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LETTER TO THE EDITOR

Schizophrenia and Plasma Homovanillic Acid in Response to Mental Stress: Methodological Considerations

Changes in blood or urine levels of catecholamine neurotransmitters including dopamine (DA) under the influence of mental stress have been a focus of several previous investigations (Januszewics et al. 1979; Frankenhaeuser et al. 1985; Rauste-von Wright and Frankenmaeuser 1989; Zemishlany and Davidson 1996). We recently studied the effect of mental stress on plasma homovanillic acid (pHVA), a major metabolite of DA, in normal control subjects and patients with schizophrenia (Sumiyoshi et al. 1998, 1999). In those studies, subjects took a modified version of the Kraepelin arithmetic task (Kuraishi et al. 1957) that requires 30 min for completion. Plasma HVA levels, immediately before and after the arithmetic test, were compared. The main finding was that mental stress resulting from the arithmetic task produced differential change patterns of pHVA levels in patients with schizophrenia and control subjects; the decrease in pHVA levels during the arithmetic task observed in normal controls was abolished in patients with schizophrenia (Sumiyoshi et al. 1998, 1999).

In this issue of the Journal, Oranje et al. (2000) presented data of pHVA levels obtained from a sequential sampling of blood using an intravenous catheter in healthy male volunteers. In their experiment, they found significantly lower pHVA concentrations at 9:30 A.M. than at 8:45 AM during which time no psychological stress such as arithmetic tasks was loaded on the subjects. Oranje et al. (2000) seem to argue that they obtained pHVA data from a "placebo condition" in this way. Based on the result, Oranje et al. claimed that the decrease in pHVA levels during the arithmetic task (in control subjects) observed in our previous studies (Sumiyoshi et al. 1998, 1999) "might have been the result of its endogenous diurnal variation."

In principle, we acknowledge the suggestion by Oranje et al. (2000) that the inclusion of data from unstressed or "placebo" condition is important for the kind of studies we performed, which has actually been mentioned in our own article (Sumiyoshi et al. 1998). Therefore, we would like to demonstrate data from a further experiment in our laboratory that examined the issue more directly with a control condition according to the suggestion by Oranje et al. (2000).

In our laboratory, we performed an experiment to evaluate the possible effect of the diurnal change in pHVA levels on the results reported in our previous studies (Sumiyoshi et al. 1998, 1999). Informed consent to participate in this study was obtained from seven normal male volunteers (mean ± SE age = 31.8 ± 1.2 yr) whose pHVA data were previously reported (Sumiyoshi et al. 1999). Subjects underwent sequential blood sampling at 9:00 and 9:30 A.M. The design of blood sampling procedure was the same as in our previous studies (Sumiyoshi et al. 1998, 1999) except that, after the initial blood sampling at 9:00 AM, the subjects were allowed to engage in activities that did not require physical movement greater than that accompanies performance of the Kraepelin arithmetic test. These included chatting with familiar people, looking over journals, etc., which did not put a psychological burden on the subjects. After completion of the study, all subjects reported that they could divert attention from being catheterized in this way. The pattern of changes in pHVA levels under the unstressed condition was compared with that of the same seven subjects in the presence of mental stress due to the arithmetic test performance (Sumiyoshi et al. 1999) by repeated measures analysis of variance (ANOVA), with testing condition (with or without mental stress) as the between subjects factor and time (9:00 or 9:30 A.M.) as the within-subjects factor. Comparisons of pHVA levels at the different sampling occasions (9:00 vs. 9:30 A.M.) in each experiment were made by two-tailed paired t-test. Significance was considered when p-value was less than .05.

Plasma HVA levels from seven subjects with (Sumiyoshi et al. 1999) or without (present study) mental

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0893-133X/00/$–see front matter
PII S0893-133X(00)00114-7

stress are shown in Figure 1. Repeated measures ANOVA revealed that the testing condition × time interaction approached significance ($F(1,12) = 4.16$, $p = .064$). Thus, while an 11.4% decrease in pHVA levels was observed under the stress condition ($t = 3.19$, $p < .02$), which is consistent with the results of our previous reports (Sumiyoshi et al. 1998, 1999), pHVA concentrations at the second blood sampling was not significantly different from those at the first sampling (a 2.4% decrease) under the unstressed condition ($t = 1.11$, $p = .31$).

In the present study, we obtained results indicating the lack of a substantial change in pHVA of normal controls during the period of 30 min in the absence of mental stress. The absence of a substantial change in pHVA related to time seems to contradict the above proposal by Oranje et al. (2000) who reported about a 18.5% decrease in pHVA levels (by comparison of values at 8:45 AM and 9:30 AM). On the other hand, about a 2% decrease in pHVA during the morning period of 30 min observed in the current study actually appears to be in agreement with a 2–4 % decline in this measure that has been reported in the studies cited by Oranje et al. themselves (Oranje et al. 2000; Davidson and Davis 1988; Stroe et al. 1997) (estimated from the figures demonstrated in these articles).

The variance in the degree of changes in pHVA levels may be a result of the difference in the "placebo" conditions employed. In the present study, we attempted to make a situation in which the subjects were almost unconscious of being catheterized, whereas Oranje et al. (2000) seemed to regard the mere absence of any psychological or mental tasks, including the arithmetic test in catheterized subjects as "a placebo condition without stressor". It is possible that the blood sampling method presented here by which the attention of the subjects was diverted may have produced a different pattern of changes in pHVA concentrations from that reported by Oranje et al. (2000). Since the mental effect of catheterization may be diminished when subjects are undergoing psychological tasks requiring mental concentration such as the arithmetic test, it could be argued that the method described here may better serve as a "unstressed" condition than that reported by Oranje et al. (2000).

It has been shown by an animal study using *in vivo* microdialysis (Pulglisi-Allegra et al. 1991) that sustained restraint stress results in decreased DA release in the nucleus accumbens, a brain region that constitutes the major component of the brain HVA output (Csernansky et al. 1990; Lambert et al. 1991). Although this finding may be in line with our previous data indicating a decrease in pHVA levels in normal control subjects during mental stress (Sumiyoshi et al. 1998, 1999), further studies using serial measurements of pHVA for a longer period of time (Zemishlany and Davidson 1996) would clarify the propriety of the interpretations of our pHVA data.

In their commentary, Oranje et al. (2000) referred to the study of Davidson and Davis (1988) who reported a diurnal variation of pHVA in healthy subjects. The

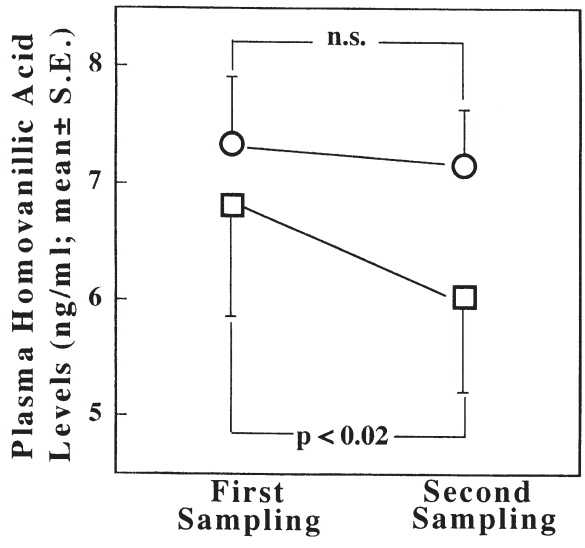

Figure 1. Plasma HVA levels from sequential blood sampling at 9:00 AM (first sampling) and 9:30 AM (second sampling) in stressed (due to the arithmetic test) or unstressed normal controls ($n = 7$). Circle, unstressed condition; square, stressed condition. Testing condition × time interaction, $F(1,12) = 4.16$, $p = .064$ (repeated measures ANOVA).

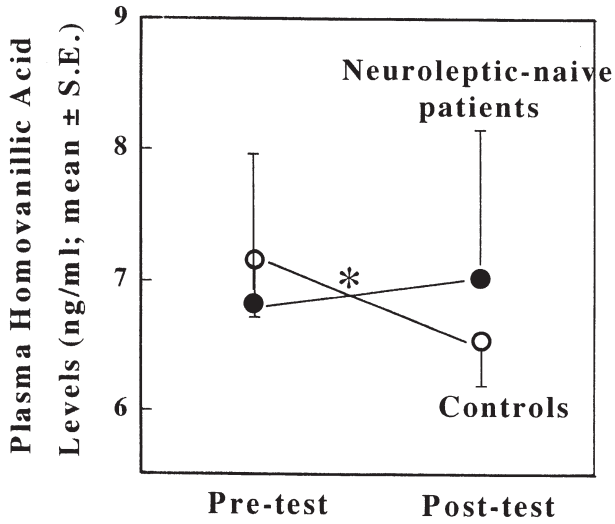

Figure 2. Effect of mental stress (due to the Kraepelin test) on pHVA levels in neuroleptic-naive patients with schizophrenia ($n = 7$) and normal control subjects ($n = 14$). * $p < .04$, significant diagnosis × test effect (repeated measures ANOVA).

same article, as well as the report by other investigators (Doran et al. 1985), also demonstrates a subtle decline in pHVA levels in neuroleptic-free patients with schizophrenia during the period of 9:00–10:00 AM. It is estimated that this pattern of changes in pHVA levels for the unstressed neuroleptic-free patients parallels that of normal healthy controls during the period of 9:00–9:30 AM (Doran et al. 1985; Davidson and Davis 1988; results of the present study). We made further analyses to compare the patterns of changes in pHVA levels under the influence of mental stress due to the Kraepelin arithmetic test in 14 normal volunteers and seven neuroleptic-naive patients with schizophrenia. Plasma HVA data of these subjects were reported in our previous study (Sumiyoshi et al. 1999). Repeated measures ANOVA revealed a significant diagnosis by the task effect ($F(1,19) = 4.89$, $p < .04$) (Figure 2); while pHVA levels in control subjects decreased by 9%, about a 3 % rise in this measure was observed for neuroleptic-naive patients with schizophrenia. At least, the opposite pattern of changes in pHVA between control subjects and neuroleptic-naive patients in the presence of mental stress seems to contrast with the parallel movement of this measure during the period of 9:00–9:30 AM when both schizophrenic patients and normal controls are in unstressed conditions.

In conclusion, although the results were based on a modest sample number, the decline in pHVA levels after mental stress in normal control subjects was found to be greater than the diurnal decrease. Moreover we have presented evidence that normal controls and neuroleptic-naive schizophrenic patients show different change patterns of pHVA in the presence of mental stress. It is expected that these findings encourage further studies investigating neurotransmitter levels such as pHVA in response to mental stress, in which Oranje et al. (2000) themselves may also be engaged.

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ACKNOWLEDGMENTS

The authors are grateful to the volunteers who participated in this study for their dedication.

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