

# Characteristics of the Dopaminergic and Noradrenergic Systems of the Pancreatic Islets

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## SUMMARY

The catecholamines norepinephrine (NE) and dopamine (DA), known inhibitors of insulin secretion, have been identified in the pancreatic islets only semiquantitatively by their histochemical fluorescence spectrum. Using collagenase digestion of golden hamster pancreas, we isolated islets and quantitated the NE and DA content with sensitive and specific radioenzymatic assays. We compared the NE and DA concentrations in islets with the median eminence and cerebral cortex of the golden hamster, tissues known to contain catecholamines. Under basal conditions, NE is in higher concentration than DA in all three tissues and is highest in the islets in a much greater concentration than is found in the median eminence. DA is present in measurable quantities in all three tissues and, in the islets, its concentration is comparable with that in the median eminence.

Treatment of the hamster with a precursor of the two catecholamines, L-dopa, produced a significant accumulation of DA in the islets and median eminence over the basal values. This L-dopa-induced increase in DA was heightened in the islets and reached significantly higher than control levels in the cortex by a pretreatment of the hamster with tranlycypromine (tran), a monamine oxidase inhibitor. Pretreatment with tran alone, however, produced no change in DA over control values.

The NE content of pancreatic islets was not altered by administration of L-dopa, tran, or L-dopa plus tran. The NE content of the median eminence was increased by the administration of tran. L-dopa plus tran did not result in an additional increase in the NE content of the median eminence. The administration of tran plus L-dopa (but not the administration of either agent alone) increased the NE content of the cortex over control levels. The present study suggests that inhibition of insulin secretion

after L-dopa administration is due to an increased concentration of DA rather than NE in the pancreatic islets. *DIABETES* 28:185-189, March 1979.

Thirty years ago, Sawyer, Markee, and Hollinshead first proposed a mechanism for hormone release in the anterior pituitary that depended on catecholamines.<sup>1</sup> Functional studies of the activity of the catecholamine system with certain endocrinologic changes<sup>2</sup> and further investigations with fluorescence-immunocytochemical techniques localizing the catecholamine storage sites<sup>3</sup> have established the importance of catecholamines in regulating hormone secretion. In 1963, biogenic amines were identified in the pancreatic islets of Langerhans of different species by formaldehyde-induced fluorescence.<sup>4</sup> Since then, precise subcellular localization of these biogenic amines to the hormone-containing granules<sup>5-7</sup> lead directly to exciting considerations of their physiologic role in the endocrine pancreas. Some investigators have demonstrated that exogenous dopamine and serotonin inhibit in vivo and in vitro insulin release stimulated by a variety of agents in certain species.<sup>6,8,9-11</sup> Other investigators have shown these two amines to stimulate insulin secretion.<sup>12-15</sup> These differences may be related to differences in dosage<sup>12</sup> or a basic species differentiation in monoamine uptake and action.<sup>12,16</sup>

Although formaldehyde-induced fluorescence data is extremely valuable for assessing the role of monoamines, the results are only semiquantitative.<sup>17</sup> To further understand the function of the monoamines, we obtained a precise quantitation of dopamine (DA)\* and norepinephrine (NE) in the golden hamster pancreatic islets with the sensitivity to determine monoamine content of the minute amount of tissue available. Using drugs that are known to change catecholamine synthesis and degradation, we then quantitated the

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\* Abbreviations used in this paper: DA, dopamine; <sup>3</sup>H-SAM, s-adenosyl-L-methyl-<sup>3</sup>H methionine; L-dopa, L-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; NE, norepinephrine; PNMT, phenylethanolamine-N-methyltransferase; tran, tranlycypromine sulfate.

possible accumulation and storage of DA and NE in the islets.

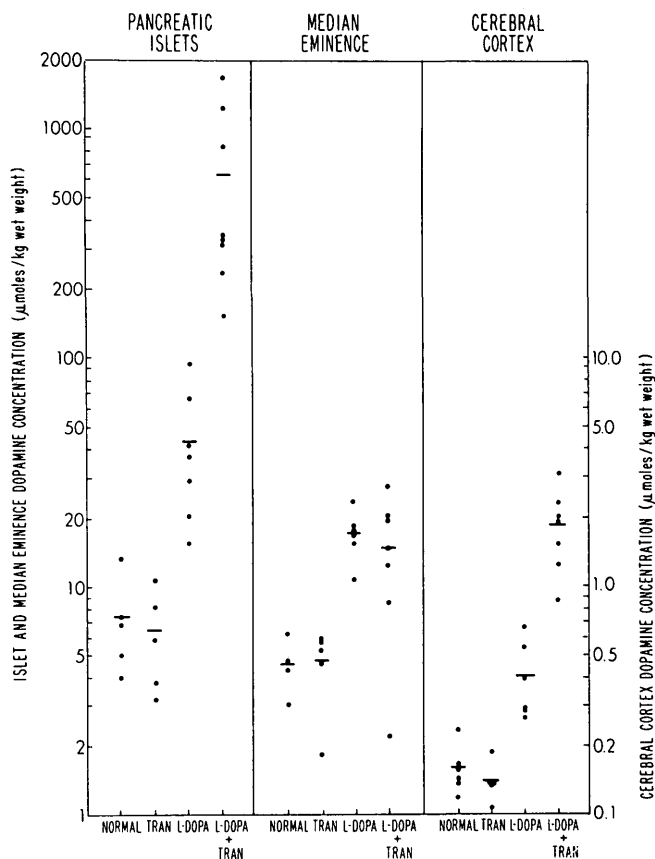
## MATERIALS AND METHODS

**Materials.** The following chemicals were purchased from commercial sources: L-3,4-dihydroxyphenylalanine (L-dopa) (Sigma Chemical, St. Louis, Missouri); collagenase, class IV (Worthington Biochemical Co., Freehold, New Jersey); tryptamine bisuccinate (side chain- $2\text{-}^{14}\text{C}$ ) with sp. act. of 60 mCi/mmol and S-adenosyl-L-[methyl- $^3\text{H}$ ]methionine ( $^3\text{H}$ -SAM) with sp. act. of 7.5 Ci/mmol (New England Nuclear, Boston, Massachusetts). The following chemicals were gifts: tranlycpromine sulfate (tran) (Smith, Kline & French, Philadelphia, Pennsylvania);  $\text{N}^1$ -(DL-seryl)- $\text{N}^2$ -(2,3,4-trihydroxybenzyl)-hydrazine (Ro-4-4602) (Hoffman-LaRoche Co., Nutley, New Jersey). Male golden hamsters (100–125 g) were purchased from Engel Laboratory Animals (Farmersburg, Indiana).

**Methods.** Preparation of hamster tissues for catecholamine measurement. Normal cerebral cortex and median eminence were dissected away immediately after the hamster was decapitated. These were then homogenized in a 1% wt/vol solution of 0.1 N perchloric acid. Typical wet weight values were cerebral cortex, 70 mg, and median eminence, 4 mg. After centrifugation, the supernatant was stored at  $-20^\circ\text{C}$  until the assay date. The pancreas also was removed immediately after the decapitation, and the islets were obtained by a modified collagenase digestion procedure.<sup>18</sup> In this procedure the islets are isolated from acinar tissue by their successive passage through three petri dishes of fresh Hanks' solution. Sample size collected varied between 300 and 500 islets. For the dopamine measurement, the isolation of islets into the third petri dish was performed in a solution in Hanks' without calcium. 0.1 N perchloric was also added to correct the volume for a final sample size of 75–125 islets per 50  $\mu\text{l}$ . It was not necessary to centrifuge the islet sample, which was also stored at  $-20^\circ\text{C}$  until assay.

Some hamsters received 6.5 mg L-dopa i.p. 1 h before being killed. Tran (25 mg/kg i.p.) was given 48 h and 24 h before killing. The L-dopa + tran-treated hamsters received the tran dose 48 and 24 h before the L-dopa treatment. Some of the animals received 300 mg/kg (50 mg) i.p. of the decarboxylase inhibitor (Ro-4-4602) 1 h before L-dopa injection.

**Dopamine measurement.** The radiometric catechol-O-methyltransferase (COMT) assay for catecholamines by Engelman and Portnoy (1970)<sup>19</sup> and modified by Coyle and Henry (1973)<sup>20</sup> was used here with a few changes, mentioned below, to measure the DA levels. The COMT methylates the hydroxyl group at the number three position of the benzene ring of the catecholamine. The [ $^3\text{H}$ -methyl] $^3$ -methoxytyramine is then separated from its [ $^3\text{H}$ -methyl]methoxy- $\beta$ -hydroxylated derivatives by selective oxidation and differential extraction. The oxidation step, cleaving the  $\beta$ -hydroxyl group of the [ $^3\text{H}$ -methyl]metanephrines, was performed at room temperature, and the reaction was then allowed to proceed for exactly 4 min before the glycerol was added to stop it. This increase in strength and length of oxidation brought the crossreactivity of NE in the DA assay down to 0%, and there was 100% recovery of DA. The crossreactivity for epinephrine (E) was found to be 7%. All drugs



**FIGURE 1.** Log scale showing DA concentration in the golden hamster islets and median eminence in micromoles per kilogram of wet weight. Cerebral cortex data plotted in micromoles per kilogram of wet weight, as shown. Each point represents the measurement from one hamster; the horizontal line represents the mean of each group.

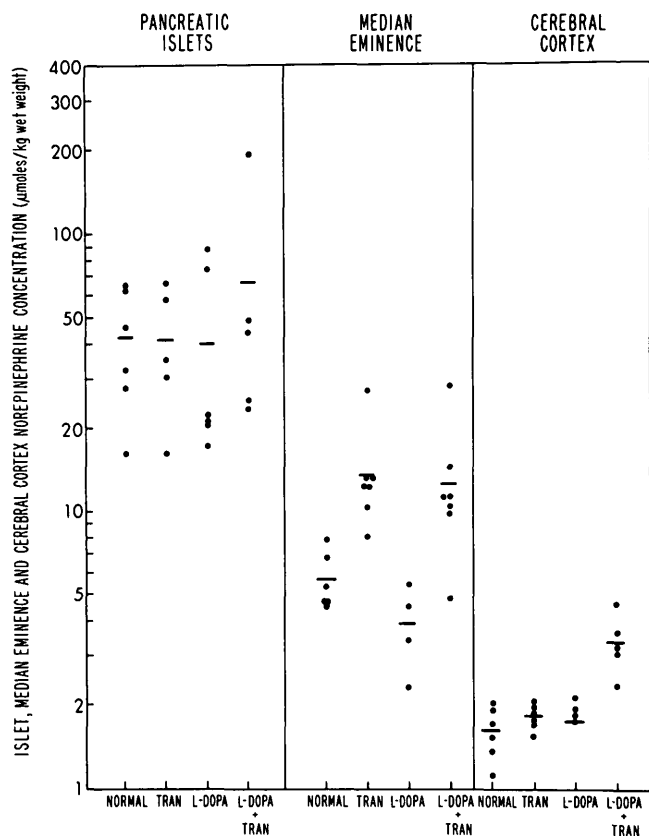
used were also tested for crossreactivity and were found to be low (tran 0.2%, L-dopa 3%, Ro-4-4602 0.2%). Due to variable inhibition of COMT in different tissues, each sample was quantitated by its own internal standard.

**Norepinephrine measurement.** The radioenzymatic method for measuring NE in tissues utilizes the enzyme phenylethanolamine-N-methyltransferase (PNMT) that we partially purified from fresh bovine adrenal glands.<sup>22</sup> The PNMT transfers a  $^3\text{H}$ -labeled methyl group from  $^3\text{H}$ -SAM to NE in tissues. Due to differences in the nonradioactive SAM content of different tissues, each sample is quantitated by its own internal standard. The assay is highly sensitive and specific; there is less than 2% crossreactivity with E, DA, serotonin, or L-dopa.

**Monoamine oxidase (MAO) Assay.** The MAO activity of the tissue homogenate was determined using tryptamine<sup>23</sup> and serotonin.<sup>24</sup> The protein content of the tissue homogenate was determined by a colorimetric method.<sup>25</sup> The specific activity of MAO is expressed as picomoles of product formed per milligram of tissue protein per minute.

**Tissue monoamine content.** The protein content of the islets were determined with the Lowry method,<sup>25</sup> and the wet weight of the islets was calculated on the basis of the protein content being 15% of their wet weight.<sup>26</sup> The median eminence and cortex were weighed. The catecholamine quantities were expressed as micromoles per kilogram of wet weight.

**Statistical methods.** The basal monoamine content of the



**FIGURE 2.** Log scale showing NE concentration in the golden hamster islets, median eminence, and cortex in micromoles per kilogram of wet weight. Each point represents the measurement from one hamster; the horizontal line represents the mean of each group.

various tissues had a small variance; however, L-dopa treatment resulted in a larger variance. Since the standard deviation in the original scale varied directly as the mean in this situation, the logarithmic transformation was used to stabilize the variance.<sup>27</sup> The data were then plotted on a semi-log graph. The logarithmic transformed data was tested for significance by analysis of variance and Scheffe's multiple comparison procedure.<sup>28</sup>

## RESULTS

**Dopamine measurement.** Figure 1 plots the dopamine quantities in the islets, cerebral cortex, and median eminence in micromoles per kilogram of wet weight. Under basal conditions, DA is present in measurable quantities in all three tissues (islets  $\bar{X} = 7.46 \pm 1.62$ , cortex  $\bar{X} = 0.60 \pm 0.20$ , median eminence  $\bar{X} = 4.62 \pm 0.67$ ) and, in the islets, is on a comparable level of concentration with the median eminence.

The DA in the islets, cerebral cortex, and median eminence showed a similarity in effect from all treatments (tran alone, L-dopa alone, and L-dopa + tran together) when compared with normal levels. MAO is present in these tissues, and the activities in picomoles of indoleacetic acid per milligram tissue protein per minute, using  $100 \mu\text{M}$  tryptamine as substrate, are: islets 2273, cerebral cortex 337, and median eminence 662. Tranylcypromine, a monoamine oxidase inhibitor, was employed in order to inactivate the major degradation pathway of the catecholamines in the tissues.<sup>29</sup> The tran dosage used inhibited islet MAO 90%, ce-

rebral cortex MAO 83%, and median eminence MAO 69%. Using tran alone, however, no build-up of DA was affected in any of these tissues over control values.

L-dopa was used in order to promote synthesis of the catecholamines. The enzyme decarboxylase is present in these tissues.<sup>29</sup> Pretreatment of the hamsters with L-dopa does demonstrate a significant rise in DA in the islets and median eminence over normal ( $P < 0.01$ ) and tran alone ( $P < 0.01$ ) values. This increase was almost two times greater in the islets than in the median eminence. Along with low crossreactivity of L-dopa, the fact that the L-dopa-induced DA rise did not occur with treatment with a peripheral decarboxylase inhibitor, Ro-4-4602,<sup>30</sup> in the islets ( $\bar{X} = 5.85$ ) and in the median eminence ( $\bar{X} = 7.15$ ), confirms that the DA is truly generated by the decarboxylation of L-dopa.

Treatment with both tran and L-dopa heightens the increase in DA in the islets and results in a significant rise in DA in the cortex over the values produced by L-dopa values alone ( $P < 0.01$ ), tran values alone ( $P < 0.01$ ), and control values ( $P < 0.01$ ). Although the mean L-dopa + tran concentration was higher in the median eminence than control values and tran alone values, it did not reach significance.

**Norepinephrine measurement.** Figure 2 plots the norepinephrine concentrations in micromoles per kilogram of wet weight in the hamster islets, cerebral cortex, and median eminence. It is easily seen that the NE is in significantly higher basal concentrations in the islets ( $\bar{X} = 42.07 \pm 8.07$ ) than in either the median eminence ( $\bar{X} = 5.66 \pm 0.55$ ) ( $P < 0.01$ ) or cerebral cortex ( $\bar{X} = 1.62 \pm 0.14$ ) ( $P < 0.01$ ). NE is also in higher concentration than DA in the islets ( $P < 0.01$ ), median eminence ( $P < 0.01$ ), and cortex ( $P < 0.01$ ). NE, however, does not exhibit a build-up in the islets with injection of the precursor or MAO inhibitor or both. The NE levels were significantly increased in the median eminence over basal values ( $P < 0.01$ ) and L-dopa values ( $P < 0.01$ ), however, by pretreatment with tran alone and to the same degree with tran + L-dopa, suggesting that, in the median eminence, the MAO inhibitor and not the precursor amine is promoting this accumulation. NE in the cerebral cortex was significantly higher when the hamster was pretreated with both tran and L-dopa than when pretreatment was control, L-dopa alone, or tran alone.

## DISCUSSION

Histochemical fluorescence studies involving either inhibition of a degradation pathway or synthesis from a precursor amine have yielded indirect evidence of the existence of biogenic amines and their uptake into the islets.<sup>4,31,32</sup> Using radioenzymatic assays that were specific, sensitive, and reproducible, we were able to measure precisely the DA and NE concentrations in golden hamster pancreatic islets. The assays were sensitive (defined as 50% over blank) down to DA (100 pg) and NE (33 pg) with linearity tested to DA (2.5 ng) and NE (5 ng). The DA assay, based on the O-methylation of the catecholamine by COMT, was modified from the method proposed by Coyle and Henry<sup>20</sup> in order to decrease the crossreactivity with NE. As has been previously noted, an unknown factor in tissue extracts affects the enzymic reaction and varies from one tissue to another.<sup>33</sup> We were unable to identify the cause of this inhibi-

tion. In order to accurately calculate the unknown sample, taking the tissue factor inhibition into consideration, we used internal standards with every sample. The quantity of  $^3\text{H-SAM}$  used was optimized for each new batch of COMT enzyme purified in order to correlate blank values with reserpined liver, a tissue depleted of catecholamines.<sup>34</sup> We avoided the use of calcium in the isolation of the islets,<sup>18</sup> since it is known that calcium inhibits the COMT reaction.<sup>33</sup>

Past studies have measured catecholamines in high concentrations (especially of dopamine) in the median eminence of the rat brain.<sup>17,35,36</sup> In the golden hamster pancreatic islets, we have discovered the islet catecholamine concentration to be significantly greater than the value found in median eminence for NE and, for DA, to be on a comparable level with the amount in the median eminence. This supports the conclusion, gained by histochemical observations, that the islets contain a very significant amount of these biogenic amines.

MAO and catechol-O-methyltransferase are the main enzymes responsible for the inactivation *in vivo* of dopamine and norepinephrine.<sup>37</sup> We have shown that MAO is present in the hamster pancreatic islets and that it is involved in catecholamine inactivation.<sup>38</sup> Although there are alternate pathways for monoamine degradation<sup>37,39</sup> and although inhibition by MAO inhibitor may enhance the use of another degradation pathway,<sup>39</sup> we wanted to quantitate whether a significant accumulation over basal values of DA or NE can occur with MAO inhibition. We also heightened the accumulation by use of L-dopa, a precursor of the catecholamines.<sup>37</sup> Since the islets are capable of L-dopa uptake<sup>16</sup> and decarboxylation,<sup>29</sup> this promotion in the monoamine synthesis combined with MAO inhibition did aid in establishing the significant DA rise. This accumulation of DA in the islets is interesting when correlated with evidence that use of MAO inhibitor (tran) potentiates the L-dopa inhibition of insulin secretion.<sup>6,8,38,40</sup>

In the median eminence, it is interesting to note that the DA values were significantly increased with L-dopa alone, the tran not increasing this effect. Whereas, the NE values in this tissue were increased significantly only by tran, the L-dopa did not increase the value. This is in contrast to the findings of a human blood pressure response study<sup>41</sup> that the DA pressor effect is augmented and prolonged to a much greater degree with MAO inhibition than is the NE pressor effect. Species difference and use of different MAO inhibitors may possibly account for this difference.

Both formaldehyde induced fluorescence techniques and electron microscopic autoradiographic techniques indicate that there is an accumulation of DA in pancreatic islets following the administration of L-dopa.<sup>4,6,31,32</sup> The accumulation of DA is prevented by the pretreatment of the animals with a decarboxylase inhibitor.<sup>6,11,31</sup> The administration of L-dopa also results in inhibition of insulin secretion in rabbits,<sup>11</sup> mice,<sup>6</sup> and human subjects.<sup>40</sup> The L-dopa-induced inhibition of insulin secretion is prevented by pretreatment of rabbits and mice with the decarboxylase inhibitor RO-4-4602.<sup>6,11</sup> In a previous study carried out with an *in vitro* golden hamster pancreas system, we demonstrated that the inhibitory potency of NE on glucose-stimulated insulin secretion was 24% that of DA and that the inhibitory potency of L-dopa on glucose stimulated insulin secretion was 5% that of DA.<sup>8</sup> In the present study we have

demonstrated that the administration of tran and L-dopa results in an 87-fold increase in islet DA with no increase in islet NE. These biochemical measurements, along with the previously noted pharmacologic and morphologic studies, support the notion that DA is responsible for L-dopa-induced inhibition of insulin secretion.

By using the differences in excitation/emission fluorescence spectrum, investigators have been able to distinguish between serotonin and catecholamines.<sup>31</sup> By utilizing the differential response of the fluorescent spectrum of NE and DA after exposure of tissues to HCl vapor these investigators can differentiate these two monoamines.<sup>31</sup> Kopin et al. recently evaluated the NE and DA content of different regions of the brain in normal rats and in rats receiving varying doses of the catecholamine depleting agent reserpine.<sup>17</sup> They compared the catecholamine content using the radioenzymatic method employed in the present study with the visual estimation of formaldehyde histofluorescence and found substantial differences in estimates of catecholamine using the two techniques. In areas of the brain with a high catecholamine content, over 50% depletion is required before there is a discernible decrease in fluorescence intensity of the fibers and most of the fluorescence disappears when 20% of the normal content remains. In areas with a normally low catecholamine content, decreased fluorescence intensity is apparent with relatively slight (20%) decrease in amine content. In such areas fluorescence disappears when about one-third to one-half of the catecholamine content is depleted.<sup>31</sup> Since it is likely that the relationship between catecholamine content and histochemical fluorescence will be only semiquantitative for other tissues, we felt it would be fruitful to evaluate the monoamine content of pancreatic islets with quantitative radioenzymatic methods of analysis.

## CONCLUSION

Using sensitive and quantitative radioenzymatic assays we have demonstrated that the pancreatic islets have a high concentration of NE and DA when compared with other monoaminergic endocrine tissues such as the median eminence. This study also shows that the concomitant administration of tran (to inhibit DA degradation) and L-dopa (a catecholamine precursor) results in a large increase in islet DA with no increase in islet NE.

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