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In vivo labeling of nicotinic acetylcholine receptors in brain with [^{3}H]epibatidine

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Abstract

In vivo imaging of nicotinic acetylcholine receptors in brain has been hampered by lack of an adequate radioligand. In the present study, [^{3}H]epibatidine was administered to mice intravenously, and its time-course in brain regions and sensitivity to blockade by nicotinic drugs were studied. The distribution of radioligand accumulation in brain, and the pharmacological characteristics of binding indicate that radiolabeled forms of epibatidine would be exceptionally promising ligands for the study of nicotinic acetylcholine receptors in vivo.

Keywords: Nicotinic acetylcholine receptor; Brain imaging; Alzheimer's disease

The nicotinic acetylcholine receptor has been implicated in various neuropathological and physiological states, including Alzheimer's disease and addiction to tobacco products. This receptor, which has been studied extensively, has been characterized by radioligand binding and electrophysiological assays; and its subunits have been cloned and sequenced (Sargent, 1993). In vivo imaging studies of nicotinic acetylcholine receptors, however, have been impeded by the lack of a suitable radioligand. Previous work has indicated that [^{3}H]*l*-nicotine (Broussolle et al., 1989) and [^{3}H]cytisine (Flesher et al., 1994) both label these receptors in brain when injected intravenously in mice. In addition, [^{11}C]nicotine has been synthesized and used in primates and human volunteers to study cerebral binding to nicotinic acetylcholine receptors in vivo (Nybäck et al., 1989). However, both radiolabeled nicotine and cytisine present limitations as in vivo tracers. A substantial proportion of their in vivo binding in brain is nonspecific (about 50% of total binding of [^{3}H]nicotine in thalamus and superior colliculus, 30% of [^{3}H]cytisine total binding in thalamus). Furthermore, [^{3}H]-nicotine administered intravenously is cleared very rapidly from the brain. Although [^{3}H]cytisine is cleared more slowly from brain than is [^{3}H]nicotine, it has a relatively low uptake in thalamus, prefrontal cortex, superior colliculus, hippocampus, and cerebellum ($< 1\%$ injected dose/gram of tissue, % ID/g).

Epibatidine (exo-2-(6-chloro-3-pyridyl)-7-azabicyclo-[2.2.1]heptane), an extract of frog skin, produces antinociception, indicating central activity after peripheral administration, and has extremely high potency for central nicotinic acetylcholine receptors in vitro (K_i about 50 pM) (Badio and Daly, 1994). These observations prompted the present investigation of the utility of [^{3}H]epibatidine for in vivo labeling of nicotinic acetylcholine receptors. In in vitro assays of membranes from rat forebrain, the radioligand binds to two populations of sites with K_d values of approximately 12 and 200 pM (Houghtling et al., 1994).

Mice received 1 μCi (~ 0.7 nmol/kg) of [^{3}H]epibatidine (specific activity 56.5 Ci/mmol, a gift to K.J.K. from S. Hurt, Dupont NEN Products, Boston, MA, USA) by tail vein injection. The time course and regional distribution of the tracer in brain (Fig. 1) and

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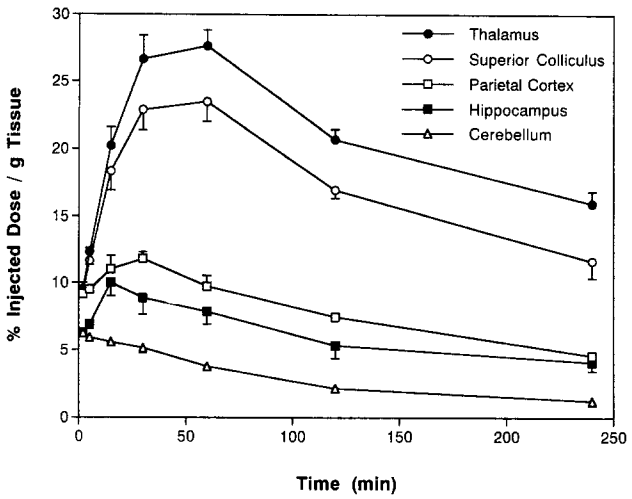

Fig. 1. Time course and regional distribution of radioactivity from [^{3}H]epibatidine, presented as percent injected dose/g tissue. Peak radioactivity was observed between 30 and 60 min after intravenous injection of the tracer, but substantial radioactivity remained for more than 4 h. The greatest concentration of radioactivity occurred in regions that are known to have high densities of nicotinic acetylcholine receptors (thalamus and superior colliculus), whereas lower concentrations were observed in regions having low to moderate densities of nicotinic acetylcholine receptors (London et al., 1985). The uptake of radiotracer could be blocked by pre-administration of nicotine or epibatidine.

susceptibility to blockade by subcutaneous administration of nicotinic agonists, prior to [^{3}H]epibatidine injection, were assessed.

[^{3}H]Epibatidine showed high uptake into whole brain (11.5% ID/g), and a regional distribution consistent with that of nicotinic acetylcholine receptors. Peak uptake occurred at 30 min and was highest in thalamus (26.7% ID/g) and superior colliculus (22.9% ID/g), intermediate in cortex (10.8% ID/g) and hippocampus (8.9% ID/g), and lowest in cerebellum (5.1% ID/g). Radioactivity declined after 30 min, but even after 4 h, a substantial amount was present in regions with high receptor densities (thalamus, 15.9% ID/g; superior colliculus, 11.7% ID/g).

Unlabeled epibatidine (5 μg/kg; −30 min), cytisine (4.5 mg/kg; −30 min) and *l*-nicotine (5 mg/kg; −5 min) each reduced the accumulation of radioactivity measured 60 min after radiotracer injection in superior colliculus from 25.5 to 7.4, 6.5, and 6.0% ID/g, respectively. The less active stereoisomer, *d*-nicotine (5 mg/kg; −5 min), and lobeline (10 mg/kg; −2 min) were less effective, reducing accumulation in the superior colliculus to ~ 13% ID/g.

[^{3}H]Epibatidine, which has high affinity for nicotinic acetylcholine receptors, also shows high uptake into brain and, compared with radiolabeled cytisine and nicotine, a slower clearance from brain, consistent with a slower dissociation from these receptors. To date, the present findings are the most promising ones obtained in studies aimed at labeling nicotinic acetylcholine receptors in vivo. Appropriately radiolabeled, epibatidine analogues should be useful for imaging studies of nicotinic acetylcholine receptors in human subjects. The structure of the epibatidine molecule presents sites for introduction of ^{11}C or ^{18}F in syntheses of radioligand probes for positron emission tomography. In addition, preparation of an iodinated analogue of epibatidine (with ^{123}I) could produce a ligand useful for imaging of nicotinic acetylcholine receptors by single photon emission computed tomography (SPECT). In this regard, such an iodinated analogue has been shown to have about one-tenth of the affinity ($K_i = 0.48$ nM) of the parent compound in in vitro assays of nicotinic acetylcholine receptors (Badio and Daly, 1994). Nonetheless, the affinity is still much greater than those of most radioligands that are used successfully for noninvasive external imaging of neurotransmitter receptors in brain. Given the slow clearance of [^{3}H]epibatidine from brain, it may be particularly convenient to perform human studies of nicotinic acetylcholine receptors with a radiolabelled iodinated analogue of epibatidine and SPECT.

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