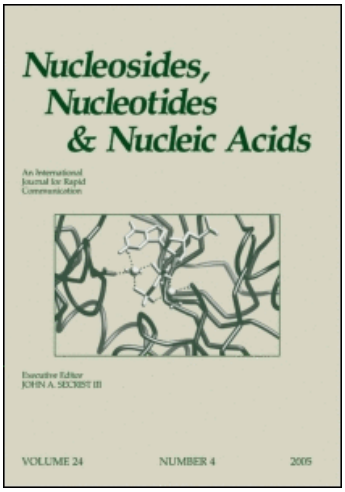

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AUTORADIOGRAPHIC LOCALIZATION AND CHARACTERIZATION OF ADENOSINE RECEPTOR SUBTYPES IN MAMMALIAN BRAIN

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Summary

Quantitative receptor autoradiography studies have shown that adenosine A_1 receptors are heterogeneously distributed in the rat brain with high concentrations found in the forebrain and cerebellum. In contrast, high affinity A_2 receptors appear to be exclusively localized in the striatum. These observations are discussed in relation to the putative neuromodulatory role of the purine in central neurotransmission.

Introduction

Quantitative receptor autoradiography has become a sensitive and reliable method for the localization and characterization of cell surface receptors in various tissues. The application of this technique to the study of brain cell surface receptors has received much experimental interest. While early autoradiographic studies offered essentially qualitative descriptions of regional receptor densities, increased sophistication in the generation and analysis of autoradiograms has allowed for quantitative comparisons of radioligand binding parameters and pharmacological profiles.

The purine riboside adenosine appears to be intimately involved in many different aspects of central and peripheral cellular function[1], however, the identification of discrete distributions of adenosine receptors in the central nervous system (CNS) has provided much support for a specific purinergic role in neuronal communication. Adenosine receptor subtypes, like many neurotransmitter receptors, are found in the greatest density in the central nervous system as compared to their relative distribution in peripheral tissues. The exact physiological significance of adenosine receptors in brain remains unknown. However, the many documented inhibitory effects of adenosine receptor activation on neurotransmitter release coupled with the psychomotor-stimulant effects of adenosine

receptor antagonists (alkylxanthines) have led to the suggestion that adenosine mediates an "inhibitory tone" upon mammalian physiology[1,2].

The application of quantitative receptor autoradiography techniques to the study of brain adenosine receptors has been particularly fruitful. The regional distribution of adenosine A_1 receptors in brain has been well characterized with several highly selective and potent agonist radioligands[3-6]. This adenosine receptor subtype is heterogeneously distributed in brain with the highest densities found in the mammalian forebrain and pyramidal motor system.

Adenosine A_2 receptors have been more difficult to study using receptor binding techniques due to the comparative lack of selective radioligands. [^{3}H]5'-N-ethylcarboxamidoadenosine (NECA) is a potent and nonselective adenosine agonist which has previously been used to study the A_2 receptor subtype following pharmacological and/or physiological interventions to block activity at the A_1 receptor[7,8]. Recently, a 2-substituted NECA derivative, CGS 21680 (2-(p-(carboxyethyl)phenethylamino)-5'-N'ethylcarboxamidoadenosine) has been developed[9] that, in tritiated form, labels the A_2 receptor with high affinity (IC_{50} = 22 nM) and with a high degree of selectivity (140-fold)[10]. The regional distribution of binding sites labeled with either [^{3}H]NECA, in the presence of 50 nM cyclopentyladenosine[7], or [^{3}H]CGS 21680, is exclusively localized in the striatal region of the rodent brain and is pharmacologically consistent with the labeling of a high affinity A_2 receptor in this structure[10-12].

In the present paper, data relevant to the regional distribution of two brain adenosine receptor subtypes, A_1 and A_2, is discussed with particular emphasis given to the use of receptor autoradiography techniques in the elucidation of purinergic contributions to central nervous system function.

Quantitative Receptor Autoradiography

With the advent of computer assisted densitometry, receptor autoradiography has evolved from an essentially qualitative endeavor to a technique that can be used for the reliable determination of receptor kinetic parameters. Two major technical problems that have been commonly associated with autoradiographic studies are the nonlinear relationship between the optical density of the exposed tritium-sensitive film and the amount of radioactivity present, and the possibility of

differential quenching obtained with tritiated ligands[13-15]. The quantitative difficulties intrinsic to these methodological problems have been variously overcome through the use of sophisticated curve fitting programs to "linearize" autoradiograms; the use of digital subtraction techniques coupled with the sampling of discrete brain regions, and the use of iodinated radioligands[16,17]. These technical advances have increased the precision and reliability of the autoradiographic analysis such that estimates of the receptor/ligand interactions do not differ significantly from those obtained with traditional membrane homogenate ligand binding techniques. As an example, several recent autoradiographic studies characterizing the regional distribution of adenosine A_1 receptors in brain, using both agonist and antagonist ligands, have produced binding parameters (i.e. Kd and Bmax) that are essentially equivalent to membrane homogenate binding data for these adenosine ligands[4,12,17,22].

Localization of Adenosine Receptor Subtypes

Initial autoradiographic studies of brain adenosine receptors involved the localization of A_1 receptors with the high affinity agonist [^{3}H]cyclyhexyladenosine (CHA)[3,18]. In these studies, the highest densities of [^{3}H]CHA recognition sites were found in the molecular layer of the cerebellum and in the CA-1 and CA-3 regions of the hippocampus. Moderate binding levels were observed in the thalamus, striatum, septum and cerebral cortex (see Fig 1). Analysis of the kinetic parameters of [^{3}H]CHA binding to cryostat sections of rat whole brain revealed a single class of high affinity (Kd = .77 nM) and limited capacity (Bmax = 420 fmol/mg protein) recognition sites[3]. Subsequent autoradiographic studies have revealed no significant regional differences in the affinity [^{3}H]CHA binding in the rodent brain[4,5,19].

While [^{3}H]CHA has been the ligand of choice for the visualization of A_1 receptor distributions, there are now a variety of novel ligands which can be used to further characterize this receptor subtype. [^{125}I]H-phenyl-isopropyladenosine (H-PIA) has been shown to label the A1 receptor in both central and peripheral tissues[20,21]. The high specific activity of this radioligand (2200 Ci/mmol) offers the added advantage of reduced quenching in tissues which contain high amounts of lipid. Several recent autoradiographic studies

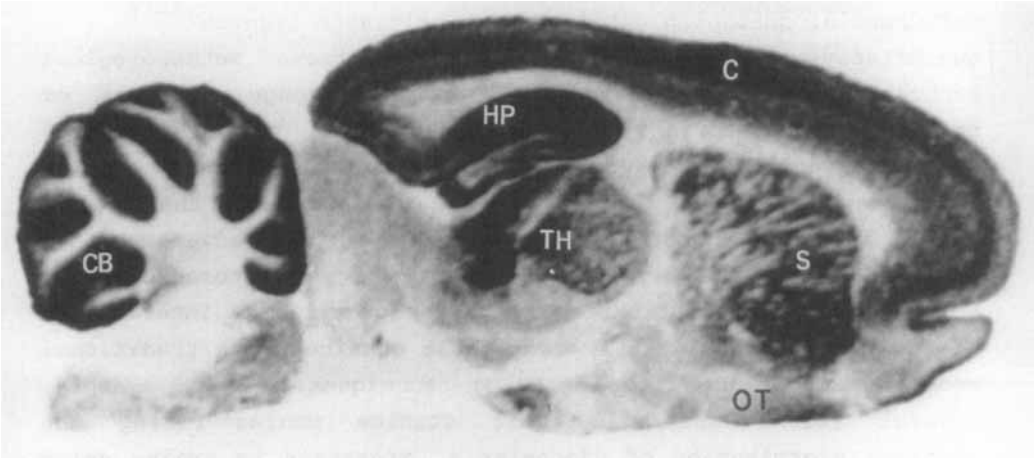

FIG. 1. Representative autoradiographic image of specific [^{3}H]CHA (1 nM) to rat brain sagittal sections. Specific binding was determined by the digital subtraction of the image on nonspecific binding from the image of total binding. Abbreviations are: C, cortex; S, striatum; OT, olfactory tubercle; TH, thalamus; HP, hippocampus; CB, cerebellum.

have used novel high affinity adenosine antagonist radioligands including the functionalized congeners [^{3}H]XCC[22] and [^{3}H]XAC[23] to selectively label the A_1 receptor in rat brain.

Until the availability of [^{3}H]CGS 21680, autoradiographic studies of the adenosine A_2 receptor involved the use of [^{3}H]NECA in the presence of an adenosine A_1-selective ligand to block [^{3}H]NECA binding to the A_1 receptor[12,19,24]. By using this procedure, [^{3}H]NECA binding has been localized in the striatum, thalamus, and cortex of the rat brain. It now appears that the choice of the A_1 receptor blocking agent may be critical for the specific visualization of high affinity A_2 receptors. The specific binding of [^{3}H]NECA, in the presence of 50 nM CPA, has been exclusively localized in the striatal region of the rodent brain and is pharmacologically consistent with the labeling of a high affinity A_2 receptor(Fig 2)[12]. However, in the presence of 1uM R-PIA or 50 nM CPX, a different distribution of [^{3}H]NECA recognition sites has been reported with significant binding densities in the thalamus and cerebral cortex[19,24]. While the

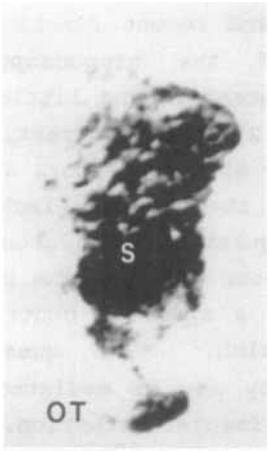

FIG 2. Representative autoradiographic image of specific 1nM [^{3}H]NECA (in the presence of 50 nM CPA) binding to high affinity adenosine A_2 receptors in rat brain sagittal sections. Abbreviations are: S, striatum; OT, olfactory tubercle.

exact nature of these additional "non-A_1" [^{3}H]NECA recognition sites remains unknown, several reports indicate that [^{3}H]NECA can also label a high-capacity "non-adenosine binding site"[8,25]. The selective localization of the high affinity A_2 receptor in the striatal region of the rodent brain obtained with [^{3}H]NECA (+ 50 nM CPA) has been recently confirmed using the highly A_2-selective agonist radioligand [^{3}H]CGS 21680[10,11]. Thus, the present data clearly indicate that while a high affinity A_2 receptor is specifically contained in the striatal region of brain, the other "non-A_1" [^{3}H]NECA recognition sites in brain may reflect binding to a low affinity A_2 receptor or a non-adenosine ribose uronamide recognition site[26].

Synaptic Localization of Adenosine Receptor Subtypes

Radioligand saturation experiments indicate that the rodent striatum contains approximately equal densities (Bmax) of both adenosine A_1 and A_2 receptors[17]. There are brain regions, however, that appear to contain a predominate concentration of only one adenosine receptor subtype. From the present

autoradiographic data and recent functional studies[27], the CA-1 and CA-3 regions of the hippocampus contain very high concentrations of A_1 receptors and little or no high affinity A_2 receptors (Figs 1 and 2). In contrast, the olfactory tubercle contains a high density of A_2 receptors and minimal quantities of A_1 receptors[17]. Given the apparent lack of regionally specific mechanisms for the regulation of adenosine availability, these differential distributions of adenosine receptor subtypes provide additional support for a specific contribution of adenosine to central neurotransmission. The question as to how this neuromodulatory activity may be mediated at the synaptic level has received some experimental attention.

Several recent studies[28-31] have employed various neurotoxins to selectively eliminate synaptic afferent and/or efferent processes in order to examine the synaptic localization of adenosine receptor subtypes (Table 1). The rodent striatum has been studied most extensively because of the relatively large concentrations of both adenosine receptor subtypes in this brain region. Striatal adenosine receptors and adenosine uptake sites are not significantly altered following the intra-striatal or intra-nigral administration of 6-hydroxydopamine which selectively destroys dopaminergic terminals, decreases steady-state dopamine concentrations, and increases postsynaptic dopamine receptor densities (Table 1)[28-31]. In contrast, the selective destruction of striatal postsynaptic terminals with excitotoxins such as kainic acid and quinolinic acid, produces profound reductions in the numbers of striatal adenosine receptors, adenosine uptake sites, and 2-deoxyglucose uptake[28-31]. These results indicate that striatal adenosine receptors are localized on intrinsic striatal interneurons and/or cortical striatal terminals.

In other brain regions, adenosine receptors appear to be localized on, or adjacent to, excitatory neurons[32]. Data from autoradiographic studies indicate that adenosine A_1 receptors are highly concentrated in brain regions, such as the hippocampus, that also contain high densities of excitatory amino acid (EAA) receptors[32-34].

Moderate concentrations of A_1 receptors are found in the granule layer of the cerebellum, an area in which N-methyl-D-aspartate (NMDA) receptors are also concentrated[33]. Direct administration of kainic acid or transient forebrain ischemia has been shown to

TABLE 1

Effects of Intrastriatal Administration of 6-OHDA and Quinolinic Acid on Purinergic and Dopaminergic Receptors[31]

LIGAND	CONTROL		6-OHDA		QUINOLINIC	
	Kd	Bmax	Kd	Bmax	Kd	Bmax
[^{3}H]CHA	1	500	1	420	2	340*
[^{3}H]NECA (+50 nM CPA)	4	700	4	720	4	290*
[^{3}H]NBI	0.2	220	0.2	210	0.2	98*
[^{3}H]SCH 23390	0.8	1045	0.9	1350*	0.6	335*
[^{3}H]SPIPERONE	0.2	900	0.4	1100*	0.4	400*

Values represent means from 8-10 saturation experiments from individually treated rats. * represents $p < .05$. [^{3}H]nitrobenzylthioinosine (NBI), [^{3}H]SCH23390, and [^{3}H]spiperone were used to label adenosine uptake sites, dopamine D-1 receptors and dopamine D-2 receptors, respectively.

markedly reduce adenosine A_1 and NMDA receptors in the hippocampal CA-1 region[33-36]. These manipulations can result in essentially complete destruction of the cell bodies of the pyramidal cell layer in the CA-1 region of hippocampus. However, a significant proportion of A_1 receptors remain unaffected in this brain region indicating that A_1 receptors are also localized postsynaptically to these excitatory neurons. These data, coupled with the observations that large amounts of adenosine and EAAs are released during an ischemic episode[37] and that adenosine can potently inhibit the release of the EAA[38], L-glutamate, suggest that adenosine may function as an endogenous anticonvulsant and may also limit the neurotoxic damage produced by excess quantities of l-glutamate.

Conclusion

There data reviewed above indicate that adenosine receptor subtypes exhibit markedly different regional distributions in the mammalian brain. These differences appear to be consistent across different species[26], however, both strain and species differences in adenosine receptor densities have been reported[26]. While an exact role for adenosine in brain function has yet to be determined, the specific regional distributions and synaptic localizations of adenosine receptors provide additional support

for the idea that adenosine may provide an homeostatic inhibitory modulation of central excitatory processes (i.e. excess L-glutamate during ischemic and/or convulsant episodes).

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