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EFFECT OF ANTICHOLINESTERASE COMPOUND PHOSALONE ON BLOOD-BRAIN BARRIER (BBB) PERMEABILITY

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Abstract : To elucidate the role of acetylcholinesterase (AChE) enzyme in BBB function, phosalone, an organophosphorous compound, was studied using rat brain micro vessels in vitro. Phosalone at 100 mg/kg b. wt. induced convulsions and caused a significant inhibition of AChE resulting in increased permeability as assessed by volume distribution. The anaesthetized phosalone treated group also increased permeability as compared to the control but the values were significantly ($P<0.05$) lower than phosalone alone treated group. The inhibition of AChE enzyme has altered the barrier function at the dose level at which it caused convulsion and had an added effect on permeability of BBB.

Key words : acetyl cholinesterase blood-brain barrier seizures

INTRODUCTION

The endothelial cells of brain capillaries form a continuous wall, collectively termed as blood-brain barrier (BBB), which restricts the entry of many substances into the brain. These endothelial cells are known to contain among others, the acetylcholinesterase (AChE) enzyme (1). The role of this enzyme in BBB function is not clearly known. Previous studies indicated that anticholinesterase compounds could induce barrier dysfunction secondary to the effect of convulsions following enzyme inhibition (2, 3). The phosalone, an organophosphorous compound, inhibits the AChE irreversibly is most widely used pesticide by the farmers. The present study was aimed to elucidate the role of AChE in BBB function by using phosalone. To assess the functional aspect of BBB, the uptake of ^{14}C labeled amino acid and sucrose was studied.

METHODS

Fourty adult male Wister/NIN rats were obtained from the NIN Hyderabad, and phosalone was supplied by Voltas India limited, Hyderabad. The animals were divided randomly into four groups. The first group served as control in which the rats were injected with glass distilled water

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intraperitonially (i/p). In-group 2, the animals were injected phosalone i/p at the rate of 100 mg/kg b. wt. The animals exhibited convulsions within few minutes after the treatment. Group 3 rats were anaesthetized with pentobarbital sodium at the rate of 50 mg/kg b. wt prior to phosalone injection. Group 4 rats were anaesthetized with nembutal.

Rats in each group were sacrificed after one hour of treatment or during the period of convulsions by decapitator (i.e. spontaneous separation of head from the body). The brains were dissected out from the skull immediately into ice-cold physiological buffer solution. The brains were then processed for isolation of metabolically active micro vessels (4). The viability of isolated micro vessels was determined by the trypan blue exclusion test and enrichment was assessed by the alkaline phosphatase (5) and gamma glutamyl transpeptidase (6) estimation. The activity of AChE was determined to evaluate the degree of inhibition in the rat brain homogenate and micro capillaries exposed to anticholinesterase compound. The AChE enzyme estimation was done by rapid colourimetric method (7). The uptake studies of labeled amino acid ^{14}C tyrosine with brain capillaries were determined (8) and in this ^{14}C Sucrose was used as marker, which is normally impermeable through BBB. Uptake was calculated and expressed as volume of distribution (V_D µl/ mg protein).

The isolated micro vessels have been useful to study in vitro permeability properties of endothelial cells. However these brain micro vessels should be free from contamination and should satisfy certain criteria such as viability by way of staining properties and enrichment through specific marker enzymes (4). In the present study trypan blue exclusion test was conducted showing morphologically normal endothelial cells for establishing viability of micro vessels. In control and as well as in experimental groups, alkaline phosphatase enriched from 14 to 18.66 folds from homogenate to isolated micro vessels. Similarly, gamma-glutamyl transpeptidase was enriched in the range of 18 to 20.77 folds indicating the present BMV preparations were satisfactory for in vitro studies. Studies have reported that alkaline phosphatase and gamma-glutamyl transpeptidase enzymes enrichment from brain homogenate to isolated brain microvessels was 14 and 20 folds respectively (4), which were almost similar to the present observation. The AChE activity in rat brain homogenate in the present study was 33.06 n mol/mg/h and the phosalone at the dose rate of 100 mg/kg b. wt caused a significant inhibition of AchE activity both in homogenate and BMV suspension (Table I).

RESULTS AND DISCUSSION

The results of the present investigation are depicted in Table I and II. The uptake studies using ^{14}C labeled tyrosine by BMV indicated that inhibition of AChE has resulted in a significant increase in V_D of tyrosine by BMV, suggestive of increased permeability of brain capillaries. Since phosalone administration has induced seizures in experimental animals, it was necessary to establish the present increase in V_D of tyrosine as a seizure-dependent

TABLE I: Activities (n mol/mg/h) of alkaline phasphase and gamma-glutamyl transpeptidase enzyme activity and their enrichment factors.

Groups	*Enzyme*	*Cereral cortex*	*BMV*	*Enrichment factor*
Group 1	1. Alkaline Phosphatase	705±76	10480±202	1:14.8
(Control)	2. Gamma-glutamyl traspeptidase	465±12	9668±157	1:20.71
Group 2	1. Alkaline Phosphatase	303.6±98	5667±228	1:18.66
(Anticholinesterase)	2. Gamma-glutamyl traspeptidase	508±84	10410±220	1:20.5
Group 3	1. Alkaline Phosphatase	367.2±76	5934±152	1:16.16
(Anticholinesterase+ Pentoarbital sodium)	2. Gamma-glutamyl traspeptidase	465±10	8920±172	1:19.1
Group 4 (Pentoarbital sodium)	1. Alkaline Phosphatase	618±33	8886±125	1:14
	2. Gamma-glutamyl traspeptidase	744±77	14880±281	1:20

Values are mean ± SD of 10 observations.

TABLE II: Acetylcholinesterase enzyme activity and volume distribution.

Groups	*AChE activity (n mol/mg/h)*		*Volume distribution of Tyrosine (µl/mg protein)*
	Brain homogenate	*Brain Microvessels*	
Group 1 (Control)	33.06 ± 3.5^{A}	10.23 ± 3.6^{A}	7.4915 ± 0.514^{A}
Group 2 (Anticholinesterase)	9.11 ± 1.5^{B}	1.2 ± 0.34^{B}	10.985 ± 0.385^{B}
Group 3 (Anticholinesterase+ Pentoarbital sodium)	10.23 ± 1.73^{B} (30.9%)	1.08 ± 0.28^{B} (10.55%)	8.9705 ± 0.188^{C}
Group 4 (Pentoarbital sodium)	35.34 ± 2.13^{A}	8.20 ± 1.12^{A}	7.49 ± 0.213^{A}

Values are mean ± SD of 10 observations.
Means with different superscripts differ significantly ($P<0.05$)

enzyme inhibition or seizure-independent inhibition. Hence pentobarbital sodium anesthesia was used prior to the use of phosalone to prevent seizures.

There was no significant difference in percent inhibition of AChE activity in the phosalone treated non-convulsive group and phosolone treated convulsive group. Uptake studies indicated that even in the absence of seizures there was a significant increase in BBB permeability over the control. However, the extent of increase in permeability was lower than that of seizures-induced group. Contrary to the present findings, the earlier research findings (2) reported that anticholinesterase compound increased the permeability of BBB provided the seizures were manifested shortly after administration of the compound and further reported that rats treated with pentobarbital sodium did not show convulsions and damage to BBB integrity was very minimum even with high degree of AChE inhibition. They concluded that the inhibition of AChE alone was not

sufficient to increase the permeability of BBB. Their findings are mostly based on *in vivo* experimentation using Evans blue and protein diffusion techniques, which are less sensitive than the *in vitro* uptake studies used in the present investigation.

In conclusion, using of rat brain micro vessels for in vitro uptake studies to elucidate the alterations in BBB permeability appear to be an ideal system. The inhibition of AChE by organophosphorus compound resulted in dysfunction of BBB indicating that this enzyme has positive role in preserving the barrier integrity. The present study also suggest that anaesthetization of animal with pentobarbital sodium appeared to have no effect on BBB.

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