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Effects of PAM, proPAM, and DFP on Behavior, Thermoregulation, and Brain AChE in Rats

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KENLEY, R. A., R. A. HOWD AND E. T. UYENO. *Effects of PAM, proPAM, and DFP on behavior, thermoregulation, and brain AChE in rats.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1001–1008, 1982.—The effects of pyridine-2 aldoxime methyl iodide (PAM), N-methyl-1,6-dihydro-pyridine-2-carbaldoxime hydrochloride (proPAM), and diisopropyl phosphorofluoridate (DFP) on performance of a conditioned avoidance response (CAR), body temperature, and in vivo acetylcholinesterase (AChE) activity in five brain regions in the rat were examined. Sublethal doses of DFP (1.5 to 2.5 mg/kg, IP) markedly degraded CAR performance. This effect was antagonized by 5 mg/kg, subcutaneously injected (SC) atropine. A 50 mg/kg, SC dose of PAM had no effect on the CAR, but an equal dose of proPAM caused a transient deterioration of performance. Given 10 min or 2 hr after DFP, 50 mg/kg proPAM initially exacerbated the behaviorally toxic effects of DFP. Neither PAM nor proPAM antagonized DFP-induced hypothermia. PAM did not reactivate DFP-inhibited brain AChE, and proPAM reactivated it by only 6 to 12% of control activity.

Oximes PAM proPAM Acetylcholinesterase reactivation DFP Rats Conditioned avoidance response

CONVENTIONAL therapy for intoxication by organophosphorus acetylcholinesterase (AChE) inhibitors consists of coadministration of atropine (to antagonize the effects of accumulated acetylcholine) and a pyridinium oxime (to reactivate inhibited AChE) [22, 45, 46]. Although the utility of this regimen is generally acknowledged, considerable controversy remains over the question of pyridinium oxime effects on the central nervous system (CNS). The so-called blood-brain-barrier [7] severely impedes the transport of the cationic pyridinium compounds into the CNS, but readily passes typically lipophilic organophosphorus antiAChE agents. Thus, although treatment of antiAChE poisoning with pyridinium oximes is highly effective for peripheral regions, it fails to restore activity to a large fraction of inhibited brain AChE [13]. Both survival rates and physiological function of saved individuals should be markedly improved by using centrally active AChE reactivators. Attempts to confirm this hypothesis [3, 4, 25, 44, 47] using lipophilic reactivators have largely failed owing to high inherent toxicity and/or low activity of the reactivators chosen. Further complicating the issue of CNS activity of AChE reactivators are reports that in some cases pyridinium oximes devoid of CNS activity are effective antidotes [29], that low concentrations of pyridinium oximes can penetrate into the CNS [15], and that some pyridinium oximes contribute to a modest, but significant, restoration of inhibited AChE activity in specific regions of the brain [1, 2, 35].

Bodor *et al.* [8, 9, 41, 42] applied the "prodrug" concept to the problem of antiAChE agent therapy, which originally promised to contribute to an understanding of CNS effects of AChE reactivators. 1-Methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride is designated proPAM because it rapidly oxidizes in vitro and in vivo to the well-known reactivator pyridine-2-aldoxime methyl chloride (PAM). In principle, as a tertiary amine, proPAM can penetrate into the CNS and oxidize to PAM, thereby reactivating brain AChE. Intravenous (IV) administration of proPAM in mice poisoned with diisopropyl phosphorofluoridate (DFP), reportedly [42] enhances the reactivation of brain AChE relative to an equivalent dose of PAM. Because proPAM converts to PAM, inherent toxicities of the two drugs should be similar, and the increased reactivation of brain AChE might make proPAM a more effective antidote than PAM.

However, recent investigations reveal that proPAM provides only a modest improvement over PAM in DFP poisoning [12] and no improvement when given as an antidote against other organophosphorus antiAChE agents [10, 12, 21].

The apparent lack of correlation between reactivation of brain AChE and antidotal efficacy, (i.e., survival rates) raises the following questions: Is CNS reactivation critical to survival? What fraction of CNS AChE is required for survival? To what degree do pyridinium oximes reactivate CNS AChE? The cited investigations also leave unanswered the important question of whether proPAM differs from PAM in improving the functional ability of saved individuals via reactivation of inhibited brain AChE.

Given these considerations we have undertaken an investigation of the behavioral effects of PAM and proPAM alone and in DFP-intoxicated rats. We elected to examine learned

behavior as an example of physiological function that is known to be affected by antiAChE agents [6, 11, 23, 34] and reactivators [16–18, 38, 39]. Because behavioral effects of antiAChE agents can be of peripheral or central origin [27,28] we also directly examined the effects of PAM and proPAM in vivo on brain AChE.

Finally, we measured the effects of DFP, atropine, PAM, and proPAM on thermoregulation in rats. Control of body temperature is at least partially of central origin [28,48] and reversal of hypothermia induced by organophosphorus antiAChE agents is known [31–33] to discriminate between cholinesterase reactivators that either do or do not readily penetrate the blood-brain-barrier.

GENERAL METHOD

Materials

Pyridine-2-aldoxime methyl iodide (PAM) (Sigma Chemical Co.), diisopropyl phosphorofluoridate (DFP) (Aldrich Chemical Co.), and atropine sulfate (Merck, Inc.) were used as supplied. A sample of l-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride (proPAM) was obtained from the U. S. Army Medical Research Institute of Chemical Defense. The purity of the sample was confirmed by elemental and uv-spectrophotometric analysis. DFP solutions in 5% aqueous ethanol) were prepared and used within one hour. Atropine, PAM, and proPAM were dissolved in 0.9% saline solution. At 25 mg/ml, the proPAM solutions in 0.9% saline exhibited pH=3.0±0.1. The stability of proPAM in the injection vehicle was checked spectrophotometrically (250 nm for aliquots removed from saline and diluted into pH 3.0 citrate buffer). The loss of proPAM followed first-order kinetics and was approximately 1% per hour. Under our experimental conditions therefore, decomposition of proPAM in the injection vehicle was not a significant source of error. Male Fischer rats (200 to 350 g) were used throughout the experiments. DFP was administered intraperitoneally (IP), while atropine, proPAM, and PAM were injected subcutaneously (SC). A standard volume of 2 ml/kg of drug solution or saline was administered in all cases.

Multisensory Conditioned Avoidance Response (CAR)

A detailed description of the apparatus and procedures for this test has been presented elsewhere [36]. The system is computer-automated and allows concurrent testing of 24 rats. The rat learns to climb or pull a pole to avoid a painful foot shock. Twenty-four test chambers—constructed of red Plexiglas—are interfaced with a DEC PDP 8/F computer (located in an adjoining room) that provides programmed stimulus control and data collection. The chambers are housed in separate, sound-attenuating cubicles in plywood cabinets (6 chambers per cabinet). Each chamber has its own air-circulation system. A response is signalled to the computer whenever the rat closes a microswitch by climbing or pulling the pole in the ceiling of the chamber. A response during a trial terminates the trial; responses that occur between trials are accumulated and recorded as intertrial responses.

Initial training consisted of giving each rat 30 trials to learn to escape an aversive current on the floor (1.0 to 1.5 mA; scrambled constant-current) by climbing or pulling a 20-cm pole. Each trial was presented randomly in time (25 to 60 sec) and lasted for 30 sec unless the rat responded earlier, in which case the response terminated the trial. Then each rat was given 3 to 6 daily, 60-trial sessions to learn to avoid the aversive foot shock by responding on a 12.5-cm pole whenever the intensity of the house light increased, or a 4 kHz tone came on, or a low-intensity, nonaversive current (<0.15 mA) was imposed on the floor. The conditioned stimulus (CS) preceded the aversive, unconditioned stimulus (US) by 10 sec. If the rat responded during this period, the trial was terminated and a successful avoidance was recorded. If the rat did not respond during the 10-sec period, the CS remained on and the US was initiated. Both the CS and the US remained on until the rat responded (an escape response) or for a maximum period of 20 sec (an escape failure). The trials were presented randomly in time (at intervals of 25 to 120 sec) to preclude temporal responding. The three stimuli were presented randomly, with only one occurring on each trial. The stimuli were pulsed at the rate of 1.25 times per second.

Rectal Temperature

Core temperatures were measured with a Yellow Springs Instrument Co. Model 44TD telethermometer and Model 402 probe. The probe was dipped in mineral oil and inserted 5 cm into the rectum. The temperature was monitored at 30 sec after insertion. Thirty minutes later, DFP was injected, followed immediately by other drugs. Temperatures were recorded at hourly intervals after injection. Ambient temperature was maintained at 22±1°C.

Brain AChE

Rats were sacrificed by decapitation 4 hr after injection of DFP. Brains were removed from the rats without the olfactory lobes and grossly dissected into five regions. The cerebellum was cut off at the cerebellar peduncles. Cerebral cortex was removed bilaterally, taking everything above the lateral ventricles, then slicing straight down on each side, avoiding the striatum. A section containing the pons was removed by an anterior incision extending from the front of the superior colliculi to just in front of the mammillary bodies, and posterior through the widest part of the fourth ventricle (approximately A16 to A7, [49]). The remaining anterior tissue was designated "rest of forebrain." The medulla was from the pontine cut to about the level of the atlas.

The rats weighed 270 to 290 g, and had mean (±S.D.) brain weights of 1.76±0.07 g. The brain parts weighed as follows: cerebellum, 0.26±0.06 g; cortex, 0.53±0.09 g; rest of forebrain, 0.61±0.08 g; pons, 0.24±0.03 g; and medulla, 0.12±0.02 g.

Rat brain sections were frozen on dry ice for weighing, then thawed and homogenized with a Polytron in 19 volumes of pH 8.0, 0.1 M phosphate buffer, except for the medulla, which was homogenized in 39 volumes of the buffer. Fifty to 100 μl of brain homogenate was pipetted into 3 ml 1-cm path-length cuvettes containing 2.82 or 2.77 ml, respectively, of pH 8.0, 0.1 M phosphate buffer. To this was added 100 μl of 0.01 M dithionitrobenzoic acid (in pH 7.0, 0.1 M phosphate buffer) and 30 μl of 0.075 M acetylthiocholine iodide (in 1:9 H_2O:ethanol).

The rate of hydrolysis of acetylthiocholine and subsequent formation of a complex with dithionitrobenzoic acid [14] was determined at ambient temperature from the increase in absorbance at 412 nm using a Gilford-D.U. spectrophotometer coupled to a MINC-11 (Digital Equipment Corp.) microcomputer for on-line slope calculation (Kenley

et al., J. mednl Chem., in press). All assays were run in duplicate. Net activities (after subtraction of a phosphate buffer blank) are expressed as absorbance change/min/g of tissue.

EXPERIMENT 1

The purpose of this experiment was to establish the validity of the CAR procedure as a model for testing the efficacy of drugs that might protect against or reverse the effects of antiAChE agents. DFP was selected as the antiAChE agent and atropine sulfate as the antagonist. Preliminary experiments established a range of doses of DFP that were effective in blocking CAR performance but not lethal. The LD_{50} of DFP in rats of the same strain, sex, and age as used in the preliminary experiments was 3.7 mg/kg (3.2 to 4.3 mg/kg, 95% confidence limits). Preliminary trials demonstrated that a dose of 2.0 mg/kg of DFP caused a severe and prolonged impairment of CAR performance, whereas a dose of 0.5 mg/kg was ineffective.

Procedure

Forty-eight rats were pretrained to perform the CAR as described in the General Method section. On the test day they were given nine warmup trials to ensure that their CAR performance was intact. Then half the rats were injected SC with saline (2 ml/kg) and the other half were injected SC with 5 mg/kg atropine sulfate (2 ml/kg in saline). Fifteen minutes later equal numbers of rats within each group were injected IP with 0.0, 1.5, or 2.5 mg/kg of DFP (2 ml/kg in 5% aqueous ethanol). All rats were tested for CAR performance in three consecutive 60-trial blocks beginning within 15 min after the second injection. Trials were presented randomly in time, about once every 2 min on the average. Thus, each block required about 2 hr and the total test sessions lasted about 6 hr.

Two measures of CAR performance were analyzed statistically: the percentage of avoidance failures (no response before the onset of the US) for each stimulus in each block of 60 trials, and the percentage of escape failures (no response throughout the trial) in each block of 60 trials. After it was determined that there were no differences among the three stimuli as to the effects of the various treatments, the data were combined for each rat to represent the percentage of total avoidance failures in each block of 60 trials.

The data were analyzed for statistical reliability using a repeated-measures analysis of variance (ANOVA) with two between-subjects factors: dose of atropine sulfate and dose of DFP. The within-subjects factor was blocks of 60 trials [26]. Significant sources of variation were explored further by *t*-tests between selected pairs of means.

Results and Discussion

Figure 1 shows that DFP in the absence of atropine sulfate severely impaired CAR performance throughout the 6-hr test session, whereas atropine sulfate was without effect when given alone. Atropine sulfate (5.0 mg/kg) completely blocked the effect of the 1.5 mg/kg dose of DFP throughout the test session, and initially blocked the effect of the 2.5 mg/kg dose of DFP. However, as the test session progressed, atropine sulfate became less effective in rats given 2.5 mg/kg DFP and by the last block of 60 trials did not give any protection. The statistical reliability of these effects was supported by the significant atropine × DFP × blocks of trials interaciton of the ANOVA, $F(4,84)=28.75$, $p<0.0001$. We observed essentially the same results in the analysis of escape failures and, therefore, have not reported these data.

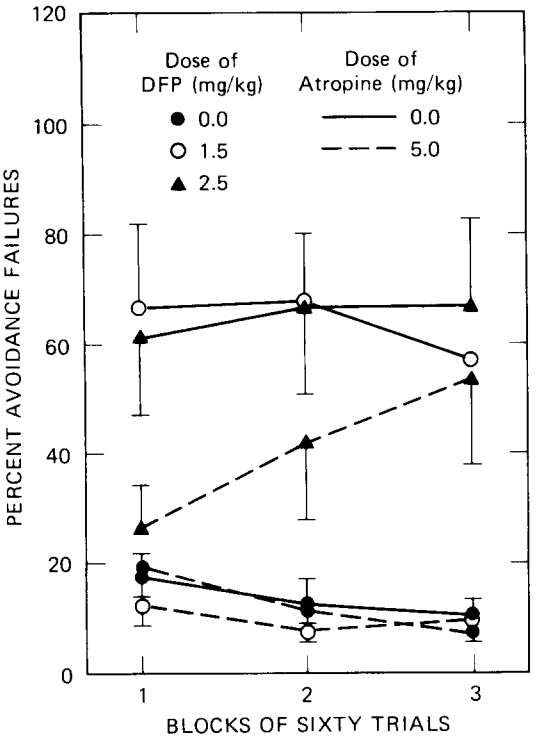

FIG. 1. Antagonism of the effects of DFP on performance of a multisensory conditioned avoidance response by atropine sulfate.

The results shown in Fig. 1 clearly demonstrate that the chosen CAR procedure can be used in monitoring the behavioral effects of DFP and that these effects can be antagonized by atropine sulfate. Although a dose-response effect of DFP on the percentage of avoidance failures was not evident in the rats given DFP alone (presumably because of a steep dose-response curve and a ceiling effect), such a relationship was evident in terms of percentage escape failures (data not shown). Moreover, the degree and duration of antagonism afforded by atropine sulfate provided further evidence for a dose-related effect of DFP. Whereas 5.0 mg/kg of atropine sulfate was able to protect completely against the effect of 1.5 mg/kg of DFP throughout the test session, this dose of atropine sulfate only afforded temporary protection against the effect of 2.5 mg/kg of DFP. Thus, as the atropine concentration declined below a critical level, the effect of accumulated acetylcholine resulting from the DFP-inhibited AChE predominated.

The protection afforded by atropine sulfate in this experiment was probably of central nervous system origin, because atropine methyl bromide is ineffective in this regard [38,39].

It is also useful to compare Experiment 1 with the previous data of Bignami *et al.* [6]. Using an automated shuttle box avoidance technique and an experimental paradigm similar to ours, Bignami and coworkers observed (as we did) a steep dose-response curve for the effects of DFP on behavior in rats. These authors also reported that effects of DFP on CAR were maximal within 1 to 2 hr and remained essentially constant over a 5-hr period. Figure 1 shows a very similar

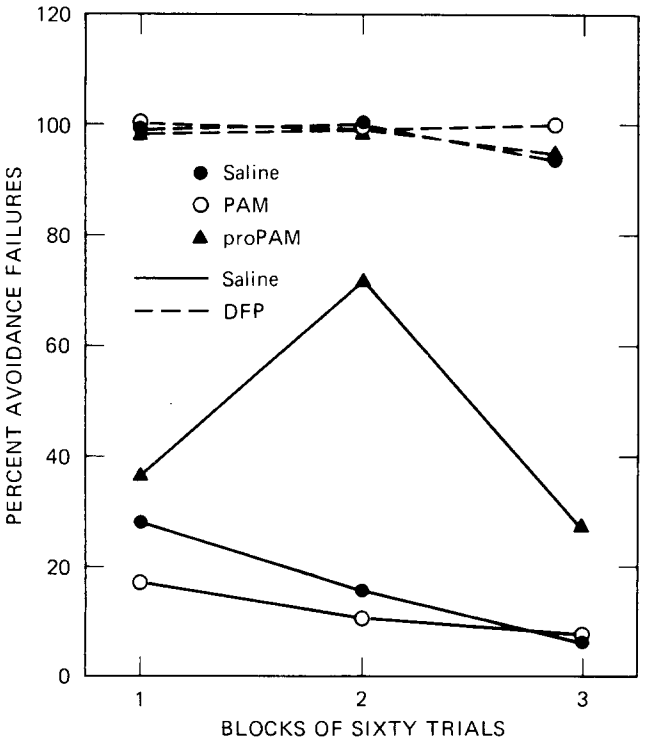

FIG. 2. Inability of PAM (50 mg/kg, SC) and proPAM (50 mg/kg, SC) to reverse the effect of DFP (2.0 mg/kg, IP) in performance of a multisensory conditioned avoidance response.

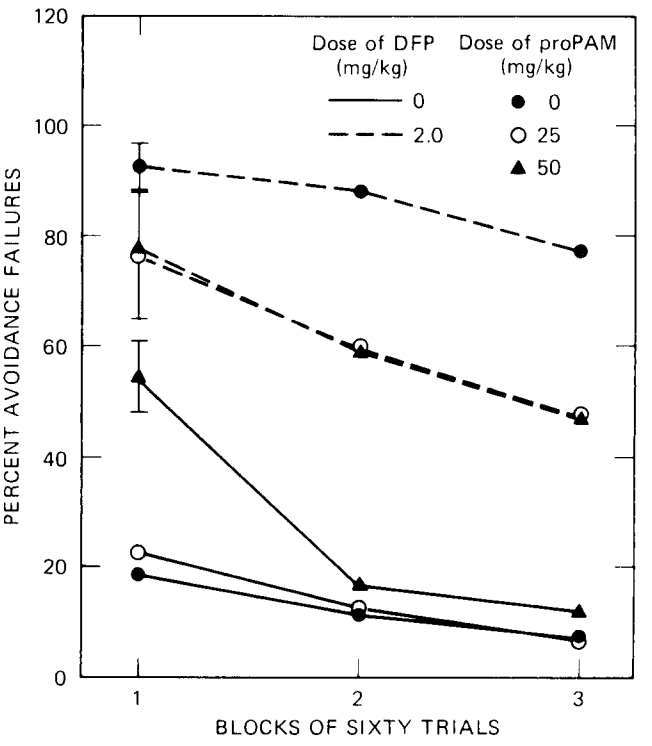

FIG. 3. Partial protection against the effect of DFP on performance of a multisensory conditioned avoidance response by proPAM.

time dependence for our study. Finally, we found that IP injection of 1.5 mg/kg (0.41 times the LD_{50}) of DFP induces a 65% CAR failure rate in otherwise untreated rats. By comparison, Bignami *et al.* administered a somewhat lower dose of DFP (0.8 mg/kg SC, 0.25 times the LD_{50}) and report a correspondingly lower CAR failure rate of approximately 40%.

Thus our data closely parallels that of Bignami and coworkers and we confirm their earlier observations. We concur with their conclusions that the CAR technique represents a generally useful method for probing the physiological effects of antiAChE agents, and the avoidance response tests are a more sensitive measure of anti-AChE effects than simpler methods such as assigning scores on the basis of symptoms or measuring spontaneous motor activity.

EXPERIMENT 2

The purpose of Experiment 2 was to compare PAM and proPAM efficacy in reversing the effect of DFP on CAR performance.

Procedure

Forty-eight rats were pretrained as described in the General Method section. After nine warmup trials on the test day, half of the rats were injected IP with saline (2 ml/kg) and the other half with 2.0 mg/kg of DFP (2 ml/kg in 5% aqueous ethanol). They were then given a 60-trial test for CAR performance. Immediately after this test (i.e., 2 hr after DFP administration), one third of the rats in each group were injected SC with saline (2 ml/kg), one third with 50 mg/kg of PAM (2 ml/kg in saline), and one third with 50 mg/kg of proPAM (2 ml/kg in saline), and tested for two additional 60-trial blocks. The data were analyzed as in Experiment 1.

Results and Discussion

Figure 2 shows that 2.0 mg/kg of DFP completely abolished CAR performance throughout the test session in this experiment. Neither PAM nor proPAM were able to reverse this effect when given about 2 hr after DFP. Indeed, proPAM caused a marked, but transient, impairment of CAR performance when given in the absence of DFP. The statistical reliability of these effects was supported by the significant 3-way interaction of the ANOVA, $F(4,74)=13.77$, $p<0.0001$. Essentially the same results were found in the analysis of escape failures, and therefore these data are not reported.

Failure of PAM to reverse the effect of DFP in CAR performance in this experiment was expected. Rosic [38] reported a similar result for toxogonin in rats treated with DFP and atropine methylbromide using a 2-way shuttle box procedure. However, because proPAM was expected to enter the CNS more readily than PAM, it was possible that reactivation of inhibited AChE might have been sufficient to at least partially reverse the behavioral effect of DFP. Such was not the case, and, indeed, proPAM at the dose used had a behaviorally toxic effect of its own, albeit of a transient nature. Whether this effect represents a direct effect of proPAM itself or the result of proPAM conversion to PAM in the brain is an interesting, but as yet unanswered, question.

We emphasize that the inability of PAM and proPAM to antagonize the behavioral effects of DFP cannot be explained by "aging" of DFP-inhibited brain AChE. The "aging" phenomenon is well-known [45] for organophosphorus

TABLE 1

EFFECTS OF DFP, ATROPINE, proPAM, AND PAM ON TEMPERATURE IN RATS

Drug Dose (mg/kg)*							
DFP	Atropine	proPAM	PAM	n	ΔT_2† °C	ΔT_{max} at t °C	hr
—	—	—	—	23	+0.2 ± 0.4	+0.4 ± 0.3	1
1	—	—	—	7	−0.6 ± 0.5	−0.6 ± 0.5	2
2	—	—	—	7	−0.8 ± 0.5	−0.8 ± 0.5	2
4	—	—	—	4	−2.7 ± 0.6	−2.7 ± 0.6	2
6	—	—	—	4‡	−3.2 ± 1.1	−4.9	5
—	20	—	—	6	+1.0 ± 0.2	+1.0 ± 0.2	2
—	—	—	25	6	+0.1 ± 0.4	+0.4 ± 0.4	1
—	—	—	50	10	+0.7 ± 0.6	0.8 ± 0.2	1
—	—	50	—	4	+0.4 ± 0.8	+0.8 ± 0.5	1
—	20	—	25	6	+0.7 ± 1.1	+1.1 ± 1.1	1
2	—	—	50	6	−1.1 ± 0.4	−1.3 ± 0.3	3
2	—	50	—	6	−1.3 ± 0.8	−1.6 ± 0.7	4
10	20	—	25	6	−1.8 ± 0.7	−1.8 ± 0.7	2
20	20	—	25	6	−1.5 ± 1.0	−1.5 ± 1.0	2

*DFP given IP, all other drugs SC alone or immediately after DFP.

†ΔT_2: temperature change at 2 hr; ΔT_{max}: maximum temperature change, at indicated time, t.

‡Two rats died in 2 hr, three died in 5 hr.

AChE inhibitors and relates to unimolecular conversion ("dealkylation") of inhibited enzyme to a species that is inert to reactivation by oximes. The phenomenon expresses itself as a decrease in the maximal percent reactivatable inhibited AChE with an increasing time interval between injection of the AChE inhibitor and the potential reactivator. For DFP-inhibited mouse brain AChE at 37°C, the half-life for the "aging" process is 4.8 hr (see Table 51 in [45]). In our experiments, PAM and proPAM were given 2 hr after DFP and during this interval only 25 percent (0.25=exp-[(0.69/half-life)·2 hr]) of the DFP-inhibited enzyme could have converted to the "aged" species. Thus a major fraction of the inhibited enzyme remained potentially susceptible to the effects of PAM and proPAM.

EXPERIMENT 3

Although proPAM (given 2 hr after DFP) was unable to reverse the effect of DFP on CAR performance in Experiment 2, it was possible that this drug might affort protection if given before DFP. Therefore, in Experiment 3 we gave one of two doses of proPAM to rats just before DFP and testing for CAR performance.

Procedure

Forty-eight rats were pretrained as described in the General Method section. After nine warmup trials on the test day, the rats were assigned to one of three groups, and each group was injected SC with 0, 25, or 50 mg/kg of proPAM (2 ml/kg in saline). Fifteen minutes later half of each group was injected IP with saline (2 ml/kg) or 2.0 mg/kg of DFP (2 ml/kg in 5% aqueous ethanol) and tested for CAR performance in three consecutive 60-trial blocks. The data were analyzed as in the first two experiments.

Results and Discussion

The results shown in Fig. 3 suggest that proPAM had a slight protective effect against the impairment of CAR performance caused by DFP. However, this effect could not be confirmed statistically by ANOVA because of the large variability among rats treated with both proPAM and DFP. Some rats in these groups appeared partially protected, whereas others did not respond throughout the 6-hr test session. Resort to nonparametric methods of analysis was also unsuccessful in demonstrating any statistical reliability of these trends. Thus, we conclude that proPAM may provide some protection against the behavioral effect of DFP, but that this effect is weak and may be idiosyncratic. As in Experiment 2, the 50 mg/kg dose of proPAM caused a transient impairment of CAR performance when given alone.

EXPERIMENT 4

The fourth set of experiments was designed to probe the effects of DFP, atropine sulfate, PAM, and proPAM on a physiological function other than behavior. We determined rectal temperatures of rats given DFP and then treated with atropine sulfate, PAM or proPAM.

Procedure

Rats were injected IP with various doses of DFP, or injected SC with other drugs or injected with DFP and then injected with other drugs. Core temperatures were recorded hourly as described in the General Method section. The temperature changes from zero time are reported at a single time point (2 hr) and expressed as ΔT_2, and are also reported at the time (t) when the temperature change reached a maximum, expressed as ΔT_{max}.

Results and Discussion

Table 1 summarizes the effects on thermoregulation of DFP, atropine, and the two reactivators. The table shows the dose-dependent DFP-induced hypothermia and the slight hyperthermic effects of atropine, PAM, and proPAM. The

TABLE 2

EFFECTS OF DFP, proPAM, AND PAM ON RAT BRAIN AChE ACTIVITY

Brain Region	AChE Activity (Abs. Unit·min^{-1}-g of Tissue)*						% Reactivation†	
	Control (Saline)	PAM (50 mg/kg)	proPAM (50 mg/kg)	DFP (2.0 mg/kg)	DFP + PAM (2.0 + 50 mg/kg)	DFP + pro PAM 2.0 + 50 mg/kg	DFP + PAM	DFP + proPAM
Cortex	20.5 ± 1.1	20.0 ± 0.7	20.4 ± 3.6	3.3 ± 1.3	3.6 ± 0.7	5.3 ± 1.3	1.7	11.6‡
Cerebellum	14.8 ± 0.7	14.2 ± 0.6	14.9 ± 1.2	3.3 ± 1.1	3.2 ± 0.5	4.4 ± 0.8	−0.9	9.6
Forebrain	57.5 ± 5.4	60.0 ± 6.6	60.0 ± 1.9	8.1 ± 3.2	8.6 ± 1.3	11.2 ± 3.0	1.0	6.3
Pons	33.3 ± 6.8	36.8 ± 1.4	35.6 ± 3.1	6.8 ± 1.6	5.4 ± 1.3	9.1 ± 1.7	−5.3	8.7
Medulla	29.6 ± 1.8	31.5 ± 2.7	32.9 ± 2.7	5.0 ± 2.0	4.9 ± 0.8	6.5 ± 1.9	−4.1	6.1

*Drug doses, routes and times of administration as in Table 1. Error limits are ±S.D.
†% Reactivation calculated from equation (1).
‡Statistically different ($p<0.05$) from DFP alone.

combined application of atropine and PAM antagonized the expected hypothermic effects of a 10 mg/kg dose of DFP, but neither proPAM nor PAM given without atropine significantly affected the temperature reduction caused by 2.0 mg/kg DFP.

EXPERIMENT 5

Because proPAM and PAM were ineffective in antagonizing the temperature decrease in DFP-poisoned rats and only marginally effective in antagonizing the behavioral effects of DFP, we determined the degree to which the reactivators actually restore AChE activity in the CNS. To check the possibility of uneven distribution of proPAM or PAM activity in the CNS [2] we measured AChE activities in five regions of the brain.

Procedure

This experiment employed some of the animals used in Experiment 4. The animals were sacrificed by decapitation immediately after recording the final temperature, i.e., 4 hr after injection of DFP. The brains were removed, dissected, and AChE activity was determined as described in the General Method section. For each brain region the percentage reactivation of AChE activity was calculated according to equation (1):

$$\% \text{ reactivation} = A_R - A_I/A_c - A_I \times 100 \quad (1)$$

where A_c, A_I, and A_R represent, respectively, enzyme activities before inhibition, after inhibition, and after reactivation. Statistical significance of mean differences in AChE activity was determined by Student's t-test.

Results and Discussion

Table 2 summarizes the results of Experiment 5 and shows that a 2.0 mg/kg dose of DFP inhibited brain AChE in all regions to approximately 15% of control activity. A 50 mg/kg dose of PAM had no significant effect on inhibited enzyme activity. The same dose of proPAM apparently provided marginal (≤12%) reactivation in all brain regions, although the reactivation was statistically significant only in the cortex.

It is useful to compare our data for in vivo reactivation of inhibited brain AChE by PAM and proPAM with analogous literature data. Table 3 gives values for percent reactivation of inhibited whole brain AChE calculated from the original literature data according to equation (1). The table also summarizes experimental details and brain enzyme activities after inhibition but before reactivation. From the data in this table the effectiveness of proPAM as a reactivator in the CNS appears to be highly dependent on the route of administration. Against DFP, 50 mg/kg proPAM given IV restores mouse brain AChE to 69% of control activity [42]. Also in mice, the same dose of proPAM given by IM injection yields only 14% reactivation of DFP-inhibited AChE [12]. Our data for SC administration of 50 mg/kg proPAM to rats revealed a similarly low (12%) reactivation of DFP-inhibited AChE. The data of Table 3 for IP injection of proPAM show intermediate levels of reactivation for this route of administration.

These observations on AChE activity indicate that the CNS activity of proPAM is a function of the proPAM to PAM oxidation rate relative to the rate of proPAM transport from the injection site to the CNS. To the extent that proPAM oxidizes before it passes the blood-brain-barrier, reactivator will not reach the CNS. Because proPAM's oxidation rate is fast (half-time in mice ≈1 min [41]) and because the rate of PAM removal from the brain is also fast (half-time ≈20 to 30 min [8]) rapid transport of proPAM to the CNS is critical to establishing effective concentrations of reactivator in the brain. The drug delivery rates for various routes of administration typically decrease in the following order: IV > IP > SC > IM. Therefore, IV administration of proPAM should reactivate the greatest percent of inhibited brain AChE.

Obviously, if slow tissue distribution retards delivery of proPAM to the CNS, proPAM will not be markedly superior to PAM as an antidote.

GENERAL DISCUSSION

ProPAM given subcutaneously shortly after DFP injection contributes to reactivation of inhibited brain AChE and ameliorates the behavioral decrements caused by DFP. These results generally support the hypothesis that CNS effects of AChE reactivators could contribute to improved

TABLE 3

REACTIVATION OF PHOSPHORYLATED AChE IN WHOLE BRAIN BY PAM AND proPAM AS A FUNCTION OF ROUTE OF ADMINISTRATION

Inhibitor	Dose mg/kg	Route	Species	Reactivator	Dose mg/kg	Route	Administration* Time min	Sacrifice Animals min	A_i %	R %	Reference
DFP	2.0	IP	Rat	PAM	50	SC	+1	+240	16†	<1†	This work
				proPAM	50	SC	+1	+240	16†	12†	
DFP	10	IV	Rat	PAM	40	IP	‡	+60	3.7	6.3	[40]
				proPAM	40	IP	‡	+60	3.7	36	
DFP	1.6	SC	Mouse	PAM	40	IP	‡	+60	8.4	5.0	[40]
				proPAM	40	IP	‡	+60	8.4	16	
DFP	25	SC	Mouse	PAM	50	IM	−10	+30	§	4	[12]
				proPAM	50	IM	−10	+30	§	14	
DFP	2.0	SC	Mouse	PAM	50	IV	+15	+90	11	12	[42]
				proPAM	50	IV	+15	+90	11	69	
Paraoxon¶	0.5	SC	Mouse	PAM	40	IP	+60	+120	15	8.2	[10]
				proPAM	40	IP	+60	+130	15	29	
Paraoxon¶	0.5	SC	Mouse	PAM	34	IP	+30	+90	31	8.7	[21]
				proPAM	34	IP	+30	+90	31	17	

*Time relative to administration of DFP or paraoxon.
†Calculated from the data of Table 2 using activities per unit weight of brain regions and average weights of individual regions.
‡Not specified.
§Not specified, but probably $A_i=0$, considering the high dose of DFP.
¶Paraoxon is diethyl *p*-nitrophenyl phosphate.

functional ability of saved individuals. The two beneficial effects (reactivation of AChE in the CNS and improved CAR performance) of proPAM observed by us were small and accompanied by pronounced transient behavioral toxicity. The limited effectiveness of proPAM given by the subcutaneous route probably relates to the short half-lives for formation ($t_{1/2}$=1 min) and elimination ($t_{1/2}$ ≈20 min) of the CNS-active species (i.e., the 1,6-dihydropyridine form) compared with slower rate of transport of the active species from the site of injection to the brain. These results provide some insight into the ultimate usefulness of proPAM as a therapeutic for antiAChE agent intoxication and support a general caveat about centrally-active therapeutics.

In the former regard, intravenous injection of an oxime is a useful route of administration in the laboratory or under certain clinical circumstances. For emergency first-aid, however, intramuscular or subcutaneous routes are more practical. In such instances, proPAM is unlikely to surpass PAM in terms of antidotal efficacy owing to minor differences in the extent of reactivation of inhibited brain AChE induced by either compound. If proPAM were given mistakenly in the absence of a background of antiAChE agent intoxication, it could produce a behaviorally toxic effect. A similar effect would be observed if proPAM were given under circumstances where the inhibited enzyme has become refractory to reactivation as a result of the "aging" phenomenon.

It is becoming increasingly clear that CNS side effects of centrally active antidotes can outweigh any therapeutic benefits. In addition to proPAM, examples of centrally active drugs that have been considered for use as antidotes but that produce performance decrements are the anticholinergic benactyzine [20, 30, 43] and the reversible AChE inhibitors Mobam [19,24] and physostigmine [5, 28, 37]. It therefore seems advisable to test the behavioral effects of CNS active drugs at an early stage in their evaluation as antidotes. The paradigm described herein could be of considerable utility in this regard.

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