# CATECHOLAMINE BIOSYNTHETIC ENZYMES IN PANCREATIC ISLET CELLS

HAROLD E. LEBOVITZ and ROBERT W. DOWNS, Jr.
Division of Endocrinology, Departments of Medicine and Physiology,
Duke University Medical Center, Durham, North Carolina 27710, U.S.A.

HISTOCHEMICAL studies using fluorescent techniques show that dopamine or serotonin or both are present in the cytoplasm of pancreatic islet cells of a number of animals, such as guinea pig, cat, dog, pig and in human fetus (CEGRELL 1968). The islet cells of other species such as albino rabbit, albino mouse and golden hamster do not ordinarily contain histochemically stainable monoamines. However, after administration of the monoamine precursors L-dopa or 5-hydroxytryptophan, to these animals, their islet cells show intense histochemical monoamine fluorescence (CEGRELL, 1970). Adrenergic nerves containing norepinephrine are present in abundance in the islets of the golden hamster but are only occasionally found in the islets of other species, such as the rabbit, mouse or man (CEGRELL, 1968).

Dopamine and serotonin significantly inhibit stimulated insulin secretion from pancreatic islets both *in vivo* and *in vitro* in many animal species (QUICKEL *et al.*, 1971a) Serotonin antagonists potentiate stimulated insulin secretion *in vitro* from pieces of pancreas from golden hamster and rabbits (LEBOVITZ and FELDMAN,1973; FELDMAN *et al.*, 1972) and *in vivo* in humans with adult onset diabetes mellitus (QUICKEL *et al.*, 1971b).

From these data it is appealing to hypothesize an important regulatory role for pancreatic $\beta$ cell monoamines as tonic inhibitors of insulin secretion. In this schema, insulin secretion could be viewed as the net result of external stimulatory agents, such as substrates and hormones, and intracellular inhibitory agents, such as dopamine or serotonin, or both (LEBOVITZ and FELDMAN, 1973).

Enigmatic in the above schema are the questions of the origin and regulation of the concentration of the pancreatic $\beta$ cell monoamines and the significance of the species variation in both the occurrence and nature of the pancreatic $\beta$ cell monoamines. The present communication is a preliminary progress report of our studies on the biosynthesis of monoamines in pancreatic islet cells.

Pancreatic islet tissue from several sources were examined for tyrosine hydroxylase and L-aromatic amino acid decarboxylase activity (EC 4.1.1.26). Isolated pancreatic islets were obtained by collagenase treatment of pancreas from albino rabbits, pigmented and albino guinea pigs and golden hamsters (LACY *et al.*, 1972). The $\beta$ cells of the albino rabbit ordinarily contain no histochemically stainable monoamines; those of the pigmented and albino guinea pig contain large quantities of dopamine and serotonin; and the $\beta$ cells of the golden hamster contain no monoamines, but their islets have an extensive adrenergic nerve innervation (CEGRELL, 1968; JAIM-ETCHEVERRY and ZIEHER, 1968). A transplantable golden hamster islet cell carcinoma whose cells contain large quantities of cytoplasmic dopamine and serotonin (CEGRELL *et al.*, 1969a) and normal hamster adrenals were also assayed for the catecholamine biosynthetic enzymes. Tyrosine hydroxylase was determined by the generation of

$^{14}CO_2$ from 1-$^{14}$C-L-tyrosine by coupled decarboxylation of the L-dopa formed (WAYMIRE *et al.*, 1971). L-Aromatic amino acid decarboxylase was measured by the conversion of 2-$^{14}$C-D,L-dopa to $^{14}$C-dopamine (CREVELING and DALY, 1971). The supernatant protein in each homogenate was determined as Lowry protein.

TABLE 1. TYROSINE HYDROXYLASE AND L-AROMATIC AMINO ACID DECARBOXYLASE ACTIVITY IN PANCREATIC ISLETS

Data are expressed as mean ± S.E. The number of determinations are given in the parentheses.

| Tissue | Tyrosine hydroxylase (n moles/hr/mg protein) | L-aromatic amino acid decarboxylase ($\mu$ moles/hr/mg protein) |
|---|---|---|
| Hamster adrenal | 10·356 | 1·937 ± 0·454 (5) |
| Hamster transplantable islet cell carcinoma | 1·590 ± 0·441 (7) | 16·413 ± 1·776 (5) |
| Isolated hamster islets | 0 | 5·058 ± 0·070 (2) |
| Isolated rabbit islets | 0 | 0·280 ± 0·073 (3) |
| Isolated guinea pig islets | 0 | 2·976 ± 0·049 (2) |

Table 1 depicts the results of these studies. All of the isolated pancreatic islets contained measureable quantities of L-aromatic amino acid decarboxylase. The difference in activity is striking. The transplantable hamster islet cell carcinoma has an extremely high activity with isolated hamster islets being about 1/3 as active, guinea pig islets about 1/6 and rabbit islets about 1/50. Tyrosine hydroxylase activity could not be detected in any of the isolated islets. The transplantable islet cell carcinoma contained significant tyrosine hydroxylase activity.

The presence of significant L-aromatic amino acid decarboxylase activity in the islets of all the species suggest that most, or all pancreatic islet tissue can readily convert L-dopa or 5-hydroxytryptophan to their appropriate monoamines and thereby increase intracellular pools of these amines when exposed to sufficient precursor.

The absence of measureable tyrosine hydroxylase activity in isolated hamster and rabbit islets might be expected, since these islets ordinarily do not show intracellular monoamines by histochemical fluorescent studies. In contrast the failure to demonstrate tyrosine hydroxylase activity in isolated guinea pig islets was unexpected, since these islets have been shown to contain considerable dopamine (0·594 ± 0·077 $\mu$g/g wet wt) and serotonin (JAIM-ETCHEVERRY and ZIEHER, 1968). The demonstration of tyrosine hydroxylase and L-aromatic amino acid decarboxylase activities in the hamster islet cell carcinoma confirm previous reports using somewhat different techniques (CEGRELL *et al.*, 1969b; AXELSSON *et al.*, 1970). The presence of these enzymes can account for the high concentrations of monoamines (dopamine 2·75 ± 0·35 $\mu$g/g wet wt; 5-hydroxytryptamine 0·87 ± 0·09 $\mu$g/g) found in these tumor cells (CEGRELL *et al.*, 1969a).

The data fail to provide a clear understanding of the origin and mechanism of species variation in pancreatic islet cell monoamines. The failure to demonstrate tyrosine hydroxylase in the guinea pig islets suggest that the dopamine normally present in the $\beta$ cells of some species may be (1) the result of tyrosine hydroxylase activity that is below the level of sensitivity of current assays, (2) the consequence of

conversion of circulating L-dopa that is taken up by the $\beta$ cells, (3) the uptake and storage of circulating dopamine by the $\beta$ cell, or, (4) the synthesis of dopamine by some as yet unidentified biosynthetic pathway. We are unable to comment about the origin of $\beta$ cell serotonin, as measurements of tryptophan hydroxylase activity in these islets have not been completed.

Further definition of the physiologic role of pancreatic $\beta$ cell monoamines in the regulation of insulin secretion must await the elucidation of the origin and control mechanisms regulating the type and quantity of these amines.

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