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A Strain Comparison of Physiological and Locomotor Responses of Mice to Diisopropylfluorosphosphate

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SMOLEN, A , T N SMOLEN, E I OH AND A C COLLINS *A strain comparison of physiological and locomotor responses of mice to diisopropylfluorophosphate* PHARMACOL BIOCHEM BEHAV 24(4) 1077–1082, 1986 —The effects of acute treatment with the organophosphate, diisopropylfluorophosphae (DFP), were studied in three inbred mouse strains, C57BL, DBA and C3H A battery of physiological and locomotor tests including respiratory rate, heart rate, body temperature, Y-maze activity and rotarod performance was used Dose-response and time course studies were carried out Approximately 15 min after injection the animals were markedly affected by the drug with maximal effects occurring approximately 2 hours after injection Strain comparisons were made at the 2 hr time point. In all strains, males and females were affected about equally except for respiratory rate and rotarod performance in which females were slightly more affected Strain comparisons revealed that for most of the tests the C57BL mice were most affected by the DFP and the C3H mice were least affected For the heart rate test the DBA mice were the most sensitive Previous studies from our laboratory have demonstrated a similar rank ordering of the strains in their responses to oxotremorine and nicotine The strain differences in response to these agents is not easily explained by differences in number or affinity of brain muscarinic or nicotinic receptors. The genetic influence on cholinergic drug response may involve receptor coupling mechanisms.

Diisopropylfluorophosphate DFP Organophosphates Genetics Hypothermia
Locomotor activity Heart rate Respiration rate

ORGANOPHOSPHATES such as diisopropylfluorophosphate (DFP) cause an irreversible inhibition of acetylcholinesterase (AChE) Inhibition of this enzyme results in the accumulation of acetylcholine in cholinergic synapses which causes intense stimulation of these pathways The excessive stimulation of muscarinic and nicotinic receptors results in a variety of physiological signs including lacrimation, urination, salivation, defecation, tremors, and finally convulsions and death.

There are genetically determined differences in the responses of animals to organophosphates. The Flinders R- (Resistant) and S- (Sensitive) rat lines developed by Overstreet and co-workers [12–15] have been used extensively to study genetic influences on response to DFP, to muscarinic agonists, and mechanisms associated with the acquisition of tolerance to organophosphates. Our approach has been to study DFP actions on various inbred mouse strains Three commonly available strains, C57BL/6, DBA/2 and C3H have been used in these studies We have shown that the C57BL mice are very sensitive to the lethal and temperature-lowering actions of DFP that C3H mice are very resistant and that the DBA strain is intermediate in sensitivity [16]. This differential sensitivity is apparently not due to strain differences in metabolism of DFP [11] or to the extent of brain AChE inhibition [16], but rather to some other CNS mechanism which is not yet known.

In the study reported here, we have assessed strain differences to DFP using several physiological and activity measurements. respiratory rate, heart rate, body temperature, Y-maze activity and rotarod performance. There is ample evidence in the literature to suggest that these kinds of measurements would be useful in quantitating genetic influences on acute responses to cholinergic agonists. The reduction of body temperature following drug treatment has been the most widely used of the physiological measurements. Strain differences in temperature response have been reported for nicotine [8], oxotremorine [7] and organophosphates [16]. In each case the C3H mice were most resistant to the temperature lowering effects of these cholinergic drugs, and the C57BL mice were found to be most sensitive [16] or equal in sensitivity to the DBA strain [7,8].

Locomotor activities have been used to study the genetics of sensitivity to muscarinic agonists [7] and antagonists [13, 17, 18] in the mouse DBA mice are very sensitive to oxo-

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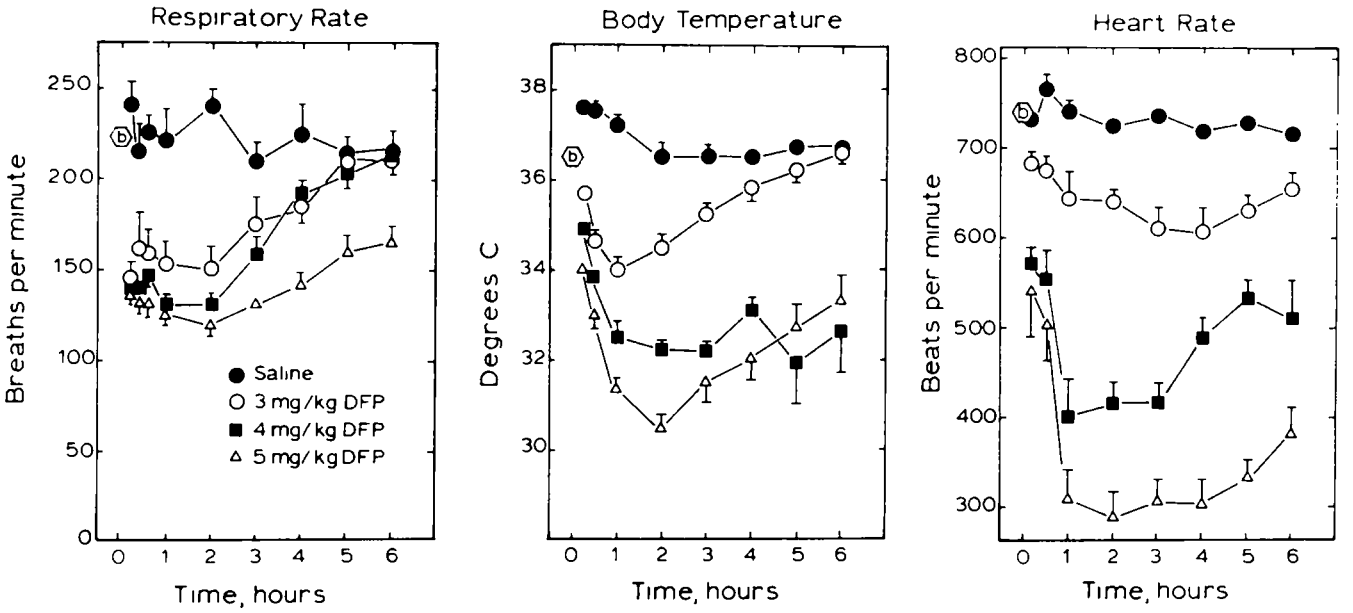

FIG 1 Dose-response and time course of DFP effects on respiratory rate, body temperature and heart rate in male DBA mice DFP (3, 4, or 5 mg/kg) was administered, IP, in saline and at the times indicated, the mice were tested <b> represents basal measurements for each test Each point is the mean±SEM for 12 animals, except temperature for which n=8

tremorine as measured by open field activity, C3H are quite resistant, and the C57BL mice are intermediate Strain differences in sensitivity to nicotine show a different pattern The C3H mice are most sensitive to the stimulating actions of nicotine as measured by open field and Y-maze activity tests The C57BL and DBA mice are about equally sensitive to the depressant properties of nicotine [6,8]

We have been studying the C57BL, DBA and C3H mice because of their differential sensitivity to nicotine and oxotremorine In a previous paper we showed that they were differentially sensitive to DFP as well In this paper we report on a series of experiments which more fully examine the acute effects of DFP in these mouse strains This battery of physiological and locomotor tests has been of value in quantitating the development of tolerance to muscarinic and nicotinic drugs [6–9] and the results presented here suggest that they will be useful in measuring tolerance to chronic DFP treatment as well

METHOD

Animals

Mice of the C57BL/6Ibg, DBA/2Ibg and C3H/2Ibg strains were bred at the Institute for Behavioral Genetics, University of Colorado, Boulder, CO The mice were housed 3–4 per cage in a room maintained at 21±2° under a 12 hour light/dark photoperiod Food and water were available ad lib Animals were 60–90 days of age when tested

Respiratory Rate

Respiratory rate was measured with a Columbus Instruments Respiration Monitor (Columbus, OH) Mice were placed inside a glass jar (10 5 cm diameter, 17 cm high), containing a small amount of aspen bedding After a 5 min acclimation period, a lid containing a pressure-sensitive transducer was placed on the jar creating a closed system The respiratory rate was recorded five times in a 1 min period starting 1 min after the jar was closed The five values were averaged and reported as the respiratory rate

Heart Rate

Heart rate was measured by placing a mouse in a restrainer to allow the insertion of needle electrodes under the skin One electrode was placed behind the left foreleg and the other in front of the right hindleg The electrodes were connected through a preamplifier to a Narco Biosystems E & M Physiograph Heart rate was measured for 6 sec and the rate was estimated by counting the number of QRS complexes

Body Temperature

Body temperature was measured with a Bailey Instruments (Saddlebrook, NJ) Thermalet THS probe The probe was lubricated with corn oil and inserted 2 5 cm into the rectum The probe equilibrates to body temperature within 10 sec

Y-Maze Activity

Both locomotor and rearing activities were determined in a symmetrical Y-maze The maze consists of three arms, 26 cm long, 6 1 cm wide and 10 2 cm high Each arm is subdivided into two equal sections The apparatus is constructed of red (top and bottom) and black acrylic plastic and is underlit through the floor with two 8 watt fluorescent lamps

Testing was conducted for 3 min during which time both crosses and rears were counted A cross was counted when an animal entered an arm (all four legs) or crossed the midpoint mark on an arm in either direction The activity score reported is the sum of the crosses during the 3 min test period Rearing was assessed as the number of times the mouse lifted its front paws off the floor during the test period

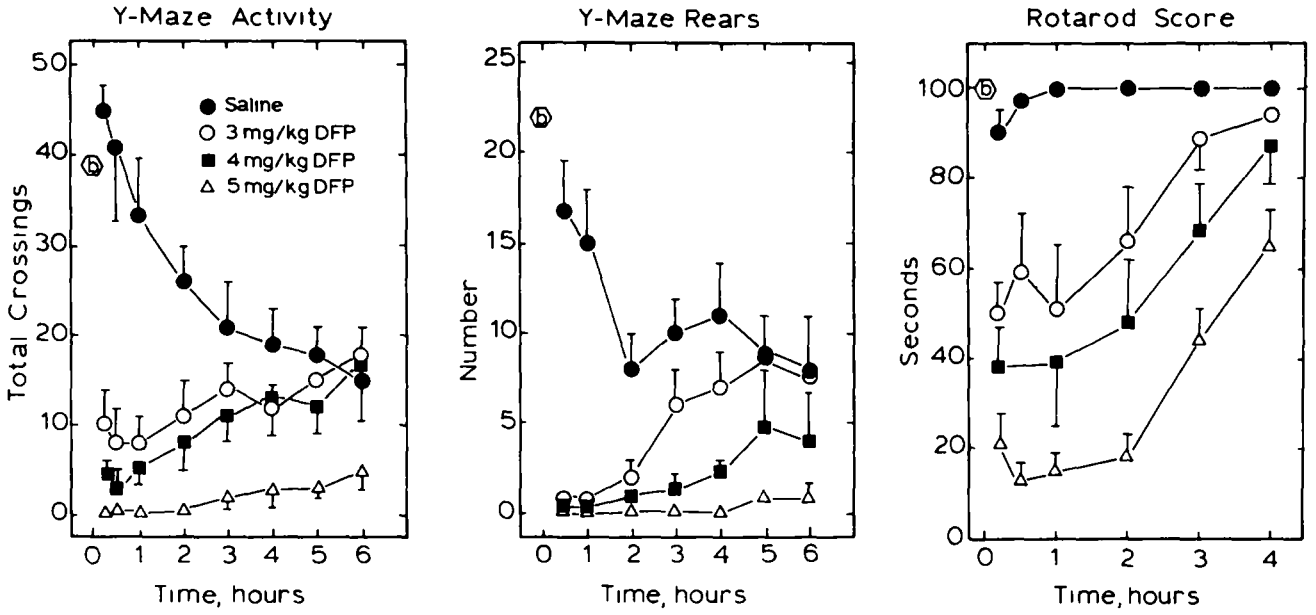

FIG 2 Dose-response and time course of DFP effects on Y-maze activity, Y-maze rears and rotarod performance in male DBA mice DFP (3, 4, or 5 mg) was administered, IP, in saline and, at the times indicated, the mice were tested for their response <b> represents basal measurements for each test Each point is the mean±SEM for 12 animals

Rotarod Test

The mice were trained to walk on a rotating rod (Rotarod, Ugo Basile Co., Milan, Italy) until they were capable of remaining on the apparatus for two successive 100 sec test periods. The rotation speed was 8 rpm Once trained, their performance remains adequate for several days. To test for drug effect, the mice were injected with DFP and, at the times indicated in the figures, were placed on the rotating rod for 100 sec or until they fell off.

Time- and Dose-Dependent Effects of DFP

Prior to injection of DFP (Sigma Chemical Co.) in saline, a baseline value was determined for each animal for each test After the baseline values were determined, the animal was injected with saline or DFP (3, 4 or 5 mg/kg) and was retested 0 25, 0.5, 1, 2, 3, 4, 5 and 6 hours later Each animal was given only one drug solution and was used for only one test.

Data Analysis

Data were analyzed by one or two way analysis of variance (ANOVA) as appropriate If a significant overall F ($p<0$ 05) was obtained, differences in individual group means were assessed using Duncan's New Multiple Range test

RESULTS

Figure 1 shows the effect of DFP on physiological measures in male DBA mice. DFP caused a dose-dependent reduction of respiratory rate, body temperature and heart rate Depending on the test and the dose of DFP used, maximum decrements occurred between 15 min (respiratory rate) and 2 hours. The effects of DFP on these physiological responses were reversible, since after 6 hours the animals were at, or were returning to, baseline levels. This is clearly evident for respiratory rate (3 and 4 mg/kg) and body temperature (3 mg/kg)

Figure 2 shows the effect of DFP on activity measurements in male DBA mice. The saline curve shows that repeated exposure to the apparatus has the effect of gradually reducing the two activity scores Total Y-maze activity and rearing in the Y-maze were markedly reduced within 15 min following DFP administration. For total activity, the dose-response curve is indicated by an initial decrement of activity which becomes more severe with increasing dose. The rate of return of activity is similar for the 3 and 4 mg/kg doses Animals treated with these doses of DFP have activities which approximate those of saline-treated controls by 6 hours. Rearing activity was virtually eliminated at each dose of DFP The dose-dependent nature of this effect is seen by the differential rate at which rearing activity increases towards baseline.

Performance on the rotarod (Fig 2) was markedly reduced within 15 min of injection of DFP. The decrement of activity was greater with increasing dose and the inability to walk on the rotarod remained at a reduced level for approximately 2 hours Four hours after treatment with DFP, the animal's performance had returned to near predrug levels.

Preliminary studies indicated that the time course of DFP responses was similar for all three strains. From the time course and dose response studies on male DBA mice shown in Figs 1 and 2, a routine procedure to screen for DFP effects in mice was devised Each animal was tested on each measurement 2 hours following a 4 mg/kg injection of DFP. The results of this testing procedure on three inbred mouse strains is shown in Fig 3.

Two way (strain-by-sex) analysis of variance showed main effects of strain on basal respiratory rate, basal heart rate and basal rearing score, $F(2,66)=6.53$, 5 04, 4.13; $p<0$ 01, 0 01, 0 05, respectively In each case, the C57BL and C3H mice were the extremes in basal measurements

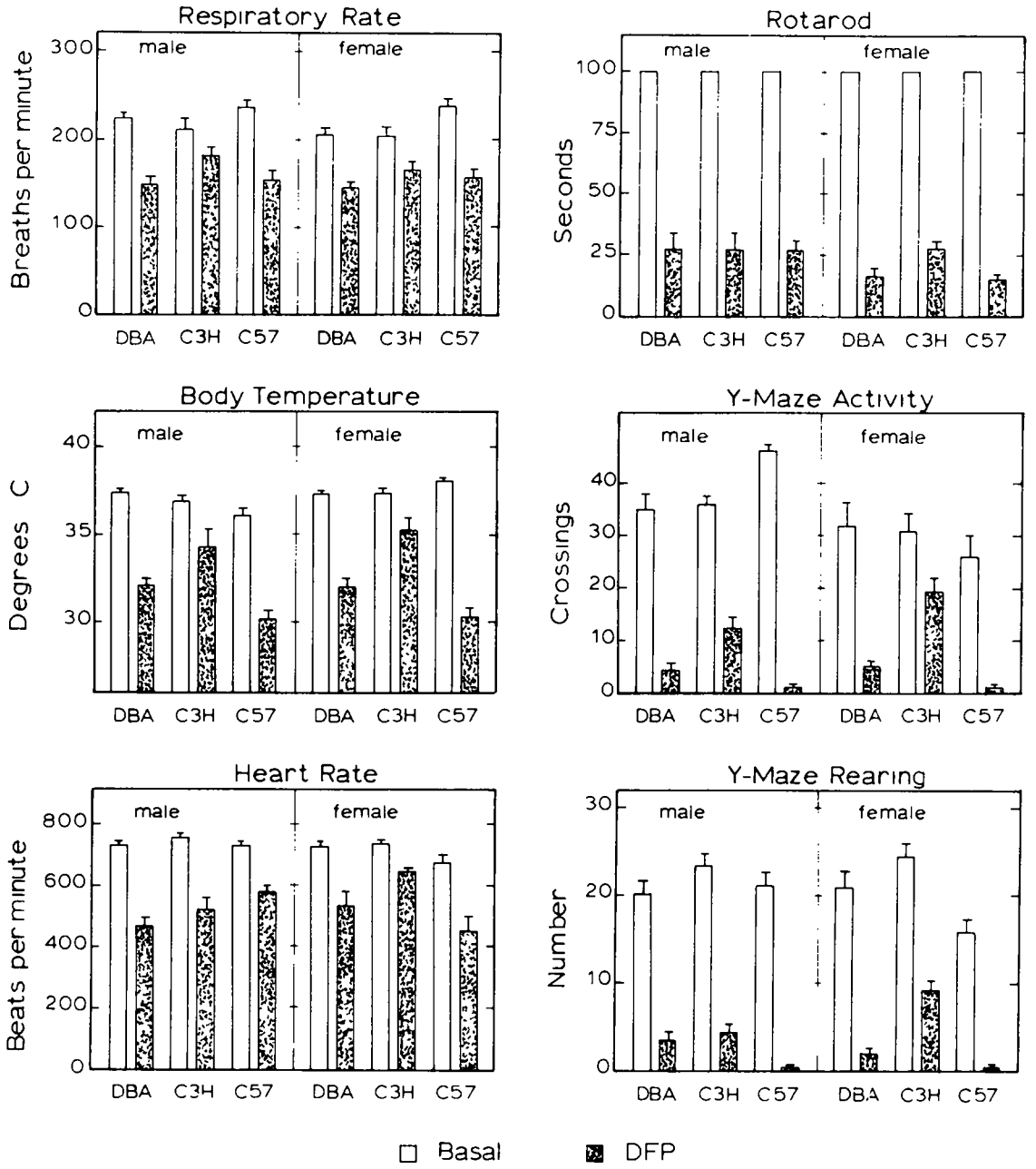

FIG 3 Strain comparison of DFP effects Following baseline measurements, 12 mice of both sexes (8 for temperature) were injected with 4 mg/kg DFP and tested on each apparatus 2 hours later

There was no strain difference detected in basal Y-maze activity or body temperature

Following a 4 mg/kg dose of DFP, main effects of strain on respiratory rate, heart rate, Y-maze activity and Y-maze rearing score, $F(2,66)=22\ 18,\ 3.79,\ 11.60,\ 5\ 35$, $p<0\ 01$, 0.05, 0 01, 0 01, respectively, were detected These significant differences were due to C3H mice having higher scores (less affected) than the other two strains. There was no strain difference on rotarod activity following DFP

Main effects of sex were found for basal heart rate, basal Y-maze activity, respiratory rate following DFP and rotarod performance following DFP, $F(1,66)=4\ 75,\ 13\ 94,\ 5\ 10,\ 5\ 74$, $p<0\ 05$, 0.01, 0 05, 0 05, respectively Females were lower than males for each of these measurements

If the data were analyzed as the percent of basal values following DFP, the results obtained were the same as those obtained using the raw score analysis except that the main effect of strain on heart rate was not significant, $F(2,66)=2\ 61$, and the main effect of sex on respiratory rate was not significant, $F(1,66)=0$. The rank ordering of the strains remained the same in the case of heart rate. For the respiratory rate test, the females were slightly more resistant than the male

A detailed study of the temperature response to DFP has

been published [16]. The response to a 4 mg/kg dose, which was used in the test battery, is shown in Fig. 3. There were no main effects of strain or sex on basal body temperature. Two hours following DFP administration, strain differences were evident, $F(2,42)=8.42$, $p<0.01$, but no sex differences were evident. The C57BL mice were most and the C3H least affected by DFP.

In general, the data of Fig. 3 show that the C57BL mice are most affected by DFP, C3H the least. The only exception is the heart rate measurement for which the male DBA mice are most affected by DFP. Sex differences are small compared to strain differences, and for all practical purposes, the two sexes can be considered identical in their responses to DFP.

DISCUSSION

These studies show significant differences in response to the acute effects of DFP among mouse strains. In nearly all cases the C3H mice are least affected by DFP, the C57BL mice are most affected. The DBA strain is generally intermediate in its response to DFP. In an earlier study [16] which concentrated on the lethal and temperature-lowering effects of DFP on these strains we found a similar rank order of these three strains in response to DFP.

Sex differences in response to DFP are not marked. In the present study, males and females were affected about equally. The males were slightly more resistant to DFP than females for respiratory rate and rotarod tests when the raw data scores were used for comparison. However, when the data were analyzed in terms of percent reduction from basal levels, the sex difference for the effect of DFP on respiratory rate disappeared. For the other four tests the females were more resistant than the males, but these differences did not reach a level of statistical significance. In general, we have found that female mice are somewhat more resistant to the debilitating effects of cholinergic drugs than are males. We have reported similar sex effects previously for DFP [16], oxotremorine [7] and nicotine [8].

Female mice of the strains used in this study have higher serum cholinestrase activity than the males [1], and Russell and Overstreet have reported that female rats of the Flinders R- and S-lines also have more serum cholinesterase activity than the males [13]. Russell and Overstreet suggested that this might contribute to the relative resistance of the females since less DFP could penetrate to the CNS. This protectant effect is not likely to occur to any significant extent in mice since we have previously shown that, for a given dose of DFP, brain AChE was reduced to the same level in both male and female mice of all three strains [16]. Thus, the relative resistance of female mice to DFP is likely due to CNS resistance to the increased synaptic acetylcholine concentrations following DFP administration and not to any sex-associated dispositional differences for DFP.

It is also unlikely that differences in DFP disposition among the three strains accounts for much of the strain differences observed. For a given dose of DFP, the brain AChE activity is reduced to the same extent in all three strains which indicates that a similar proportion of the administered drug reaches the CNS [16]. We have also presented preliminary evidence that there is little difference in the serum enzyme responsible for DFP hydrolysis, DFPase (paraoxonase), in these inbred mouse strains [11]. In addition, if differences in metabolism of DFP are responsible for the strain differences in response, the relative rank order of the three strains for the various tests should be the same. This is not the case in that the DBA mice were most responsive in the heart rate test and intermediate in response for the other tests. All of these observations lead to the conclusion that strain and sex differences in response to acute DFP administration are centrally mediated, with the likely exception of heart rate, and that metabolic factors play a minor role in determining sensitivity to organophosphates in the mouse.

The strain differences reported here are similar to the rank order of the strains for sensitivity to the muscarinic agonist oxotremorine [7] and to the nicotinic agonist nicotine [8]. These earlier studies from our laboratory showed the C3H strain to be least affected and the C57BL strain to be most affected by oxotremorine. For nicotine, the C57BL and DBA are about equally sensitive, the C3H mice are, again, quite resistant. It is interesting that brain muscarinic receptor numbers measured by ^{3}H-L-quinuclidinyl benzilate binding are very similar in the three strains [7]. Similarly, brain nicotinic receptors measured with ^{3}H-nicotine binding are virtually identical in the three strains [8]. Differences in brain, primarily hippocampal and midbrain, ^{125}I-α-bungarotoxin binding have been observed but the strain which is least sensitive to DFP, the C3H, has the greatest number of these brain nicotinic receptors [8]. The strains did not differ in K_D values for any of these ligands in any of the seven brain regions tested. These results indicate that the strain differences in acute responses to DFP cannot be easily explained by differences in receptor density. However, these receptor density measurements were made in homogenates obtained from seven brain regions. Differences in specific brain nuclei may not have been detected using this method. Clearly, additional studies of the relationship between cholinergic drug response and brain cholinergic receptors must be carried out before an unequivocal answer concerning the relationship between drug response and receptor number can be obtained.

Chronic treatment with organophosphates is known to result in a decrease in the number of brain muscarinic receptors [3, 4, 5] which occurs in parallel with the acquisition of tolerance. Thus, an argument could be made that strain differences in response to an acute dose of DFP should be relatable to differences in brain muscarinic receptors. However, as discussed above, we have not detected such differences [7] nor have we detected differences in brain nicotinic receptors [8] which explain the strain differences in response to DFP. In view of the fact that the strain differences in response to DFP parallel the strain differences in response to oxotremorine and nicotine, it seems reasonable to suggest that the genetic influence on cholinergic drug response is linked to cholinergic receptors or pathways in some manner. Perhaps the genetic influence on cholinergic drug response relates to a receptor coupling mechanism. There are a number of mechanisms through which receptor activation can lead to neuronal and behavioral responses [10]. Each of these represents a potential site for regulation of differences in responses to cholinergic drugs. Clearly, additional studies are needed to provide an explanation for the strain differences in response to DFP. Such studies could be useful in the rational design of antidotes to the organophosphates and in understanding mechanisms which allow for the selection of resistant strains of target organisms.

ACKNOWLEDGEMENT

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