Adenosine Deaminase of Cultured Brain Cells

By EBERHARD G. TRAMS and CARL J. LAUTER

Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20014, U.S.A.

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Two types of adenosine deaminase (EC 3.5.4.4) were found in cultured cells of central-nervous-system origin. The predominant and more active enzyme was obtained in soluble form from the cytosol of mouse neuroblastoma (N-18), neonatal hamster astrocytes (NN), human oligodendroglioma (HOL) and human astrocytoma (Cox Clone). Particulate adenosine deaminase was probably associated with the plasma membrane. When radioactive adenosine was added to superfusates of monolayer cultures it was rapidly converted into inosine and hypoxanthine. The metabolic conversion required adenosine uptake by the cells, a probable transition through the intracellular ATP pool(s) and a rapid excretion into the superfusate of the catabolic products. We discuss the evidence that points to adenosine and its derivatives as neurohumoral modulators of central-nervous-system function.

We have postulated that plasma membrane $Mg^{2+}+Ca^{2+}$ -activated adenosine triphosphatase (EC 3.6.1.3) and 5'-nucleotidase (EC 3.1.3.5) are ecto-enzymes and that these cell-surface enzymes are widely distributed among eukaryotic cells (Trams & Lauter, 1974). Observations made in other laboratories appear to support this conclusion, and it now seems likely that other phosphoester hydrolases also function as ecto-enzymes (DePierre & Karnofsky, 1974; Mandel *et al.*, 1974; Evans, 1974; Reichert *et al.*, 1974). We offered an explanation for the existence of some ecto-enzymes when we presented evidence for ATP action on the cell surface (Trams, 1974). We also observed that ATP produced a biphasic change in membrane permeability; for instance, an increase in the permeation rate of $^{42}K^{+}$ or $^{32}P_i$ was followed by a decrease when $1\mu M$ -ATP was applied to monolayer cultures of astrocytes. Under some experimental conditions, adenosine inhibited the ATP signal or by itself produced a temporary decrease in permeability. Conceivably ATP and adenosine are linked in a physiological sequence of reactions that modulates membrane permeability (or excitability thresholds). In this scheme we visualized the deamination of adenosine to inosine as the step that terminated the sequence. The prospect that an adenosine deaminase (EC 3.5.4.4) might prove to be yet another ecto-enzyme was underscored by a quite rapid conversion of added adenosine into inosine in the medium of neuroblastoma and astrocyte cultures.

Various pharmacological effects of adenosine administration have been described, but of particular interest to us were the experiments of Pull & McIlwain

(1972) who observed that guinea-pig brain slices that had been pulse labelled with radioactive adenosine or adenine, released adenosine and inosine into the medium; this release was markedly increased by electrical stimulation of the slices. McIlwain (1972a) has reviewed the regulatory significance and action of adenosine in the central nervous system in some detail and he proposed that adenosine actually subserved a neurotransmitter function. He further suggested that the prebiotic nature of the adenine derivatives might have qualified them for some neurohumoral transmitter role in the early stages of evolution. We consider this an attractive hypothesis because the ecto-enzymes and the effects produced by ATP on the cell surface may represent the vestiges of a primitive (and still functioning) intercellular communications system. A further contribution by Pull & McIlwain (1974) deals with cerebral-cortex adenosine deaminase, and we shall discuss their observations in the appropriate sections below.

Experimental

Tissue cultures

The following cell lines served as enzyme sources: neonatal Syrian hamster astrocytes (NN); mouse-neuroblastoma C 1300 (N-18); human oligodendroglioma (HOL) and human grade II–III astrocytoma (Cox Clone). The cells were propagated in 75 cm^2 plastic tissue-culture flasks (Falcon Plastics, Oxnard, CA., U.S.A.) in the appropriate media. When reference is made to intact cells, experiments were performed on monolayer cultures in these culture flasks. Cell-free preparations were obtained by

removing the cells from the flasks (either mechanically or by trypsin treatment; no differences were noted in results) and subjecting them to ultrasonic treatment. After exposure to 3×1 s bursts from a micro-tip of a Branson Sonifier Model L 575, microscopic observation revealed nearly complete cell disruption.

Assays of adenosine deaminase

Optical method (modified from that of Kalckar, 1947). Before the experiments, the cells were rinsed several times with an iso-osmotic phosphate-free buffer (Trams & Lauter, 1974) to remove all traces of serum proteins and indicator dyes from the medium. Whole cells or cell-free preparations were incubated with adenosine in phosphate-free buffer at 37°C in an atmosphere of air + CO_2 (95:5). Monolayer cultures were rotated horizontally for 6 s at 50 rev./min once every 150 s to ensure adequate superfusion. Cell-free preparations were incubated in 15 ml conical test tubes in a Dubnoff metabolic shaker. Incubations were terminated by the addition of ice-cold 0.5 M- HClO_4 to a final concentration of 0.25 M- HClO_4 . The absorbance of the acidified incubation medium (after centrifugation, if necessary) was measured at 265 nm. The extinction coefficient of adenosine in 0.25 M- HClO_4 was estimated to be $14.3 \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ and that of inosine under these conditions was estimated to be $7.8 \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

Radioisotope assay. Intact cells or cell-free preparations were incubated with $[8\text{-}^{14}\text{C}]$ adenosine or $[2,8\text{-}^3\text{H}]$ adenosine (New England Nuclear Corp., Boston, MA, U.S.A.) which had been purified by t.l.c. and was adjusted to the desired specific radioactivity with unlabelled adenosine (Eastman, Rochester, NY, U.S.A.). At the end of the incubation period the reaction was stopped by the addition of HClO_4 (final concn. 0.25 M). The mixture was centrifuged in the cold and 50–200 μl portions were mixed with known amounts of carrier adenosine, inosine, hypoxanthine and occasionally other metabolites. Adenosine was separated from its metabolites by t.l.c. either on MN 300F cellulose (250 μm thick; Analtech, Newark, DE, U.S.A.) or on microcrystalline cellulose F (250 μm thick, Analabs, Inc., North Haven, CT, U.S.A.). Separation of inosine from hypoxanthine was better on MN 300F plates, but the sharpness of separation for all other adenosine congeners was better with microcrystalline cellulose F. T.l.c. plates were developed in propan-1-ol–28% NH_3 –water (13:7:1 or 3:1:1, by vol.). The first system gave better separation of the nucleosides, whereas the second favoured separation of nucleotides. The areas on the t.l.c. plates that contained the various adenosine metabolites were scraped into scintillation vials and suspended in 3 ml of water, to which was added 10 ml of Aquasol (New England Nuclear Corp.). This mixture formed a stable gel in which the cellulose particles were suspended. The radioisotope was

assayed by scintillation spectrometry; counting efficiency ($^{14}\text{C} = 55\text{--}65\%$, $^3\text{H} = 25\text{--}38\%$) was determined with an external standard after calibration of the system with internal standards.

Kinetic constants. These were estimated by the Direct Linear Plot of Eisenthal & Cornish-Bowden (1974). We have found their graphical procedure for estimating enzyme kinetic parameters a simple and elegant method.

Miscellaneous methods

For the determination of protein content, cells were suspended in 1 M-NaOH until digested. Proteins were determined as described by Lowry *et al.* (1951), with human serum albumin (fraction V) as standard. Cell counts were made with an improved Neubauer-Levy corpuscle-counting chamber (V. A. Hausser, Philadelphia, PA, U.S.A.). Resolution of nucleotides, nucleosides and their derivatives by two-dimensional t.l.c. was as described by Pataki (1967). Tissue-culture media were Hank's minimum essential medium (hereafter referred to as Hank's medium) and Dulbecco's phosphate balanced-salt solution (Dulbecco's medium) (both from Grand Island Biological Co., Grand Island, NY, U.S.A.).

Results

When a confluent culture of neonatal hamster astrocytes (NN) was superfused with Hank's medium containing 1.0 mM- $[8\text{-}^{14}\text{C}]$ adenosine it could be observed that adenosine disappeared rather rapidly from the medium. The disappearance of ^{14}C from the medium, however, proceeded at a much slower rate. This suggested that metabolic conversion was the cause of the disappearance of adenosine from the medium, rather than its uptake by the cells. Chromatography of samples of the medium at intervals showed that inosine and hypoxanthine were the principal metabolites. This pattern was reminiscent of the metabolic conversion of ATP and AMP by plasma-membrane ecto-enzymes.

Fig. 1 depicts the metabolic fate of 2 μmol of $[^{14}\text{C}]$ adenosine which were superfused (in 2 ml of medium) on to 75 cm^2 of confluent NN-cell cultures (about 6 mg of protein) over a period of 60 min. Nearly 25% (0.5 μmol) of the adenosine from the medium disappeared within the first 5 min of incubation. After 60 min of incubation a substantial amount of label was fixed in the NN cells as acid-insoluble ^{14}C . Of the acid-soluble cytoplasmic ^{14}C , over 90% was present as ATP. The quantities of adenosine and inosine found in the cytoplasmic compartment were small.

In the medium, ^{14}C derived from $[8\text{-}^{14}\text{C}]$ adenosine was recovered mainly as inosine, hypoxanthine, xanthine and uric acid. Identity of these products was established by two-dimensional t.l.c. and radio-

Fig. 1. Metabolic fate of 1 mM-[8- ^{14}C]adenosine in astrocyte monolayer cultures

Confluent NN-cell cultures were superfused with 2 ml of 1 mM-adenosine in Hank's medium for 10, 20, 30 and 60 min. Adenosine and metabolites in the superfusate and in the cells were determined by the radioisotopic method. Shaded areas indicate ^{14}C distribution (as % change with time) in adenosine and metabolites in the superfusate. Clear areas below the broken line indicate ^{14}C distribution in the cells.

autography. The excretion of uric acid into the medium may be peculiar to NN-cell culture, since uric acid was not found in the superfusates of N-18-cell cultures. Inosine accumulation in the medium proceeded initially at about 8.5 nmol/min per mg of cell protein.

This rapid conversion of adenosine into inosine in the superfusate could have occurred either by (a) the action of a plasma-membrane ecto-adenosine deaminase or (b) the rapid uptake of adenosine by the cells, intracellular deamination and nearly equally rapid excretion of inosine into the superfusate. We knew that adenosine could be taken up by the cells and that it was immediately converted into ATP. The absence of a strong permeability barrier for the substrate made it difficult to apply the methods and criteria used for the demonstration of ecto-adenosine triphosphatases and ecto-5'-nucleotidases (Trams & Lauter, 1974; DePierre & Karnofsky, 1974). Because cells that had been pulse-labelled with trace amounts of [^{14}C]adenosine excreted [^{14}C]inosine into the medium, it was clear that cases (a) and (b) would have to be assessed with respect to their quantitative contributions.

A simple pulse label-chase experiment indicated

that the conversion of adenosine into inosine in the superfusate proceeded probably via mechanism (b): 12 culture flasks with confluent NN cells were exposed for 2 h to 20 μM -[8- ^{14}C]adenosine (2.85×10^6 d.p.m.) in 2 ml of Hank's medium. During this period 1.98×10^6 d.p.m. (69%) were taken up by the astrocytes. The cells were then washed three times with phosphate-free buffer (Trams & Lauter, 1974) and incubated with 2 ml of buffer containing from 0 to 5 mM unlabelled adenosine. Samples of medium were removed at 5, 10 and 20 min for liquid-scintillation counting and t.l.c. The cells were washed three times with cold 0.9% NaCl and extracted with 2 ml of 0.2M-HClO₄. The results of the experiment are

Fig. 2. Effect of various concentrations of adenosine in the superfusate on the rate of ^{14}C efflux (inosine + hypoxanthine) from [8- ^{14}C]adenosine pre-labelled NN astrocytes

Cultures had been labelled for 2 h with 20 μM -[^{14}C]adenosine. They were then rinsed several times and superfused with various concentrations of unlabelled adenosine in Hank's medium for 5 (.....), 10 (----) and 20 (—) min. Inset shows cytoplasmic ATP at the end of the 20 min incubation as a function of adenosine concentration in the medium. Inset abscissa also shows medium adenosine concentrations (mM). Bars indicate $\pm$ s.e.m. values.

shown in Fig. 2. Accumulation of ^{14}C in the superfusate during the first 5 min of incubation was independent of external adenosine concentration. After this time-lag, however, it became apparent that extrusion rates of radioisotope into the superfusate were markedly accelerated, even at low adenosine concentrations in the medium. Maximum ^{14}C extrusion was observed when external adenosine was 0.2 mM. More than 90% of the ^{14}C in the medium was accounted for by inosine and hypoxanthine. Assay of cytoplasmic [^{14}C]ATP content as a function of external adenosine concentration showed a minimum at 20 min, when external adenosine was 0.2 mM (Fig. 2, inset). Our interpretation of this experiment was that adenosine was taken up from the medium and entered the intracellular nucleotide/nucleoside pools. Uptake and turnover rates increased as the external adenosine concentration reached an optimum. The only significant source of labelled inosine in the medium was cytoplasmic [^{14}C]ATP; we estimated that the decrease in this [^{14}C]ATP was sufficient to accommodate a sweep-through of 0.4 mmol of adenosine through an ATP pool of 0.18–0.27 mmol in 20 min. It was not proved, however, that conversion of adenosine into inosine required a transition through the ATP pool.

The following experiment was designed to obtain data on the movement of adenosine through the pertinent intracellular metabolic compartments. Although it is probable that more than one pool exists for each metabolite (see Shimizu *et al.*, 1970), we have based our calculations on the assumption of single pools. Six 75 cm² culture flasks containing neonatal hamster astrocytes (passage 88, 11 days old) were incubated for 60 min with 0.5 mM-[8- ^{14}C]-adenosine (10.76×10^6 d.p.m./ μmol) in 2 ml of Hank's medium. After removal of this medium the cells were washed three times with 5 ml of Hank's medium at 37°C and then superfused with 2 ml of Hank's medium containing 0.5 mM-[2,8- ^3H]adenosine (38.11×10^6 d.p.m./ μmol). At the end of 0.5, 5 and 10 min incubation periods the medium was removed from two flasks each and stored at 0°C. The monolayers were washed twice with ice-cold Hank's medium and extracted with 2 ml of cold 0.5 M-HClO₄. The HClO₄ was removed by aspiration and the cells were digested in NaOH for protein determination.

Portions of superfusates and HClO₄ extracts were chromatographed on MN-cellulose plates (in propan-1-ol-28% NH₃-water, 3:1:1, by vol.) to separate the nucleotides and adenosine and its metabolites. Under these conditions, inosine and hypoxanthine were not resolved by t.l.c. The areas containing ATP, adenosine and inosine (+hypoxanthine) were scraped off the plates and ^{14}C and ^3H contents were determined by scintillation counting.

After the 60 min [^{14}C]adenosine labelling period, 16% of the radioisotope ($=0.16 \mu\text{mol}$) had been

converted into ATP; 11% was fixed in the HClO₄-insoluble fraction and 57% was recovered in the medium, predominantly as inosine, xanthine and uric acid. Some 16% was not accounted for and some of it presumably had been oxidized to $^{14}\text{CO}_2$.

During the subsequent [^3H]adenosine chase periods it was found that about 60% of the inosine that accumulated in the superfusate during the first 0.5 min was labelled with ^{14}C (Table 1). If an ecto-adenosine deaminase had contributed substantially to the formation of inosine in the superfusate, the predominant label in the inosine in the medium at the beginning of the incubation period would have been ^3H . The efflux of [^{14}C]inosine into the medium continued at a substantial rate during the next 9.5 min, and the proportion of ^3H label in inosine became dominant. During the same periods, the labelling pattern of the cytoplasmic pools of adenosine and inosine remained rather steady (Table 1). We interpret this as a constant inward/outward flow of adenosine/inosine through pools that were small in comparison with the ATP pool. The relative sizes of the cytoplasmic compartments for ATP, inosine and adenosine were estimated to be about 550, 13 and 1 respectively. The turnover of adenosine in the cytoplasmic pool was estimated to be about 60 nmol/min per mg of protein, a value that is quite compatible with the V_{max} value calculated for adenosine deaminase from NN cells (see below).

The cytoplasmic adenosine deaminase constituted from two-thirds to three-quarters of total cell adeno-

Table 1. [^{14}C]Adenosine pulse label, [^3H]adenosine chase experiment with NN astrocyte monolayer cultures

Cultures were pulse-labelled with 0.5 mM-[^{14}C]adenosine for 60 min. After removal of ^{14}C from the medium, cultures were exposed to 0.5 mM-[^3H]adenosine for 0.5, 5 and 10 min. For experimental details, see the text. Results are expressed as nmol/2 ml of medium or nmol/mg of protein as appropriate.

Metabolic compartment	Time after addition of [^3H]adenosine to medium (min)		
	0.5	5	10
Medium	-	-	-
[^{14}C]Inosine	6.44	24.2	53.3
[^3H]Inosine	4.6	55.0	113
[^{14}C]Adenosine	0.40	1.81	2.62
Intracellular			
[^{14}C]Adenosine	0.048	0.055	0.039
[^3H]Adenosine	0.040	0.078	0.068
[^{14}C]Inosine	0.08	0.84	1.01
[^3H]Inosine	0.25	0.48	0.48
[^3H]ATP	1.92	4.15	5.96

Table 2. Adenosine deaminase and 5'-nucleotidase of neonatal hamster astrocyte particulate fractions

NN-cell monolayer cultures were scraped from the flasks and washed with Hank's medium and phosphate-free buffer (Trams & Lauter, 1974) and suspended in 2 ml of the buffer. Cells were disrupted by sonication (see the Experimental section) and the sonicate was centrifuged in the Sorvall SS-34 rotor at 20000 rev./min for 30 min. The supernatant was decanted and the sediment, resuspended in 1 ml of buffer, was layered on a 15 ml discontinuous sucrose gradient [3.0 ml of 50%, 4.0 ml of 35%, 4.0 ml of 21% and 4.0 ml of 10% (w/v) sucrose] or on a 15.5 ml continuous sucrose gradient (14 ml of a linear 10–50% sucrose gradient on a 1.5 ml cushion of 50% sucrose). The subcellular fractions were separated by centrifugation in a Spinco SW-27.1 rotor at 25000 rev./min for 60 min. Particle bands were collected separately, diluted with about 20–30 ml of buffer, sedimented at high speed (Sorvall SS-34 rotor, 20000 rev./min, 10 min), resuspended in 1 ml of buffer and assayed for enzyme activity.

Fraction	Sucrose (%, w/w)	Density (g/ml)	Activity (nmol/min per mg of protein)	
			Adenosine deaminase	5'-Nucleotidase
Sonicated cells	—	—	9.3–10.3	2.7–3.8
High-speed supernatant	—	—	68.7	4.9
High-speed pellet			1.3	5.4
High-speed pellet fractionation				
Discontinuous sucrose gradient	0.0	1.02	0	8.1
	10.0	1.038	0	4.8
	21.0	1.085	13.2	14.1
	35.0	1.151	2.5	6.9
	50.0	>1.229	0	3.4
Continuous sucrose gradient	10–21	1.038–1.085	0.8	3.8
	21–35	1.085–1.151	4.7	6.6
	35–50	1.151–1.229	2.7	3.7
	50	>1.229	0.7	1.2

Fig. 3. Formation of inosine by soluble adenosine deaminases from cultured brain cells

After sonic disintegration in iso-osmotic phosphate-free buffer, pH 7.4 (Trams & Lauter, 1974), cytoplasmic adenosine deaminase activity was determined on 25000g-h supernatant fractions. Incubation was for 10 min at 37°C; the optical assay was used. □, Cox Clone; ○, N-18, neuroblastoma; △, NN, neonatal hamster astrocyte; ●, HOL, human oligodendroglioma.

sine deaminase activity, and the enzyme was obtained as a soluble protein from high-speed supernatant. In all cell lines tested, the soluble enzyme had no sharp pH optimum; the activities over a pH range from 5.5 to 8.5 were nearly constant.

The insoluble adenosine deaminase(s) tended to be associated with particulate zonal fractions that were rich in plasma-membrane fragments. Table 2 shows the results of separating fragmented neonatal astrocyte cells on continuous and discontinuous sucrose gradients. There was a moderate enrichment of particulate adenosine deaminase in those fractions that also showed an enrichment of 5'-nucleotidase, a presumptive plasma-membrane marker enzyme. The specific activity of the soluble supernatants from various cell lines was 50–100-fold higher than that of the residual high-speed pellets. Except for the soluble enzyme obtained from the Cox Clone (human grade II–III astrocytoma), the activity of the enzymes (mol/unit time per mg of protein) from the other brain-derived cell lines was very similar (Fig. 3).

The kinetic constants that we have obtained from various cell lines were approximations. The data varied somewhat with the age and cell density of the cultures. They were in the range of K_m values for adenosine from 0.045 to 0.15 mM and V_{max} values from 35 to 150 nmol/min per mg of protein. An example of an estimate of kinetic constants of the soluble adenosine deaminase from the Cox Clone by the direct linear plot is shown on Fig. 4.

Fig. 4. Graphic estimate (Eisenthal & Cornish-Bowden, 1974) of kinetic constants of soluble adenosine deaminase obtained by high-speed centrifugation from sonicates of Cox Clone (human astrocytoma) monolayer cultures

Incubation was for 10 min at 37°C, pH 7.4, in the presence of 27 μ g of protein and an incubation volume of 1.0 ml; the optical assay was used. $K_m \sim 48 \mu M$, $V_{max} \sim 0.13 \mu mol/min$ per mg of protein.

Discussion

We developed an adenosine deaminase assay based on a radioactive substrate because of the limitations of the spectrophotometric method. In the meantime, an assay was described by Pull & McIlwain (1974) which was very similar to the one that we have used. Our results with brain-cell tissue-culture lines, using both the optical and the radioisotopic assay, were similar to those obtained by Pull & McIlwain (1974) with the rat cerebral cortex.

We investigated adenosine deaminase with the possibility in mind that this enzyme was a terminal link in a chain of ecto-enzymes that catabolized translocated ATP (Trams, 1974). Our observations on adenosine metabolism in superfusates of monolayer cultures did not eliminate the existence of an ecto-adenosine deaminase. On a quantitative basis, however, the deamination of exogenous adenosine was predominantly catalysed by a soluble cytoplasmic enzyme. In intact cells (and presumably in tissue slices) the rate of adenosine deamination is probably limited by the uptake rate. Considering that the

superfusate volume exceeded that of the cell cultures by a factor of 50 or more, the uptake of adenosine by the cytoplasmic compartment was very rapid indeed. We assume that, at least in part, adenosine was transferred from the superfusate into the cytosol by facilitated diffusion (Plagemann, 1971). In the absence of adenosine accumulation in the cytoplasm and because of rapid conversion into ATP, we conclude that the brain-derived cells possess an efficient nucleoside salvage pathway.

Pull & McIlwain (1974) had been unable to determine what the distribution of adenosine deaminase was among different cell types of the brain. Our data suggest an ubiquitous distribution. Pull & McIlwain (1974) also had proposed that the particulate adenosine deaminase present in crude mitochondrial fractions of brain was of synaptosomal origin. We found particulate enzyme in cells of glial origin as well (neonatal astrocyte, astrocytoma, oligodendroglia), and we propose the plasma membrane as an alternative source for particulate adenosine deaminase. The presence of a plasma-membrane adenosine deaminase, though of lower specific activity than the cytoplasmic enzyme, could be a corollary to a postulated adenosine receptor (Huang & Daly, 1974). The term 'receptor', of course, has the implied meaning of chemical transmission and a substantial body of information now points to adenosine and its derivatives as neurohumoral transmitters.

One of the most comprehensive studies actually implicates ATP as an inhibitory transmitter in what is generally referred to as the 'purinergic nerves' (Burnstock, 1972). The role of adenosine, however, is less clearly defined. It may modulate the response of the second messenger system by either stimulating cyclic AMP synthesis (Sattin & Rall, 1970; McIlwain, 1972b; Blume *et al.*, 1973; Huang & Daly, 1974), or by inhibiting adenylate cyclase (Fain *et al.*, 1972; McKenzie & Bär, 1973). Adenosine was reported to have a powerful depressant effect on single cortical neurons (Kostopoulos *et al.*, 1975) and a possible hypnogenic role has been ascribed to it (Haulíca *et al.*, 1973). It can act as a potent vasodilator (Berne, 1963) and also as a vasoconstrictor (Thomas *et al.*, 1975). The mode of action of adenosine as a metabolic mediator, agonist or pharmacological agent, however, is still poorly understood. Many effects that have been observed are seemingly unrelated. For instance, the intraperitoneal injection of 200 mg of adenosine/kg produced in rats an eightfold increase in liver glycogen synthetase activity (Chagoya De Sanchez *et al.*, 1974) and a 23-fold increase in liver glycogen turnover (Chagoya De Sanchez & Piña, 1972). The mechanisms by which adenosine deaminase stimulated lipolysis in fat-cells (Schwabe & Ebert, 1974) and the reason why patients with severe combined immunodeficiency exhibit the phenomenon

of adenosine deaminase deficiency in their erythrocytes and serum (Giblett *et al.*, 1972; Parkman *et al.*, 1975) remain equally unexplained yet. The overall impression is that adenosine and the adenine nucleotides play a general role in cellular excitability. A key to their mode of action may be found in further studies of their specific effects on particularly excitable tissues, such as the brain or the heart.

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