Thesis Title THE EFFECT OF AF64A ON CHOLINERGIC NEURONS IN DIFFERENT REGIONS OF RAT BRAIN

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Date of Graduation 10 July B.E. 2534 (1991)

ABSTRACT

Ethylcholine aziridinium (AF64A), a cholinergic neurotoxin that induces a selective cholinergic hypofunction was utilized to develop an animal model of Alzheimer's disease in which a cholinergic hypofunction has been implicated. However, there is some discrepancy about the selectivity and specificity of this agent. An attempt was made to establish the appropriate concentration and the mode of administration in order to obtain selective neurotoxicity at a given site. AF64A was bilaterally injected ICV in male rats in a dosage range of 0.025-6.0 nmoles/side. By day 7, ChAT activity was significantly reduced in the hippocampus to 61.35% of control ($P<0.05$) and 15.15% of control ($P<0.01$) at 3.0 nmoles and 6.0 nmoles, respectively. The values of ED_{50}

(median effective dose) and LD_{50} (lethal dose) were 2.8 and 5.2 nmoles, respectively. The effect of AF64A on ChAT activity in the hippocampus was determined at 7, 21, 35 and 82 days after bilateral injection (3 nmoles/3 ul/side, ICV). The ChAT activity was maximally reduced at day 21 (47.88% of control, $P<0.01$). In addition, when the effect of AF64A on calbindin D_{28k} immunoreactivity (CaBP-Ir) was studied, it was found that the number of CaBP-Ir neurons in hippocampus (especially dentate gyrus) was not significantly reduced within 7 days following ICV injection of AF64A. The effect of AF64A in vitro on ChAT activity was studied by adding 0.3 mM AF64A to the incubation mixture, the result demonstrating a significant decrease in the enzyme activity in a homogenate of all brain regions. This study suggests that AF64A can substantially impair cholinergic presynaptic neurotransmission by, perhaps, serving as a directed inhibitor of ChAT activity besides the high affinity of choline uptake. The effect of vitamin E on AF64A-lesioned rats was studied in two different conditions. First, rats were treated with vitamin E (50 mg/kg, IP), both at 24 hrs and then again 15 mins prior to bilateral injection of AF64A (3 nmoles/3 ul/side, ICV). Two weeks later, ChAT was measured. It was then demonstrated that vitamin E attenuated significantly the toxic effects in the hippocampus of AF64A-lesioned rats (from 0.230 ± 0.039 nmoles/min/mg protein to 0.403 ± 0.062 nmoles/min/mg protein, $P<0.01$) and there were significant differences between the sham and

AF64A-lesioned groups (from 0.472±0.027 nmoles/min/mg protein to 0.230±0.039 nmoles/min/mg protein, $P<0.01$). In the second condition, following AF64A administration (3 nmoles/3 ul/side, ICV), rats were then treated daily with vitamin E for 30 days (50 mg/kg, IP).ChAT activity was measured in hippocampus, striatum, and frontal cortex. There was no difference in ChAT values between the AF64A and AF64A + vitamin E groups in any brain region. In aged rats (18-28 months), it appeared that ChAT activity was significantly reduced ($P<0.01$) in the hippocampus, striatum, and frontal cortex (0.321±0.001, 0.739±0.064, and 0.274±0.009 nmoles/min/mg protein, respectively) when compared to adult rats (2 months) (0.472±0.027, 1.620±0.160, and 0.338±0.028 nmoles/min/mg protein, respectively). When aged rats were treated daily with vitamin E (50 mg/kg, IP) for 30 days, they showed a significant increase in ChAT activity in striatum (from 0.791±0.059 nmoles/min/mg protein to 0.970±0.063 nmoles/min/mg protein, $P<0.01$). The mechanism underlying the effect of pre-treated of vitamin E on AF64A-lesioned and on the aged brain is not clearly understood. However, this finding suggests a particularly high vulnerability of the cholinergic system to age-related changes.