AMINES AND THE RAT EXOCRINE PANCREAS: (2)
EFFECTS OF RECEPTOR BLOCKERS ON 
TURNOVER OF L-5HTP

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Abstract—The metabolism of L-5HTP by the rat exocrine pancreas, and effects of blockers on the metabolism were studied by fluorescent histochemical and chemical methods. Histochemically, 5-hydroxytryptamine (5-HT) blockers (methysergide and cyproheptadine) and dopamine (DA) blockers (haloperidol and sulpiride) produced no apparent changes in fluorescence pictures after injection of L-5HTP. α-blockers (phenoxybenzamine and phentolamine) and monoamine oxidase (MAO) inhibitor (iproniazide) produced an increased accumulation of 5-HT fluorescence in the apical regions of acinar cells where the zymogen granules are stored. Chemically, the 5-HT blockers decreased the 5-HT content after injection of L-5HTP. Sulpiride had no effect. Haloperidol decreased the 5-HT content. MAO inhibitor resulted in a vast accumulation of 5-HT. Some differences were noted between the L-5HTP metabolism and that of L-dopa: e.g. (1) L-5HTP was more slowly eliminated, and (2) 5-HT blockers produced a decreased content of 5-HT after injection of L-5HTP, in contrast to the finding that DA-blockers produced an increased content of DA after injection of L-dopa. The mechanism responsible for the differences is discussed in relation to the possible pharmacological effects of L-5HTP and L-dopa on the secretion from the exocrine pancreas of rats.

The exocrine pancreas has a considerable capacity to take up amino acids and analogues of amino acids (1-3) from the blood, presumably because of its high protein synthesizing capacity. By histochemical and chemical methods it has been shown that the amino acids L-dopa and L-5HTP are taken up and handled by the acinar cells in a special way (4-8). Our preliminary studies showed that L-dopa was metabolized rapidly by the exocrine pancreas of the rat and the turnover of L-dopa was decreased significantly by pretreatment with DA- or α-blockers. It was suggested that there was a dopaminergic or α-adrenergic mechanism in the rat exocrine pancreas which regulated the enzyme secretion.

In the present study, we studied histochemically and chemically the L-5HTP metabolism by the exocrine pancreas of the rat and effects of receptor blockers on the metabolism, in an attempt to determine whether or not there are differences between L-dopa and L-5HTP metabolism, or whether there is a serotonergic mechanism.

MATERIALS AND METHODS

Animals used: Male Sprague-Dawley rats were used. Animals used for histochemical
and chemical studies weighed 230–290 g while animals used for collection of the pancreatic juice weighed 350–420 g. The animals were given a standard diet and water *ad libitum* before experiments. In the histochemical studies, 3–6 animals were used per group. The number of animals used in the chemical studies are shown in the Results.

**Histochemical and chemical studies:** Drugs were given i.v. to rats, and after various times L-5HTP was given i.v. Control animals were given vehicles only. The animals were sacrificed by a blow on the neck at various times after the injection of L-5HTP, and the pancreas was excised. Fluorescent histochemistry was performed according to the method of Falck and Hillarp (9). The chemical determination of 5-HT was made fluorometrically according to the method of Snyder et al. (10).

**Fluorescence microscopy:** After injection of L-5HTP, there appeared specific yellowish fluorescence in the exocrine pancreatic cells. The fluorescent picture was determined by two criteria; The intensity of the fluorescence and the cellular localization. If the fluorescence appeared more or less uniformly over the cells and could not be restricted to any cellular structure, it was referred to as diffuse fluorescence. When the fluorescence was confined to zymogen granules and appeared coarse, it was referred to as granular fluorescence.

**Chemical determination of 5-HT and 5-HIAA in the pancreatic juice:** Under pentobarbital Na (30 mg/kg i.p.) anesthesia, bile free pancreatic juice was obtained according to the method of Grossman (11). A bypass for the bile to enter the duodenum was provided. After the operation the animals were placed in restraining cages of Bollman type with access to food and water, and were used for the experiments in the next morning. After i.v. administration of L-5HTP 60 mg/kg, the juice was collected in a test tube for 90 min. Test tube contained 1 ml of 0.4 N perchloric acid (PCA), to which EDTA (0.1%) and ascorbic acid (0.4 mg/ml) had been added. The samples were stored frozen until assay. The concentrations of 5-HT and 5-HIAA were determined chemically according to the methods of Snyder et al. (10) and Udenfriend et al. (12), respectively.

**Substances used:** L-5HTP (Sigma) was dissolved in 0.9% saline with the aid of a minimum amount of 1N HCl and gentle warming. Sulpiride (Delagrange) and haloperidol (Janssen) were dissolved in 0.9% saline with the aid of a minimum amount of 1N H2SO4 and 50% (v/v) acetic acid, respectively. Iproniazide phosphate (Aldrich), cyproheptadine hydrochloride (Nihon Merck Banyu), methysergide bimaleate (Sandoz) and phenoxybenzamine hydrochloride (Tokyo Kasei) were dissolved in 0.9% saline. Phentol amine mesylate (Regitin®, CIBA-Geigy) was diluted with 0.9% saline.

**Treatments with drugs:** When effects of drugs on the L-5HTP metabolism by the exocrine pancreas were studied, the drugs were given by respective schedules as following: iproniazide (150 mg/kg) 3 hr, cyproheptadine (4 mg/kg) 10 min, methysergide (1 mg/kg) 10 min, sulpiride (32 mg/kg) 10 min, haloperidol (2 mg/kg) 10 min, phenoxybenzamine (8 mg/kg) 10 min, and phentolamine (8 mg/kg) 10 min before injection of L-5HTP (60 mg/kg). Doses were expressed in terms of salts. Iproniazide was given s.c. in a volume of 0.5 ml/100 g body weight, and other drugs into one of the lateral veins, in a volume of 0.2 ml/100 g body weight.
**RESULTS**

**Fluorescence microscopy:** In the untreated pancreas, the specific yellow fluorescence due to 5-HT occurred only in a few cells in the largest pancreatic ducts, and no specific fluorescence was observed in the pancreatic acinar cells (Fig. 1a). After injection of L-5HTP, there appeared a yellowish fluorescence in the exocrine pancreatic cells. Little or no specific fluorescence occurred in the connective tissue. In a pilot study, L-5HTP was given in two different doses to rats (60 and 120 mg/kg) and the animals were sacrificed after 10, 20, 40, 60, 90, and 120 min. Up to 40 min after the injection, the intense specific fluorescence was diffusely distributed throughout the acinar cells. Specific fluorescence also occurred in the apical parts of some acini as densely aggregated coarse granules 60-120 min after the injection. In contrast to the evidence obtained after injection of L-dopa (our preliminary study) in which granular fluorescence predominated at those time intervals studied, diffuse fluorescence in the cytoplasm always predominated after injection of L-5HTP and only some acini contained granular fluorescence. Both doses of 60 and 120 mg/kg produced nearly the same morphological pictures. Therefore, in the following experiments, L-5HTP was injected at a dose of 60 mg/kg in order to study effects of drugs (Fig. 1b).

**Histochemical study of effects of drugs:** Pretreatment with MAO inhibitor iproniazide produced a very strong and wide-spread granular fluorescence as well as diffuse fluorescence all over the acini at 20 and 60 min after the injection of L-5HTP (Fig. 1c). Neither 5-HT blockers (methysergide and cyproheptadine), nor DA-blockers (haloperidol and sulpiride) exerted any influence on the fluorescent pictures after injection of L-5HTP (Fig. 1d, e). Pretreatment with α-blockers (phenoxylbenzamine and phentolamine) resulted in strong granular fluorescence in a few acini, and weak granular fluorescence in the remaining wider area of acini at 20 and 60 min after injection of L-5HTP (Fig. 1f). The diffuse fluorescence in the cytoplasm was also stronger than that of control.

**Chemical determination of the pancreatic 5-HT contents:** Results are shown in Table 1. The untreated pancreas contained only a very small amount of 5-HT. Effects of drugs were studied in two series of experiments. In the first series of experiments, the times of observation were the same as those of the histochemical study. In the control group, the 5-HT content decreased gradually from 20 to 60 min after injection of L-5HTP. The reduction in this period was about 35%. Methysergide produced a slight, not significant decrease at 20 min, and a marked and significant decrease at 60 min. The reduction during this period was about 65%. Haloperidol produced a significant decrease of the 5-HT content at both times. On the contrary, iproniazide produced a remarkable increase in the 5-HT content. In the second series of experiments, the 5-HT contents at 5 and 10 min after injection of L-
5HTP were also studied to obtain more detailed time courses. As is evident in the Table, the pancreatic 5-HT content in the control group gradually decreased over a period of 60 min. For unknown reasons, the control values at both 20 and 60 min after injection of L-5HTP were consistently lower than those in the first series of experiments. Cyproheptadine produced a significant decrease at 5, 10, and 60 min, whereas sulphiride was without effect.

Chemical determinations of the 5-HT and 5-HIAA contents in the pancreatic juice: Results are shown in Table 2. The pancreatic juice collected from untreated rats contained no detectable amounts of 5-HT and 5-HIAA. In the juice collected from rats given L-5HTP 60 mg/kg, the concentrations of 5-HT and 5-HIAA were 0.73 ± 0.17 and 1.59 ± 0.60 µg/ml, respectively.
DISCUSSION

Our results showed that there were differences between L-dopa and L-5HTP metabolism in the exocrine pancreas of rats. First, L-5HTP appears to be more slowly eliminated than L-dopa. The pancreatic 5-HT concentrations of untreated rats at 20 and 60 min after injection of L-5HTP 60 mg/kg were 38.15±3.83 (n=11) and 22.55±2.85 (n=11) μg/g, respectively. On the other hand, the concentrations of DA at the corresponding times after injection of L-dopa 50 mg/kg were 10.50±0.97 (n=10) and 2.26±0.54 (n=10) μg/g,
respectively. Moreover, when the amounts of metabolites of L-5HTP and L-dopa in the pancreatic juice collected during the same period after injection of roughly an equal dose of L-5HTP and L-dopa, respectively, were compared, the amount of metabolite of L-5HTP was much smaller.

Table 1. Effects of drugs on the pancreatic 5-HT contents after i.v. administration of L-5HTP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Contents of 5-HT (µg/g wet weight)</th>
<th>Time after injection of L-5HTP (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>—</td>
<td>0.07 ± 0.03 (5)</td>
<td>5</td>
</tr>
<tr>
<td>L-5HTP alone</td>
<td>60</td>
<td>Not assayed</td>
<td>48.7 ± 4.5 (5)</td>
</tr>
<tr>
<td>Methysergide</td>
<td>1</td>
<td>Not assayed</td>
<td>44.6 ± 4.5 (5)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>2</td>
<td>Not assayed</td>
<td>21.4 ± 3.5 (5)*</td>
</tr>
<tr>
<td>Iproniazide</td>
<td>150</td>
<td>Not assayed</td>
<td>120.8 ± 10.9 (5)**</td>
</tr>
<tr>
<td>L-5HTP</td>
<td>60</td>
<td>37.0 ± 0.6 (6)</td>
<td>29.4 ± 2.5 (6)</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>4</td>
<td>29.4 ± 2.7 (6)*</td>
<td>21.6 ± 1.6 (6)*</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>32</td>
<td>34.6 ± 2.9 (6)</td>
<td>34.8 ± 4.5 (6)</td>
</tr>
</tbody>
</table>

Drugs, except iproniazide, were given i.v. 10 min before L-5HTP. Iproniazide was given s.c. 3 hr before injection of L-5HTP. Values are mean ± S.E. Number of animals in parentheses. Significantly different from corresponding values; * at p < 0.05, ** at p < 0.01.

Table 2. Concentrations of 5-HT and 5-HIAA in the pancreatic juice after i.v. administration of L-5HTP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>N</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>5</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>
| L-5HTP    | 60         | 9 | 0.73 ± 0.17           | 1.59 ± 0.60

The pancreatic juice was collected for 90 min after i.v. administration of L-5HTP or saline. N: Number of animals. Values are mean ± S.E.

respectively. Moreover, when the amounts of metabolites of L-5HTP and L-dopa in the pancreatic juice collected during the same period after injection of roughly an equal dose of L-5HTP and L-dopa, respectively, were compared, the amount of metabolite of L-5HTP was much smaller.

Second, immense granular fluorescence as after injection of L-dopa did not develop after injection of L-5HTP. Third, the effect of iproniazide, a MAO inhibitor, was more remarkable in the L-5HTP metabolism. Actually, granular fluorescence due to 5-HT, which had a wide distribution and high density comparable to that of DA after L-dopa injection, was obtained only after the pretreatment with iproniazide. Fourth, 5-HT was excreted mainly after being metabolized to 5-HIAA, whereas a considerable amount of DA was found in the juice. Finally, 5-HT blockers produced a decrease in 5-HT contents after injection of L-5HTP, in contrast to the finding that DA-blockers produced an increase in DA contents after injection of L-dopa.

The third and fourth differences indicate that MAO is more important in the enzymatic degradation of 5-HT than in that of DA. This may be partly because COMT is available as an alternate enzyme in the case of DA. The pancreas contains both MAO (13) and COMT

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However, the difference between the enzymatic degradation of DA and 5-HT cannot explain why 5-HT did not densely accumulate in the apical part of acinar cells as did DA, despite the extended presence of 5-HT in the pancreas. Moreover, the longer 5-HT remains in the pancreas, the more 5-HT is apt to be deaminated by MAO, and 5-HIAA could be the main metabolite.

The majority of DA and its metabolites appeared to be excreted from the acinar cells by the same route as the digestive enzymes, or by mediation of the discharge of zymogen granules. Thus, shortly after injection of L-dopa, the DA formed from L-dopa was found to be diffusely distributed in the cytoplasm, somewhat later confined to the zymogen granules, and gradually disappeared leaving large amounts of DA and metabolites in the pancreatic juice. The paucity of granular 5-HT fluorescence, and of 5-HT and its metabolite in the juice raises the possibility that the emptying into the acinar lumina is not the main route of excretion of 5-HT from the acinar cells.

The apical regions of almost all acinar cells had a fluorescence intensity comparable to that seen in the cytoplasm. A certain amount of 5-HT was detected in the pancreatic juice. Iproniazide produced an intense accumulation of 5-HT at the apical part of acinar cells. From these results it seems probable that 5-HT is also confined, to a certain extent, to zymogen granules. The 5-HT blockers decreased the 5-HT contents after injection of L-5HTP, and such probably occurs by inhibiting the uptake of L-5HTP by exocrine pancreas. However, the inhibition of L-5HTP uptake may not be so important, because cyproheptadine and methysergide reportedly have only a weak effect on the amine uptake by platelets (15, 16, 17). There is the possibility that the blockers induced an increased turnover of L-5HTP. If 5-HT has an inhibitory effect on the secretion of zymogen granules (carriers of 5-HT), and the blockers inhibited the action of 5-HT, there would be a decrease in 5-HT contents. In fact, in our preliminary experiments, L-5HTP did significantly suppress the protein secretion from the exocrine pancreas of rats.

Sulpiride, a DA-blocker, did not modify the 5-HT concentration after injection of L-5HTP. However, pretreatment with another DA-blocker, haloperidol, resulted in a significant reduction of the 5-HT content. Recently, some neuroleptics, including haloperidol, have been reported to block 5-HT receptors as well as DA-receptors (18, 19). Therefore, it can be speculated that haloperidol with its antiserotonergic activity induced the change. Haloperidol also produced a decrease in the 5-HT content by inhibiting the uptake of L-5HTP by the exocrine pancreas; haloperidol is reported to have an inhibitory effect on the amine uptake by neuronal membrane (20, 21) or adrenal chromaffin granules (22). Sulpiride appears not to have such an effect (23, and our unpublished data).

It is noteworthy that with administration of α-blockers there was an increase in the accumulation of granular fluorescence of both 5-HT and DA, although the effect was more conspicuous in that of DA. To explain the differences between the L-5HTP and L-dopa metabolism, and the effects of blockers, we are now studying the effects of L-dopa, L-5HTP, and the blockers on the exocrine pancreatic secretion.

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REFERENCES


8) ALM, P.: Fluorescence microscopy of the 5HTP turnover in the exocrine pancreas of mice and rats. Z. Zellforsch. 96, 212-221 (1969)


