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VARIATION BETWEEN THREE STRAINS OF RAT: INHIBITION OF NEUROTOXIC ESTERASE AND ACETYLCHOLINESTERASE BY TRI-*o*-CRESYL PHOSPHATE

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The present study is concerned with the involvement of strain differences in rodent sensitivity to organophosphorous compound-induced delayed neurotoxicity (OPIDN). The inhibitory effect of three doses of tri-o-cresyl phosphate (TOCP) on neurotoxic esterase (NTE) and acetylcholinesterase (AChE) in brain was compared in three strains of rat: Long-Evans (LE) animals, which have been reported to be sensitive to the neurotoxic effects of TOCP, and Sprague-Dawley (SD) or Fischer 344 (F344) strains, with which negative results have been obtained. Differences in basal levels were found for NTE (LE > F344) > SD, with a range of 4.87–7.47 nmol phenylvalerate hydrolyzed/mg protein), but not AChE. Strain differences in inhibition by TOCP were found with both assays, with Sprague-Dawley animals being much less sensitive to esterase inhibition than either Long-Evans or Fischer 344 rats. The ED50 values for NTE inhibition were estimated to be 458, 209, and 288 mg/kg for SD, F344, and LE rats, respectively. The ED50 values for AChE inhibition were estimated to be 1007, 408, and 420 mg/kg for SD, F344, and LE rats, respectively. Liver microsomes from the Fischer animals had less cytochrome P-450 than those from the other two strains. Differences in the ability of the strains to either form or inactivate the active metabolite of TOCP may account for the variation observed. While metabolism may play a role in the differences in the level of NTE inhibition in SD rats compared to the LE strain, it cannot account for the lack of sensitivity of the F344 animals to OPIDN. These results may be important in selecting a strain for the study of the toxic effects of organophosphorous compounds in rats.

INTRODUCTION

Upon the discovery that tri-*o*-cresyl phosphate was responsible for the outbreak of "ginger jake" paralysis (Smith et al., 1930), now known as organophosphorous compound-induced delayed neurotoxicity (OPIDN) (Abou-Donia, 1981), the hen was quickly established as the animal model most useful for assessing the neurotoxicity of organophosphorous compounds. Hens are widely available, and demonstrate clinical and histopathological signs of neuropathy that are easily

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observed. Rodents, more commonly used laboratory animals, are less sensitive to the neurotoxic effects of organophosphorous compounds (Smith et al., 1932; Abou-Donia, 1981).

More recent evidence has shown that although rodents are less sensitive than the chicken, they develop delayed neurotoxicity following exposure to TOCP or mipafox (*N,N*-diisopropylphosphorodiamidic fluoride). First, some neurotoxic organophosphorous compounds can slow axonal transport in rat optic nerve (Reichert and Abou-Donia, 1980). Second, chronic exposure to TOCP can produce clinical signs of a neuropathy in the mouse (Lapadula et al., 1985) and rat (Veronesi, 1984). Third, low levels of nerve damage can be detected in rats after single doses of either TOCP or mipafox (Veronesi, 1984; Veronesi and Padilla, 1985).

Other studies have found that exposures of rodents to doses of organophosphorous compounds that would be extremely neurotoxic in the hen have no apparent clinical effect beyond the acute toxicity attributable to acetylcholinesterase inhibition. For instance, subchronic (14-d) exposure to high levels of *O,O*-diisopropyl phosphorofluoridate (DFP) produced no clinical signs of delayed neurotoxicity (Russell et al., 1975; Lim et al., 1983, 1986). A TOCP dosing regimen that resulted in considerable testicular pathology produced no evidence of nerve damage using either histopathological or sensitive behavioral techniques (Somkuti et al., 1988).

One difference in the many reports concerned with the toxic effects of organophosphorous compounds on rats is the strain of animal used. Most of the recent documentation of neuropathology in the rat employed the Long-Evans (hooded) variety (Veronesi, 1984; Padilla and Veronesi, 1985; Veronesi and Padilla, 1985). However, minimal histopathological alterations with no clinical signs have also been reported in the Sprague-Dawley strain after chronic exposure to TOCP (Veronesi and Abou-Donia, 1982). The negative result reported by Somkuti et al. (1988) was obtained with Fischer 344 rats. Other experiments have been done with the Sprague-Dawley variety (Reichert and Abou-Donia, 1980; Carrington and Abou-Donia, 1984; Patton et al., 1986), while other papers specify that "albino" rats were used (Smith et al., 1932).

This report describes a study designed to examine the possibility that the strain of rat may affect the results of a test for neurotoxicity. Three strains were used that have commonly been used to assess the toxic effects of organophosphorous compounds: Sprague-Dawley, Long-Evans, and Fischer 344. Animals were exposed to three doses of TOCP that were selected on the basis of their established delayed neurotoxicity in the Long-Evans rat (Veronesi, 1984). The inhibition of neurotoxic esterase (NTE) and acetylcholinestrase (AChE) in brain was used as end points for OPIDN and acute toxicity, respectively. To help assess the involvement of metabolic differences between strains in suscepti-

bility to TOCP, levels of cytochrome P-450 were measured in microsomes prepared from the livers of the control animals.

METHODS

Animals and Materials

All rats were male and were obtained from Harlan Sprague-Dawley, Indianapolis, Ind. The Sprague-Dawley and Long-Evans animals weighed between 225 and 250 g and were about 7 w old at the time of the experiment. The Fischer 344 rats weighed between 200 and 225 g and were about 10 w old.

The tri-*o*-cresyl phosphate (97%) was obtained from Eastman Kodak, Rochester, N.Y.

Dosing

Animals were randomly assigned to 4 groups, which were dosed with 0, 290, 580, or 1160 mg/kg TOCP. These doses were selected on the basis of their demonstrated neurotoxicity in the Long-Evans rat (Padilla and Veronesi, 1985). All doses were given orally by gavage. The volume of each dose was adjusted with corn oil so that they were equal to the volume of the highest dose (1 ml/kg). There were initially 4–6 animals in each of the 12 groups. Mortality (5 of 59 died) reduced the range to between 3 and 5 animals per group.

Biochemical Assays

The animals were sacrificed 48 h after dosing. The brain was removed and homogenized with a Polytron in 10% (w/v) sucrose. Livers were taken from the control animals only. Liver microsomes were prepared from fresh tissue using the method of Schenkman and Cinti (1978) in 0.25 *M* sucrose/10 m*M* Tris-HCl. The brain homogenates and liver microsomes were frozen for 1 d to 1 w at −20°C prior to assay for NTE, AChE, cytochrome P-450, and protein. NTE was assayed using the method of Jonson (1977). AChE was assayed using the method of Ellman et al. (1961). Cytochrome P-450 was assayed using the method of Omura and Sato (1964). Protein was assayed using the method of Lowry et al. (1951).

Statistical Analysis

Enzyme activity was calculated per milligram protein for both NTE and AChE. Analysis of covariance was then performed by fitting each of the three data sets with different models through the use of nonlinear regression and weighted least squares (Carrington and Abou-Donia, 1986). The four models employed were based on a simple first-order

dose-response curve with one of the following sets of assumptions: (1) all three strains were equivalent, (2) the basal (control) levels differed but the inhibition constants were equivalent, (3) the basal levels were equal but the inhibition constants differed, or (4) both the basal levels and the inhibition constants differed. The significance of the added variables in models 2–4 was tested by using the "extra sum of squares" principle (Draper and Smith, 1981). Control values were also compared using analysis of variance in conjunction with a Duncan's test (Duncan, 1955).

RESULTS

Basal Activities

There were small, but significant, strain differences in the NTE activities measured in brain (Table 1), with LE having the highest activity (7.47 nmol phenylvalerate hydrolyzed/min·mg protein) followed by F344 (5.76 nmol/mg·min) and SD (4.87 nmol/mg·min). There were no significant differences in the activity of AChE.

Inhibition of NTE by TOCP

Comparison of the data from all three strains using the extra sum of squares principle yielded significant strain differences for both the ED50 ($F = 5.13$ with 2,48 df; $p < 0.02$) and the basal levels ($F = 5.67$ with 2,48 df; $p < 0.01$). The ED50 for NTE inhibition was estimated to be 458, 209, and 288 mg/kg for SD, F344, and LE, respectively. The statistical difference is mainly attributable to the greater NTE inhibition in the Sprague-Dawley animals relative to the other two strains (Table 1; Fig. 1). Two-way comparison of the ED50 of the strains resulted in a significant difference between the SD and F344 strains ($F = 16.6$ with 1,28 df,

TABLE 1. Activities and ED50 for Inhibition by TOCP for NTE and AChE in Brain

Enzyme	Sprague-Dawley	Fischer 344	Long-Evans
NTE			
Activity[a]	4.87 ± 0.23	5.76 ± 0.16*	7.47 ± 0.14**
ED50[c]	458	209	288
AChE			
Activity[b]	68.6 ± 5.9	68.3 ± 5.6	69.3 ± 5.6
ED50[c]	1007	408	420

[a]The units for neurotoxic esterase activity are nmol phenylvaleratere hydrolyzed/min·mg protein (±SEM).

[b]The units for acetylcholinesterase activity are nmol acetylthiocholine hydrolyzed/min·mg protein (±SEM).

[c]See text for statistical comparisons.

*$p < 0.05$ (SD vs. F344). **$p < 0.01$ (SD vs. LE and F344 vs. LE).

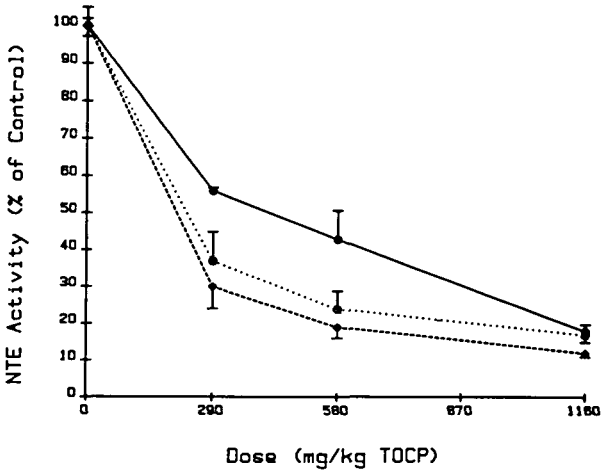

FIGURE 1. Dose-response curve for the inhibition of neurotoxic esterase in brain by TOCP *in vivo* with three different strains of rat (Sprague-Dawley, solid circles and solid line; Long-Evans, open circle with dotted middle line; Fischer 344, solid diamonds with dashed line). Values (±SEM) are given as a percentage of the control means. The ED50 values were calculated to be 458, 209, and 288 mg/kg for SD, F344, and LE, respectively.

$p < 0.001$) but not for the other two comparisons (for SD vs. LE, $F = 3.12$ with 1,32 df, $p < 0.1$ but > 0.05).

Acute Toxicity and AChE Inhibition

The pattern for AChE inhibition in brain was very similar to that for NTE (Table 1; Fig. 2). There were significant strain differences in sensitivity to TOCP ($F = 4.31$ with 2,50 df; $p < 0.02$) but not in initial activity levels ($F = 0.73$ with 2,50 df; $p > 0.05$). The Sprague-Dawley animals were by far the least sensitive to AChE inhibition (ED50 = 1007 mg/kg), while the Fischer 344 (408 mg/kg) and LE (420 mg/kg) rats were similarly affected. The mortality rates corresponded. Of the Fischer animals, 2 of 5 administered 1160 mg/kg and 1 of 5 given 560 mg/kg TOCP died prior to termination. Only one of five Sprague-Dawley and one of six Long-Evans animals died from the high-dose groups, while there was no mortality among those given lower doses. The Fischer rats may be even more sensitive than inidicated by the dose-response curves since the greater losses from the Fischer groups may have shifted the curve to the right. Two-way comparison of the ED50 of the strains resulted in a significant difference between the SD and F344 strains ($F = 6.20$ with 1,30 df; $p < 0.02$) but not for the other two comparisons (for SD vs. LE, $F = 3.76$ with 1,33 df, $p < 0.1$ but > 0.05; for LE vs. F344, $F = 1.16$ with 1,33 df, $p > 0.1$).

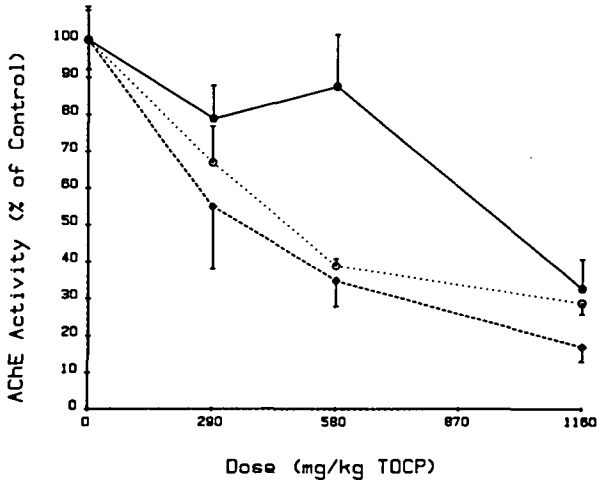

FIGURE 2. Dose-response curve for the inhibition of acetylcholinesterase in brain by TOCP *in vivo* with three different strains of rat (Sprague-Dawley, solid circles and solid line; Long-Evans, open circle with dotted middle line; Fischer 344, solid diamonds with dashed line). Values (±SEM) are given as a percentage of the control means. The ED50 values were calculated to be 1007, 408, and 420 mg/kg for SD, F344, and LE, respectively.

Cytochrome P-450 Content

The cytochrome P-450 content (Table 2) of the liver microsomes prepared from the Fischer 344 rats (1.05 nmol/mg protein) was significantly less than the other two strains (1.5 and 1.46 nmol/mg for SD and LE, respectively; $p < 0.02$ for both comparisons).

TABLE 2. Cytochrome P-450 Levels in Rat Liver Microsomes

	Cytochrome P-450 levels[a]		
Study[b]	Sprague-Dawley	Fischer 344	Long-Evans
Present study (n = 5)	1.50 ± 0.16	1.05 ± 0.07[c]	1.46 ± 0.17
Gold and Widnell (1975)	0.75 ± 0.16	0.59 ± 0.08	—
Creel et al. (1976)	0.26 ± 0.04	0.33 ± 0.05	0.40 ± 0.05
Dent et al. (1980)	0.84 ± 0.03	0.67 ± 0.04	—

[a]Units are nmol cytochrome P-450/mg protein ± SEM.

[b]The results of three similar studies are given for comparison. Three out of four studies found lower levels in Fischer 344 rats, while the other found the reverse to be true. Differences in microsomal preparation or the age of the animals might account for the variation between studies of both the absolute and relative levels of P-450.

[c]The F344 rats differ from the other two with $p < 0.05$.

DISCUSSION

The data presented in this report indicate that there are differences in the sensitivity of Sprague-Dawley, Long-Evans, and Fischer 344 rats to esterase inhibition by TOCP, with the F344 strain being the most sensitive, and the SD rats the least sensitive. This result should be considered when comparing experiments involving the effect of TOCP and different strains of rats, particularly if one of the two is Sprague-Dawley. For instance, Patton et al. (1986) found that a test dose of 750 mg/kg TOCP, which is profoundly neurotoxic in the hen, produced no evidence of neurotoxicity in Sprague-Dawley rats. On the basis of our results, this dose would result in 67%, 78%, or 90% inhibition of brain NTE in SD, LE, and F344 rats, respectively. If the criterion of approximately 70% inhibition for the induction of OPIDN that has been established for hens (Johnson, 1982) were also applicable to rats, this should be a borderline neurotoxic dose in the SD strain, but frankly neurotoxic in the other two.

The strain variation in esterase inhibition by TOCP could be due either to differences in the esterases themselves, or in the metabolic or pharmacokinetic characteristics of the strains (Abou-Donia and Nomeir, 1986). Considering the slight variation in the sensitivity to inhibition of NTE by organophosphorous compounds in various species (Johnson, 1982), it does not seem likely that there would be any significant variation in the sensitivity of the enzyme between strains of the same species. On the other hand, the ability to metabolize neurotoxic organophosphorous compounds has been implicated in differences in species sensitivity to OPIDN (Lasker et al., 1982; Abou-Donia, 1983). Furthermore, the relative sensitivity of the strains is identical for two enzymes. This suggests that there is a variation in some process occurring prior to the arrival of the inhibitor to the target issue. Therefore, the strain differences are probably related to their ability to metabolize xenobiotics, although other pharmacokinetic factors such as absorption or tissue or protein binding could also be involved. Since the metabolite saligenin cyclic-*o*-tolyl phosphate is known to be responsible for the induction of OPIDN by TOCP (Eto et al., 1966), differences in either the rate of activation or inactivation could affect the sensitivity of the animal to TOCP.

In studies where the metabolism of xenobiotics in these or other strains has been compared differences have routinely been found. First, differences in the metabolites formed in vivo from nitrobenzene were found for Fischer 344 and Sprague-Dawley (CD) strains (Rickert et al., 1983). Second, strain differences in the ability of liver microsomes to catalyze metabolite formation in vitro have been noted [F344 vs. SD (CD), Dent et al. (1980) and McClellan-Green et al. (1987); SD vs. dark agouti, Churchill et al. (1987)]. Third, several P-450 subtypes have been

found to have distinguishing immunological characteristics in different strains (Guengrich et al., 1981; Churchill et al., 1987; McClellan-Green et al., 1987). Finally differences in the relative amounts of either specific or total P-450 enzymes responsible for biotransformation have been detailed (Table 2) (Churchill et al., 1987; McClellan-Green et al., 1987).

Since the mechanism underlying the strain differences is unknown, it is not clear to what extent the present results may be generalized to other compounds. Organophosphorous compounds that require no metabolic activation and act rapidly, such as DFP, might behave similarly in all three strains. However, it seems likely that at least some strain differences would exist for other compounds as well.

A level of 70% NTE inhibition has been reported to correlate with histopathological damage induced by organophosphorous compounds in the LE rat (Veronesi and Padilla, 1985; Padilla and Veronesi, 1985). The lack of sensitivity of the SD rat to the neurotoxic effects of organophosphorous compounds may be attributed at least in part to metabolic differences between the strains, which affect the amount of neurotoxicant reaching the tissue. However, the high level of NTE inhibition in the F344 rat stands in distinct contrast to the sensitivity of this strain to the neurotoxic effects of TOCP. If NTE inhibition is an accurate marker for the F344 rat, then the lack of neurotoxicity must be due either to an inability of the neurotoxicant to damage the tissue once it has reached it, or to the ability of the nerve to recover from the damage after it has occurred. The same factors that make rodents a less susceptible species to OPIDN may also be operating to varying extents in different strains of the rat.

Although intraspecies variation has not been studied in the adult chicken, hens have routinely been found to be suitable for testing compounds for their ability to cause OPIDN throughout the world (Abou-Donia, 1981). The variation between strains of rat makes this species much less suited for the study of OPIDN.

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