Quantitative *In Vivo* Receptor Binding

II. Autoradiographic Imaging of Muscarinic Cholinergic Receptors¹

K. A. FREY,* R. L. E. EHRENKAUFER,§ AND B. W. AGRANOFF^{2*‡}

**Neuroscience Laboratory, ‡Department of Biological Chemistry, and §Division of Nuclear Medicine, The University of Michigan, Ann Arbor, Michigan 48109*

Abstract

The *in vivo* distribution of [³H]scopolamine in rat brain following establishment of constant, saturating arterial tracer concentrations was examined with the use of quantitative autoradiography. The equilibrium drug distribution, studied 240 min after initiation of tracer infusion, was highly correlated with the regional density of muscarinic receptor sites determined *in vitro* in the same animals by autoradiographic analysis of [³H]quinuclidinyl benzilate binding. Brain regions of highest receptor density were generally correlated with known terminal fields of cholinergic neurons, and they demonstrated a protracted time course of *in vivo* labeling. An exception was noted in the basal pons, where a receptor population of high density without documented cholinergic innervation was rapidly labeled. It is suggested that synaptic muscarinic receptors are labeled slowly, as a consequence of either restricted tracer accessibility or competition between tracer and endogenous acetylcholine for available binding sites, and that the pontine receptors may be functionally distinct from those in other brain regions. The *in vivo* equilibrium binding technique used in the present study results in regional tissue radioligand concentrations directly proportional to receptor density and may, thus, provide a basis for receptor imaging in the human brain by means of positron emission tomography.

Considerable progress toward an integrated understanding of the structure and function of the central nervous system may be attributed to the use of radioligand binding techniques to study the regional distributions and properties of neurotransmitter receptors. Application of combined neurochemical and neuroanatomic analysis to postmortem studies of human neurological disorders, as well as to *in vitro* studies in animal models of these diseases, has led to the implication of specific, neurochemically defined pathologic alterations in Parkinson's disease (Reisine et al., 1977; Hornykiewicz, 1982), Huntington's disease (Enna et al., 1976; Wastek and Yama-

mura, 1978), schizophrenia (Lee et al., 1978), and Alzheimer's disease (Perry et al., 1977; Whitehouse et al., 1981; Terry and Katzman, 1983). If *in vitro* ligand binding techniques could be extended to noninvasive clinical research using positron emission tomography (Raichle, 1979), it would undoubtedly provide additional insight into the relationship between synaptic neuropharmacology and the progression of these diseases or their response to therapeutic agents.

In a previous report (Frey et al., 1985) a general model for interpretation of *in vivo* radioligand distribution was presented and validated with the use of [³H]scopolamine to measure muscarinic cholinergic receptors in the rat brain. An equilibrium solution to the model accurately predicted regional brain tracer distribution 4 hr after establishment of constant arterial plasma levels of approximately 20 nM. Under these conditions, specific as well as total *in vivo* scopolamine binding in dissected brain regions was highly correlated with *in vitro* muscarinic receptor density measurements. It was further demonstrated that scopolamine metabolites accounted for less than 10% of the nonvolatile brain radioactivity. These findings suggested the feasibility of autoradiographic imaging of the ligand distribution. In the present study, quantitative autoradiography with a tritium-sensitive film is employed to further define the relationship between *in vivo* scopolamine binding and muscarinic receptor density in rat brain at a histological level of resolution.

Materials and Methods

Scopolamine was purchased from Sigma Chemical Co. (St. Louis, MO). Ibotenic acid was obtained from Regis Chemical Co. (Morton Grove, IL). Tritiated scopolamine (specific activity, ~90 Ci/mmol) was obtained by custom synthesis from Amersham Corp. (Arlington Heights, IL) and was adjusted to a specific activity of 30 Ci/mmol by addition of unlabeled scopolamine. Tritiated (–)-quinuclidinyl benzilate (QNB) (specific activity, 33.1 Ci/mmol) and En³hance, a fluorographic enhancing agent for autoradiography, were obtained from New England Nuclear (Boston, MA). Male Sprague-Dawley rats weighing between 180 and 200 gm were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN).

In vivo scopolamine distribution. Cannulations of a femoral artery and vein were performed in rats under light diethyl ether anesthesia. Animals were allowed to recover for 3 to 4 hr before initiation of binding experiments. [³H]Scopolamine was administered intravenously as a bolus of unit magnitude followed by infusion at a rate of 0.04 unit/min. A total of 15 mCi/kg (0.5 μmol/kg) was given over 240 min. This protocol has been shown previously to result in constant arterial plasma levels of scopolamine of approximately 20 nM, which saturate cerebral muscarinic receptors (Frey et al., 1985). Timed samples of arterial plasma were obtained for measurement of [³H]scopolamine and labeled metabolites. At the termination of the infusion period, animals were killed by decapitation and samples of whole blood, plasma, and liver were obtained. The brain was rapidly dissected and a portion of the frontal pole was removed for chromatographic analysis. The remainder was divided coronally, caudal to the inferior colliculus, and the two tissue blocks were mounted on brass microtome chucks, frozen with crushed dry ice, and stored at –70°C.

Received October 12, 1984; Revised January 25, 1985;
Accepted February 8, 1985

¹ This work was supported by National Institutes of Health Grant 15655, The Wiley T. Buchanan Research Fund for the Study of Alzheimer's Disease and Related Dementias, and by a grant from the Michigan Memorial Phoenix Fund, University of Michigan. K. A. F. was a trainee under National Institutes of Health Grant 1 T32 GM07863.

² To whom correspondence should be addressed, at Neuroscience Laboratory Building, The University of Michigan, 1103 East Huron, Ann Arbor, MI 48109.

Coronal sections of the brains were cut in a cryostat at -15°C and mounted on subbed microscope slides. Tissue sections for autoradiography of *in vivo* scopolamine binding were rapidly desiccated on a hotplate at 70 to 80°C to minimize diffusion of tracer. Immediately adjacent sections were thaw-mounted on slides, air-dried, and stored at -20°C for subsequent *in vitro* muscarinic receptor autoradiography.

***In vitro* muscarinic receptor autoradiography.** *In vitro* muscarinic receptor binding to intact tissue sections was performed as described previously (Fisher et al., 1981; Wamsley et al., 1981) with minor modifications. Slide-mounted sections were washed for a minimum of 90 min at 25°C in phosphate-buffered saline (PBS), pH 7.4, containing 1 mM EDTA. Slides were then incubated in buffer containing 1 nM [^3H]QNB for 90 min at 25°C , followed by two successive 5-min rinses in fresh buffer at 0°C . Slides were dipped briefly in distilled water to remove excess buffer salts and then air-dried at room temperature. Under these conditions, more than 95% of the [^3H]scopolamine present from the *in vivo* tracer infusion was removed during the wash and incubation with QNB. Specific binding of QNB was greater than 95% of the total activity present as determined by addition of $1\text{ }\mu\text{M}$ atropine to the incubation buffer.

Quantitative ^3H autoradiography. Representative sections from the *in vivo* and *in vitro* ligand distribution protocols described above were mounted in x-ray cassettes and apposed to LKB Ultrathin film for 14 to 21 days. Plastic radioactive standards which had been calibrated against ^3H -containing tissue standards were included in the cassettes to allow densitometric quantitation of the autoradiograms as described previously (Pan et al., 1983). Isotope concentrations in brain regions underlying the autoradiographic film were determined by means of a computer-assisted spot densitometer (Dauth et al., 1983). Densitometric readings from the images produced by the standards were used to construct a standard curve relating film density to tissue isotope concentration. Regional brain isotope concentrations were calculated based on the densities of the autoradiographic images overlying the regions of interest in comparison to labeled standards on the same film. Selected sections were additionally evaluated with a computer-based scanning densitometer similar to the system described by Gooch et al. (1980).

Determination of [^3H]scopolamine and metabolites. Tissue concentrations of [^3H]scopolamine were determined as described previously (Frey et al., 1985). Brain samples were weighed and homogenized in 10 vol of 80% ethanol and centrifuged at $15,000 \times g$ for 10 min. Plasma samples were mixed with 3 vol of 95% ethanol and centrifuged. Aliquots of the ethanol supernatants were analyzed for nonvolatile radioactivity following drying at 80°C . Scopolamine content of the nonvolatile fraction was determined by thin-layer chromatography. Aliquots of the ethanol supernatants were concentrated by vacuum desiccation and chromatographed with carrier scopolamine (0.3 mg) on silica gel plates coated with a fluorescent indicator (Merck F 254), using the solvent system tetrahydrofuran-diisopropylethylamine, 95:5. Scopolamine was located by fluorescence quench and quantitated by scintillation counting. Selected plates were sprayed with En 3 hance and exposed to Kodak XRP-5 x-ray film for 1 to 4 weeks.

Striatal lesions. Unilateral striatal lesions were produced by stereotaxic injection of the excitatory neurotoxin ibotenic acid. Animals were anesthetized with diethyl ether and mounted in a stereotaxic frame with reference planes according to the atlas of König and Klippel (1963). Twenty micrograms of ibotenic acid in a volume of $1\text{ }\mu\text{l}$ were injected over a period of 8 min through a 30 gauge steel cannula positioned at the following coordinates: 1.0 mm anterior to the bregma, 2.6 mm lateral to the midline, and 5.5 mm below the dura. The cannula was left in place for an additional 2 min following the injection to minimize reflux of ibotenate up the cannula track.

Results

Autoradiography of *in vivo* scopolamine distribution. *In vivo* binding of scopolamine 240 min after initiation of the tracer infusion revealed regional variations consistent with previous regional dissection experiments (Frey et al., 1985). As anticipated, further heterogeneity in tracer distribution was observed within discrete anatomic regions (Fig. 1). In the rostral forebrain, accumulation of tracer was highest in the striatum, nucleus accumbens, olfactory tubercle, nucleus of the lateral olfactory tract, and neocortex, with lower levels of uptake observed in the septal region and globus pallidus. Tracer uptake in myelinated fiber tracts including the corpus callosum, anterior commissure, and internal capsule was considerably lower than in gray matter.

Within the cortex, the distribution of label was densest over the most superficial layers (layers I to III), lowest over the internal granular and ganglionic layers (layers IV and V), and intermediate in the

multiform layer (layer VI). This relationship was maintained throughout the neocortex irrespective of local cytoarchitectonic differences between motor, sensory, or associative areas.

The distribution of tracer within the hippocampal formation was heterogeneous based on the cytological stratification of cells, fibers, and synapses, as well as on position along the longitudinal axis. The greatest density of labeling was thus observed over the stratum oriens within the hippocampus proper and over the molecular layer within the dentate gyrus. Relatively little binding was observed over cellular regions, such as the hippocampal stratum pyramidale or the granule cell layer in the dentate gyrus. Along the longitudinal axis of the hippocampal formation, the density of tracer accumulation was greater in the dorsal than in the ventral portion. Labeling was heavier in subiculum, CA1, and the dentate gyrus than in CA3.

Within the amygdala, the corticomedial portion demonstrated considerably less tracer labeling than did the basolateral division.

The hypothalamus and thalamus possessed relatively uniform and modest levels of tracer uptake. An exception to this generalization was dense labeling observed over the anteroventral nucleus and portions of the intralaminar thalamic nuclei.

Like the pattern observed in the diencephalon, the majority of brainstem structures were modestly labeled. Notable exceptions with denser labeling included the superficial layer of the superior colliculus, the periaqueductal region, the nuclei of cranial nerves supplying motor innervation, and the basal pontine nuclei. Tracer uptake in the cerebellum was relatively low and diffuse.

Although the results of *in vitro* receptor autoradiographic experiments (Fig. 2) appeared to be in striking agreement with the observed distribution of [^3H]scopolamine described above, a difference between the two distributions was seen in brain regions containing relatively low densities of binding sites, such as in white matter and cerebellar cortex. In these regions, the density observed in the *in vitro* receptor autoradiograms was considerably lower than that in the corresponding *in vivo* sections.

A relationship between local muscarinic receptor density and *in vivo* tracer distribution was supported by quantitative microdensitometry of the autoradiograms (Table I). The correlation between *in vivo* binding of [^3H]scopolamine 240 min after initiation of the infusion and the *in vitro* binding of [^3H]QNB was linear over a 10-fold range of receptor densities (see Fig. 4A). By contrast, the correlation between the same *in vivo* tracer distribution and rates of local cerebral blood flow (Sakurada et al., 1978) was poor ($r^2 = 0.21$).

The relationship between *in vivo* tracer accumulation and *in vitro* receptor binding was additionally evaluated after 60 min of tracer infusion. The results of these autoradiographic experiments differed significantly from those obtained 240 min after initiation of the infusion, in that the distribution of [^3H]scopolamine at 60 min was more homogeneous (Fig. 3). Comparison of this tracer distribution with regional values of *in vitro* receptor density indicated that the regions of highest binding capacity (i.e., cerebral cortex, striatum) were relatively further from the line of regression calculated from the relationship observed at 240 min than were regions with lower receptor densities (Fig. 4B). An exception was noted in the basal pontine nuclei, which were equally labeled at 60 and 240 min.

Effect of striatal lesion. Three weeks after striatal ibotenic acid lesion, the *in vivo* accumulation of [^3H]scopolamine in the lesioned striatum was markedly reduced compared to the contralateral side. Autoradiographic imaging of the tracer distribution in a lesioned animal demonstrated excellent correlation between the region of decreased *in vivo* binding and the loss of muscarinic receptors determined *in vitro* (Fig. 5).

Metabolism of scopolamine. A detailed study of the nonvolatile metabolites of [^3H]scopolamine was conducted in animals used for autoradiographic binding experiments. Samples of plasma, whole blood, brain, and liver were analyzed by thin layer chromatography followed by autoradiography of the chromatograms (Fig. 6). More than 95% of the nonvolatile activity recovered from cerebral cortex was accounted for by [^3H]scopolamine. In plasma, scopolamine

FIG 1

Figure 1. Autoradiographic distribution of $[^3\text{H}]$ scopolamine in rat brain following a 240-min infusion. Coronal sections from levels between the head of the caudate nucleus and the medulla are shown. Computer-assisted scanning densitometry was used to convert autoradiographic density to pseudocolor images representing regional tissue isotope concentration in dpm/ μg of protein according to the color key at the lower right.

Figure 2. Autoradiographic comparison of *in vivo* and *in vitro* muscarinic binding in rat brain. The *top* row of coronal brain sections represents distribution of [^3H]scopolamine (specific activity, 30 Ci/mmol) after a 240-min *in vivo* infusion. The *bottom* row shows adjacent sections from the same animal processed for *in vitro* muscarinic receptor autoradiography using [^3H]QNB (specific activity, 33.1 Ci/mmol). Computer-assisted scanning densitometry was used to convert autoradiographic density to pseudocolor images representing regional tissue isotope concentration in dpm/ μg of protein according to the color keys (*top* row, key to right; *bottom* row, key to left).

Figure 3. Autoradiographic distribution of [^3H]scopolamine following a 60-min infusion. Coronal sections of rat brain from levels between the head of the caudate nucleus and the medulla are shown. Computer-assisted scanning densitometry was used to convert autoradiographic density to pseudocolor images representing regional tissue isotope concentration in dpm/ μg of protein according to the color key at the lower right.

TABLE 1

Quantitative comparison of *in vivo* and *in vitro* muscarinic receptor autoradiography

Isotope concentrations were determined by quantitative densitometry of autoradiograms obtained following *in vivo* infusion of [³H]scopolamine for 240 min and from adjacent tissue processed for *in vitro* receptor autoradiography using [³H]QNB. Values represent the mean $\pm$ SEM of three animals.

	<i>In Vivo</i> Binding	<i>In Vitro</i> Binding
	nmol/gm of protein	
Striatum	1.92 $\pm$ 0.09	2.41 $\pm$ 0.05
Frontal cortex	1.70 $\pm$ 0.03	2.04 $\pm$ 0.04
Lateral septal nucleus	1.14 $\pm$ 0.06	1.31 $\pm$ 0.05
Globus pallidus	0.29 $\pm$ 0.02	0.22 $\pm$ 0.01
Ventral thalamus	0.68 $\pm$ 0.03	0.60 $\pm$ 0.04
Cortical amygdala	1.08 $\pm$ 0.03	1.27 $\pm$ 0.03
Lateral amygdala	1.79 $\pm$ 0.03	2.29 $\pm$ 0.04
Posterior thalamus	0.98 $\pm$ 0.02	1.03 $\pm$ 0.05
Lateral geniculate nucleus	0.87 $\pm$ 0.03	0.99 $\pm$ 0.07
Medial geniculate nucleus	0.87 $\pm$ 0.05	0.98 $\pm$ 0.03
Substantia nigra	0.51 $\pm$ 0.03	0.48 $\pm$ 0.04
Periaqueductal gray	0.96 $\pm$ 0.03	1.12 $\pm$ 0.03
Pontine nuclei	1.61 $\pm$ 0.09	1.71 $\pm$ 0.10
Cerebellar cortex	0.26 $\pm$ 0.02	0.16 $\pm$ 0.01
Facial nucleus	1.22 $\pm$ 0.17	1.33 $\pm$ 0.04
Hypoglossal nucleus	1.41 $\pm$ 0.03	1.62 $\pm$ 0.05

represented approximately 50% of the nonvolatile activity and in samples of liver it represented less than 10%. The time courses of labeled scopolamine as well as of two major metabolites in plasma are presented in Figure 7. Both labeled metabolites approached apparent steady-state levels after 60 min, suggesting a balance between production on the one hand and excretion or further metabolic degradation on the other.

Discussion

Relationship between *in vivo* scopolamine binding and muscarinic receptor density. Results obtained in the present autoradiographic experiments confirm the findings of previous regional dissection studies (Frey et al., 1985) and extend them to a level approaching light microscopic resolution. Both the pattern and levels of *in vivo* [³H]scopolamine binding are highly correlated with *in vitro* muscarinic receptor densities determined with [³H]QNB. However, several potential sources of error may influence the interpretation of the present results. First, although the global ratio of specific to total *in vivo* scopolamine binding was expected to be high, nonspecific binding may have contributed substantially to tracer uptake in regions with low receptor densities. Since rinsing of tissue sections

following *in vitro* [³H]QNB binding removes the bulk of nonspecific ligand binding, the line of regression relating *in vivo* and *in vitro* binding does not pass through the origin.

Although quantitative autoradiography has been employed extensively in the past in the study of the cerebral distribution of β^- -emitting radiotracers (Freygang and Sokoloff, 1958; Reivich et al., 1969; Sokoloff et al., 1977), application of the method to measurement of tritiated compounds has emerged only recently (Faraco-Cantin et al., 1980; Penney et al., 1981). A major distinction between quantitation of tritium and higher energy isotopes is that the β^- -range of the former is short compared to the thickness of tissue sections, compared with that of ¹⁴C, ³⁵S, ³²P, or ¹³¹I. Thus, although less important in quantitation of higher energy isotopes, regional differences in tissue density may influence the apparent concentrations of tritium in brain underlying the autoradiographic film. In particular, it has been demonstrated that the differential lipid contents of gray and white matter contribute to a relative underestimation of tritium concentration of approximately 60% in the latter tissue (Alexander et al., 1981). Thus, a portion of the apparent anatomic correlation between *in vivo* and *in vitro* tracer autoradiograms presented here may reflect regional lipid content. Additionally, it may be speculated that rinsing of unfixed, slide-mounted tissue sections alters tissue attenuation properties by removal of water-soluble substances. This effect may explain in part a greater apparent binding of [³H]QNB than of [³H]scopolamine in regions of high receptor density. Notwithstanding these considerations, the most significant influence on equilibrium binding of scopolamine appears to be the local muscarinic receptor concentration. The autoradiographic results obtained in the present study demonstrate a linear relationship between muscarinic receptor density and the *in vivo* equilibrium distribution of [³H]scopolamine over a 10-fold range of receptor densities in the various brain regions examined.

Metabolism of scopolamine. Several labeled metabolites of [N-methyl-³H]scopolamine have been observed following intravenous administration. In a previous study, the predominant metabolite fraction in plasma and brain was detected as volatile radioactivity (Frey et al., 1985). The probable source of volatile ³H from the [N-methyl-³H]scopolamine used is the result of N-demethylation and subsequent oxidation of labeled formaldehyde to [³H]H₂O and formate, although tritium exchange as a source of labeled water cannot be ruled out. In the present study, several nonvolatile metabolites were observed by thin layer chromatography of tissue extracts. Although none were characterized further, the migration pattern of these compounds indicates increased polarity, suggesting oxidation to acidic forms, glucuronide conjugation, or both. Since more polar compounds than the parent molecule are essentially excluded from entry into the brain, we interpret nonvolatile activity in the brain as indicative of [³H]scopolamine content.

Figure 4. Relationship between muscarinic receptor density and *in vivo* scopolamine binding. A, Data represent quantitative densitometric measurements of *in vitro* receptor density using [³H]QNB and *in vivo* accumulation of [³H]scopolamine following a 240-min infusion within structures in adjacent sections from the same experimental animals ($N = 3$). Data points were fit by linear regression to the following equation: $QNB = -14.9 + 1.47(\text{scopolamine})$, $r^2 = 0.99$. B, Data represent quantitative densitometric measurements of *in vitro* receptor density using [³H]QNB and *in vivo* accumulation of [³H]scopolamine following a 60-min infusion within structures in adjacent sections from the same experimental animals ($N = 2$). The lines of regression calculated for the relationship between *in vivo* and *in vitro* receptor autoradiography following 240 min of tracer infusion (A) is shown for comparison.

Figure 5. Autoradiographic comparison of *in vivo* and *in vitro* muscarinic receptor binding 3 weeks after striatal lesion. A, The top coronal brain section represents distribution of [^3H]scopolamine (specific activity, 30 Ci/mmol) after 240 min of *in vivo* infusion. B shows an adjacent section from the same animal processed for *in vitro* muscarinic receptor autoradiography using [^3H]QNB (specific activity, 33.1 Ci/mmol).

Relationship of muscarinic receptors to cholinergic innervation. The regional distribution of muscarinic receptors determined both *in vitro* and *in vivo* in the present work is in general agreement with previous regional dissection (Kobayashi et al., 1978), as well as autoradiographic *in vitro* studies (Rotter et al., 1979a, b; Wamsley et al., 1980, 1981). A close correspondence between areas of high receptor density and the distribution of terminal fields of cholinergic neurons is observed in most brain regions. Thus, receptors observed in the olfactory tubercle, neocortex, hippocampus, and basolateral amygdala may represent the anticipated postsynaptic sites innervated by the basal forebrain cholinergic system (Johnston et al., 1981; Fibiger, 1982; McKinney et al., 1983; Mesulam et al., 1983). Similarly, the observed receptor density in the striatum correlates well with the presence of cholinergic interneurons, the presence of which accounts for the high levels of choline acetyltransferase activity and acetylcholine in the basal ganglia (McGeer et al., 1971). Although receptor densities in the thalamic nuclei are generally lower than those discussed above, the findings again appear to correlate with the reported density of cholinergic terminals (Mesulam et al., 1983). Thus, nuclei with documented cholinergic innervation, e.g., the anteroventral and intralaminar nuclei, have significantly greater receptor densities than do the surrounding nuclei. In the brainstem and spinal cord, the receptor densities associated with the motor neuron pools may correspond to synapses of recurrent collaterals of the motor neurons on inhibitory interneurons (Renshaw cells).

Figure 6. Chromatographic analysis of nonvolatile radioactivity in various tissues following infusion of [^3H]scopolamine. A representative autoradiogram of the distribution of scopolamine (band V) and various nonvolatile metabolites following extraction from tissues and thin layer chromatography. The animal received 3 mCi of [^3H]scopolamine at a specific activity of 30 Ci/mmol over 240 min.

Figure 7. Time course of nonvolatile radioactivity in plasma during *in vivo* infusion of [^3H]scopolamine. The time courses of scopolamine (●) and two metabolites or groups of metabolites (○, band I; and ◐, band IV, from Fig. 6) are shown from a representative animal over a 240-min infusion period.

The binding observed in the basal pontine nuclei does not correspond to any known cholinergic innervation. It is interesting to note, however, that these binding sites are predominantly of the high affinity agonist type when studied by *in vitro* homogenate techniques, as opposed to the former receptor locations which demonstrate a large proportion of the low affinity receptor conformation (Birdsall et al., 1980). The binding sites in the pons thus may be functionally distinct from those in the forebrain.

Time course of equilibration of scopolamine with muscarinic receptors. In the autoradiographic imaging studies performed at 60 min after initiation of the infusion, it was observed that the brain regions of greatest *in vitro* receptor density diverged more from the expected labeling levels at 240 min than did regions of intermediate

and low receptor density. A notable exception was the rapid binding observed in the basal pons, which appeared to have reached equilibrium within 60 min. These observations, combined with evidence discussed previously on the correlation between receptor density and cholinergic innervation, support the notion that presumptive synaptic receptors are labeled slowly, either as a consequence of restricted tracer access or because of the presence of endogenous transmitter.

Equilibrium binding method. The static equilibrium approach to quantitation of regional receptor density has several theoretical advantages. The method is based on relatively few assumptions with regard to tracer distribution within the blood and tissue compartments. It is assumed that the only saturable tracer environment in brain samples represents specific binding to the receptor of interest, and that all other compartments are not saturable over the range of ligand concentrations used. Perhaps the principal advantage of this experimental approach is that the tracer distribution is directly proportional to receptor density under conditions which minimize the contribution of nonspecific binding, as was achieved in the present study, demonstrating the autoradiographic distribution of [^3H]scopolamine in the rat brain. With some ligand-receptor pairs, the method may be limited by the necessity to employ saturating ligand concentrations for a protracted period of time in order to approach equilibrium. This is the case for scopolamine and muscarinic receptors in the brain. Thus, the equilibrium approach to imaging muscarinic binding is of limited value from the standpoint of clinical research applications because of the toxicity associated with saturating anticholinergic drug levels in the human, as well as the relatively short isotopic half-lives of potential positron-emitting forms of scopolamine. Positron-labeled scopolamine may nevertheless prove to be a useful imaging agent in humans when coupled with non-equilibrium methods (Mintun et al., 1984; K. A. Frey, R. D. Hichwa, R. L. E. Ehrenkaufer, and B. W. Agranoff, manuscript in preparation). A favorable property of positron emission tomography scanning is the ready availability of kinetic data from multiple regions required for these dynamic studies. This possibility is rendered attractive by a reported [^{11}C]scopolamine synthesis (Vora et al., 1983) and by the present results, since they validate specificity of ligand binding under *in vivo* conditions.

The rate of equilibration between plasma and receptor-bound pools of tracer may be substantially greater for ligand-receptor pairs other than scopolamine and the muscarinic receptor. Additionally, significant levels of receptor occupancy may be tolerated in the clinical estimation of binding of ligands such as opiate antagonists or benzodiazepines, based on the general clinical safety of these agents. The equilibrium receptor imaging approach presented here may therefore have application to the clinical investigation of other receptor-ligand interactions in the central nervous system.

References

- Alexander, G. M., R. J. Schwartzman, R. D. Bell, J. Yu, and A. Renthal (1981) Quantitative measurement of local cerebral metabolic rate for glucose utilizing tritiated 2-deoxyglucose. *Brain Res.* 223: 59–67.
- Birdsall, N. J. M., E. C. Hulme, and A. Burgen (1980) The character of the muscarinic receptors in different regions of the rat brain. *Proc. R. Soc. Lond. (Biol.)* 207: 1–12.
- Dauth, G. W., K. A. Frey, and S. Gilman (1983) A densitometer for quantitative autoradiography. *J. Neurosci. Methods* 9: 243–251.
- Enna, S. J., J. P. Bennett, Jr., D. B. Bylund, S. H. Snyder, E. D. Bird, and L. L. Iverson (1976) Alterations of brain neurotransmitter receptor binding in Huntington's chorea. *Brain Res.* 116: 531–537.
- Faraco-Cantin, F., J. Courville, and J. P. Lund (1980) Methods for ^3H -2-deoxyglucose autoradiography on film and fine-grain emulsions. *Stain Technol.* 55: 247–252.
- Fibiger, H. C. (1982) The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res. Rev.* 4: 327–388.
- Fisher, S. K., K. A. Frey, and B. W. Agranoff (1981) Loss of muscarinic receptors and of stimulated phospholipid labeling in ibotenate-treated hippocampus. *J. Neurosci.* 1: 1407–1413.
- Frey, K. A., R. L. E. Ehrenkaufer, S. Beaucage, and B. W. Agranoff (1985) Quantitative *in vivo* receptor binding. I. Theory and application to the muscarinic cholinergic receptor. *J. Neurosci.* 5: 421–428.
- Freygang, W. H., and L. Sokoloff (1958) Quantitative measurements of regional circulation in the central nervous system by the use of radioactive inert gas. *Adv. Biol. Med. Physiol.* 6: 263–279.
- Gooch, C., W. Rasband, and L. Sokoloff (1980) Computerized densitometry and color coding of [^{14}C]deoxyglucose autoradiographs. *Ann. Neurol.* 7: 359–370.
- Hornykiewicz, O. (1982) Brain neurotransmitter changes in Parkinson's disease. In *Movement Disorders*. Vol. 2: *Neurology*, C. D. Marsden and S. Fahn, eds., pp. 41–58, Butterworth Scientific, London.
- Johnston, M. V., M. McKinney, and J. T. Coyle (1981) Neocortical cholinergic innervation: A description of extrinsic and intrinsic components in the rat. *Exp. Brain Res.* 43: 159–172.
- Kobayashi, R. M., M. Palkovits, R. E. Hruska, R. Rothschild, and H. I. Yamamura (1978) Regional distribution of muscarinic cholinergic receptors in rat brain. *Brain Res.* 154: 13–23.
- Konig, J. F. R., and R. A. Klippel (1963) *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brainstem*, Robert E. Krieger Publishing Co., Inc., New York.
- Lee, T., P. Seeman, W. W. Tourtellotte, I. J. Farley, and O. Hornykiewicz (1978) Binding of ^3H -neuroleptics and ^3H -apomorphine in schizophrenic brains. *Nature* 274: 897–900.
- McGeer, P. L., E. G. McGeer, H. C. Fibiger, and V. Wickson (1971) Neostriatal choline acetylase and cholinesterase following selective brain lesions. *Brain Res.* 35: 308–314.
- McKinney, M., J. T. Coyle, and J. C. Hedreen (1983) Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. *J. Comp. Neurol.* 217: 103–121.
- Mesulam, M.-M., E. J. Mufson, B. H. Wainer, and A. I. Levy (1983) Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* 10: 1185–1201.
- Mintun, M. A., M. E. Raichle, M. R. Kilbourn, G. F. Wooten, and M. J. Welch (1984) A quantitative model for the *in vivo* assessment of drug binding sites with positron emission tomography. *Ann. Neurol.* 15: 217–227.
- Pan, H. S., K. A. Frey, A. B. Young, and J. B. Penney, Jr. (1983) Changes in [^3H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. *J. Neurosci.* 3: 1189–1198.
- Penney, J. B., Jr., H. S. Pan, A. B. Young, K. A. Frey, and G. W. Dauth (1981) Quantitative autoradiography of [^3H]muscimol binding in rat brain. *Science* 214: 1036–1038.
- Perry, E. K., R. H. Perry, G. Blessed, and B. E. Tomlinson (1977) Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* 1: 189.
- Raichle, M. E. (1979) Quantitative *in vivo* autoradiography with positron emission tomography. *Brain Res. Rev.* 1: 47–68.
- Reisine, T. D., J. Z. Fields, H. I. Yamamura, E. D. Bird, E. Spokes, P. S. Schreiner, and S. J. Enna (1977) Neurotransmitter receptor alterations in Parkinson's disease. *Life Sci.* 21: 335–344.
- Reivich, M., J. Jehle, L. Sokoloff, and S. S. Kety (1969) Measurement of regional cerebral blood flow with antipyrine- ^{14}C in awake cats. *J. Appl. Physiol.* 27: 296–300.
- Rotter, A., N. J. M. Birdsall, A. S. V. Burgen, P. M. Field, E. C. Hulme, and G. Raisman (1979a) Muscarinic receptors in the central nervous system of the rat. I. Technique for autoradiographic localization of the binding of [^3H]propylbenzylcholine mustard and its distribution in forebrain. *Brain Res. Rev.* 1: 141–165.
- Rotter, A., N. J. M. Birdsall, P. M. Field, and G. Raisman (1979b) Muscarinic receptors in the central nervous system of the rat. II. Distribution of binding of [^3H]propylbenzylcholine mustard in the midbrain and hindbrain. *Brain Res. Rev.* 1: 167–183.
- Sakurada, O., C. Kennedy, J. Jehle, J. D. Brown, G. L. Carbin, and L. Sokoloff (1978) Measurement of local cerebral blood flow with iodo- ^{14}C antipyrine. *Am. J. Physiol.* 234: H59–H66.
- Sokoloff, L., M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, and M. Shinohara (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* 28: 897–916.
- Terry, R. D., and R. Katzman (1983) Senile dementia of the Alzheimer type. *Ann. Neurol.* 14: 497–506.
- Vora, M. M., R. D. Finn, T. E. Boothe, D. R. Liskowsky, and L. T. Potter (1983) [^3H]-N-methyl- ^{11}C -scopolamine: Synthesis and distribution in rat brain. *J. Labeled Compd. Radiopharm.* 20: 1229–1236.

- Wamsley, J. K., M. A. Zarbin, N. J. M. Birdsall, and M. J. Kuhar (1980) Muscarinic cholinergic receptors: Autoradiographic localization of high and low affinity agonist binding sites. *Brain Res.* 200: 1-12.
- Wamsley, J. K., M. S. Lewis, W. S. Young, III, and M. J. Kuhar (1981) Autoradiographic localization of muscarinic cholinergic receptors in rat brainstem. *J. Neurosci.* 1: 176-191.
- Wastek, G. J., and H. I. Yamamura (1978) Biochemical characterization of the muscarinic cholinergic receptor in human brain: Alterations in Huntington's disease. *Mol. Pharmacol.* 14: 768-780.
- Whitehouse, P. J., D. L. Price, A. W. Clark, J. T. Coyle, and M. R. DeLong (1981) Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* 10: 122-126.