secondary stimulation. Furthermore, cells secondarily stimulated with an optimal antigen dose 2, 5, and 11 weeks after priming contained less than 100 PFC per million cells for the first 3 days of culture in diffusion chambers (*8*). Finally, primary stimulation in vitro followed by culture in diffusion chambers gave rise to only 80 direct PFC per million cells and no indirect PFC, indicating that there was no accidental priming by cross-reacting environmental antigens in our system.

If complexing of antigen with cell surface receptors is the initial obligatory step for the induction of memory cells, and if induced cells and their progeny are restricted to the production of antibodies which are identical to their receptors, a limiting dose of antigen should have induced only cells with high-avidity receptors whose progeny cells should have produced only high-avidity antibodies. Since this was not the case, we conclude that one or both of the above premises are incorrect; complexing of antigen with cell receptors may not be the only critical determinant step in induction of memory cells and/or such cells and their progeny may synthesize antibodies which are at least functionally different from precursor cell receptors.

Others have reported findings which are in conflict with the hypothesis of cell selection by antigen. Harel *et al.* (*13*) found that antigenic competition reduced both the amount and the affinity of anti-DNP antibodies in guinea pigs. They suggested that antibody affinity may increase during the differentiation of a single clone, and that antigenic competition may block the differentiation of the antibody-producing cells at an early stage. A similar suggestion was made by Macario *et al.* (*14*), who found that small fragments of lymph nodes from primed rabbits, believed to contain one or very few clones of memory cells, elaborate antibodies of progressively higher affinity during a 40-day period of cultivation in vitro. Werblin and Siskind (*15*) found that in rabbits showing a progressive increase in average antibody affinity, low-affinity antibodies persisted in approximately constant amounts from 7 days to 1 year after immunization. The persistence of low-affinity antibodies for such a long time is in conflict with the hypothesis of cell selection by antigen, which postulates that maturation of the immune response results from preferential stimulation of high-affinity cells, and lack of stimulation of low-affinity cells, by decreasing amounts of administered antigen.

We have presented here the results of a direct experimental test of the hypothesis of cell selection by antigen. We find that the experimental evidence does not support the hypothesis.

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Diisopropylfluorophosphate: Suppression of Ionic Conductance of the Cholinergic Receptor

Abstract. *When frog sartorius muscles were exposed to diisopropylfluorophosphate, the amplitude and half-decay time of the end-plate current decreased; the half-decay time became almost potential-independent and the equilibrium potential for the end-plate current was more negative than during control conditions. When the excess reagent was removed by washing so that only the phosphorylated acetylcholinesterase remained, the amplitude of the end-plate current was restored, while its half-decay time was markedly increased. These findings reveal that this organophosphate significantly affects the receptor-ionic conductance modulator complex in addition to its well-known anticholinesterase activity.*

At the vertebrate motor end plate, when an inhibitor of acetylcholinesterase (AChE) such as neostigmine is present (*1, 2*) or when AChE is removed by proteolysis (*3*), the time course of the end-plate potential (EPP) and, even more significantly, of the end-plate current (EPC) (*4, 5*), is prolonged. It has been suggested (*2, 4*) that the effects of the anti-AChE drugs are due to their slowing of conformational change in the acetylcholine (ACh) receptor, rather than to the synaptic persistence of ACh. To study the course of activation by ACh of the receptor complex we have now measured by a conventional voltage-clamp technique (*6*) the EPC of the frog sartorius muscle (*7*) during and after treatment with an irreversible AChE inhibitor, diisopropylfluorophosphate (DFP). Acetylcholinesterase was then quantitatively reactivated by treatment with pyridine-2-aldoxime methiodide (2-PAM) (*8*) to provide a subsequent internal control. The results indicate that DFP, in addition to inhibiting AChE, interacts with the complex of the ACh receptor and the ionic conductance modulator (ICM) (*9*) in a reversible manner.

Contrary to expectation, the half-decay time of the EPC in the presence of DFP (0.9×10^{-3} to $1.1 \times 10^{-3} M$) decreased to 50 percent. The amplitude of the EPC was decreased to 70 percent of the control (Fig. 1A, and Table 1). In contrast to the control, the falling phase of the EPC showed two distinct exponential components (2 in Fig. 2A), a fast phase followed by a slow residual current. Thus these effects of DFP on the EPC are similar to those of local anesthetics (*10*) and different from those of reversible AChE inhibitors (*4, 5*). At a lower concentration of DFP ($2.5 \times 10^{-4} M$), the half-decay time of the EPC was decreased by 20

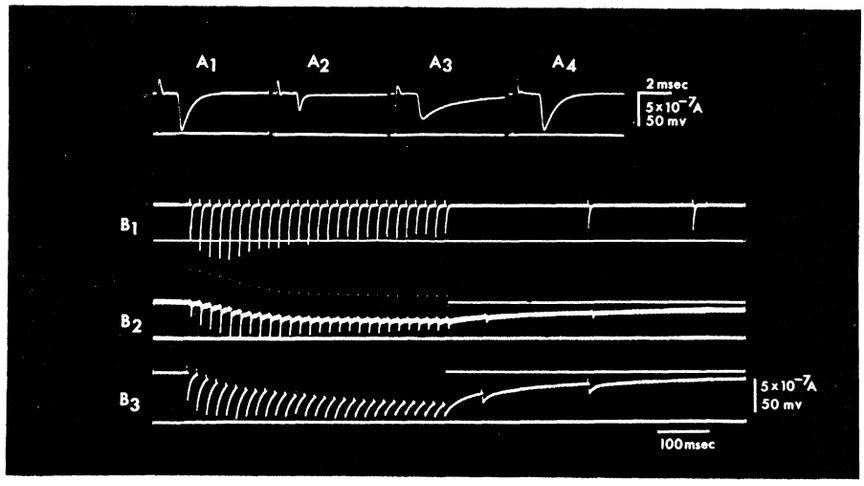


Fig. 1. (A) Representative EPC's recorded under various experimental conditions. A_1, control; A_2, during exposure to DFP ($1.1 \times 10^{-3} M$); A_3, 30 minutes after washing out the excess DFP; and A_4, after exposure to 2-PAM ($9 \times 10^{-3} M$) and washing for 30 minutes. (B) Series of EPC's elicited by repetitive nerve stimulation under control conditions (B_1), in the presence of DFP (B_2), and after washing out the excess DFP for 30 minutes (B_3). The membrane potential in both (A) and (B) was preset at — 90 mv. Upper sweeps are currents; lower sweeps are membrane potentials.

percent. In our DFP reaction conditions the active centers of AChE at the end plate became fully blocked (*8*).

After exposure to DFP for 30 minutes, the muscles were washed with Ringer solution for at least 30 minutes, which removed the excess DFP. By the end of this initial period of washing, the time course of the EPC was greatly prolonged and the amplitude of the current was restored to 90 percent of the control; the half-decay time of the EPC after washing was then 127 percent greater than the control (Fig. $1A_3$ and Table 1). The logarithmic curve of the falling phase of the EPC now departed only slightly from a single exponential function (3 in Fig. 2A).

The configuration of the EPC returned almost to that of the control preparation after the muscles were exposed to 2-PAM ($9 \times 10^{-3} M$) for 30 minutes and subsequently washed with normal Ringer solution for another 30 to 45 minutes (Fig. $1A_4$, 4 in Fig. 2A, and Table 1).

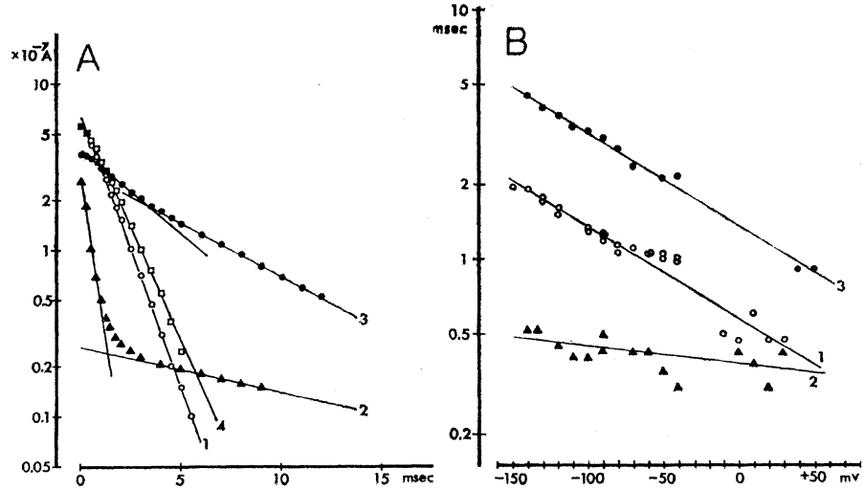


Fig. 2. Semilogarithmic plots of the falling phase of EPC's and the relation between the half-decay time and the membrane potential obtained under various experimental conditions. (A) The abscissa refers to the time of the falling phase of the EPC in milliseconds and the ordinate designates the amplitude of the EPC on a log scale. The membrane potential was kept at — 90 mv. (B) The abscissa refers to the membrane potential and the ordinate designates the half-decay time on a log scale. In (A) and (B) 1 indicates control; 2 indicates in the presence of DFP ($1.1 \times 10^{-3} M$); 3 indicates after washing out the excess DFP; and 4 [only for (A)] indicates after treatment with 2-PAM ($9 \times 10^{-3} M$) and subsequent washing for 30 minutes.

The potential-dependent nature of the half-decay time of the EPC seems to be due to the fact that the rate of the conformational change of the receptor during the action of the transmitter is a membrane potential-dependent event (*4*, *5*). Normally, the logarithm of the half-decay time is linearly related to the membrane potential; that is, the half-decay time increases as the membrane potential becomes more negative (1 in Fig. 2B). The magnitude of the half-decay time notably decreased in the presence of DFP, and the falling phase of the EPC became almost potential-independent (2 in Fig. 2B). After the excess DFP was washed away from the bath, the slope returned to that of the control, but with an increased magnitude of the half-decay time (3 in Fig. 2B). These latter effects are, in fact, the same as those in the presence of a reversible anti-AChE (*4*).

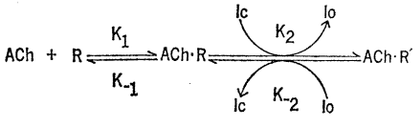
Aside from blocking AChE, DFP (when maintained in the medium) is also affecting the ACh receptor–ICM complex. Therefore, the equilibrium potential of the EPC (E_{EPC}) was measured under various experimental conditions. When the values of the E_{EPC} in the normal Ringer solution (-1.4 ± 1.4 mv; six preparations) were compared with those observed in the presence of DFP (-5.4 ± 1.6 mv; $N = 6$), a significant difference ($P < .01$) was obtained. This result indicates that DFP decreased conductance ratio of sodium to potassium of the end-plate membrane. After removal of excess DFP, the E_{EPC} was $+1.0 \pm 0.4$ mv ($N = 6$), which was not significantly different from control.

A further indication of action of an anti-AChE agent can be observed by analyzing the behavior of the EPC during repetitive stimulation (*2*). In comparison with control (Fig. $1B_1$), the decrease of the EPC amplitude during a series of such stimulations was significantly greater in the presence of DFP (Fig. $1B_2$) and also after washing away the excess of the drug (Fig. $1B_3$). The half-decay time of the initial phase of each EPC in the presence of DFP was almost unchanged throughout tetanic stimulation. After the excess DFP was removed, the half-decay time of each EPC was progressively increased up to the seventh EPC, and thereafter reached a steady value which was twice that of the first EPC. Apparently each EPC summated during a tetanic stimulation, and the baseline of the current recording was increased.

Upon cessation of a tetanus, the recovery of the current to the original level was very slow with a half-decay time of 0.5 to 1 second (Fig. 1, B_2 and B_3). Even 0.5 second after cessation of the tetanic stimulation, single stimulation generated an EPC with an increased half-decay time (Fig. $1B_3$).

DFP (0.25×10^{-3} to $1.1 \times 10^{-3} M$) had no significant effect on the membrane potential, or on the amplitude, overshoot, and rate of rise of the directly or indirectly elicited action potential of the muscle fiber.

In order to account for the sites of action of DFP at the ACh receptor–ICM system, the following simplified model may be used:



where R and R′ represent the receptor in the resting or activated conformation, respectively, and Ic or Io the closed or opened conformation of the ICM, respectively. The symbols k_1, k_{-1}, k_2, and k_{-2} are the rate constants for the reactions indicated. Under normal conditions the velocity of the reaction of ACh with R would be much faster than the resultant conformational changes of the receptor and of the ICM. Thus, k_{-2} would be a rate-limiting constant for the falling phase of the EPC (*4*). The following conclusions are drawn.

1) The shortening of the EPC associated with a decrease in its amplitude in the presence of DFP may reflect an apparent increase in k_{-2}, or destabilization of Io by the drug. The shift of the equilibrium potential to more negative values (Table 1) in the presence of DFP indicates that the sites of the action of the drug are at the level of the ICM. Furthermore, the main phase of the EPC decay has become virtually independent of the membrane potential, and this indicates that a different rate-determining process is involved. Therefore, one may conclude that the drug affects the ICM in the Io condition. This reversible phase of the action of DFP is similar to that of local anesthetics (*10*). Weber *et al.* (*11*) have reported that procaine antagonizes the effects of cholinergic agonists and antagonists by an action other than at their site of binding to the receptor, and we have reported data indicating that perhydrohistrionicotoxin interacts specifically at the ICM moiety (*12*).

2) Normally, the time course of cleft ACh concentration will not be longer than that of the EPC (since the latter is determined by the level of ACh·R′), and simulations (*5*) show that it is shorter. In the presence of DFP, under the conditions used here, AChE is fully blocked (*8*) and the ACh lifetime must be as extended as it is in state 3 of Figs. 1 and 2. Since, however, a shortening of the EPC occurs, this suggests that the destablization by DFP is an uncoupling of Io from the ACh·R′ complex, so that Io can decay rapidly, independent (initially) of ACh persistence. Under this condition, conformational change of the receptor-ICM complex is determining most of the falling phase of the EPC.

Table 1. The mean values (± S.E.M.) for the amplitude and half-decay time (HDT) of the EPC obtained under various experimental conditions. For the control and experimental studies at least 30 single surface fibers from ten sartorius muscles were used. For all fibers the holding potential was maintained at − 90 mv.

Condition	Amplitude (10^{-7} amp)	HDT (msec)
Control	4.08 ± 0.33	1.34 ± 0.03
DFP ($9 \times 10^{-3} M$)	2.83 ± 0.31	0.67 ± 0.06
DFP removed	3.60 ± 0.35	3.04 ± 0.22
2-PAM removed ($9 \times 10^{-3} M$)	3.42 ± 0.47	1.82 ± 0.12

3) The increase in the half-decay time of the EPC after the free DFP is removed is partly attributed to the lack of ACh hydrolysis by AChE. This conventional concept is confirmed by the present results (state 3, Figs. 1 and 2) because AChE is then fully blocked but the receptor active centers are not affected by DFP (*13*) and the 2-PAM unblocking of AChE (*8*) restored the original properties here. In agreement, proteolytic removal of AChE similarly prolongs the EPP (*3*). Although the half-decay time of the EPC progressively increased with the initial responses of tetanus after washing out the excess DFP, this was not seen for the fast decay phase in the presence of DFP. Yet, the recovery of the summated EPC after cessation of the stimulation both in the presence of DFP (state 2) and also after washing (state 3) was much slower than can be expected for the escape of transmitter if freely diffusing (*2, 14*). This further supports the idea that local concentration of ACh controls a slower rate of conformational change of the receptor-ICM unit (*2, 5*). This relationship could account for the prolongation of the entire EPC in state 3, and of its terminal phase in state 2.

Our study indicates that DFP acts reversibly on the receptor-ICM complex, modifying the EPC time course, in addition to its well-known irreversible effect as an anticholinesterase agent. Even under the condition of AChE blockade, the lifetime of ACh in the junctional cleft could be shorter than the time course of the EPC. Conformational change of the receptor-ICM unit is thought to determine the rate of decay of the conductance change at the end-plate membrane.

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6. The voltage-clamp circuit was similar to that described by A. Takeuchi and N. Takeuchi [*J. Neurophysiol.* **22**, 395 (1959)] and modified by E. X. Albuquerque and K. Kuba. The techniques for recording at the end-plate region were similar to those reported (*4, 5*). Microelectrode resistance was from 2 to 15 megohm.
7. Sciatic nerve–sartorius muscle preparations were dissected from frogs (*Rana pipiens*). In order to eliminate the muscle contraction, the muscles were treated with Ringer solution containing 400 m*M* glycerol and then washed as described by P. W. Gage and R. S. Eisenberg [*Science* **158**, 1702 (1967)]. The Ringer solution used is similar to that described by E. X. Albuquerque, J. E. Warnick, F. M. Sansone, J. Daly [*J. Pharmacol. Exp. Ther.* **184**, 315 (1973)]. DFP (Sigma) was dissolved in propylene glycol. Propylene glycol (13 m*M*) has no significant effect on the EPC. 2-PAM (Sigma) was dissolved in Ringer solution. All experiments were performed at 21° to 24°C. P values $< .01$ were considered statistically significant. All values are expressed as the mean ± S.E.M.
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Insomnia with Sleep Apnea: A New Syndrome

Abstract. *A new clinical syndrome, sleep apnea associated with insomnia, has been characterized. Repeated episodes of apnea occur during sleep. Onset of respiration is associated with general arousal and often complete awakening, with a resultant loss of sleep. An important clinical implication is that patients complaining only of insomnia may be suffering from this syndrome.*

Although patients who complain of insomnia have been studied in this laboratory for several years, we only recently began to include respiratory studies routinely. Using these procedures, we discovered a new syndrome —insomnia with sleep apnea—which is associated with dramatic sleep disturbances.

Sleep apneas have been reported in the cardiopulmonary syndrome of obesity ("Pickwickian") and in other syndromes involving hypersomnia, such as narcolepsy (*1*, *2*). The apneas in hypersomniacs seem to be temporally associated with sleep. Several distinct types of sleep apnea have been defined in these conditions (*2*). They include a "central" type, characterized first by cessation of breathing and then, after the apnea, by a simultaneous resumption of diaphragmatic movements and oral airflow; an "obstructive" or "peripheral" type, characterized by the interruption of airflow secondary to upper airway obstruction, but with continuance of diaphragmatic and thoracic muscle contraction; and a "mixed" type, characterized by an initial central apnea followed by temporary upper airway obstruction at the subsequent resumption of diaphragmatic movements.

We now report observations in two insomnia patients who presented symptoms we have designated as a syndrome of sleep apnea and insomnia. This syndrome can most readily be diagnosed with continuous all-night polygraphic recordings of sleep and respiration.

The patients were males, 57 and 54 years of age. Patient 1 gave a 20-year history of several arousals during the night and difficulty in maintaining his sleep, particularly between 3 and 6 a.m. He noted that he awakened himself and disturbed his wife by his snoring. After we completed our work-up, his wife mentioned that, on several occasions in past years, she had noticed a cessation of her husband's respiratory movements while he was asleep. This patient had taken many types of sleeping pills and reported that barbiturates and chloral hydrate were not only ineffective, but also increased his sleep problem. The sleep problem of patient 2 progressively developed over the past 25 years. He complained of several awakenings during the night and early morning arousals from which he found it very difficult to return to sleep. Finally, his wife complained about his snoring.

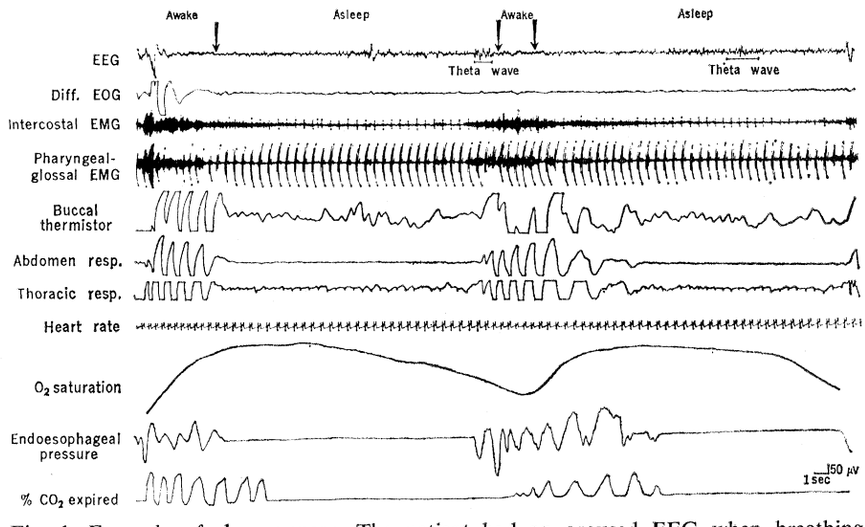

Fig. 1. Example of sleep apneas. The patient had an aroused EEG when breathing and sleep apnea when he fell asleep. There was no real increase in the endoesophageal pressure when he was breathing again. This is a central type apnea. The percentage of expired CO_2, determined from the nostril, shows that the air is expired with a delay directly related to physiological "dead space." Abbreviations: *Diff. EOG*, differential electrooculogram; *EMG*, electromyogram; and *resp.*, respiration.

Our studies during wakefulness included determinations of lung volume, ventilatory mechanics, elastic recoil, airflow resistance, and arterial blood gases. Patient 1 also participated in a more extensive cardiopulmonary study. A multilevel exercise study was conducted, with measurements of the ventilatory response to CO_2. Arterial blood was collected by means of a catheter introduced by percutaneous technique into the brachial artery, with P_{O_2}, P_{CO_2}, and *p*H determined by standard methods. Results of all these tests were well within normal limits during wakefulness. In addition, a posthyperventilation breathing test as described by Plum *et al.* (*3*) was also normal. Cardiac catheterization was performed by floating a Swan-Ganz catheter into the pulmonary artery through an antecubital vein. The catheter was positioned at 3:00 p.m., and continuous recording of pulmonary artery pressure was made over the next 18 hours. As in the other tests, these results were normal during wakefulness.

While the patient was in bed for the night, an ear oxymeter continuously measured arterial oxygen saturation. Arterial blood samples were drawn periodically throughout the night so that the oxymeter could be accurately calibrated. A catheter-tip pressure transducer (Bio-Tec-BT5F) was positioned in the lower esophagus for the recording of intrathoracic pressure changes (*4*), which facilitated accurate determination of the type of apnea involved. Several 0.2-mm wire electrodes were inserted in cricopharyngeal and intercostal muscles for electromyographic recording. A four-channel Sanborn polygraph and a Grass model 7 polygraph were used for the continuous all-night recordings.

Before the respiratory studies, documentation of the insomnia in each patient had been obtained during several all-night or 24-hour sleep studies. However, in the absence of ventilation recordings, the respiratory abnormalities reported here were not appreciated. During the most recent studies with