Homovanillic and 5-Hydroxyindoleacetic Acids in Cerebrospinal Fluid After Probenecid: Measurement of Brain Monoamine Oxidase Inhibition *in Vivo* (34678)

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There is now considerable evidence that there are several forms of mitochondrial monoamine oxidases (1). In addition, the existence of more than one kind of monamine oxidase (MAO) in brain has been suggested (2–4) and disputed (5). Finally, only a small fraction of the total MAO in heart and other tissues is localized in sympathetic nerve endings (6, 7) and it has been suggested that this fraction is partly localized in NE-containing vesicles (8, 9).

A number of methods have been used to measure the inhibition of MAO *in vivo* produced by inhibitors. The methods used are based on the urinary excretion of amines, such as tryptamine or serotonin and their acid metabolites (10, 11), or in the measurement of enzyme activity in intestinal biopsies (12). It is evident that changes in the activity of MAO measured by the above methods may not reflect changes in the activity of the enzyme at nerve endings.

Metabolites of dopamine (DA) and serotonin (5-HT), such as homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), are found in the cerebrospinal fluid (CSF) of various mammalian species including man (13, 14). The concentrations of these acids are markedly increased after probenecid treatment, presumably because this drug competes with the transport of these acid metabolites out of the CSF to the blood (15). In this study, we have measured the suppression of HVA and 5-HIAA levels in the CSF by pargyline in normal dogs and in animals treated with probenecid as an index of the MAO activity in brain. Inhibition of the probenecid effects was related to the dose of pargyline and was evident in doses as low as 6.25 mg/kg. Pargyline inhibited the metabolism of dopamine more effectively than that of serotonin.

Methods. The experiments were carried out in 9 male beagle dogs, 1–6 yr old, weighing 9–17 kg, over a period of 5 months. Cerebrospinal fluid (2 ml) was taken by a percutaneous puncture from the cisterna magna a few seconds after the onset of light anesthesia with sodium thiamylal (12.5 mg/kg, iv). The samples were frozen immediately and stored at −20° until analysis. This was done within 1 week after sampling.

Control levels of 5-HIAA and HVA were considered the values determined on samples of CSF withdrawn from each dog at the beginning of the experimentation and 2 months after administration of drugs. One week after the first CSF sampling, the 9 dogs were treated intraperitoneally with probenecid (150 mg/kg). This was dissolved in 0.1 *N* NaOH, and the pH of the solution was adjusted with HCl to 7.4.

At various times after the administration of probenecid, HVA and 5-HIAA concentrations in the CSF were determined. Each dog served as a donor of two CSF samples, taken 18–24 hr apart. Probenecid treatment was repeated at weekly intervals until each dog served as a donor of the 1-, 3-, 4-, 6-, and 24-hr CSF samples.

One week after the last control adminstration of probenecid, the 9 dogs were divided into three groups and treated subcutaneously with pargyline in doses of 6, 12.5, and 25 mg/kg, respectively daily for 4 days.

Six hr after the third administration of pargyline, CSF was withdrawn for the assay of 5-HIAA and HVA. Two hr after the fourth dose of pargyline, the animals received probenecid (150 mg) and 4 hr later CSF sam-

pling was repeated. At weekly intervals after stopping pargyline treatment, the basal levels and the probenecid-induced rise of acid metabolites were determined.

Pargyline treatment was repeated 6 weeks after the first cycle. In these experiments, dogs which had received 25, 12.5, and 6.25 mg/kg of pargyline were treated with 6.25, 25, and 12.5 mg/kg, respectively.

Chemical assays. Samples of 2 ml of CSF were acidified with 0.2 ml of 6 *N* HCl, and the HVA and 5-HIAA were extracted into 5 vol of redistilled butyl acetate saturated with solid NaCl and then returned to an aqueous phase by shaking with 2 ml of 0.2 *M* phosphate buffer pH 7.0. The 5-HIAA and HVA were measured fluorometrically according to methods of Giacalone and Valzelli (16) and Anden *et al.* (13), respectively.

Results. Levels of 5-HIAA and HVA in the CSF of control dogs and of dogs treated with probenecid. Figure 1 shows that after the administration of probenecid (150 mg/kg, ip), the concentrations of 5-HIAA and HVA in the CSF rose progressively and reached a peak in about 4 hr. The concentration of 5-HIAA rose from 40 ng /ml to 420 ng/ml, while that of HVA rose from 100 ng/ml to about 725 ng/ml. In 24 hr the levels of these acids had declined to almost the control values.

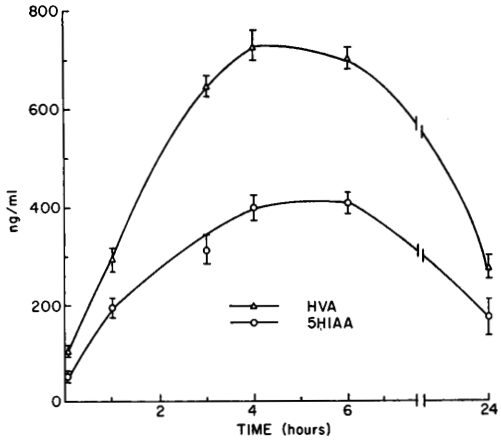

FIG. 1. Basal levels of HVA and 5-HIAA in the CSF of the dog. Time course of the effect of probenecid on the concentrations of these acid metabolites. Probenecid was given intraperitoneally at the dose of 150 mg/kg. Each value is the average ± SE of 9 values obtained from different dogs.

Effect of pargyline on 5 HIAA and HVA concentrations in the CSF and on the probenecid-induced rise of these metabolites. The results of these experiments are summarized in Figs. 2 and 3. The administration of pargyline reduced the control levels of HVA and 5-HIAA and prevented the rise in the levels induced by probenecid. The degree to which pargyline prevented the rise in levels of HVA and 5-HIAA elicited by probenecid

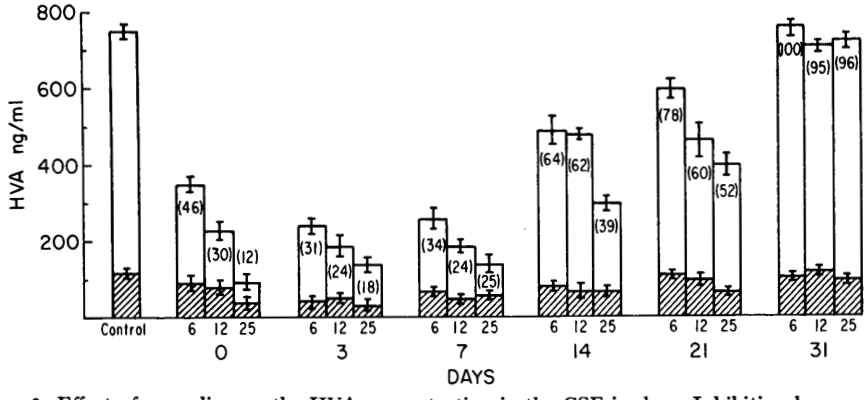

FIG. 2. Effect of pargyline on the HVA concentration in the CSF in dogs. Inhibition by pargyline of the probenecid-induced rise of this metabolite: (shaded columns), basal levels; (open columns), 4 hr after probenecid (150 mg/kg ip.) Numbers in parentheses represent the percentage rise in HVA in respect to controls. Numbers at the foot of each column indicate the dose of pargyline (mg/kg). 0 = during pargyline treatment; 3, 7, 14, 21, and 31 = days after stopping pargyline treatment. Each value is the average ± SE of 6 values obtained from different dogs.

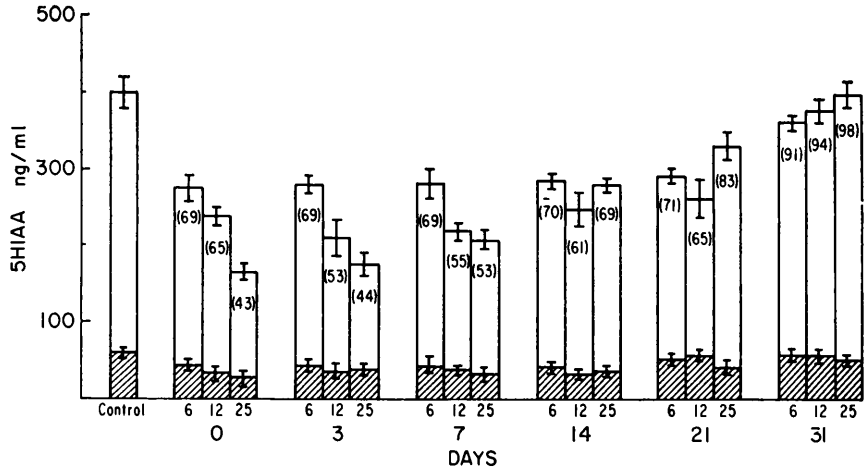

FIG. 3. Effect of pargyline on the 5-HIAA concentration in the CSF in dogs. Inhibition by pargyline of the probenecid-induced rise of this metabolite: (shaded columns), basal levels; (open columns), 4 hr after probenecid (150 mg/kg ip). Numbers in parentheses represent the percentage rise in 5-HIAA in respect to controls. Numbers at the foot of each column indicate the dose of pargyline (mg/kg). 0 = during pargyline treatment; 3, 7, 14, 21, and 31 = days after stopping pargyline treatment. Each value is the average ± SE of 6 values obtained from different dogs.

was dose dependent. A clear effect was evident with doses of pargyline as low as 6.25 mg/kg.

Pargyline prevented the rise in HVA produced by probenecid to a greater extent than the rise in 5-HIAA. The inhibition by pargyline of dopamine and serotonin metabolism was very persistent as shown by the time required for the HVA and 5-HIAA to return to the control values after stopping pargyline treatment. The basal levels of HVA and 5-HIAA returned to normal only after 3–4 weeks and the probenecid-induced rise in HVA and 5-HIAA returned to control levels in about 30 days.

Discussion. Considerable evidence supports the view that HVA and 5-HIAA in CSF originate from brain DA and 5-HT (14, 17) and that the probenecid-induced accumulation in the CSF of these acid metabolites results from a blockade of their efflux from CSF (15). The effect of pargyline in decreasing the basal levels of these acid metabolites, as well as the rise in levels elicited by probenecid, is a reflection of MAO inhibition in brain tissue. The effect of pargyline on probenecid-induced rise in the acid metabolites was dose related and was still measurable in a dose of 6.25 mg/kg. These results indicate that the measurements of the effects on CSF levels can be used as a sensitive indicator of the MAO inhibition in brain *in vivo*.

Since DA is a better substrate for brain MAO than 5-HT (5), the preferential inhibition by pargyline of the probenecid-induced rise in HVA levels relative to that in 5-HIAA suggests the possibility that more than one kind of MAO is present in brain, and that the MAO metabolizing DA is more readily inhibited by pargyline. However, further research would be needed to prove this point. After pargyline treatment ended, the recovery to normal values of the probenecid-induced rise in acid metabolites required several weeks, suggesting a regeneration of brain MAO. This fact is consistent with the time course of recovery of MAO in brain after irreversible MAO inhibitors (18).

The measurements of acid metabolites in the CSF after probenecid are an index not only of brain MAO activity but of the degree of metabolism of a specific monoamine. Indirect methods for measuring MAO inhibition do not give information about MAO activity in brain. Direct measurements of MAO activity in whole brain homogenates also fail to reflect the metabolism of a specific monoamine. It would be interesting to study whether

the central stimulant or depressant activity of certain MAOI, like iproniazid or 1-benzyl-2-methyl-5-methoxytrpytamine (BAS) (19), respectively, orginates from a preferential inhibition of the metabolism of one monoamine.

It seems possible to apply the described method to clinical practice since the levels in the lumbar CSF of HVA and 5-HIAA in man (20) are in the range of the levels in the cisternal CSF of dogs, and probenecid also causes the levels of these acid metabolites to rise in patients (21).

Summary. The effects of inhibition of monoamine oxidase (MAO) by pargyline on the concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) of dogs are reported. Administration of pargyline reduced control levels of HVA and 5-HIAA and inhibited the probenecid-induced rise of these acid metabolites. The magnitude of this inhibition was related to the dose of pargyline. Pargyline inhibited the probenecid-induced rise in HVA more effectively than the rise in 5-HIAA, suggesting the existence of different monamine oxidases in dog brain. After completion of the pargyline treatment, the probenecid-induced rise of 5-HIAA and HVA returned to normal in about 1 month. Measurement of the acid metabolites in the CSF is a direct index not only of the MAO activity in brain but of the degree to which the metabolism of a specific monoamine is inhibited. Probenecid treatment makes this method sensitive enough that it might be applied for measurement of brain MAO activity in clinical practice.

For the generous supply of pargyline and probenecid, we thank Abbott Laboratories, Chicago, Illinois, and The Merck Institute for Therapeutic Research, West Point, Pennsylvania. We also acknowledge the technical assistance of Mrs. M. A. Williams.

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Received Nov. 24, 1969. P.S.E.B.M., 1970, Vol. 133.