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NOREPINEPHRINE BIOSYNTHESIS IN THE RAT PANCREAS

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Abstract—The biosynthesis *in vivo* of norepinephrine was studied in rat pancreas with ^{14}C-dopamine as precursor. It was shown that after i.m. injection ^{14}C-dopamine was taken up by the pancreatic tissue, where this catecholamine was metabolized. Norepinephrine was the major transformation product found in the pancreas. Dopamine was metabolized faster in the pancreas than in the heart and the spleen. On the other hand, the concentration of the radioactivity at 30-min and 120-min time intervals was higher in the heart and spleen than in the pancreas.

THE PANCREAS has been shown to be innervated by sympathetic and parasympathetic nerve fibers.[1] Lever *et al.*[2] demonstrated that uptake of norepinephrine by periarteriolar nerves of the pancreas is restricted to adrenergic nerve fibers. More recently, Esterhuizen *et al.*[3] provided histochemical and autoradiographic evidence for adrenergic and cholinergic innervation of the alpha and beta cells of the islets of Langerhans of the cat. The presence of noradrenaline and 5-hydroxytryptamine (5-HT) has been demonstrated in the guinea pig pancreas by Cegrell *et al.*,[4] and dopamine was found in varying amounts in the pancreas of fetal and newborn animals.[4,5] It was also shown[6] that mouse and rat pancreas have a highly efficient mechanism for uptake and storage of L-dihydroxyphenylalanine (DOPA). These authors have further indicated a very rapid appearance of dopamine in the mouse pancreas after DOPA administration.

Since the pancreas has a sympathetic innervation and several authors[7–9] postulated a neurogenic mechanism in the regulation of insulin secretion, it was of interest to examine quantitatively the metabolic fate of catecholamines in this organ.

The present communication will show that dopamine-^{14}C is taken up by rat pancreas and converted to the neurotransmitter, norepinephrine. Dopamine uptake and the rate of norepinephrine synthesis will be compared with selected sympathetically innervated tissues.

METHODS

Male albino rats (CFE) were obtained from Carworth Farms. The animals were housed under laboratory conditions for at least 1 week prior to use. The experiments were initiated by injecting (i.m.) the animals, weighing on the average 250 g, with 0·26 mg/kg of 1-^{14}C-labeled dopamine (New England Nuclear Corp.; sp. act., 7·07 mc/m-mole). At selected time intervals after dopamine injection, the rats were killed by cervical dislocation. Heart, spleen and pancreas (the portions located in the splenic,

stomach and duodenal areas) were dissected out as rapidly as possible, blotted to remove excess blood and immediately frozen on a block of dry ice. The detailed experimental procedure for the determination of catecholamines and the metabolites has been described elsewhere.[10] Briefly, the tissues were homogenized in 0·01% hydrochloric acid in ethanol and the precipitated protein was removed by centrifugation at 70,000 *g*. Aliquots of clear supernatant were counted in a Packard liquid scintillation counter. The remaining supernatant was concentrated at low temperature and high vacuum and the dry residue was extracted with 0·1 to 0·2 ml absolute ethanol and applied on Whatman No. 1 paper for chromatography. Dopamine (DA), norepinephrine (NE), 3-*O*-methyl-dopamine (3MDA), 3-*O*-methyl-norepinephrine (3MNE) and homovanillic acid (HVA) carriers were routinely cochromatographed.

Two chromatographic systems were used for developing the chromatograms; *n*-butanol (redistilled) saturated with 1 N HCl, and phenol:H_2O (88:12). The latter system was particularly useful in differentiation between DA and 3MNE, which had similar R_f values in butanol:HCl. The air-dried chromatograms, 1·5 inches wide, were scanned in a 4π chromatogram counter (Nuclear Chicago) connected to an automatic integrator. This permitted quantitative determination of areas under peaks. The radioactivity peaks were identified by their correspondence to the carriers on the paper strip, visualized by ferricyanide spray under u.v. light.

RESULTS

Metabolic fate of dopamine-^{14}C in pancreas. The results obtained in the pancreas are summarized in Table 1. The quantitative evaluation of the chromatographic

TABLE 1. FATE OF DOPAMINE-^{14}C IN RAT PANCREAS*

	Chromatographic pattern (Per cent of radioactivity on paper strip)						Radioactivity content of tissue (cpm/g)
	System: Butanol–HCl			System: Phenol–H_2O			
	DA	NE	Metab	DA	NE	Metab	
30 min	33·4 ± 2·3	40·2 ± 6·6	26·3 ± 5·4	34·4 ± 1·1	34·5 ± 5·2	31·1 ± 4·3	5809 ± 267
60 min	22·3 ± 1·4	67·2 ± 3·4	10·5 ± 2·2	19·8 ± 1·3	67·1 ± 1·4	13·1 ± 1·5	5643 ± 320
120 min	5·6 ± 1·4	83·4 ± 2·0	10·9 ± 1·6	2·5 ± 2·2	83·1 ± 2·7	14·4 ± 1·0	4008 ± 259

* Mean ± S.E. of 4 values. DA = dopamine; NE = norepinephrine; Metab = metabolites.

pattern yielded almost identical results in the two different solvent systems. After 30 min, about 34 per cent of the radioactivity found in the pancreas could be identified as DA. At 120 min, only about 4 per cent (average for both chromatographic systems) of the radioactive DA remained unchanged. NE was the major product found in the tissue at all time periods. While chromatographic data show a rapid conversion of DA to NE and a few unidentified metabolites, the total radioactivity tissue content decreased only slightly with time, as seen on the right of Table 1.

Fig. 1 illustrates a characteristic pattern of the metabolic pathway of DA in the pancreas. At the early time period, the radioactivity not associated with DA or NE represents about 26 per cent of the total radioactivity present. Some of the peaks

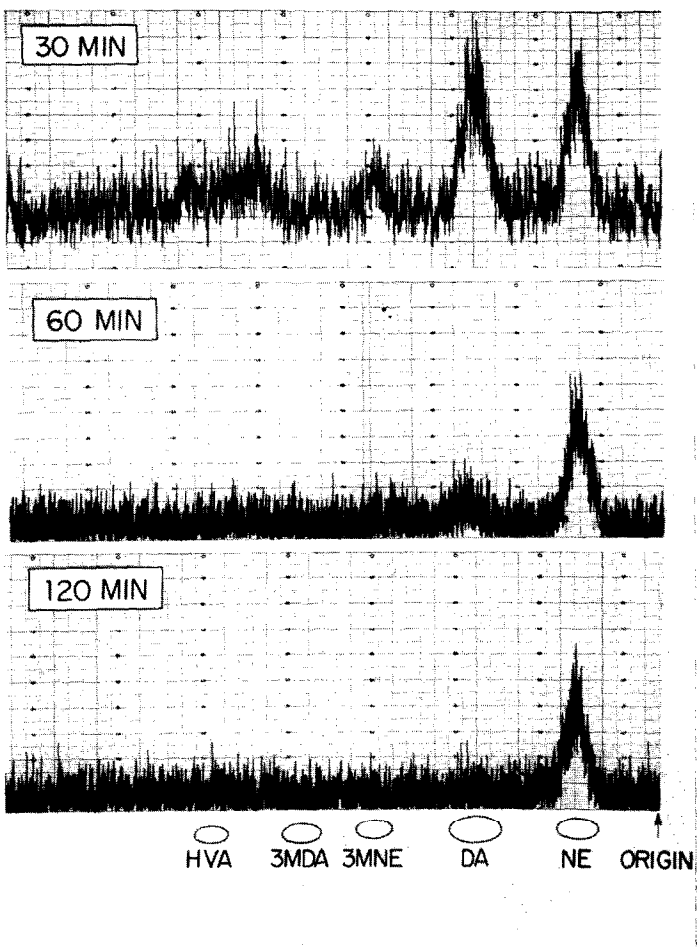

FIG. 1. Chromatographic pattern of DA metabolism in the rat pancreas. Phenol:H_2O (88:12) was used to develop the chromatograms. The areas outlined on the bottom denote the position on paper strips of cochromatographed carriers.

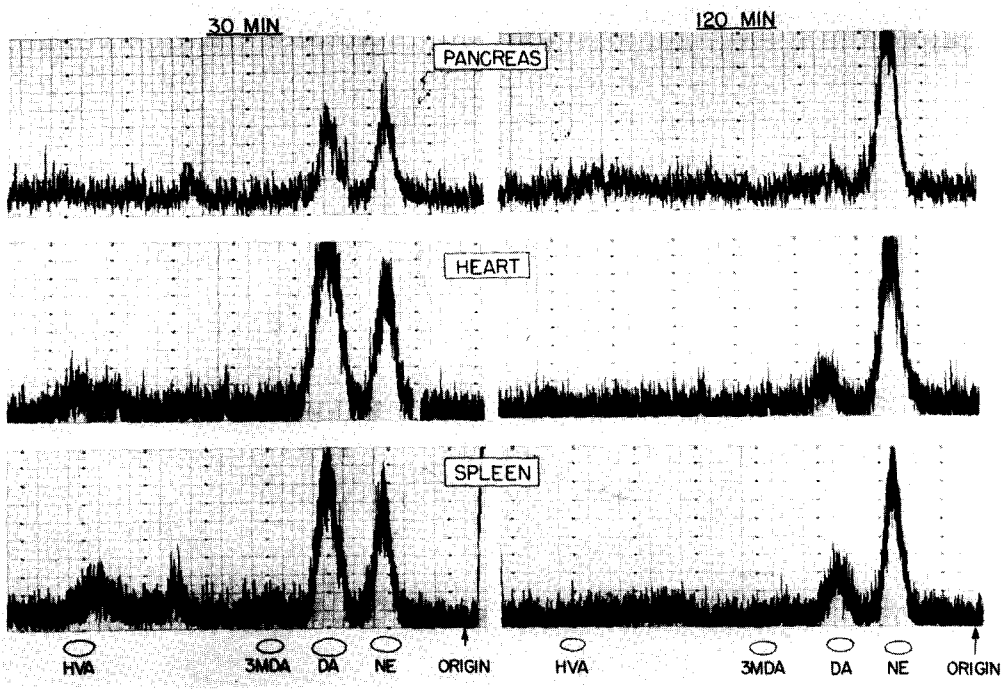

FIG. 2. Chromatographic pattern of DA metabolism in the rat pancreas, heart and spleen. Butanol:HCl was used to develop the chromatograms. The areas outlined on the bottom denote the position on paper strips of cochromatographed carriers.

correspond chromatographically to the position on the paper strip of 3MDA and HVA carriers. Relative amounts of these metabolites decrease with time.

Comparison of the fate of dopamine-^{14}C in the pancreas, heart and spleen. Table 2 shows the content of radioactivity in three sympathetically innervated tissues at two time intervals after i.m. injection of DA-^{14}C. At 30 min the highest concentration

TABLE 2. RADIOACTIVITY CONTENT OF TISSUE (cpm/g)*

Tissue	30 min	120 min
Pancreas	5,317 ± 238	3,175 ± 135
Spleen	11,296 ± 457	7,972 ± 427
Heart	18,800 ± 1060	13,516 ± 393

* Mean ± S.E. for 8 values.

of radioactivity is present in the heart, followed by the spleen and finally the pancreas. The same pattern is also observed after 120 min. It is interesting that at both time intervals the heart contains 3–4 times as much radioactivity per gram of tissues as the pancreas.

The metabolic fate of DA-^{14}C in the pancreas, heart and spleen is shown in Table 3. It is apparent that radioactive DA is metabolized most rapidly in the pancreas. At 30

TABLE 3. METABOLIC FATE OF DOPAMINE-^{14}C

	Chromatographic pattern (System: butanol–HCl) (Per cent of radioactivity on paper strip)*					
Tissue	30 min			120 min		
	DA	NE	Metab	DA	NE	Metab
Pancreas	31·2 ± 1·1	38·8 ± 2·0	29·9 ± 2·1	5·9 ± 0·5	78·7 ± 1·7	14·9 ± 1·8
Spleen	45·3 ± 1·9	31.0 ± 0·7	23·6 ± 1·8	23·2 ± 1·6	64·5 ± 2·5	12·3 ± 1·1
Heart	58·0 ± 2·5	27·6 ± 1·3	14·3 ± 2·4	17·2 ± 0·7	76·8 ± 1·4	5·8 ± 1·4

* Mean ± S.E. for 8 values.

min, about 69 per cent of the DA-^{14}C was metabolized in the pancreas, compared to 55 per cent in the spleen and 42 per cent in the heart. At 120 min, we see again a larger portion of DA-^{14}C being metabolized in the pancreas than in the heart and spleen.

Fig. 2 illustrates a characteristic chromatographic pattern of radioactive catecholamines and their metabolites in the pancreas, heart and spleen at 30 and 120 min. A number of small areas of radioactivity were detected by scanning the paper strips. Some of the peaks, probably acidic metabolites, are visible in this figure. These metabolites of catecholamines show a similar pattern in all tissues and their relative amount in each organ decreases with time.

DISCUSSION

The pancreas was shown to be innervated by sympathetic nerve fibers[1,3] which should be able to take up, release, store, synthesize and metabolize NE.[11] Synthesis *in situ* appears to be the main source of NE in sympathetically innervated tissues.[12]

Recent experiments using fluorescent techniques[6] have demonstrated that mouse pancreas has a highly efficient mechanism for uptake and storage of L-DOPA. The authors observed moderate intensity of green, and sometimes yellow-green, fluorescence in the islets after i.p. administration of L-DOPA. This fluorescence increased greatly as a result of pretreatment with monoamine oxidase inhibitors.[7] The green fluorescence indicates the presence of primary catecholamines and yellow fluorescence, the presence of 5-HT.[4] The presence of catecholamines has also been shown in the guinea pig pancreas[4,5] by the same technique.

While the histochemical fluorescence techniques have proven of great value in detecting the presence of biogenic amines in tissues, their metabolic transformations cannot be determined quantitatively, and at present, there is no reliable histochemical method to distinguish between DA and NE.[4] The present study has quantitatively evaluated the metabolic pathway of catecholamines in the pancreas. It was shown that rat pancreas can take up dopamine and convert it to norepinephrine. The metabolism of dopamine in the pancreas is faster than in the heart and spleen, while the concentration of this biogenic amine per gram of tissue is smaller in the pancreas than in the other tissues.

The biological function of catecholamines in the pancreas has not as yet been established. However, it has been shown under conditions both *in vitro* and *in vivo*, that norepinephrine[8,13–15] and, to a lesser degree, dopamine[13] block the insulin release from the pancreas. Porte and Williams[8] suggested that the sympathetic nervous system may play a role in the regulation of insulin release. Similarly, Cegrell *et al.*[7] suggested that a monoaminergic mechanism is operating in the endocrine pancreas.

On the other hand, the cholinergic mechanism may counteract the inhbitory effect of catecholamines on pancreatic insulin release. Daniel and Henderson[16] have shown, in the baboon, that subdiaphragmatic stimulation of the cut right vagal trunk produced an increase in insulin concentration in the inferior vena cava and the splenic vein. Kaneto *et al.*[9] have found that stimulation of both cervical vagi and the dorsal vagal trunk of the dog induced elevation in immunoreactive insulin (IRI) in the plasma effluent of the pancreas. Frohman *et al.*[17] confirmed the influence of the vagus nerve on insulin secretion in the dog. However, Nelson *et al.*[18] did not enhance the pancreatic IRI release upon stimulation of the vagus nerve at the level of the diaphragm or in the neck.

The results presented in this communication show that the pancreas is capable of uptake and synthesis of catecholamines to a degree comparable to other sympathetically innervated tissues, thus further strengthening the belief that catecholamines may have an important biological function in the pancreas.

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