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# The Adenosine Hypothesis of Epilepsy

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Abstract — The present communication summarizes a variety of diverse observations indicating that adenine ribo-nucleoside (ARN), or adenosine, may play an important role as an endogenous anti-epileptic compound in the central nervous system. From such observations has evolved an hypothesis which states that defects in the synthesis, release, action and/or degradation of ARN may be a causative factor in some forms of epilepsy. Of particular interest is the emerging realization that the adenosine system may be a common factor in the mechanism of action of many otherwise unrelated anticonvulsant compounds. Thus, a more detailed understanding of the ARN system and its role in the control of cerebral activity may lead to rational strategies for the development of efficacious theraputic agents having greater specificity and fewer side effects.

## Introduction

The various pharmacological agents used in the treatment of epilepsy have for the most part been developed either through a "me-too" process of altering structures of known active agents or through a serendipitous process of finding anticonvulsant activity in agents being cross-screened for theraputic application not related to epilepsy. Given the limitations in our understanding of the causes of epilepsy this reliance on good fortune in the development of anticonvulsant agents is not at all surprising. Furthermore, the minimal amount of information currently available regarding the mechanisms of action of anticonvulsants has been of little use in developing theories as to the causes of epilepsy. Thus, this field has suffered for its relative lack of theories and hypotheses with which experimentation can be guided. Certainly, no hypothesis has appeared strong enough to influence the field of epilepsy research in the way that the dopamine hypothesis of schizophrenia and the monoamine hypothesis of depression have served as focal points for directing, interpreting and interrelating clinical and laboratory findings in these respective fields of scientific endeavor. However, this situation may be changing.

In the last few years a number of diverse lines of investigation have yielded data suggesting that endogenous adenine-ribose nucleoside (ARN), commonly known as adenosine, has the ability to prevent or arrest seizure activity. From such observations has evolved an hypothesis, implied in the publications of a number of laboratories, which states that defects in the metabolism or action of endogenous ARN may be a causative factor in some forms of seizure activity. The

present article traces the development of the major lines of evidence which lead to this adenosine hypothesis of epilepsy.

*Endogenous Adenosine as an Anticonvulsant Agent*

In 1969 Rall and co-workers (2) reported that a stable substance elaborated from electrically stimulated brain slices caused large accumulations of cAMP in those slices. Unexpectedly, when a methylxanthine, theophylline, was added to the system to block phosphodiesterase and thereby enhance the cAMP accumulation, the unknown substance lost its activity. The following year Sattin and Rall (1) published evidence identifying the active agent as ARN. Sattin and Rall also demonstrated for the first time the ability of methylxanthines to block the stimulatory effects of ARN. On the basis of these observations Sattin and Rall postulated the existence of a methylxanthine-blockable ARN receptor linked to adenylate cyclase. Since this historic discovery evidence in support of the existence of the ARN receptor has accrued to the point that its existence is now beyond any doubt and receptor sub-classes of the ARN receptor are currently being identified. A number of recent reviews discussing the identification, classification and nomenclature of ARN receptors have appeared (3, 4, 5, 6, 7). The present author prefers the nomenclature of Londos et al. (6).

Characterization of ARN receptors in the CNS has progressed on the basis of two experimental approaches: 1) the ability of ARN to activate (via $R_a$ receptors) or inhibit (via $R_i$ receptors) adenylate cyclase (7), and 2) the nearly universal ability of ARN to depress neuronal firing (8). The relationship between the electrophysiological effects of ARN and its dichotomous effects on cAMP metabolism has yet to be elucidated. At present the strongest case seems to be in favor of activation of adenylate cyclase being the means by which ARN inhibits neuronal activity. Thus, Phyllis (9) has reported that $R_a$ agonists are more potent than $R_i$ agonists in inhibiting neuronal activity in intact brain. Furthermore, ARN and bioamines act synergistically in both stimulating cAMP accumulation (1) and in inhibiting neuronal firing (37) in the CNS. These observations suggest that it is the activation of adenylate cyclase via the $R_a$ receptor that is the initial step in the inhibition of neuronal activity by ARN. However, investigators using brain slice preparations have found the electrophysiological effects of adenosine not well correlated with current receptor sub-type classifications, and it has been suggested that there may be more than two sub-types of the ARN receptor (10, 11).

The issue of whether ARN is released within the brain during seizures is, of course, of paramount importance with respect to any hypothesis that purports to relate endogenous ARN to seizure activity. It appears from a large amount of data that active neurons either release ARN/ARN precursors or cause such ARN/ARN precursors to be released by other cells. Electrical stimulation, L-glutamate, veratridine, hypoxia and potassium depolarization have all been shown to be effective in stimulating release of ARN and adenine nucleotides from intact brain, brain slices and CNS synaptosomal preparations (14–17). Due to highly active extracellular nucleotidases, adenine nucleotides elaborated from neural tissue are an important potential source of extracellular ARN. Sattin (18) concluded from cAMP studies with mice that ARN is released in the brain during seizures. Winn et al. (19) corroborated this finding by directly measuring increased ARN levels in the brains of chemically convulsed rats.

The foregoing biochemical and electrophysiological observations demonstrate that ARN is elaborated by the brain during seizure activity and that it has the ability to produce or promote a pronounced neuronal quiescence. Thus, there have been established the elements of a prima facie case that the release of ARN during neuronal activity acts as a feedback to prevent that activity from developing into seizure activity. (On a more speculative level, the waves of the electroencephalogram may represent the periodic moderation of neuronal activity as a result of cycles of ARN release and uptake. This speculation and observations supporting it will be developed further in a subsequent publication.) Additional support of an anticonvulsant role of ARN comes from a number of behavioral observations which have established that ARN, ARN agonists and ARN uptake inhibitors are effective anticonvulsant agents (21–24). It is also well established from clinical (25) and experimental (21) observations that antagonists of the ARN receptors (i.e., methylxanthines) are proconvulsive agents. The important implication of these diverse observations has been noted by a number of investigators who have postulated an anticonvulsant role for endogenous, extracel-

lular ARN working through ARN receptors (21, 32–34). An extension of this postulate leads directly to the present hypothesis which states that seizure activity may be initiated, or the seizure threshold may be lowered, by defects which cause a diminution of the normal anticonvulsant activity of endogenous ARN.

### *The CNS "Adenosine system" and epilepsy*

By the CNS "ARN system" is meant the collective mechanisms responsible for: 1) the accumulation of ARN at its CNS receptors; 2) the depression of neuronal activity by ARN action at those receptors; 3) the removal of ARN from the vicinity of its receptors. According to the ARN hypothesis, seizure activity may be produced or promoted by any agent or defect that alters this system in such a way as to decrease the ability of ARN to depress neuronal activity.

For instance, if the rate of accumulation of ARN in the extracellular space adjacent to a locus of activated neurons is not sufficient to keep up with the increased firing rate of those neurons, then the locus could become hyperactive and such hyperactivity could spread across or through the brain to normal areas faster than ARN could be elaborated to contain it. Many ways can be envisioned in which the accumulation of ARN could be compromised. Glial cells that proliferate in response to local injury or disease processes could clear ARN from the extracellular space too rapidly. Alternatively, an increase in the activity of ARN deaminase, the major enzyme of ARN degradation, could lead to insufficient levels of ARN in the extracellular space. In this regard it is of interest to note a recent report that genetic mutations in fibroblasts result in a hyperactive ARN deaminase which protects those fibroblasts from the inhibitory effects of ARN (26). Similarly, an increase in ARN kinase or a decrease in nucleotidases could result in decreased levels of extracellular ARN during periods of enhanced neuronal activity.

The loss or blockade of ARN receptors might also lead to seizure activity, according to the adenosine hypothesis. As mentioned above, methylxanthines, antagonists of the ARN receptors, cause seizure activity in humans when taken in excess. On these grounds it has been suggested that caffeine and other methylxanthines are contraindicated in epileptic patients (29). The need for controlled clinical observations in this regard is obvious. It is also theoretically possible that ARN receptor agonists will be discovered which will have clinically useful anticonvulsant activity. Already a specific $R_a$ agonist has been reported to offer protection against seizures in kindled rats (31). This observation is consistent with Phyllis' suggestion that neuronal depression is mediated by the $R_a$ receptor (9). Also, Skerrit et al. (30) have reported ligand binding studies that suggest that the anticonvulsant agent carbamazepine is an ARN agonist or partial agonist. However, complimentary studies characterizing carbamazepine as an ARN agonist in biochemical and electrophysiological paradigms must be undertaken before its mechanism of action can be attributed to an interaction with the ARN receptor.

The anticonvulsant activity of ARN also suggests that agents which enhance the rate of accumulation of ARN should have an indirect anticonvulsant activity, even in situations where seizure activity is not caused by some defect in the ARN system. This may explain why papaverine, an inhibitor of ARN uptake, is both potent and efficacious in suppressing the afterdischarges associated with seizure activity in kindled rats (22). Furthermore, it has been demonstrated that a variety of anticonvulsant benzodiazepines are potent inhibitors of ARN uptake in synaptosomal preparations (27), and it is therefore possible that one class of "benzodiazepine receptor" is a component of the ARN uptake system. This possibility is supported by the observation that dipyridamole, an inhibitor of ARN uptake, is, relatively speaking, quite potent in displacing $^3$H-diazepam from binding sites in rat brain (28). Thus, it is not unreasonable to suggest that the anticonvulsant activity of diazepam may arise from its ability to inhibit the uptake of ARN and thereby accentuate the accumulation of ARN in the extracellular space. This reasoning can now be extended to the mechanism of action of diphenylhydantoin whose anticonvulsant activity has recently been attributed to its ability to inhibit ARN uptake (37).

## Conclusions

The adenosine hypothesis has found support from both clinical and laboratory observations, and the ARN system appears to be emerging as an important key to the understanding of seizures and the mechanisms of action of some

important anticonvulsant agents. Thus, the adenosine hypothesis has the potential of becoming a theoretical nucleus around which further experimentation can crystallize.

However, it must be noted that there are many possible ways in which seizure activity could be initiated and even if a defect in the ARN system is demonstrated to be a causative factor in some types or cases of epilepsy, it does not necessarily follow from the adenosine hypothesis that all forms or cases of epilepsy have their origin in a defective ARN system. Nor does it follow that all convulsant or anticonvulsant agents must interact with the ARN system. On the other hand, it does follow from the above hypothesis that agents which increase ARN levels or stimulate the appropriate ARN receptors in the appropriate area of the brain will have anticonvulsant activity regardless of the etiology of the specific disorder. This may well be the aspect of the hypothesis that is most accessible to experimental verification, as well as the aspect most applicable to the development of new concepts and strategies for controlling epilepsy. Even where the ARN system is not the site of the primary defect which leads to seizure activity, it may be possible to control such activity by enhancing or mimicking the anticonvulsant activity of this important nucleoside.

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