INTRODUCTION

Vacor (N-3-pyridylmethyl- N'- p-nitrophenylurea)-poisoned patients had complained of various neurological symptoms such as urinary retention, burning sensation of the foot, areflexia and orthostatic hypotension. Various neurotoxicities of Vacor were reported in the acute toxic phase. However, the cause of neurotoxicity is not well known.

In the electrophysiologic studies of the peripheral nerve, Vacor did not affect the conduction velocity. However, the decrease of action potential amplitudes was developed on the sciatic-tibial nerve of a Vacor-treated rat. The decrement of action potential amplitude indicates that axonal or muscular dysfunction might be induced by Vacor.

To define these electrophysiologic abnormalities in the Vacor-induced peripheral neuropathy, an ultrastructural study was carried out in the neuromuscular junction of the interosseous muscle in Vacor-treated rats.

MATERIAL AND METHODS

Male Wistar rats (n=30), weighing 200-250g, were used in this study. A single dose of Vacor (N-3-pyridylmethyl- N'- p-nitrophenylurea, MW 272), 80 mg/kg of body weight, was administrated by an orogastric cannule, while distilled water was given in controls.

The experimental animals were sacrificed at 1, 3, 7, 14 days after administration of Vacor. The animals were perfused through the jugular vein with 5% glutaraldehyde in 0.1 M sodium phosphate buffer.

To examine the morphological changes of the neuromuscular junction, tissues were taken from the interosseous muscles of the hind foot.

The tissue fragments were divided into small blocks, 2×2×1 mm each, and subsequently immersed in 2.5% glutaraldehyde for 12 hours at 10 °C. They were postfixed in 2% osmium tetroxide for 4 hours at room temperature. After dehydration in graded ethanol, they were
embedded in epon mixture for three days at 50°C.

Thick sections, 1 μm in thickness, stained with toluidine blue were prepared for light microscopy. Thin sections, 80 nm in thickness, were obtained from the representative areas and stained with uranyl acetate and lead citrate. The sections were examined with a Zeol 100 CXII electron microscope.

RESULTS

In control rats, myelinated axons and axon terminals at neuromuscular junctions of the hind foot interosseous muscle maintained their structural integrity well. No degenerative changes were seen in myelinated nerve fibers (Fig. 1). The axon terminal contained mitochondria, concentrically lamellated endoplasmic reticulum (ER) and numerous small round presynaptic vesicles (Fig. 2), and closely contacted to sarcoplasmic membrane of myocyte.

Morphological change of the axon terminals in neuromuscular junctions was not evident within one day after Vactor administration (Fig. 3). The synaptic cleft between the axon terminal and postsynaptic folds of sarcoplasm was not widened. At 3 days after Vactor administration, there was remarkable loss of presynaptic vesicles and swollen ER in the axon terminal (Fig. 4).
ULTRASTRUCTURE OF NEUROMUSCULAR JUNCTION IN VACOR-INDUCED DIABETIC RATS

Fig. 4. Neuromuscular junction days after Vacor administration. Note mild degenerative changes consisting of significantly reduced number of presynaptic vesicles (SV) and dilatation of endoplasmic reticulum (ER) in the axon terminal, and flattening of postsynaptic folds (PS) (× 52,000).

Fig. 5. Neuromuscular junction 7 days after Vacor administration. Note progressive degenerative changes consisting of marked loss of presynaptic vesicles (SV) and focal disruption of membrane (arrow) in the axon terminal with myeloid body (MB) formation and flattened postsynaptic folds (PS) (× 52,000).

Fig. 6. Neuromuscular junction 14 days after Vacor administration. Some presynaptic vesicles were disappeared, and the lesion is replaced by irregularly elongated processes of Schwann cell (S) which engulfed a few presynaptic vesicles (SV). Note thin delicate basal lamina along the processes (arrow). Postsynaptic folds (PS) were simplified (× 22,400).

Fig. 7. Neuromuscular junction 14 days after Vacor administration. Note markedly decreased number of presynaptic vesicles (SV) in the axon terminal surrounded by collagenous fibers (C) and flattened postsynaptic folds (PS) of sarcoplasm of myocyte (× 40,000).

The papillary postsynaptic folds were partially flattened. Progressively decreased number of presynaptic vesicles and disruption of membrane of the axon terminal with myeloid body formation were seen at 7 days after Vacor administration (Fig. 5). The synaptic cleft was widened. The axon terminals significantly reduced in number at 14 days after Vacor administration. A few presynaptic vesicles were engulfed in irregular Schwann cell processes identified by a delicate lining of their basal lamina (Fig. 6).

DISCUSSION

Degenerative changes and segmental demyelinations were developed in diabetic neuropathy with long duration.
In the acute phase of experimental diabetes induced by streptozotocin, pathologic changes of the nervous system were not found, and it required more days or weeks to develop.

The diabetes induced by Vacor has various neurological complications such as urinary retention, burning sensation of the foot, areflexia and orthostatic hypotension, etc. Lee et al. had reported that rats given Vacor show various morphological changes such as necrosis of axon, myelin sheaths and glia cells in the spinal cord with unchanged organelles of the peripheral nerves, but its correct mechanism is not clear.

Park reported that the amplitude of the compound muscle action potential decreased but conduction velocity of sciatic-tibial nerve of a Vacor treated rat was unchanged, and Na⁺, K⁺-ATPase activity and inositol content of sciatic nerve were unchanged, which were different from those of streptozotocin diabetes.

However, neurotoxicity was reported in the acute toxic phase of Vacor poisoning. Lewitt reported the active breakdown of myelin sheath in a streptozotocin diabetic animal.

In this study morphological changes of axon terminals at neuromuscular junction were not evident within one day after Vacor treatment. However, loss of presynaptic vesicles in axon terminal and swollen endoplasmic reticulum were developed at 3 days after Vacor administration. These abnormalities of axon terminal progressed at 7 and 14 days after Vacor treatment.

These findings may provide us the evidence that decreased amplitude of compound muscle action potential caused by axonal degeneration.

REFERENCES

1. Lee TH. Diabetes mellitus by rodenticide, RH-787 poisoning. Korean J Intern Med 1976; 19:618-624.
2. Lee TH, Kang JC, Kim JS, Rho JW, Chung MY, Choi BI. Clinical observation of rodenticide, RH-787 poisoning. J Korean Diab Assoc 1977; 4:43-51.
3. Prosser PR, Karam JH. Diabetes mellitus following rodenticide ingestion in man. JAMA 1978; 239:1148-1150.
4. Karam JH, Lewitt PA, Young CW, Nowlin RE, Frankel BI, Fujita H, Freedman ZR, Grodsky GM. Insulinopenic diabetes after rodenticide(Vacor) ingestion. Diabetes 1980; 29:971-978.
5. Lee TH, Doi K, Yoshida M, Baba S. Morphological study of nervous system in Vacor-induced diabetic rats. Diabetes Res Clin Pract 1988; 4:275-279.
6. Park CJ, Ryu HK, Chung MY, Lee TH. Functional study on peripheral neuropathy in Vacor-treated rat(abstract). Korean J Intern Med 1995; 47(Suppl.1):63.
7. Felig P. Disorders of carbohydrate metabolism. In: Bondy PK, Rosenberg LE, ed. Duncan’s Metabolic Control and Disease. 8th ed. Saunders: Philadelphia, 1980; 348-350.
8. Sharma AK, Thomas PK. Peripheral nerve structure and function in experimental diabetes. J Neurol Sci 1974; 23:1-15.
9. Lewitt PA. The neurotoxicity of the rat poison Vacor. N Engl J Med. 1980; 302:73-77.