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Distribution of nucleoside transport sites in guinea-pig brain

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Inhibition of the cellular uptake of adenosine may be achieved by impairment of the function of the membrane nucleoside transport mechanism which mediates the entry of adenosine into cells. Such inhibition, which might be expected to potentiate the effects of adenosine deriving from interaction with extracellular adenosine receptors, has been suggested to be involved in the central (Phillis et al 1980) and peripheral (Clanachan & Marshall 1980) actions of a variety of agents including dipyridamole and benzodiazepines (BDZs). The affinities of a series of recognized nucleoside transport inhibitors and BDZs for the nucleoside transport system of human erythrocytes have been estimated through the inhibition of the binding of [G-^{3}H]nitrobenzylthioinosine (NBMPR), a potent and specific inhibitor of nucleoside transport (Hammond et al 1981). Nucleoside transport activity ceases when specific membrane sites, evidently on the nucleoside transporter elements, are occupied by NBMPR, certain NBMPR congeners, or by dipyridamole. NBMPR binding sites appear to be present only on functional nucleoside transport elements (Jarvis & Young 1980; Cass et al 1981). This report describes a study of the regional distribution of nucleoside transport sites, identified by the site-specific binding of NBMPR, in guinea-pig brain.

Method

Female guinea-pigs (250–300 g) were decapitated and brain regions (Table 1) were homogenized in 10 volumes of sucrose (0·32 M) at 4 °C. Membrane fractions (P_2) were prepared by differential centrifugation (Gray & Whittaker 1962). Membranes (0·2–0·4 mg protein) were incubated for 20 min at 22 °C in Krebs-Tris buffer (1 ml final vol) containing [G-^{3}H]NBMPR (0·06–1·25 nM). Binding assays, conducted in 1·5 ml polypropylene centrifuge tubes, were initiated by the addition of membranes and terminated by centrifugation for 2 min in an Eppendorf 5412 microcentrifuge. The pelleted material was washed once with ice-cold Krebs-Tris buffer (1 ml) and dissolved in 0·5 M KOH (250 μl) before the assay of ^{3}H activity by liquid scintillation. Non-specific NBMPR binding was defined as the concentration (fmol mg^{-1} protein) of [G-^{3}H]NBMPR which remained membrane-associated when binding assays were conducted in the presence of a second transport inhibitor, dipyridamole (40 μM). Non-specific binding was never more than 30% of total binding in all brain regions tested. NBMPR binding parameters (K_D and B_{max}) were determined by mass law analysis (Scatchard plot) of the binding data. To determine inhibitor K_i values, membrane preparations were incubated with [G-^{3}H]NBMPR (0·2–0·8 nM) alone and in the presence of two or three concentrations of each inhibitor; data for the site-specific binding of NBMPR were subjected to mass law analysis by the double reciprocal plot method. The protein content of membrane preparations was estimated by the method of Lowry et al (1951).

Results

In each brain area examined, site-specific binding of NBMPR was saturable; in contrast, concentrations of the non-specific component of membrane-associated NBMPR were proportional to NBMPR concentration. Mass law analysis of the binding data indicated that in each brain region, NBMPR molecules were bound to a single class of high affinity sites and dissociation constants (mean ± s.e. mean) for NBMPR (Table 1) bound at these sites ranged from 0·15 ± 0·02 nM (olfactory lobe) to 0·38 ± 0·04 nM (pons/medulla). These differences are probably not attributable to competition with higher extracellular concentrations of adenosine because preincubation of membranes with adenosine deaminase (70 μg ml^{-1}; final concentration) did not influence NBMPR binding constants. It is clear that specific binding sites for NBMPR (expressed as fmol mg^{-1} protein) were not distributed uniformly throughout the brain (Table 1). As has been shown previously (Hammond & Clanachan 1982), dipyridam-

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Table 1. Maximal specific binding (B_{max}) and apparent dissociation constant (K_D) of nitrobenzylthioinosine (NBMPR) in various regions of guinea-pig brain.

Brain Region	B_{max} (fmol mg^{-1} protein)	K_D (nM)
Pons/medulla	644 ± 12	0·38 ± 0·04
Lower brain stem	623 ± 22	0·34 ± 0·02
Thalamus/hypothalamus	440 ± 6	0·28 ± 0·01
Cortex	433 ± 15	0·31 ± 0·02
Olfactory lobe	353 ± 14	0·15 ± 0·02
Caudate nucleus	310 ± 7	0·22 ± 0·02
Cerebellum	299 ± 18	0·31 ± 0·03
Hippocampus	201 ± 10	0·20 ± 0·02

Values are the mean ± s.e. mean of three experiments performed in duplicate. Each experiment was performed using pooled brain regions from 5 or 6 guinea-pigs.

ole and diazepam inhibited the binding of NBMPR to cortical membranes with K_i values of 11 ± 2 nM and 16 ± 2 μM, respectively. Double reciprocal plots of specifically bound NBMPR versus the equilibrium concentration of free NBMPR indicated that this inhibition was competitive. Dipyridamole and diazepam also competitively inhibited the binding of NBMPR to membranes prepared from areas of high (pons/medulla) and low (hippocampus) binding site density; observed were K_i values of 6 and 7 nM, respectively, for dipyridamole and 13 and 21 μM, respectively, for diazepam.

Saturable, high affinity binding sites for NBMPR have been demonstrated on cells of various types (Lauzon & Paterson 1977; Jarvis & Young 1980). Similar sites are also present on guinea-pig brain membranes. As these sites are believed to represent functional nucleoside transporters possessing a constant maximum translocation capacity (Jarvis & Young 1980), their non-uniform distribution in guinea-pig brain suggests that differences exist in the capacity of membranes in various brain areas to transport nucleosides. The highest number of sites was found in lower brain areas consisting of the pons/medulla and lower brain stem. Interestingly, the distribution of these sites appears unrelated to that for adenosine receptors. The latter, identified by the site-specific binding of 2-chloroadenosine, have a low density in spinal cord, hypothalamus and pons/medulla and a high density in the caudate nucleus, cerebellum and hippocampus (Williams & Risley 1980). The transport sites in the areas examined displayed no marked differences in their affinities for the transport inhibitors tested; areas of high and low site density had high affinity for dipyridamole and low affinity for diazepam. The low (relative to NBMPR or dypridamole) affinity of diazepam for the nucleoside transport system and the lack of correlation between the distribution of nucleoside transport sites and BDZ recognition sites (Mohler & Okada 1978) further supports our previous studies (Hammond et al 1981; Hammond & Clanachan 1982) which suggested that these sites are distinct entities. It is therefore unlikely that inhibition of nucleoside transport by BDZs contributes to their anxiolytic action (Phillis et al 1980).

This demonstration of regional differences in nucleoside transporter density may have important consequences in the measurement of drug action in the c.n.s. For example, significant regional differences should be expected in the apparent potency of adenosine receptor agonists and antagonists (Clanachan & Muller 1980) as the concentration of agonist in the vicinity of its extracellular receptors may be influenced by its rate of removal by the transport system. Similarly, regional variations should be expected in the efficacy of nucleoside transport inhibitors as modulators of extracellular nucleoside concentrations.

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