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BRAIN RESEARCH

Research Report

Fluorophilia: Fluorophore-containing compounds adhere non-specifically to injured neurons

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ABSTRACT

Ionic (free) zinc (Zn^{2+}) is implicated in apoptotic neuronal degeneration and death. In our attempt to examine the effects of Zn^{2+} in neurodegeneration following brain injury, we serendipitously discovered that injured neurons bind fluorescein moieties, either alone or as part of an indicator dye, in histologic sections. This phenomenon, that we have termed "fluorophilia", is analogous to the ability of degenerating neuronal somata and axons to bind silver ions (argyrophilia — the basis of silver degeneration stains). To provide evidence that fluorophilia occurs in sections of brain tissue, we used a wide variety of indicators such as Fluoro-Jade (FJ), a slightly modified fluorescein sold as a marker for degenerating neurons; Newport Green, a fluorescein-containing Zn^{2+} probe; Rhod-5N, a rhodamine-containing Ca^{2+} probe; and plain fluorescein. All yielded remarkably similar staining of degenerating neurons in the traumatic brain-injured tissue with the absence of staining in our sham-injured brains. Staining of presumptive injured neurons by these agents was not modified when Zn^{2+} in the brain section was removed by prior chelation with EDTA or TPEN, whereas staining by a non-fluorescein containing Zn^{2+} probe, N-(6-methoxy-8-quinolyl)-p-toluenesulfonamide (TSQ), was suppressed by prior chelation. Thus, certain fluorophore-containing compounds nonspecifically stain degenerating neuronal tissue in histologic sections and may not reflect the presence of Zn^{2+}. This may be of concern to researchers using indicator dyes to detect metals in brain tissue sections. Further experiments may be advised to clarify whether Zn^{2+}-binding dyes bind more specifically in intact neurons in culture or organotypic slices.

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Abbreviations: Ca^{2+}, (ionic) calcium; CA1, 2 and 3, *Cornu Ammonis regions 1, 2 and 3*; F, fluorescein; FJ, Fluoro-Jade; FPI, fluid percussion injury (an experimental model of TBI); FZ3, FluoZin-3; NG, Newport Green; OCT, optimum cutting temperature (for embedding); Rhod-5N, rhod-5N tripotassium salt (Ca^{2+} indicator); TBI, traumatic brain injury; TPEN, N, N, N′, Nr-tetrakis-[2-pyridylmethyl]-ethylenediamine; TSQ, N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide; Zn^{2+}, (ionic) zinc

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1. Introduction

Ischemia-induced neuronal degeneration has been associated with the appearance of histochemically-reactive or "free" zinc (Zn^{2+}) in degenerating neuronal somata (Choi, 1996; Medvedeva et al., 2009; Tonder et al., 1990). Data also indicate that chelation of Zn^{2+} dramatically reduces neuronal loss after ischemia–reperfusion injury in animals (Calderone et al., 2004; Koh et al., 1996). Therefore, the role of Zn^{2+} in apoptotic cell death has been intensively studied (Bossy-Wetzel et al., 2004; Capasso et al., 2005; Kim et al., 1999; Levenson, 2005; Li et al., 2010). Indeed, the recent demonstration that zinc-buffering therapy yields improved recovery from clinical stroke in humans (Diener et al., 2008) has encouraged the notion that Zn^{2+} signaling pathways in neuronal death are an attractive target for therapeutic intervention.

The present report summarizes a serendipitous finding about the detection of Zn^{2+} by fluorescent probes in degenerating hippocampal neurons in histologic sections after traumatic brain injury (TBI). These observations were confined to hippocampal neurons both because of the abundance of histochemically-reactive Zn^{2+} in the hippocampus (Frederickson and Danscher, 1990; McLardy, 1964; Slomianka, 1992) and the vulnerability of hippocampal neurons (Friede, 1966) to ischemic and TBI-induced degeneration (Clifton et al., 1989; Jenkins et al., 1989; Lowenstein et al., 1992; Royo et al., 2006). Two peculiarities about the staining triggered the current investigation. First, we observed that the staining of serial sections for degenerating neurons with Fluoro-Jade (FJ), a probe considered specific for neuronal degeneration, and Newport Green (NG), a probe considered specific for Zn^{2+}, produced surprisingly high correspondence between the two staining patterns (Hellmich et al., 2006). We were surprised by the high correlation although it was previously noted that the qualitatively high correspondence between N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ) and eosinophilic neurons occurred after TBI (Suh et al., 2000) and after *status epilepticus* (Frederickson et al., 1988; Frederickson et al., 1989). Second, the staining for Zn^{2+} by NG was occurring under conditions that would be expected to eliminate specific staining for Zn^{2+} in the cytosol, namely fixation of the sections in ethanol (Frederickson et al., 1987) and immersion of fixed sections in a Coplin staining jar filled with a solution of the fluorescent Zn^{2+} probe, in which the probe could be saturated by incidental Zn^{2+} in solution. Therefore, we hypothesized that, after TBI or pilocarpine-induced seizures, fluorescein (or rhodamine)-containing dyes might bind nonspecifically to injured neurons in fresh frozen or fixed histopathologic sections as much as silver is known to do, thus rendering fluorescein-containing substances ineffective for identifying Zn^{2+} in injured neurons in fresh frozen or fixed histopathologic sections.

2. Results

2.1. *Non-specific binding of fluorescein-based indicators to injured neurons*

Cell-impermeant Newport Green (NG), a di-2-picolylamine derivative bound to dichlorofluorescein, is a relatively weak-binding albeit selective Zn^{2+} indicator with a K_D for Zn^{2+} of 1 μM. NG stained neurons in the rat hippocampus, ipsilateral to the injury site, following fluid percussion traumatic brain injury (Fig. 1). Due to the absence of NG positive neurons in the sham rat's brains, photos were omitted. Removal of metals with the chelator, TPEN (100 μM or 1 mM; 5 s to

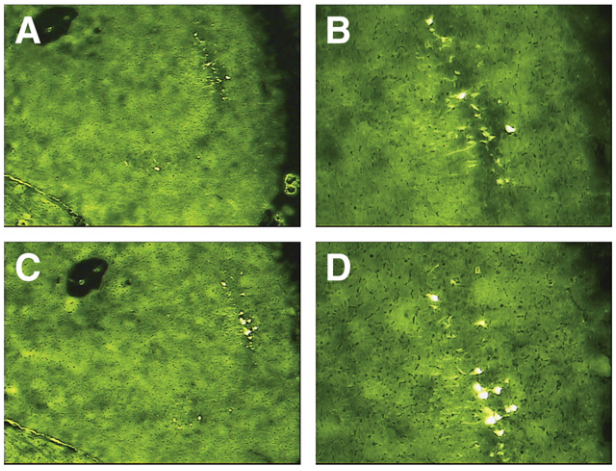

Fig. 1 – Effect of TPEN on Newport Green. Newport Green DCF stained brain sections (right hippocampus shown) from traumatic brain injured rat viewed at 4× (A) and 10× (B). Serial sections from the same rat were treated with TPEN prior to Newport Green DCF staining, viewed at 4× (C) and 10× (D).

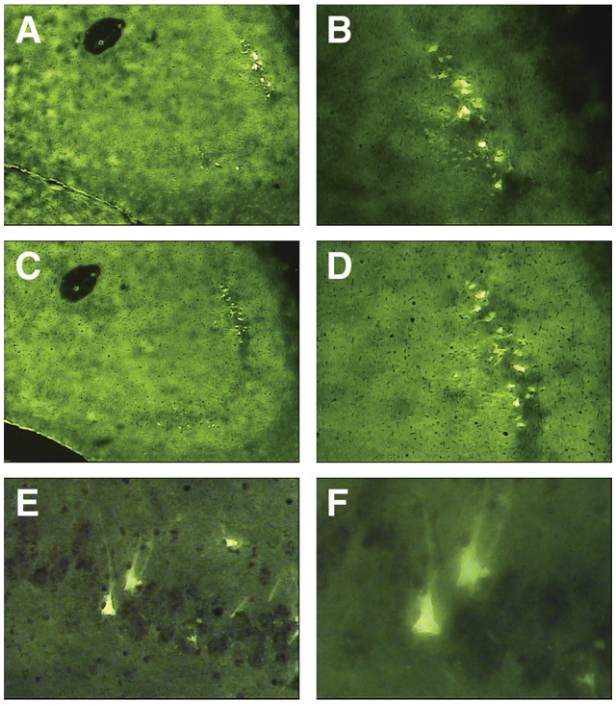

Fig. 2 – Effect of TPEN on Fluoro-Jade. Fluoro-Jade stained brain sections (right hippocampus shown) from traumatic brain injured rats viewed at 4× (A) and 10× (B). Serial sections from the same rats were treated with TPEN prior to Fluoro-Jade staining, viewed at 4× (C), 10× (D), 20× (E) and 40× (F).