Triphenyl Phosphite and Diisopropylphosphorofluoridate Produce Separate and Distinct Axonal Degeneration Patterns in the Central Nervous System of the Rat

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Exposure to organophosphorus (OP) compounds such as diisopropylphosphorofluoridate (DFP) and tri-o-tolyl phosphate (TOTP) results in a neurological condition known as organophosphorus-induced delayed neurotoxicity (OPIDN) (Abou-Donia, 1981). OPIDN is categorized by substantial inhibition of neuropathy target esterase (NTE) (Johnson, 1982, 1990), a characteristic pattern of axonal and terminal degeneration in the central and peripheral nervous systems (Cavanagh, 1954, 1964; Cavanagh and Patanga, 1965; Tanaka and Bursian, 1989; Tanaka et al., 1990a). A delayed onset ataxia is the predominant clinical manifestation (Abou-Donia, 1981; Baron, 1981). Exposure to the OP chemical triphenyl phosphate (TPP) also results in a delayed onset neurotoxicity (Veronesi and Dvergsten, 1987; Tanaka et al., 1990b) characterized by a species-dependent inhibition of NTE (Veronesi et al., 1986; Carrington and Abou-Donia, 1988) and an extensive distribution of neuronal degeneration in the central nervous system (Tanaka et al., 1992a). After a relatively short delay period, TPP-exposed animals also express a variety of behavioral deficits including ataxia, bidirectional circling, and spasticity (Smith et al., 1932; Veronesi and Dvergsten, 1987).

Because some aspects of TPP-induced neurotoxicity are similar to those seen in OPIDN, it has been proposed that TPP-induced neurotoxicity should be classified as a second type of OPIDN (Abou-Donia and Lapadula, 1990; Abou-Donia, 1992). However, these similarities are evident mainly in the chicken, a species which is highly sensitive to OPIDN (Carrington and Abou-Donia, 1988; Carrington et al., 1988). In contrast, comparison of the neuropathic effects produced by TOTP and TPP in species which are clinically less sensitive to OPIDN, such as the Japanese quail (Francis et al., 1980; Bursian et al., 1983; Varghese et al., 1995a) and rat (Smith et al., 1932; Barnes and Denz, 1953; Abou-Donia, 1981), indicates that the two neuropathies are quite different from each other (Veronesi and Dvergsten, 1987; Varghese et al., 1995a,b). Based on this lack of correspondence in less sensitive OPIDN species, we and others have suggested that TPP-induced neurotoxicity should not be considered a type of OPIDN (Veronesi et al., 1986; Padilla et al., 1987; Veronesi et al., 1986).
of DFP-injected rats, preliminary studies had indicated that the 28-day exposure period resulted in the greatest density of silver-impregnated axonal degeneration.

Following perfusion, brains and spinal cords were removed and placed in fresh fixative for 1–2 days. Brains were then transferred to a solution of 30% sucrose in a 10% formalin–saline solution for 2–3 days, frozen, and cut in the coronal plane at a thickness of 40 μm. Every fifth (200 μm apart) or 10th (400 μm apart) section through the entire brain and cervical spinal cord was processed with the Fink-Heimer silver impregnation method for degenerating axons and terminals (Tanaka, 1976). Adjacent sections were stained with cresyl violet to delineate nuclear boundaries and assess neurotoxicant effects on cell somata. Selected adjacent silver-impregnated and cresyl violet-stained sections were photographed at low power using a Wild M400 Photomacroskop. Areas containing degeneration were mapped directly onto the photographs with the aid of a compound microscope and line drawings were traced from the photographs. Nuclei and fiber tracts were identified according to the atlases of Zilles (1985) and Paxinos and Watson (1986).

RESULTS

Clinical Signs

TPP-injected rats. The latency to onset of clinical signs from the last injection in TPP-injected rats was 2 days (two rats), 5 days (one rat), 13 days (two rats), and 18 days (one rat). Although there was a high degree of variability with regard to the time of onset of clinical signs, once they appeared the animal’s condition deteriorated rapidly over 1–2 days such that the rat became severely incapacitated. In each case, the TPP injection affected primarily the hindlimbs, resulting in onset of ataxia, a flaccid paresis, and an inability to use the hindlimbs for forward progression or rearing. In all but one rat, a peculiar stereotyped side-to-side repetitive movement involving the upper part of the body and/or circling behavior were also noted.

DFP-injected rats. Both rats treated with DFP showed signs typical of anticholinesterase toxicosis, including labored breathing, tremors, and reduced activity, within 2–3 hours after dosing. However, these signs diminished in severity by 24 hr postdosing and by 48 hr the animals had recovered. No additional signs of neurological impairment were observed in the DFP-injected rats at any time during the 28-day observation period.

Control rats. Control rats did not exhibit any neurological deficits during the 28-day observation period.

Neuropathology

Degeneration characteristics. Silver-impregnated degenerating axoplasm appeared as black debris against a light brown or yellow background. Axonal degeneration appeared as linearly arranged black fragments located within known fiber tracts while terminal degeneration appeared as black irregularly arranged fragments and punctate debris located within nuclei or cortical laminae. Results from previous electron microscopic studies of silver impregnation methods indicate that the silver preferentially impregnates degenerating axons and terminals (Heimer and Peters, 1968; Heimer,
Deformation was distinguished from artifact (impregnation of reticular fibers, silver deposition within cell somata, and nonspecific silver deposition) on the basis of its general appearance, circumscribed location, continuity from section to section, and bilateral occurrence.

**TPP-exposed brains.** The general pattern and distribution of deformation were similar for all rats exposed to TPP with some variation in density occurring among animals. To illustrate the location and extent of deformation in the TPP-injected rats, cross-sectional illustrations were prepared through 10 levels of the forebrain, midbrain, hindbrain, and cervical spinal cord of a representative rat (Figs. 1-4).

In the forebrain, deformation was observed in the cerebral cortex, thalamus, hippocampus, septal region, and hypothalamus (Figs. 2, 5, and 6). Heavy deformation was present in the lateral orbital cortex while a mixed pattern of light axonal and terminal deformation was noted in the medial prefrontal and agranular retrosplenial cortices. Deformation was also detected in the sensorimotor, auditory, and visual cortical areas. Heavy terminal deformation occurred throughout layer IV of the primary and supplementary somatosensory cortices and sensorimotor cortex while lighter deformation was present in layer IV of the auditory and visual cortices. Heavy terminal deformation was present in the mediodorsal, ventromedial, and medial geniculate thalamic nuclei. Lighter axonal deformation also passed through the central medial nucleus on its way to terminating in the paracentral and central lateral thalamic nuclei. The hippocampal region contained light axonal deformation scattered throughout the dentate gyrus and fields CA1–CA3 of Ammon’s horn. Light axonal and terminal deformation were also noted in the presubiculum. In the septum and hypothalamus, moderate terminal deformation was detected in the nucleus of the horizontal limb of the diagonal band, the magnocellular preoptic nucleus, and the medial mammillary nucleus (Figs. 3 and 6).

Silver impregnated deformation was also detected in several areas of the midbrain (Fig. 3). Light terminal deformation was present in the subthalamic nucleus and in the substantia nigra, pars compacta. Light to moderate deformation was also present in the intermediate gray and white layers of the superior colliculus and in the pretectal area. The central nucleus of the inferior colliculus also contained moderate deformation while its external cortex contained lighter dorsoventrally oriented axonal deformation. Light deformation was also noted in the oculomotor nucleus and in the dorsal raphae nuclei.

In the brainstem, deformation was present in the cerebellum, vestibular nuclear complex, cochlear nuclei, and the reticular formation (Fig. 4). The cerebellum contained light scattered deformation in the granule cell layer of all vermal and hemispherical lobules. Moderate to heavy axonal deformation was present in the medial and spinal vestibular nuclei. Light terminal deformation was also located in the superior vestibular nuclei and in the parasolitary nucleus of the vestibular nuclear complex.
ular complex. The dorsal cochlear nucleus and ventral cochlear nucleus, pars posterior, contained light terminal and axonal degeneration, respectively, while the gracile fasciculus and nucleus contained only small numbers of degenerating axons.

**DFP-exposed brains.** In contrast to the findings in TPP-injected rats, moderate amounts of axonal and terminal degeneration were noted in the rostral part of the gracile fasciculus and in the gracile nucleus, respectively, of both DFP-exposed rats (Fig. 4). However, no axonal, terminal, or somatic degeneration was detected in any other region of the spinal cord or brain in these animals.

**Control brains.** No degeneration was detected in any area of the CNS examined in control rats.

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**FIG. 3.** Line drawings of cross-sections through the midbrain depicting the extent and density of axonal and terminal degeneration (dots) in a rat injected with 1184 mg TPP/kg body wt on Days 1, 4, and 7 and killed on Day 15. Note the dense degeneration present in both the superior and inferior colliculi and in the medial mammillary nuclei. Cross-sections are arranged from rostral (E) to caudal (F) and correspond to the lettered levels shown in Fig. 1.

**FIG. 2.** Line drawings of cross-sections through the cerebral hemispheres depicting the extent and density of axonal and terminal degeneration (dots) in a rat injected with 1184 mg TPP/kg body wt on Days 1, 4, and 7 and killed on Day 15. Note the dense degeneration present in the prefrontal, motor, and somatosensory cortices and the axonal and terminal degeneration located in the mediodorsal, intralaminar, and ventral medial thalamic nuclei. Cross-sections are arranged from rostral (A) to caudal (D) and correspond to the lettered levels shown in Fig. 1.

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**DISCUSSION**

**DFP-Induced Neuropathology**

Exposure of the rat to a single high subcutaneous dose of DFP resulted in no clinical signs and minimal CNS degeneration. Even with the use of the sensitive Fink-Heimer method, DFP-induced CNS axonal degeneration was detected only in the gracile fasciculus at the level of the cervical spinal cord and in the gracile nucleus. Axonal degeneration in the gracile fasciculus is consistent with that found in other studies in which rats were administered a single neuropathic dose of TOTP (Padilla and Veronesi, 1985, 1988; Inui et al., 1993) or mipafox (Veronesi and Padilla, 1985; Carboni et al., 1992; Dyer et al., 1992). We also have used the Fink-Heimer technique to map DFP-induced CNS degeneration in the chicken (Tanaka et al., 1990a) and ferret (Tanaka et al., 1991), species which are highly sensitive to OPIDN. In these species, axonal degeneration is observed in the gracile fasciculus and nucleus as it is in the rat. However, the chicken and ferret also develop axonal and terminal degeneration in the ascending spinocerebellar, spinoloivary, spinovestibular, and spinoreticular pathways and in the descending corticospinal tract (ferret) or its avian homologue, the medial pontine-spinal tract (chicken). Thus, when compared to the neuropathology observed in species which are highly sensitive to OPIDN, it is evident that the extent of
DFP-induced degeneration in the CNS of the rat is much less extensive.

The gracile fasciculus consistently undergoes degeneration following exposure to DFP or TOTP. Although this tract has been shown to mediate a number of cutaneous and proprioceptive sensory modalities, organophosphorus ester-induced degeneration localized to these fibers alone does not appear to result in any detectable clinical signs. Rather, it seems that additional involvement of fibers composing the ascending spinocerebellar tracts as well as possibly the terminal degeneration of these fibers in the cerebellar cortex is necessary to evoke ataxia (Tanaka et al., 1991; Pope et al., 1992). Results of a recent study by Funk and her colleagues (1994), which examined the neuropathology of OPIDN in domestic chicks, support this view. It should be noted, however, that a study in the mouse reported that high doses of TOTP produced moderate damage in lateral as well as dorsal columns of the cervical spinal cord (Veronesi et al., 1991). It is not clear whether the damaged fibers in the lateral columns included spinocerebellar fibers but these mice did not display any signs of ataxia during postexposure observation times.

**TPP-Induced Neuropathology**

Although the results of this and other studies (Veronesi 1984; Carboni et al., 1992) show that the rat is relatively resistant to OPIDN, this species appears to be highly susceptible to TPP intoxication. Subacute, subcutaneous exposure to TPP resulted in numerous clinical signs including ataxia, flaccid paresis, stereotyped alternating side-to-side movements, and circling behavior. In addition, axonal and/or terminal degeneration were detected throughout the CNS. Heavy degeneration was noted in visual, auditory, and senso-
rimotor systems at multiple levels of the neuraxis, including associated thalamic nuclei and cerebral cortical areas. Severe terminal degeneration also occurred in the mammillary nuclei of the hypothalamus and in the vestibular nuclear complex. These results show that TPP-induced CNS degeneration patterns and clinical signs observed in the rat are similar
FIG. 6. Photomicrographs illustrating terminal degeneration in (A) the mediodorsal thalamic nucleus and (B) the medial mammillary nucleus of a rat injected with 1184 mg TPP/kg body wt on Days 1, 4, and 7 and killed on Day 15. Scale bar, 50 μm.

to those seen in the ferret (Tanaka et al., 1990b, 1994), chicken (Tanaka et al., 1992b), and Japanese quail (Varghese et al., 1995a,b), although individual CNS tracts and nuclei affected by TPP may vary slightly among species. In contrast to that seen with DFP, TPP exposure resulted only in very light axonal terminal degeneration in the gracile fasciculus and nucleus. Thus, it is apparent that the distribution of CNS neuropathology induced by exposure to TPP or DFP is quite distinct in the rat.

TPP and OPIDN

It is clear from the results of our studies that susceptibility to OPIDN has little influence on whether a species will show susceptibility to TPP. Indeed, two of the least sensitive OPIDN species, the Japanese quail and the rat, display some of the most severe TPP-induced CNS neuropathology and clinical signs. This contrasting species sensitivity indicates that TPP neuropathy differs from OPIDN. In fact, because of striking differences in onset and type of clinical signs, magnitude of in vivo NTE inhibition, and the extent and type of neuropathology, we and other investigators (Veronesi et al., 1986; Padilla et al., 1987; Veronesi and Dvergsten, 1987; Varghese et al., 1995a,b) have proposed that OPIDN- and TPP-induced delayed neurotoxicity are not two different types of OPIDN as has been suggested (Abou-Donia and Lapadula, 1990; Abou-Donia, 1992), but rather are entirely separate categories of organophosphorus-induced neurotoxicity. The severity of TPP-induced clinical signs, along with the extensive patterns of CNS degeneration, may be more closely related to neurotoxicity resulting from exposure to certain organophosphorus nerve agents. For example, forebrain degeneration patterns observed in the rat following exposure to TPP are similar to those induced by soman (McLeod et al., 1984; Switzer et al., 1988; Petras, 1994). However, TPP intoxication does differ from that of soman in that soman does not appear to cause degeneration in medullary or cerebellar structures (McLeod et al., 1984; Switzer et al., 1988; Petras, 1994). Thus, it appears that TPP is able to affect more widespread areas of the CNS than either OPIDN-producing compounds or soman. This ability to affect neurons at all levels of the neuraxis may result not only in the onset of motor clinical signs as seen in the present study, but also in heretofore undefined behavioral and cognitive deficits caused by disruption of neural activity associated with higher order processing and integrative functions. Along these lines, results of a recent study indicate that TPP-injected rats are deficient in the acquisition of a spatial alternation task (Levin et al., 1995).

In summary, we have shown in the present and previous studies (Varghese et al., 1995a,b) that CNS degeneration patterns and clinical signs induced by exposure to the organophosphorus compounds TPP and DFP are markedly different in two species less sensitive to OPIDN—the rat and the Japanese quail. Based upon these results, we suggest that TPP-induced neurotoxicity should not be classified as a type of OPIDN, but rather as a separate and distinct category of organophosphorus-induced neurotoxicity.

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REFERENCES

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