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Genetically Determined Differences in Acute Responses to Diisopropylfluorophosphate

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SMOLEN, A., T. N. SMOLEN, J. M. WEHNER AND A. C. COLLINS. *Genetically determined differences in acute responses to diisopropylfluorophosphate*. PHARMACOL BIOCHEM BEHAV **22**(4) 623–630, 1985.—The acute effects of diisopropylfluorophosphate (DFP) were assessed in DBA/2Ibg, C57BL/6Ibg and C3H/2Ibg mice. The DFP was administered by intraperitoneal injection in saline. Brain acetylcholinesterase (AChE) activity was maximally inhibited within 5 min after injection. All mice showed signs of organophosphate intoxication including salivation, lacrimation, diarrhea, respiratory distress, tremor and, at high doses, seizures. The C57BL mice were most susceptible to these effects of DFP. The LD_{50} values for DFP were 8.0, 7.6, and 6.8 mg/kg for male DBA, C3H, and C57BL mice, respectively. The LD_{50} values for females were nearly the same. Body temperature and brain AChE activity decreased in a dose-dependent manner following injections of DFP of 3.17, 4.22, 5.28, and 6.33 mg/kg. Maximum temperature depression occurred 2 hours after DFP administration; by 24 hours temperatures had returned to normal except for C57BL mice treated with the highest dose of DFP. The C57BL strain was most susceptible to the DFP-induced hypothermia, the C3H strain was the most resistant, and the DBA strain was intermediate. Maximum temperature depression and residual AChE activity, as measured 24 hours after injection, were linearly related. These strain differences do not seem to be explained easily by a differential inhibition of AChE activity.

Diisopropylfluorophosphate Strain differences Brain AChE activity Body temperature

ORGANOPHOSPHATES produce many of their effects by reacting with, and irreversibly inhibiting, acetylcholinesterase (AChE, EC 3.1.1.7), which results in the accumulation of acetylcholine in cholinergic synapses. Thus, the actions of organophosphates are due largely to excess cholinergic stimulation. This excess stimulation presumably occurs at both muscarinic and nicotinic cholinergic sites. Included among the responses seen following organophosphate administration are salivation, lacrimation, urination and defecation as well as manifestations of central nervous system excitation such as tremors and convulsions.

Recent reports indicate clearly that the actions of organophosphates are regulated by genetic factors. Overstreet and coworkers [20] have selectively bred two lines of rat that differ in response to a single injection of diisopropylfluorophosphate (DFP). These two rat lines, the Flinders R- (resistant) and S- (sensitive) lines, were selectively bred for differences in DFP-induced changes in body temperature, water consumption and body weight. More recent studies indicate that the S-line is also more sensitive to the effects of DFP on locomotor activity, FR5 responding for a water reward, and analgesia which indicates that some degree of generalization exists [23]. The S-line is also more responsive than is the R-line to the actions of muscarinic agonists and antagonists. This finding argues that some factor related to receptor occupancy may underlie the genetically determined difference in sensitivity to DFP.

Ample evidence exists to support the notion that genetic factors influence the response of rats and mice to muscarinic agents. For example, studies by Anisman and coworkers [2,3] indicate that the muscarinic antagonist, scopolamine, affects locomotor activity to a different degree and/or manner in different mouse strains. Similarly, reports by van Abeelen and coworkers [26, 27, 28] demonstrate that the effects of muscarinic antagonists (scopolamine and methylscopolamine) on exploratory behavior in mice are genetically controlled, possibly via an influence on a cholinergic mechanism in the hippocampus [28]. Oliverio *et al.* [18] have also presented evidence which indicates that exploratory behavior in the mouse is mediated by cholinergic mechanisms that are genetically controlled. More recently, a report from our laboratory indicates that the effects of the muscarinic agonist, oxotremorine, on locomotor activity and body temperature are also genetically determined in the mouse [12]. We found that the C57BL strain was affected more by the hypothermia-producing and locomotor activity-impairing effects of oxotremorine than were DBA mice. Animals of the C3H strain were least sensitive to oxotremorine. We also

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measured muscarinic receptors, in seven brain regions, in these mouse strains. Although modest differences in receptor number were found, these differences did not easily explain the strain differences in acute responses to oxotremorine. We, therefore, suggested that the genetic influences on response to oxotremorine are due to some other factor such as a genetic influence on receptor coupling processes.

Genetic influences on the responses of rats and mice to nicotinic agonists also exist. An early report by Garg [7] demonstrated that nicotine facilitated rearing activity in rats and that this facilitation varied in intensity in different rat strains. Nicotine apparently can either increase or decrease activity in the rat and the direction of response is apparently genetically mediated. For example, Morrison and Lee [17] noted that the activity of rat strains with high basal activity (Lister and Sprague-Dawley) is decreased by nicotine whereas the activity of a low basal activity strain (Wistar) is increased. We [10,14] have observed genetic influences on nicotine-induced alterations in Y-maze and open field activity in the mouse. These differences in response are not apparently due to differences in the rates of metabolism of nicotine. Our studies have also demonstrated genetic influences on the effects of nicotine on such behavioral and physiological measures as heart rate, respiration rate, rotarod performance and acoustic startle response. For some of these measures (open field activity and startle response) both the magnitude and direction (stimulation or inhibition) are genetically influenced [14]. In general, we find that C3H mice are most sensitive to the stimulant effects of nicotine [14,16] while C57BL and DBA mice are most affected by depressant effects. We have also measured brain nicotinic receptors in these mouse strains. Only the strain difference in response to nicotine-induced seizures seems to correlate with brain nicotinic receptors [16].

Although, as noted above, evidence exists that mice differ in response to muscarinic and nicotinic agonists or antagonists, little evidence is available that indicates whether this genetic control extends to cholinesterase inhibitors. This paper presents the results of a study of the effects of the organophosphate, DFP, in three of the inbred mouse strains that we have been studying for their relative responses to muscarinic and nicotinic agonists. The results obtained indicate, as expected, that genetic factors do, indeed, influence the response of mice to DFP. The relative strain sensitivity resembles that seen for oxotremorine [12]. This strain difference is not readily explained by a difference in cholinesterase inhibition.

METHOD

Animals

Male and female DBA/2Ibg, C57BL/6Ibg and C3H/2Ibg mice, 60–80 days of age were used in these studies. Mice were raised at the Institute for Behavioral Genetics, kept on a 12 hour light cycle and allowed free access to food (Wayne Lab Blox) and water.

Drug Treatment

Diisopropylfluorophosphate (Sigma) was prepared in saline and injected intraperitoneally. It is common for DFP to be prepared in an oil vehicle, yet DFP is stable for several hours in saline [9,29]. Because a saline solution is more easily administered and was better received by the animals, the DFP was administered in a saline vehicle. Solutions were used within 1 hour of preparation.

LD_{50} Determinations

The lethal effects of DFP were assessed in male and female mice of each of the strains listed above. Mice were injected with 0.01 ml of drug solution per gram of body weight. Six DFP doses were used: 4.22, 5.28, 6.33, 7.39, 8.44 and 10.55 mg/kg. Mice were kept 2 or 3 to a cage and observed continuously for 3 hours. Time of injection and death were noted. The LD_{50} values were calculated from linear regression analyses of log dose vs. probit plots of the data.

Hypothermic Response to DFP

The experiments were conducted in a room which was constantly maintained at 23±0.1 degrees. Mice were weighed and placed back in their home cage. Two hours later their basal rectal temperatures were measured with a Bailey TH-5 temperature probe. Immediately after the basal temperature was taken, the animals were injected with DFP or an equal volume of saline (controls). Temperatures were taken at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 24 hours post injection. Twenty-four hours after receiving DFP or vehicle, brain AChE activity was measured.

Brain Acetylcholinesterase Activity

Mice were sacrificed by cervical dislocation, the brain removed rapidly and homogenized in 10 volumes of 50 mM potassium phosphate, pH 7.4, in an all-glass (Dounce) homogenizer. Whole brain AChE activity was measured using a modification of Ellman's method [6], as described previously [11]. In some experiments regional AChE activity was measured. In these experiments, mice were injected with DFP (6.33 mg/kg) or an equal volume of saline. The animals were sacrificed 24 hours later, their brains removed, and dissected, on ice, into cortex, cerebellum, hippocampus, hypothalamus, striatum, midbrain (the tissue remaining after removal of the hippocampus, hypothalamus and striatum from the midbrain area; primarily thalamus) and hindbrain (pons-medulla). Homogenates were prepared, as described above, and brain AChE activity measured.

Analysis of Data

Data were analyzed by linear regression analysis or analysis of variance, as appropriate. Temperature profiles were analyzed by three-way analysis of variance of both the maximum temperature depression and the area under the time-temperature curves. For maximum temperature depression, the body temperature 2 hours after DFP administration was used, without regard to initial body temperature. Area under the curve was calculated by taking the difference in the initial body temperature and the temperature at each time point. The area was calculated by integration (Simpson's rule) using a TI-59 programmable calculator. Thus, the hypothermia data were analyzed by two methods, one which depended on the initial body temperature, and one which did not. For all analyses, an alpha value of 0.05 was considered significant.

RESULTS

Figure 1 shows the time course of inhibition of whole brain AChE activity in each of the three strains of mouse following

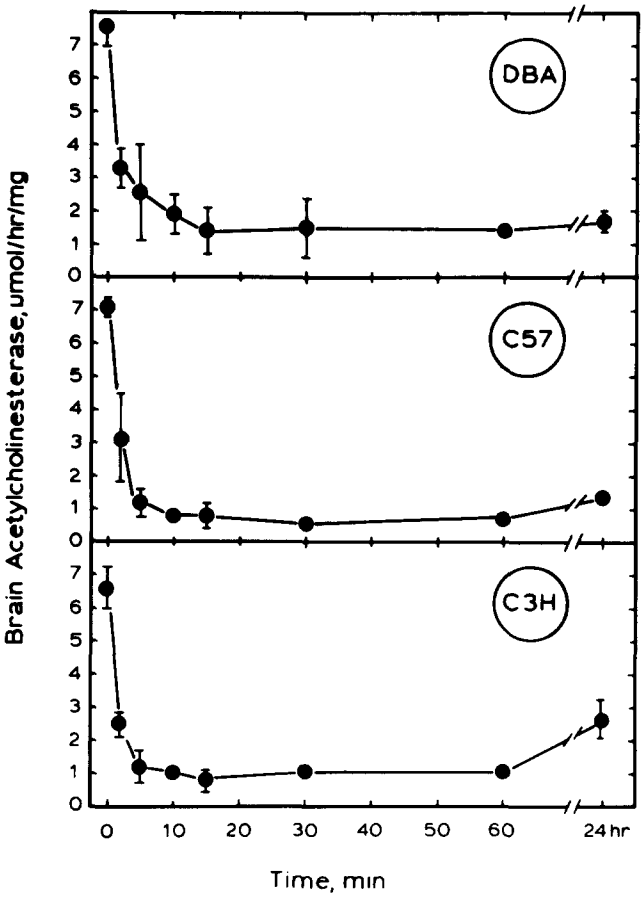

FIG. 1. Time course of inhibition of brain acetylcholinesterase activity following a single intraperitoneal injection of DFP. Male DBA, C57BL, and C3H mice were injected with DFP, 5.28 mg/kg, prepared in saline. At the times indicated, mice were killed and their brain acetylcholinesterase activity measured. Each point is the mean±SEM of 4 to 6 determinations. Where not indicated, standard errors are contained within the plotted points. At all time points, enzyme activity was significantly lower than control in DFP treated animals.

the injection of DFP (5.28 mg/kg). Brain AChE activity was maximally inhibited within 5 minutes of administration, and the activity was reduced for a minimum of 24 hours. The degree of inhibition was similar in all three strains. These results indicate that DFP can be administered in a saline vehicle and that the difference in response to DFP cannot be easily ascribed to a difference in inhibition of whole brain AChE activity.

The LD_{50} for DFP was estimated in the three strains of mice using the data depicted in Fig. 2. The data are plotted as DFP dose vs. % mortality at each dose. The plot shown in Fig. 2 is shown for clarity. The LD_{50} values were actually calculated from log dose vs. probit plots of the data. The DBA strain was the most resistant and the C57BL strain the most sensitive to the lethal effects of DFP. At sublethal doses of DFP, the C57BL mice exhibited marked signs of DFP intoxication including salivation, lacrimation (eyes white and opaque), severe diarrhea, extreme respiratory distress, copious respiratory tract secretions, and severe tremors. The C3H and DBA mice were not as visibly affected by the drug. Although not easily quantified, the general impression was that DBA mice were slightly more affected than C3H mice with the exception of lacrimation.

The average time of death at each DFP dose is shown next to the data points in Fig. 2. At any given dose, the DBA mice survived longer than did the other two strains, a further demonstration of their relative resistance. Death was often preceded by a hyperactivity phase consisting of increased locomotor activity (not wild running), tail rattling, tremor (often severe) and tonic seizures. Seizures, however, were not always seen. This phase could last for as short a period as 15 seconds or as long as 2 minutes.

Sex differences may also exist. In the DBA and C3H strains, females appeared to be slightly more resistant to the lethal effects of DFP than were the males. In the C57BL strain, the males may be more resistant. However, these sex differences are less than the strain differences that were detected.

The effects of DFP on body temperature are depicted in Fig. 3. The sexes did not differ in this response to DFP since an analysis of variance revealed no main effect of sex for maximal temperature depression (2 hours post DFP administration), $F(1,168)=1.04$, NS, or area under the time-temperature curve, $F(1,168)=1.17$, NS. Strain differences were evident for both maximal temperature depression, $F(2,168)=50.3, p<0.01$, and area under the time-temperature curve, $F(2,168)=133, p<0.01$. The C57BL mice were markedly affected by DFP as is evidenced by the large decrease in temperature while the C3H strain was least sensitive. The DBA strain was intermediate in response.

A dose-response relationship was evident in each of the three mouse strains for hypothermia. As the DFP dose increased, both the maximal temperature change, $F(3,168)=63, p<0.01$, and area under the time-temperature curve increased, $F(3,168)=145, p<0.01$. In the case of the C57BL mice, temperature decreased to nearly 25° at the highest dose used. Even with such severe drug effects, most of the animals survived, and by the next day body temperatures had returned to control levels. At each of the doses, the rank order of the strains remained the same with the C3H strain being the least, and the C57BL strain being the most affected. Neither sex-by-strain nor sex-by-dose interactions were significant in the analysis of variance. However, strain-by-dose interactions were significant for maximal temperature change seen at 2 hours, $F(6,168)=2.84, p<0.01$, and area under the time-temperature curve, $F(6,168)=5.67$, $p<0.01$. These significant differences were not further analyzed. Three way interaction terms were not significant.

Figure 4 shows the brain AChE activity remaining in the animals used in the temperature profile experiment. Enzyme activity was measured in these animals the morning after the effects of DFP on temperature were measured. As DFP dose increased, a modest increase in inhibition of AChE activity was evident. The reduction in brain AChE activity in the C3H strain was 5 to 10 percent less than that seen in the other two strains at the lower doses. This difference had disappeared at the higher doses. The small difference at the lower doses may partially explain the lesser sensitivity of C3H mice to the temperature lowering effects of DFP seen at these lower doses.

The analysis of variance of the data reported in Fig. 4 failed to detect any sex differences in control brain AChE activity when the data were pooled across strain, $F(1,46)=2.45$, NS. There were no strain differences in whole brain AChE activity in males, $F(2,21)=3.44$, NS, but a strain difference in female AChE activity was detected,

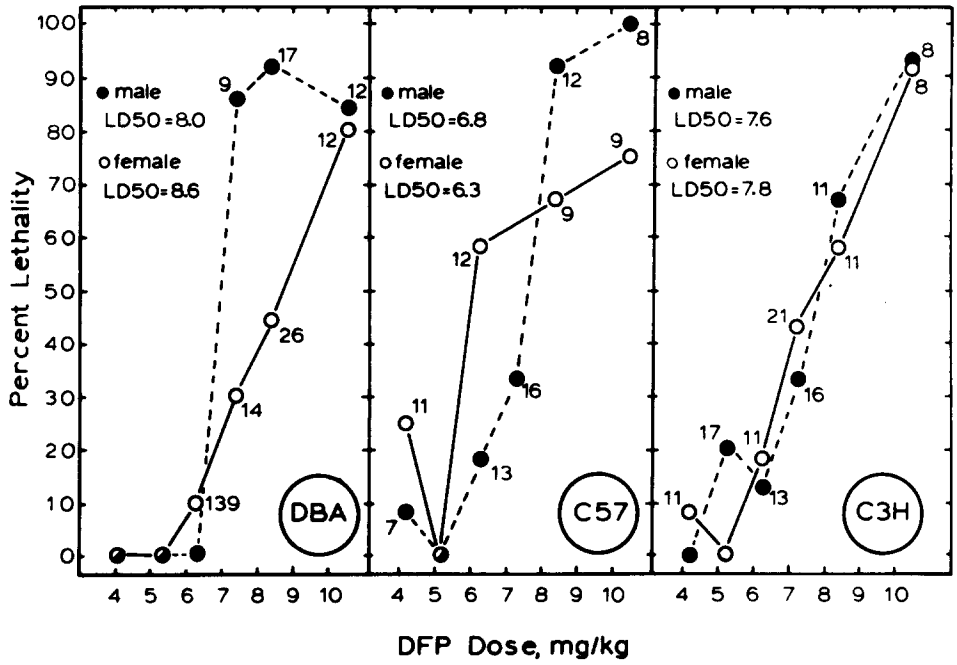

FIG. 2. LD_{50} for DFP. Male (●) and Female (○) DBA, C57BL and C3H mice were injected with various doses of DFP prepared in saline. Time of injection and death were noted. The average time for death to occur is listed next to the data point in the body of the figure. Percent lethality was determined for 7 to 20 animals at each point.

$F(2,21)=8.76$, $p<0.01$. This appears to be due to the lower basal activity seen in C57BL females. As noted above, strain differences in the effect of various doses of DFP on total brain AChE activity were not evident.

Table 1 presents the effects of a 6.33 mg/kg DFP dose on AChE activity in the seven brain regions measured 24 hours after DFP treatment. Strain differences in control AChE activity were detected in several brain regions with the DBA strain generally having the greatest activity. While the DBA strain had what appeared to be slightly higher activity in every brain region, statistically significant differences were detected only in cortex, hindbrain, and hippocampus, $F(2,18)=16.34$, 8.31, 7.82; $p<0.01$ for cortex, hindbrain, and cerebellum, respectively. Differences between brain regions in sensitivity to DFP were evident with cortex and striatum being markedly affected while hypothalamus and cerebellum were relatively unaffected. All three strains exhibited a similar degree of inhibition in the various brain regions when the data are compared on a percent inhibition basis. However, if residual activity is compared, the DBA strain appears to have more activity after DFP treatment than do the other two strains due, most probably, to the slightly greater basal activity seen in controls. DBA mice had greater enzyme activity in cortex, midbrain, and cerebellum than did the other two strains after DFP treatment ($F(2,20)=16.68$ and 8.18 for cortex and cerebellum, $p<0.01$; and $F(2,20)=3.58$ for midbrain, $p<0.05$). The total residual activity seen in the C57BL and C3H strains after DFP treatment is virtually identical in the seven brain regions.

Figure 5 is a plot of body temperature 2 hours post DFP treatment (maximal temperature decrease) vs. total brain AChE activity measured 24 hours after DFP treatment. The effects of all four doses used in the experiment described in Fig. 4 are plotted for each of the mouse strains. Males and females are plotted separately. No statistically significant sex differences were detected for DBA and C3H mice as determined by a comparison of the slopes of the lines. A sex difference ($p<0.05$) was detected in the C57BL strain. Figure 5 indicates that a linear relationship exists between effects of DFP on body temperature and brain AChE activity in all three mouse strains. The relationship is virtually identical in the three strains.

DISCUSSION

The results reported here show a clear genetic influence on the responses of mice to the acute effects of DFP. Strain differences of a quantitative nature were seen. In nearly all cases the C57BL strain appears to be the most affected by DFP. The C3H strain is least affected and the DBA strain is intermediate between the two. The only exception to this rank order is the lethality data where the DBA strain was found to be least sensitive. In addition to strain differences, sex differences were also evident with females being slightly less debilitated by DFP in all three mouse strains.

It does not seem reasonable to ascribe the strain differences in response to DFP to differences in degree of (% of control) AChE inhibition. When total brain activity was measured (Fig. 4) no strain differences in inhibition were detected. However, when regions were examined (Table 1) a slightly different picture emerged. When the effects of DFP on regional AChE activity were compared, no significant strain differences were observed when the data were calculated on a percent inhibition basis. However, if total residual activity comparisons are made, strain differences are evident. In several brain regions (e.g., cortex, midbrain, cerebellum) the DBA strain had greater activity after DFP treatment. This may explain why the DBA strain has the highest

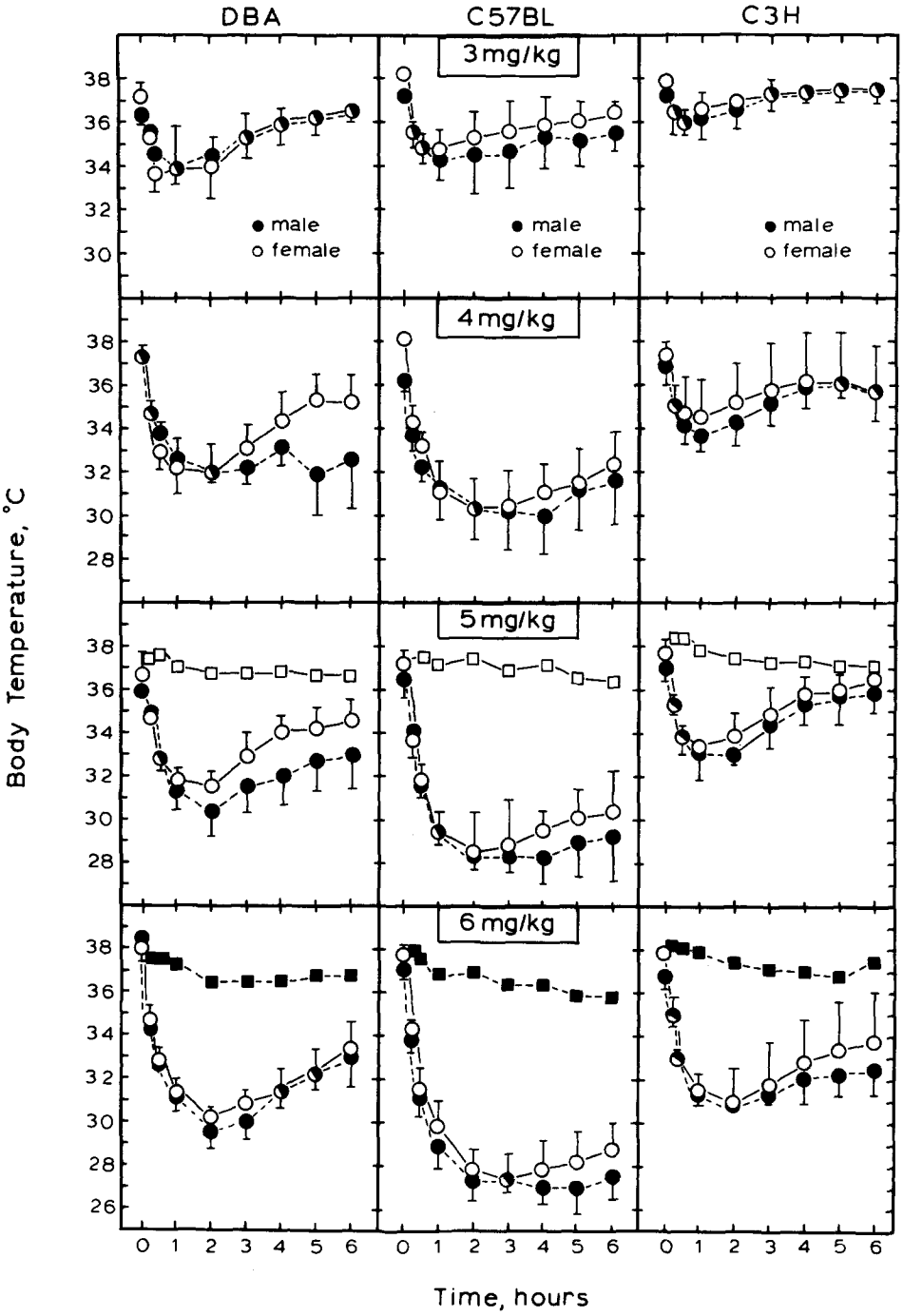

FIG. 3. Effects of DFP on body temperature. Male (●) and Female (○) DBA, C57BL, and C3H mice were injected with DFP, 3.17, 4.22, 5.28 or 6.33 mg/kg (designated as their integer values in the figure) prepared in saline. Male (■) and Female (□) controls were tested along with the DFP-treated animals at each dose but the results obtained are plotted in only one panel for clarity. Each point is the mean±SD of 8 animals per point. Standard deviation bars are directed down for males and up for females, except where obvious. Where not plotted, standard deviations lie within the plotted point. Standard deviations, rather than standard errors, are presented because the standard errors were often within the plotted points. Standard deviations, therefore, provide a better representation of the variability of the data.

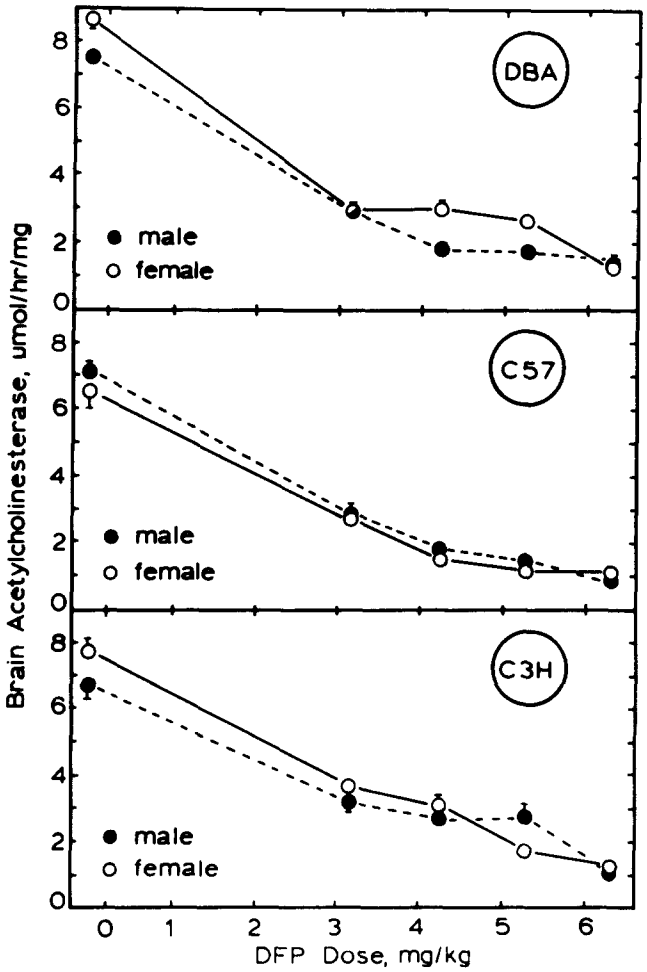

FIG. 4. Effect of varying doses of DFP on brain acetylcholinesterase activity. Total brain acetylcholinesterase activity was measured 24 hours after injection with 3.17, 4.22, 5.28 or 6.33 mg/kg of DFP prepared in saline. Each point is the mean±SEM of 8 animals per point. Males (●) and females (○) are plotted separately. Where not indicated, the standard errors are contained within the plotted data point. All doses of DFP elicited significant reductions, from control, of enzyme activity.

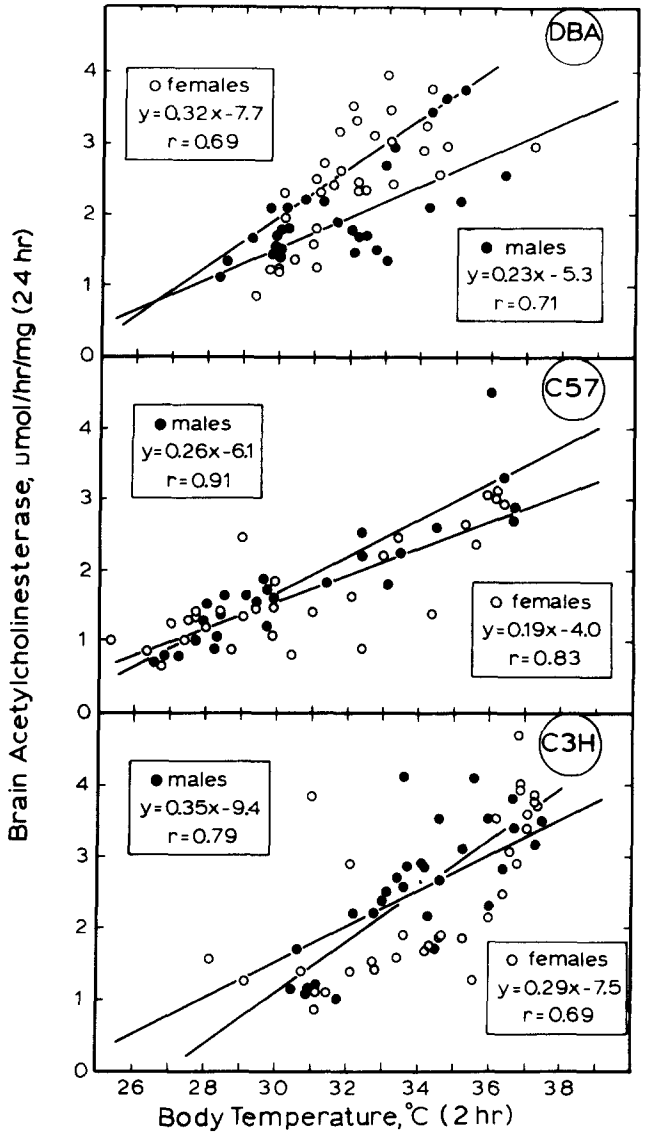

FIG. 5. Relationship between maximal temperature depression (2 hours after DFP treatment) and residual brain acetylcholinesterase activity measured 24 hours after DFP administration. Each of the slopes is significantly different from zero. Males (●) and females (○) are plotted separately. The slopes of the regression lines are not significantly different in the DBA and C3H strains but a significant ($p<0.05$) sex difference was detected in the C57BL strain.

LD_{50} for DFP. However, the DBA strain was intermediate between the other two strains for the hypothermic response while hypothalamic AChE activity which, presumably, could be very important with respect to a hypothermic response, was greatest in this strain. Thus, it seems reasonable to suggest that some factor other than relative inhibition of AChE activity is responsible for the strain differences in response to DFP.

The observation that sex differences exist, in rodents, in response to DFP is not a new one. Two studies, using the Flinders R- and S- rat lines, have demonstrated sex differences in acute response to DFP [21] and in the development of tolerance to DFP's actions [24]. The first of these studies [21] presented evidence that female rats are less sensitive to DFP and that females have greater serum cholinesterase (ChE) activity which may result in less DFP penetrating the CNS. We did not measure peripheral ChE activity but the data presented in Fig. 4 allow a partial assessment of the possibility that differences in peripheral ChE activity exist. Only in the DBA strain does it appear as though brain AChE activity is uniformly less affected in the females. No consistent sex differences were detected in the C57BL and C3H mice as estimated by the experiment reported in Fig. 4. However, as noted in Fig. 5, the AChE activity-hypothermia regression lines consistently had a greater slope for males but only in the C57BL strain was this apparent difference in slope between the sexes statistically significant. Thus, it is possible that a sex difference in response to DFP exists but sex differences are small in comparison to the strain differences. An explanation other than differences in inhibition of enzyme activity seems necessary to explain the strain and, perhaps, the sex differences.

As noted previously, we [10, 12, 14, 16] and others [2, 3,

TABLE 1

REGIONAL DISTRIBUTION OF ACETYLCHOLINESTERASE ACTIVITY IN MOUSE BRAIN FOLLOWING A SINGLE INJECTION OF DFP

Strain	Treatment	Acetylcholinesterase Activity (μmoles/hr/mg) Cortex	Midbrain	Hindbrain	Hippocampus	Striatum	Hypothalamus	Cerebellum
DBA	Saline (9)	7.26 ± 0.30	6.61 ± 0.65	5.46 ± 0.23	4.06 ± 0.25	16.06 ± 1.55	4.00 ± 0.37	1.51 ± 0.07
	DFP (8)	0.95 ± 0.04	1.32 ± 0.11	1.43 ± 0.15	0.75 ± 0.06	1.85 ± 0.19	1.55 ± 0.10	0.52 ± 0.02
	% Remaining	13.1	20.0	26.2	18.4	11.5	38.8	34.4
C57BL	Saline (6)	4.37 ± 0.25	4.84 ± 0.56	3.60 ± 0.48	2.81 ± 0.17	15.60 ± 1.76	3.07 ± 0.24	1.28 ± 0.13
	DFP (8)	0.60 ± 0.07	1.01 ± 0.15	1.23 ± 0.16	0.52 ± 0.08	1.41 ± 0.26	1.28 ± 0.15	0.37 ± 0.03
	% Remaining	13.7	20.9	34.2	18.5	9.0	41.7	28.9
C3H	Saline (6)	5.69 ± 0.53	5.44 ± 0.43	4.47 ± 0.32	3.18 ± 0.24	13.63 ± 1.04	3.17 ± 0.30	1.33 ± 0.12
	DFP (7)	0.59 ± 0.03	0.88 ± 0.08	1.17 ± 0.14	0.53 ± 0.09	1.19 ± 0.14	1.27 ± 0.16	0.39 ± 0.03
	% Remaining	10.4	16.2	26.2	16.7	8.7	40.1	29.3

Mice were injected with DFP (6.33 mg/kg) or an equal volume of saline. Approximately 24 hours later acetylcholinesterase activity was measured in each of the seven brain regions listed above. Tabled values are mean ± S.E.M. Numbers in parentheses are the number of animals in each group.

7, 17, 18, 26, 27, 28] have noted strain differences in response to muscarinic and nicotinic agonists and antagonists. In our study [12] of strain differences in response to the muscarinic agonist, oxotremorine, we noted that the rank order of strain sensitivity to the hypothermia-producing effects of oxotremorine was C57BL>DBA>C3H; an order which exactly matches the relative strain sensitivities found for DFP in the present study. We have also measured the effects of nicotine on body temperature in these three mouse strains [14] and found that the relative strain sensitivities are C57BL=DBA>C3H. The marked similarity in rank order of hypothermic response for oxotremorine and DFP suggests the possibility that muscarinic systems are more important than are nicotinic systems in regulating the hypothermic actions of DFP. This makes sense if for no other reason than muscarinic receptors outnumber nicotinic receptors by approximately 20:1 in brain [13,25]. The C57BL mice were also more sensitive than were the other strains to the salivation-, lacrimation-, urination-, and defecation-producing effects of DFP. These latter responses presumably arise as a consequence of activation of muscarinic receptors in the peripheral autonomic nervous system. Thus, it may be that the C57BL strain has a different level of muscarinic activity than do the other two strains in both the central and autonomic nervous systems.

A number of studies (see, for example [4, 5, 8]) have demonstrated that chronic treatment with cholinesterase inhibitors results in a decrease in brain muscarinic receptors. These changes in receptor number parallel the development of tolerance to several of the behavioral and physiological effects of the cholinesterase inhibitors [4]. Similarly, we have demonstrated that chronic infusion of the muscarinic agonist, oxotremorine, into the mouse results in a decrease in the number of muscarinic receptors [11,15]. We also found that considerable tolerance develops to oxotremorine before detectable changes in receptor number develop thereby suggesting that changes in receptor coupling processes are responsible for the early stages of tolerance to oxotremorine [15].

The strain differences in response to DFP observed in the present study may arise because of genetic influence on some receptor-related process. As mentioned above, we [12] have measured muscarinic receptors in seven brain regions in these strains and have detected modest strain differences in muscarinic receptor number, as measured by ^{3}H-L-quinuclidinyl benzilate binding. C3H mice have greater QNB binding than do the other two strains in striatum and DBA mice have greater QNB binding in the hippocampus than do C57BL mice; C3H mice are intermediate between the other two strains in hippocampal QNB binding but not significantly different from either. These differences are small (less than 20%) and, of importance to the present study, do not parallel the apparent strain differences in sensitivity to DFP. Thus, some explanation other than differences in muscarinic receptor number must be sought to explain the strain differences in response to DFP.

Acetylcholine also has nicotinic receptors which have classically been measured using alpha-bungarotoxin as a ligand [19,25]. More recently, we [13] and others [1,22] have demonstrated that ^{3}H-L-nicotine may also bind to brain nicotinic receptors. We have measured bungarotoxin and nicotine binding in the C57BL, DBA, and C3H mice [14]. The only significant strain difference found was C3H mice have a greater number of hippocampal and midbrain bungarotoxin binding sites than do the other two strains. No strain differences in bungarotoxin binding were found in the other five regions that we routinely measure nor were any differences in nicotine binding in any brain region found. Thus, the strain differences in response to DFP are not easily explained by differences in nicotinic receptors.

In summary, the present study demonstrates genetic influences on the response of mice to DFP and corroborates the finding of others [20, 21, 23, 24] who have demonstrated that genetic factors influence the response of rats to DFP. The strain differences in response to DFP seen in the mouse are not easily explained by differential inhibition of AChE nor are they easily explained by differences in muscarinic or nicotinic receptors. Perhaps differences in some process such as receptor coupling underlie these strain differences in response to DFP.

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