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Histamine response in developing chick oesophagus

A pharmacological analysis of the response to histamine in the oesophagus of developing chick

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Abstract

Developmental changes in response to histamine were investigated in the oesophagus isolated from the chick between 15 days of incubation and 15 days after hatching. The contraction could be already caused by histamine (20 μM) in the chick oesophagus from 15 days of incubation. The pD_2 values for histamine and acetylcholine did not change during the period between 17 days of incubation and 5 days after hatching. The response to histamine (20 μM) was extremely resistant to tetrodotoxin (0.78 μM), procaine (0.2 mM) and atropine (1 μM) during the period between 17 days of incubation and 20 days of incubation. The tetrodotoxin-resistant response was replaced by a tetrodotoxin-sensitive response after hatching. On the other hand, the neuronal response induced by transmural electrical stimulation (20 Hz) or dimethylphenylpiperazinium (20 μM) was uniformly sensitive to tetrodotoxin throughout the periods. Mepyramine (2 μM), but not metiamide (20 μM), inhibited the histamine-induced responses in every age tested. These results suggest that the myogenic receptivity of histamine transiently increases during the terminal period of embryonic development and declines after hatching.

Introduction

The response to histamine in alimentary tracts is known to differ greatly between species and tissues [1, 2]. Particularly, the receptor of histamine is divided into H_1 and H_2 subtypes and histamine may act either directly on the smooth muscle or indirectly via nerve elements depending on the species and organs [2]. However, only little is known concerning age- or development-related change in the action of the biogenic amine histamine, though much information has been available about other several drugs including neurotransmitter [3, 4] and neuropeptide [5]. Botting [6] showed in the neonatal rabbit ileum that the contractile response caused by histamine decreased in both amplitude and sensitivity as the age of the rabbit increased, a phenomenon which the author explained by a loss of the myogenic histamine H_1 receptor.

In the chick oesophagus, the action of histamine seems to be different in nature from the action in other portions of the chick alimentary tract [2]. Histamine has been reported to contract the chick oesophagus indirectly via cholinergic nerve elements, as the contraction was sensitive to atropine, cocaine [7] or morphine [8] besides mepyramine. Autonomic innervation in this preparation has been reported to be predominantly cholinergic in nature [9, 10]. Little is known on the development change in the neuronal action of histamine, especially in the period of embryonic development.

In the present study, therefore, the developmental change of the response induced by histamine was investigated in the oesophagus isolated from the chick before and after hatching. In the course of this investigation, it was noticed that histamine-induced response in the preparation during terminal period of embryonic development was remarkably insensitive to tetrodotoxin (TTX), suggesting a direct action of histamine on the smooth muscle in contrast to the preparation after hatching. This suggests that the mode of action of histamine changes during the short period of the development of the chick.

Materials and methods

Fertilized eggs and hatched chicks (Shaber Star Cross) were used. The eggs were maintained in a humidified incubator at 38 ± 0.5 °C until the time of experiments. The chicks examined were from 15 days of incubation to 15 days after hatching.

The segment of oesophagus upward from the crop, about 1–2 cm long, was isolated from the embryos or the chicks, which had been killed by stunning. The preparation was immersed in a 5 or 20 ml organ bath filled with modified Krebs solution. The composition of the solution was (m*M*): NaCl 118.0; KCl 4.75; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0 and glucose 11.5. The solution was saturated with mixture of 95% O_2 and 5% CO_2 (pH 7.4) and kept at 37 ± 1 °C. The preparation was allowed to equilibrate for 60 min before the commencement of any other experimental procedure. The mechanical response of the muscle segment was recorded on isotonically or isometrically on a pen writing recorder with mechanoelectrical transducers. Electrical field stimulation was applied to the muscle segment via coaxial Ag/AgCl electrodes [11] with trains of rectangular pulse (supramaximal voltage, 0.5 msec duration) at various frequencies. Drugs were applied to the organ bath with a micropipette. As the amplitudes of contractile responses induced by drugs and electrical field stimulation greatly changed with the developmental age of the chick, the degree of all responses were expressed as a percentage of hypertonic potassium (K^+, 80 m*M*) contracture. The statistical significance of the differences of means was analyzed by Student's *t*-test and a *P* value of 0.05 or less was considered statistically significant.

Drugs used and their source were; acetylcholine chloride (ACh), atropine sulphate, eserine sulphate (physostigmine), procaine hydrochloride (Wako Chemical, Japan), 1-1-dimethyl 4-phenyl piperazinium iodide (DMPP, Tokyo Kasei, Japan), histamine dihydrochloride, mepyramine maleate, tetrodotoxin (TTX, SIGMA, USA) and metiamide (Smith Kline & French).

Results

Histamine-induced responses in several developmental ages

Histamine caused a contraction in the chick oesophagus isolated from 15 days of incubation to 15 days after hatching in a dose dependent manner (1–100 μ*M*). The maximal contraction induced by histamine was obtained at about 30 μ*M*. Acetylcholine (ACh, 0.1–100 μ*M*) also produced a similar contraction. The developmental changes of calculated pD_2 values for histamine and ACh are shown in Fig. 1. Because of the difficulty in distinguish between spontaneous contractions and contractions evoked by lower concentrations of histamine and ACh, an attempt to make a dose response curve was not successful in preparations earlier than 15 days of incubation. The pD_2 values of histamine at various ages of the chick were not significantly different from 17 days of incubation to 5 days after hatching and the mean value was about 5.8. But these values decreased gradually from 5 day after hatching as described by Bartlet and Hassan [7]. On the other hand, the pD_2 values for ACh were not significantly different in the chick 17 days of incubation, 21 days of incubation and 15 days after hatching, suggesting no change in sensitivity to ACh until 15 days after hatching. These findings suggest that the sensitivity of chick oesophagus to histamine and ACh did not greatly change in the period around hatching, at least during the period between 17 days of incubation and 5 days after hatching.

Effect of tetrodotoxin on histamine-induced responses during the period around hatching

In the oesophagus isolated from the chick after hatching, the contraction induced by histamine was observed to be sensitive to atropine, cocaine [7] and morphine [8], suggesting preferentially the

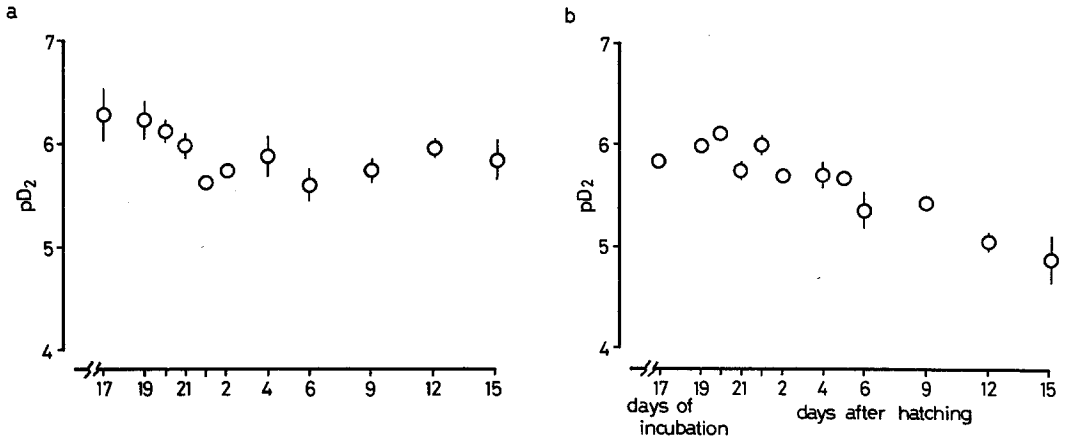

Figure 1
The pD_2 values for histamine (His) and acetylcholine (ACh) in the preparation at different ages. Ordinates indicate pD_2. Mean pD_2 values (○) for ACh (a) and His (b), calculated from each dose-response curve, in 3–8 preparations are plotted as a function of age (day). The SEM bars are only shown when they exceed the dimensions of the symbols used.

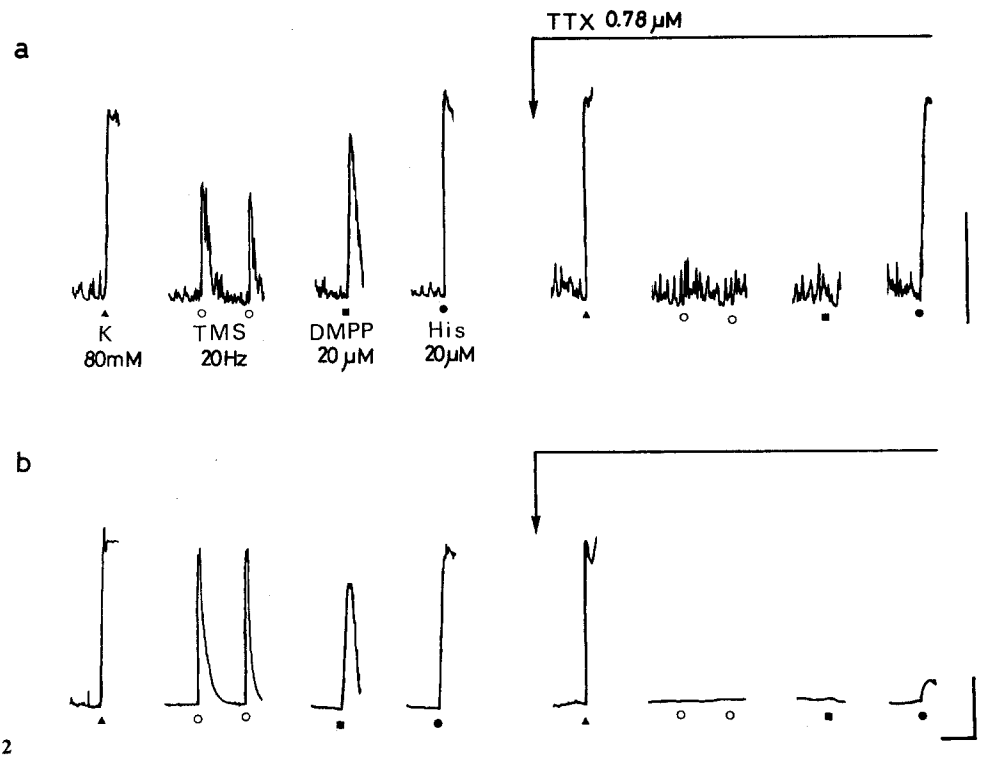

Figure 2
Effect of TTX on contractile responses induced by the application of 80 m*M* potassium chloride (K^+ 80 m*M*, ▲), dimethyl phenyl piperazinium (DMPP 20 μ*M*, ■) and histamine (His 20 μ*M*, ●), and electrical field stimulation (TMS, 20 Hz, ○) in the preparation at 19 days of incubation (a) and 3 days after hatching (b). Arrows (↓) indicate the exposure of tetrodotoxin (TTX, 0.78 μ*M*). Note that His response in the preparation at 19 day of incubation was resistant to the treatment of TTX. Vertical scales, 5 mm; horizontal scale, 2 min (isotonic recording).

indirect action of histamine via some cholinergic link. We investigated the response caused by histamine in the oesophagus from the chick before and after hatching using tetrodotoxin (TTX) and we found that the sensitivity of histamine-induced contractions to TTX was greatly different in the preparations before and after hatching. Fig. 2 shows typical traces of the effect of TTX (0.78 μM) on the histamine responses at two different stages. In the preparation at 19 days of incubation, the preceding treatment of TTX was not effective in reducing the contraction induced by histamine (20 μM), though histamine response at 3 days after hatching was markedly suppressed in the presence of TTX. On the other hand, nerve mediated contractions induced by electrical field stimulation (20 Hz) or DMPP (20 μM) application were completely prevented by TTX in both preparations, suggesting that the effect of TTX on the nerve excitation was not greatly different between these preparations. The same effects of TTX on the histamine response were observed in several preparations before ($n=5$) and after hatching ($n=3$). The extent of inhibition of histamine response by TTX did not change by an increase in the concentration of TTX up to 1.56 μM.

Furthermore, procaine (0.2 mM) which has similar inhibitory effects on the nerve excitation was also effective in reducing the histamine response only in the preparations after hatching as shown in Table 1. The response to histamine was resistant to the treatment of procaine in the 20 days of incubation (about 90% to control response) and sensitive to the treatment in 5 days after hatching (about 8% to control response). The remarkable resistance of histamine response to TTX and procaine suggested that the mode of action of histamine on the chick oesophagus was different in the development age and that in the oesophagus during the terminal period of embryonic development, histamine was capable of causing a contractile response without the excitation of neuronal elements.

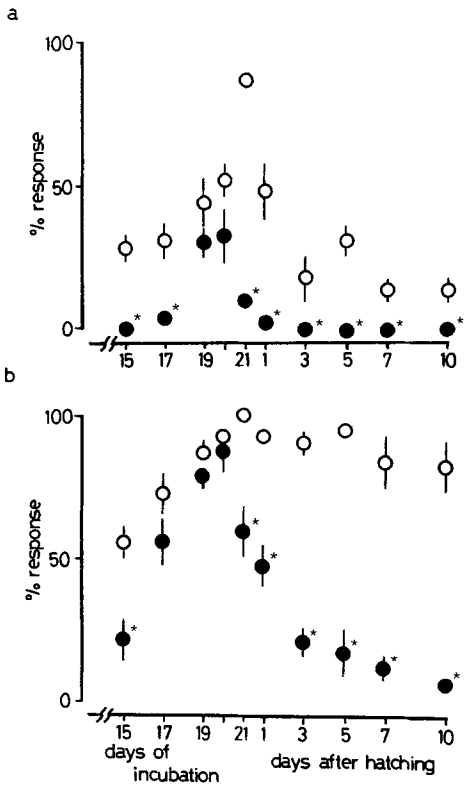

Figure 3
Effect of TTX on responses to histamine (His) at different ages. a: His (2 μM). b: His (20 μM). Ordinates indicate the contraction induced by His as a percentage of the response of the tissue to 80 mM K^+. The abscissa indicates the developmental ages as days. Each point is the mean amplitude of contraction in the control (○) or TTX (0.78 μM)-treated (●) preparations ($n=3-9$) and the SEM bars are only shown when they exceed the dimensions of the symbols used. * $P<0.05$ compared to control response at different age.

Table 1
The effect of procaine (0.2 mM) on the responses to histamine (His, 20 μM), DMPP (10 μM) and ACh (5 μM) in the oesophagus of developing chick. The responses were expressed as percentages (mean ± SEM) of the response induced by 80 mM KCl. ID20 and 5AH represent the age of chick at 20 days of incubation and 5 days after hatching.

Age	(Control)			(Treated with procaine)		
	His 20 μM	DMPP 10 μM	ACh 5 μM	His 20 μM	DMPP 10 μM	ACh 5 μM
ID20 ($n=3$)	99.2 ±3.1	76.6 ±10.8	97.6 ±4.1	89.1 ±6.6	0 –	71.9 ±9.1
AH5 ($n=5$)	97.1 ±1.9	98.0 ± 2.2	96.0 ±2.0	8.1* ±2.0	0 –	74.4* ±2.3

* $P<0.05$ compared to control response.

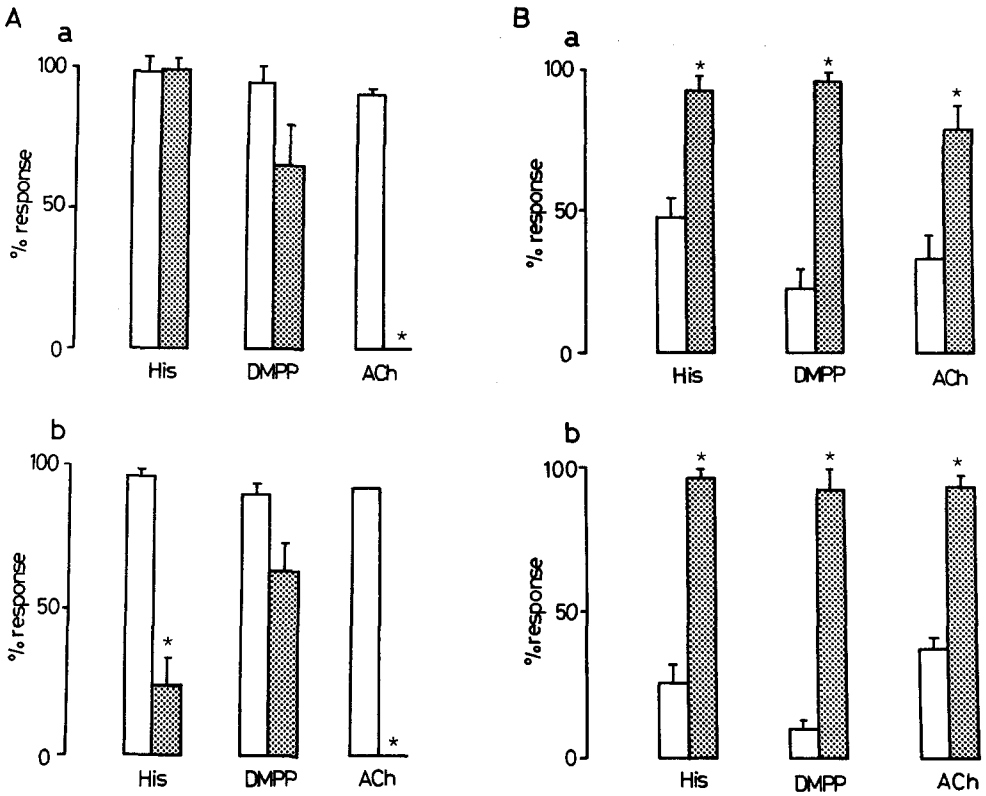

Figure 4
Effects of atropine and physostigmine on responses to histamine (His), dimethyl phenyl piperazinium (DMPP) and acetylcholine (ACh) in the preparation before and after hatching. Open column: control responses to His (20 and 1 μM), DMPP (20 and 2 μM) and ACh (5 and 0.2 μM) in A and B, respectively. Dotted column: the responses after treatment of atropine (1 μM, A) or physostigmine (50 nM, B). a: 20 days of incubation. b: 3 days after hatching. Ordinates indicate the contraction induced by each drug as a percentage of the response of the tissue to 80 mM K^+. Each column is the mean amplitude of responses in 3–6 preparations and vertical bars show SEM. * $P < 0.05$ compared to control response.

Developmental change of TTX-resistant response to histamine

The degree of resistance of the histamine response to TTX was examined throughout the development of the chick. The histamine (2 and 20 μM)-induced responses and the effect of TTX on these responses at several ages is shown in Fig. 3. The response caused by 20 μM of histamine, at which concentration about maximum effect was obtained, increased with development until 19 days of incubation (Fig. 3b). The degree of histamine response thereafter was maintained at a relatively constant level and was compatible with that of an 80 mM K^+ contracture. TTX (0.78 μM) failed to inhibit significantly the response in the preparations between 17 and 20 days of incubation, while it inhibited the response by about 50% in the preparation at 15 days of incubation. In the preparations after hatching, histamine response came to be greatly inhibited by TTX, again. The degree of inhibition gradually increased with age and was $90 \pm 1\%$ at 10 days after hatching (Fig. 3b). The effect of TTX on the response evoked by a low concentration (2 μM) of histamine, the amount needed to induce about half maximum effect, was qualitatively similar to that in a higher concentration of histamine, though the responses tended to be more sensitive to TTX (Fig. 3a). Thus, TTX-resistant responses

of histamine was remarkable at the terminal period of embryonic development and it then rapidly decreased with the age of the chick.

Effects of atropine and physostigmine on TTX-resistant responses caused by histamine in the preparation before hatching

The effects of atropine and physostigmine on the histamine response were compared between preparations before and after hatching in order to further elucidate whether TTX-resistant contractions to histamine before hatching was not due to the release of ACh from cholinergic nerves.

Atropine (1 μM), which completely prevented the maximum contraction caused by ACh (20 μM) did not inhibit the contraction induced by histamine (20 μM) in the preparation at 20 days of incubation ($n=3$, Fig. 4Aa), whereas the response to histamine at 3 days after hatching was inhibited remarkably by $78.5 \pm 8.8\%$ ($n=3$, Fig. 4Ab) as earlier observations [7, 8]. In addition, the contraction produced by DMPP application (20 μM), which resulted from the excitation of nerve elements was reduced to the same extent (about 70%) by the treatment of atropine in both preparations. On the other hand, the pretreatment of physostigmine (50 nM) potentiated all of the responses to the three drugs (histamine 1 μM, DMPP 2 μM, ACh 0.2 μM) in the preparations both before and after hatching ($n=3$, Fig. 4B).

In connection with these findings on the effect of TTX, the results of this experiment suggest that in the preparations immediately before hatching, histamine at a high concentration was capable of causing a contraction mostly without the release of ACh by the excitation of cholinergic nerve elements in contrast to the preparations after hatching.

Effects of histamine antagonists on TTX-resistant responses induced by histamine

The effect of receptor antagonists on the TTX-resistant contraction induced by histamine was observed to clarify the receptors by which the TTX-resistant response was mediated.

Mepyramine (2 μM), a specific H_1-antagonist, almost completely blocked the response produced

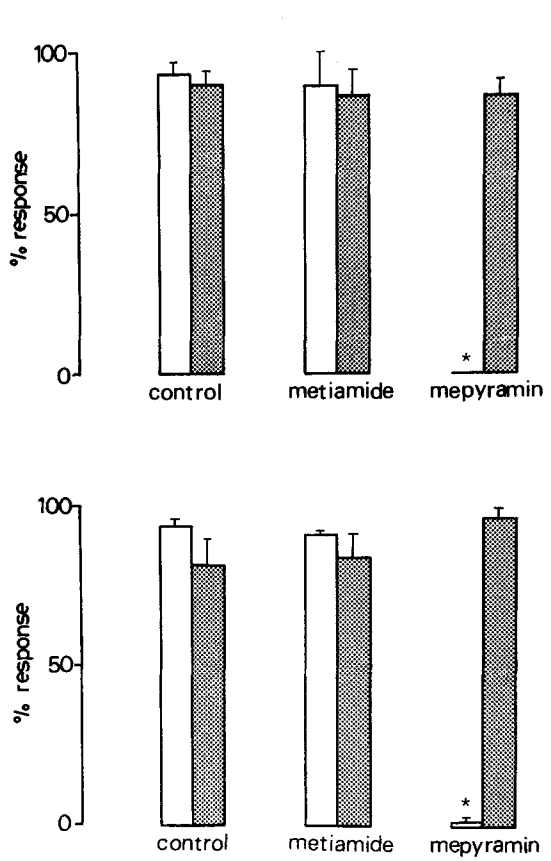

Figure 5
Effects of the anti-histaminergic drugs on the response to histamine (His). a: 19 days of incubation. b: 3 days after hatching. Open column: His response (20 μM). Dotted column: dimethyl phenyl piperazinium (DMPP) response (20 μM). The responses after the treatment of mepyramine (2 μM) and metiamide (20 μM) are shown. Ordinates indicate the contraction induced by His or DMPP was expressed as a percentage of the response of the tissue to 80 mM K^+. Each column is the mean amplitude of His or DMPP responses in three preparations and vertical bars show SEM. * $P<0.05$ compared to control response.

by histamine in the preparations both before and after hatching without an any effect on the DMPP (20 μM) response. On the other hand, metiamide (20 μM), an H_2-blocker, was not effective in the inhibition of the histamine responses in either before and after hatching (Fig. 5).

It is evident from these experiments that TTX-resistant responses of histamine on the chick oesophagus were mediated by histamine H_1-receptors, as TTX-sensitive response was so.

Discussion

In the present experiment, the development of histamine-induced response on the oesophagus isolated from the chick was examined between the period from 15 days of incubation to 10 days after hatching. A histamine-induced contraction was already possible in the oesophagus prepared from 15 days of incubation. On the basis of the pD_2 values calculated from dose-response curves, it seemed likely that the sensitivity of histamine on the oesophagus did not change during the period from 17 days of incubation to 5 days after hatching. The sensitivity, thereafter, declined progressively with age, in agreement with the earlier description [7] that the oesophagus from older birds (more than 2 weeks) were much less sensitive to histamine. The decrease in sensitivity to histamine in the postnatal periods was also reported in the smooth muscle of the ileum [6] and trachea [12] of the rabbit. On the other hand, the sensitivity (pD_2 value) to ACh did not change during the same periods examined. This observation was consistent with the many reports on the smooth muscles of the gastrointestinal tract of the guinea-pig [3], rabbit [13] and rat [4].

The mode of action of histamine observed in the period was, however, different in nature. In the oesophagus isolated from the chick after hatching, the contraction produced by histamine was greatly inhibited by TTX and atropine, in agreement with the earlier findings [7, 8] that the action of histamine on the chick oesophagus was predominantly indirect via cholinergic nerve elements. However, we observed here that the contractile response induced by histamine in the oesophagus immediately before the time of hatching was extremely insensitive to TTX, procaine and atropine. There are several possible mechanisms whereby the action of histamine was insensitive to these drugs. One possible explanation is that the sensitivity of nerve elements to TTX is very low in the oesophagus immediately before hatching. This possibility could be ruled out, however, since the response to nerve excitation induced by DMPP application or electrical nerve stimulation was similarly abolished in both the preparations before and after hatching. Furthermore, the degree, to which TTX caused a reduction of the histamine response did not change by an increase in the concentration of TTX. As another alternative, the increase in the sensitivity of ACh also may be involved in the difference in sensitivity to these agents before and after hatching. However, in the preparations during the period examined, the sensitivity of ACh remained constant. The most reasonable explanation, therefore, appears to be that most of the histamine response in the oesophagus immediately before hatching is not due to an indirect action via the excitation of cholinergic nerves as in the case after hatching, but to a direct action on the smooth muscle itself. On the other hand, the TTX-resistant responses to histamine were sensitive to mepyramine, but not metiamide, indicating that both pre- and post-hatching responses to histamine were elicited by the excitation of H_1-receptors on the smooth muscles or cholinergic nerves.

The resistance of histamine response to TTX was remarkable in the preparations immediately before hatching (specially between 19 and 20 days of incubation) in two different concentrations (2 and 20 μM) of histamine. However, the degree of the resistance was somewhat lower in a low concentration (2 μM) of histamine. Physostigmine could potentiated the response elicited by a low concentration (1 μM) of histamine in the oesophagus even before hatching. It is likely that the nerve-mediated action also partially contributes to the response induced by histamine especially in low concentrations. In the preparations immediately before hatching, neurogenic action of histamine may not be noticed, since histamine acts predominantly on the smooth muscle. After hatching, by way of contrast, the myogenic action may be reduced and the neurogenic action may become predominant. Such a change in the receptive site of drugs in relation to development has been observed in the effect of two neuropeptides, though the direction of the change was the reverse of our observations. On the basis of the sensitivity to TTX, Tonoue et al. [5] reported that the effect of the thyrotropine releasing hormone on the rat duodenum changed a neurogenic contraction to a myogenic relaxation during postnatal development. And, the relaxation induced by methionine enkephaline was also reported to change from neurogenic to myogenic during the course of postnatal development in the same preparation [14].

Data on factors by which the change in the receptive site occurs are not available. In the rat duodenum, Tonoue et al. [5] presumed that suckling may be related to the disappearance of the neurogenic contraction induced by the thyrotropin-releasing hormone. In the chick embryo, a transient subsensitivity to catecholamine during the period immediately before hatching was known to occur widely in the cardiovascular system [15]. Higgins and Pappano [16] have proposed a hormonal effect on the sensitivity of tissues to catecholamine as one of the factors.

The TTX-resistant response of the histamine, which seemed to be myogenic, increased in the terminal period of embryonic development and decreased drastically after hatching. This decline of myogenic histamine response was compatible with the observation that in the rabbit ileal smooth muscle, histamine-induced myogenic contraction, while remarkable during neonate, declined with age and became only slight even in a high concentration after the rabbit was 37 days old [6]. From the radioligand binding experiments, the qualitative and quantitative changes in the binding sites to drugs on the course of development was reported in several tissues involving chick heart [17], rat vas deferens [18], and rat pancreas [19]. So, the decline in the TTX-resistant response may be explained in terms of a reduction in number of histamine H_1-receptor on the smooth muscle. Indeed, it was reported in the rat pancreas [19] and chick expansor secundariorum muscle [20] that a number of muscarinic binding sites changed in correlation with responses to agonists. However, this is not the case for the vas deferens [18], and rabbit heart [21].

In conclusion, the present results have demonstrated that a contraction in the chick oesophagus can be already caused by histamine from 15 days of incubation. In the course of development, the mode of action of histamine appeared to change without great alteration of the sensitivity of the oesophagus to histamine. Myogenic receptivity of histamine was observed during the time immediately before hatching (specially between 19 and 20 days of incubation), then it declined and was replaced thereafter by neurogenic receptivity.

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References

[1] E. E. Daniel, *Pharmacology of adrenergic, cholinergic, and drugs acting on other receptors in gastrointestinal muscle.* In *Handbook of experimental pharmacology,* Vol. 59/II, (Ed. G. Bertaccini) pp. 249–322, Springer-Verlag, New York 1982.

[2] M. E. Parsons, *Histamine receptors in alimentary and genitro-urinary smooth muscle.* In *Pharmacology of Histamine Receptors,* (Eds. G. R. Ganellin and M. E. Parsons) pp. 323–350, Wright. PSG, London 1982.

[3] L. O. Boréus and D. M. McMurphy, *Ontogenetic development of cholinergic receptor function in guinea pig ileum,* Acta physiol. scand. *81,* 143–144 (1971).

[4] H. Miyazaki, A. Ohga and K. Saito, *Development of motor response to intramural nerve stimulation and to drugs in rat small intestine,* Br. J. Pharmac. *76,* 531–540 (1982).

[5] T. Tonoue, K. Furukawa and T. Nomoto, *Transition from neurogenic to myogenic receptivity for thyrotropin-releasing hormone (TRH) in the duodenum of the neonatal rat,* Endocrinology *108,* 723–725 (1981).

[6] J. H. Botting, *Sensitivity of neonatal rabbit ileum to histamine,* Br. J. Pharmac. *53,* 428–429 (1975).

[7] A. L. Bartlet and T. Hassan, *Some actions of histamine and 5-hydroxytryptamine on isolated chicken oesophagus,* Br. J. Pharmac. *32,* 156–163 (1968).

[8] J. O. Olubadewo and S. L. Bodhankar, *A study of histamine tachyphylaxis on isolated chick oesophagus,* Pharmac. Res. Commu. *14,* 551–565 (1982).

[9] H. Ohashi and A. Ohga, *Transmission of excitation from the parasympathetic nerve to the smooth muscle,* Nature *216,* 291–292 (1967).

[10] H. Ohashi, *An electrophysiological study of transmission from intramural excitatory nerves to the smooth muscle cells of the chicken oesophagus,* Jap. J. Pharmac. *21,* 585–596 (1971).

[11] W. D. M. Paton, *The response of guinea-pig ileum to electrical stimulation by coaxial electrodes,* J. Physiol. *127,* 40P–41P (1955).

[12] S. Hayashi and N. Toda, *Age-related alterations in the response of rabbit tracheal smooth muscle to agents,* J. Pharmac. exp. Ther. *214,* 675–681 (1980).

[13] M. D. Gershon and E. B. Thompson, *The maturation of neuromuscular function in a multiple 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 (1973).

[14] K. Furukawa, T. Tonoue and T. Nomoto, *Postnatal change in receptivity for methionine-enkephalin in rat duodenum: transition from neurogenic to myogenic receptivity,* Eur. J. Pharmac. *82,* 161–166 (1982).

[15] A. J. Pappano, *Ontogenetic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts,* Pharmac. Rev. *29,* 3–33 (1977).

[16] D. Higgins and A. J. Pappano, *Developmental changes in the sensitivity of the chick embryo ventricle to β-adrenergic agonist during adrenergic innervation,* Circ. Res. *48,* 245–253 (1981).

[17] M. M. Hosey and J. Z. Fields, *Quantitative and qualitative differences in muscarinic cholinergic receptors in embryonic and newborn chick hearts,* J. Biol. Chem. *256,* 6395–6399 (1981).

[18] H. Higuchi, K. Takeyasu, Y. Watanabe, S. Uchida and H. Yoshida, *Developmental changes in α-adrenergic and muscarinic receptor-mediated contractions of rat vas deferens,* Jap. J. Pharmac. *32,* 671–678 (1982).

[19] Y. Dumont, L. Larose, J. Morisset and G. G. Poirier, *Parallel maturation of the pancreatic secretory response to cholinergic stimulation and the muscarinic receptor population,* Br. J. Pharmac. *73,* 347–354 (1981).

[20] M. F. Crouch, C. P. Morris, T. B. Cheah and R. A. Rush, *Developmental loss of a smooth muscle muscarinic receptor population,* Brain Res. *232,* 212–215 (1982).

[21] H. W. Dalrymple, C. A. Hamilton and J. L. Reid, *The effect of age on peripheral α-adrenoceptors in vivo and in vitro in the rabbit,* Br. J. Pharmac. *77,* 322 p (1982).