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A Possible Mechanism for Diisopropylfluorophosphate-Induced Memory Loss in Rats

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GARDNER, R., R. RAY, J. FRANKENHEIM, K. WALLACE, M. LOSS AND R. ROBICHAUD. *A possible mechanism for diisopropylfluorophosphate-induced memory loss in rats.* PHARMACOL BIOCHEM BEHAV **21**(1) 43–46, 1984.—Passive avoidance retention and cortical [H^3]-quinuclidinyl benzilate (QNB) binding were examined in rats that were chronically treated with diisopropylfluorophosphate (DFP), an irreversible acetylcholinersterase inhibitor. Retention of a passive avoidance response in DFP-treated rats was significantly lower when compared to vehicle-treated controls. Passive-avoidance retention decreased from 93% in control animals to 68% in DFP-treated rats. QNB binding studies revealed the density of muscarinic receptors in cortical homogenates was significantly reduced from 0.95±0.04 pmole/mg protein in controls to 0.72±0.04 pmole/mg protein in DFP-treated rats. Scatchard analysis of QNB binding curves did not reveal a decrease in affinity of muscarinic receptors for QNB. Based on data that DFP causes a reduction in cholinergic receptors, this study supports the hypothesis that central cholinergic receptors are associated with mechanisms involved in memory storage.

Quinuclidinyl benzilate Diisopropylfluorophosphate Central cholinergic receptors
Memory and down-regulation of central cholinergic receptors

AGING can be characterized as an inevitable, progressive deterioration of intrinsic biological functions. Senile dementia of the Alzheimer's type occurs increasingly with age and is associated with cognitive impairment and a decrease in central cholinergic function. Anatomical [19], neurochemical [5,6] and pharmacological [1,7] evidence support the concept that central cholinergic hypoactivity underlies the deficit in memory that occurs in the elderly. Numerous investigators [8,18] suggest that memory deficits can be treated with centrally acting parasympathomimetic agents.

Chronic administration of DFP, an irreversible acetylcholinesterase inhibitor, has been shown to reduce central cholinergic activity as reflected by altered responsiveness to cholinergic agonists and antagonists [4,18]. Evidence generated from such studies has shown that altered physiological and behavioral responses to cholinergic agents were due to a reduction in the number and perhaps affinity of central muscarinic cholinergic receptors [11,22].

Although previous studies have demonstrated that cholinergic drug treatment can alter either short-term memory [1,5] or the number of central cholinergic receptors [11,21], the experimental conditions such as the type of drug treatment, drug dosage, duration of treatment, and the species of experimental animal used in each experiment varied considerably from one study to the next. Since evidence of an association between central cholinergic receptors and memory formation is only circumstantial, this study was designed to determine if chronic administration of DFP could alter both short-term memory formation and central cholinergic receptors in the same group of experimental animals.

METHOD

Adult male Hooded, Wistar rats (200–250 g) were used in this study. Rats were housed in stainless-steel cages; were given food and water ad lib and kept on a 12-hr-light/12-hr-dark cycle (6 a.m., 6 p.m.) at 21°±2°C.

Treated rats received 1.0 mg/kg, S.Q. doses of DFP (Calbiochem) in a dose volume of 0.1 ml % body weight. A 10% propylene glycol solution dissolved in 0.9% saline served as the vehicle. Control animals received injections of the vehicle. After the first injection which was designated as day one, rats were injected every 72 hours and treatment was continued for 20 administrations.

Behavioral training and testing [12] was initiated 24 hours following the last injection (20th administration). Behavioral training occurred over two days. Day one included three pretraining trials for each rat at two hour intervals. Each trial consisted of placing the animal on a lighted runway leading to a darkened box which contained a metallic grid on the floor. Due to handling, rats were provoked with air currents to enter the box by placing an electric fan (10 cm blade) 90

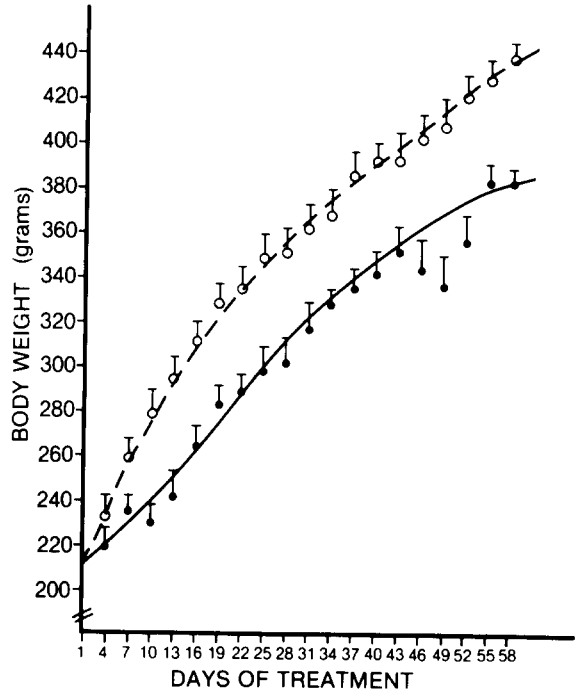

FIG. 1. Growth curves of vehicle-treated control rats (○) and rats injected with 1.0 mg/kg diisopropylfluorophosphate, DFP (●). Injections began on day one. Each point represents the mean±S.E.M. of body weights (grams) measured from 42 vehicle-treated and 38 DFP-treated rats.

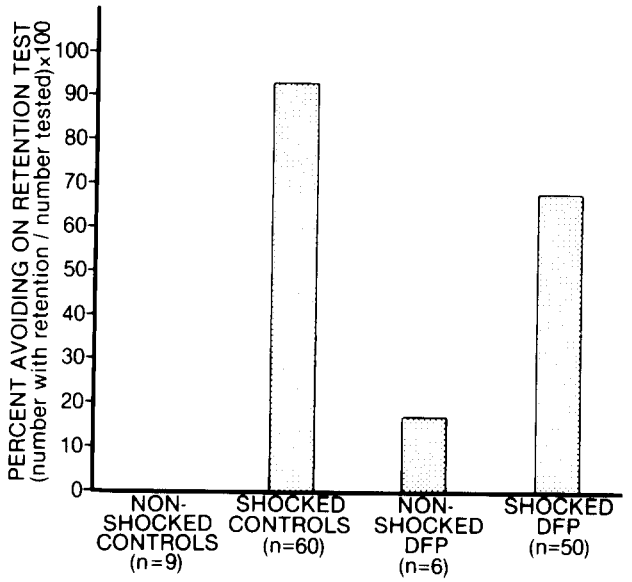

FIG. 2. The percent of rats avoiding a footshock during a retention test utilized in a one-trial passive-avoidance paradigm. Vehicle-treated or DFP-treated rats that did not receive a footshock during training served as non-shocked retention control animals. Rats from vehicle-treated or DFP-treated groups that did receive a footshock (0.5 mA/10 sec) were utilized to measure retention. Retention in DFP-treated rats was significantly lower ($p<0.05$) than in vehicle-treated control animals.

cm from the runway. Once the animal had all four feet on the grid floor, the trap door was closed and each rat remained in the darkened box for 10 seconds. Each rat was carefully removed from the box and placed in the home cage. On the second day, the training began by placing each rat on the lighted runway with electric fan in operation. When the rats stepped through the trap door and onto the metallic grid the door was closed. Ten seconds after entering the box, rats received a footshock of 0.5 mA for a duration of 3 seconds. A small group of animals did not receive a footshock and served as non-shock controls. Rats that were placed on the runway were given 120 seconds to enter the box. Animals not entering the box within this time period were not utilized for experimentation. In the retention test (third day), a latency of 120 seconds for rats remaining on the aerated, lighted runway was taken as evidence that there was retention of the experience of having previously received a footshock.

Rats were sacrificed by decapitation within 60 minutes following retention studies. Brains were rapidly dissected on ice and the cortex of each animal was quickly frozen. Rats (n=5–7) were randomly selected from each experimental group and QNB binding assays were performed [23]. A Scatchard analysis of QNB binding was utilized to determine the affinity (dissociation constants) of muscarinic binding sites in the cortex. The binding of QNB was studied at six concentrations. Protein levels were determined by the methods of Lowry *et al.* [15].

The results for the OTPA experiments are expressed as the percentage of rats which, during the retention test, did not walk into the darkened compartment in 120 seconds. A 2×2 Chi-square analysis was utilized to estimate statistical significance ($p<0.05$), for retention between experimental groups. A student *t*-test analysis was utilized to estimate significance ($p<0.05$) for body weights and QNB receptor number and affinity between experimental groups.

RESULTS

The initial injections of 1.0 mg/kg DFP produced exaggerated parasympathomimic symptoms such as lacrimination, salivation, tremors, ataxia, and diarrhea. These symptoms remained severe following the first three doses of DFP but became less intense after doses 4–6. Vehicle-treated rats showed no parasympathomimic symptoms. Figure 1 illustrates the growth curves for rats utilized in the first series of experiments. Following the second dose, the body weights of rats receiving DFP injections remained significantly lower than control rats through the 20 administrations.

For the OTPA paradigm, a latency of 120 seconds for remaining on the runway was taken as evidence that there was retention of the experience of having previously received a footshock. Figure 2 shows that percent retention in rats receiving DFP injections were significantly lower than control rats ($p<0.05$). Retention in non-shocked controls was 17% and 0% for DFP treated and control rats respectively.

The effects of DFP treatment on the binding of QNB to cortical homogenates are summarized in Fig. 3A and 3B. Specific QNB binding utilizing 0.05 pmole QNB was significantly reduced ($p<0.05$) in cortical homogenates taken from rats that were chronically treated with DFP. The density of QNB binding sites decreased from 0.95±0.04 pmole/mg protein to 0.72±0.04 pmole/mg protein. Scatchard plots were utilized to determine the affinity of cholinergic receptors for

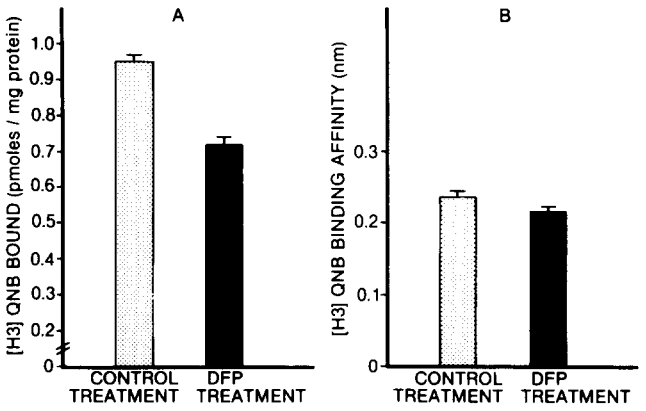

FIG. 3. (A) illustrates the amount of [H³] QNB bound to cortical homogenates taken from vehicle control or DFP-treated rats. Each value represents the mean±S.E.M. of [H³[QNB binding in 5–7 rats. Cortical [H³] QNB in DFP-treated rats was significantly lower ($p<0.01$) than those found in vehicle control rats. (B) illustrates the affinity of [H³] QNB binding to cortical muscarinic receptors in homogenates taken from vehicle control or DFP-treated rats. Scatchard analysis was used to determine the affinity (dissociation constant) of [H³] QNB binding in experimental animals. Each value represents the mean±S.E.M. of [H³] QNB binding affinity in 5 rats. Cortical [H³] QNB affinity was not significantly altered ($p>0.05$) in DFP-treated rats as compared to vehicle-control rats.

QNB in cortical homogenates. The affinity of cholinergic receptors for QNB was not significantly altered by DFP treatment. The affinity of QNB for muscarinic receptors in control rats and DFP-treated rats were 0.215±0.01 nM and 0.237±0.01 nM respectively.

DISCUSSION

The results of this study show that an association exists between a decrease in the density of muscarinic receptors in the cortex and a loss of short-term memory, as measured by percent retention in an OPTA paradigm. Numerous studies support the association of central cholinergic activity with cognitonic processes [14,20]. Central cholinergic activity appears to be involved in memory encoding, storage, or retrieval [3,17]. Since the hippocampus both receives important cholinergic inputs [23], and is associated with memory processes [21], it appears likely that hippocampal cholinergic activity controls some aspects of memory. Anatomical [21], neurochemical [15] and pharmacological [10] evidence supports the concept that central cholinergic hypoactivity underlies the deficit in memory that occurs in the elderly. This concept is further supported by studies which show that memory deficits can be treated with dietary cholinergic precursors [1] and centrally acting cholinergic agents [9].

The results of this study are similar to other reports [11,22] which have shown that chronic administration of DFP reduces the number, but not affinity, of postsynaptic muscarinic receptors in the brain. It is proposed [11] that DFP induced changes in QNB binding are caused by acetycholinesterase inhibition and the resulting accumulation of acetylcholine at cholinergic receptor sites.

Retention of memory found in animals chronically treated with DFP may have also reflected a diminished performance that was the result of elevated acetycholine (ACh) levels in the brain. Parasympathomimic agents which were administered to experimental animals in increasing doses produced both increased and decreased retention of passive avoidance behavior in a OTPA paradigm [13]. Impairment of retention by the cholinergic agonists may have resulted from cholinergic receptor desensitization or indiscriminate activation of cholinergic neurons. Elevated levels of ACh in DFP-treated animals in this study may have also altered cholinergic pathways not associated wtih memory formation and thus altered retention of passive avoidance behavior by non-specific mechanisms.

This study has demonstrated that chronic DPF administration can diminish retention in the OTPA paradigm and this loss in memory can be associated with a decrease in the number of cortical cholinergic receptors.

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