Characterization, Distribution, and Ontogenesis of Adenosine Binding Sites in Cat Visual Cortex

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In vitro autoradiographic techniques were used to characterize binding sites for ^3H -cyclohexyladenosine (CHA) and ^3H -5'-*N*-ethylcarboxamidoadenosine (NECA) in cat and kitten visual cortex. ^3H -CHA binding sites in adult cat have a B_{max} of 1363 fmol/mg protein and a K_d of 6.8 nM. Displacement experiments indicate that ^3H -CHA binds to an adenosine receptor similar to the A_1 -adenosine receptor described by other investigators. ^3H -NECA binding sites in adult cat have a B_{max} of 518 fmol/mg protein and a K_d of 15.4 nM. Displacement experiments do not allow us to identify this binding site unambiguously. B_{max} values increase during postnatal development for both binding sites, peaking in adulthood for ^3H -CHA and at 30 d for ^3H -NECA. K_d values show neither consistent nor significant differences during postnatal development for either binding site. ^3H -CHA and ^3H -NECA binding sites are concentrated in layers 1-3 and upper layer 5 in the visual cortex of adult cats. These laminar patterns, however, change during postnatal development, showing the densest binding in the deep cortical layers (5 and 6) in kittens younger than 30 d of age and a fairly homogeneous binding in older kittens before achieving the adult distribution.

The visual cortex plays a critical role in the analysis of visual signals concerned with form, motion, and depth. The mechanisms by which the cortex processes visual information have been the subject of numerous investigations at the physiological (Hubel and Wiesel, 1962), anatomical (Lund, 1973), and neurochemical (Emson and Hunt, 1979) levels. In particular, the last few years have witnessed a dramatic increase in our understanding of the chemistry of the cortex. Accumulating evidence gathered with immunocytochemical, iontophoretic, and receptor binding techniques has greatly expanded the list of neurotransmitters/neuromodulators that may be active in cerebral cortex, in particular, visual cortex (Emson and Hunt, 1979; Parnavelas and McDonald, 1983). These findings point toward a complex chemical circuitry of neocortex in which various neurotransmitters can modulate each other's actions in a variety of different ways.

One such modulatory substance may be adenosine. This purine derivative decreases the firing rates of both central and peripheral neurons (Phillis and Kostopoulos, 1975; Phillis and Wu, 1981; Stone, 1982; Stone and Perkins, 1979). Adenosine modulates adenylate cyclase activity (Ribeiro et al., 1979; Stone, 1981; Van Calker et al., 1979) and the release of several neu-

rotransmitters (Fredholm and Hedqvist, 1980; Ginsborg and Hirst, 1972; Harms et al., 1979; Hollins and Stone, 1980; Michaelis et al., 1979; Ribeiro, 1979; Snyder, 1985). There is evidence for at least 2 classes of adenosine receptors in the nervous system (Burnstock, 1978; Londos and Wolff, 1977; Londos et al., 1980; Van Calker et al., 1979). A_1 -adenosine receptors, generally labeled with ^3H -cyclohexyladenosine (CHA) (Bruns et al., 1980; Goodman and Snyder, 1982; Goodman et al., 1983), may have a presynaptic location on axon terminals (Goodman et al., 1983) and are associated with inhibition of adenylate cyclase activity (Van Calker et al., 1979). ^3H -5'-*N*-ethylcarboxamidoadenosine (NECA) may be a more selective agonist of the A_2 class of adenosine receptors (Londos and Wolff, 1977; Londos et al., 1980), which are associated with activation of adenylate cyclase (Van Calker et al., 1979) and may have a postsynaptic location. This last point is controversial, however, since ^3H -NECA can label both A_1 and A_2 receptors (Snyder, 1985), and species differences in the subtypes of adenosine receptors have been described (Barnes and Thampy, 1982; Bruns et al., 1980; Murphy and Snyder, 1982).

Our own studies have been directed to the examination of the receptors for various neurotransmitters and neuromodulators thought to be active in the cat visual cortex (for a summary, see Shaw et al., 1984c). These data, gathered primarily with the methods of receptor autoradiography (Snyder, 1984; Young and Kuhar, 1979), have provided information on the characteristics and laminar distributions of the various receptor populations. Special attention has been directed towards the study of receptor alterations during normal postnatal development, since several receptor populations show differing characteristics and/or distributions in infant and adult animals (Shaw et al., 1984a, c, 1985). In these cases, receptor alterations take place during the physiologically defined critical period (Cynader et al., 1980; Hubel and Wiesel, 1970; Olson and Freeman, 1980), the period in the first few months of postnatal life of greatest neuronal plasticity. Our working hypothesis is that alterations of receptor distribution, number, and/or affinity may play a role in the mechanism by which visual exposure modifies cortical function during the critical period.

In the present study, we have examined adenosine binding sites in visual cortex using ^3H -CHA and ^3H -NECA as ligands (Bruns et al., 1980; Goodman et al., 1983). The adenosine binding sites are characterized and their postnatal development charted in terms of number, affinity, and laminar distribution.

While this paper was being revised, a report by Aoki (1985) appeared describing adenosine binding site distribution in normal and dark-reared cats.

Materials and Methods

Fourteen male colony cats were maintained on a 14 hr/10 hr light/dark cycle and were sacrificed at approximately the same time of day (1600-1830). Following an overdose of sodium pentobarbital, they were perfused through the heart with cold (4°C) phosphate buffer solution (PBS; 0.1 M, pH 7.4). In some animals, the PBS was followed by a 0.1-0.2%

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formaldehyde/PBS solution in order to lightly fix the tissue. This procedure had no discernible effect on the characteristics of adenosine binding sites. The brains were quickly removed and frozen in liquid Freon. Sixteen micron coronal sections were cut on a cryostat and thaw-mounted onto subbed glass slides, then stored at -20 to -25°C until used. In experiments designed to compare animals of different ages, sections were cut within the same 24 hr period and stored for the same length of time before being used.

^3H -CHA (New England Nuclear; specific activity, 25 Ci/mmol), an adenosine agonist, was used to study binding at A_1 -adenosine binding sites, and ^3H -NECA (New England Nuclear; specific activity, 30 Ci/mmol) was employed in an attempt to label both A_1 and A_2 binding sites.

The slide-mounted sections were allowed to warm to 4°C . The slides were then preincubated for 10 min in dishes containing 4°C Tris-HCl buffer (50 mM, pH 7.7). A small amount of formaldehyde (0.2%) was added to lightly fix the tissue. Two additional 5 min washes in buffer alone removed the formaldehyde. Reduction of endogenous adenosine was accomplished through the addition of adenosine deaminase, Sigma type VIII (Goodman and Snyder, 1982), to all 3 preincubation rinses at a concentration of 0.06 IU/ml. In preliminary experiments we determined that raising the preincubation concentration of adenosine deaminase to 1 IU/ml gave, at most, 20% higher binding. For this reason, preincubation rinses contained the lower concentration. All incubation media, however, contained adenosine deaminase at a concentration of 1 IU/ml (Goodman and Snyder, 1982). After the preincubation washes, the slides were laid out on a black plastic tray to aid visualization of the sections. The incubation medium containing the tritiated ligand (0.4–52 nM for both ligands) was dripped onto the sections. Nonspecific binding was determined by including 1×10^{-4} M CHA or L-PIA for ^3H -CHA or ^3H -NECA, respectively, to the incubation medium. Incubations for both ligands were for 120 min at 23°C . The incubations were terminated with two 5 min washes in 4°C buffer. In characterization studies, areas 17–19 identified from the cortical maps of Tusa et al. (1981) were scraped off the slides onto Whatman GB/F filter paper, suspended in Aquasol II and the bound ligand was counted in a scintillation counter. For autoradiography, sections were incubated at optimal conditions as determined in the characterization experiments. The sections were dried under a stream of cool air, allowed to dry further for at least 12 hr over desiccant at 4°C , and then apposed to LKB tritium-sensitive Ultrofilm for 1–4 weeks. The film was conventionally developed and fixed.

Protein content was determined in alternate sections from each animal using the methods of Lowry et al. (1951) and used for computing B_{max} values. Saturation binding data were analyzed by Eadie-Hofstee plots as described by Zivin and Waud (1982). Cortical lamination was determined for comparison with the autoradiograms by staining alternate sections with cresyl violet or processing for cytochrome C oxidase activity (Wong-Riley, 1979).

Results

Characterization of adenosine binding sites using ^3H -CHA and ^3H -NECA

Figures 1 and 2 illustrate biochemical experiments in which we characterized the binding sites for ^3H -CHA and ^3H -NECA, respectively. For each experiment, each point represents at least 3 separate determinations for total binding. Determination of nonspecific binding was always performed in duplicate. All experiments for these characterizations were performed at least twice. All time course and displacement curves thus represent average values for 2 or more experiments.

Figure 1. *Top*, Measurements of association and dissociation time constants for ^3H -CHA binding sites in the visual cortex (areas 17–19) of an adult cat. Incubation was at 23°C with 9 nM ^3H -CHA. Total binding values (TB) are given for the association points, while total, specific (SB), and nonspecific (NSB) values are shown for the dissociation points. For association rate measurements, the rinse was for 1 min. Arrow indicates the beginning of the postincubation rinse. Two 5 min rinses were adopted for subsequent autoradiographic studies. This time point left the specific binding quite high while virtually eliminating nonspecific

binding. *Center*, Saturation binding for ^3H -CHA in the visual cortex (areas 17–19) of an adult cat. The ^3H -CHA incubation was for 120 min at 23°C . Nonspecific binding was measured by coincidence of alternate sections with 1×10^{-4} M unlabeled CHA. Filled circles, total binding; open circles, specific binding; triangles, nonspecific binding. *Inset*, Eadie-Hofstee plot. *Bottom*, Displacement studies for ^3H -CHA in the visual cortex (areas 17–19) of an adult cat. IC_{50} values are given in Table 1. Incubation in each case was in 20 nM ^3H -CHA for 120 min at 23°C with the appropriate displacers present.

Figure 2. *Top*, Time constant measurements for ³H-NECA binding sites in the visual cortex (areas 17–19) of an adult cat. Incubation was at 23°C using 8 nM ³H-NECA. All other details as in Figure 1. *Center*, Saturation binding for ³H-NECA in the visual cortex (areas 17–19) of an adult cat. The ³H-NECA incubation was for 120 min at 23°C. Nonspecific binding was measured by coincubation of alternate sections with 1×10^{-4} M L-PIA. *Filled circles*, total binding; *open circles*, specific binding; *triangles*, nonspecific binding. *Inset*, Eadie-Hofstee plot. *Bottom*, Displacement studies for ³H-NECA in the visual cortex (areas 17–19) of an adult cat. IC_{50} values are given in Table 1. Incubation in each case was in 20 nM ³H-NECA for 120 min at 23°C with the appropriate displacers present.

The time course of association and dissociation was examined for ³H-CHA binding (Fig. 1, top panel). At 23°C, equilibrium binding was achieved by 120 min, remaining stable for at least another hour. The association rate constant (K_{+1}) was cal-

culated to be $2.06 \times 10^{-3} \text{ min}^{-1} \text{ nM}^{-1}$. Rinsing the sections in an "infinite" dilution led to a rapid decline in specific binding over 10 min, followed by a slower decline. The dissociation rate constant (K_{-1}) was calculated to be $1.93 \times 10^{-2} \text{ min}^{-1}$. The

Figure 3. Laminar binding patterns for ^3H -CHA (*A*) and ^3H -NECA (*B*) in the visual cortex of adult cats. In both cases, incubation was for 120 min at 23°C . Ligand concentrations were 26 nM (*A*) and 30 nM (*B*); exposure period for LKB Ultrofilm was 14 d (*A*) and 21 d (*B*). Adjacent sections in each instance were processed for cytochrome c oxidase activity (Wong-Riley, 1979). Photographs of the autoradiograms and the cytochrome c-stained sections were made to the same magnification. A portion of a photograph of a cytochrome c-stained section (medial bank of area 17) has been superimposed on the photograph of the autoradiogram. Dark arrows indicate layer 4 which stains most densely for cytochrome c oxidase; white arrow in *A* points to the labeled sublayer in layer 5. Calibration bar, 1 mm. Dorsal (*D*) and medial (*M*) directions are as indicated.

ratio of these rate constants gave a K_d ($= K_{-1} - 1/K_{+1} + 1$) of 9.34 nM.

A representative saturation binding experiment for ^3H -CHA is illustrated in Figure 1 (middle panel). Increasing the concentration of ^3H -CHA resulted in saturable binding. Nonspecific binding was less than 7% of total binding at the highest ligand concentration used. Total binding is represented by filled circles, while specific and nonspecific binding are indicated by open circles and triangles, respectively. Eadie-Hofstee analysis of these data (insert) gave a B_{max} of 1363.17 ± 89.04 fmol/mg protein and a K_d of 6.83 ± 0.7 nM. A Hill plot of these data had a slope of 1.02, suggesting the existence of a single population of binding sites.

Figure 1 (bottom panel) illustrates a series of displacement experiments in which we determined the IC_{50} (concentration required to displace 50% of the bound ^3H -CHA) for various agonists and antagonists of adenosine. CHA had the lowest value of the compounds tested, with an IC_{50} of 5×10^{-9} M. This value is in good agreement with the previously determined K_d 's derived from the rate constant measurements and with other reports in the literature (Aoki, 1985; Bruns et al., 1980). The IC_{50} values for all of the compounds tested are shown in Table 1. The order of potency of the various compounds suggests that ^3H -CHA labels a binding site similar to the A_1 -adenosine receptor described by other investigators in other preparations (Bruns et al., 1980).

Figure 2 illustrates the association and dissociation time courses for binding sites labeled with ^3H -NECA (top panel). At 23°C , ^3H -NECA binding reached equilibrium by 120 min, remaining relatively stable for at least another hour. The association rate constant was calculated to be $7.66 \times 10^{-4} \text{ min}^{-1} \text{ nM}^{-1}$, almost 3 times slower than the K_{+1} for ^3H -CHA. Postincubation rinses produced a rapid early decline in specific binding over 10 min, followed by a slower decline that was similar to that described above for ^3H -CHA. The dissociation rate constant was calculated to be $1.51 \times 10^{-2} \text{ min}^{-1}$, a value close to that for ^3H -CHA. The K_d calculated from these rate constants was

19.71 nM. In Figure 2 (middle panel) the results of a representative saturation binding experiment using ^3H -NECA are illustrated. Specific binding was saturable. Eadie-Hofstee analysis (insert) gave a B_{max} of 517.77 ± 7.05 fmol/mg protein and a K_d of 15.41 ± 1.84 nM. Nonspecific binding was about 25% of total binding at the highest concentration. A Hill plot of these data gave a slope of 1.02, suggesting the presence of a single population of binding sites.

In Figure 2 (bottom panel) a series of displacement experiments is shown (see also Table 1) using ^3H -NECA. NECA had an IC_{50} of 14 nM, in very good agreement with the 2 K_d values determined above for ^3H -NECA. The relative potencies of the various displacers for ^3H -NECA resembled those for ^3H -CHA with exceptions only in the order of potency of CHA and L-PIA. These observations were repeated in several experiments. Their similar displacement profiles raise the question of whether ^3H -CHA and ^3H -NECA are labeling separate binding sites. This issue is further addressed in the Discussion.

Both the binding sites labeled by ^3H -CHA and ^3H -NECA were

Table 1. IC_{50} values (M) of various adenosine analogs displacing ^3H -CHA and ^3H -NECA

Displacer	^3H -CHA binding sites	^3H -NECA binding sites
CHA	5×10^{-9}	1.6×10^{-9}
L-PIA	1×10^{-8}	8×10^{-10}
D-PIA	4×10^{-8}	5.6×10^{-9}
NECA	1.6×10^{-7}	1.4×10^{-8}
DPX	1×10^{-6}	1×10^{-6}
Theophylline	3×10^{-4}	4×10^{-5}

Displacement experiments were performed for ^3H -CHA and ^3H -NECA using various adenosine analogs. The numbers shown represent IC_{50} values (the concentration required to displace 50% of total binding) for each compound. PIA, phenylisopropyl adenosine; DPX, 1,3 diethyl-8-phenylxanthine.

Figure 4. Distribution of ^3H -CHA binding sites in the visual cortex of cats of different postnatal ages: *A*, 3 d; *B*, 15 d; *C*, 30 d; *D*, 60 d; *E*, 95 d; *F*, adult. Ligand concentration, 26 nM. Photographs are from LKB Ultrofilm autoradiograms photographed for actual contrast; film exposure, 14 d. Note the changes in laminar distribution during development, from deep layers in the young kittens to superficial layers in adults. For each cat the different laminae were identified in alternate sections stained with cresyl violet or cytochrome c oxidase. Calibration (all panels), 1 mm. Dorsal is up, medial is left for all panels.

impervious to additions of up to 1×10^{-6} M dipyrindamole, a blocker of facilitated diffusion of adenosine into cells (Wu et al., 1981). Neither the number of binding sites, nor their laminar distributions, was altered for either ligand. These data suggest that neither ^3H -CHA nor ^3H -NECA is labeling high-affinity adenosine transport sites in cat visual cortex.

Distributions of ^3H -CHA and ^3H -NECA binding sites in cat visual cortex

The laminar binding patterns for ^3H -CHA and ^3H -NECA are illustrated in Figure 3. These photographs of the autoradiograms have been combined with inserted slices of photographs from

Figure 5. Distribution of ³H-NECA binding sites in the visual cortex of cats of different postnatal ages: *A*, 3 d; *B*, 15 d; *C*, 30 d; *D*, 60 d; *E*, 95 d; *F*, adult. Ligand concentration, 30 nM. Photographs are from LKB Ultrofilm autoradiograms photographed for actual contrast; film exposure, 21 d. Changes in laminar distribution are similar to those seen for ³H-CHA binding sites (see Fig. 4). Calibration (all panels), 1 mm. Dorsal is up, medial is left for all panels.

Figure 6. $B_{\max}$ values for different postnatal ages for $^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding sites. All incubations were at 23°C for 120 min.

alternate sections processed for cytochrome c oxidase. Cytochrome c oxidase labels layer 4 most densely in cat visual cortex (Wong-Riley, 1979), offering a convenient laminar landmark with which to identify laminar patterns of receptor binding. In the adult cat, $^3\text{H-CHA}$ (Fig. 3A) showed the densest binding in the supragranular layers (layers 1–3). Moderate binding was also seen in the upper portion of layer 5. $^3\text{H-NECA}$ labeled binding sites with essentially the same laminar distribution as that of $^3\text{H-CHA}$ in the adult cat cortex (Fig. 3B).

Postnatal development of $^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding patterns

Figure 4 illustrates the laminar distribution of $^3\text{H-CHA}$ binding sites in kittens of various postnatal ages. The illustrations shown here have been photographed from the original autoradiograms at the same contrast level in order to allow direct comparisons among the different panels of Figure 4. At 3, 15, or 30 d after birth (panels A–C), layers 5–6 were most densely labeled, while the superficial cortical layers were relatively lightly labeled. By 60 d of age, the deep cortical layers were still labeled, but moderate binding had appeared in the superficial layers as well, resulting in a relatively laminarly homogeneous, albeit slightly patchy, binding pattern. By 95 d postnatal (panel E), the adult binding pattern became more apparent: Layers 1–3 exhibited dense binding, with layers 4–6 much lighter in comparison. In the adult, $^3\text{H-CHA}$ strongly labeled layers 1–3, with an additional sublayer in layer 5 (Fig. 4F), a pattern very different from that of the young kittens (Fig. 4, A–C).

$^3\text{H-NECA}$ binding shows a developmental pattern (Fig. 5) similar to that of $^3\text{H-CHA}$. Again, the deep cortical layers were heavily labeled early in postnatal life. A more homogeneous binding pattern then developed, with the superficial layers becoming heavily labeled only late in postnatal development.

Figure 6 illustrates the changes in receptor number ($B_{\max}$) during postnatal development for $^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding sites. The number of $^3\text{H-CHA}$ binding sites increased more than 3-fold from low early values to adulthood. $^3\text{H-NECA}$ binding increased until 30 d postnatally, remaining relatively constant thereafter.

Figure 7 shows K_d values during postnatal development for

Figure 7. K_d values for different postnatal ages for $^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding sites. Incubation as in Figure 6.

both populations of binding sites. $^3\text{H-CHA}$ showed a generally constant K_d during postnatal development. $^3\text{H-NECA}$ K_d values were quite variable but showed no clear trend during development.

$^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding in other cortical and subcortical areas

Studies of other receptors in cat and raccoon cortex reveal that receptor binding in primary sensory cortices is often denser and can show different lamination patterns than that in adjacent association cortices (Sampson et al., 1984; Shaw et al., 1984a). $^3\text{H-CHA}$ and $^3\text{H-NECA}$ binding in cat visual cortex likewise follows this pattern: Areas 17 and 18 have denser binding and more distinct lamination than nearby association cortex. Similarly, auditory cortex shows a highly laminar-specific binding pattern, with the highest binding densities in the supragranular laminae (data not shown). Binding also becomes less dense and more homogeneous across cortical layers in nonsensory cortical areas. Figure 8 illustrates a clear transition between the binding pattern in the ventral-most part of the striate cortex and that of the cingulate cortex.

$^3\text{H-CHA}$ and $^3\text{H-NECA}$ have similar distributions in subcortical structures. Stratum oriens and stratum radiatum of the CA1 to CA3 fields of the hippocampus label densely. The subiculum and the inner molecular layers of the dentate gyrus also label densely for both ligands. The lateral geniculate nucleus (LGN) shows moderate and homogeneous binding in the cell layers, with the interlaminar zones relatively free of label. The medial geniculate nucleus labels moderately with some inhomogeneities. Both inferior and superior colliculi show low-moderate binding in the superficial layers only. Figure 8 shows $^3\text{H-CHA}$ binding sites in the cat diencephalon midbrain to illustrate adenosine receptor distribution in some of the regions mentioned above.

Discussion

The identification of the $^3\text{H-CHA}$ binding site in the cat visual cortex as an A_1 -adenosine receptor seems relatively straightforward.

Figure 8. Montage of 2 coronal sections of cat diencephalon midbrain and cortex are used to illustrate ³H-CHA binding in the visual cortex and other cortical and subcortical areas. The sections were incubated with 26 nM ³H-CHA for 120 min at 23°C. Note in general the dense binding in cortex compared with most of the diencephalon. *A*, The most densely labeled diencephalic areas are the hippocampus (*hipp.*; see text for further description) and the lateral and medial geniculate bodies (*lgn.*, *mgn.*). A clear laminar difference is observable between the binding in visual cortex (*vc*) and cingulate cortex (*cing.*). *B*, Low to moderate binding is observed in the superficial layers of the superior colliculus (*sc*). Film exposure, 14 d. Calibration bar, 1 mm. Dorsal is up, medial is right in *A* and left in *B*.

ward. The binding pattern is similar to that reported for A₁-adenosine receptors in the cat (Aoki, 1985); in rat cortex the binding pattern differs (Goodman and Snyder, 1982; Goodman et al., 1983). Additionally, the *K_d* and displacement characteristics are generally similar to those reported for A₁-adenosine receptors previously described (Aoki, 1985; Bruns et al., 1980; Goodman and Snyder, 1982), although Aoki (1985) reports 2 distinct binding sites in cat visual cortex using ³H-CHA.

The identification of the cortical ³H-NECA binding site is more problematic. ³H-NECA binds with lower affinity than ³H-CHA, in agreement with reports of A₂-adenosine receptor binding in other preparations (Bruns et al., 1980). A Hill plot of the saturation binding data suggests the existence of a single population of binding sites, although ³H-NECA in other preparations is reported to bind to both A₁ and A₂ adenosine sites (Fredholm, 1982; Snyder, 1985). The higher *K_d* for ³H-NECA is attributable to an almost 3-fold slower association rate constant. The dissociation rate constants for the 2 ligands are approximately the same, suggesting that the differences in *B_{max}* (³H-CHA nearly 3 times that of ³H-NECA) are due to different

numbers of distinct binding sites. If ³H-NECA were binding to both A₁ and A₂ sites, we would expect a higher *B_{max}* than for ³H-CHA, unless the *K_{off}* were appreciably faster, which is not the case. In addition, the postnatal time course of variation in *B_{max}* is quite different for the 2 ligands. Taken together, these data argue for a cortical ³H-NECA binding site distinct from the A₁ site labeled by ³H-CHA. The difficulty with this interpretation is that the order of effectiveness for the various displacer substances tested is very similar, although not identical, for the 2 ligands (Figs. 1; 2; Table 1). Previous reports in other systems have indicated major differences in displacer potencies at the 2 sites. Another difficulty is the nearly identical laminar distribution of ³H-CHA and ³H-NECA binding patterns. These 2 reservations, especially the first, raise questions concerning the identification of the ³H-NECA binding site in the cat cortex as the A₂-adenosine receptor described by other investigators. Further experiments are required to establish definitively whether ³H-NECA binds to the same sites as does ³H-CHA or to a distinct subclass of adenosine receptors in cat visual cortex.

The binding patterns exhibited for adenosine receptors in the

Figure 9. Schematic representation of the laminar binding patterns for 9 receptor populations in 3-d-old and adult cats. Note that binding densities are given only in qualitative values: very high, high, moderate, low, and no binding. Using this scale, it is possible to compare the relative binding density for any receptor population across the cortical laminae but not to compare different receptors to each other or the 3-d-old to the adult. Abbreviations: ACh receptors (acetylcholine, muscarinic sites) were labeled using ^3H -quinuclidinyl benzilate (QNB) and ^3H -N-methyl-scopolamine (NMS; Shaw et al., 1984a with carbachol competition); β -adrenergic receptors were labeled using ^3H -dihydroalprenolol (DHA; Shaw et al., 1984c); N-methyl-D-aspartate (NMDA) kainate and quisqualate receptors were labeled using ^3H -L-glutamate (C. Shaw and M. Cynader, unpublished observations); quisqualate receptors were labeled using ^3H -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA; C. Shaw and M. Cynader, unpublished observations); cholecystokinin (CCK) receptors were labeled using ^3H -pentagastrin (CCK-5; Shaw and Cynader, 1985a); GABA receptors were labeled using ^3H -muscimol (Shaw et al., 1984b); and benzodiazepine (BDZ) receptors were labeled with ^3H -flunitrazepam (FNZ); for further details, see Shaw et al. (1984c).

adult cat resemble those previously described for muscarinic ACh receptors (Shaw et al., 1984a), cholecystokinin receptors (Shaw and Cynader, 1985), β -adrenergic receptors (Shaw et al., 1984c), and, to a lesser extent, binding sites labeled by ^3H -L-glutamate and ^3H -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (C. Shaw and M. Cynader, unpublished observations).

Both ^3H -CHA and ^3H -NECA binding sites show alterations in their laminar binding patterns during postnatal development. Similar changes, or even reversals, of the original neonatal laminar binding pattern have been found in the majority of cortical receptor populations we have studied to date (Shaw and Cynader, 1985; Shaw et al., 1984a, b, 1985). These data are summarized in schematic form for young kittens and adult cats in Figure 9.

Most of the previously studied receptors that have exhibited changes in developmental laminar binding patterns (muscarinic/cholinergic, cholecystokinin, and β -adrenergic binding sites) have shown dense concentrations in layer 4 initially, and subsequent alterations to favor the superficial and/or deep layers. Other receptors (GABA, benzodiazepine) are concentrated in layer 4 at all postnatal ages. Adenosine binding sites differ in that layers 5 and 6 show the densest binding initially, layers 1–3 are most heavily labeled in the adult, and layer 4 is not a zone of highest concentration at any stage of development. The pattern of alteration of binding sites (from deep to superficial cortical layers) parallels that previously reported for the ontogeny of neurons in the different cortical layers (Rakic, 1974). The relative absence of adenosine receptors in layer 4 at all ages suggests that adenosine does not exert its effects at the first stage of cortical information processing, but rather that it acts on higher-order

cortical functions. This reduced presence in layer 4 also characterizes muscarinic acetylcholinergic binding sites of the adult cat (Shaw et al., 1984a), and it is interesting to note that these 2 "modulatory" substances seem to have similar effects on cortical EEG and behavioral arousal. It has long been known that administration of the muscarinic antagonist scopolamine results in cortical EEG synchronization in the absence of behavioral sleep (Steriade and Hobson, 1976). Administration of L-PIA, an adenosine agonist, results in behavioral manifestations of sleep, but with a desynchronized cortical EEG (Meltzer et al., 1984).

As was the case for several other receptors studied in cat visual cortex (Jonsson and Kasamatsu, 1983; Shaw and Cynader, 1985, unpublished observations; Shaw et al., 1984a–c; Wilkinson et al., 1983) and the cortex of other species (Candy and Martin, 1979; Pittman et al., 1980), adenosine receptor density (B_{max}) was initially low but increased during postnatal development. ^3H -CHA binding density peaked in adulthood, while ^3H -NECA binding density peaked at 30 d postnatally. Geiger et al. (1984) have reported an increase in the number of A_1 -adenosine receptors during postnatal development in rat cerebral cortex.

K_d values for the 2 binding sites showed some developmental variations, but no clear or significant trends were seen, unlike those observed for rat adenosine receptors (Geiger et al., 1984) and for other cortical receptor populations (Shaw et al., 1984c, 1985). Of the other receptors studied to date in cat visual cortex, only the β -adrenergic receptors, also purported to be modulatory in action, show no consistent K_d changes during postnatal development (Shaw et al., 1985).

The issue of the cellular location of adenosine receptors is a subject of some controversy. Goodman et al. (1983) have sug-

gested a presynaptic locus for A₁-adenosine receptors in rat superior colliculus but not for the LGN or visual cortex. In other preparations, adenosine receptors are probably postsynaptic (Geiger et al., 1984). We have examined these issues in a preliminary way in adult cats subjected to unilateral enucleation (3 month survival), unilateral decortication (2–3 week survival), or unilateral LGN lesion (2 week survival). In no case have we noted a significant change in adenosine receptor density or pattern of binding in either the LGN (following enucleation), superior colliculus, or visual cortex. Results were the same whether ³H-CHA or ³H-NECA was employed as a ligand. These results suggest that if adenosine receptors are associated with the retinogeniculate, retinocollicular, geniculocortical, or callosal pathways, then they are not presynaptic in location, at least not in the cat. Any presynaptic loci for adenosine receptors in cat visual cortex are thus likely to arise from intracortical circuits.

The functional role of adenosine receptors within the cortex remains unclear. The evidence that adenosine modulates the synaptic release of a wide variety of different substances (Harms et al., 1979; Hollins and Stone, 1980; Michaelis et al., 1979; Snyder, 1985) allows for a number of different roles. It is interesting, in view of recent findings, that adenosine blocks release of glutamate from cortical slices (Dolphin and Archer, 1983), and that adenosine receptors and binding sites labeled by L-glutamate (Monaghan et al., 1985) show somewhat similar laminar distributions within the visual cortex (C. Shaw and M. Cynader, unpublished observations). We note, however, that the distributions of adenosine receptors and glutamate-related binding sites both alter their laminar distribution during development and that these patterns of changes are clearly different. The developmental patterns, which are specific and different for these, and other, receptors may allow for maximum modulation of the functions of any particular neurotransmitter system by adenosine at a specific time during postnatal development.

The identification of adenosine receptors in cat visual cortex and the description of their laminar binding pattern and ontogenesis add important information to the broadening picture of the chemical circuitry of this region of the brain. In conjunction with the description of the other receptor populations thus far studied, these data reveal a complex system with the possibility of multiple interactions among different neurotransmitters. Unraveling these interactions promises to be a major task for the future.

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