Br. J. Pharmac. (1979), **65**, 257–260

THE APPEARANCE OF NORADRENALINE AND ADRENALINE AND THE DEVELOPMENTAL CHANGES IN THEIR CONCENTRATIONS IN THE GUT OF THE CHICK

S. KONAKA, H. OHASHI, T. OKADA & T. TAKEWAKI

Department of Pharmacology, Faculty of Agriculture, Gifu University, Gifu, Japan

1 The times of appearance and the concentrations of noradrenaline and adrenaline were determined fluorimetrically in the gut of the embryonic and developing chick.

2 In the duodenum and jejunum, noradrenaline was detected initially in embryos on the 12th day of incubation. The concentration continued to increase throughout the embryonic stage and attained a maximum within 3 days of hatching. Afterwards, it decreased to about one-third of the peak level. No adrenaline was detectable in most stages of development.

3 In the rectum, noradrenaline was detected in embryos on the 12th day of incubation; both noradrenaline and adrenaline were invariably found on the 15th day of incubation. The concentrations of both amines fluctuated after hatching, but the amount of adrenaline was always approximately 40% of the amount of noradrenaline, except in the adult.

Introduction

Proof that the neurotransmitter of adrenergic fibres is present in the tissues would serve as important evidence for demonstrating sympathetic innervation in organs. It has been generally accepted that, in mammals, the predominant catecholamine found in tissues receiving a sympathetic nerve supply is noradrenaline, although variable amounts of adrenaline may be present.

The assay of catecholamines in the brain, heart and vas deferens of the chicken demonstrated that both noradrenaline and adrenaline were present and the concentration ratio of adrenaline to noradrenaline was observed to be considerably higher than that in mammals (Sjöstrand, 1965; Callingham & Cass, 1966; DeSantis, Längsfeld, Lidmar & Löffelholz, 1975).

The present experiments were undertaken to determine the amount of noradrenaline and adrenaline in the chick gut, a tissue which is richly supplied by a number of adrenergic fibres derived from Remak's nerve and from the coeliac and mesenteric plexuses (Nolf, 1934a, b). In addition, an attempt has been made to describe the times at which catecholamines appeared and the changes in concentrations of noradrenaline and adrenaline that occurred with increasing age in the embryonic and developing chick.

Part of the present paper has been communicated to the Japanese Pharmacological Society (Takewaki, Ohashi & Okada, 1976).

Methods

Embryos and hatched chicks (White Leghorn) of various ages were used. After they were decapitated or stunned and bled, the intestines were removed. The isolated intestines were divided into the small intestine (i.e., duodenum, jejunum) and rectum in embryos, and into the duodenum, jejunum and rectum in hatched chicks. The intestinal segments were freed from mesentery, rinsed with ice-cold Tyrode solution, blotted on filter paper to remove blood and then weighed on a torsion balance. The segments from the same region were collected from 20 to 100 embryos and 1 to 6 hatched chicks, and were subjected to the following treatments: the tissues were homogenized in ice-cold 0.4 N perchloric acid and kept at −20°C overnight; the homogenate was centrifuged to remove precipitated protein.

The catecholamines of the tissue samples were adsorbed on alumina at pH 7.8 and then eluted with 0.2 N acetic acid as described by Chang (1964). Noradrenaline and adrenaline in the eluates were determined fluorimetrically by a modification of the method described by Anton & Sayre (1962; 1964).

Internal standards for noradrenaline and adrenaline were prepared as follows: the stock solutions of these amines were freshly diluted with 0.4 N perchloric acid to give solutions of at least four different concentrations embracing the expected range. The same procedure was followed for the solutions as for the re-

0007-1188/79/020257-04 $01.00

spective tissue samples. Tissue blanks were prepared by following the same procedures as for the sample except that the oxidizing agent was added after the exposure to 10 N acetic acid. Recoveries of noradrenaline and adrenaline varied from 70 to 80%. The values given are uncorrected for the percentage recovery.

The concentration of noradrenaline and adrenaline in samples was calculated by the use of simultaneous equations as described by Anton & Sayre (1962) and expressed as μg per g of tissue protein, since it is well known that the water content of tissues is higher in embryos than in hatched chicks. Tissue protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Results

Noradrenaline and adrenaline concentrations in the duodenum and jejunum

Noradrenaline and adrenaline concentrations in the small intestine of embryos are shown in Table 1. Noradrenaline was not found in samples from embryos up to the 10th day of incubation. In three out of four samples from embryos on the 12th day of incubation a small amount of noradrenaline was detected; this level increased markedly between the 12th and 15th day of incubation. Adrenaline was not detected in most samples from embryos. Two samples contained a very small amount of adrenaline which was very close to the lower limit (0.2 μg/g protein) of the sensitivity of the fluorimetric method used.

Figure 1 shows changes in the concentrations of

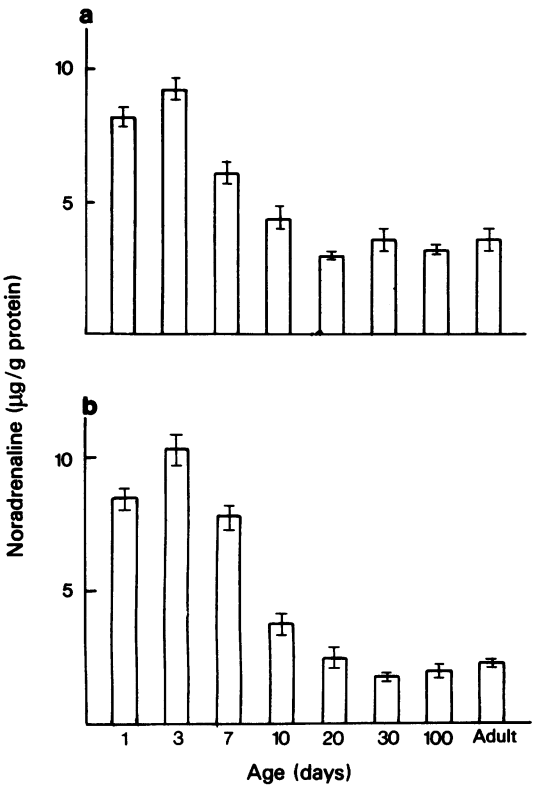

Figure 1 Changes in the concentration of noradrenaline with age in the duodenum (a) and jejunum (b) of the developing chick. Each column represents the mean of six to ten separate determinations; vertical lines indicate s.e. means.

Table 1 Noradrenaline and adrenaline concentrations in the small intestine and rectum of chick embryo measured fluorimetrically

Embryonic age (days)	*Noradrenaline (μg/g protein)*		*Adrenaline (μg/g protein)*	
	Small intestine	*Rectum*	*Small intestine*	*Rectum*
10	<0.2; <0.2; <0.2	—	<0.2; <0.2; <0.2	—
12	0.7; 0.9; 0.9; <0.2	2.2; 4.2; 4.0; 2.4; 2.8; 3.6	<0.2; <0.2; <0.2; <0.2	<0.2; <0.2; 0.8; 0.4; <0.2; <0.2
15	5.5; 4.1; 6.7; 5.5; 6.3	6.1; 7.0; 8.8; 5.3; 5.9; 4.6; 4.9	0.2; <0.2; <0.2; <0.2; <0.2	1.2; 0.8; 1.3; 1.9; 2.1; 1.6; 1.5
18	7.0; 7.5; 7.8; 6.4	7.5; 8.2; 5.6; 5.7; 8.8; 5.0; 6.1	0.5; <0.2; <0.2; <0.2	3.4; 2.6; 3.4; 3.7; 2.0; 3.7; 5.4

Each value represents a sample prepared from 20 to 100 embryos.

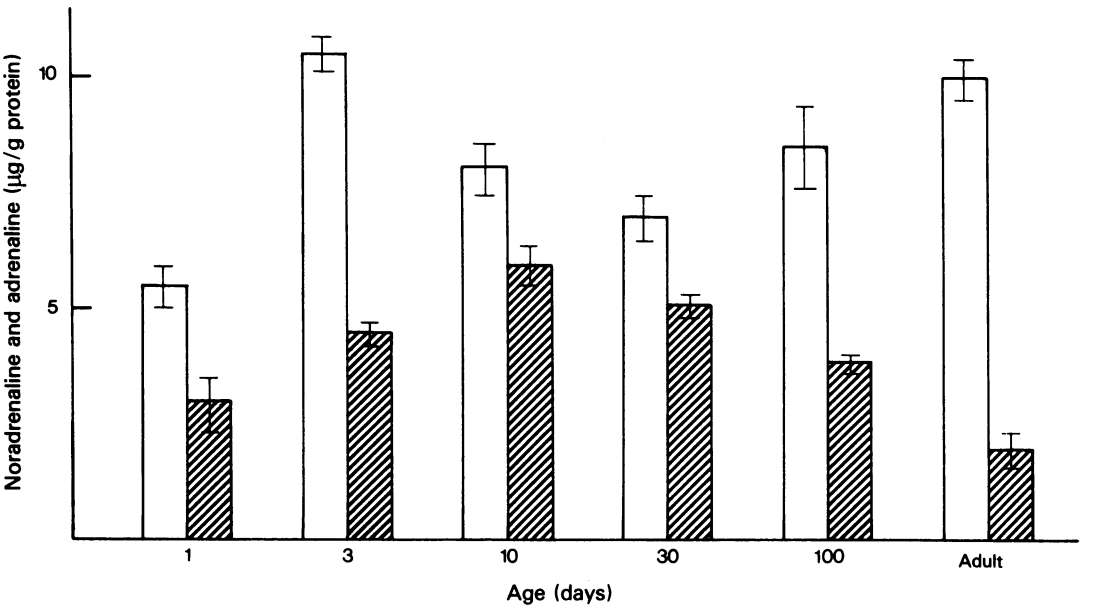

Figure 2 Concentrations of noradrenaline and adrenaline (μg/g protein) in the rectum of the chick at different ages after hatching. Each column represents the mean of five to ten separate determinations; vertical lines show s.e. means. Open columns: noradrenaline; hatched columns: adrenaline.

noradrenaline after hatching in chick duodenum and jejunum. The noradrenaline level increased to a maximum on the 3rd day and then gradually declined up to the 20th day by which time it had decreased to about one-third of the peak level. The decreased level was maintained during the remainder of life. Adrenaline was not detected in the duodenum. Similar changes in the noradrenaline concentration with age in the jejunum to those seen in the duodenum were observed.

Noradrenaline and adrenaline concentrations in the rectum

A detectable amount of noradrenaline was present in all six samples from embryos on the 12th day of incubation (the earliest embryonic stage examined), whereas adrenaline was detected in only two out of six samples (Table 1). Figure 2 shows changes in the noradrenaline and adrenaline levels at various days after hatching. The noradrenaline concentration attained a maximum on the 3rd day after hatching, declined and then rose gradually. The adrenaline concentration, on the other hand, increased more slowly after hatching, attaining a maximum on the 10th day and then progressively declining during the remaining period of life.

Discussion

These results indicate that the noradrenaline and adrenaline concentrations in the intestine of the chick vary greatly with the region and with age. The predominant catecholamine in the duodenum and jejunum is noradrenaline, with a barely detectable trace of adrenaline. In contrast, both adrenaline and noradrenaline are present in the rectum from the 15th day of incubation and subsequently at all ages after hatching. The adrenaline concentration is approximately 40% of the noradrenaline concentration in young chicks. In the rectum, noradrenaline appeared before adrenaline; the same order of appearance as in the embryonic heart (Ignarro & Shideman, 1968). No extraneuronal source of catecholamines in the chick gut has been demonstrated by fluorescent histochemistry (Everett & Mann, 1967). If a large proportion of catecholamines found in the chick gut is neuronal in origin, then adrenaline as well as noradrenaline could act as a neurotransmitter in the rectum since both are found here but only noradrenaline in other regions of the intestine. Adrenaline is liberated from the chicken rectum by stimulation of Remak's nerve (Takewaki, Ohashi & Okada, 1977a). Adrenaline also functions as a neurotransmitter in adrenergic fibres in the chicken heart (DeSantis *et al.*, 1975). Interestingly, there are apparent differences in the origin

of adrenergic fibres innervating the duodenum and jejunum from those supplying the rectum (Bennett & Malmfors, 1970). This could account for the above-mentioned regional differences in the catecholamine distribution.

The early appearance of catecholamines in the duodenum and rectum (Enemar, Falck & Hånkanson, 1965) and in the gizzard (Bennett & Cobb, 1969), of chick embryos has been observed with the fluorescent histochemical technique. The age at which they appeared was similar to that observed in the present experiments. If the level of either catecholamine in peripheral tissues reflects the extent of ingrowth of adrenergic fibres (Enemar *et al.*, 1965; Iversen, de Champlain, Glowinski & Axelrod, 1967), the present results suggest that catecholamine-containing fibres differentiate and develop to some extent during the third week of embryonic development. On the other hand, the appearance of neuroeffector transmission in the chick heart is delayed for several days after the ingrowth of autonomic nerves to the heart (Pappano & Löffelholz, 1974). If this is also the case in the gut, the adrenergic innervation of the intestine may become functional about the time of hatching, when the noradrenaline concentration approaches the peak level. This assumption is supported by the fact that well-developed varicose nerve plexuses are present in the gut of chicks soon after hatching (Bennett & Malmfors, 1970), and by findings which show that an adrenergic component is involved in relaxation of the rectum in response to nerve stimulation (Takewaki, Ohashi & Okada, 1977b).

The concentrations of noradrenaline in the small intestine decreased in the adult to less than 50% of the peak level at the 3rd day. This suggests that there may be a decrease in the number of adrenergic fibres per g protein and/or a decrease in the concentration of noradrenaline in each fibre. This problem remains to be solved.

We are grateful to Dr T. B. Bolton for his helpful criticism of the manuscript.

References

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

ANTON, A.H. & SAYRE, D.F. (1964). The distribution of dopamine and DOPA in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.*, **145**, 326–336.

BENNETT, T. & COBB, J.L.S. (1969). Studies on the avian gizzard: The development of the gizzard and its innervation. *Z. Zellforsch.*, **98**, 599–621.

BENNETT, T. & MALMFORS, T. (1970). The adrenergic nervous system of the domestic fowl (*Gallus domesticus* (L.)). *Z. Zellforsch.*, **106**, 22–50.

CALLINGHAM, B.A. & CASS, R. (1966). Catecholamines in the chick. In *Physiology of the Domestic Fowl*. ed. Horton-Smith, C. & Amoroso, E.C. Edinburgh: Oliver & Boyd Ltd.

CHANG C.C. (1964). A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmac.*, **3**, 643–649.

DESANTIS, V.P., LÄNGSFELD, W., LIDMAR, R. & LÖFFELHOLZ, K. (1975). Evidence for noradrenaline and adrenaline as sympathetic transmitter in the chicken. *Br. J. Pharmac.*, **55**, 343–350.

ENEMAR, A., FALCK, B. &. HÅNKANSON, R. (1965). Observations on the appearance of norepinephrine in the sympathetic nervous system of the chick embryo. *Develop. Biol.*, **11**, 268–283.

EVERETT, S.D. & MANN, S.P. (1967). Catecholamine release by histamine from the isolated intestine of the chick. *Eur. J. Pharmac.*, **1**, 310–320.

IGNARRO, L.J. & SHIDEMAN, F.E. (1968). Appearance and concentrations of catecholamines and their biosynthesis in the embryonic and developing chick. *J. Pharmac. exp. Ther.*, **159**, 38–48.

IVERSEN, L.L., DE CHAMPLAIN, J., GLOWINSKI, J. & AXELROD, J. (1967). Uptake, storage and metabolism of norepinephrine in tissues of the developing rat. *J. Pharmac. exp. Ther.*, **157**, 509–516.

LOWRY, O.H., ROSEBROUGH, N.R., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

NOLF, P. (1934a). Les nerfs extrinsèques de l'intestin chez l'oiseau. II. Le nerf coeliaques et mésentériques. *Archs int. Physiol.*, **39**, 156–226.

NOLF, P. (1934b). Les nerfs extrinsèques de l'intestin chez l'oiseau. III. Le nerf de Remak. *Archs int. Physiol.*, **39**, 227–256.

PAPPANO, A.L. & LÖFFELHOLZ, K. (1974). Ontogenesis of adrenergic and cholinergic neuroeffector transmission in chick embryo heart. *J. Pharmac. exp. Ther.*, **191**, 468–478.

SJÖSTRAND, N.O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta physiol. scand.*, **65**, Suppl. 257.

TAKEWAKI, T., OHASHI, H. & OKADA, T. (1976). Ontogenetic changes in sympathetic innervation of the gastrointestinal tract in the chicken. *Jap. J. Pharmac.*, **26**, Suppl. 162.

TAKEWAKI, T., OHASHI, H. & OKADA, T. (1977a). Evidence for that epinephrine is the transmitter of adrenergic fibers in Remak's nerve extending to the chicken rectum. *Jap. J. Pharmac.*, **27**, Suppl. 113.

TAKEWAKI, T., OHASHI, H. & OKADA, T. (1977b). Non-cholinergic and non-adrenergic mechanisms in the contraction and relaxation of the chicken rectum. *Jap. J. Pharmac.*, **27**, 105–115.

(*Received May 31, 1978. Revised August 1, 1978.*)