A FISHY EPILEPSY MODEL

Pentylenetetrazole-Induced Changes in Zebrafish Behavior, Neural Activity, and *c-fos* Expression

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Rodent seizure models have significantly contributed to our basic understanding of epilepsy. However, medically intractable forms of epilepsy persist and the fundamental mechanisms underlying this disease remain unclear. Here we show that seizures can be elicited in a simple vertebrate system, for example, zebrafish larvae (*Danio rerio*). Exposure to a common convulsant agent (pentylenetetrazole, PTZ) induced a stereotyped and concentration-dependent sequence of behavioral changes culminating in clonus-like convulsions. Extracellular recordings from fish optic tectum revealed ictal- and interictal-like electrographic discharges after application of PTZ, which could be blocked by tetrodotoxin or glutamate receptor antagonists. Epileptiform discharges were suppressed by commonly used antiepileptic drugs, valproate and diazepam, in a concentration-dependent manner. Upregulation of *c-fos* expression was also observed in CNS structures of zebrafish exposed to PTZ. Taken together, these results demonstrate that chemically induced seizures in zebrafish exhibit behavioral, electrographic, and molecular changes that would be expected from a rodent seizure model. Therefore, zebrafish larvae represent a powerful new system to study the underlying basis of seizure generation, epilepsy, and epileptogenesis.

COMMENTARY

Research into epilepsy mechanisms primarily involves studies of rodents. Other mammalian species are occasionally investigated, as is mammalian tissue grown as individual neural cells or brain slices in the dish. Simpler vertebrates have not been used as model systems to study epilepsy. Many argue that their nervous systems are too primitive to generate seizures or that potential seizure-like activity in these models has little relevance to human epilepsy. The conservation of many neural functions among vertebrates, however, suggests that fish and other simpler vertebrates may provide useful models for a variety of neurological disorders. Indeed, studies of simpler vertebrates, such as zebrafish, have shed light on brain developmental mechanisms in mammals. In this regard, Baraban and colleagues examine the feasibility of using developing zebrafish larvae as an epilepsy model system.

Why zebrafish? Zebrafish are freshwater teleosts that are readily available and exceptionally useful organisms for developmental and genetic studies (1). Adults are easy to house and maintain, reach sexual maturity in 2–3 months, breed year-round, and can produce several hundred progeny at weekly intervals. Embryos develop externally and are optically clear, allowing visualization of brain development; the fish remain semitranslucent with respect to many organ systems, including the brain. The zebrafish genome shares 70–80% homology with that of humans, and zebrafish increasingly are being used to model human disease (2). The use of zebrafish for genetic screens by random mutagenesis is well characterized and has led to the identification of many genes important for brain development (3,4). Zebrafish also offer the potential for high throughput chemical and behavioral screens, as pharmacological agents can be added to the tank water and swimming behavior easily characterized.

In the present study, which serves as a foundation for planned epilepsy genetic studies, Baraban and colleagues examine the influence of the chemoconvulsant pentylenetetrazol (PTZ) on the behavior and neural activity of 7-day postfertilization zebrafish larvae. They first show that PTZ treatment elicits dose-dependent seizure-like behaviors in the fish. Three seizure stages are characterized. Stage I involves increased swimming activity, followed by rapid circular, "whirlpool-like" swimming

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(Stage II). The seizure-like activity then progresses to clonic-like convulsions followed by brief loss of posture, termed Stage III. As with rodents, PTZ-evoked behaviors show dose-dependent effects in terms of latency to onset and severity. The investigators then perform field potential recordings from the midbrain optic tectum (chosen for its large size) of live, immobilized fish exposed to PTZ. The addition of PTZ evokes brief interictal- and ictal-like discharges that are attenuated by tetrodotoxin or glutamate receptor antagonists, but potentiated by the $GABA_B$-receptor agonist baclofen. PTZ-induced seizure-like activity also is inhibited by antiepileptic drugs, with an efficacy profile similar to that seen for PTZ-induced seizures in rodents (5). In addition, the authors show that PTZ-evoked seizures in the fish are associated with increased mRNA expression for *c-fos*, an immediate early gene, particularly in optic tectum and cerebellum.

These findings support the feasibility of the zebrafish as a potential model organism for the study of epilepsy. The fish show many similarities to chemoconvulsant-induced seizures in rodents: progressive stages of seizure-like activity; electrographic discharges resembling interictal, ictal and postictal activity; similar profiles of pharmacologic and anticonvulsant responsiveness; and induction of immediate early gene expression. The zebrafish model, however, also has some differences in comparison to human and experimental rodent epilepsy, which raise uncertainties that need to be addressed. First, the electrographic discharges are very brief (ictal-like discharges averaged about 5 seconds) and do not clearly evolve. Second, *c-fos* expression appears mainly in tectum and cerebellum but not the telencephalon. Recordings from the telencephalon will need to be performed. Third, the fish used in this study were 7-day postfertilization larvae. The experiments, therefore, will need to be repeated using older fish. From a technical standpoint, the small size makes recording from multiple sites extremely difficult. Despite these concerns, the data represent an impressive first step to validating the zebrafish epilepsy model. The potential yield from such a model, which includes discovery of epilepsy genes and high throughput pharmacological screens, certainly makes the attempt worthwhile.

by Jack Parent, MD

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