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EFFECT OF PROBENECID ON THE CONCENTRATION OF THE LUMBAR CEREBROSPINAL FLUID ACIDIC METABOLITES OF TYRAMINE, OCTOPAMINE, DOPAMINE AND NOREPINEPHRINE

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Abstract—Mass fragmentography was employed to measure the concentrations of the acidic metabolites of tyramine (*p*-hydroxyphenylacetic acid), octopamine (*p*-hydroxymandelic acid), dopamine (3,4-dihydroxyphenylacetic and homovanillic acid) and norepinephrine (vanilmandelic acid) in lumbar cerebrospinal fluid (CSF) of schizophrenic patients. Treatment with oral probenecid (100 mg/kg) increased the concentrations of *p*-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic and homovanillic acid by over 3-fold compared to the baseline levels. The concentration of *p*-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency toward being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

The ability of probenecid to block the transport of homovanillic acid (HVA) [1] and 5-hydroxyindoleacetic acid (5-HIAA) [2] from the brain and from the cerebrospinal fluid (CSF) [3] has been exploited by a number of workers in their attempts to gain some insight into the dynamics involved in the production of these metabolites within the brain [4–11]. Although there is much to be learned about the mechanisms within which probenecid induces an accumulation of acidic metabolites in the CSF and about the relationship between the dose of probenecid and the degree of transport blockade, the probenecid test [7–9] is a useful clinical tool. It has been used to demonstrate differences in CSF concentrations of HVA and 5-HIAA among normal, neurologically ill [12] and mentally ill persons [4, 7, 10, 13, 14].

Of the various acidic metabolites that can enter the CSF from the metabolism of brain biogenic amines and hence may be studied by the probenecid test, only HVA and 5-HIAA have been extensively investigated. In an effort to enlarge the number of metabolites that might be studied by the probenecid test, we have undertaken the task of critically evaluating the effect of probenecid on the transport of the acidic metabolites derived from four closely interrelated biogenic amines [15]. These amines are: tyramine, octopamine, dopamine and norepinephrine.

MATERIALS AND METHODS

Twelve schizophrenic patients (four male and eight female) were studied on a research ward at the Clinical Center of the National Institute of Mental Health. The patients were drug-free for at least 2 weeks prior to lumbar puncture. The lumbar punctures were performed in the lateral decubitus position after 9 hr bed rest at 9:00 a.m. (baseline) and 15 hr of bed rest at 3:00 p.m. (probenecid) the following day. During the intervening period, the patients received orally 100 mg/kg of probenecid in four divided dosages over 18 hr according to previously described methods [7]. In seven of the patients, lumbar punctures were repeated after 1–5 months. The data were analyzed by the paired two-tailed *t*-test, which assumes in each data set, that the baseline is probenecid independent. The assays were performed on 0.5 ml CSF obtained after the withdrawal of the first 16 ml CSF, and frozen at −60° until assayed.

The acid metabolites of tyramine (*p*-hydroxyphenylacetic acid), octopamine (*p*-hydroxymandelic acid), dopamine [dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)], and norepinephrine [vanilmandelic acid (VMA)] were measured by a slightly modified mass fragmentographic method which has been previously described [16]. In brief, 0.5 ml CSF was mixed with 0.5 ml of 0.5 N HCl and the metabolites were extracted into ethyl acetate. Aliquots of the ethyl acetate extracts were transferred and evaporated *in vacuo*. The metabolites were then converted to their methyl ester/pentafluoropropionyl derivatives. Deuterated isomers of all five metabolites were added to each sample as internal reference standards prior to extraction. In each batch of analyses, a standard curve was constructed from four samples of pooled CSF, three of which contained different amounts of the non-deuterated metabolites (5, 10 and 20 ng for HVA and 1, 2 and 5 ng for the other metabolites). Mass fragmentography was carried out on a Finnigan model 3000D quadrupole gas chromatograph mass spectrometer employing an 8 ft $\frac{1}{8}$ in. i.d. steel column packed with 3% SE54. The oven temperature was maintained isothermally at 200°.

Table 1. Lumbar CSF concentration of biogenic amine acidic metabolites in schizophrenics before and after probenecid*

	p-Hydroxyphenylacetic acid		Homovanillic acid		Dihydroxyphenylacetic acid		Vanilmandelic acid		*p*-Hydroxymandelic acid	
	Baseline	Probenecid	Baseline	Probenecid	Baseline	Probenecid	Baseline	Probenecid	Baseline	Probenecid
Mean ± S.E.M.	6.6 ± 0.8	38.1 ± 8.3	58.2 ± 2.7	274 ± 19	0.81 ± 0.09	6.4 ± 0.61	0.66 ± 0.09	1.2 ± 0.3	3.0 ± 0.4	4.7 ± 0.5
P value comparing before and after probenecid		$P < 0.001$		$P < 0.001$		$P < 0.001$		NS		$P < 0.02$

* Results are expressed as ng ml. Probenecid (100 mg kg) was administered orally in four divided doses over 18 hr. NS = not significant.

RESULTS AND DISCUSSION

For the relatively small number of subjects, there was no statistically significant difference by sex for the five acid metabolites except for the concentration of *p*-hydroxyphenylacetic acid after probenecid; the mean ± S.E.M. for the males was 17.2 ± 2.2 while that for the females was 48 ± 13.8 ng/ml ($P < 0.05$). A larger sample will be necessary before we can compare our results with those of Fyrö *et al.* [17] who reported a higher HVA concentration in female schizophrenics than in males. The mean baseline concentration of the acidic metabolites for this group of patients (Table 1) is comparable to those previously observed from a limited number of CSF samples collected from patients with a variety of neurological disorders [16]. Here, however, we are making no attempt to compare values across disorders. Furthermore, because of the small number of subjects studied, no attempt was made to correlate the levels of the metabolites measured with the clinical histories of the patients. We have also not determined the probenecid levels in the CSF analyzed. Work is now in progress aimed at fulfilling the above two omissions.

The baseline concentrations of the acidic metabolites reported in the lumbar CSF are considerably lower than those observed in the ventricular fluid in a different group of patients [16] employing the same method. It is, therefore, likely that a concentration gradient exists for these metabolites along the ventriculo-lumbar space. A concentration gradient has already been reported by other workers for HVA and 5-HIAA in man [18–20], cats [21], and recently for HVA and possibly for VMA in monkeys [5]. In this connection, a contribution from spinal cord to the lumbar CSF levels of norepinephrine and possibly dopamine metabolites should also be considered [22, 23].

Although there is little doubt about the identity of HVA in the CSF as concluded from multiple ion detection studies [15, 16], the mean baseline concentrations of HVA reported in the literature are very inconsistent. The means of lumbar HVA concentrations reported in the literature for a variety of neurological disorders tend to fall within three categories. These categories cannot be explained solely in terms of their associated disorders. In the first, the means range from 15 to 30 ng/ml [4, 6, 7, 19, 24–26]; in the second, from 45 to 60 ng/ml including the results reported in Table 1 [10, 13, 18–20, 27]; and in the third, the means tend to be above 60 ng/ml [28, 29]. It is interesting to note that, in each of the above three categories, there are values determined by mass fragmentography. Thus, it appears that mass fragmentography may be prone to produce the same inconsistencies as other methods. Although mass fragmentography is potentially a very sensitive and specific technique [15], the choice of gas chromatographic column and the selection of the atomic mass unit for mass fragmentography together with the procedures employed (which include the mode of extraction, the derivative used, how the internal standard was prepared and at what stage of the procedure the internal and deuterated standards are added) play very important parts in the overall accuracy of the method.

Other factors that can contribute to the above inconsistencies may also include differences in the physical activity of the subjects [30], volume of CSF withdrawn (the current samples came after 16 ml CSF had been withdrawn to be examined for other purposes), position of the patients, as well as differences in handling the samples between the time of withdrawal and assay. It is worth mentioning here that the levels of HVA reported in the lumbar CSF of patients with a variety of neurological disorders [16] were measured in the first 5–10 ml CSF withdrawn. Therefore, the withdrawal of the first 16 ml CSF in the present study was not expected to markedly affect the concentration of HVA in the CSF. In a recent report [31], HVA was measured in the first 10 ml and in the subsequent 10–20, 20–30 and 30–40 ml fractions of lumbar CSF withdrawn. The mean concentrations observed were 46.4, 63.4, 71.2 and 80.1 ng/ml respectively. Therefore, the concentrations of HVA reported here should not be expected to be more than 20 per cent higher than the actual normal concentrations. In any event, even after allowing for this discrepancy, the results reported in Table 1 will still fall within the range cited in the above second category of HVA concentrations.

In contrast to HVA, information on the other four acidic metabolites is very scanty. The mean concentration of VMA observed here is comparable to those recently reported in the literature with a range between 0.5 and 1.2 ng/ml [6, 25, 32, 33]. Except for the report previously published from one of our laboratories [16], there is no quantitative work published on the CSF concentrations of *p*-hydroxyphenylacetic acid and *p*-hydroxymandelic acid. In this connection, the mean lumbar CSF concentration of DOPAC previously reported [16] from the analysis of five random samples is higher than what had since been observed. The DOPAC concentrations shown in Table 1 are similar to those recently reported by other workers [24, 34].

As shown in Table 1, probenecid significantly increased the CSF concentrations of *p*-hydroxyphenylacetic acid, HVA, DOPAC and *p*-hydroxymandelic acid. Our finding on the ability of probenecid to block the transport of DOPAC differs from the observation of Wilk [26], who reported that probenecid had no effect on DOPAC concentration in the CSF. Furthermore, in a recent study, probenecid was found capable of increasing DOPAC concentrations in certain areas of the rat brain.*

The mean concentration of VMA almost doubled after probenecid treatment; the changes were not, however, statistically significant, due to the wide variation encountered among individuals. While about one-third of the CSF samples collected after probenecid treatment showed either little or no change from their corresponding baselines, the VMA increased in the rest of the samples by 50–200 per cent. Recently, two reports using larger sample sizes of CSF, described a moderate, but significant, increase in VMA in the CSF after probenecid treatment [6, 25].

In rats [35], endogenous VMA was recently discovered to be almost completely eliminated from the brain by a transport mechanism insensitive to pro-

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benecid. The comparatively modest rise in VMA levels in the CSF reported previously in man [6, 25] as well as our observation on the highly variable effect of probenecid, together suggest that a probenecid-insensitive mechanism in the human central nervous system, comparable to that present in the rat brain, may be the predominant route for the removal of VMA from the CSF. Similar transport mechanisms as those suggested for VMA may also be involved in the elimination of *p*-hydroxymandelic acid from the CSF which was also only moderately increased by probenecid.

It is interesting to note that both VMA and *p*-hydroxymandelic acid have β-hydroxyl radicals in their side chains. Whether or not the presence of such a β-hydroxyl radical is responsible for the moderate effect of probenecid on the accumulation of these metabolites in the CSF requires further study. It is now clear that, whatever the reason, the existence of a carboxyl group in the molecule of a metabolite is not the only criterion required for its transport to be blocked by probenecid.

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