L-α-Methyl-α-hydrazino-β-(3,4-dihydroxyphenyl)propionic Acid: Relative Lack of Antidecarboxylase Activity in Adrenals

Abstract. *In rats previously treated with a monoamine oxidase inhibitor, the administration of 5-hydroxytryptophan results in increases in concentrations of 5-hydroxytryptamine in kidney, brain, and adrenal glands. When the peripheral* L-*aromatic amino acid decarboxylase inhibitor,* L-α-*methyl-α-hydrazino-β-(3,4-dihydroxyphenyl) propionic acid (HMD) is administered prior to 5-hydroxytryptophan, the concentration of 5-hydroxytryptamine in kidneys does not rise, that of the brain increases slightly, and that of the adrenal rises markedly. This indicates that although the adrenal gland is a peripheral organ, it does not respond in the typical manner to the antidecarboxylase action of HMD. These results suggest that HMD does not gain free access into the adrenal medulla and that a possible "blood-adrenal barrier" may exist to this compound.*

In order to decrease the peripheral side effects and the large doses of L-dopa needed in the treatment of Parkinson's disease, a current trend in clinical investigations has been to employ a peripheral decarboxylase inhibitor in conjunction with L-dopa (*1*). While the initial results appear encouraging, the effects of the peripheral decarboxylase inhibitors themselves are not fully known. Presently, two compounds are prominent as peripheral inhibitors; these are L-α-methyl-α-hydrazino-β-(3,4-dihydroxyphenyl) propionic acid (often designated as L-α-hydrazino-α-methyldopa or HMD) and *N'*-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (RO 4-4602). This report is concerned with the former compound, HMD, and its relative lack of antidecarboxylase activity in the adrenal gland of rats.

Female Sprague-Dawley rats (200 to 250 g) were pretreated with pargyline HCl (25 mg per kilogram of body weight, intraperitoneally) as the base, a monoamine oxidase inhibitor. Eighteen to 24 hours later the animals were given either 20 mg/kg of DL-5-hydroxytryptophan (5HTP), L-α-methyldopa plus 5HTP, or HMD plus 5HTP. L-α-Methyldopa and HMD were given intraperitoneally in doses of 50 mg/kg and 25 mg/kg, respectively, and the time interval between the inhibitors and 5HTP was usually 45 minutes. Animals were killed 90 minutes after the administration of the 5HTP, unless otherwise specified. In this study 5HTP, rather than dopa, was employed since it is decarboxylated by the same enzyme and since 5-hydroxytryptamine (5HT) is more readily measurable than dopamine, which can undergo further transformation to other catecholamines. The 5HT was assayed by the fluorimetric method as described by Udenfriend *et al.* (*2*). Care was taken to remove excess 5HTP that might be present by washing the butanol extract twice with borate buffer. Preliminary determinations with added 5HTP assured us that the washings were adequate and that the precursor was not interfering with the 5HT assay.

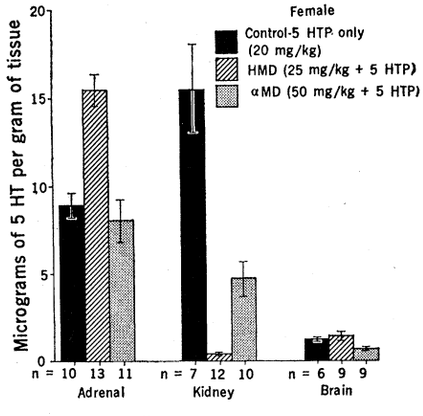

Fig. 1. The effect of HMD and α-methyldopa (*αMD*) on concentrations of 5HT in rat adrenal, kidney, and brain after administration of 5HTP. Animals had been treated with pargyline HCl (25 mg/kg of base) 18 to 24 hours previously. Vertical lines indicate the standard deviation of mean values.

The adrenal, and especially the kidney, of animals given 5HTP exhibit elevated concentrations of 5HT of 9.0 and 15.6 μg per gram of tissue, respectively (Fig. 1). The brain concentration amounts to some 1.3 μg per gram of tissue, which represents about a 150 percent increase above control animals (those not treated with 5HTP). After treatment with HMD plus 5HTP, the adrenal 5HT rises to almost twice control values (to 15.5 μg/g). Kidney 5HT falls almost to zero, and the concentration of brain 5HT remains approximately the same as the 5HTP controls. After administration of α-methyldopa the adrenal concentrations of 5HT do not change from those of the controls, but the kidney concentrations were decreased to 30 percent of controls. Brain 5HT exhibited essentially no change. The unusual finding in this data is the apparent lack of action of HMD on the adrenal gland. In fact, rather than inhibition there is a marked increase in 5HT concentrations in the gland, most probably resulting from a greater amount of 5HTP becoming accessible because of inhibition of aromatic amino acid decarboxylase in other peripheral tissues, such as the kidney, liver, and others. Surprisingly, the concentrations of 5HT in the brains of animals treated with HMD were not significantly higher than those in 5HTP controls, although the behavioral effects in these animals were much more severe than in the controls. This minimal difference in 5HT concentrations between animals treated with HMD plus 5HTP and those treated with 5HTP agrees with the findings of Bartholini *et al.* (*3*).

Our first impression was that the HMD was not reaching the adrenal gland to inhibit aromatic amino acid decarboxylase, or that the adrenal enzyme was not sensitive to the agent. In order to eliminate the latter possibility, in vitro determination of brain, kidney, and adrenal homogenates, in which we used concentrations of HMD and α-methyldopa ranging from $10^{-7}M$ to $10^{-5}M$, were carried out. All of these tissues exhibited similar sensitivities to the inhibitory effects of HMD

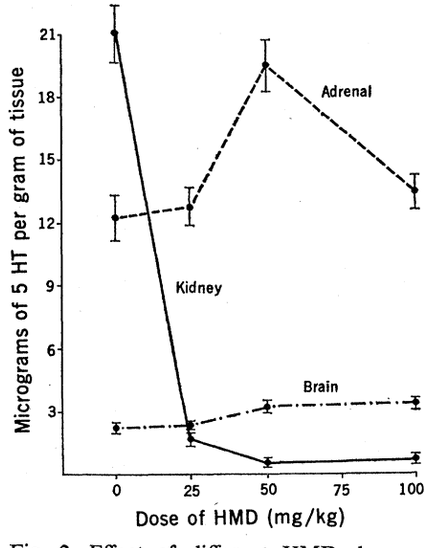

Fig. 2. Effect of different HMD dosages on the concentrations of 5HT in rat adrenal, kidney, and brain after administration of 5HTP. Each point represents mean values obtained from four to eight animals ± the standard error.

and α-methyldopa, although in all cases HMD was a much more active inhibitor.

When doses of HMD of 25, 50, and 100 mg/kg were compared for their antidecarboxylase activities in vivo, again it was the adrenal that displayed an unusual dose response curve, while the kidney and brain exhibited the expected responses (Fig. 2). In these experiments, HMD at 25 mg/kg followed by 5HTP (20 mg/kg) was ineffective in lowering adrenal and brain 5HT below 5HTP concentrations of controls, but kidney 5HT was decreased almost completely with this and all higher doses. At a dose of HMD of 50 mg/kg, adrenal 5HT increased markedly, while brain 5HT increased only slightly. When the high dose of HMD of 100 mg/kg was employed the adrenal 5HT again returned to near 5HTP control levels, and brain 5HT was raised slightly further (Fig. 2). Although the brain concentrations of 5HT were not raised greatly under these conditions, the turnover rate must have been greatly increased, for the animals exhibited extreme agitation and excitation.

When 5HTP is administered to rats, even those previously treated with monoamine oxidase inhibitors, peak brain concentrations of 5HT are reached 1 to 2 hours after 5HTP and subsequently fall over the following 14 to 16 hours (*4*). The effect of time, therefore, was measured in animals pretreated 18 to 24 hours with pargyline HCl followed by 25 mg of HMD and 20 mg of 5HTP per kilogram of body weight and compared with animals with the same regimen, but without HMD treatment. As shown in Fig. 3, adrenal and brain 5HT concentrations in 5HTP controls peaked at 90 minutes, and thereafter began to descend, while kidney 5HT concentrations peaked early (30 minutes) and then fell rapidly. In animals given HMD the brain and adrenals followed the initial rise in 5HT concentrations that was observed in control animals, but these concentrations continued to increase even after 180 minutes. The adrenals exhibited the greatest increase at this time, reaching levels of about 25 μg of 5HT per gram of tissue. Kidney 5HT concentrations in these animals, as expected, remained low throughout the experimental period, although there was a concentration of up to 5 μg/g at the initial 30-minute period, which fell rapidly thereafter. Control kidneys, while beginning at a high concentration, fell rapidly to concentrations similar to those of animals treated with HMD by 180 minutes.

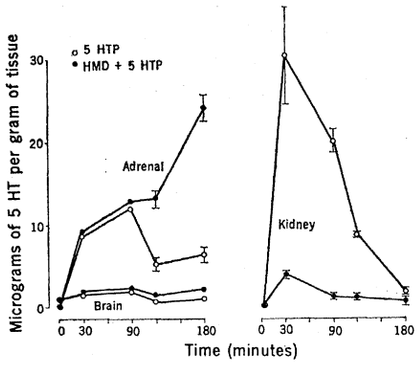

Fig. 3. Effect of varying time on the concentrations of 5HT in rat adrenal, kidney, and brain after administration of 5HTP. Each point represents mean values obtained from three to nine animals ± the standard error.

It should be noted that while brain 5HT concentrations in the HMD animals do not exhibit marked increases, the behavioral events are quantitatively different during the 3-hour period. Initial symptoms of increased locomotion, tremors, pawing, salivation, and hyperthermia are clear at 90 minutes, most severe at 120 minutes, and become less intense at 180 minutes. It again points out the lack of correlation between brain concentrations of 5HT and the behavioral effects produced by 5HTP.

These studies indicate that the adrenal gland, presumably the medulla, does not respond to HMD, a peripherally acting decarboxylase inhibitor, like a typical peripheral organ. That HMD inhibits primarily in the periphery is well documented (*5*). It appears that the adrenal medulla may present an exception in this case. Not only is there an absence of inhibition of adrenal decarboxylase, especially at doses up to 50 mg/kg, but there is an actual enhancement of 5HT synthesis, probably resulting from an increased availability of 5HTP to the adrenals. Whether at the dose of 100 mg/kg HMD penetrates the adrenals has yet to be determined. It may represent inhibition, but it may also be acting to interfere with the transport of 5HTP into the adrenals.

If we assume that human and rat adrenals are similar in this regard, these findings may also be of clinical importance if HMD is to be used extensively as an adjunct to dopa therapy in Parkinson's disease. While HMD has been demonstrated to have beneficial effects in this disorder, the increased synthesis of amines in the adrenals as described above may contribute to an indirect secondary action of HMD. It should be pointed out, however, that our experiments were carried out in rats previously treated with a monoamine oxidase inhibitor.

Finally, and perhaps most important, is the indication of a possible "blood-adrenal barrier" to certain drugs. To our knowledge, there has not been a formal proposal of a "blood-adrenal barrier" to drugs, although there are many known examples of drugs that act at peripheral adrenergic nerve terminals but not on the adrenal medulla. Thus, compounds such as bretylium, guanethidine, and 6-hydroxydopamine, being poorly lipid soluble compounds, do not significantly penetrate the blood-brain barrier. However, they are active at adrenergic nerve terminals but not on the adrenal medulla. Therefore, HMD resembles these compounds in these properties. Whether all of these compounds are unable to act on the adrenals because of a common "blood-adrenal barrier" remains to be investigated. In any event, HMD represents one agent that appears unable to penetrate the adrenal medulla to the extent that it penetrates other peripheral structures.

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6. We thank Dr. C. C. Porter of the Merck Sharp and Dohme Research Laboratories, West Point, Pa., for generous supplies of HMD. This research was supported by grant MH-02435 from the National Institute of Mental Health.

1 December 1971