Br. J. Pharmac. (1978), **64**, 247–251.

SOME OBSERVATIONS ON THE DEVELOPMENT OF ADRENERGIC INNERVATION IN RABBIT INTESTINE

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1 Stimulation of periarterial nerves to the ileum of 1 to 12 day old rabbits with supramaximal voltages and frequencies of 1, 2, 5, 10 and 20 Hz with square wave pulses of 2–5 ms duration for 30–40 s produced responses that were initially contractor. In the course of the first week, the responses changed from motor to inhibitory, the change occurring first at the highest rates of stimulation. By the 7th day of life, almost all responses were inhibitory.

2 The motor responses were potentiated by physostigmine and blocked by hyoscine suggesting that they were mediated by acetylcholine.

3 In preparations from rabbits older than 3 days, motor responses could be converted to inhibitory ones by prior exposure to noradrenaline (NA, 1 μg/ml) for 20 min. This procedure also significantly increased the responses which were already inhibitory.

4 The ability of the ileum to take up NA increased with age. This uptake was blocked by cocaine.

5 The following explanations are possible: (a) changeover from cholinergic to adrenergic transmission in sympathetic fibres; (b) existence of 'parasympathetic' splanchnic nerves or a permanent cholinergic 'sympathetic' component of splanchnic nerves and (c) temporal delay in the development of adrenergic nerves (compared with cholinergic nerves) in the intestine.

Introduction

In teleost fishes and chickens the splanchnic innervation of the intestine is motor (Young, 1936; Burnstock, 1958; Burn, 1968b). The motor effect is cholinergically mediated (Burnstock, 1958; Burn, 1968b). In the adult rabbit, sympathetic nerve stimulation causes relaxation of the intestine which is due to the release of noradrenaline (NA). Burn (1968a) argued 'if it is true that there has been a change in sympathetic function in the course of evolution and that the motor effect on the intestine seen in the trout and the chicken has become changed to an inhibitory action in the adult rabbit then since ontogeny recapitulates phylogeny, some sign of this should be seen in the new-born rabbit'. He did indeed observe that stimulation of sympathetic innervation of new-born rabbit intestine elicited a motor effect which was cholinergic in nature, while slightly older rabbits exhibited an adrenergic mediated inhibitory response (Burn, 1968b). In the present study an attempt is made to determine the time course of the development of functional adrenergic innervation in the intestine of the new-born rabbit. The ability of the intestine to accumulate NA is also investigated.

Methods

All experiments were performed on pieces of distal ileum obtained from albino rabbits of either sex and of various ages.

Pharmacological experiments

Pieces of ileum (approx. 3 cm long) together with the adjacent mesentery and the arteries running in it were obtained from 1 to 12 day old rabbits and prepared according to the method of Finkleman (1930). The preparations were set up in a 35 ml organ bath containing McEwen's (1956) solution of the following composition (g/l of distilled water): NaCl 7.6, KCl 0.2, $CaCl_2$ 0.24, NaH_2PO_4 0.143, $NaHCO_3$ 2.0, dextrose 2 and sucrose 4.5. The solution was maintained

at 35°C ± 1°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The movement of the ileum was recorded by an isotonic frontal writing lever (magnification: 8 fold and load: 300 mg) on a smoked drum. The periarterial nerves were stimulated by a pair of platinum ring electrodes (Burn & Rand, 1960) at supramaximal voltages and frequencies of 1, 2, 5, 10 and 20 Hz with square wave pulses of 2.5 ms duration for 30–40 s every 5 min. In all experiments, after control responses had been elicited at different frequencies the preparations were exposed to NA (1 μg/ml) for 20 min, washed and re-stimulated.

Biochemical experiments

The lumen of a piece of ileum (approx 2 cm in length) was cut open and washed with chilled McEwen's solution. It was then divided into two equal pieces which were used for studying the accumulation of exogenously added NA in the presence and absence of cocaine. One piece was incubated at 35°C in 35 ml of McEwen's solution containing disodium edetate (10 μg/ml) and bubbled with 95% O_2 and 5% CO_2 for 10 min. The other piece was similarly incubated in medium which additionally contained cocaine (3.4 μg/ml). Ten min later, the cocaine-medium was replaced by one containing both cocaine (3.4 μg/ml) and NA (1 μg/ml). The medium in which the other piece was incubating was replaced by one containing only NA (1 μg/ml). After incubating for an additional 20 min, each tissue was washed three times with normal McEwen's solution and dried by gently pressing between filter paper. Both the pieces were weighed. The NA content of the tissue was estimated by the method of Anton & Sayre (1962). The fluorescence was read on a Turner Fluorometer (Model 110). The NA content of the medium (M) at the end of the incubation was not estimated but was derived by subtraction of the amount accumulated in the tissue (T) from the total amount (35 μg) added to the medium at the start of the experiment. The T/M ratio was used as an index of the accumulation in the tissue of exogenously added NA. In separate experiments, the recovery of NA added to alumina was found to be 70%. The fluorimetric readings obtained were, therefore, corrected for 70% recovery. The values of NA are expressed as μg/g of the wet weight of the tissue.

Drugs

The following drugs were used: cocaine hydrochloride (May & Baker); hyoscine hydrobromide (E. Merck); (−)-noradrenaline (NA) (Rhone Poulenc) and physostigmine salicylate (E. Merck). A stock solution of NA was prepared in 0.01 N HCl containing 0.05% sodium metabisulphite. Dilutions of the stock solution and solutions of other drugs were made in McEwen's medium before the start of the experiments. Concentrations of NA are in terms of its base and those of other drugs are in terms of their respective salts.

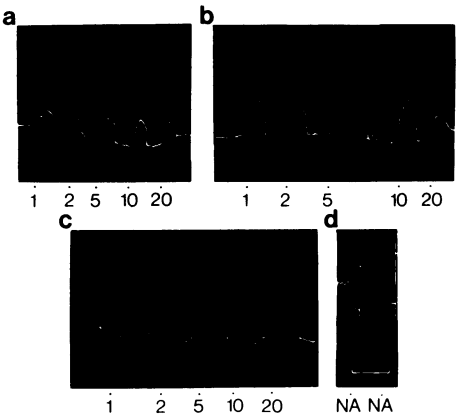

Figure 1 Finkleman preparations (in McEwen's solution) from 2 day old rabbits. Responses to periarterial nerve stimulation at different frequencies are indicated in Hz below each panel. (a) Shows control responses. Between (a) and (b) the preparations was exposed to physostigmine (0.1 μg/ml) for 5 min and washed. (b) Shows responses to nerve stimulation in the presence of physostigmine. Between (b) and (c) the preparation was exposed to hyoscine (0.1 μg/ml) for 5 min and washed. (c) Shows responses to nerve stimulation and (d) responses to noradrenaline (NA: 200 ng/ml, first response and 500 ng/ml, second response) of the intestine from a different 2 day old rabbit. Time mark, 1 min.

Results

Responses of isolated preparations from young rabbits to periarterial nerve stimulation: modification by noradrenaline

Ileum preparations from 1 to 3 day old rabbits exhibited contractor responses to nerve stimulation at all frequencies (Figure 1a); 200 to 500 ng/ml of NA failed to elicit any relaxation in preparations from 1 day old rabbits but did so in those from 2 to 3 day old rabbits (Figure 1d). In preparations from 2 day old rabbits, after eliciting control responses at different frequencies, the preparations were re-stimulated in the presence of physostigmine (0.1 μg/ml) or hyoscine (0.1 μg/ml). Motor responses were potentiated by physostigmine (Figure 1b), particularly at lower frequencies and were completely blocked by hyoscine (Figure 1c).

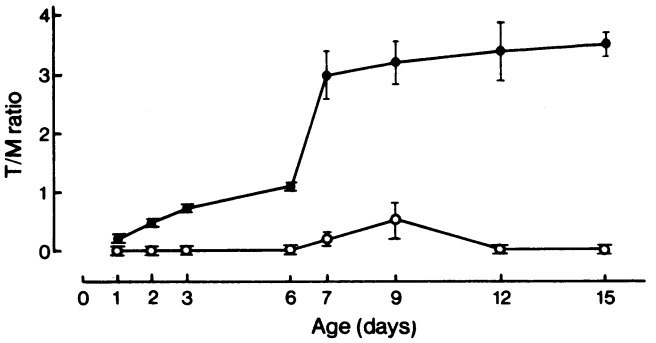

Figure 2 Changes in the amount of noradrenaline (NA) accumulated by rabbit ileum with increasing age. The ordinate scale depicts accumulation (expressed as T/M ratios) of NA (1 μg/ml) by rabbit ileum while the abscissa scale depicts the age of the animals. (●) Control T/M ratios; (○) ratios obtained in the presence of cocaine (3.4 μg/ml). Each point is the mean of 4 to 6 observations. Vertical lines indicate s.e. mean.

Thus in the presence of physostigmine, the motor responses were 275 ± 12.5%, 172 ± 16.2%, 154 ± 22.9%, 186 ± 29.1% and 134 ± 22.5% (mean ± s.e. mean, $n = 4$) of the control responses at 1 Hz, 2 Hz, 5 Hz, 10 Hz and 20 Mz respectively.

With increasing age the responses of rabbit ileum preparations to nerve stimulation changed from motor to inhibitory, the change occurring first at the highest frequencies of stimulation (10 and 20 Hz). By the 7th day of life almost all responses were inhibitory (Table 1). Subsequent immersion of these preparations in a solution containing 1 μg/ml of NA for 20 min had one of two effects: (a) the motor responses were converted to inhibitory responses; (b) in preparations with an inhibitory response, this response was significantly increased (Table 1). In rabbits younger than 4 days, NA eliminated responses to nerve stimulation completely (Table 1).

Accumulation of exogenously added noradrenaline by ileum of rabbits of various age groups

The accumulation of NA by rabbit ileum rose steadily in the first 6 days (0.2 to 1.0 μg/g wet wt. tissue) but there was a sudden increase on the 7th day (3.0 μg/g) followed again by a steady increase till the 15th day (3.5 μg/g). The accumulation was reduced to zero, or thereabout in the presence of cocaine (Figure 2).

Table 1 Effect of exogenously added noradrenaline (NA) on responses to periarterial nerve stimulation of the isolated ileum preparations from young rabbits

Age	*Group*[b]	*Responses*[a] *(mm)* 1 Hz	2 Hz	5 Hz	10 Hz	20 Hz
2 days (6)	C	10.0 ± 4.0	14.3 ± 5.3	16.6 ± 5.1	21.0 ± 5.1	26.6 ± 5.8
	NA	0	0	0	0	0
4 days (3)	C	13.0 ± 6.0	15.3 ± 4.9	3.3 ± 9.8	−11.6 ± 1.7	−18.0 ± 5.7
	NA	0	−5.0 ± 0.6[c]	−11.0 ± 1.0	−18.0 ± 2.1	−20.0 ± 2.7
7 days (4)	C	1.0 ± 3.4	−5.0 ± 2.8	−14.5 ± 1.4	−33.0 ± 3.4	−37.5 ± 6.7
	NA	0	−9.7 ± 3.9	−32.2 ± 4.2[c]	−43.2 ± 5.9	−47.0 ± 6.5
11 days (3)	C	−5.0 ± 1.5	−7.2 ± 2.0	−11.3 ± 1.0	−28.6 ± 3.2	−31.0 ± 3.6
	NA	−8.1 ± 1.1	11.6 ± 1.3	−16.0 ± 1.0[c]	−35.0 ± 3.5	−40.0 ± 1.5

Figures in parentheses indicate the number of observations; values are mean ± s.e. mean.
[a] Lack of response is indicated by zero: inhibitory response is indicated by the minus sign preceding it; all other responses were contractor. [b] Group C—controls; Group NA—preparations exposed to NA (1 μg/ml) for 20 min. [c] $P < 0.05$ (compared to control response at corresponding frequency).

Discussion

Stimulation of periarterial nerves to the intestine of 1 to 3 day old rabbits elicited motor responses at all frequencies. The responses were potentiated by physostigmine and blocked by hyoscine suggesting mediation by acetylcholine. These findings are in accord with those of Burn (1968b). Stimulation of the lumbar sympathetic chain in 1 to 14 day old dogs produces a fall in perfusion pressure in the perfused hind leg despite the fact that the blood pressure is very low; however, adrenaline causes a rise in perfusion pressure (Boatman, Shaffer, Dixon & Brody, 1965). In the present experiments, NA failed to relax the intestine of 1 day old rabbits but did so in preparations from 2 to 12 day old rabbits. Thus, it appears that in the intestine of the young rabbit as in the case of the hind leg of young dog, stimulation of sympathetic fibres elicits cholinergic responses.

In preparations from 1 to 3 day old rabbits, prior exposure to NA extinguished motor responses to nerve stimulation. It is possible that nerve stimulation subsequent to exposure to NA released both acetylcholine and NA in amounts just sufficient to balance their opposing effects. That the intestinal nerves did possess some ability to accumulate exogenously added NA even in this period of life is amply demonstrated by the results shown in Figure 2. The lack of motor responses after exposure to NA might also be explained by the interaction of NA with α-adrenoceptors on the periarterial nerves, resulting in a block of the release of acetylcholine (Kosterlitz, Lydon & Watt, 1970; Knoll & Vizi, 1971).

With increasing age, lower frequencies of stimulation could elicit relaxation, and exposure to NA converted motor responses to inhibitory ones. Possibly, the amount of noradrenaline available for release by stimulation of the periarterial nerves of rabbit ileum may increase with age.

The accumulation by the intestine of exogenously added NA (1 μg/ml) was found to increase gradually from day 1 to day 6 of life. On the 7th day, there was a sudden increase in the ability of the intestine to accumulate NA. The accumulation was blocked by cocaine suggesting that it may have been neuronal uptake (uptake$_1$). However, uptake$_2$ into smooth muscle cannot be ruled out since the concentration of NA used was high. It is possible that the neuronal uptake and or binding mechanisms are poorly developed in the first 6 days of life. Although the T/M ratios of less than unity obtained for 1 to 6 day old rabbits would indicate passive diffusion of NA (Ignarro & Shideman, 1968), the accumulation was blocked by cocaine. This suggests that the uptake mechanism may be developed but that the ability of the nerves to store NA may be poor. This is corroborated by the failure of NA to convert motor responses to inhibitory ones. The small amount of NA retained may have been responsible for the block of the motor responses.

The ability of the intestine from rabbits older than 7 days to accumulate NA was indicated by T/M ratios of 3.0 and above and would suggest that the uptake was fully developed from the 7th day onwards. Further, the relaxant responses to periarterial nerve stimulation were obtainable at all frequencies implying a functional adrenergic neurone, a conclusion in accord with the full development of innervation in the intestine of 7 day old rabbits (Burn, 1968b).

One explanation for the findings as suggested by Burn (1968b) may be that in the first week of life there is a change over from cholinergic to adrenergic transmission in sympathetic fibres. Other possibilities are (i) there are 'parasympathetic' splanchnic nerves; (ii) the adrenergic nerves may grow into the intestine at a later time than the cholinergic nerves with a few adrenergic nerves present at birth; (iii) there is a permanant cholinergic 'sympathetic' component of the splanchnic nerve which is eventually overshadowed by developing adrenergic sympathetic fibres. Histochemical examination of adrenergic fibres in the periarterial nerves, the myenteric plexus and the nerve fibres in the muscle coats of rabbits of different ages may be helpful in resolving the different possibilities.

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(Received February 6, 1978.
Revised April 20, 1978.)