Pyridoxine Megavitaminosis: An Analysis of the Early Changes Induced with Massive Doses of Vitamin B$_6$ in Rat Primary Sensory Neurons

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Abstract. The early effects of high toxic doses of vitamin B$_6$ (pyridoxine) on the peripheral sensory neurons were studied in laboratory rats. The animals were treated with 600 mg/kg of pyridoxine hydrochloride by intraperitoneal injection twice daily. Thereafter they were killed by perfusion-fixation at periods ranging from one to 14 days and the tissues were examined by light and electron microscopy. The primary change consisted of the formation of swollen membranous profiles in both the axon hillock and the initial axonal segment of the large dorsal root cyeons. This change occurred within 24 hours of exposure, and was followed by an axonal reaction of the nerve cell bodies and by secondary degeneration of their processes. These findings identify the probable target site for pyridoxine toxicity, and establish a simple animal model for studying not only sensory denervation, but also the axonal reaction and secondary degeneration.

Key Words: Pyridoxine; Rat; Sensory neurons; Toxicity; Vitamin B$_6$.

INTRODUCTION

The neurotoxic effects of massive doses of vitamin B$_6$ (pyridoxine) in experimental animals were reported more than 40 years ago, at the time of its introduction into clinical medicine (1). Since the amount of pyridoxine needed to intoxicate animals was far in excess of that recommended, at the time, to treat human patients, the results of experimental neurotoxicity did not seem to have any bearing on the possible adverse effect of vitamin B$_6$ on man.

Over the years, pyridoxine has been used as an experimental tool in toxicological research (2, 3). The lesion produced has been proposed as an animal model for human sensory neuropathy (4) and has been characterized through histopathological and electrophysiological studies in dogs (5, 6). It is a purely sensory neuropathy with selective damage to the large primary sensory neurons, and their central and peripheral processes, within the spinal nerves, the trigeminal nerve, and the spinal cord.

Faith in the relative safety of pyridoxine for man has been challenged by the recent observation that ataxia and severe sensory abnormalities occur after daily, high-level consumption (7). The potential vulnerability of human peripheral sensory neurons, along with the theoretical interest in its action on mammalian sensory neurons, warrant further investigations into the pathogenesis of pyridoxine neurotoxicity.

This report describes the effect of massive doses of pyridoxine on the primary sensory neurons of the rat. We provide evidence that the initial changes may occur
1 day treatment (2 × 600 mg/kg)
3 rats examined 1 day after last administration: early change restricted to initial axonal segment and axon hillock of occasional large dorsal root cyton; nerve fibers intact
1 rat examined 2 days after last administration: nerve fibers intact
1 rat examined 3 days after last administration: nerve fibers intact
1 rat examined 4 days after last administration: nerve fibers intact

2 day treatment (4 × 600 mg/kg)
1 rat examined 1 day after last administration: about 7% vacuolated cytons and 8% cytons with features of axonal reaction; nerve fibers intact
1 rat examined 2 days after last administration: nerve fibers intact
1 rat examined 3 days after last administration: nerve fibers intact
1 rat examined 4 days after last administration: nerve fibers intact

3 day treatment (6 × 600 mg/kg)
3 rats examined 1 day after last administration: about 2% vacuolated cytons and 6% cytons with features of axonal reaction; first degenerating nerve fibers
1 rat examined 2 days after last administration: frequent degenerating nerve fibers

during the first few days of intoxication, and identify the probable target site as the axon hillock and the initial axonal segment.

MATERIALS AND METHODS

The study was carried out on young, adult male, albino laboratory rats of a Sprague-Dawley-derived strain (TifRAf) (body weight 250 to 500 g). A freshly prepared solution of pyridoxine hydrochloride (Vitamin B₆ purum, Fluka AG), dissolved in distilled water, was administered intraperitoneally in doses of 600 mg/kg body weight, twice daily. Six rats were treated for one day, two sets of four rats for two and three days respectively, and a further three sets of two rats for 4, 8, and 14 days respectively; two rats remained as untreated controls. To test for the reversibility of the early primary changes and to detect the onset of nonspecific secondary degeneration, those rats treated for either 1, 2 or 3 days were examined after various, treatment-free periods of survival (Table 1). Those rats treated for four or more days were invariably examined on the day following the last treatment.

A light and electron microscopic examination was carried out on tissues from perfusion-fixed animals; either 4% formaldehyde alone for teased fiber preparations, or 4% formaldehyde followed by 5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) under deep barbiturate anesthesia; the tissues were osmicated in Dalton’s solution, embedded in Spurr epoxy resin, and either stained with toluidine blue or with uranyl acetate and lead citrate. The portions of peripheral nerve used for examination of teased-fibers were stained with Sudan black, then transferred into glycerin. The areas sampled included the dorsal root ganglia, the dorsal and ventral roots in the lumbar region (L₁ and L₂), and the spinal cord and peripheral sciatic nerve (with its distal, tibial and plantar branches) at various levels. The relative number of dorsal root cytons affected by early changes was assessed by cell counts on photographic prints under a final magnification of ×220 (Table 1).

RESULTS

1. Clinical Findings

Peripheral ataxia was first noticed after three days of treatment and by the eighth day the rats showed severe paralysis. Although muscle force was preserved they were unable to walk (Fig. 1). By the fourteenth day they had lost much weight (a reduction of approximately 40%) and were debilitated.
Fig. 1. Severe pyridoxine toxicity after eight days of treatment. A rat, deliberately placed on its back (A), corrects its position by a vigorous swing of the tail and paddling movements of the hind legs (B); it is unable to walk (C).

2. Microscopic Features

The initial lesion could be determined by microscopic examination as early as 24 hours after treatment. Occasional, large dorsal root neurons contained swollen, smooth membranous profiles and focal clearing of the cytoplasmic matrix (Figs. 2, 3) in
Fig. 2. Dorsal root (L₆) ganglion from a rat examined the day following the first day of treatment. The initial axonal segment (IA) shows focal vacuolation (arrow) by light microscopy (top). × 508. The presence of membrane fragments and rarefaction of axoplasm are evident by electron microscopy (double arrows, bottom). ×9,860.
either the region of the axon hillock or the initial axonal segment close to this region. This initial change proved to be reversible. The only abnormality encountered in some dorsal root cytons following two days without exposure and after one day of treatment, consisted of a slight focal accumulation of neurofilaments; after another day without exposure the cytons appeared normal.

The advanced lesion in the dorsal root ganglia of rats treated for two days consisted of many large cytons with vacuolated cytoplasm (Fig. 3). The ganglia also contained many other cytons with features of the axonal reaction, especially dispersion of the Nissl bodies and peripheral displacement of the nucleus. The number of vacuolated cytons declined after three days of treatment; after four days of treatment they were no longer apparent. Some large cytons were lost while the persisting ones revealed signs of the axonal reaction (Fig. 4).

Secondary Degeneration of the Nerve Cell Process. No breakdown of fibers was observed in the peripheral nerves and spinal tracts examined up to four days following the first and second days of treatment. This apparent absence of damage to the nerve cell processes was in contrast to the axonal reaction observed in numerous nerve cell bodies after only two days of treatment. The only abnormalities detected in the axoplasm at this time were lysosome-like lamellar bodies, restricted to the area close to the dorsal root ganglia (Fig. 5).

Degenerating peripheral nerve fibers were first observed on the day following the third day of treatment; after a further day without exposure, they became quite frequent at all levels of the peripheral sciatic nerve especially in the distal branches (tibial and plantar nerves). Teased nerve fibers at this stage invariably showed the appearance of early secondary (Wallerian-like) degeneration, with fragmentation most advanced in the mid-internodal region close to the Schwann-cell nucleus (8, 9) (Fig. 6).

In the dorsal spinal columns, the onset of degeneration also occurred following the third day of treatment; the earliest change was present in the middle portion of the dorsal columns (gracile tract), and was most frequent in the cervical region. As in the peripheral nerve, the spinal lesion resembled a nonspecific secondary degeneration: it was characterized by an initial axonal swelling and distention of the myelin sheaths, with consecutive loss of axoplasm and the collapse of myelin (Fig. 7).

The late lesion in the animals examined after prolonged periods of treatment (up to 14 days) was a widespread damage to the sensory nerve fibers in the dorsal columns, including their lateral portion (cuneate tract). Corticospinal motor fibers in the deep middle portion of the dorsal spinal columns, motor neurons in the ventral horns and the ventral spinal roots remained intact.

Following the eighth day of treatment, the dorsal root ganglia exhibited a marked loss of large cytons that were replaced by nodules of Nageotte. Some of the larger cell bodies remaining in the ganglion had features of the futile axonal reaction (Fig. 8). A number of small cytons were still present in the dorsal ganglia after the fourteenth day of treatment.

DISCUSSION

The rats in our study were given extremely large quantities of pyridoxine each day to induce a marked toxic effect within a short period of time. This treatment resulted in damage to, and loss of, large primary sensory neurons. Since the initial changes involved the nerve cell bodies, the lesion may be called a primary sensory neuropathy (10). Previous studies in dogs intoxicated with high doses of pyridoxine have revealed similar effects (5, 6).
Fig. 3. Dorsal root (L₄) ganglion from a rat examined the day following the second day of treatment. Intracytoplasmic vacuoles (arrows) contain swollen membranous profiles (double arrows) in the area of the axon hillock (AH). (top) × 508. (bottom) × 7,648.
Fig. 4. Dorsal root ganglion (L₆) from a rat examined the day following the fourth day of treatment with numerous cytons showing the axonal reaction (arrows, top). A control dorsal root ganglion (L₆) in which the cytons show a normal distribution of Nissl bodies, with centrally placed nuclei (bottom). ×508.

Lower doses of pyridoxine used in other experiments, induced a sensory neuropathy in both rats and dogs with the predominant damage in the distal portions of the nerve fibers (3, 11, 12). This change resembles more closely the adverse effects of pyridoxine in human patients who showed distally accentuated symptoms, with a dramatic reversal after cessation of intake (7). The severe loss of nerve fibers observed in the sural nerve of one of these patients, however, indicated extensive damage to the sensory neurons.
We consider two morphological features of the nerve cell to be pertinent to an understanding of the distally accentuated symptoms. Firstly, the volume of the cyton is determined by the extent of the peripheral field innervated (13). Secondly, the size of the cyton is, in general, proportional to that of its axon (14). If, therefore, the axonal length were proportional to the volume of its cyton, distally accentuated symptoms could be accounted for by a particular susceptibility to pyridoxine of the large dorsal root cytons supplying the extremities.
Our study has furnished evidence that the primary changes in pyridoxine neuropathy may occur in the axon hillock and the initial axonal segment. It is unclear whether the effect on the axon hillock is due to either a higher concentration of pyridoxine or a particular local vulnerability at this site. The presence of abnormal membranous structures may indicate a disturbance in the assembly of membranous material employed in fast axoplasmic transport. Abnormal membrane fragments could be displaced into the initial axonal segment and cause obstruction.
Fig. 7. The cervical gracile tract from a rat examined the day following the third day of treatment. Axonal swelling and distention of the myelin sheath are the most frequent change at this stage (top). ×9,860. A less frequent alteration is axonal shrinkage (arrow), and wide distention ("bubbling") of the myelin sheath, a change known to occur after compression of nerve tissue and axonal atrophy (bottom). ×9,744.

Fig. 8. Dorsal root (L2) ganglion from a rat examined the day following the eighth day of treatment. The cytons show an advanced axonal reaction, with cell shrinkage, undulation of the nuclear membrane (N = nucleus), and masses of neurofilaments (arrows in top figure, NF in bottom figure). (top) ×1,312. (bottom) ×6,467.
tively, the axoplasmic reticulum itself, within the initial axonal segment, could be a target. The initial axonal segment of the dorsal root neurons is equipped with a richly folded axolemma, which provides a disproportionately large surface area (15). This anatomical feature could, therefore, facilitate the entry of exogenous substances that damage the neuron.

The disruption of continuity between the ganglionic cell bodies and their processes was followed by secondary changes in both the proximal and distal portions of the neuron. The cytons that were not destroyed by the initial vacuolar swelling developed characteristic of axonal transection, while the central and peripheral axons underwent secondary degeneration. The promptness of the axonal reaction, which had already occurred in the cytons before the breakdown of the distal cell processes, may be due to the short distance between the cyton and the site of axon severance. This reaction obviously represents an instant response to the loss of feedback control, normally mediated by retrograde axoplasmic transport (16).

The accumulation of neurofilaments within the dorsal root cytons is a characteristic feature of the axonal reaction (17). Our finding of an early proximal separation of the cell body from its processes in pyridoxine neuropathy, indicates that the accumulation of neurofilaments is due to an inability to transport them into the axon.

The effect of pyridoxine on the rat dorsal root ganglia, presented in this study, may be considered as a "partial chemical gangliectomy." Although this lesion was induced with an excessive dose of pyridoxine, there is no reason to expect a different neuronal target site in lower toxic doses. We presume that a critical amount of pyridoxine can damage the metabolism of the neuron in the axon hillock area, and thereby impair axonal maintenance by the cyton. Pyridoxine toxicity would then exemplify the importance of the neuronal cell body in neurotoxic injury. Such a role has been contemplated by other investigators (18). Lower toxic doses of pyridoxine administered over a prolonged period of time may, however, have a direct action on the distal axon or the sensory nerve endings. The design of our experiments did not permit us to explore this question further.

Experimental pyridoxine neurotoxicity in rats appears to offer a suitable animal model for studying not only the sensory denervation, but also the axonal reaction and secondary degeneration, with the possibility of influencing these conditions by means of therapy. In considering its relevance in human neurotoxicity two facts must be taken into account. Firstly, the relative susceptibility to pyridoxine seems to be inversely proportional to body size, e.g. a daily intake of 1,200 mg/kg body weight in the rat is approximately equivalent to 300 mg/kg body weight in the dog. Secondly, excessive amounts of pyridoxine are still administered in treating certain specific conditions in man (19). Our findings, however, in no way cast doubt on the prophylactic and therapeutic value of pyridoxine in reasonable amounts.

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