

TARGETING THE BRAIN: FOCAL DELIVERY OF NATURAL ANTICONVULSANT MOLECULES

Seizure Suppression and Lack of Adenosine A₁ Receptor Desensitization after Focal Long-term Delivery of Adenosine by Encapsulated Myoblasts

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Adenosine is an important inhibitory modulator of brain activity. In a previous *ex vivo* gene-therapy approach, local release of adenosine by encapsulated fibroblasts implanted into the vicinity of an epileptic focus was sufficient to provide transient protection from seizures (Huber A, Padrun V, Deglon N, Aebischer P, Mohler H, Boison D. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. *Proc Natl Acad Sci USA* 2001; 98:7611–7616). Long-term seizure suppression beyond 2 weeks was precluded by limited life expectancy of the encapsulated fibroblasts. To study the feasibility for long-term seizure suppression by adenosine-releasing brain implants, in the present contribution, mouse C₂C₁₂ myoblasts were engineered to release adenosine by genetic inactivation of adenosine kinase. After encapsulation, the myoblasts were grafted into the lateral brain ventricles of epileptic rats kindled in the hippocampus. Although seizure activity in animals with wild-type implants remained unaltered, 1 week

after grafting, all rats with adenosine-releasing implants ($n = 25$) displayed complete protection from convulsive seizures and a corresponding reduction of afterdischarges in EEG recordings. The duration of seizure suppression was maintained for a period of 3 weeks in 50% of the animals, ranging to a maximum of 8 weeks in one animal. During the course of these experiments, adenosine A₁ receptors remained responsive to selective agonists and antagonists, indicating a lack of desensitization of A₁ receptors after local long-term exposure to adenosine. Furthermore, local release of adenosine did not affect locomotor activity, whereas systemic application of the A₁ agonist 2-chloro-N⁶-cyclopentyladenosine caused strong sedation. Thus, the local release of adenosine by cellular implants provides a feasible option for a potential side effect-free approach for the long-term treatment of focal epilepsies.

COMMENTARY

A number of naturally occurring brain substances are capable of suppressing seizure activity and may function as endogenous anticonvulsants. Appealing as it may seem, clinical application of unmodified endogenous anticonvulsants for epilepsy therapy faces substantial challenges. For example, anticonvulsant neuropeptides, such as somatostatin, neuropeptide Y, and galanin, have limited bioavailability and poorly penetrate the blood–brain barrier. Furthermore, cellular targets for many endogenous compounds, including neuropeptides and openers of adenosine triphosphate (ATP)-sensitive potassium channels, are expressed both in the brain and in peripheral tissues, so that systemic administration of the ligands are likely to cause undesirable systemic side effects. Development of synthetic analogues of endogenous anticonvulsants might overcome some of these limitations. An example of such an approach might be the

synthesis of chemically stable, low-molecular-weight ligands for neuropeptide receptors. However, any modification applied to a native molecule often leads to the loss of biochemical and physiologic efficacies.

An alternative method might be delivery of an unmodified endogenous anticonvulsant directly into the brain. Such focal drug delivery would allow bypassing gut metabolism and the blood–brain barrier, as well as avoiding peripheral side effects. However, such an intervention obviously cannot be repeated on a frequent basis. Thus, investigators have been prompted to develop a system of drug application that can be grafted into the brain, allowing continuous release of an ample amount of therapeutic agent for a prolonged period.

The ribonucleoside adenosine is an endogenous anticonvulsant that could potentially be useful in epilepsy therapy. Adenosine exerts neurophysiologic actions through four receptors, designated A₁, A_{2A}, A_{2B}, and A₃ (1). Adenosine, acting predominantly through the A₁ receptor, inhibits excitatory glutamatergic neurotransmission. In addition, adenosine can induce G protein-coupled inhibition of Ca²⁺ channels as well as inhibit Ca-independent release of glutamate and

G protein-dependent, inwardly rectifying K^+ channels (2). All of these actions could contribute to anticonvulsant activity. Adenosine inhibits seizures in several experimental models (2–4) and exerts a neuroprotective effect, which further enhances its merit for use in epilepsy therapy (2,5). However, clinical use of adenosine in epilepsy has been limited because adenosine receptors are widely distributed, not only in the brain but also in peripheral tissues, particularly in the cardiovascular system. Thus, systemic administration of adenosine in the doses necessary to mitigate seizures would likely lead to untoward cardiovascular side effects.

For the past several years, studies have been under way to develop systems for focal delivery of adenosine into the brain. The study by Güttinger and colleagues represents an attempt to optimize local adenosine delivery and to characterize its anticonvulsant and safety profiles. As a delivery system, the authors chose mouse C_2C_{12} myoblasts, which are committed precursors of differentiated skeletal muscle cells. The authors exploited the fact that myoblast cell lines, although having the advantage of being dividing cells and having unlimited availability, can be differentiated into a nonmitotic stage on exposure to a low serum-containing medium. Furthermore, on differentiation, myoblasts survive for prolonged periods (6).

Güttinger and coworkers optimized the delivery system in two ways. First, they ensured that the myoblasts afforded high concentration and release of adenosine. This effect was achieved through the knockout of the enzyme adenosine kinase, a key regulator of intracellular adenosine concentration, which converts adenosine to adenosine 5'-monophosphate (5'-AMP). Thus, although the adenosine synthesis was not affected and occurred through a natural pathway, adenosine metabolism was inhibited. With regard to epilepsy, seizures have been shown to lead to overexpression of adenosine kinase in astrocytes, a phenomenon that may be an epileptogenic factor (7). Furthermore, transgenic animals with overexpression of adenosine kinase exhibit spontaneous seizures and have increased spiking frequency after injection of kainic acid (8). Thus, inhibition of adenosine kinase activity seems to be a reasonable method for increasing the anticonvulsant efficacy of endogenous adenosine.

Second, by encapsulating the cells into semipermeable polymer membranes, Güttinger and colleagues designed a delivery system that avoided the integration of transplanted cells into neuronal networks and the disruption of these networks. Another advantage of using encapsulated cells and of the preservation of intact tissue around the graft is that it provides the option of surgical removal of the transplant should the treatment be discontinued.

The developed system proved to represent a viable approach. Adenosine kinase knockout myoblasts showed a 17-

fold increase in adenosine concentration, as compared with that of wild-type cells. On transplantation into the lateral brain ventricle, the cells exhibited a strong anticonvulsant effect in the kindling model of epilepsy. The antkindling effect was the most pronounced during the first 2 weeks, although some animals were protected for as long as 8 weeks after transplantation. The specificity of the anticonvulsant effect of the transplant was demonstrated by its reversal, by using a selective A_1 -receptor antagonist. Importantly, although the transplant did suppress seizures, it did not induce behavioral side effects.

Remarkably, continuous delivery of adenosine did not lead to the desensitization of A_1 receptors and to an associated loss of anticonvulsant effect. The authors attributed the absence of A_1 -receptor desensitization to the fact that the amount of adenosine released by the transplants, although sufficient to suppress seizures, was not great enough to cause desensitization. Although it was significantly more successful than earlier efforts, the C_2C_{12} myoblast system was still not perfect. Suppression of kindled seizures gradually declined starting at week 3, as a result of death of the transplanted cells.

Despite the drawbacks, the cell-transplant system described by Güttinger and coworkers provides an interesting potential approach for the delivery of antiepileptic substances. Such an approach might be applied to a variety of agents that cannot be administered by conventional drug-delivery methods. Because myoblast precursors (muscle satellite cells) are present in human adult muscle tissue, these cells could be engineered in a similar way as are the mouse cells, which would reduce the possibility of immune rejection compared with that with a xenograft.

by Andrey Mazarati, MD, PhD

References

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