Noradrenaline concentration and turnover in different regions of the gastrointestinal tract of the rat: an approach to the evaluation of sympathetic activity in the gut

H. L. TAUBIN[1], B. DJAHANGUIRI, AND L. LANDSBERG with the statistical analysis by E. LERNER

From the Catecholamine Laboratory, West Haven Veterans Hospital, West Haven, Connecticut, and the Departments of Medicine (Section of Gastroenterology) and Pharmacology, Yale University School of Medicine

SUMMARY   The endogenous noradrenaline concentration, fractional turnover, half-life, and turnover rate of tritiated (^{3}H) noradrenaline were determined in the oesophagus, non-glandular and glandular portions of the stomach, duodenum, jejunum, ileum, and colon of the rat. The highest concentration of endogenous noradrenaline was present in the duodenum and colon. The ^{3}H-noradrenaline fractional turnover rates and half-lives were significantly greater in the small and large intestines as compared with the oesophagus and stomach. The noradrenaline turnover rate, which is an estimate of the level of sympathetic activity, was greatest in the colon and duodenum. This method of assessing sympathetic activity in various tissues by direct measurement of the noradrenaline turnover rate may be applied to the study of the adrenergic nervous system in the physiology and pathophysiology of the gastrointestinal tract.

The influence of the sympathetic nervous system on gastrointestinal function has long been the subject of investigation. Information about the sympathetic innervation of the gastrointestinal tract has accumulated gradually from diverse techniques such as nerve sectioning and nerve stimulation, the application of autonomic agonists and antagonists, and the preparation of histochemical stains specific for neurotransmitters. Nevertheless, the role played by the adrenergic nervous system in normal physiological mechanisms of the gut is poorly understood. Even more obscure is the contribution of adrenergic factors to the pathogenesis of gastrointestinal disease.

Noradrenaline is the adrenergic neurotransmitter. All the noradrenaline in periphral tissues is located in the sympathetic nerve endings. The concentration of noradrenaline in a particular tissue has a characteristic level which reflects the extent of sympathetic innervation. Noradrenaline, which is released in

response to sympathetic nerve impulses, is rapidly restored by uptake again and biosynthesis. Thus, alterations in sympathetic activity do not change the level of the neurotransmitter and more sophisticated methods must be used to assess sympathetic activity. Noradrenaline turnover may be measured by the use of tracer doses of tritiated noradrenaline (^{3}H) (Montanari, Costa, Beaven, and Brodie, 1963; Neff, Tozer, Hammer, Costa and Brodie, 1968; Costa, Boulin, Hammer, Vogel, and Brodie 1966; Landsberg and Axelrod, 1968). Since the axonal membrane of the sympathetic nerve actively takes up noradrenaline from the circulation, tracer ^{3}H-noradrenaline is taken up by the nerve ending after intravenous injection. The tracer rapidly equilibrates with the endogenous noradrenaline stores and serves as a valid marker of noradrenaline release (Costa *et al*, 1966). Thus, the turnover rate of noradrenaline for a particular tissue may be calculated from the rate of decline of specific activity over time (Costa *et al*, 1966; Neff *et al*, 1968). Changes in impulse traffic in the sympathetic nerves result in corresponding changes in biosynthesis and release of noradrenaline (Sedvall, Weise, and Kopin,

[1]Please address reprint requests to Howard L. Taubin, M.D., Section of Gastroenterology, Yale University School of Medicine, New Haven, Conn. 06510.

1968). These changes are reflected by alterations in the turnover rate of noradrenaline. The turnover rate, therefore, reflects the degree of sympathetic activity. The present study examines the endogenous concentration and the turnover rate of noradrenaline in various portions of the gastrointestinal tract of the rat. It also provides the methodology required for evaluating the role of the sympathetic nervous system in normal physiological control and pathological disorders of the gastrointestinal tract.

Materials and Methods

Female Sprague-Dawley rats weighing 170 g were purchased from the Charles River Company, Wilmington, Mass, and used in these experiments. D,L-noradrenaline-7-^{3}H (13c/mmole) was obtained from New England Nuclear Corporation. It was purified before use by adsorption onto an alumina column at pH 8·6 and eluted with 0·2N acetic acid. After appropriate dilution with isotonic saline, the tracer was administered to unanaesthetized animals via the tail vein, in a dosage of 150 μc/kg (325 ng noradrenaline per rat). After administration of the tracer the animals were divided into five groups of five animals each and killed by a blow at the base of the skull at one and a half, four, eight, 14, and 24 hours. The organs were rapidly removed, rinsed with cold water, blotted dry, weighed, and quickly frozen on dry ice. The hearts and various portions of the gastrointestinal tract were removed as illustrated in Figure 1. The portions of the gut studied included the oesophagus (distal 4 cm); the squamous or non-glandular portions of the stomach, which was carefully dissected free of the glandular portion; the glandular portion of the stomach; the duodenum

(the first 10 cm of the small bowel distal to the pylorus); the jejunum (a 10 cm portion beginning 10 cm distal to the duodenum); the ileum (distal 10 cm of the small bowel); and the colon (10 cm following the caecum). The specimens of oesophagus were pooled at each time period because of their small size. The other specimens were processed individually.

At the time of analysis (within one week) the specimens were homogenized in ice-cold 0·4N perchloric acid and the precipitated protein was removed by centrifugation. Noradrenaline was isolated from the perchloric acid extract by adsorption onto alumina at pH 8·6 (Anton and Sayne, 1962; de Champlain, Krakoff, and Axelrod, 1967) and eluted with 0·2N acetic acid. Aliquots of the alumina eluate were counted for ^{3}H-noradrenaline by liquid scintillation spectrometry in a Packard Tri-Carb scintillation counter at an efficiency of about 18% for tritium. Endogenous noradrenaline was determined on the alumina eluate spectrofluorometrically (Aminco Bowman spectrofluorometer) by the trihydroxyindole method of von Euler and Lishajko (von Euler and Lashajko, 1961). Values were corrected for a recovery of 80 to 90%, as determined for each experiment.

Rate constants for the disappearance of ^{3}H-noradrenaline were determined as follows: the specific activity of noradrenaline (millimicrocuries per microgram) was plotted semilogarithmically against time according to the method of least squares. The specific activity for each tissue was converted into a logarithm and linear least square equations were plotted on semilogarithmic charts with the standard errors about the mean at each time interval. In this manner, a slope with the standard
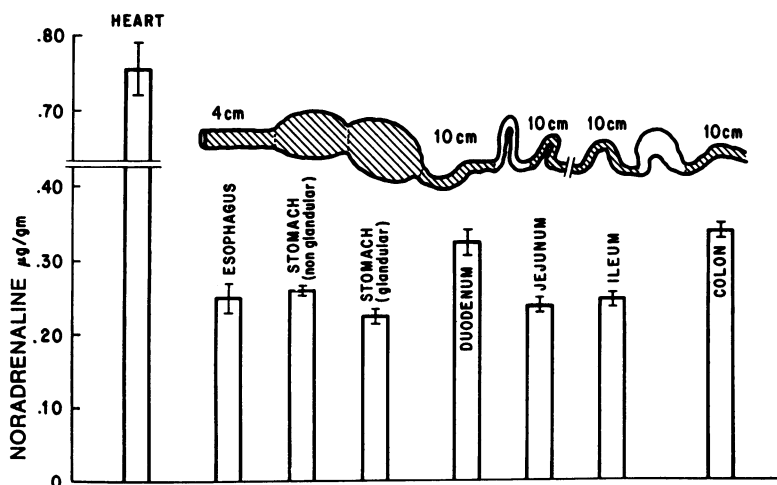

Fig. 1 *Endogenous noradrenaline concentrations in different regions of the gut (mean ± SEM). The concentration was significantly different in the colon and duodenum (P < 0·005) from that in all other regions. The concentration was significantly lower (P < 0·01) in the glandular portion of the stomach than in the non-glandular portion.*

error about the slope was generated. The slope, or rate constant of decline, represents the fractional turnover rate of noradrenaline or the percentage of the pool declining per hour. The half-life was calculated from the equation $t_\frac{1}{2} = 0.693$ divided by the slope (Costa et al, 1966). The turnover time for the entire pool was considered equal to the endogenous concentration divided by the turnover rate. The turnover rate was calculated from the product of the slope and the endogenous noradrenaline concentration (Costa et al, 1966). The 95% confidence intervals were determined for the turnover rates as follows: a confidence interval of one standard error about the slope and the endogenous concentration was established. The lower limits of the slope and the lower limits of the endogenous intervals were multiplied to obtain the lower 95% confidence limits for the mean turnover rates. In a similar manner, 95% confidence limits were determined for the upper intervals. Comparison of the endogenous concentrations, slopes, and half-lives were made by the Student t test (Armitage, 1971).

Results

ENDOGENOUS NORADRENALINE CONTENT IN DIFFERENT PORTIONS OF THE GUT (FIG. 1, TABLE)
The noradrenaline concentration in the gastrointestinal tract varied from 0.226 ± 0.010 to 0.340 ± 0.012 $\mu g/g$ of tissue. The highest concentrations were present in colon and duodenum. The concentration in these two tissues differed significantly from that in the other portions of the gastrointestinal tract. The content of the heart was 0.755 ± 0.034 $\mu g/g$ of tissue.

FRACTIONAL TURNOVER RATE AND NORADRENALINE HALF-LIFE IN VARIOUS PORTIONS OF THE GUT (FIGURES 2,3; TABLE)
The fractional turnover rate and half-life of nor-

adrenaline, which are dependent primarily on impulse traffic in the sympathetic nerves, were similar in the heart (k = 0.068, $t_\frac{1}{2}$ = 10.2 hr), oesophagus (k = 0.044, $t_\frac{1}{2}$ = 15.7 hr), non-glandular stomach (k = 0.058, $t_\frac{1}{2}$ = 11.9 hr), and glandular stomach (k = 0.064, $t_\frac{1}{2}$ = 10.8 hr) (Fig. 2). They differed significantly from the fractional turnover rate and half-life in the duodenum (k = 0.093, $t_\frac{1}{2}$ = 7.3 hr), jejunum (k = 0.090, $t_\frac{1}{2}$ = 7.7 hr), ileum (k = 0.093, $t_\frac{1}{2}$ = 7.4 hr), and colon (k = 0.102 $t_\frac{1}{2}$ = 6.8 hr) (Fig. 3).

TURNOVER RATE OF NORADRENALINE IN VARIOUS PORTIONS OF THE GUT (FIGURE 4, TABLE)
The turnover rate, which reflects the amount of noradrenaline released per gram of tissue per unit of time, is dependent on both the rate constant of decline and the endogenous noradrenaline concentration for a particular tissue. Thus, the turnover rate is an estimate of the level of sympathetic activity in a particular organ in a given time. The colon (0.035 $\mu g/g/hr$) and duodenum (0.031 $\mu g/g/hr$) were found to have the highest turnover rate. Next were ileum (0.023 $\mu g/g/hr$) and jejunum (0.022 $\mu g/g/hr$) followed by glandular stomach (0.014 $\mu g/g/hr$), non-glandular stomach (0.015 $\mu g/g/hr$), and oesophagus (0.011 $\mu g/g/hr$).

Discussion

Noradrenaline is known to be the neurotransmitter at postganglionic adrenergic synapses (von Euler, 1966). The methods utilized here in the study of the turnover rate in the gastrointestinal tract have been successfully applied to the study of sympathetic activity in the heart (Landsberg and Axelrod, 1968). The values determined for cardiac noradrenaline turnover in these experiments agree well with published results (Costa et al, 1966). The rates of decline of specific activity in the tissues of the

Tissue	Endogenous Noradrenaline ($\mu g/g$)	Fractional Turnover[1] (% hr)	Turnover Time[2] (hr)	Half-life $0.693/k$ hr (hr)	Turnover Rate[3] ($\mu g/g \times k$) ($\mu g/g/hr$)
Heart	0.755 ± 0.034	6.8 ± 0.5	14.7 ± 1.1	10.2 ± 0.8	0.051
Oesophagus[4]	0.251 ± 0.023	4.4 ± 1.5	22.7 ± 8.8	15.7 ± 6.1	0.011
Non-glandular stomach	0.261 ± 0.007	5.8 ± 0.7	17.2 ± 2.1	11.9 ± 1.5	0.015
Glandular stomach	0.226 ± 0.010	6.4 ± 0.6	15.6 ± 1.5	10.8 ± 1.0	0.014
Duodenum	0.326 ± 0.018	9.5 ± 0.7	10.5 ± 0.8	7.3 ± 0.6	0.031
Jejunum	0.241 ± 0.010	9.0 ± 0.6	11.1 ± 0.8	7.7 ± 0.6	0.022
Ileum	0.248 ± 0.009	9.3 ± 0.7	10.7 ± 0.8	7.4 ± 0.6	0.023
Colon	0.340 ± 0.012	10.2 ± 0.8	9.8 ± 0.8	6.8 ± 0.6	0.035

Table   *Kinetic analysis of noradrenaline turnover*

[1]Fractional turnover rate (k) represents percentage of noradrenaline pool lost per unit time (k × 100%).
[2]Turnover time, 1/k or endogenous noradrenaline/turnover rate, represents time required for turnover of entire pool.
[3]Turnover rate represents amount of noradrenaline released per gram of tissue per unit time. The 95% confidence intervals are found in Figure 4.
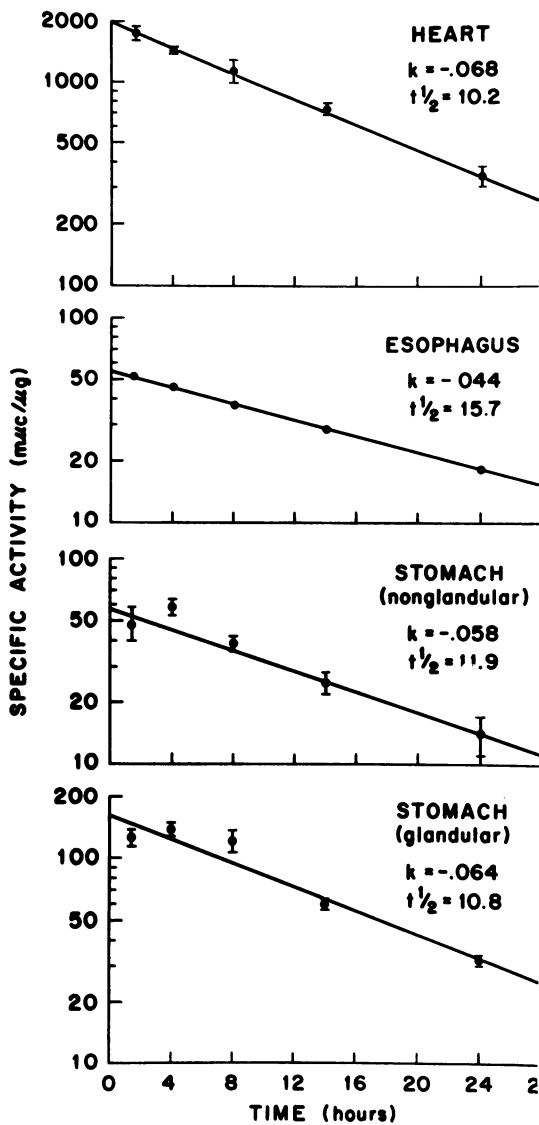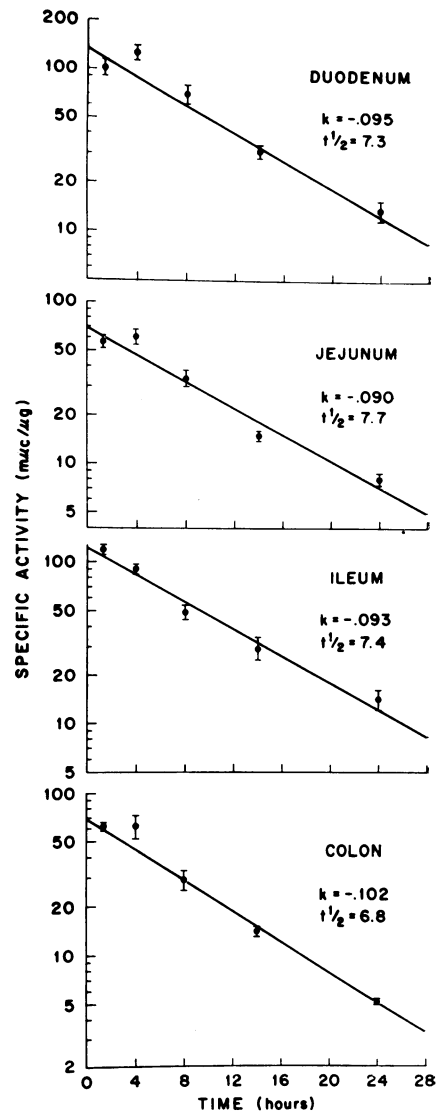[4]Values represent results of pooled specimens in each time period.

Fig. 2

Fig. 3

Fig. 2 *Fractional turnover rate (k) and noradrenaline half-life (t$_{\frac{1}{2}}$) in the heart, oesophagus, non-glandular, and glandular stomach. Each point represents the mean specific activity (millimicrocuries per microgram) $\pm$ SEM for five organs at the particular time interval. Correlation coefficient (r) for heart $= 0.94$, oesophagus $= 0.87$, non-glandular stomach $= 0.86$, and glandular stomach $= 0.92$. The slopes for the tissues represented in this figure did not differ significantly, but were significantly different (P < 0.01) from those shown in Figure 3. Each point, for the oesophagus, represents the result of five pooled specimens.*

Fig. 3 *Fractional turnover rate (k) and noradrenaline half-life (t$_{\frac{1}{2}}$) in the duodenum, jejunum, ileum, and colon. Each point represents the mean specific activity (millimicrocuries per microgram) $\pm$ SEM for five organs at the particular time interval. Correlation coefficient (r) for duodenum $= 0.94$, jejunum $= 0.96$, ileum $= 0.94$, colon $= 0.94$. The slope for the tissues represented in the figure did not differ significantly but were significantly different (P < 0.01) from those shown in Figure 2.*
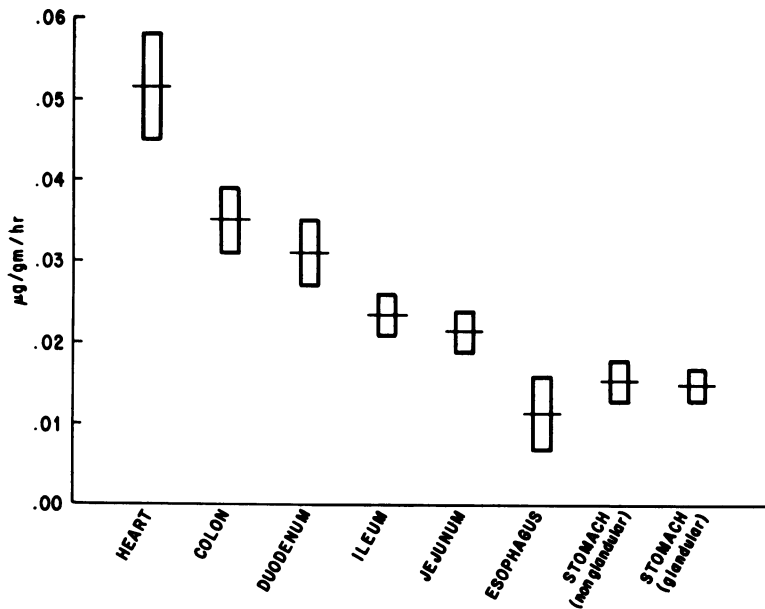
Fig. 4 *Noradrenaline turnover rate (slope × endogenous concentration). The horizontal bar represents the mean turnover rate. The bars above and below represent 95% confidence intervals about the mean, computed as described in the methods. Statistically significant differences in noradrenaline turnover rates (P = < 0·05) are found in those tissues where 95% confidence intervals do not overlap.*

gastrointestinal tract display a high correlation with time over 24 hours (correlation coefficients 0·86-0·96) (Figs. 2, 3). Thus, the rate of disappearance of noradrenaline is best described by a monoexponential function and implies a functional single homogeneous pool in each of the tissues for the entire 24-hour period (Costa *et al*, 1966; Neff *et al*, 1968).

The sympathetic innervation of the gut occurs via the splanchnic nerves and the para-aortic plexuses. Preganglionic fibres originate in the thoracic segment of the spinal cord, traverse the paravertebral sympathetic ganglia, coalesce to form the splanchnic nerves, and terminate in the preaortic abdominal plexuses. Postganglionic fibres, originating in the preaortic plexuses, are distributed to all parts of the gastrointestinal tract in close association with blood vessels. Fluorescent histochemical studies have shown that noradrenaline is localized in the myenteric plexus of Auerbach, the submucosal plexus of Meissner, and in fine fibres coursing through the muscle layer and ramifying on the basal aspect of the glandular epithelium. The greatest concentration of noradrenaline, as judged from histochemical fluorescent studies, appears to be in the duodenum and colon (Jacobowitz, 1965; Hollands and Vanov, 1965). The results of the present study substantiate the histochemical findings; noradrenaline determined biochemically was also greatest in the duodenum and colon.

Studies employing sympathetic agonists and blocking agents (Neely and Catchpole, 1967; Bennet and Whitney, 1966) have provided evidence that sympathetic activity is greater in the duodenum,

small bowel, and colon than it is in the stomach. The direct measurement of noradrenaline turnover rate in the present study confirms these results. It should be emphasized that the values obtained comprise the sum of endogenous noradrenaline and its turnover in the intramuscular plexus, submucosal plexus, and the vasculature. Since the turnover in these areas may vary independently from organ to organ, comparison between organs may be of questionable validity. Fluorescent histochemical studies, however, indicate that most of the noradrenaline in the gut is associated with smooth muscle and nerve plexuses rather than blood vessels (Hollands and Vanov, 1965). These studies have also indicated a basic similarity in the distribution of noradrenaline in different regions of the gut. Thus, it seems likely that meaningful comparisons of noradrenaline turnover can be made between different portions of the gastrointestinal tract.

The colon deserves special comment. Previous observers have suggested a prominent role for the sympathetic nervous system in the control of colonic motility (Bucknell and Whitney, 1964; Fishlock and Parks, 1966). In the present study the colon was found to have a rich supply of sympathetic fibres, as indicated by the high endogenous noradrenaline concentration, and a high degree of sympathetic tone, as indicated by the high noradrenaline turnover rate. Thus, the sympathetic nervous system may be the dominant influence in the physiological control of colonic motility.

The physiological effects of sympathetic stimulation upon the gastrointestinal tract are largely

inhibitory. Activation of the adrenergic nervous system or the administration of sympathomimetic amines results in decreased motility, decreased secretion, decreased blood flow, and depressed rates of cell renewal of the mucosa (Bullough and Laurence, 1964; Bass and Patterson, 1967; Jacobson, 1968; Daniel, 1969). These and other findings suggest that the sympathetic nervous system may play a part in the pathogenesis of stress ulceration of the stomach and paralytic or postoperative ileus (Catchpole, 1969). The role of the sympathetic nervous system in the pathogenesis of these disorders could be profitably investigated by the use of the techniques described here.

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