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A Study on the Relationship Between Event-Related Potentials and Plasma Homovanillic Acid Concentration in Schizophrenics

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Abstract

Aiming to examine the relationship between attentional-cognitive deficits and dopamine system dysfunction in schizophrenia, we measured P300 amplitudes, P300 latencies, P300 areas, and Nd areas in event-related potentials for two paradigms (a syllable discrimination paradigm and an oddball paradigm) and plasma homovanillic acid (pHVA) concentration in twenty-two schizophrenic patients. Among these, twenty-one patients were on antipsychotic medication at the time of the investigation. The schizophrenic patients exhibited reduced P300 amplitudes, P300 areas and Nd areas, and prolonged P300 latencies compared with normal controls. Plasma HVA concentrations were distributed in a narrow range within normal limits, which was assumed to be due to the effects of the antipsychotic medication. None of the ERP indices were significantly correlated with the pHVA concentration. Further studies are warranted on the effects of antipsychotic medication on the dynamic aspects of the dopamine system function in order to reach clearer conclusions concerning the relationship between attentional-cognitive deficits and dopamine-system dysfunction in schizophrenia.

Introduction

The "attentional-cognitive deficit hypothesis" 15,20) has been proposed in the fields of physiology and psychology to explain the basic dysfunctions in schizophrenia, while the "dopamine hypothesis" has been proposed in the fields of biochemistry and pharmacology. Animal experiments have pointed to facilitation in reward-related learning 3) as a function of the dopamine system, while studies on humans have suggested that this system functions to facilitate switching between channels of activity 16) as well as in goal-directed behavior 21). These roles are believed to be closely related to the attentional cognitive function. However, surprisingly little research has been conducted on the relationship between the attentional-cognitive hypothesis and the dopamine hypothesis.

Information processing in the brain, such as in attention and cognition, is believed to be reflected in the P300 component (mainly the cognitive function) and the Nd component (mainly selective attention) 12) of event-related potentials (ERPs). Abnormalities such as a reduced P300 amplitude and prolonged P300 latencies are reported especially in schizophrenics 13,17,18). Thirty to fifty percent of plasma homovanillic

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acid (pHVA) originates from the dopamine system of the central nervous system. It is acknowledged that pHVA concentration is useful as an index of the central dopamine function of schizophrenics. 8). The present study used the P300 and Nd components in ERPs as the physiological index and the pHVA concentration as a biochemical index to investigate the relationship between the "attentional-cognitive deficit hypothesis" and the "dopamine hypothesis" by examining the correlation between these indices in the same patients.

Measurements of event-related potentials and pHVA density were authorized by the Research Morals Investigation Committee of Tokyo University, Faculty of Medicine. Written consents were obtained from the patients to be examined before the investigation was begun.

Subjects

The subjects of this experiment were twenty-two schizophrenics who were diagnosed according to the DSM-III-R (the diagnostic and statistical manual of mental disorders, the third revised edition; American Psychiatric Association) criteria. Fourteen were males and eight were females, and their average age was 44.0±10.7 (Table 1). These patients were chosen because they were stable in their psychiatric symptoms. Their total scores on the scaled-expanded brief psychiatric rating scale (scaled-expanded BPRS) 4) averaged 17.8±94. Twenty-one of the patients were on antipsychotic medication; this dose was essentially constant throughout the study period, and its chlorpromazine (CPZ) equivalents (by Kinoshita 14) conversion table) were 210±217 mg. The patients were categorized in terms of disease types on the basis of the DSM-III-R as follows: disorganized type (six), paranoid type (three), undifferentiated type (two) and residual type (eleven). In terms of the course of their disease, one patient was of the subchronic type, eighteen patients were of the chronic type, one patient was of the chronic type with acute exacerbation. Two patients were in remission.

Methods

(1) Recording of event-related potentials

i) Paradigms

In our recording of the event-related potentials, two paradigms--the syllable discrimination paradigm and the oddball paradigm--were employed.

In the dichotic syllable discrimination paradigm (using Hayashida's 9) method) employed in the present study, male and female voices were used to present the syllables (/te/ and /ga/) to the subjects via headphones, with the male voices being presented to one ear and the female voices to the other ear with no change between the stimuli within a session. The frequency-ratio for the appearance of /te/ and /ga/ was 1:3. The stimuli were presented in a random, alternative pattern to one side or the other. The subjects

were required to press one of two response-keys to differentiate between "targets", that is the syllable /te/ in one designated ear, and other categories of stimuli. The duration of each stimulus was 150msec; the stimulus intensity being set approximately at 60dBSL. The inter-stimulus intervals varied randomly between 1800-2000 msec. All of the subjects performed 4 runs, that is, one target syllable X two voices (male or female) X two attended channels (left or right ear). The number of the target stimuli for each run was 30.

In the oddball task, 1000 and 2000 Hz tone bursts were presented binaurally via headphones, at a respective frequency-ratio of 1:3. The detailed characteristics of the stimuli were the same as in the above task, and the subjects were required to press a response-key upon the detection of the 1000 Hz tone in one run which consisted of 117 stimuli.

ii) Recording of electroencephalograms (EEG)

The subjects were seated in an sound-proof anechoic room with eyes closed. According to the International 10-20 Electrode System, EEGs were derived from the Fz and Pz regions and monopolarly referenced to linked ear-lobe electrodes. Vertical and horizontal eye-movements were also recorded from the right eye. The EEGs were amplified using DC amplifiers (bandpass down 6dB at 0.15 and 300 Hz) and processed on-line through a microcomputer with a sampling frequency of 250Hz/Ch. EEG data contaminated by peak to peak potentials of more than 100 microvolts or accompanied by electrooculograms (EOGs) of more than 150 microvolts during the period from 40msec prestimulus to 800 msec post-stimulus were eliminated from the averaging. After a smoothing process using a digital filter with a window-width of 33.3 msec, the ERP waveforms for the individual subjects were averaged separately into four (in the dichotic syllable discrimination task) or two (in the oddball task) categories.

iii) Identification of ERP components

The ERP components analyzed included the P300 and Nd component in the dichotic syllable discrimination paradigm, and the P300 component in the oddball paradigm. The Nd was defined as the negative component of a difference wave in the dichotic syllable discrimination task -- in the period 0-400 msec after the stimulus onset -- between the ERPs elicited by non-target syllables in the attended ear and those elicited by non-target syllables in the non-attended ear. For the Nd index, those areas were employed which were negative between 0-400 msec in the difference wave described above. The P300 was defined as the positive component of the ERP, in the period of 260-600 msec after stimulus onset. The P300 area, P300 amplitudes, and P300 latencies for the target stimuli in both tasks were employed as indices representing the P300. The P300 ares were defined as the positive areas in the target ERPs between 260-600 msec, with the P300 amplitude and P300 latencies corresponding to those measures of the most positive peaks in the relevant latency periods.

(2) Measurement of plasma homovanillic acid concentration

i) Pretreatment

Blood was drawn from the patients for the measurement of the plasma homovanillic acid concentration at 10 A.M. Since it is known that diet, exercise and antipsychotic medication influence pHVA concentration 8), patients were asked to fast for twelve hours before the test, taking nothing except water after 10 P.M. of the night before the test. Furthermore, they were asked to take as little exercise as possible on the morning of the test, and to stop taking their antipsychotic medication in the morning. Blood was cooled to 4 degrees centigrade immediately after the samples were drawn in order to prevent the homovanillic acid from decomposing, and the plasma was then separated.

ii) Determination of concentration

The homovanillic acid concentration was determined by means of high-performance liquid chromatography with the electrochemical detection method (HPLC-ECD method).

One-ml of each samples was deprotenized in a neutral condition, then centrifuged at 35000 rpm for 20 minutes. The resulting supernatant was extracted twice with ethyl acetate in acidic conditions. The ethyl acetate thus obtained was reverse-extracted after the addition of 1ml of 0.1M Tris buffer (pH 8.5), then the Tris buffer layer after the removal of the ethyl acetate layer was applied to the HPLC-ECD system. The HPLC-ECD system consisted of a combination of a KSST-601 pump (Kyowa-Seimitsu Industries, Japan) and an ECD-100 electrochemical detector (EICOM, Japan). A 0.075M citric acid buffer (pH3.3) was used as the moving phase of the HPLC, and the ODA column of the Cosmosil 5C18 (Nakarai Tesc company, Japan; 4.6 x 250mm) was used as the fixed column. The flow speed of the moving phase was 0.6ml/min, and the temperature of the column was 37 degrees centigrade. The voltage applied to the ECD was 800 mV. The determination of the plasma homovanillic acid concentration was performed by SRL, Inc., Tokyo, Japan.

In many cases, there was a half-year delay between blood sampling for the measurement of the plasma homovanillic acid concentration and ERP recording. However, the schizophrenics examined were chosen because they had shown no changes in their symptoms throughout this period, and there was no change in the dosage of their antipsychotic drug except for two patients. In those two patients, the fluphenazine dose was changed only within 1mg. In the remaining twenty patients (including the one without medication), no change in medication was recorded.

Results

(1) Results of event-related potentials

i) Syllable discrimination paradigm

In the dichotic syllable discrimination task, the P300 amplitude was 5.8+4.3

microvolts, the P300 latency was 375±73 msec, the P300 area was 1227±1108 microvolts-msec and the Nd area was 406±342 microvolts-msec. The data on the P300 amplitude further broke down into 4.6±4.1 microvolts for the males and 8.0±3.9 microvolts for the females. Thus, P300 amplitudes tended to be higher in the females, while there were no differences between males and females on the other indices. No significant main effect was obtained in one-way analyses of variance as to whether the differences in disease type and course according to the DSM-III-R were related to the event-related potential indices.

The correlations with age, total scores on the BPRS, and the CPZ-equivalent antipsychotic dosage were examined for the P300 component index and the Nd component index. The P300 amplitude exhibited a positive correlation with age ($r=0.32$, $p=0.07$) and a negative correlation with antipsychotic dosage ($r=-0.34$, $p=0.06$). The P300 area showed a negative correlation with BPRS scores ($r=-0.30$, $p=0.08$) and a significant negative correlation with the antipsychotic dosage ($r=-0.38$, $p=0.04$).

Compared to the data on one-hundred healthy subjects reported by Hayashida 10) (P300 amplitude: 8.5±4.7 microvolts, P300 latency: 343±32 msec, P300 area: 2149±1261 microvolts-msec, Nd area: 554±308 microvolts-msec), the P300 amplitudes, the P300 areas and the Nd were reduced, and the P300 latency was prolonged (However, since the distribution of the indices from these healthy subjects did not follow a normal distribution, accurate evaluations were not possible. Even if a t-test were conducted, a significant deviation would have appeared in the P300 amplitudes).

iii) Oddball task

In the oddball task, the P300 amplitude was 8.1±4.3 microvolts, the P300 latency was 343+53 msec, and the P300 area was 1581±1091 microvolts-msec. There were no differences in any indices between males and females. When the correlations between these indices and age, the total BPRS scores, and the CPZ-equivalent antipsychotic dosage were examined, only the P300 amplitudes were found to have a negative correlation with the BPRS scores ($r=-0.35$, $p=0.06$); the other indices showed no significant correlations.

When a one-way analysis of variance was applied to the differences in disease type and course according to the DSM-III-R, a main effect for disease type was found in the P300 amplitude ($F=4.9$, $df=3.17$, $p=0.01$). This was due to the fact that the amplitude in the residual type (10.6±3.6 microvolts) was significantly larger (Duncan's multiple range test) than that in the disorganized type (4.0±3.5 microvolts) or the paranoid type (5.5±1.9 microvolts). No significant correlation between the P300 latency or the P300 area and disease type or course was observed.

Amplitudes and areas of the P300 were reduced and P300 latencies were prolonged compared to those of the forty-seven healthy subjects reported by Hayashida et al. 11), (P300 amplitudes: 11.7±4.6 microvolts , P300 latencies: 336±27 msec, P300 areas: 2337±1146 microvolts-msec). The difference in the amplitude of the P300 between the two groups was significant.

(2) Results for plasma homovanillic acid concentration

The average plasma homovanillic acid concentration was 10.7±3.0 ng/ml, with the minimum and the maximum concentrations of 6.1 ng/ml and 17.9 ng/ml, respectively. When the results obtained in the current study were compared with those reported for healthy subjects--10.0±5.5 ng/ml (33 healthy subjects; HPLC-ECD method) 22); males 13.3±4.7 ng/ml, females 14.0±5.8 mg/ml (65 males and 122 females; gas chromatographic and mass spectrometric method 5)-- the pHVA concentration values were found to be within a range of 1.6SD. Furthermore, when two cases -- 17.9 and 17.7 ng/ml -- were excluded, the remaining 20 values fell within a narrower range of less than 12.7 ng/ml (one of the high values was a patient who took the test before medication).

There was no difference in the concentration of the homovanillic acid with respect to sex or disease type or course according to the DSM-III-R (one-way analysis of variance). Also, no correlation with the total BPRS score and the CPZ-equivalent antipsychotic dosage was found, but a tendency toward a positive correlation with age was observed ($r=0.32$, $p=0.07$) (see Fig. 1).

(3) Relationship between ERP index and plasma homovanillic acid concentration

With respect to the correlation between the index of the event-related potentials and the plasma homovanillic acid concentration in the two tasks, there was a tendency toward a positive correlation only between the P300 latency and the pHVA concentration with $r=0.30$ ($p=0.10$). No other indices showed a significant correlation or tendency toward correlation (Figs. 2 and 3).

Discussion

From the results obtained in the current research, it can be concluded that there was no overall relationship between the P300 and Nd indices of the ERPs on the one hand and the plasma homovanillic acid concentration on the other. This result can be interpreted as indicating that the relationship between the attentional-cognitive deficit and the dopamine system in schizophrenia is negative. This is similar to the results reported in other articles; there seems to be no correlation between the C-T ratio of the P50 component and pHVA concentration in schizophrenics 1, 2). This fact is considered as suggesting that the attentional-cognitive deficit and the dopamine system are not directly related in schizophrenia. However, in order to make a conclusive determination, further study taking into account the following points will be necessary.

The first point is that our patients were on medication (except for one case). In the past, it has been reported 6,7) that patients who had a high pHVA concentration without medication exhibited a lower concentration when they were put on antipsychotic medication for a long period; conversely, patients who had a low pHVA concentration without medication exhibited no change in concentration when they were put on antipsychotic medication. Hence, the pHVA concentration distribution in our study was obtained from

a mixture of patients whose pHVA concentrations before medication varied. This might have produced data which fell within a relatively narrow range. In fact, the patient who exhibited the highest value, 17.9 ng/ml, was not on medication. Thus, the distribution of the pHVA concentrations was within a relatively narrow range; this may possibly be related to our result that no correlation with the ERP indices was found.

The second point to be considered is that there was a half year's delay between the measurement of the event-related potentials and the measurement of the pHVA concentration. As described previously, we selected patients who exhibited little change in symptoms and dosage of medication in the period between the two measurements. However, the fact that these measurements did not take place on the same day may have contributed to the absence of a correlation.

The third point is that we measured the pHVA concentrations only once in this research. We have found that in some cases, when the pHVA concentration of healthy subjects was measured several times on different days, more than a twofold difference in concentration was observed 8). Furthermore, in animal studies, the application of weak electric shocks and stress or medication with anxiety-inducing beta-carboline increases the activity of the dopamine system selectively 19). Judging from these results, it is possible that the activity levels of the dopamine system are not stable and constant, but variable, and that the dopamine system is a dynamic system whose level of activity varies with environmental conditions. If this dynamic aspect is related to the attentional-cognitive deficit, one time measurement will not be sufficient to obtain a correlation between the attentional-cognitive deficit and ERP indices.

Hence, in order to obtain definitive conclusions concerning the relationship between attentional-cognitive deficits and the function of the dopamine system in schizophrenia, further studies on the following points are required. 1) In order to exclude the effect of antipsychotic medication, studies must be conducted using subjects who are not on medication. 2) Measurements of ERPs and pHVA concentration should be carried out on the same day. 3) In order to clarify the dynamic aspect of the dopamine system, measurements of pHVA concentration should be carried out several times throughout any study.

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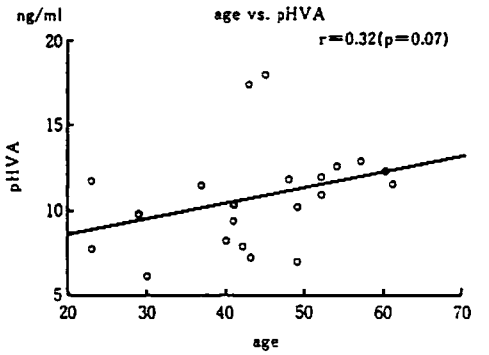

Fig. 1 Correlation between age and plasma homovanillic acid concentration (pH VA) in schizophrenics.

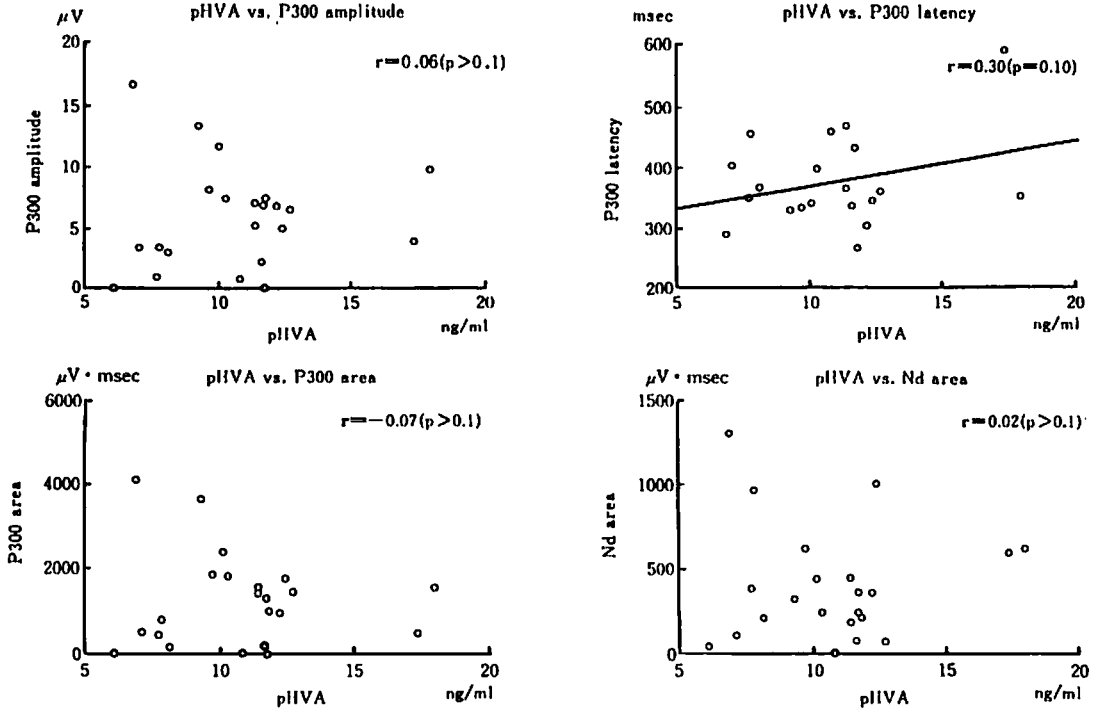

Fig. 2 Correlations between plasma homovanillic acid concentration (pHVA) and event-related potential indices (P300 amplitude, P300 latency, P300 area and Nd area) for the syllable discrimination paradigm in schizophrenics.

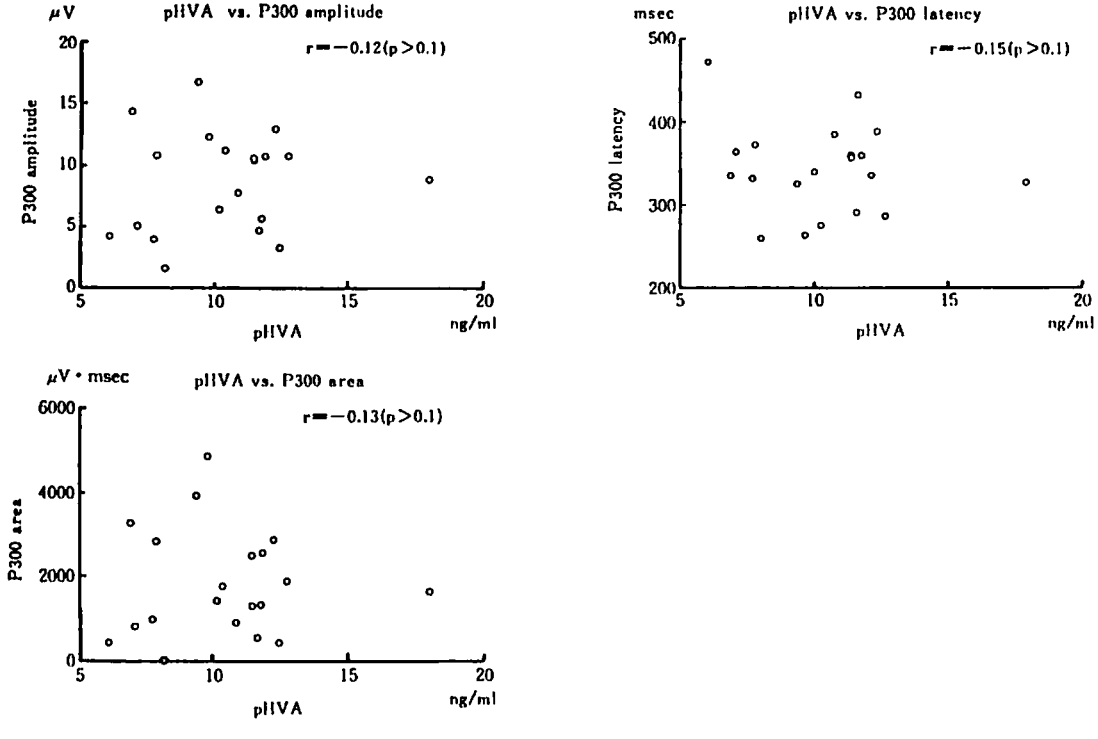

Fig. 3 Correlations between plasma homovanillic acid concentration (pIIVA) and event-related potential indices (P300 amplitude, P300 latency and P300 area) for the oddball paradigm in schizophrenics.