Neuroprotective actions of pyridoxine

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Abstract

Electroencephalographic recordings in cerebral cortex of mice given a single sub-convulsive dose of domoic acid exhibited typical spike and wave discharges. Administration of the anti-epileptic drugs sodium valproate, nimodipine, or 5α-pregnan 3α-ol-20-one as well as pyridoxine simultaneously with or after domoic acid treatment resulted in significantly less spike and wave activity. Administration of these same drugs 45 min prior to the administration of domoic acid also significantly reduced EEG background. Mechanistically, sodium valproate and pyridoxine significantly attenuated domoic acid-induced increase in levels of glutamate, increase in levels of calcium influx, decrease in levels of γ-aminobutyric acid and increase in levels of the protooncogenes c-fos, jun-B and jun-D. In hippocampal cells, domoic acid-induced increases in glutamate and calcium influx were significantly decreased by pyridoxal phosphate or nimodipine. Similarly in neuroblastoma–glioma hybrid cells (NG 108/15), pyridoxine attenuated domoic acid-induced increases in glutamate, influx of extracellular calcium, and enhanced induction of oncoproteins regardless of whether cells were undifferentiated, differentiated or de-differentiated. Pyridoxine has anti-seizure and neuroprotective actions mediated through mechanisms similar to those targeted by current therapeutic strategies.

Keywords: Pyridoxine; Neuroprotection; Domoic acid; Neurotoxicity; Calcium influx; Oncoprotein

1. Introduction

Early reports suggested a relationship between infants fed a special formula diet, apparently deficient in vitamin B₆, and seizures. We were the first to report that induction of congenital pyridoxine deficiency in rats resulted in seizures [1]. The relationship between pyridoxine and the development of seizure activity was associated with decreased the synthesis and secretion of γ-aminobutyric acid [2]. Subsequently, we showed that pyridoxine, through its role in the synthesis and secretion of various neurotransmitter amines was involved in the normal functioning of the hypothalamo-pituitary-end organ axes [3]. Together, this work showed that in addition to correcting a number of defects associated with vitamin B₆ deficiency, high doses of pyridoxine can ameliorate degenerative diseases and protect against neurotoxicity under conditions not associated with vitamin B₆ deficiency [4].

Domoic acid, like kainic acid, is a rigid structural analog of glutamic acid. It is a toxic contaminant of cultivated and contaminated mussels. There have been several reports of neurotoxic outbreaks due to domoic acid poisoning through ingestion of such mussels. We reported that administration of γ-aminobutyric acid directly into rat hippocampus protected against domoic acid-induced seizures [5]. Using domoic acid as a prototype neurotoxin and because we showed previously that pyridoxine can increase levels of γ-aminobutyric acid and decrease seizure activity, we tested the hypothesis that pyridoxine would protect against domoic acid-induced seizures. Here we report our findings using whole animal,
and cultures of mouse hippocampus and a neuroblastoma glioma cell line.

2. Materials and methods

The methods used to administer domoic acid, and conduct EEG recordings in mice and analyze the computerized data were reported previously[6–8]. The methods for determining levels of glutamate[9] and calcium influx[10] were reported previously. Protooncogene expression determinations were conducted using Northern blotting and immunoblotting procedures[11].

3. Results

Domoic acid at three doses (0.6, 1.5 or 2.4 mg/kg body weight) were administered to separate groups of mice to determine the medium (sub-convulsive) dose. The medium dose of domoic acid was determined to be 1.5 mg/kg and produced typical acute phase seizure activity lasting 4 to 6 h, which consisted of body shivering, tonic–clonic body movements, increased cardiorespiratory activity, and vibratile tail movements. Falling, convulsive seizures and loss of consciousness were not observed in these animals. Only the highest dose of domoic acid (2.4 mg/kg) produced severe tonic–clonic seizure discharge activity and more than 80%
of these animals died at this dose. For all subsequent studies, the medium dose was used.

To determine the effectiveness of anti-epileptic drugs as well as pyridoxine in preventing domoic acid-induced seizure activity, sodium valproate (10 mg/kg), nimodipine (100 μg/kg), 5α-pregnan 3α-ol-20-one (100 μg/kg) or pyridoxine (10 mg/kg) were administered to mice after the EEG pattern was recorded following domoic acid administration. Sodium valproate, nimodipine, 5α-pregnan 3α-ol-20-one or pyridoxine when administered following domoic acid, significantly suppressed the domoic acid-induced spike and wave activity (Fig. 1). Various parameters of electrographic seizure activity such as seizure latency, interictal duration, ictal duration, burst duration, burst frequency and burst amplitude measured after domoic acid administration alone or after therapeutic administration of the drugs are illustrated in Fig. 2. The seizure latencies were increased and all other parameters were decreased significantly when the anti-seizure treatments were administered prior to domoic acid treatment. When the same four agents at the same doses were administered 45 min prior to injection of domoic acid similar anti-seizure effects were observed including consistent reduction in EEG background frequency represented by intermittent zones of electro silence and occasional burst discharge activity within the first 20 min of domoic acid administration. Administration of a second dose of these drugs completely suppressed the domoic acid-induced burst discharge activity.

Domoic acid at a single sub-convulsive dose was found to increase the mRNA expression of the protooncogenes c-fos, jun-B and jun-D dose-dependently within 20 min of domoic acid administration. The increase in c-fos expression was inhibited significantly by prophylactic treatment with either sodium valproate or pyridoxine (Fig. 3). This observation of the inhibition of mRNA expression was extended using immunoprecipitation and immunoblotting techniques which showed that levels of c-fos protein were reduced significantly by sodium valproate or pyridoxine.

![Fig. 2. Quantitative analysis of domoic acid-induced electrographic seizure discharge activity and the effect of therapeutic drug administration.](image-url)
To determine the underlying mechanisms by which pyridoxine decreases domoic acid-induced seizures, we used primary cultures of mouse hippocampal neurons. Under normal conditions, the hippocampal neurons exhibited extensive neuritogenesis. However, following treatment with domoic acid, these cells exhibited decreased neuritogenesis and increased cellular aggregation. Domoic acid significantly increased levels of glutamate in perchloric acid extracts of these cells and pretreatment with pyridoxine effectively attenuated the increases in glutamate. Domoic acid also increased in a concentration- and time-dependent manner calcium influx in cultured hippocampal neurons. As with glutamate, pre-incubation of hippocampal neurons with nimodipine or pyridoxal phosphate significantly inhibited domoic acid-induced increases in calcium influx (Fig. 4). Neither nimodipine nor pyridoxal phosphate by themselves produced any significant changes in calcium influx.

The above experiments with hippocampal neurons were repeated using a neuroblastoma–glioma hybrid cell line (NG108/15) in order to extend the findings into other cell types.
types. Similar to the effects on hippocampal neurons, domoic acid increased levels of glutamate, calcium influx, and expression of the protooncogene c-fos. When the cells were pretreated with pyridoxine 45 min prior to the addition of domoic acid, NG108-15 cells whether undifferentiated, differentiated or de-differentiated, exhibited reduced levels of calcium influx (Fig. 5A) and c-fos protein expression (Fig. 5B).

4. Discussion

The results presented indicate that domoic acid-induced seizure activity and measures of excitotoxicity in whole animals as well as cultured cells were attenuated by pyridoxine when administered prior to or after domoic acid. One significant feature of the effect of domoic acid under these conditions was increased levels of cellular glutamate. This increase in glutamate can lead to increased levels of intracellular calcium and enhanced expression of oncoproteins. Pyridoxine, acting through pyridoxal phosphate, appears to decrease intracellular levels of glutamate by increasing glutamic acid decarboxylase activity and decrease calcium influx through actions on cell surface calcium channels. Such actions of pyridoxal phosphate were established previously in smooth muscle and cardiac sarcolemma [12]. In the former, voltage-gated L-type calcium channels were the major calcium transporters, whereas in cardiac sarcolemma, purinergic P2X receptors activated by ATP seems to be the major pathway of calcium transfer. Pyridoxal phosphate inhibits both calcium transport mechanisms and is central to the neuroprotective actions of pyridoxine.

References