation, the length of the smooth muscle segment used and the wet weight of the stomach are shown in Table 1. Contractile responses to CCh and SP were seen at all stages beyond ED 15, the earliest stage tested. The response to CCh was abolished by atropine (1 μM) at all stages examined, indicating that the contractile response to CCh is due to activation of muscarinic receptors. The contractile activity of the circular muscle seemed to begin to increase from ED 18 because the amplitude of the response to three different types of stimulation increased simultaneously between ED 17 and ED 18 (CCh and SP, $P < 0.001$; transmural stimulation, $P < 0.01$). It should be noted that the amplitude of the contractile response after ED 20 did not reflect the development of the contractility because the isotonic response depended on the length of the preparation at these stages. It is suggested that the increase in the contraction in response to various stimuli is due to the development of the contractile machinery of the circular smooth muscle of the stomach. Although there was a significant difference between the amplitude of

Table 1 The wet weight of the stomach, the length of the circular muscle preparation and the maximum contractile response to various stimuli at various stages

	ED 15	*16*	*17*	*18*	*19*	*20*	*21*	*1-DP*	*2-DP*
Wet weight (mg)	1.2 ±0.1	1.7 ±0.1	3.5 ±0.2	4.9 ±0.2	9.8 ±0.3	17.1 ±1.0	26.8 ±1.4	28.8 ±0.7	34.9 ±3.7
Length of preparation (mm)	2–3.5	3–4.5	3.5–5	5–6	6–9	6–9	6–9	6–9	6–9
CCh (mm)	0.19 ±0.02 (4)	0.29 ±0.03 (6)	0.41 ±0.05 (8)	1.31 ±0.11 (8)	1.46 ±0.2 (5)	1.90 ±0.16 (7)	2.20 ±0.55 (3)	2.29 ±0.21 (8)	2.32 ±0.39 (8)
TMS (mm)		0.14 ±0.03 (5)	0.27 ±0.04 (6)	0.69 ±0.11 (5)	1.13 ±0.19 (6)	1.89 ±0.3 (5)	1.77 ±0.18 (4)	1.83 ±0.24 (6)	2.22 ±0.69 (5)
SP (mm)	0.08 ±0.02 (4)	0.12 ±0.03 (5)	0.22 ±0.02 (3)	0.66 ±0.04 (4)	0.66 ±0.11 (4)	0.83 ±0.08 (5)	1.26 ±0.5 (3)	1.15 ±0.17 (5)	1.75 ±0.37 (4)

The lengths of the preparation (mm) indicate the range of tissue lengths determined after the end of the experiment at each stage. The maximal amplitude (mm) of contraction induced by carbamylcholine (CCh), transmural nerve stimulation (TMS) and substance P (SP) are summarized. ED and DP indicate foetus and neonate, respectively. Values in parentheses indicate the number of samples. For further explanation see text.

contraction induced by CCh and transmural stimulation until ED 18 ($P < 0.05$), differences between them disappeared thereafter, suggesting that the increase in the contractile response to transmural stimulation was probably due to the development of the intrinsic cholinergic innervation. On the other hand, differences between the responses to CCh and SP were observed until 1-DP. The development of contractile responses to SP seems to lag behind that to CCh.

Contractile response to transmural nerve stimulation

Transmural nerve stimulation at 5 or 10 Hz for 10 s elicited a small contraction in 3 out of 7 preparations at ED 15. From ED 16, it led to a frequency-dependent contractile response which attained a maximum during or slightly after stimulation in all preparations. At ED 16 and ED 17, the contractile response to transmural stimulation appeared at 1 Hz and reached a maximum at 10 Hz. Following this period, the transmural stimulation-induced contraction appeared effective at 0.2 Hz and attained a maximum at 5 Hz. All transmural stimulation-induced contractions were abolished by tetrodotoxin (TTX, 0.2 μM).

Forty min after treatment with physostigmine (0.1 μM), transmural stimulation caused a small but frequency-dependent contraction in all preparations at ED 15. From ED 16 to 2-DP, the contractile response to transmural stimulation appeared at lower frequencies (0.1 or 0.2 Hz) but reached a maximum at a similar frequency to that in the absence of the drug. Physostigmine potentiated the amplitude of the contractile response (Figure 1a) and/or prolonged the duration of the contractile response to nerve stimulation (Figure 1b). The potentiating effect of physostigmine on the amplitude of the contraction at various stages is shown in Figure 2a. The amplitude of the contractile response to nerve stimulation was significantly potentiated by physostigmine until ED 18. The potentiating effect on the amplitude of contraction decreased thereafter and almost disappeared after birth. In the neonate, physostigmine was more effective in prolonging the duration than in increasing the amplitude of the contractile response of the circular smooth muscle to transmural stimulation (Figure 1).

Atropine-resistant contractile response to transmural nerve stimulation

At ED 15, 30 min treatment with atropine (0.1 μM) abolished the contractile response to transmural nerve stimulation even in the presence of physostigmine (0.1 μM). Atropine (0.3–3 μM) inhibited, but failed to abolish the contraction at all stages after ED 16. TTX (0.2 μM) abolished the contractile responses, indicating that these responses were neural in nature. From ED 16 to ED 19, an atropine-resistant contractile response to transmural nerve stimulation appeared during stimulation and had a longer duration than the contraction in the absence of atropine (Figure 3a). In subsequent stages to ED 20, the contraction was observed after the cessation of transmural stimulation in most of the preparations (Figure 3b).

The effect of atropine on the amplitude of nerve-

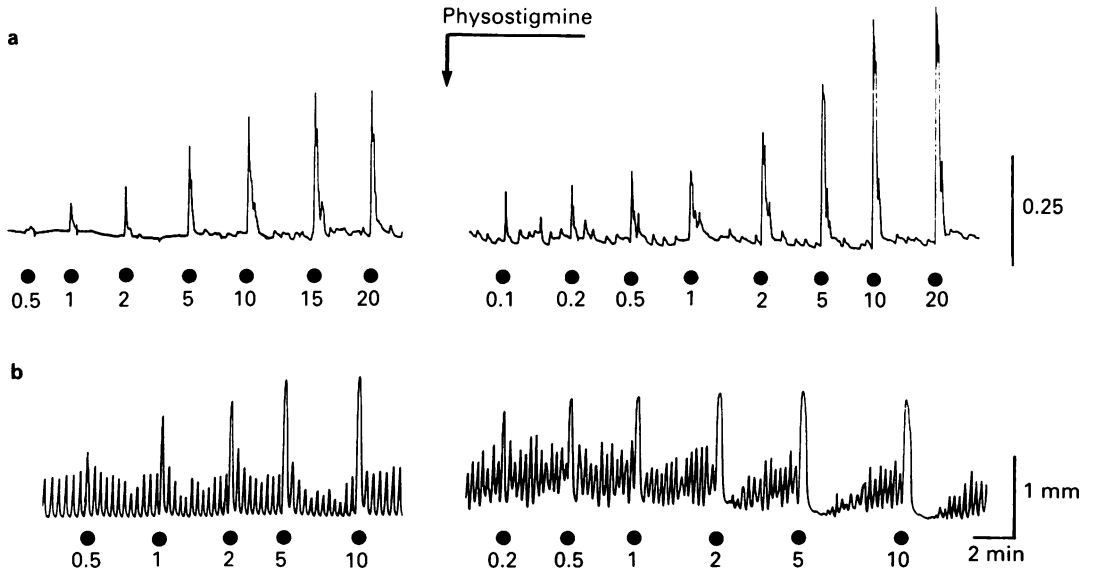

Figure 1 Contractile response of the circular muscle of the rat stomach to transmural nerve stimulation in the presence and absence of physostigmine (0.1 μM). Transmural nerve stimulation (●) was applied to the circular smooth muscle for 10 s at ED 18 (a) and 2-DP (b). Numbers associated with symbols refer to frequencies (Hz) of stimulation.

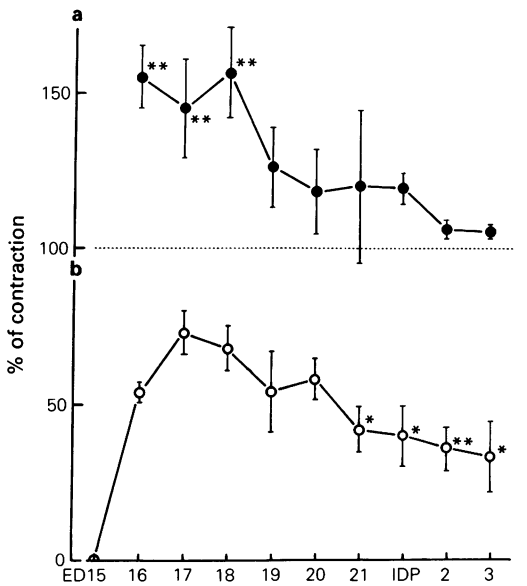

Figure 2 The amplitude of the contractile response of the circular muscle of the rat stomach to transmural nerve stimulation in the presence of physostigmine or atropine at various developmental stages. The abscissa scale indicates the stages of the foetus (ED) and neonate (DP). The ordinate scale represents the % of the contractile response to transmural nerve stimulation (10 Hz) in the absence of drugs at each developmental stage. Points show the mean value obtained in the presence of (a) physostigmine (●, n = 4–9, 0.1 μM) and of (b) atropine (○, n = 6–13, 0.1–3 μM). Vertical lines show s.e. mean. $^{*}P < 0.05$; $^{**}P < 0.01$, significantly different from the values obtained at ED 17 (b) and at 3-DP (a).

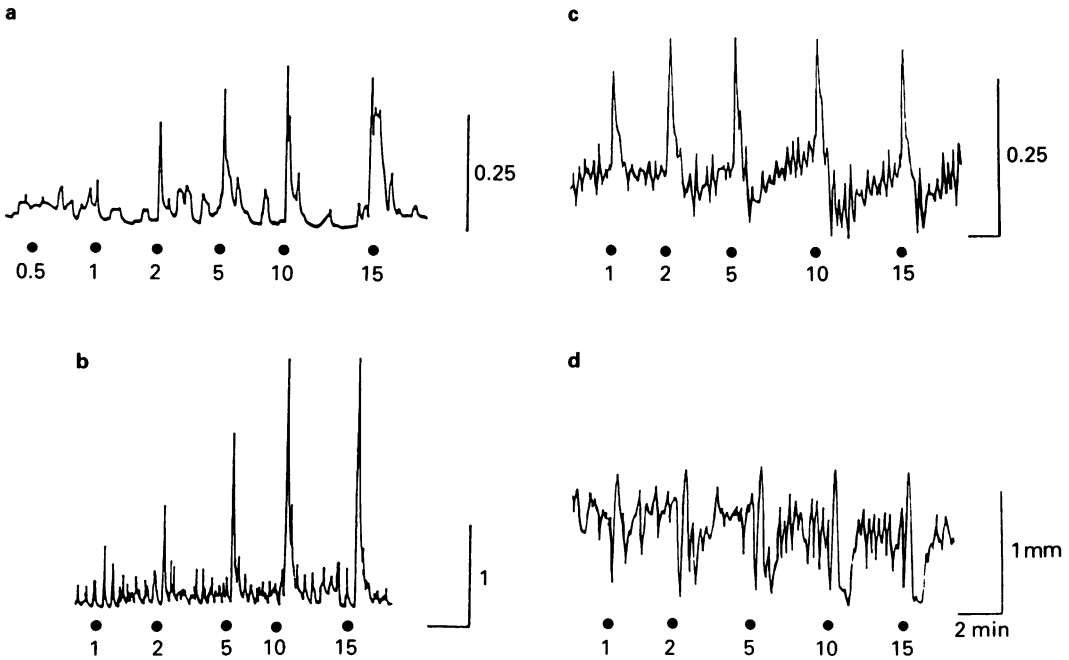

Figure 3 Atropine-resistant contractile responses and two types of relaxant responses to transmural nerve stimulation in the circular muscle of the rat stomach. Transmural nerve stimulation (●) was applied to the circular muscle for 10 s in the presence of atropine (0.3 μM) at ED 18 (a) and ED 20 (b), and to the circular smooth muscle contracted by carbamylcholine at ED 18 (c, a biphasic response) and ED 21 (d, a triphasic response). Numbers associated with the symbols refer to frequencies (Hz) of nerve stimulation.

mediated contractions at various stages of development is shown in Figure 2b. The atropine-resistant component of the contraction appeared from ED 16, reached a maximum at ED 17 and then decreased gradually thereafter. Guanethidine (1 μM) did not affect the atropine-resistant contraction at any stage. The effect of the SP antagonist, (D-Pro^2, D-Trp^{7,9})-SP (Leander *et al.*, 1981), on the atropine-resistant contraction was examined in the muscle strips from ED 21 to 2-DP specimens. The SP antagonist at a concentration of 10 μM caused a small contraction in the presence of atropine. The contractile responses to SP and transmural nerve stimulation were decreased by about 40% and 25%, respectively, in 2 out of 7 preparations.

Relaxant response to transmural nerve stimulation

A relaxation to transmural nerve stimulation was only observed, in some preparations, at stages later than 2-DP. It is possible that the tone of the smooth muscle at early stages is so low that the relaxation could not be detected. In the following experiments, therefore, the tone of the circular muscle strip was increased by adding CCh, at a concentration which caused a contraction of more than 70% of the maximal contraction induced by transmural nerve stimulation.

At ED 17, transmural nerve stimulation elicited a small but perceptible relaxation in one out of 7 preparations. After ED 18, two types of response were evoked by transmural nerve stimulation, a biphasic response consisting of a contraction followed by a long-lasting relaxation (Figure 3c) and a triphasic response consisting of a rapid relaxation preceding the biphasic response described above. (Figure 3d). The type of response did not vary with different stimulus frequencies in the same preparation. However, the precise frequency-relaxation relationship was difficult to measure because the contraction also increased with increasing frequencies. The relaxation induced by transmural stimulation was not affected by guanethidine (1 μM) but blocked by TTX (0.2 μM) at all stages examined, indicating that the relaxation was mediated by the non-adrenergic inhibitory neurones. Table 2 shows the number of the preparations showing each type of response at each developmental stage. The biphasic response was

Table 2 The number of preparations in which biphasic and triphasic mechanical responses were observed to transmural nerve stimulation in the pre-contracted circular smooth muscle of the rat stomach

	ED 17	*18*	*19*	*20*	*21*	*1-DP*	*2-DP*	*7-DP*
Biphasic response	1	5	9	12	2	1	0	0
Triphasic response	0	1	1	4	6	6	10	5

For explanation see text. The number of experiments is 7 at ED 17. Only one preparation responded to nerve stimulation at this stage. After ED 18, transmural nerve stimulation elicits the biphasic and/or triphasic responses in all preparations, so that the number of preparations represents the number of experiments.

observed in most of the preparations until ED 19 and the triphasic response was observed at the neonatal stage. Both types of response were encountered at ED 20 and ED 21. The results indicate that the number of the preparations showing the biphasic response decreases, while that showing the triphasic response increases with development.

Response to carbamylcholine, substance P and vasoactive intestinal polypeptide

In order to estimate the development of the sensitivity of the circular muscle to CCh, SP and VIP, concentration-response curves were constructed at various stages. The concentrations of CCh and SP giving about 50% of their respective maximal contraction (EC_{50}) are shown in Figure 4. Ranges of EC_{50} values for CCh were from 1 to 10 μM between ED 15 and ED 19 and from 0.1 to 1 μM after birth. The mean EC_{50} values for CCh at ED 19 and 1-DP were $2.1 \pm 0.2\,\mu$M ($n = 3$) and $0.53 \pm 0.5\,\mu$M ($n = 4$), respectively and were significantly different ($P < 0.01$) (Figure 4a). It is suggested that the affinity of muscarinic receptors for CCh increases significantly during the 3 days before birth. On the other hand, the EC_{50} value for SP did not change significantly during ontogeny in both the presence and absence of atropine (1 μM) and guanethidine (1 μM) (Figure 4b). However, it should be noted that an atropine-sensitive component of the contraction was observed with lower concentrations (0.03 and 0.1 μM) of SP after but not before birth.

The sensitivity of the circular smooth muscle to VIP was examined at various stages in the preparations, of which the tone was raised by CCh. The earliest foetal stage examined was ED 16, at which a response to VIP had already been observed. In most of the preparations, VIP at lower concentrations decreased muscle tone without affecting spontaneous contractile activity, but higher concentrations of VIP elicited a pronounced relaxation accompanied by the inhibition of spontaneous activity. The long-lasting relaxation induced by VIP made it difficult to maintain the tension in some preparations. Therefore, VIP was applied to the preparation successively by increasing the concentration at intervals, and the concentration of VIP giving about a 50% inhibition of the tone just before each application of VIP was considered to be a 50% effective concentration (EC_{50}). EC_{50} values for VIP were obtained successfully in 22 out of 41 preparations from ED 16 to 5-DP and were plotted against the developmental stage (Figure 4c). In the other preparations, VIP at concentrations of up to 0.32 μM failed to cause a 50% inhibition of the tone because the muscle tone was gradually decreased by each application of VIP. The sensitivity of the circular smooth muscle to VIP tended to increase with development.

Discussion

Development of cholinergic excitatory innervation

Transmural nerve stimulation produced an atropine-sensitive contraction in about half of the preparations at ED 15. The cholinergic excitatory response was unmasked by physostigmine in the preparations not contracting to nerve stimulation, indicating that functional cholinergic innervation is already present but significantly modified by the enzymatic activity of acetylcholine esterase (AChE) at this stage. It is suggested that the cholinergic innervation in the rat stomach is established by ED 16 similar to the rat intestine (Miyazaki *et al.*, 1982). This suggestion is supported by the fact that AChE-positive enteric ganglion cells in the rat stomach increase in number during this period (Boekelaar *et al.*, 1985).

Physostigmine potentiated the amplitude of contraction in response to transmural nerve stimulation between ED 16 and ED 18. This potentiating effect of physostigmine was replaced by an increase in the duration of contraction after birth. The ratio of the amplitude of contraction induced by transmural nerve stimulation to that induced by CCh increased at ED 19. Therefore, in earlier foetal stages, transmural stimulation seemed unable to elicit the

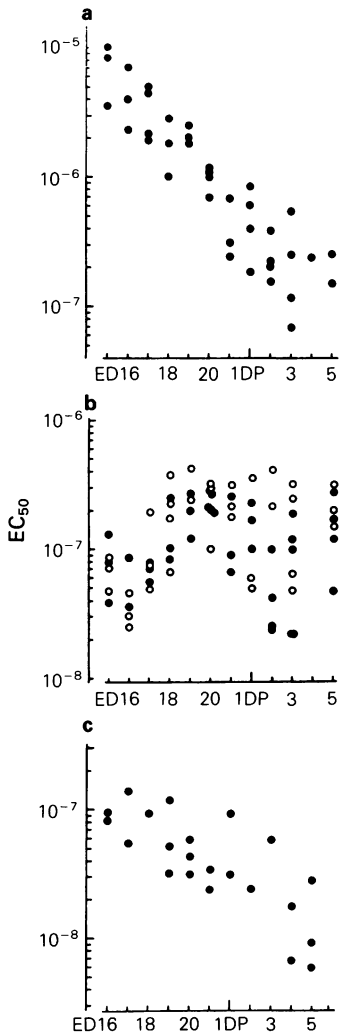

Figure 4 Sensitivity of the circular smooth muscle to carbamylcholine (CCh), substance P (SP) and vasoactive intestinal polypeptide (VIP) at various developmental stages. The ordinate scales represent the EC_{50} values (M) for CCh (a), SP (b) and VIP (c) in foetus (ED) and neonate (DP). (○) In (b) indicate the value obtained in the presence of atropine (1 μM) and guanethidine (1 μM). For further explanation see text.

maximal contractile response. This may be due to the immature state of the cholinergic innervation and/or to electrical coupling between circular smooth muscles. The low sensitivity of muscarinic receptors in circular smooth muscles might also contribute to this effect because of the higher EC_{50} values for CCh at earlier stages.

Development of non-cholinergic excitatory innervation

An atropine-resistant, TTX-sensitive contraction in response to transmural nerve stimulation appeared at ED 16. It is unlikely that the atropine-resistant contraction is due to the inaccessibility of atropine to junctional muscarinic receptors, because atropine is expected to diffuse easily into the intercellular space of the circular smooth muscle of the foetal stages (Maggi *et al.*, 1984). It may be possible that the contraction is a post-inhibitory rebound effect (Bennett, 1966). However, the inhibitory response could not be detected until ED 18. Therefore, the atropine-resistant contraction may be attributed to activation of non-cholinergic excitatory neurones. If this is the case, the functional non-cholinergic excitatory nerve develops at the same stage as the cholinergic nerve established by ED 16. It seems unlikely that the developmental changes in the magnitude of the non-cholinergic excitatory response, which attained a maximum at ED 17 and decreased thereafter, reflect the ontogeny of the non-cholinergic excitatory innervation to the circular muscles. If the transmural nerve stimulation-induced contraction in the presence of physostigmine is considered to be 100% at each stage instead of that in the absence of the drug, as shown in Figure 2, the ratios of the atropine-resistant contraction between ED 16 and ED 20 will be less than 50%, which are not statistically different from values obtained at postnatal periods. Therefore, the relative magnitude of the atropine-resistant contraction at foetal stages seems to be affected by the immature state of the cholinergic nerve-mediated response. The neurotransmitter of the non-cholinergic excitatory neurones might be a substance more resistant to enzymatic degradation than ACh.

Development of non-adrenergic inhibitory innervation

Transmural nerve stimulation began to elicit a biphasic response consisting of a contraction followed by a long-lasting relaxation from ED 18. The biphasic response seemed to be replaced by a triphasic response consisting of a rapid relaxation followed by the biphasic response for the period from ED 20 to ED 21. These responses to transmural nerve stimulation were not affected by guanethidine but blocked by TTX, indicating the involvement of activation of non-adrenergic inhibitory neurones. Therefore, it is proposed that the non-adrenergic inhibitory innervation starts to function at ED 18. The establishment of the non-adrenergic inhibitory innervation to the circular muscle layer may account for the rapid relaxant response to transmural nerve stimulation, and the slow relaxation might be due to the immature state of inhibitory innervation.

Putative transmitter of non-adrenergic, non-cholinergic neurones

It has been proposed that SP or a related tachykinin is responsible for the non-cholinergic contraction in the stomach of the adult rat (Hunt *et al.*, 1983) and that VIP mediates a non-adrenergic relaxation in the guinea-pig fundic strip (Grider *et al.*, 1985). Changes in the sensitivity of the gastric smooth muscle to drugs occurred during ontogeny in a different manner. A similar phenomenon has also been observed in the spinal neurones of the developing rat for SP, glutamate and *r*-aminobutyric acid (Seno *et al.*, 1984; Seno & Saito, 1985). Although the developmental changes in peptide sensitivity of the rat stomach do not provide any information about transmitter candidates of the intramural neurones, the results indicate that the circular muscle already responded to CCh, SP and VIP at the stage before the establishment of functional innervation of these intramural nerves. The non-cholinergic excitatory innervation seems to start functioning simultaneously with cholinergic innervation. All SP immunoreactive nerve cell bodies in the submucosa have been proposed to contain choline acetyltransferase immunoreactivity in the guinea-pig ileum, suggesting that SP co-exists with ACh (Furness *et al.*, 1984). If SP mediates the non-cholinergic response, this may explain the functional appearance of both excitatory neurones at the same stage. The functional non-adrenergic inhibitory innervation developed prenatally in the rat stomach in this experiment, although VIP immunoreactive fibres were detectable after birth (Larsson, 1977; Sikoro *et al.*, 1984). It should be noted that VIP immunoreactivity was detected by radioimmunoassay in the rat duodenum from ED 16 (Emson *et al.*, 1979), at which neither VIP immunoreactive cells nor neurones were seen (Larsson, 1977). The SP antagonist failed to exert a constant inhibitory effect on the non-cholinergic and SP-mediated contraction in the circular muscle of the rat stomach. However, the sensitivity of the circular smooth muscle to SP or its antagonist in the rat stomach appeared to be lower than that of the circular smooth muscles of the guinea-pig intestine (Costa *et al.*, 1985). Further studies are required to evaluate the transmitters of the non-cholinergic, non-adrenergic intramural nerves.

We wish to thank Drs Y. Nakazato and T. Ohta for their advice and continual encouragement. This work was supported in part by Grants from the Japanese Ministry of Education, Science and Culture, and from Foundation for the Promotion of Research on Medical Resources.

References

BARTHÓ, L. & HOLZER, P. (1985). Search for a physiological role of substance P in gastrointestinal motility. *Neuroscience* **16**, 1–32.

BENNETT, M.R. (1966). Rebound excitation of the smooth muscle cells of the guinea-pig taenia coli after stimulation of intramural inhibitory nerves. *J. Physiol.*, **185**, 124–131.

BENNETT, M.R., BURNSTOCK, G. & HOLMAN, M.E. (1966). Transmission from intramural inhibitory neurones to the smooth muscle cells of the guinea-pig taenia coli. *J. Physiol.*, **182**, 541–558.

BOEKELAAR, A.B., DRUKKER, J., GROEN, G.J. & BALJET, B. (1985). The development of the peripheral autonomic nervous system in relation to the gastro-intestinal tract. *Acta Morphol. Neerlando-Scand.*, **23**, 157–180.

BURN, J.H. (1968). The development of adrenergic fibre. *Br. J. Pharmacol. Chemother.*, **32**, 575–582.

COSTA, M., FURNESS, J.B., PULLIN, C.O. & BORNSTEIN, J. (1985). Substance P enteric neurons mediate non-cholinergic transmission to the circular muscle of the guinea-pig intestine. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 446–453.

EMSON, P.C., GILBERT, R.F.T., LOREN, I., FAHRENKRUG, J., SUNDLER, F. & SCHAFFALITZKY DE MUCKADELL, O.B. (1979). Development of vasoactive intestinal polypeptide (VIP) containing neurones in the rat brain. *Brain Res.*, **177**, 437–444.

FAHRENKRUG, J., GALBO, H., HOLST, J.J. & SCHAFFALITZKY DE MUCKADELL, O.B. (1978). Influence of the autonomic nervous system on the release of vasoactive intestinal polypeptide from the porcine gastrointestinal tract. *J. Physiol.*, **280**, 405–422.

FURNESS, J.B. & COSTA, M. (1980). Types of nerves in the enteric nervous system. *Neuroscience*, **5**, 1–20.

FURNESS, J.B., COSTA, M. & KEAST, J.R. (1984). Choline acetyltransferase- and peptide immunoreactivity of submucous neurons in the small intestine of the guinea-pig. *Cell Tissue Res.*, **237**, 329–336.

GERSHON, M.D. & THOMPSON, E.B. (1973). The maturation of neuromuscular function in a multiply innervated structure: Development of the longitudinal smooth muscle of the foetal mammalian gut and its cholinergic excitatory, adrenergic inhibitory, and non-adrenergic inhibitory innervation. *J. Physiol.*, **234**, 257–277.

GRIDER, J.R., CABLE, M.B., SAID, S.I. & MAKHLOUF, G.M. (1985). Vasoactive intestinal peptide as a neural mediator of gastric relaxation. *Am. J. Physiol.*, **248**, G73–G78.

GULATI, O.D. & PANCHAL, D.I. (1978). Some observations on the development of adrenergic innervation in rabbit intestine. *Br. J. Pharmacol.*, **64**, 247–251.

HUNT, W.B., O'HAGAN, D.T. & WILKINSON, J. (1983). Inhibition of the rebound contraction of the rat gastric corpus strip to field stimulation following substance P (SP) desensitization. *J. Physiol.*, **342**, 26P–27P.

LARSSON, L.-I. (1977). Ontogeny of peptide-producing nerves and endocrine cells of the gastro-duodeno-pancreatic region. *Histochemistry*, **54**, 133–142.

LEANDER, S., HÅKANSON, R., ROSELL, S., FOLKERS, K., SUNDLER, F. & TORNQVIST, K. (1981). A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation. *Nature*, **294**, 467–469.

MAGGI, C.A., SANTICIOLI, P. & MELI, A. (1984). Postnatal development of myogenic contractile activity and excitatory innervation of rat urinary bladder. *Am. J. Physiol.*, **247**, R972–R978.

MIYAZAKI, H., OHGA, A. & SAITO, K. (1982). Development of motor response to intramural nerve stimulation and to drugs in rat small intestine. *Br. J. Pharmacol.*, **76**, 531–540.

SENO, N., ITO, S. & OHGA, A. (1984). The development of responsiveness to substance P and glutamate in the spinal motoneurons of rat fetuses. *Brain Res.*, **298**, 366–369.

SENO, N. & SAITO, K. (1985). The development of the dorsal root potential and the responsiveness of primary afferent fibers to *r*-aminobutyric acid in the spinal cord of rat fetuses. *Dev. Brain Res.*, **17**, 11–16.

SIKORA, L.K.J., BUCHAN, A.M.J., LEVY, J.G., McINTOSH, C.H.S. & BROWN, J.C. (1984). VIP-innervation of rat intestine: A developmental study with monoclonal antibodies. *Peptides*, **5**, 231–237.

(Received July 28, 1987.
Revised October 13, 1987.
Accepted October 24, 1987.)