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Distribution of Adenosine Deaminase Activity in Rat Brain and Spinal Cord

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The activity of adenosine deaminase (ADA) was measured in 62 discrete regions of the CNS, and in some autonomic and sensory ganglia, peripheral nerves, and peripheral tissues of the rat using an automated high-pressure liquid chromatography (HPLC) method. The formation of inosine and hypoxanthine as a measure of ADA activity in homogenates of brain was optimal at pH 7.0, linear for up to 60 min at 37°C using 500 μM adenosine as substrate, and linear with protein concentrations ranging from 0.05 to 0.8 mg. The K_m and V_{max} values for ADA activity in homogenates of whole brain were 47 μM and 107 nmol/mg protein/30 min, respectively. Among the CNS regions examined, the highest activity was found in posterior hypothalamic magnocellular nuclei and the lowest in hippocampus. In general, spinal cord contained relatively low levels of ADA activity, with that in dorsal cord approximately 40% higher than ventral cord. In the periphery, parasympathetic ganglia contained higher levels of ADA than sensory ganglia and brain. Most peripheral tissues—including adrenal gland, lung, liver, and anterior and posterior pituitary—exhibited activity comparable to levels in the posterior hypothalamus. ADA activity in thymus was about 10 times higher than any other tissue examined.

The uneven distribution of ADA activity in the rat CNS corresponds well with the immunohistochemical localization of this enzyme in discrete neural systems of this species. Structures that contain high ADA activity exhibit intense ADA immunostaining of neuronal perikarya and/or fibers. The 13-fold difference between brain regions containing the highest and lowest ADA activity suggests that, in addition to its involvement in intermediary metabolism, this enzyme may have an important role in regulating the putative neuromodulatory actions of adenosine in the CNS.

Adenosine deaminase (ADA; Adenosine aminohydrolase, EC 3.5.4.4) is responsible for the deamination of adenosine to the physiologically less active compound inosine. Various forms of this cytosolic enzyme have been identified with molecular weights of 36,000, 114,000, and 298,000 (Van der Weyden and Kelley, 1976). By complexing with a membrane-bound binding protein, low-molecular-weight ADA is convertible to the large form of the enzyme. The binding protein does not itself express enzymatic activity, and its physiological function remains unclear (Andy and Kornfeld, 1982). Several lines of investigations on the cellular metabolism of purines in peripheral tissues have generated considerable interest in ADA. The recognition that elevated levels of adenosine and deoxyadenosine are cytotoxic to certain cell types suggests an important contribution by ADA to the maintenance of appropriate cellular adenine nucleoside and nucleotide concentrations (Fox and Kelley, 1978). The discovery that some patients with severe combined immunodeficiency disease (SCID) are genetically deficient in ADA activity points to a crucial role of this enzyme in cells of the immune system (Daddona et al., 1983; Giblett et al., 1972). The use of adenine nucleoside analogs as chemotherapeutic agents has led to the development of potent ADA inhibitors, which, given in conjunction with these agents, prevent their deamination and prolong their effectiveness (Agarwal et al., 1975). The availability of such inhibitors together with the consequences of ADA deficiency in patients suffering from SCID prompted clinical trials of these inhibitors as treatments for certain forms of leukemia (Major et al., 1981).

Our interest in the function of ADA in neural tissue was kindled by the recent flood of reports supporting the notion that adenosine serves in a neuromodulatory capacity in both the PNS and CNS (Phillis and Wu, 1981; Stone, 1981). Indirect evidence suggests that ADA may have a role analagous to other catabolic enzymes that participate in the regulation of both synaptoplasmic levels and the extracellular actions of transmitter substances. Thus, the depressive electrophysiological actions of adenosine when applied to central neurons are potentiated by the concomitant application of potent ADA inhibitors such as 2′-deoxycoformycin (DCF) or erythro-9-(2-hydroxy-3-nonyl) adenine and mimicked by the application of these inhibitors alone (Phillis and Wu, 1981; Phillis et al., 1979). Moreover, ADA inhibitors administered to animals produce behavioral effects similar to those of adenosine agonists and cause physiological responses that are thought to be mediated through the action of adenosine on its receptors (Phillis et al., 1984, 1985; Radulovacki et al., 1983). Anatomical evidence for a relationship between ADA and the neuromodulatory actions of adenosine is derived from recent immunohistochemical demonstrations of the uneven distribution of ADA-immunoreactive neurons in discrete neural systems in the rat CNS (Nagy and Daddona, 1985; Nagy et al., 1984a, b, 1985). Among these were ADA-containing preganglionic parasympathetic neurons (Senba et al., 1985a) which in the cat have been shown to have an adenosine neurotransmitter component (Akasu et al., 1984). Moreover, structures immunoreactive for ADA in rat brain were found to correspond closely to those exhibiting the greatest density of binding sites for ^{3}H-nitrobenzylthioinosine (^{3}H-NBI) (Nagy et al., 1984a, b), a putative ligand for adenosine uptake sites.

The above findings, together with the now established concept that adenosine acts as a neuromodulator and/or neurotrans-

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mitter in CNS and some peripheral systems, warrant investigations aimed at elucidating the biochemical pathways governing the metabolism of adenosine in neural tissue. As a first step towards this goal we analyzed the characteristics and regional distribution of ADA in the rat CNS.

Materials and Methods

Tissue dissections

Male Sprague-Dawley rats weighing approximately 300 g were sacrificed by decapitation. The brain, spinal cord, and peripheral tissues were removed, placed on an ice-cold metal tray, and dissected freehand or from thick frozen sections as previously described (Geiger and Nagy, 1984, 1985), unless otherwise indicated.

Cortical areas were subdivided according to the atlas of Paxinos and Watson (1982), and all other areas were dissected according to the atlas of Koning and Klippel (1963). No attempt was made to dissect specific subnuclei of the thalamus other than medial and lateral geniculate nuclei. The rest of the thalamus was divided into anterior and posterior regions at a rostrocaudal level demarcated by the anterior border of the habenula, and each of these regions was subdivided into medial and lateral areas. The anterior thalamic regions contained nucleus medialis, parstenialis, anterior medialis, rhomboideus, and reuniens medially, and the remaining nuclei laterally. The posterior thalamic regions contained the caudal extensions of these nuclei as well as nucleus posteriormedianus, ventralis medialis, and parafascicularis medially; and nucleus posterior, posterior lateralis, and pretectalis laterally.

The hypothalamus was excised from whole brain and taken for assay as such or divided into anterior and posterior regions containing all of the preoptic–anterior hypothalamic areas and posterior hypothalamic nuclei, respectively. In addition, frozen sections of whole brain were used to further subdivide these regions into medial and lateral preoptic and anterior hypothalamic areas. The posterior hypothalamic magnocellular nuclei consisting of the tuberal, caudal, and postmammillary caudal magnocellular cell groups were dissected from more posterior sections. Samples of habenula contained both its medial and lateral subnuclei. Samples of reticular formation were obtained at the midbrain level. The hippocampal formation was dissected according to its CA1, CA2, CA3, and dentate gyrus subfields or with respect to its septotemporal axis. In the latter dissection, the hippocampus was cut into 6 segments at right angles to its long axis. Tissue samples from the septal pole progressively to the temporal pole were designated segments 1–6, respectively. All tissues for the regional analyses were immediately frozen on dry ice following dissection and stored at −80°C until taken for assay. The dissection time was always less than 15 min.

Adenosine deaminase assay

The assay method of Hartwick et al. (1978) was adapted for measurement of ADA activity. For characterization of the assay, experiments were conducted using either fresh whole brains or previously frozen CNS or peripheral tissues. Samples were homogenized with a Polytron (setting 6, 10 sec) in ice-cold Tris buffer (pH 7.0, 50 mM) containing 10 mM $MgCl_2$. It was found that for dorsal and ventral roots and ganglia, preincubation for 30 min at 37°C with 10 units of collagenase (Type VII, Sigma Chem. Co., St. Louis, MO) was necessary to ensure disruption of connective tissue and uniform homogenization. The collagenase did not itself affect ADA activity or protein measurements. Homogenate sufficient for measurable ADA activity but that did not produce greater than 20% substrate depletion was added to reaction vessels (250 μl microcentrifuge tubes) containing 500 μM adenosine in a total volume of 100 μl Tris-$MgCl_2$ buffer. Following incubation for 30 min at 37°C, the reaction was stopped with 10 μl of 20% trichloroacetic acid (TCA) and the incubation tubes centrifuged at 14,000 × *g* for 2 min. The reaction supernatant was transferred to microcentrifuge tubes, neutralized with a tri-*n*-octylamine : freon mixture (55:145, vol/vol), agitated briefly using a vortex mixer, and recentrifuged. Filtration of the final aqueous phase through 0.2 μm filters to remove debris that might interfere with the HPLC analysis was found to be unnecessary since over 5000 sample injections were accomplished before deterioration of the HPLC column occurred.

The aqueous layer (top) containing the compounds to be measured was chromatographed using a Waters HPLC system consisting of a WISP 710B autosampler, Automated Gradient Controller, 510 pump, 441 UV detector with a 254 nm filter, and a Shimadzu C-R3A plotter/printer/integrator. The mobile phase consisting of 88% KH_2PO_4 (0.01 M, pH 5.0) and 12% methanol was filtered through a 0.45 μm filter, degassed under reduced pressure, and pumped at 1.5 ml/min through a reverse-phase C_{18} analytical column (Spheri-5, OD-MP, 10 cm, Brownlee Lab., Santa Clara, CA) fitted with a 2.5 cm guard column of the same material. This solvent was not recycled. Unless otherwise indicated these conditions were used throughout the present investigations. Injection volumes of 25 μl were chromatographed every 5 min. Chromatographic peaks were identified according to retention times of standards containing inosine, hypoxanthine, and adenosine. The identity of the peaks was verified by incubating samples of adenosine with adenosine deaminase (Type VII, Sigma) or inosine with purine nucleoside phosphorylase (Sigma). Standard concentrations of hypoxanthine, inosine, and adenosine were used to calibrate the integrator at the beginning of every set of assays. Calibration was rechecked midway through each assay and at the end. ADA activity was determined as nmol of product (inosine plus hypoxanthine) formed/mg protein/30 min rather than as adenosine utilized since product formation was found to be a more sensitive and reproducible measure of enzyme activity. Depending on the amount of tissue used, however, adenosine utilization was found to equal inosine plus hypoxanthine formation using the assay conditions routinely employed. Assay blanks were calculated from samples in which endogenous levels of adenosine, inosine, and hypoxanthine were measured by adding tissue homogenates to reaction vessels already containing 20% TCA. For each brain region and peripheral tissue assayed, reaction blanks were subtracted from the levels of products formed after incubation with 500 μM adenosine. Protein was measured according to the method of Lowry et al. (1951) using BSA as standard.

Perfused versus nonperfused brain regions

The contribution of ADA activity in red blood cells to that in brain tissue was determined by measuring the activity in 6 brain regions (hypothalamus, superior colliculus, cerebellum, cerebral cortex, striatum, and hippocampus) taken from 8 animals that were either perfused transcardially with physiological saline or decapitated without perfusion. All 16 animals were anesthetized with chloral hydrate and 8 were perfused with 50 ml 0.9% saline at room temperature over a period of 2 min.

Optimization of ADA assay

The establishment of the HPLC assay and characterization of ADA in CNS tissue required determination of the following: (1) completeness of purine separation under optimal HPLC conditions; (2) linearity with which the HPLC procedure detected purines; (3) efficiency of purine recovery from tissue homogenates; (4) effect of the detergent Triton X-100 on extraction of ADA activity during the homogenization procedure; (5) optimal substrate (adenosine) concentration and K_m and V_{max} values; (6) linearity of enzyme activity with respect to protein concentration in homogenates; and (7) optimal pH of the enzyme.

Chromatography of hypoxanthine, inosine, and adenosine was examined over the concentration range of 0.1–16.6 nmol (5.2–667 μM). The linearity of UV detection was determined by comparing the known concentrations chromatographed with the integrated areas under the identified peaks using least-squares regression analyses. The efficiency of purine extraction from assay examples was determined for whole-brain homogenates after inactivation of enzymatic activity with 20% TCA. Hypoxanthine, inosine, and adenosine at 500 μM concentrations were added to these inactivated samples, which were then neutralized and chromatographed. The peak areas were compared with those of standards of the same concentration, and the extraction efficiency was incorporated into all subsequent calculations.

Homogenization of brain tissue in the presence, as compared with the absence, of Triton X-100 has been found to enhance the activity of ADA (Pull and McIlwain, 1974). The effect of up to 2% Triton X-100 on ADA activity was therefore tested using the present assay incubation and homogenization conditions. Whole brain—as well as cerebellum, hypothalamus, hippocampus, midbrain, and thalamus—was homogenized with and without Triton X-100 using a Polytron and processed as described above. The brain subregions were tested in order to assess whether the detergent treatment had differential effects on ADA activity in brain regions previously reported to contain ADA-immunostaining of primarily perikarya or in those densely innervated by ADA-immunoreactive fibers (Nagy et al., 1984a).

The optimal substrate concentration for ADA was determined in brain by incubating homogenates containing 15.6, 31.2, 62.5, 125.0,

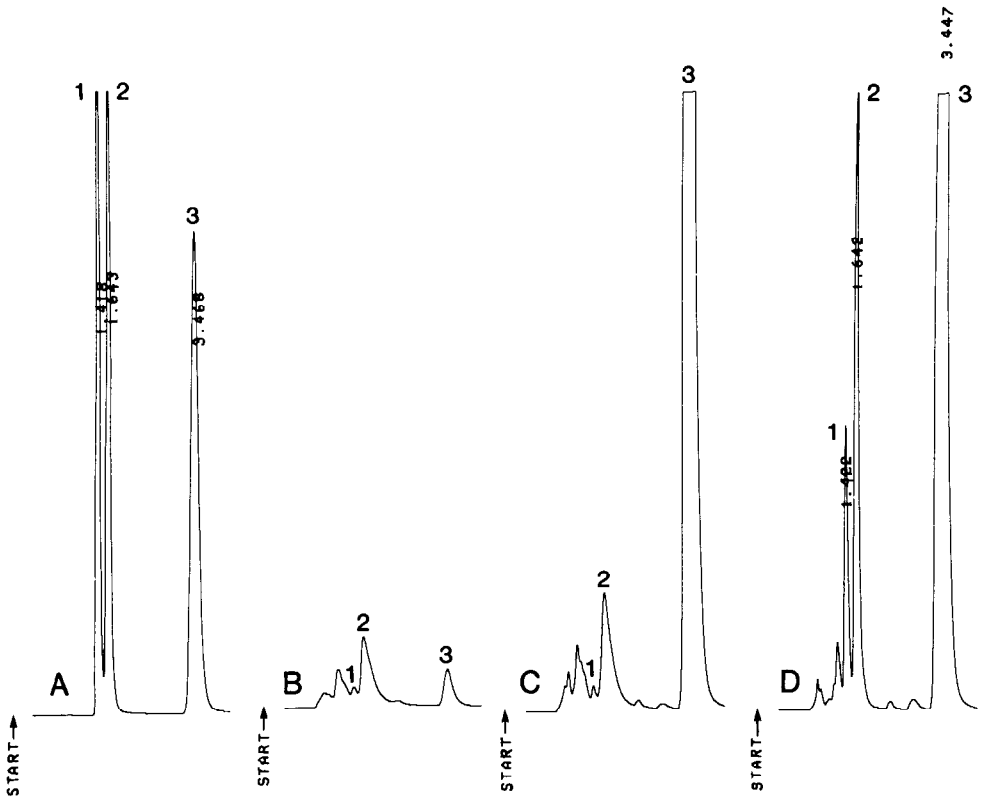

Figure 1. Series of chromatograms illustrating the HPLC separation of hypoxanthine (*1*), inosine (*2*), and adenosine (*3*) under 4 different conditions. *A*, Solution of 2.1 nmol (83 μM) of standards; *B*, sample of endogenous levels in brain homogenate; *C*, sample of endogenous levels in brain homogenate plus 12.5 nmol (500 μM) of adenosine; *D*, levels following incubation of brain homogenate with 500 μM adenosine at 37°C for 30 min. The Shimadzu C-R3A plotter was run at 10 mm/min at an attenuation level of 2^7 mV.

250.0, or 500 μM adenosine for 5, 15, 30, and 60 min. The initial velocities for these reactions were determined graphically, and these data were plotted as reciprocals of velocity (nmol product formed/mg protein/30 min) versus substrate concentration (μM). The K_m and V_{max} values were determined for whole brain, cerebellum, hippocampus, and anterior and posterior hypothalamus using a weighted least-squares analysis version of the Michaelis-Menten equation. The linearity of the assay with respect to enzyme concentration was examined by varying whole-brain homogenate protein content from 0.05 to 0.8 mg in assays containing 500 μM adenosine and adjusting incubation times so that not greater than 20% conversion of substrate occurred. The optimal pH for ADA activity in homogenates of whole brain was established using a "universal" buffer consisting of 20 mM each of sodium acetate, sodium phosphate, and Tris, the pH of which was adjusted to between 3.0 and 11.0 with phosphoric acid or NaOH.

Results

Characterization of the ADA assay

The HPLC separation of purines using the solvent system employed is shown in Figure 1. Adequate separation of endogenous (blank) and assay levels of hypoxanthine, inosine, and adenosine was obtained in homogenates. The retention times (min) were consistently 1.4 for hypoxanthine, 1.6 for inosine, and 3.5 for adenosine. The identity of the chromatographic peaks was confirmed by the metabolism of adenosine to inosine following incubation with purified ADA, and the metabolism of inosine to hypoxanthine by purine nucleoside phosphorylase. Purines were detectable at concentrations as low as 0.1 nmol (5.2 μM) (Fig. 2) and their UV detection was linear to 16.6 nmol (667 μM). The efficiency of adenosine, inosine, and hypoxanthine extraction from homogenates and HPLC measurement of the extract was found to be 91%. The activity of ADA in brain homogenates was linear in the range of 0.05–0.8 mg protein (Fig. 3) and optimal at pH 7.0 (Fig. 4) when incubations were for 30 min at 37°C using 500 μM adenosine (Fig. 5). For every tissue, preliminary studies were conducted to verify that, at the protein concentration required for assay linearity (routinely less than 0.15 mg), a measurable amount of product was formed during the incubation period. All samples were measured in duplicate, and the variability between duplicates was typically

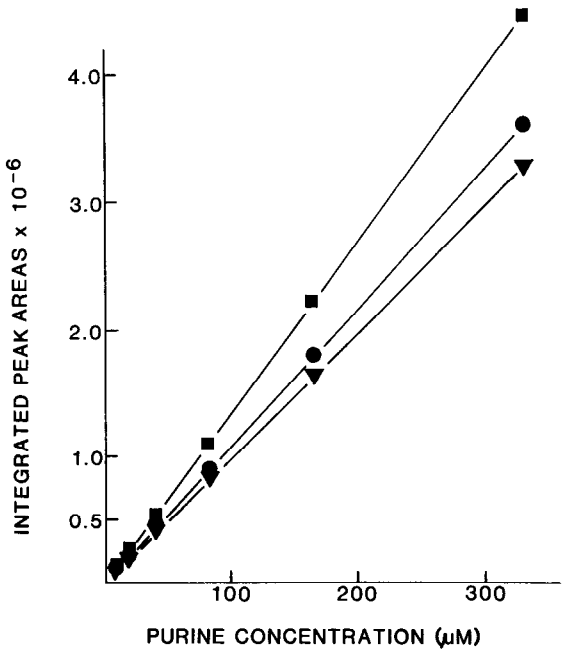

Figure 2. Linear relationship between the chromatography of different concentrations of adenosine (■), inosine (●), and hypoxanthine (▼) and their detection using the described HPLC technique. Illustrated points represent mean values from 3 determinations at each concentration. Variability among the triplicate samples was less than 1% for each purine.

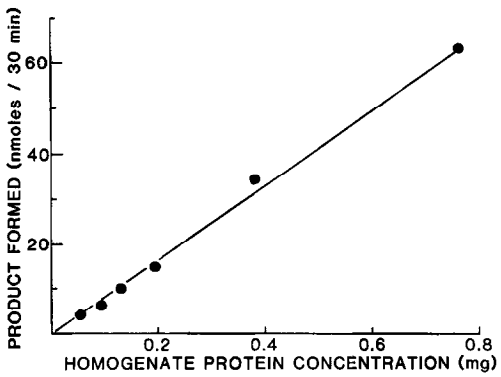

Figure 3. Linearity of ADA activity as a function of protein concentration in brain homogenates. Data points represent mean values of an assay performed in triplicate.

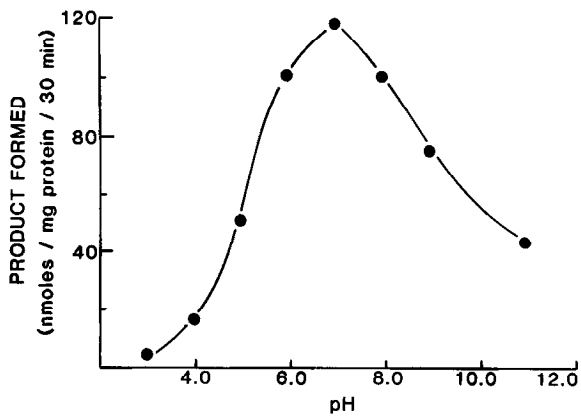

Figure 4. pH dependence of ADA activity in homogenates of whole brain. Values represent 2 separate experiments that yielded similar results.

less than 10%. Preliminary studies showed that freezing the tissue at −80°C for up to 2 weeks had no effect on the levels of ADA activity as compared with those observed in freshly dissected tissue. On rare occasions it was necessary to store the final aqueous layer from the enzymatic assay. Purines in these samples were found to be stable during storage at −20°C for 2–3 d, as indicated by calculations of enzymatic activity. The levels of ADA activity in homogenates of whole brain or of the 5 brain regions examined were not significantly different in the absence or presence of Triton X-100 at concentrations between 0.2 and 2% (vol/vol) in the homogenization medium (data not shown).

The results of ADA activity measurements of 6 brain regions taken from normal or saline-perfused rats are shown in Table 1. Perfusion did not significantly change ADA activity in any area other than cerebral cortex, where a small reduction of 14% was found. Therefore, this procedure was not employed in the subsequent regional analysis.

The derived K_m and V_{max} values taken from 3 independently conducted measures of ADA activity in whole brain, cerebellum, hippocampus, and anterior and posterior hypothalamus homogenates are shown in Table 2. Except for anterior hypothalamus, the K_m values for these tissues were not significantly different. Clearly, the posterior hypothalamus, as reflected by the V_{max} values, contains the highest ADA activity of the brain regions examined.

Regional distribution of ADA activity (Table 3)

Within the forebrain about a 9-fold difference in ADA activity was found between the highest level of 279.2 in olfactory bulbs and the lowest activity of 31.6 nmol/mg protein/30 min in whole hippocampus. Very little variation in ADA activity was noted among the CA1, CA2, CA3, and dentate gyrus subregions of the hippocampus. However, when dissected into segments along its septotemporal axis, ADA activity in the segment at the extreme temporal pole was nearly twice that observed in most of the other more dorsomedial hippocampal areas analyzed. Of the 7 cortical regions examined, the activities ranged from 84.0 in parietal cortex to 39.4 nmol/mg protein/30 min in frontal cortex. The levels in olfactory and entorhinal areas were approximately equal to the level in parietal cortex. The enzymatic activity in the amygdala, septal nuclei, accumbens, and globus pallidus was roughly equal to 60 nmol/mg protein/30 min. Slightly lower activity was found in whole striatum, irrespective of whether the head, tail, or whole region was taken for assay. Corpus callosum was among the CNS areas containing the lowest levels of ADA activity.

In the diencephalon, a high level of activity was observed in hypothalamus; the posterior aspects and the posterior magnocellular nuclei, in particular, contained the highest ADA activity

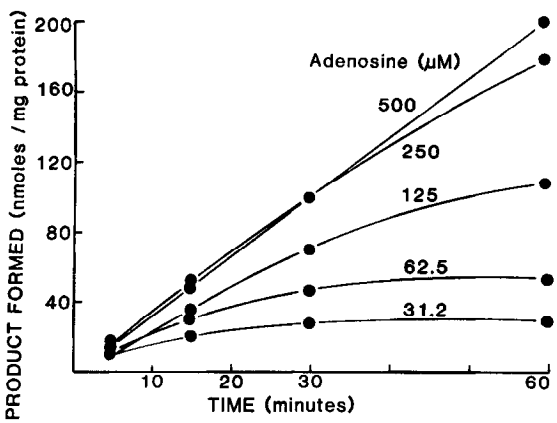

Figure 5. ADA activity in whole-brain homogenates incubated with adenosine concentrations of 31.2, 62.5, 125, 250, or 500 μM for the time periods indicated. Each point is the mean of triplicate determinations, and the values are representative of 2 separate experiments.

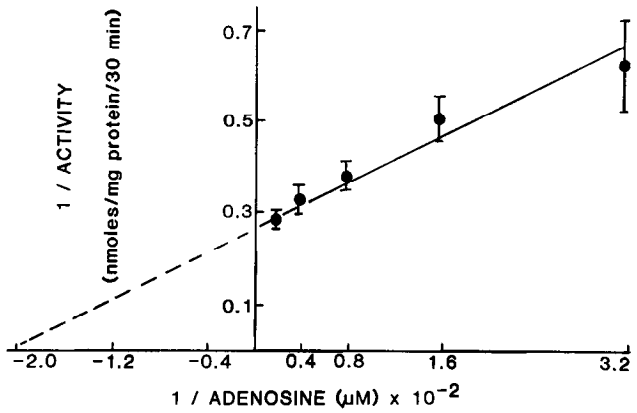

Figure 6. Double-reciprocal plot of ADA activity in whole-brain homogenates versus adenosine concentration. Incubations were for 30 min at 37°C using 0.3 mg homogenate protein. Values represent means ± SEM of 3 separate experiments. Regression line was drawn according to a weighted least-squares analysis.

Table 1. Effect of animal perfusion on adenosine deaminase activity in discrete brain regions

Brain region	Adenosine deaminase activity (nmol product formed/mg protein/30 min) Nonperfused	Perfused
Hypothalamus	277.6 ± 14.0 (6)	290.2 ± 8.0 (6)
Superior colliculus	142.0 ± 9.4 (6)	151.6 ± 22.0 (6)
Cerebellum	59.4 ± 1.8 (8)	66.2 ± 3.2 (8)
Cortex	56.0 ± 2.4 (6)	48.4 ± 2.2 (6)[a]
Striatum	39.6 ± 3.2 (8)	42.2 ± 2.6 (8)
Hippocampus	39.2 ± 2.8 (8)	39.6 ± 2.4 (8)

Brains were obtained from rats anesthetized with chloral hydrate and sacrificed by decapitation prior to or following perfusion transcardially with 50 ml of 0.9% NaCl (22°C). Values represent means ± SEM of the number of animals indicated in parentheses. Statistical analyses were conducted using a 2-tailed Student's t test.
[a] $p < 0.05$.

in brain, with the exception of choroid plexus. The heterogeneity of ADA activity in hypothalamus was further indicated by the 4-fold difference between levels in the lateral portion of the preoptic area and the posterior magnocellular nuclei. The level in habenula was about 50% higher than that in the thalamus, where activity ranged from 51.4 in the anterior lateral region to 86.8 nmol/mg protein/30 min in the lateral geniculate nucleus.

In the mesencephalon and brain stem, the highest ADA activity—147.2 nmol/mg protein/30 min—was found in superior colliculus, within which the superficial layers had nearly twice the activity of deeper collicular layers. The lowest activity in the mesencephalon, nearly 4 times lower than superficial layers of superior colliculus, was observed in the inferior colliculus. The rest of the tissues examined in the mesencephalon and brain stem had ADA levels ranging from 104.4 in the substantia nigra to 64.0 nmol/mg protein/30 min in cerebellum.

In spinal cord, the ADA levels in dorsal halves were consistently about 40% higher than levels in ventral halves at all rostrocaudal regions examined, including cervical, thoracic, and lumbar segments. The activity in lumbar white matter, 34.6 nmol/mg protein/30 min, was substantially less than either lumbar dorsal or ventral gray matter. The activity in dorsal and ventral roots was about equal at 133.8 and 116.0 nmol/mg protein/30 min, respectively. The parasympathetic sphenopalatine ganglia demonstrated an activity of 834 nmol/mg protein/30 min, which was twice that of the sympathetic superior cervical ganglia, 40% higher than pelvic ganglia, and 2–4 times higher than sensory ganglia. The ADA activity in optic, sciatic, saphenous, and vagus nerves was fairly uniform, ranging from 255.8 to 276.0 nmol/mg protein/30 min. Except for the very high activity in thymus (4200 nmol/mg protein/30 min), the other peripheral tissues examined contained ADA activity ranging from 211.4 for anterior pituitary to 1450 nmol/mg protein/30 min for tongue epithelium.

Discussion

ADA activity in brain homogenates was maximum at pH 7.0, a result similar to that previously reported (Arch and Newsholme, 1978; Mustafa and Tewari, 1970; Van der Weyden and Kelley, 1976). In the present study, treatment with 0.2–2.0% Triton X-100 did not affect levels of ADA activity. This finding is in agreement with reports by Arch and Newsholme (1978) and Van der Weyden and Kelley (1976), but not with the findings that homogenization of CNS tissue with 2% Triton X-100 augmented ADA activity by 48% (Mustafa, 1972; Pull and McIlwain, 1974). Therefore, it appears that the more vigorous homogenization using a Polytron as performed here obviates the need for treatment with this detergent. The K_m and V_{max} values for ADA activity in homogenates of whole brain were 47 μM and 107 nmol/mg protein/30 min, respectively. Previously reported K_m values for CNS tissue included 17, 34, and 100 μM for whole brain and 54–57 μM for cerebral cortex (Arch and Newsholme, 1978; Phillips and Newsholme, 1979; Pull and McIlwain, 1974; Skolnick et al., 1978). These same authors reported V_{max} values of 174, 54, 115, and 105 nmol/min/g wet weight tissue, respectively. Our calculated V_{max} value for whole brain was 308.2 nmol/mg tissue/min. Thus, the K_m and V_{max} values obtained here are similar to those previously reported for brain and agree well with the K_m values reported for peripheral tissues, which ranged from 24 to 65 μM in rat liver, heart, and gastrocnemius muscle (Arch and Newsholme, 1978); human mononuclear white blood cells (Morisaki et al., 1985); and bovine skeletal muscle (Martinez et al., 1984). In the present study, the K_m value for whole brain was not significantly different from that of the individual brain regions examined except for anterior hypothalamus, where it was found to be lower. Although repeatable, the reason for this difference is presently unclear, but it may be related to the very high density of axons as compared with cell bodies immunoreactive for ADA in the anterior hypothalamus; the kinetic characteristics of ADA may be dependent on its cellular localization. In this context, it should be noted that V_{max} values, where determined, were very similar to the levels of ADA activity measured using a single concentration (500 μM) of adenosine.

Table 2. Kinetic parameters of ADA activity in rat brain

Region	Adenosine deaminase activity K_m (μM)	V_{max} (nmol/mg protein/30 min)
Whole brain	46.9 ± 8.1	106.8 ± 6.2
Cerebellum	32.6 ± 8.1	66.6 ± 6.0
Hippocampus	39.1 ± 2.4	35.8 ± 8.0
Anterior hypothalamus	24.4 ± 4.9	180.4 ± 12.0
Posterior hypothalamus	37.6 ± 4.5	477.8 ± 27.6

Values represent means ± SEM of 3 determinations.

The present results concerning the distribution of ADA activity in the rat CNS are in general agreement with the results of Phillips and Newsholme (1979), who examined this enzyme in 9 regions of human brain and found that hypothalamus contained nearly an order of magnitude greater activity than hindbrain, which contained the lowest activity. These results, however, are in marked contrast to the regional activities reported by several others (Davies and Taylor, 1979; Norstrand et al., 1984; Sun et al., 1976; Van der Weyden and Kelley, 1976). Sun et al. (1976) and Davies and Taylor (1979) examined brain regions in the rat and found ADA activity in the hypothalamus to be about 2 times higher than in striatum or neocortex. Van der Weyden and Kelley (1976) reported 3 times higher activity in human cerebrum than in cerebellum or spinal cord. Norstrand et al. (1984) found that white matter in frontal, orbital, and temporal cortical regions in human brain contained the highest ADA activity, with a 9-fold difference noted between these areas and spinal cord, the area with the lowest activity. They also reported relatively high levels of activity in the hippocampus compared with somewhat lower levels in the hypothalamus. These disparate findings may be due to species variations in ADA activity or differences in dissection procedures or assay methodologies. For example, Norstrand et al. (1984) employed a 1200 × g supernatant as the source of ADA from human brain tissue, calculated activities on the basis of wet weight tissue, and measured ADA activity indirectly by quantitation of ammonia formation.

Table 3. Distribution of adenosine deaminase activity in rat brain and spinal cord

Area	Adenosine deaminase activity (nmol/mg protein/30 min)	
Telencephalon		
Olfactory bulb	279.2 ± 13.6	(6)
Olfactory cortex	77.8 ± 4.0	(6)
Entorhinal cortex	80.8 ± 16.8	(4)
Frontal cortex	39.4 ± 4.8	(4)
Cingulate cortex	67.0 ± 4.0	(4)
Temporal cortex	56.6 ± 7.6	(4)
Striate cortex	58.8 ± 6.8	(4)
Parietal cortex (sensory, motor)	84.0 ± 11.0	(4)
Amygdala	61.4 ± 6.6	(6)
Septal nuclei	72.2 ± 3.4	(6)
Hippocampus (whole)	31.6 ± 1.6	(6)
CA1	40.4 ± 4.8	(5)
CA2	33.6 ± 2.8	(5)
CA3	35.2 ± 3.0	(5)
Dentate gyrus	31.8 ± 2.0	(5)
Septal pole		
Segment 1	44.2 ± 3.0	(5)
Segment 2	34.0 ± 1.0	(5)
Segment 3	35.6 ± 1.6	(5)
Segment 4	34.4 ± 1.0	(5)
Segment 5	39.6 ± 1.8	(5)
Temporal pole, segment 6	63.2 ± 2.8	(5)
Accumbens	60.2 ± 3.0	(6)
Striatum (whole)	37.2 ± 2.0	(10)
Head of striatum	40.2 ± 2.0	(5)
Tail of striatum	48.2 ± 2.4	(5)
Globus pallidus	60.6 ± 4.4	(5)
Bed nucleus of stria terminalis	83.2 ± 2.6	(5)
Corpus callosum	38.8 ± 2.4	(6)
Diencephalon		
Thalamus (whole)	61.4 ± 3.4	(7)
Anterior medial	74.6 ± 5.6	(5)
Anterior lateral	51.4 ± 2.8	(5)
Posterior medial	80.4 ± 2.6	(4)
Posterior lateral	66.6 ± 1.6	(4)
Lateral geniculate nucleus	86.8 ± 3.8	(5)
Medial geniculate nucleus	85.0 ± 5.6	(5)
Hypothalamus (whole)	207.8 ± 11.4	(11)
Anterior half	168.0 ± 12.6	(5)
Posterior half	390.6 ± 9.6	(5)
Preoptic area, medial	138.2 ± 5.6	(5)
Preoptic area, lateral	98.0 ± 6.6	(5)
Anterior hypothalamic area, medial	134.6 ± 7.8	(4)
Anterior hypothalmic area, lateral	125.0 ± 13.2	(4)
Posterior magnocellular nuclei	420.4 ± 52.0	(4)
Habenula	122.0 ± 6.8	(5)
Mesencephalon		
Midbrain (whole)	70.2 ± 3.6	(5)
Superior colliculus (whole)	147.2 ± 7.4	(9)
Superficial layers	181.0 ± 13.6	(6)
Deep layers	106.4 ± 6.4	(6)
Inferior colliculus	52.8 ± 3.2	(6)
Periaquaductal gray	92.0 ± 4.6	(6)
Substantia nigra	104.4 ± 4.0	(5)
Reticular formation	82.4 ± 7.8	(6)
Brain stem		
Pons	71.2 ± 6.6	(8)
Medulla	94.8 ± 7.2	(8)
Cerebellum	64.0 ± 3.2	(9)
Choroid plexus	466.2 ± 10.0	(4)
Spinal cord		
Spinal cord (whole)	42.0 ± 2.8	(6)
Cervical		
Dorsal	83.2 ± 2.8	(6)
Ventral	58.8 ± 4.2	(6)
Thoracic		
Dorsal	96.6 ± 9.0	(6)
Ventral	62.8 ± 8.6	(6)
Lumbar		
Dorsal	103.2 ± 7.8	(6)
Ventral	73.8 ± 5.6	(5)
Dorsal gray	103.6 ± 7.2	(5)
Ventral gray	72.2 ± 6.2	(5)
White matter	34.6 ± 2.6	(5)
Dorsal roots	133.8 ± 24.0	(6)
Ventral roots	116.0 ± 11.4	(5)
Ganglia		
Sensory		
Trigeminal	218.8 ± 18.2	(6)
Lumbar	394.6 ± 24.0	(6)
Parasympathetic		
Sphenopalatine	834.0 ± 112.0	(7)
Pelvic	600.0 ± 38.0	(7)
Sympathetic		
Superior cervical	407.6 ± 21.8	(5)
Nerves		
Optic	255.8 ± 28.4	(5)
Sciatic	276.0 ± 34.6	(6)
Saphenous	250.8 ± 40.6	(5)
Vagus	450.8 ± 40.6	(5)
Peripheral tissues		
Retina	80.6 ± 3.6	(8)
Anterior pituitary	211.4 ± 10.8	(10)
Posterior pituitary	302.8 ± 18.2	(5)
Pineal gland	350.4 ± 23.0	(4)
Adrenal gland	460.4 ± 21.2	(4)
Lung	492.2 ± 14.8	(8)
Liver	337.0 ± 22.0	(7)
Epithelium (tongue)	1450.0 ± 79.0	(6)
Thymus	4200.0 ± 268.0	(7)

Values represent means ± SEM of the number of determinations indicated in parentheses.

Interpretation of the possible functional significance of the markedly heterogeneous distribution of ADA activity within the CNS and among peripheral tissues requires consideration of the role of ADA in the intracellular metabolism of adenosine.

Additionally, it necessitates comparison of the present results with previous reports on the immunohistochemical localization of ADA in the CNS and the distribution of ^{3}H-NBI binding to putative nucleoside transport sites in brain.

Observations that high concentrations of adenosine are cytotoxic indicate the requirement to maintain subtoxic levels of this nucleoside (Fox and Kelley, 1978). Intracellularly, adenosine can be incorporated into *S*-adenosylhomocysteine via the enzyme *S*-adenosylhomocysteine hydrolase (AH), phosphorylated by adenosine kinase (AK) to 5'-AMP, or deaminated by ADA. Although AK has a higher affinity than ADA for adenosine, under basal conditions the enzyme appears to be close to saturation (Arch and Newsholme, 1978). Also, the formation of *S*-adenosylhomocysteine has been shown to be only a minor product in brain slices even after they are exposed to relatively high concentrations of adenosine (Reddington and Pusch, 1983). Therefore, ADA may play a greater role than AK or AH in regulating adenosine concentrations under conditions where adenosine levels are markedly increased. This notion is supported by reports showing that seizure activity in animals or exposure of CNS tissues to depolarization or hypoxic conditions causes marked increases in inosine and hypoxanthine levels with little change in 5'-AMP levels (Daval and Barberis, 1981; Lewin and Bleck, 1981, 1983).

In the present study, a heterogeneous distribution of ADA activity was found within the CNS and among peripheral tissues examined, and particularly high activities were observed in discrete and diverse regions of the brain. For CNS tissues, the highest levels were found in the choroid plexus, posterior hypothalamic magnocellular nuclei, olfactory bulbs, and superficial layers of superior colliculus. The lowest levels were in subregions of the hippocampus and the corpus callosum. In spinal cord, the activity was relatively low, with that in dorsal regions significantly greater than in ventral regions. In contrast, the activity in ganglia, nerves, and other tissues was higher than that observed in CNS tissues, with thymus clearly containing the highest ADA activity of any tissue examined. This latter finding is in concert with previous reports (Chechik et al., 1983). Additionally, the ADA levels reported in rat liver and lung were similar to those previously reported for human tissue (Van der Weyden and Kelley, 1976). Consistent with studies on the immunohistochemical localization of ADA in rat CNS (Nagy and Daddona, 1985; Nagy et al., 1984, 1985), the regions that contain ADA-immunoreactive perikarya or fibers also contain the highest activity of ADA. This is especially true for the posterior basal region of the hypothalamus, where immunoreactive cell bodies are found in the tuberal, caudal, and postmammillary caudal magnocellular nuclei, and in the anterior hypothalamus, where ADA-immunostaining of fibers is particularly dense in medial areas. The relatively higher activities in superficial layers of the superior colliculus, habenula, olfactory bulbs, and dorsal as compared with ventral spinal cord, is also consistent with observations of intense ADA immunostaining of fibers and/or cell bodies in these areas. The greater ADA activity in the temporal compared with the septal pole of the hippocampus coincides with findings that ADA-immunostained fibers are more abundant in the former than the latter region (W. S. Staines and J. I. Nagy, unpublished observations). Due to limitations imposed by dissection techniques, however, high ADA activity in very small nuclei that contain ADA-immunoreactive elements is not reflected in the present measurements of ADA; in some instances, the samples taken for assay contained a disproportionately greater amount of tissue that lacks or exhibits sparse ADA-immunostaining relative to a constituent subregion within which intense immunoreactivity is observed. It is likely, for example, that the medial habenula, which displays very dense ADA immunostaining of what we suspect to be axons and terminals, contains much greater ADA activity than indicated by the present measurements of whole habenula since ADA-immunoreactive elements in the relatively larger lateral subnucleus are scarce. Similarly, because of its dispersed anatomical configuration (Senba et al., 1985b), it was difficult to dissect posterior hypothalamic magnocellular nuclei from surrounding tissue. The ADA activity in the ADA-immunopositive cell groups comprising this nucleus may be substantially higher than presently reported.

The diverse physiological actions of adenosine are believed to be mediated extracellularly through its receptors. Access of extracellular adenosine to ADA, which appears to be primarily a cytoplasmic enzyme, is controlled mainly by the nucleoside transport system and, to a limited extent, by passive diffusion through membranes (Plagemann and Wohlhueter, 1980; Wu and Phillis, 1984). The nucleoside transport system is potently inhibited by NBI in peripheral tissues, and recently ^{3}H-NBI binding sites have been demonstrated in CNS tissues (Geiger, 1986; Geiger et al., 1985; Hammond and Clanachan, 1984; Marangos et al., 1982). In a previous report, we found a close correspondence between brain areas containing structures immunoreactive for ADA and those containing high levels of ^{3}H-NBI binding sites as demonstrated by autoradiographic and membrane binding methods (Geiger and Nagy, 1984; Nagy et al., 1985). Here, we found a significant correlation of $r = 0.830$ between levels of ADA activity and ^{3}H-NBI binding sites when comparisons involved discrete brain regions richly endowed with fibers immunoreactive for ADA. However, a correlation coefficient of $r = 0.239$ was obtained if comparisons included brain regions, such as whole thalamus or midbrain, that are more heterogeneous with respect to their subnuclei organization. In the thalamus, for example, the extreme medial nuclei contain greater quantities of ADA-immunoreactive fibers, ADA activity, and ^{3}H-NBI binding sites compared with the bulk of the lateral areas. Thus, although there is strong circumstantial evidence pointing to a biochemical and anatomical relationship between ADA and ^{3}H-NBI binding sites in the rat CNS, more direct methods and a finer level of analysis are now required to establish the exact nature of this relationship.

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