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Department of Neuropathology, Harvard Medical School and Department of Neuroscience, Children's Hospital Medical Center, Boston, Mass, 02115, U.S.A.

RALPH A. NIXON

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Extraneuronal uptake and metabolism of [^{3}H]L-norepinephrine by the rat duodenal mucosa*

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Recent work in our laboratory has demonstrated that rat duodenal mucosa avidly takes up circulating L-dopa and stores dopa metabolites largely in the form of glucuronide conjugates [1]. The studies reported here indicate: (i) that norepinephrine, like L-dopa, is concentrated by duodenal mucosa; (ii) that the uptake process is outside the sympathetic nerve endings; and (iii) that NE is stored in the mucosal cells as noncatechol metabolites, principally the glucuronide conjugate of NE.

The methods utilized have been previously described in detail [1]. Rats weighing 180–220 g (Charles River) were used in all experiments. L-norepinephrine-7-[^{3}H] (4–7 Ci/m-mole) and L-3,4-dihydroxyphenylalanine (6–9 Ci/m-mole) (New England Nuclear Corp.) were purified prior to use by column chromatography on alumina. They were

Table 1. NE and dopa uptake by duodenal mucosa: effect of 6-OHDA

	Total ^{3}H (nCi/g)	
	Duodenal mucosa	Heart
[^{3}H]L-NE		
Control	478.9 ± 42.8	1560.5 ± 96.4
6-OHDA	623.9* ± 46.8	307.7‡ ± 16.4
[^{3}H]-dopa		
Control	1264.7 ± 101.7	171.4 ± 6.3
6-OHDA	1675.0† ± 78.1	168.2 ± 11.4

* $P < 0.02$ † $P < 0.01$ ‡ $P < 0.001$ compared with control 5–7 animals per group; 6-OHDA administered i.v. 24 hr before (100 mg/kg). Controls received diluent. Rats were given [^{3}H]L-dopa (400 μCi (13.6 μg) per kg) or [^{3}H]L-NE (200 μCi (9 μg) per kg) i.v. and killed 10 min later. Results are means ± S.E.M. Hearts of 6-OHDA treated rats had no detectable NE by fluorescent assay.

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injected, after appropriate dilution with isotonic saline, into the tail veins of unanesthetized rats. 6-hydroxydopamine (6-OHDA) (Sigma) was injected i.v. 24 hr prior to [^{3}H]L-NE injection in a dose of 100 mg/kg. The animals were killed by a blow at the base of the skull, the hearts removed, the duodenum rinsed in cold tap water, blotted dry, and the mucosa separated from muscularis by the sharp edge of a glass slide. Samples of mucosa weighed between 150 and 200 mg. Chromatographic separation of dopa and NE metabolites was performed on alumina and Dowex columns as described previously [1], with the following exception: when [^{3}H]NE was administered the alumina eluate containing catechols was not passed over Dowex. The catechol acids were extracted from the alumina eluate into ethyl acetate at pH 1 [2] resulting in a catechol acid rather than a 'deaminated' fraction. Endogenous NE was determined on the alumina eluate by the trihydroxyindole method of Von Euler and Lishajko [3] and the fluorescence determined in an Amico Bowman spectrophotofluorimeter. Recovery of NE averaged 85 per cent, dopa 75 per cent. The results reported here are not corrected for recovery. Incubations with Glusulase (Endo) were as described previously [1] except that the time of incubation was reduced to 2 hr as compared with 18 in the previous study. The recovery of added [^{3}H]L-NE was 65 per cent after incubation (not corrected). In the experiments involving analysis of the deaminated noncatechol fraction for conjugates, perchlorates were moved by adjusting the pH of the Dowex effluent to 5.4 with 2N KOH and freezing overnight. Statistical significance was determined by Student's *t*-test for unpaired variables.

In the experiment shown in Table 1 the effect of 6-hydroxydopamine on [^{3}H]L-NE and [^{3}H]L-dopa uptake in heart and duodenum was examined. In the heart, 6-OHDA markedly inhibited the accumulation of ^{3}H after [^{3}H]L-NE but had no effect after [^{3}H]L-dopa. This demonstrates the efficacy of 6-OHDA pretreatment in destroying the sympathetic nerve endings and indicates that dopa uptake in the heart is predominantly extraneuronal. In duodenal mucosa, 6-OHDA pretreatment actually increased the accumulation of both [^{3}H]L-dopa and [^{3}H]L-NE. This is consistent with an extraneuronal site for the uptake process. The reason for the increase in 6-OHDA pretreated animals is not clear but may reflect alterations of blood flow and hence delivery of the tracer.

The effect of 6-OHDA pretreatment on the metabolism of both [^{3}H]L-NE and [^{3}H]L-dopa in duodenal mucosa is shown in Table 2. There was no difference; the 6-OHDA pretreated rats produced the same pattern of metabolites as the controls. The metabolism of [^{3}H]L-NE resembled that of [^{3}H]L-dopa; over 90 per cent of the total ^{3}H was in the form of noncatechols with a large portion (25 per cent in this experiment, 40 per cent in the experiment depicted in Table 3) in the neutral buffer eluate from Dowex (amino acid fraction). Comparison with duodenal muscularis and heart is shown in Table 3. The muscularis, which is not completely free of mucosa by this technique

Table 2. Effect of 6-OHDA on [^{3}H]L-NE and [^{3}H]L-dopa metabolism in duodenal mucosa

[^{3}H]L-NE Administered	Per cent total ^{3}H	
	Control	6-OHDA
[^{3}H]catechols	2.8 ± 1.1	2.1 ± 1.5
[^{3}H]noncatechols		
Amino acid fraction	26.6 ± 3.7	25.3 ± 3.9
Amine fraction	8.9 ± 1.9	6.2 ± 1.1
Deaminated fraction	61.8 ± 4.8	66.4 ± 4.2
[^{3}H]L-dopa Administered		
[^{3}H]catechols		
Amino acid fraction	2.5 ± 1.5	1.9 ± 0.4
Amine fraction	1.9 ± 0.8	1.0 ± 0.4
Deaminated fraction	0.5 ± 0.3	1.4 ± 0.6
[^{3}H]noncatechols		
Amino acid fraction	34.6 ± 1.8	37.4 ± 1.2
Amine fraction	3.1 ± 1.2	2.5 ± 0.2
Deaminated fraction	58.9 ± 2.5	55.8 ± 2.2

Protocol is in legend to Table 1. Rats treated with 6-OHDA do not differ from controls. Amino acid fraction refers to the neutral buffer Dowex eluate in this and subsequent tables.

Table 3. [^{3}H]L-NE metabolism in duodenal mucosa, muscularis and heart

	Duodenal mucosa		Duodenal muscularis		Heart	
	(nCi/g)	(per cent ^{3}H)	(nCi/g)	(per cent ^{3}H)	(nCi/g)	(per cent ^{3}H)
Total radioactivity	703.2 ± 87.9		737.4 ± 79.0		2353.6 ± 303.3	
Catechols						
Amines	23.9 ± 1.3	(3.6) (± 0.4)	320.5 ± 49.1	(43.1) (± 3.8)	1819.9 ± 190.7	(74.1) (± 2.2)
Acids	0		3.2 ± 0.4	(0.4) (± 0.4)	18.1 ± 3.0	(0.8) (± 0.1)
Noncatechols						
Amino acid fraction	296.8 ± 64.2	(40.8) (± 5.5)	136.8 ± 33.5	(17.6) (± 2.7)	17.9 ± 2.8	(0.8) (± 0.2)
Amine fraction	0		26.1 ± 2.5	(3.6) (± 0.1)	71.4 ± 13.4	(3.2) (± 0.6)
Deaminated fraction	381.3 ± 43.3	(55.5) (± 5.2)	250.8 ± 17.3	(35.3) (± 4.0)	550.9 ± 80.8	(21.2) (± 2.2)

Five animals per group (mean ± S.E.M.). Animals killed 10 min after [^{3}H]L-NE. Mucosa differs significantly from muscularis ($P < 0.05$) for catecholamines, noncatecholamine acid fraction, and noncatechol deaminated fraction. Endogenous NE in duodenal mucosa 45.6 ± 5.8 ng/g as compared with 245.6 ± 26.2 ng/g in muscularis ($P < 0.001$). The metabolic pattern in heart is shown for comparison.

Table 4. Effect of incubation of the noncatechol amino acid fraction with glusulase

	[^{3}H]NE (per cent)	
	No enzyme	With glusulase
Control	0	19.4 ± 1.9
6-OHDA	0	22.9 ± 2.4

Protocol as in Table 1. Noncatechol amino acid fraction of duodenal mucosa was incubated with and without glusulase as described in Methods. No [^{3}H]NE was recovered in samples incubated without enzyme. 6-OHDA pretreated rats do not differ from controls. Results are expressed as [^{3}H]NE per cent of total ^{3}H (means ± S.E.M.).

Table 5. Effect of glusulase on noncatechol amino acid and deaminated fraction

	[^{3}H]NE (per cent)	
	No enzyme	Glusulase
Noncatechol amino acid fraction	0.2 ± 0.2	27.2 ± 1.9*
Noncatechol deaminated fraction	4.9 ± 3.4	20.7 ± 5.2†

Protocol as in Table 3. * $P < 0.05$ † $P < 0.001$ vs. No enzyme [^{3}H]NE as per cent total ^{3}H (means ± S.E.M.).

of separation [1] contained 43 per cent of the tritium in the catecholamine fraction.

Similarly, the endogenous NE content of the muscularis was 245.6 ± 26.2 as compared with 45.6 ± 5.8 ng/g in the mucosa. It thus appears that most of the sympathetic nerve endings in duodenum are in the muscularis. The uptake and metabolism of [^{3}H]L-NE by the duodenal mucosa resembles that of [^{3}H]L-dopa and is predominantly extraneuronal.

The major metabolite of [^{3}H]L-dopa formed by the duodenal mucosa has been shown to be the glucuronide conjugate of dopamine [1]. The glucuronide conjugates appear, at least partially, in the noncatechol neutral buffer Dowex eluate [1, 4] and can be identified after hydrolysis of the conjugate by Glusulase or β-glucuronidase [1]. Since a major portion of the [^{3}H]L-NE metabolites appear in the noncatechol neutral buffer Dowex eluate (amino acid fraction—Tables 1 and 2) it seemed likely that conjugates of NE were formed in duodenal mucosa as well. The results of incubating the noncatechol neutral buffer eluate with Glusulase, from 6-OHDA and control rats, is shown in Table 4. Samples incubated with Glusulase contained about 20 per cent of the ^{3}H in the catechol fraction. Pretreatment with 6-OHDA did not influence the formation of conjugates. In Table 5 further evidence of conjugate formation is presented. The noncatechol deaminated fraction was analyzed for conjugates along with the amino acid noncatechol fraction. Significant amounts of conjugates were recovered from the samples incubated with Glusulase, thus indicating that retention of conjugates on Dowex is not complete. The results indicate that conjugation of [^{3}H]L-NE is a major pathway in duodenal mucosa just as it is for [^{3}H]L-dopa.

The studies reported here in duodenal mucosa, are most consistent with [^{3}H]L-NE uptake in an extra-neuronal site. This is consistent with the observation of Iversen *et al.* [5], that immunosympathectomy did not impair the accumulation of ^{3}H by the jejunum after the administration of i.v. [^{3}H]NE. The previously reported uptake of [^{3}H]L-dopa by duodenal mucosa [1] is thus not unique for the catechol amino acid. These results imply that duodenal mucosa may possess an important inactivating mechanism for the catechol group. Although the data shown in Table 1 suggest that the mechanism is more avid for L-dopa than L-NE, this cannot be concluded from the present study. Since the volume of distribution and metabolic fate of these two compounds is different, simultaneous plasma levels are required with plasma–tissue ratios before an assessment of the relative affinity can be made.

These findings support the concept that the gut is a potentially important site in the metabolic disposition of circulating catechols of endogenous origin.

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Thorndike Laboratory, LEWIS LANDSBERG*
Harvard Medical School and
Department of Medicine,
Beth Israel Hospital, Boston, Mass., U.S.A.

* Address reprint requests to Dr. Landsberg, Beth Israel Hospital, 330 Brooklin Ave., Boston, Mass. 02215.

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