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Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients

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Abstract

The catecholamines dopamine (DA), noradrenaline (NA) and adrenaline (A), their aminoacid precursors tyrosine (Tyr), L-3,4-dihydroxyphenylalanine (L-DOPA), two of their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxy phenyl glycol (MHPG), serotonin (5-HT) and its precursor tryptophan (Trp), were measured by high pressure liquid chromatography (HPLC) with electrochemical detection in seven regions (globus pallidus, putamen, nucleus amygdalae, nucleus caudatus, substantia nigra, gyrus cinguli and raphe) of postmortem brains from eight histologically verified cases with Alzheimer's disease (AD) and six histologically normal controls. Concentrations of L-DOPA, DA, DOPAC, NA and 5-HT were significantly reduced, while Tyr and MHPG concentrations were significantly increased in AD versus control patients. The concentrations of Trp and A in AD patients were not significantly different from controls. Furthermore, for most brain regions examined, significant negative correlations between Tyr and DA as well as between NA and MHPG levels were found. These data confirm and extend findings of monoaminergic systems disturbances in AD, emphasize the significance of dopaminergic deficit for AD and suggest that in pharmacotherapy of AD, attempts to restore deficits of the transmitter systems should be directed to the monoaminergic, in particular the dopaminergic system.

Keywords: Alzheimer's disease; Postmortem brain; Catecholamines; 5-Hydroxytryptamine; Tyrosine; Tryptophan

Although a number of biochemical studies of Alzheimer's disease (AD) have been reported, there is no agreement about the underlying pathogenic mechanism. The major and most widely accepted modification in AD is the deficiency of the cholinergic system due to the progressive loss of cholinergic presynaptic neurons located in the basal forebrain [10]. In addition to generalized cortical cholinergic deficits, several reports have found structural and functional disturbances of monoaminergic systems, including serotonin (5-HT), dopamine (DA) and noradrenaline (NA) [3,8,16,]. As the evidence of a dysfunction of dopaminergic neurons in AD has been less conclusive than that for the involvement of the serotonergic and noradrenergic systems [15], our study was concentrated on DA metabolism disturbances in particular brain regions of AD patients. Concerning functional, cognitive and behavioral disorders which AD causes, we examined the following brain regions: globus pallidus (GP), putamen (P), nucleus amygdalae (AM), nucleus caudatus (CA), substantia nigra (SN), gyrus cinguli (GC) and raphe (R), of AD and of control patients for determination of catecholamines adrenaline (A), NA and DA, their precursors aminoacids tyrosine (Tyr) and L-3,4-dihydroxyphenylalanine (L-DOPA), and two of their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxy phenyl glycol (MHPG). Concentrations of 5-HT and its precursor aminoacid tryptophan (Trp) were also determined.

In the autopsy study there were eight clinically and histologically verified AD patients, three females and five males. The mean age ± SD of the patients at time of death was 61.8 ± 12.9 years, ranging from 46–79 years. The control group consisted of six patients (three females and three males) which had showed no signs or records of neurological or psychiatric disorder. The mean age ± SD of the controls at time of death was 69.8 ± 5.4 years, ranging from 63–78 years. The postmortem delay was 9 ±

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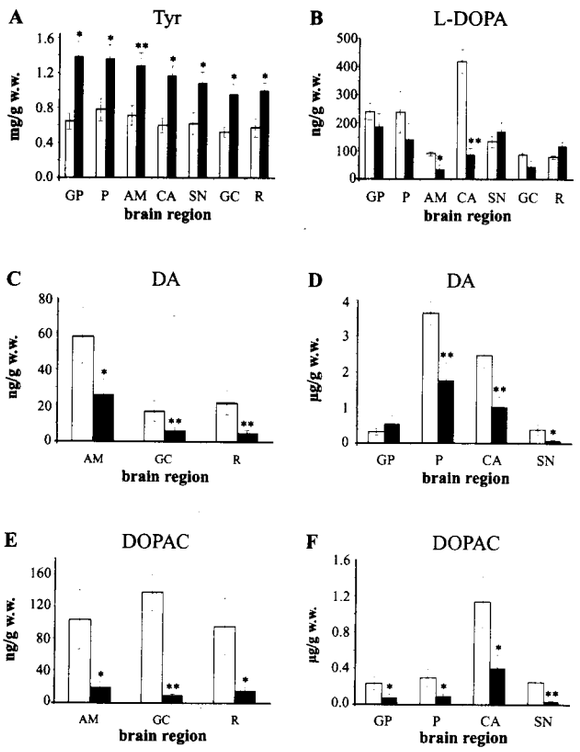

Fig. 1. Differences between controls (white bars) and Alzheimer's patients (black bars) through seven brain regions (see text for abbreviations) of (A) Tyr, (B) L-DOPA, (C,D) DA, (E,F) DOPAC. Data are presented as mean ± SEM. Differences between controls and patients are analyzed by Mann–Whitney U-test; $*P < 0.05$, $**P < 0.01$.

3 h for the controls and 10 ± 2 h for AD patients. There were no significant differences between the groups with respect to postmortem delay or age. None of the controls had received neuroleptic treatment; six out of eight AD patients were on neuroleptic treatment at the time of death. The brain regions used in the study were: left putamen, left globus pallidus, left nucleus amygdalae, left nucleus caudatus, left gyrus cinguli, right substantia nigra and raphe. After dissection the brain samples were placed in airtight plastic vials and stored at −70°C until analyzed. The contents of DA, L-DOPA, DOPAC, A, NA and MHPG as well as of 5-HT, Tyr and Trp in the samples of brain regions were determined by high pressure liquid chromatography (HPLC) using electrochemical detection according to the method of Yang [20]. The Coulochem electrochemical detector Model 5100A from ESA (Bedford, MA) was used in connection with a high sensitivity analytical cell ESA-Model 5011. For catecholamines, separation was achieved by a catecholamine HR-80 column (4.6 × 80 mm, packed with the micron C-18 stationary phase) purchased from ESA. For 5-HT, Tyr and Trp, separation was achieved by a reversed phase column for VMA-test (150 × 4.6 mm) purchased from BIO RAD (Munich, Germany). All standards were purchased from Sigma (St. Louis, MO). All other reagents used were obtained from MERCK (Darmstadt, Germany) and were of reagent grade.

As is evident from Fig. 1A, there were increased mean values of Tyr in the AD group in all seven brain regions analyzed, and all the differences between patients and controls were significant. The L-DOPA concentrations were significantly reduced in AM and CA of AD patients (Fig. 1B). The levels of DA were significantly reduced in P, AM, CA, SN, GC and R of AD patients (Fig. 1C,D). In all seven brain regions investigated, DA's metabolite DOPAC was significantly depleted in AD patients (Fig. 1E,F). Significant negative correlation (Spearman's rank correlation coefficient, R_S) between Tyr and DA was found for P, $R_S = -0.49$ ($P < 0.05$); AM, $R_S = -0.72$ ($P < 0.005$); CA, $R_S = -0.74$ ($P < 0.005$); SN, $R_S = -0.67$ ($P < 0.01$); GC, $R_S = -0.45$ ($P < 0.05$); and R, $R_S = -0.71$ ($P < 0.005$). Furthermore, significant positive correlations were observed between DA and DOPAC concentrations for P, $R_S = 0.57$ ($P < 0.025$); AM, $R_S = 0.58$ ($P < 0.025$);

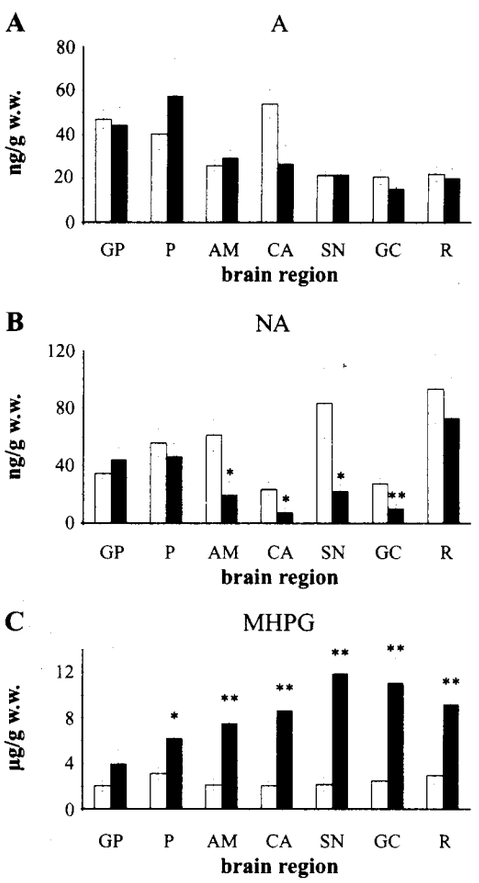

Fig. 2. Differences between controls (white bars) and Alzheimer's patients (black bars) through seven brain regions (see text for abbreviations) of (A) A, (B) NA, (C) MHPG. Data are presented as mean ± SEM. Differences between controls and patients are analyzed by Mann–Whitney U-test; $*P < 0.05$, $**P < 0.01$.

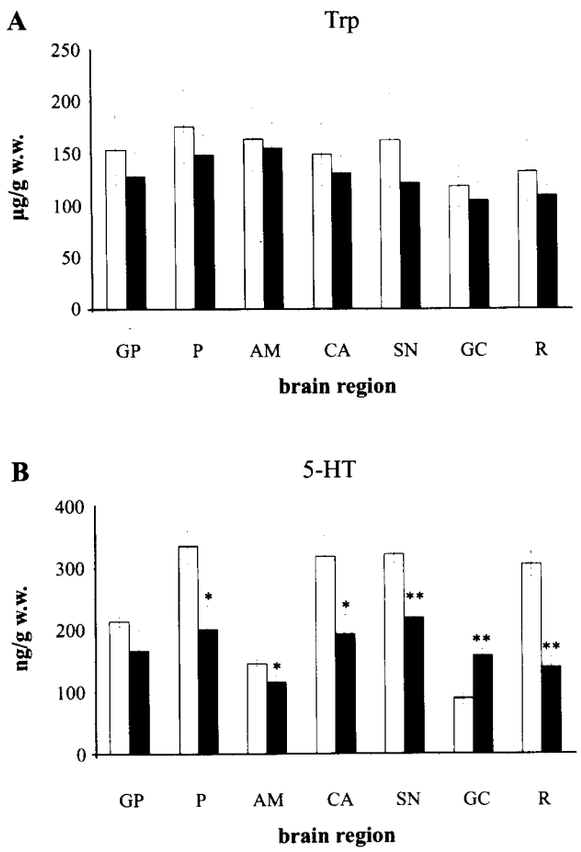

Fig. 3. Differences between controls (white bars) and Alzheimer's patients (black bars) through seven brain regions (see text for abbreviations) of (A) Trp and (B) 5-HT. Data are presented as mean ± SEM. Differences between controls and patients are analyzed by Mann–Whitney *U*-test; $*P < 0.05$, $**P < 0.01$.

CA, $R_s = 0.65$ ($P < 0.01$); SN, $R_s = 0.84$ ($P < 0.001$); GC, $R_s = 0.56$ ($P < 0.025$); and R, $R_s = 0.77$ ($P < 0.001$). Two negative correlations between Tyr and L-DOPA were found for AM, $R_s = -0.49$ ($P < 0.05$), and CA, $R_s = -0.54$ ($P < 0.025$); and one positive correlation for R, $R_s = 0.70$ ($P < 0.005$). L-DOPA versus DA positive correlations were observed for CA, $R_s = 0.67$ ($P < 0.005$), and GC, $R_s = 0.67$ ($P < 0.005$); and a negative correlation for R, $R_s = -0.64$ ($P < 0.01$). Concentrations of A were not significantly changed in AD patients when compared to controls (Fig. 2A). The concentrations of NA declined significantly in AM, CA, SN and GC of AD patients (Fig. 2B) while concentrations of NA's major metabolic product MHPG were significantly increased in six out of seven brain regions investigated (Fig. 2C). Correlation between NA and MHPG was significantly negative for AM, $R_s = -0.68$ ($P < 0.01$); CA, $R_s = -0.49$ ($P < 0.05$); SN, $R_s = -0.45$ ($P < 0.1$); GC, $R_s = -0.73$ ($P < 0.005$); and R, $R_s = -0.53$ ($P < 0.025$), but positive and significant for GP, $R_s = 0.62$ ($P < 0.025$). Correlation between DA and NA was significantly positive for AM, $R_s = 0.76$ ($P < 0.005$); CA, $R_s = 0.40$ ($P < 0.1$); SN, $R_s = 0.56$ ($P < 0.05$); GC, $R_s = 0.62$ ($P < 0.01$); and R, $R_s = 0.73$ ($P < 0.005$). The concentrations of Trp in AD patients were not significantly different from controls (Fig. 3A). The levels of 5-HT were reduced in all seven brain regions investigated and in six of them the reduction was significant (Fig. 3B). No correlation between Trp and 5-HT concentrations was found.

In general agreement with previous reports [1,2,11,13, 16] the present study demonstrated significant reductions in the concentrations of DA, 5-HT and NA in certain brain regions of AD patients. Our study differs from previous reports in that we systematically investigated seven brain regions of AD and control patients, where we determined not only the neurotransmitters content but also their metabolic precursors as well as their metabolites. Therefore, many of our results represent new findings: DA deficit in AM, SN, GC and R; DOPAC decrease in GP, AM, CA, SN, GC and R; Tyr increase in GP, P, AM, CA, SN, GC and R; L-DOPA deficit in AM and CA; NA deficit in AM and SN; MHPG increase in P, AM CA, SN GC and R; 5-HT decrease in R. A and Trp concentrations showed no significant change when compared to controls. DA and its metabolite DOPAC were significantly reduced in most AD brain regions. On the other hand the DA precursor Tyr was significantly increased. The latter observation is in accordance with elevated Tyr concentrations in serum and CSF of AD patients [12]. If the concentration of Tyr is elevated and that of DA is diminished the conversion process of Tyr to DA is not functioning to its full extent. The cause could be a reduced activity of tyrosine-hydroxylase, the rate limiting enzyme in DA biosynthesis [17]. If Tyr is not completely converted to DA, an increased level of Tyr and a reduced DA concentration is the consequence. A lack of DA leads to a decreased DOPAC levels as well. Our findings explain the significantly negative correlations between Tyr and DA concentrations versus significantly positive correlations between DA and DOPAC concentrations.

The increased MHPG concentrations found in our investigations are in agreement with Palmer et al. [14] and Bierer et al. [4], but in contrast with Cross et al. [7], who reported reduced concentrations of MHPG in AD brains. The increase in MHPG concentration could be explained by reduced activity of dopamine β-hydroxylase [6] and by the already decreased concentration of its substrate DA, which lead to the observed lower NA concentrations. On the other hand the already reduced NA is degraded more rapidly, due to the significantly increased monoamine oxidase-B activity [9], leading to an elevated level of NA's major metabolite MHPG. This finding is in accordance with the significantly negative correlation between NA and MHPG concentrations and significantly positive correlation between DA and NA concentrations. As the control group was matched to AD group with respect to age and postmortem delay, it seems unlikely that

the neurochemical differences between the groups were either age-related or postmortem artifacts. A possible further argument that drug treatment of AD patients may be responsible for the reported differences also seems unlikely, as it had been reported that drug treatment does not alter the levels of monoamines in postmortem human brains [1,18]. In spite of the widely accepted opinion that the deficiency of the cholinergic system is the most likely cause of the cognitive dysfunction in patients with AD, our results confirm and extend previous findings that the monoaminergic system, in particular DA production, is also widely affected in AD. Our findings suggest that these disturbances in catecholamine metabolism are not only due to loss of synapses or degeneration of the particular neurons, but are caused by a dysfunction in biosynthesis and degradation of monoaminergic neurotransmitters. Our results point to the significance of the dopaminergic system as one of the biochemical causes of AD, and suggest that attempts to restore the DA deficits should be considered as a therapeutic approach. In this regard, the coenzyme nicotinamide adenine dinucleotide (NADH) was found to increase tyrosine hydroxylase activity and DA production in pheochromocytoma cells [19]. NADH has also been applied to 17 AD patients and all of the patients included in the trial exhibited a distinct improvement of certain cognitive impairment [5]. The clinical trial was mainly based on our biochemical findings and confirms their importance.

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