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NICOTINIC CHOLINERGIC RECEPTORS IN RAT BRAIN

Annual Report Number 2

Kenneth J. Kellar

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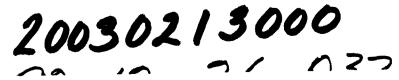
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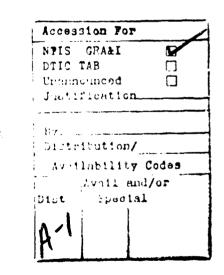
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In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (DHHS, PHS, NIH Publication No. 86-23, Revised 1985).



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During the past year we have conducted studies to:

1. Determine if the nicotinic cholinergic agonist binding site is located presynaptically on catecholamine axons where it might be involved in the regulation of release of catecholamine neurotransmitters.

2. Determine if the nicotine-induced increase in nicotinic receptors measured by binding studies results in increased behavioral responses to nicotine.

This report describes the methods that we used and the results of those studies.

1. Presynaptic location of $[{}^{3}H]$ acetylcholine recognition sites in rat brain. To determine if $[{}^{3}H]$ acetylcholine ($[{}^{3}H]$ ACh) recognition sites are located on catecholamine or serotonin axons in rat brain, male Sprague-Dawley rats (250-300 g) were anesthetized with equithesin and placed in a stereotaxic instrument. Either 6-hydroxydopamine (6-OHDA; 250 μ g) or 5,7-dihydroxytryptamine (5,7-DHT; 250 μ g) disselved in 10 μ l saline containing 0.1% ascorbic acid was then infused intraventricularly over a 5 min period. These neurotoxins selectively lesion catecholamine and serotonin axons, respectively. Control rats were infused similarly with vehicle. The rats infused with 5,7-DHT were pretreated with desipramine (25 mg/kg) 30 min before intraventricular infusions to ensure selective lesions of serotonin neurons. All rats were sacrificed by decapitation 7-10 days after surgery and the brains were dissected, frozen on dry ice, and stored at -80°C until assayed for nicotinic cholinergic receptors using the [${}^{3}H$]ACh binding assay (Schwartz <u>et al.</u>, 1982).¹ The extent and selectivity of the lesions were assessed by measuring the content of dopamine and serotonin in the striatum by high performance liquid

¹Schwartz, R.D., R.M. McGee, Jr. and K.J. Kellar: Nicotinic cholinergic receptors labeled by [³H]acetylcholine in rat brain. Mol. Pharmacol. <u>22</u>: 56-62 (1982).

chromatography (HPLC) with electrochemical detection and by measuring the uptake of [³H]norepinephrine and [³H]serotonin in synaptosomes prepared from fresh hypothalamus. These measurements indicated that 6-OH? produced a selective lesion of catecholamine axons and that 5,7-DHT produced a selective lesion of serotonin axons. Statistical analyses of data were carried out using Duncan's new multiple range test.

Following the 6-OHDA lesions of catecholamine axons, [³H]ACh binding in the striatum and hypothalamus was decreased by 30 percent and 60 percent, respectively (Table 1). Similarly, following 5,7-DHT lesions of serotonin axons, [³H]ACh binding in the striatum and hypothalamus was decreased by 30 percent and 43 percent, respectively (Table 1). In contrast to the striatum and hypothalamus, binding in the cortex and thalamus was not significantly affected by either of these lesions (Table 1).

Table 1. [³H]ACh binding in brain regions from control rats and from rats lesioned with 6-OHDA or 5,7-DHT injected intraventricularly. Assays were conducted using 10 nM [³H]ACh. Values are means \pm SEM from the number of animals indicated in parentheses².

| Brain area | | Specific binding (fmol mg protein | |
|--------------|-----------------|--------------------------------------|-----------------|
| | Control | N OHD N | S."-DHT |
| Cortex | 184 - 26141 | 362 + 19(4) | 36 () = 2.1 (4) |
| Thulamus | N 7 - 12/40 | 566 - 21 (4) | \$4.0 + 2.1 (4) |
| Striatum | 47.7 + 2.2 (8) | 33 (0 + 3 21 (6) | 112 + 1 75 (7) |
| Hypothalamus | 41 5 - 2 5 1 51 | 169 - 1 1" (6) | 250 - 30" (6) |

²Please note that [³H]ACh binding values are expressed on a per mg protein basis rather than the per mg wet weight basis used in Annual Report No. 1.

To determine whether there was a loss of binding sites or a change in affinity of the sites for [³H]ACh, saturation binding of [³H]ACh was measured in the striatum. Table 2 shows that following both lesions, the decreased binding was due to a loss of binding sites (decreased B_{max}) with no significant enange in affinity (K_d).

Table 2. Binding constants of [¹H]ACh in striatum from control, 6-OHDA, and 5,7-DHT lesioned rats. Striatal homogenates were incubated with [¹H]ACh (1-30 nM) for 40 min at 0°C and then filtered through Whatman GF/C filters. Values are the mean \pm SEM from 3 experiments.

| • | | | |
|---|------------|----------------|----------------|
| | Control | 6-OHDA | <u>5.7-DHT</u> |
| K _d (nM) | 13.0 ± 1.4 | 11.8 ± 1.3 | 10.3 ± 0.3 |
| B _{max} (fmol/mg protein) | 108.4 ± 7 | 80.9 ± 1.8" | 84.5 ± 5.5* |
| • p < 0.05. | | | |

The results of these studies indicate that nicotinic cholinergic recognition sites are located on catecholamine and serotonin axons in the striatum and in the hypothalamus but not in the cortex or thalamus. These receptors may be involved in the modulation of dopamine, norepinephrine, or serotonin release. Although nicotine has been reported to stimulate catecholamine and serotonin release from brain slices, in most of the reported studies, very high concentrations of nicotine were utilized and the pharmacology of the effects was not investigated. Nevertheless, the presence of nicotinic cholinergic recognition sites on catecholamine and serotonin axons in the striatum and hypothalamus suggest that regulation of release of neurotransmitters may be one function of these sites. In addition, the presence of these

recognition sites on axons in the hypothalamus suggests the possibility that they may also be involved in the release of hypothalamic hormones that regulate pituitary function.

2. Behavioral effects of repeated administration of nicotine. To determine whether there was a correlation between the nicotine-induced increase in [³H]ACh binding sites and behavioral effects of nicotine, the effects of repeated administration of nicotine on nicotine-induced locomotor activity were measured and compared to changes in [³H]ACh binding sites in brain.

Male Sprague-Dawley rats (250-300 g; approximately 3 months of age) were injected subcutaneously with nicotine once daily for up to 8 days. Following each injection, the rats were tested in identical cages equipped with two infrared photocell beams which were monitored by a microcomputer. Alternately breaking the two beams was counted as a cage-crossing. The rats were allowed to habituate to the photocell cages for 1 hour before each injection.

In the first experiment, the rats were injected and tested each day with 0.2 mg/kg nicotine. On two days, either mecamylamine (1 mg/kg), a nicotinic antagonist that crosses the blood-brain barrier, or hexamethonium (2 mg/kg), a nicotinic antagonist that does not readily enter the brain, was injected 20 min before the nicotune injection. The results of these benavioral studies are shown in Figure 1. Repeated treatment with nicotine induced progressively increased locomotor responses. These responses to nicotine were completely blocked by mecamylamine, but were unaffected by hexamethonium.

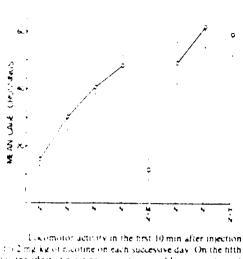
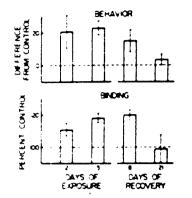


Figure 1

102 mg/kg of nucline on each successive day. On the fifth day be effect of nucline way antigonized by an injection of -mg/kg of mecany-amine (M + N) = Hexamethonium2 mg/kg was administered prior to nucline on the eighth day $(H + N) (n \neq 0)$

In the second experiment, nicotine (0.2 mg/kg) or saline was administered for 2 days or 5 days before measuring nicotine-induced locomotor activity. Other rats were treated with nicotine or saline for 5 days and the behavioral measurements were made either 8 days or 21 days after the last injection to test for recovery. Following the behavioral tests, the rats were sacrificed and the brains were dissected and frozen until assayed for nicotinic cholinergic receptor binding sites. The results of these experiments are shown in Figure 2.



Comparison of the behavioral effects of nicotine and nicotinic receptor binding under various conditions after 2 or 5 days of exposure to 0.2 mg/kg of nicotine or, after 5 days of exposure to 0.2 mg/kg of nicotine and then either 8 or 21 days without nicotine ($\alpha = 5$ for 2 and 5 day exposure groups), $\alpha = 0$ for 8 and 21 recovery groups)

Both 2 days and 5 days of nicotine treatment resulted in an increase in nicotine-induced locomotor behavior. This increased response to nicotine persisted for at least 8 days after the last of 5 daily nicotine injections, but the response returned to control levels 21 days after the last nicotine injection. [³H]ACh binding sites in the cerebral cortex were increased after both 2 days and 5 days of nicotine treatment. This increase in binding was still seen 8 days after the last of 5 daily nicotine injections, but like the behavioral response, the binding returned to control values 21 days after the last injection. Thus, there appears to be at least some correspondence between nicotine-induced increases in a behavioral response and increases in nicotinic cholinergic recognition sites 'abeled by [³H]ACh.

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