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Overview

Nicotine, in tobacco smoking concentrations, is a powerful psychoactive drug (Domino 1973; Kumar and Lader 1981; Balfour 1984). A wide variety of stimulant and depressant effects is observed in animals and humans that involves the central and peripheral nervous, cardiovascular, endocrine, gastrointestinal, and skeletal motor systems. These heterogeneous effects, along with behavioral and psychological variables, result in self-administration of tobacco, tobacco dependence, and withdrawal phenomena with abrupt cessation of tobacco smoking. This Chapter discusses sites and mechanisms of nicotine actions that may help to explain why tobacco products are self-administered.

The first Section of this Chapter provides general summaries of several major effects of nicotine in the body. Following this broad overview, the Chapter presents detailed discussions of sites and mechanisms of nicotine action that may be particularly important to understand tobacco use. Tissue distribution of nicotine, cerebral metabolic effects, and nicotine receptor binding are reviewed. Next, neuroendocrine and endocrine effects of nicotine are discussed. Then, electrophysiological effects of nicotine are presented. Finally, the effects of smoking on psychophysiological reactivity are discussed.

Peripheral Effects of Nicotine

Nicotine exerts its action on the cardiovascular, respiratory, skeletal motor, and gastrointestinal systems through stimulation of peripheral cholinergic neurons via afferent chemoreceptors and ganglia of the autonomic nervous system (ANS) (Ginzel 1967b). Inasmuch as both sympathetic and parasympathetic ganglia are stimulated by levels of nicotine derived from tobacco smoking, the end result depends on the summation of the effects of autonomic ganglion stimulation and reflex effects. The resulting peripheral physiological changes generally resemble sympathetic nervous system (SNS) arousal, but there are also some effects of nicotine and smoking that lead to physiological relaxation. For example, there is usually an increase in heart rate and blood pressure immediately following cigarette smoking. In addition, there is cutaneous vasoconstriction of the distal extremities. In contrast, nicotine can relax skeletal muscles (e.g., reduce patellar reflex) in humans and animals via effects on Renshaw cells (Domino and Von Baumgarten 1969; Ginzel and Eldred 1972; Ginzel 1987). But it also can enhance tension in some muscles (e.g., trapezius muscle) (Fagerström and Gotestam 1977). Nicotine in small doses can enhance respiration through stimulation of peripheral chemoreceptors. Yet, high nicotine doses can cause respiratory failure. (See Appendix B for a discussion of

nicotine toxicity.) The gastrointestinal effects of nicotine are complex, involving an increase in secretions and reduced motility for a short period of time.

The peripheral actions of nicotine as a cholinergic agonist have made it a valuable pharmacologic tool for studying nicotinic cholinergic actions and functioning in many physiological systems. This Chapter focuses on the mechanisms of nicotine's actions relevant to tobacco use. Several peripheral actions of nicotine, for instance muscular relaxation, may contribute to the habitual use of tobacco products (see smoking and stress in Chapter VI). However, because the central nervous system (CNS) actions of nicotine and resulting neurochemical and electrical effects mediate subsequent biological and behavioral responses, a review of these actions contributes to an understanding of the reinforcing effects of nicotine.

Central Sites of Nicotine Actions

Nicotinic binding sites or receptors in the brain have been differentiated as very high, high, and low affinity types (Shimohama et al. 1985; Sloan, Todd, Martin 1984; Sloan et al. 1985). In the rat brain, when cholinergic muscarinic receptors are blocked, the autoradiographic distribution of ^3H -acetylcholine (ACh) and ^3H -nicotine are essentially identical (Clarke and Kumar 1984; Clarke, Pert, Pert 1984). However, these brain binding sites differ from peripheral nicotinic receptors in ganglia and skeletal muscle.

Chronic nicotine administration results in up-regulation in regional rat brain ^3H -ACh binding sites measured in the presence of atropine to block the muscarinic sites (Schwartz and Kellar 1985). Up-regulation of ^3H -nicotine binding sites also has been reported after continuous nicotine infusions in mice (Marks, Burch, Collins 1983a). In contrast, most agonists that act on receptor sites in the body, when given chronically, produce a reduction (or down-regulation) in the number of receptors. Both Marks, Burch, and Collins (1983b) and Schwartz and Kellar (1983, 1985) have suggested that nicotinic cholinergic receptors undergo a functional blockade but that sufficient recovery would allow enhanced behavioral responses to low doses of nicotine to occur within 24 hr, as has been shown behaviorally by Clarke and Kumar (1983) and Ksir and coworkers (1985). This phenomenon may help to explain the tolerance to nicotine that develops with repeated exposure. However, the time course of changes in receptor number and other biological effects of nicotine must be carefully compared to determine mechanisms underlying tolerance. (See Chapter II for additional discussion.)

Several investigators have used *in vitro* autoradiography to identify ^3H -nicotine binding sites in the rat brain. These autoradiographic binding studies suggest where nicotine is acting. London, Waller, and Wamsley (1985) have found the most intense localization

of ^3H -labeled nicotine in the interpeduncular nucleus and medial habenula.

Cerebral metabolism studies also suggest key sites of action. London and colleagues (1985) have reported that nicotine stimulated local cerebral glucose utilization (LCGU) by 139 percent over that of the control in the medial habenula and by 50 to 100 percent in the superior colliculus and the anteroventral thalamic and interpeduncular nuclei. Other areas of the brain showed moderate or no significant changes. These effects of nicotine were blocked by mecamylamine, a nicotinic receptor antagonist, confirming that they acted via nicotinic receptors. Furthermore, they correlated well with the distribution of ^3H -nicotine binding in the brain except in layer IV of the neocortex, which showed nicotine binding but no change in LCGU. Sites that show increased glucose utilization after nicotine administration are probably functionally important loci of nicotinic actions. When nicotine binding and increased energy utilization both occur at a given site, it is likely to be involved in nicotine's actions.

Neuroendocrine Effects of Nicotine

Some of the actions of nicotine result from the release of ACh and other neurotransmitters, including norepinephrine (NE). Nicotinic cholinergic agonists including nicotine, carbachol, and 1,1-dimethyl-4-phenylpiperazinium (DMPP) release endogenous ACh from the presynaptic cholinergic nerve terminals in addition to stimulating postsynaptic nicotinic receptors (Chiou 1973; Chiou and Long 1969). Nicotinic agonists also release ACh from rat cerebral cortical synaptic vesicles and can release newly synthesized ^3H -ACh from synaptosomes prepared from the myenteric plexus of guinea pig ileum and from mouse cortical synapses (Briggs and Cooper 1982; Rowell and Winkler 1984). These effects are Ca^{2+} -dependent and are blocked by hexamethonium, a quaternary nicotinic receptor antagonist. In addition, nicotine-induced release of ACh in the hippocampal synaptosomes is blocked by the ion channel blocker, histrionicotoxin (Rapier et al. 1987). There is good evidence that nicotine releases ACh by a presynaptic mechanism. In contrast, presynaptic muscarinic receptors, mostly of the M_2 -subtype, inhibit ACh release. Nicotine administration increases the amounts of other chemicals in the blood and brain, including serotonin, endogenous opioid peptides, pituitary hormones, catecholamines, and vasopressin (Domino 1979; Gilman et al. 1985; Marty and colleagues 1985). These chemicals may be involved in reinforcing effects of nicotine (see Chapters IV, VI).

Electrophysiological Effects of Nicotine

Nicotine administration is accompanied by brain wave or electroencephalogram (EEG) activation in animals (Domino 1967). The EEG-activating effects of small doses of nicotine occur in intact as well as

brainstem-transected animals. Nicotine acts primarily directly on brainstem neuronal circuits to produce these effects (Domino 1967). However, stimulation of peripheral afferents (Ginzel 1987) and release of catecholamines and possibly neurotransmitters and modulators, such as serotonin or histamine, may enhance the direct central effects of nicotine.

The EEG-activating effects of nicotine result in behavioral arousal (Domino, Dren, Yamamoto 1967). In cigarette smokers, nicotine produces sedative and stimulant effects (Kumar and Lader 1981). Aceto and Martin (1982) have reviewed the large variety of nicotine effects on behavior including facilitation of memory, the increase in spontaneous motor activity, nicotine's antinociceptive properties, and its suppression of irritability. These behavioral and psychological effects are discussed in Chapters IV and VI.

Distribution and Cerebral Metabolic Effects of Nicotine

Nicotine, administered by various routes, rapidly enters the brain and also distributes to specific, peripheral organs. Nicotine produces a distinct pattern of stimulation of cerebral metabolic activity that suggests where nicotine acts in the brain. This Section reviews studies on the distribution of nicotine after its administration to experimental animals, data on the relationship between tissue levels of nicotine and the drug's biological effects, and studies on mapping the cerebral metabolic effects of nicotine in the rat brain.

Distribution of Nicotine

Tissue Distribution of Nicotine: Time Course and Other Considerations

The distribution in the body of exogenously administered nicotine has been a topic of interest for more than a century and has been reviewed several times (Larson, Haag, Silvette 1961; Larson and Silvette 1968, 1971). As early as 1851, Orfila described experiments in which he detected nicotine in various organs (e.g., liver, kidney, lungs) and in the blood of animals after nicotine administration. In the 1950s the development of radiotracer methods led to a reexamination of nicotine distribution in the body.

Werle and Meyer (1950) found that the brain, compared with other organs, contained the highest nicotine levels immediately after injection of a lethal dose in guinea pigs. Tsujimoto and colleagues (1955) found a high concentration of nicotine in the brain after the drug was administered to rabbits and dogs. Yamamoto (1955) observed that 1 hr after a subcutaneous (s.c.) injection of 5 mg/kg in the rabbit, the nicotine content was highest in the kidney. The pancreas, ileum, ventricular muscle, skeletal muscle, lung, spleen, cerebral cortex, omental fat, and liver showed progressively lower

levels of nicotine at 1 hr. None of the tissues had detectable levels at 6 hr. In the dog, the highest level at 1 hr was in the kidney, followed by the pancreas, brain, ileum, liver and omental fat, spleen, heart, muscle, and lung.

Schmitterlöw and colleagues used radiolabeled nicotine and whole-body autoradiography to study the distribution of nicotine in several species (Hansson and Schmitterlöw 1962; Appelgren, Hansson, Schmitterlöw 1962, 1963; Hansson, Hoffman, Schmitterlöw 1964; Schmitterlöw et al. 1965; Schmitterlöw et al. 1967). After radiolabeled nicotine was administered, radioactivity representing nicotine and its metabolites was concentrated in some organs, particularly the brain. Hansson and Schmitterlöw (1962) injected (S)-nicotine-methyl-¹⁴C intramuscularly or intravenously (i.v.) in mice. Within 5 min, high concentrations were found in the brain, adrenal medulla, stomach wall, and kidney. Lower concentrations were observed in the liver, skeletal muscle, and blood, but all concentrations were higher in tissue than in blood. Activity was high in the kidney from 5 min to 4 hr after the nicotine injection, with the highest activity occurring within the first hour. The adrenal medulla maintained a high concentration at 1 hr and 4 hr after injection, but little or no activity was observed at 24 hr. At 30 min, the levels were high in the walls of large blood vessels and in the bone marrow. Radioactivity disappeared rapidly from the brain.

Appelgren, Hansson, and Schmitterlöw (1962) prepared whole-body autoradiograms of mice and cats given i.v. injections of ¹⁴C-nicotine. An initial, heterogeneous accumulation of radioactivity occurred in the CNS. Fifteen minutes after the radiotracer injection, the cat brain showed distinctly more intense labeling of grey than of white matter. Also apparent was a regional distribution within grey matter areas, particularly in the hippocampus. By 30 min, radioactivity was reduced. Studies of mice demonstrated a high concentration of label in the brain at 5 min. By 30 min, the concentration was high in salivary glands, stomach contents, liver, and kidneys, while the brain was almost devoid of radioactivity. The same group also showed the accumulation of ¹⁴C-nicotine in the retina of the eye after i.v. administration (Schmitterlöw et al. 1965).

Fishman (1963) reported that in rats given randomly labeled ¹⁴C-nicotine intraperitoneally (i.p.) and killed 3 hr later, the kidney contained the highest concentration of radioactivity, followed by the lung, liver, brain, skeletal muscle, spleen, and heart. In the dog, more ¹⁴C-nicotine was present in the stomach wall than in any other tissue analyzed 3 hr after i.v. injection of radioactive nicotine.

Yamamoto, Inoki, and Iwatsubo (1967) gave mice s.c. injections of 5 mg/kg methyl-¹⁴C-nicotine. Five minutes later, they found 0.5 to 1 µg/g (wet weight) of nicotine in various brain regions, including the cerebral cortex, superior and inferior portions of the brain stem, and

the cerebellum. Highest levels were detected 5 to 10 min after injection. Maximum levels in liver and whole blood were observed 2 and 10 min, respectively, after the injection.

Yamamoto, Inoki, and Iwatsubo (1968) studied penetration of ^{14}C -nicotine in rat tissues *in vivo* and *in vitro*. They found that 5 mg/kg, *i.p.*, in male Wistar rats produced the following maximum tissue-to-blood ratios of ^{14}C -nicotine activity after 10 to 20 min: kidney, 8.7; liver, 6.7; submaxillary gland, 6.2; cerebral cortex, 3.5; brainstem, 2.4; and heart, 1.8. When they incubated tissue slices with 10^{-4} M ^{14}C -nicotine for 30 min at 37°C , the relative uptake of the label was similar: kidney cortex, 2.6; liver, 2.1; submaxillary gland, 2.1; and cerebral cortex, 2.0. Penetration in slices was unaffected by uncoupling oxidative phosphorylation or blocking metabolic pathways, indicating that the uptake was not by active transport. *In vivo*, tissue-to-blood ratios were greater than slice-to-medium ratios, indicating that a process other than passive diffusion was involved.

Because the respiratory tract is a major route by which nicotine from tobacco smoke enters the body, Schmitterl w and coworkers (1965) sprayed ^{14}C -nicotine solution directly onto the trachea of mice. Autoradiograms from mice killed at 2 min exhibited a high amount of radioactivity in the respiratory tract and lungs and showed that nicotine enters the CNS rapidly by this route as well. At 15 min, radioactivity still persisted in the lungs, was reduced in the brain, and appeared in large amounts in the kidneys and stomach.

Uptake and distribution of nicotine from tobacco smoke have also been assessed. Harris and Negroni (1965) exposed mice to cigarette smoke and extracted nicotine from the lungs (5 to 25 μg). Mattila and Airaksinen (1966) exposed guinea pigs to the smoke of one 4-g cigar over a period of 40 min, with intermittent ventilation with fresh air, and found that the same tissues which concentrated nicotine administered by other routes also showed nicotine uptake from smoke. Organ-to-blood ratios were lung, 2.0; spleen, 3.0; intestine, 2.9; and brain, 1.1.

The use of positron-emitting radiotracers permits *in vivo* estimation of nicotine uptake into the brain and other organs, offering the potential of eventually relating nicotine action in the living human brain to behavioral and disease states. Maziere and coworkers (1976) prepared (S)-nicotine-methyl- ^{11}C , which they administered by *i.v.* injection to mice and rabbits. The time course of the radiotracer confirmed earlier studies and showed a maximum concentration in the 5 min following injection, except in the liver and spleen. Highest radioactivity was in kidneys and brain, followed by liver and lungs. The brain activity dropped rapidly, whereas the kidney concentration remained high (8 percent of injected dose) at 50 min after the injection. External imaging by a γ camera showed considerable

radioactivity in the head, kidneys, and liver. Brain activity decreased sharply over 1 hr, while activity remained high in liver and kidneys.

Maziere and coworkers (1979) used ^{11}C -nicotine and positron emission tomography (PET) in baboons and found that ^{11}C -nicotine readily penetrated into the brain and then dropped sharply with time. Radioactivity was high in the temporal lobe, cerebellum, occipital cortex, pons, and medulla oblongata. There was also a high, stable radioactivity level in the retina, consistent with the earlier observation that radioactivity from ^{14}C -nicotine is found in the retina after i.v. administration (Schmitterl w et al. 1965).

Heterogeneity of Nicotine Uptake: Microautoradiographic and Subcellular Studies

Appelgren, Hansson, and Schmitterl w (1963) used a microautoradiographic method to study the localization of nicotine within the superior cervical ganglion of the cat. Most of the radioactivity was localized in the ganglion cells, with little labeling of satellite cells and connective tissue.

Schmitterl w and coworkers (1967), using microautoradiograms of mouse brains after injection of ^{14}C -nicotine and ^3H -nicotine, reported that nicotine is concentrated in nerve cells. Brain areas with a high density of nerve cells, such as the molecular and pyramidal cell layers of the hippocampus and the molecular layer of the cerebellum, contained high amounts of radioactivity.

Yamamoto, Inoki, and Iwatsubo (1967) studied accumulation of ^{14}C -nicotine into subcellular fractions (nuclear, mitochondrial, nerve ending, microsomal, soluble) of mouse brain after i.p. injection of 5 mg/kg (20 $\mu\text{Ci}/\text{kg}$). Most of the radioactivity was in the soluble fraction. Less than one-tenth of the radioactivity in the soluble fraction was found in microsomes and nerve endings; however, radioactivity levels in microsomes were somewhat higher than in nerve endings.

Effects of Nicotine on Cerebral Metabolism

Following the demonstration that ^3H -nicotine binds stereoselectively and specifically in preparations of rat brain (Yoshida and Imura 1979; Martin and Aceto 1981; Marks and Collins 1982), brain binding sites were visualized (Clarke, Pert, Pert 1984) and quantified (London, Waller, Wamsley 1985) by light microscopic autoradiography. However, mapping nicotinic binding sites or identifying specific binding sites for any drug or neurotransmitter does not necessarily mean that receptors are coupled to pharmacologic actions. An example of nonfunctional, stereoselective, specific binding is that of ^3H -naloxone to glass fiber filters (Hoffman, Altschuler, Fex 1981). In addition, because the brain is a highly interconnected organ, drugs

may produce effects in brain regions remote from their initial receptor interactions. Receptor maps would show primary binding sites but not sites where important secondary actions might occur.

Functional mapping procedures, such as the use of autoradiographic techniques to measure rates of LCGU and regional cerebral blood flow, are another way to determine the sites of the *in vivo* effects of nicotine in the brain. The 2-deoxy-D-[1-¹⁴C]glucose (2-DG) method for measuring LCGU (Sokoloff et al. 1977) has been used to demonstrate a relationship between local cerebral function and glucose utilization under a wide variety of experimental conditions, including pharmacologic treatments (Sokoloff 1981; McCulloch 1982). The effects of acute, *s.c.* injections of nicotine on LCGU were examined by London and colleagues (1985, 1986) and by London, Szikszay, and Dam (1986), while Grünwald, Schröck, and Kuschinsky (1987) measured the effects on LCGU of constant plasma levels of nicotine produced by *i.v.* infusion.

Subcutaneous injections of nicotine stimulated LCGU in specific brain regions (Table 1, Figure 1), including portions of the visual, limbic, and motor systems. Effects of nicotine infusion generally paralleled those obtained with *s.c.* injections. The greatest increase in response to *s.c.* nicotine occurred in the medial habenula. Marked increases in LCGU were noted in the anteroventral thalamic nucleus, interpeduncular nucleus, and superior colliculus. Moderate increases were seen in the retrosplenial cortex, interanteromedial thalamic nucleus, lateral geniculate body, and ventral tegmental area. No significant effects were observed in the frontoparietal cortex, lateral habenula, or central grey matter. LCGU responses to *s.c.* injection of nicotine were completely blocked by mecamylamine, indicating the specificity of nicotine effects.

The effects of nicotine on LCGU correlate well with the distributions of ³H-nicotine binding sites (Clarke, Pert, Pert 1984; London, Waller, Wamsley 1985). Areas such as the thalamic nuclei, the interpeduncular nucleus, medial habenula, and the superior colliculus, where there is dense labeling with ³H-nicotine, show moderate to marked nicotine-induced LCGU increases. Areas with less specific binding show smaller LCGU responses to nicotine, and the central grey matter, which lacks specific ³H-nicotine binding, shows no LCGU response. Similarly, nicotine dramatically increases LCGU in the medial but not the lateral habenula, reflecting different densities of ³H-nicotine binding sites. In general, ³H-nicotine binding sites visualized autoradiographically in the rat brain are functional nicotine receptors. However, layer IV of the neocortex displays significant ³H-nicotine binding, but lacks an LCGU response.

In most brain areas, significant LCGU stimulation was obtained with 0.3 mg/kg of nicotine *s.c.* (London et al. 1986), a dose similar to one used successfully in training rats to distinguish nicotine from

TABLE 1.—R,S-Nicotine effects on glucose utilization in the rat brain

Brain region	Local cerebral glucose utilization ($\mu\text{mol}/100 \text{ g tissue/minute}$)	
	Saline control	Nicotine (1.75 mg/kg)
Frontoparietal cortex, layer IV	110 \pm 8.1	108 \pm 6.5
Retrosplenial cortex, layer I	98 \pm 6.5	123 \pm 5.1 ¹
Thalamic nuclei		
Anteroventral	109 \pm 6.5	201 \pm 6.1 ¹
Interanteromedial	125 \pm 8.6	175 \pm 12.3 ¹
Lateral geniculate body	82 \pm 6.8	106 \pm 4.4 ¹
Interpeduncular nucleus	99 \pm 9.8	182 \pm 9.3 ¹
Medial habenula	70 \pm 5.2	167 \pm 3.7 ¹
Superior colliculus	72 \pm 5.2	142 \pm 4.6 ¹
Central grey matter	66 \pm 4.0	77 \pm 4.3

NOTE: Results are expressed as the means plus or minus standard deviation for four rats per group.

¹Significantly different from saline control ($p \leq 0.05$).

SOURCE: London et al. (1985).



FIGURE 1.—Effect of subcutaneous R,S-nicotine (1 mg/kg, 2 min before 2-deoxyglucose) on autoradiographic grain densities, representing glucose utilization

NOTE: Photographs of x-ray film exposed to 20- μm brain sections from control rat (A) given 0.9 percent sodium chloride (1 mL/kg) and another rat (B) given nicotine; note the increased density in medial habenula (mh) and fasciculus retroflexus (fr).

SOURCE: London et al. (1986).

saline in a T-maze apparatus (0.4 mg/kg, s.c.) (Overton 1969). Nicotine-induced stimulation of LCGU in the ventral tegmental area

and the habenular complex (London et al. 1985, 1986) may relate to the reinforcing properties of the drug (see Chapter IV). These regions of the brain have been implicated in drug- and stimulation-induced reward systems, respectively (Wise 1980; Nakajima 1984). Additional studies, using specific conditions under which nicotine is reinforcing, are needed to elucidate the anatomical loci involved in nicotine-induced reward and to identify the neurophysiological mechanisms by which nicotine acts as a reinforcer.

Nicotine Receptors

Nicotine exerts diverse pharmacologic effects in both the peripheral nervous system (PNS) and CNS. The peripheral actions of nicotine are important, and some may reinforce the self-administration of nicotine. For example, stimulation in the trachea (Rose et al. 1984) seems to be involved in some of the pleasurable effects of smoking. Skeletal muscle relaxation and electrocortical arousal, both stimulated by actions of nicotine in the lung (Ginzel 1967a,b, 1975, 1987), may contribute to habitual tobacco use (Chapter VI). However, it is generally believed that the central actions of nicotine are of primary importance in reinforcing tobacco use (Chapter IV). In animals, the neuropsychopharmacologic effects of this drug are, with few if any exceptions, mediated through central sites of action. These effects are likely to contribute to the drug's reinforcing properties in animals and humans (Clarke 1987b). In addition, the effects of nicotinic antagonists on tobacco smoking in humans (Stolerman et al. 1973) and in rhesus monkeys (Glick, Jarvik, Nakamura 1970) suggest a central site of reinforcement, but do not rule out a peripheral site. To understand these actions, it is important to know exactly where nicotine acts in the body. This Section discusses evidence for nicotine receptors.

Peripheral Nicotine Receptors

In the mammalian PNS, nicotine and muscarine mimic different actions of ACh by acting at different types of cholinergic receptors. Nicotinic cholinergic receptors (nAChRs) have been subdivided according to location and sensitivity to nicotinic antagonists. Receptors of the C6 or "ganglionic" type are found principally at autonomic ganglia, in the adrenal medulla, and at sensory nerve endings; nicotinic cholinergic transmission in autonomic ganglia is selectively blocked by hexamethonium and certain other compounds. Receptors of the "neuromuscular" type (sometimes referred to as C10 type) are located on the muscle endplate, where transmission is selectively blocked by compounds such as decamethonium and alpha-bungarotoxin (α -BTX).

Higher doses of nicotine are required to stimulate nAChRs in skeletal muscle than at autonomic ganglia. Ganglionic nAChRs appear to be more sensitive than their neuromuscular counterparts, not only to the stimulant but also to the desensitizing actions of nicotine (Paton and Savini 1968). Doses of nicotine obtained by smoking cigarettes do not appear to affect the muscle endplate directly. Therefore, if the CNS were to possess both types of nAChR, doses of nicotine obtained by normal cigarette smoking might affect only the C6-receptor population. Accordingly, many of the central effects of nicotine in vivo and in vitro are reduced or blocked by nicotinic antagonists that are C6-selective in the periphery. The most widely used C6-selective antagonist is mecamylamine, which passes freely into the CNS after systemic administration. Mecamylamine antagonizes actions of nicotine in the brain and spinal cord, as revealed by behavioral (Collins et al. 1986; Goldberg, Spealman, Goldberg 1981) and electrophysiological experiments (Ueki, Koketsu, Domino 1961) and also by studies of neurotransmitter release (Hery et al. 1977; Chesselet 1984). There have been few attempts to determine whether these central nicotinic actions are also blocked by neuromuscular antagonists, while several studies support the existence of central C6 nAChRs (Aceto, Bentley, Dembinski 1969; Brown, Docherty, Halliwell 1983; Caulfield and Higgins 1983; Egan and North 1986).

The search for putative central α -BTX nAChRs has been hindered by several factors, including the central convulsant actions of α -BTX antagonists (Cohen, Morley, Snead 1981) and the probable need to deliver locally high concentrations of nicotine. Nevertheless, several studies have demonstrated actions of nicotine or cholinergic agonists that can be reduced or blocked by α -BTX, which acts selectively at neuromuscular nAChRs (Zatz and Brownstein 1981; Farley et al. 1983; de la Garza et al. 1987a).

Radioligand Binding to Putative Nicotine Cholinergic Receptors in Mammalian Brain

Many receptors for neurotransmitters in the brain have been identified through the use of radiolabeled probes (radioligands). Attempts to label putative brain nAChRs have used compounds with known potency at peripheral sites (see Table 2).

Agonist Binding

The stereospecific, saturable, and reversible binding of ^3H -nicotine to rodent brain is well-described (Romano and Goldstein 1980; Marks and Collins 1982; Costa and Murphy 1983; Benwell and Balfour 1985a; Clarke, Pert, Pert 1984). Most studies have demonstrated the existence of a population of high-affinity binding sites (reflected by a dissociation constant in the low nanomolar range) that is potently

TABLE 2.—Radioligands for putative nicotinic cholinergic receptors in mammals

Antagonists	Bind	Functional antagonism	Sites examined	Agonists
¹²⁵ I-BTX	Yes	Yes	Muscle endplate	³ H-nicotine
	Yes	Yes	Autonomic ganglia, spinal cord	
	Yes	Yes	Brain (certain sites only)	³ H-methyl-carbachol
¹²⁵ I-naja toxin	Yes	Yes	Muscle endplate	³ H-ACh (with excess muscarinic antagonist and AChE inhibitor)
	Yes	ND ¹	Brain	
³ H-dTC	ND	Yes	Muscle, spinal cord, ganglia	
	Yes	Yes	Brain	
³ H-DHBE	ND	Yes	Muscle, autonomic ganglia	
	Yes	Yes	Brain, spinal cord	
Neosurugatoxin	ND	No	Muscle endplate	
	ND	Yes	Autonomic ganglia	
	Yes	Yes	Brain (inhibits ³ H-nicotine)	

¹ ND=no data.

inhibited by nicotinic agonists including ACh. In contrast, most nicotinic antagonists have very low affinity for this site. Binding with similar characteristics has been reported in rat brain tissue with ³H-methyl-carbachol (Abood and Grassi 1986; Boksa and Quirion 1987) and with ³H-ACh in the presence of excess atropine to prevent binding to muscarinic receptor sites (Schwartz, McGee, Kellar 1982).

In the presence of atropine, tritiated nicotine and ³H-ACh probably bind to the same population of high-affinity sites in rat brain. Thus, the two radioligands share the same neuroanatomical distribution of binding (Clarke, Schwartz et al. 1985; Marks et al. 1986; Martino-Barrows and Kellar 1987). Binding of both ligands is inhibited with similar potency by a range of nicotinic agents, is up-regulated by chronic nicotine treatment in vivo, is down-regulated by chronic treatment with acetylcholinesterase inhibitors, and is diminished by disulfide reducing agents in vitro (Marks et al. 1986; Martino-Barrows and Kellar 1987; Schwartz and Kellar 1983). Although less well studied, it appears that sites labeled by ³H-methyl-carbachol are the same as those labeled by ³H-ACh and ³H-nicotine (Abood and Grassi 1986; Boksa and Quirion 1987). High-affinity nicotine binding sites have been found in brain tissue of mice (Marks and Collins 1982), rats (Romano and Goldstein 1980), monkeys (Friedman et al. 1985), and humans (Shimohama et al. 1985; Flynn and Mash 1986; Whitehouse et al. 1986).

Some investigators have reported a second class of sites which are characterized by lower binding affinity and higher capacity for ³H-

nicotine. With no demonstrated differential anatomical distribution or stereoselectivity (Romano and Goldstein 1980; Marks and Collins 1982; Benwell and Balfour 1985b), these low-affinity sites are of questionable pharmacologic significance, but may be the result of post mortem proteolysis (Lippiello and Fernandes 1986). Careful analysis of ^3H -nicotine binding conducted in the absence of protease inhibitors has revealed the existence of five affinity sites or states (Sloan, Todd, Martin 1984). Functional studies (Martin et al. 1986) suggest that some of these different sites may represent in vivo sites of action for nicotine, although it is not clear which if any would be activated by nicotine doses obtained from typical cigarette smoking.

Radioligand Binding

Many receptors of different nicotine binding affinities have been reported. It is unclear whether these reflect different conformational states or binding sites of a single type of receptor, distinct receptor populations, or a single type of high-affinity site which has undergone proteolytic degradation. Preliminary evidence supports the existence of distinct receptor subtypes labeled by agonists. Two components of high-affinity ^3H -nicotine binding, differing in their affinity for neosurugatoxin, can be distinguished in rat brain. The relative proportion of these two components differs in different regions of the rat brain, suggesting that they are physically distinct receptors (Yamada et al. 1985).

Antagonist Binding

Most studies of nicotine binding in mammalian brain have used radioiodinated α -BTX (^{125}I -BTX), which binds with high affinity and in a saturable manner to sites in mammalian brain (Schmidt, Hunt, Polz-Tejera 1980; Oswald and Freeman 1981). This binding is selectively inhibited by nicotinic agents, including nicotine and ACh. Cobra (naja) alpha-toxin, like α -BTX, is a selective neuromuscular blocker in the mammal, and appears to label the same sites as α -BTX in mammalian brain. Binding is potently inhibited by unlabeled α -BTX and has a regional distribution resembling that of ^{125}I -BTX binding (Speth et al. 1977). The antagonist dihydro-beta-erythroidine (DHBE) binds to two sites in rat brain, but the regional distribution of binding differs from that of ^{125}I -BTX (Williams and Robinson 1984). DHBE acts with similar potency at both types of peripheral nAChR in vivo. It is not clear whether ^3H -d-tubocurarine binding is selectively inhibited by nicotinic agents. In rat brain, ^{125}I -BTX binds to a distinct population of sites that are not labeled with high affinity (nanomolar K_D) by tritiated nicotinic agonists. Radioiodinated α -BTX sites have a different neuroanatomical distribution (Marks and Collins 1982; Schwartz, McGee, Kellar 1982; Clarke, Schwartz et al.

1985) and can be physically separated from tritiated agonist binding sites by affinity chromatography (Schneider and Betz 1985; Wonnacott 1986). This type of study helps to determine the location and numbers of nicotine binding sites.

Functional Significance of Nicotinic Binding Sites

High-Affinity Agonist Binding Sites

Brain sites which bind $^3\text{H-ACh}$ and $^3\text{H-nicotine}$ with high affinity represent nAChRs that respond in some ways like the C6 type of receptor found in the periphery (Clarke 1987a). Studies using the 2-DG technique have revealed that the neuroanatomical pattern of cerebral activation following the systemic administration of nicotine in rats is strikingly similar to the distribution of high-affinity agonist binding demonstrated autoradiographically (London et al. 1985; Grunwald, Schrok, Kuschinsky 1987). Pretreatment with mecamylamine blocks the effects of nicotine on LCGU, suggesting that putative ganglionic (C6-type) receptors in the brain are associated with high-affinity agonist binding.

Most of nicotine's actions on central receptors are blocked by the C6-selective antagonist mecamylamine. The relevant nAChRs are probably those which are labeled with high affinity by tritiated agonists. However, the absence of high-affinity agonist binding sites in PC12 cells (derived from a pheochromocytoma cell line) known to express C6-type receptors (Kemp and Morley 1986) indicates that although central and ganglionic nAChRs have pharmacologic similarities, they may not be identical at the molecular level.

High-affinity agonist binding sites are relevant to long-term effects of human tobacco smoking. Recently, Benwell, Balfour, and Anderson (in press) observed that the density of high-affinity $^3\text{H-nicotine}$ binding in post mortem human brain is higher in smokers than in nonsmokers. The increased density of sites in smokers is consistent with studies in animals that show that chronic treatment with nicotine leads to an increased number of nicotinic receptors in the brain (Schwartz and Kellar 1983; Marks, Burch, Collins 1983b).

Alpha-Bungarotoxin Binding Sites

Although $\alpha\text{-BTX}$ does not block nicotinic actions in all areas of the CNS (Duggan, Hall, Lee 1976; Egan and North 1986), there are several reports of antagonism (Zatz and Brownstein 1981; Farley et al. 1983; de la Garza et al. 1987a). In the rat cerebellum, locally applied nicotine alters single-unit activity in a manner dependent on cell type: nicotine excites interneurons but inhibits Purkinje cells. Both actions are directly postsynaptic (de la Garza et al. 1987, in press(b)). The inhibitory effects of nicotine are blocked by hexame-

thonium but not by α -BTX, which does block the excitatory effects (de la Garza et al., in press(a)).

Strain differences exist in mice in the physiological and behavioral effects of nicotine, in the development of tolerance to these effects, and in the regional distribution of ^{125}I -BTX binding density (Marks, Burch, Collins 1983a; Marks, Stitzel, Collins 1986). The genetically determined variation in response is not readily explained by differences in brain nicotinic receptors. However, a classical genetic analysis indicates that the density of ^{125}I -BTX binding sites in mouse hippocampus correlates with susceptibility to seizures induced by high doses of nicotine (Miner, Marks, Collins 1984). These and other considerations (Clarke 1987a) suggest that ^{125}I -BTX may label a subtype of nAChR in the brain and that this receptor is pharmacologically akin to the nAChR found in muscle.

Although ^{125}I -BTX binding sites are found in human brain, the available evidence suggests that nicotine at doses obtained from cigarette smoking does not activate this population of brain nAChRs. Rather, the pattern of neuronal activation that follows the in vivo administration of nicotine in animal experiments, even in doses far greater than those likely to occur during smoking, resembles the neuroanatomical distribution of high-affinity agonist binding sites (London et al. 1985; Grunwald, Schrok, Kuschinsky 1987). However, this issue is not conclusively resolved, and a potential role for bungarotoxin binding receptors in mediating effects of smoking cannot be completely excluded.

Behavioral and Physiological Studies

The effects of mecamylamine on several responses elicited by nicotine in mice have been examined (Collins et al. 1986). The responses are of two major classes: those blocked by low doses of mecamylamine (inhibitory concentrations for 50 percent of mice tested (IC_{50}) < 0.1 mg/kg) (seizures and startle response) and those blocked by higher doses (IC_{50} approximately 1 mg/kg) (effects on respiratory, heart rate, body temperature, and Y-maze activity). Strain differences are also apparent in the sensitivity to mecamylamine blockade. These findings are consistent with the existence of at least two types of central nAChR.

The Neuroanatomical Distribution of Nicotinic Binding Sites in the Brain

High-Affinity Agonist Binding Sites

Rodent

Autoradiographic maps of high-affinity nicotinic binding sites in rat brain are essentially identical for ^3H -nicotine, ^3H -ACh, and ^3H -methyl-carbachol (Clarke, Pert, Pert 1984; Clarke, Schwartz et al.

1985; London, Waller, Wamsley 1985; Boksa and Quirion 1987). Dense labeling is observed (1) in the medial habenula and interpeduncular nucleus, which appear to belong to a common cholinergic system; (2) in the so-called specific motor and sensory nuclei of the thalamus and in layers III and IV of cerebral cortex with which they communicate; (3) in the substantia nigra pars compacta and ventral tegmental area, where labeling is associated with dopaminergic cell bodies (Clarke and Pert 1985); and (4) in the molecular layer of the dentate gyrus, the presubiculum, and the superficial layers of the superior colliculus. Labeling is sparse in the hippocampus and hypothalamus.

Monkey

The autoradiographic distribution of high-affinity ^3H -nicotine binding in rhesus monkey brain is similar to that in the rat (Friedman et al. 1985). Dense labeling has been noted in the anterior thalamic nuclei and in a band within cerebral cortex layer III. The latter band is densest and widest in the primary sensory areas. Several other thalamic nuclei are moderately labeled, but as in the rat, the label is sparse in the midline thalamic nuclei. In contrast to findings for the rat, the medial habenula appears unlabeled.

Human

High-affinity agonist binding has not been mapped autoradiographically in human brain. However, assays of a few dissected brain areas suggest the following pattern: nucleus basalis of Meynert > thalamus > putamen > hippocampus, cerebellum, cerebral cortex, and caudate nucleus (Shimohama et al. 1985). Two affinity sites for ^3H -nicotine have been detected, and the regional distribution observed reflects the presence of both sites.

Alpha-Bungarotoxin Binding Sites

Because ^{125}I -BTX sites may not be relevant to tobacco smoking, they will be discussed only briefly here. There are clear differences of regional distribution not only between mice and rats, but also between different strains of mice (Marks et al. 1986). The autoradiographic distribution of ^{125}I -BTX labeling in rat brain is strikingly different from the pattern of ^3H -agonist labeling, with highest site density in hippocampus, hypothalamus, and superior and inferior colliculi (Clarke, Schwartz et al. 1985). An attempt to map ^{125}I -BTX binding in human brain was hampered by a high degree of nonspecific binding, with diffuse specific labeling in the hippocampus and cerebral cortex (Lang and Henke 1983).

Molecular Biology

Goldman and colleagues have mapped regions in the brain which contain cell bodies expressing RNA that codes for putative nAChRs. The RNA identified is homologous to cDNA clones encoding the alpha subunits of the muscle nAChR and a putative neuronal nAChR (Goldman et al. 1986; Goldman et al. 1987). These and related findings show that a family of genes exists that codes for proteins similar to, but not identical with, the muscle nAChR. The functional role of these putative nAChR subtypes in the CNS is not clear.

Central Nicotinic Cholinergic Receptors: Pre- or Postsynaptic?

Presynaptic Regulation of Neurotransmitter Release

The release of ACh from some nerve terminals in the CNS (Rowell and Winkler 1984; Beani et al. 1985) and periphery (Briggs and Cooper 1982) is increased by activation of presynaptic nicotinic "autoreceptors." Preliminary evidence from lesion experiments suggests that some nicotinic autoreceptors in the brain may be high-affinity ^3H -nicotine binding sites (Clarke et al. 1986).

Nicotine also modulates the release of certain other neurotransmitters by acting at receptors located on nerve terminals. This form of regulation has been shown for dopaminergic, noradrenergic, and serotonergic terminals (Starke 1977; Chesselet 1984). Lesion studies suggest that these receptors are labeled by ^3H -agonists (Schwartz, Lehmann, Kellar 1984; Clarke and Pert 1985; Prutsky, Shaw, Cynader 1987).

Somatodendritic Postsynaptic Actions

Much of ^3H -agonist labeling probably represents nAChRs located on neuronal cell bodies or dendrites. For example, nicotine excites neurons postsynaptically in the medial habenula, locus coeruleus, and interpeduncular nucleus, all areas of moderate to dense ^3H -agonist binding (Brown, Docherty, Halliwell 1983; Egan and North 1986; McCormick and Prince 1987).

Neuroendocrine and Endocrine Effects of Nicotine

Nicotine has direct and indirect effects on several neuroendocrine and endocrine systems (Balfour 1982; Clarke 1987a; Hall 1982). This Section reviews research on the effects of nicotine in animals and humans that are relevant to understanding cigarette smoking. Nicotine effects on cholinergic and noncholinergic nicotinic receptors, as well as on the release of catecholamines, monoamines, pituitary hormones, cortisol, and other neuroendocrine chemicals,

are discussed. Effects on single neuroregulators are emphasized, but it is important to recognize that there are extensive interrelationships among these substances (Tuomisto and Männistö 1985).

Nicotine has effects on peripheral endocrine as well as on central neuroendocrine functions. In the early 1900s researchers discovered that nicotine stimulated autonomic ganglia (ganglia were painted with tobacco solutions), inducing such effects as the release of adrenal catecholamines (Larson, Haag, Silvette 1961). As the health consequences of cigarette smoking have become clearer, many investigators have sought to determine tobacco's effects on the endocrine system, with the possibility that understanding such effects may help to explain smoking behavior. Nicotine is regarded as the major pharmacologic agent in tobacco and tobacco smoke responsible for alterations in endocrine function. However, there has not been a systematic evaluation of the effects of metabolites of nicotine or constituents of tobacco other than nicotine on the endocrine system.

The functional significance of nicotine-induced perturbations in hormonal patterns and the role of neuroregulators in smoking are poorly understood. Extensive literature using nicotinic agonists and antagonists indicates relationships between cholinergic activity and particular behavioral effects (Henningfield et al. 1983; Kumar, Reavill, Stolerman, in press). Similar strategies have been employed to explore the contributions of catecholamines to smoking-related behavior. However, the exploration of the importance of neuroregulators in the reinforcement of cigarette smoking is still at an early stage.

Cholinergic Effects

Nicotine has cholinergic effects in the PNS and CNS. Nicotine is a cholinergic agonist at peripheral autonomic ganglia and somatic neuromuscular junctions at low doses and becomes an antagonist at high doses (Volle and Koelle 1975). Nicotine also releases ACh in the cerebral cortex (Armitage, Hall, Morrison 1968; Rowell and Winkler 1984) and in the myenteric plexus of the peripheral ANS (Briggs and Cooper 1982). Balfour (1982) has suggested that cortical arousal (see *Electrophysiological Actions of Nicotine* for a detailed discussion) is mediated by ACh release but that behavioral stimulation (see Chapter IV) either is not mediated by ACh release or does not depend on the action of ACh at a muscarinic receptor.

Studies involving intracerebral administration of nicotine have been used to determine the loci of nicotine's action (Kammerling et al. 1982; Wu and Martin 1983). The injection of nicotine into the cerebral ventricles of cats, dogs, and rats produces a variety of effects including changes in cardiovascular activity, body temperature, respiration, salivation, muscle reflex tone, and electrocortical indices

of sleep and arousal; the direction and duration of effects depend on dosage and on baseline response parameters (Hall 1982).

Nicotine's cholinergic actions can affect other neuroregulators in the body (Andersson 1985). Nicotine stimulates NE release in the hypothalamus by a Ca^{2+} -dependent process that can be inhibited by prior administration of hexamethonium or ACh (Hall and Turner 1972; Westfall 1974). The mechanism resembles nicotine's effects on peripheral adrenergic nerve terminals (Westfall and Brasted 1972). At high dose levels, nicotine stimulates NE release by displacing it from vesicle stores at sites outside the hypothalamus (Balfour 1982). These actions are relevant to understanding the reinforcing effects of nicotine. For example, using drug discrimination procedures, Rosecrans (1987) has demonstrated that intact central NE and dopamine (DA) function were required to elicit the cue properties of nicotine.

Intravenous administration of nicotine modulates the release of both neurohypophyseal and adenohypophyseal hormones (Bisset et al. 1975; Hall, Francis, Morrison 1978). Hillhouse, Burden, and Jones (1975) found that the *in vitro* application of ACh to the hypophysiotropic area of the rat caused a significant increase in the basal secretion of corticotropin-releasing hormone (as measured by bioassay), which in turn controls, via the anterior pituitary, the release of the pro-opiomelanocortin (POMC) group of hormones— β -endorphin, β -lipotropin, melanocyte-stimulating hormone-releasing factor, and adrenocorticotrophic hormone (ACTH) (Meites and Sonntag 1981). The humoral mechanism for the release of vasopressin has been traced from the medulla to the paraventricular nuclei of the hypothalamus (Bisset et al. 1975; Castro de Souza and Rocha e Silva 1977). Similarly, Risch and colleagues (1980) have demonstrated a cholinergic mechanism for the release of β -endorphin.

Modulation of Catecholamine and Serotonin Activity

Dale and Laidlaw (1912) found that the pressor response of the cat to nicotine was due in part to the release of epinephrine from the adrenal glands. Over the past 75 years, a large body of research has confirmed and further investigated this phenomenon. Stewart and Rogoff (1919) quantified the effect of nicotine on adrenal epinephrine release. Kottogoda (1953) observed that nicotine releases catecholamines from extra-adrenal chromaffin tissues. Watts (1961) demonstrated the effect of smoking on adrenal secretion of epinephrine. Hill and Wynder (1974) reported that increasing the nicotine content in cigarette smoke progressively increased the serum concentration of epinephrine, but not NE. Winternitz and Quillen (1977) found that the excretion of urinary catecholamines tended to be higher on smoking days than on nonsmoking days. Several recent studies have focused on the role of nicotine and the mechanisms involved in the

release of catecholamines from cultured chromaffin cells (Forsberg, Rojas, Pollard 1986). Earlier experiments by Douglas and Rubin (1961), using denervated perfused cat adrenal glands, indicated that nicotine augments catecholamine release from chromaffin cells by promoting an influx of extracellular calcium. Forsberg, Rojas, and Pollard (1986) suggested that nicotine-induced catecholamine secretion may be mediated by phosphoinositide metabolism in bovine adrenal chromaffin cells.

The anatomical localization and importance of biogenic monoamines such as serotonin (5-HT [5-hydroxytryptamine]), DA, and NE have been the subject of intense research for the past 30 years. The classic studies of Dahlstrom and Fuxe (1966) revealed that neurons containing these amines were localized in specific ascending projection systems; descending monoaminergic neurons have also been described. The physiological integrity of these systems was further demonstrated by Aghajanian, Rosecrans, and Sheard (1967), who observed that stimulation of 5-HT cell bodies localized in the midbrain raphe nucleus released 5-HT from nerve endings located in the more rostral forebrain. The recognition that these amine systems constitute a unique interneuronal communication system has played a central role in understanding underlying neurochemical and behavioral mechanisms.

The cholinergic system has undergone a similar analysis (Fibiger 1982), but the delineation of specific cholinergic pathways has been more difficult because no histochemical method has been available for ACh. It does appear, however, that the cholinergic system is similarly organized and interacts with specific biogenic amine pathways. For example, Robinson (1983) has clearly shown that both 5-HT and DA systems exert tonic inhibitory control over ACh turnover in both the hippocampus and frontal cortex regions. Lesions of the medial raphe nuclei increase the ACh turnover rate in hippocampal sites, while lesions of the dorsal raphe elicit a similar effect in frontal cortical areas. Evidence of DA control comes from the observation that the catecholamine neurotoxin, 6-OHDA, when injected into the DA-rich septal area, facilitated hippocampal ACh turnover. The research of Kellar, Schwartz, and Martino (1987) and others also suggests that nicotinic receptors may occupy a presynaptic site on select DA and 5-HT nerve endings. Westfall, Grant, and Perry (1983), using a tissue slice preparation, have shown that the DMPP-induced stimulation of nicotinic receptors in the striatum will facilitate the release of both 5-HT and DA. This preparation is devoid of cell bodies or 5-HT- and DA-containing axon terminals, suggesting that these nicotinic cholinergic receptors are primarily presynaptic. Further, hexamethonium, but not atropine, attenuated nicotine-induced amine release, confirming that these effects are nicotinic in nature.

Nicotine may have simultaneous actions on many types of neurons. Even though only one kind of receptor may be stimulated, either activation or inhibition of a particular 5-HT, NE, or DA neuron may be the ultimate outcome. Conversely, the activity of specific cholinergic neurons may also be controlled by one of these biogenic-amine-containing projection systems. Nicotine appears to produce its discriminative stimulus effect in at least one major brain area, the hippocampus. This site is rendered insensitive if DA neurons innervating this area are destroyed (Rosecrans 1987). The interrelationships of these amine pathways are important to understand nicotine's effects on behavior and its effects on the neuroendocrine system because of the central role that these amine systems play in the hypothalamic control of the pituitary.

Effects on Serotonergic Neurons

Research evaluating the relationship between nicotine and 5-HT has involved several different approaches. Hendry and Rosecrans (1982) compared the effects of nicotine on conditioned and unconditioned behaviors in rats selected for differences in physical activity and 5-HT turnover. Balfour, Khuller, and Longden (1975) observed that acute doses of nicotine were capable of attenuating hippocampal 5-HT turnover, an effect specific to the hippocampus. Fuxe and colleagues (1987) did not observe any acute changes in 5-HT function following acute nicotine dosing but did observe a significant reduction of 5-HT turnover following repeated doses (3 x 2 mg/kg/hr). This effect, however, was suggested to be due to cotinine, the primary metabolite of nicotine.

In addition to attempts to correlate 5-HT function with some pharmacologic effect of nicotine, investigators have evaluated potential links between 5-HT and neuroendocrine function. Balfour, Khuller, and Longden (1975) showed a relationship between 5-HT and nicotine's ability to induce the release of plasma corticosterone, presumably by activation of the pituitary-adrenal axis. Following acute nicotine injections in the rat, a reduction in 5-HT turnover correlated with an increase in plasma corticosterone. Rats exhibited tolerance to pituitary activation following repeated nicotine doses, but not to the attenuation of hippocampal 5-HT turnover. Stress antagonized nicotine-induced reductions of hippocampal 5-HT. Also, nicotine was reported to inhibit the adaptive response to adrenocortical stimulation following chronic stress (Balfour, Graham, Vale 1986). One interpretation of these data is that nicotine can modify how rats adapt to stress, which may be mediated by changes in hippocampal 5-HT function. At this point, however, it is difficult to draw firm conclusions concerning how nicotine affects 5-HT neurons and whether this neurotransmitter is involved in any of nicotine's

effects on neuroendocrine function. Hippocampal 5-HT turnover appears to be selectively attenuated by nicotine.

Effects on Catecholaminergic Neurons

Studies of the effects of nicotine on NE-containing neurons have produced mixed results. Earlier work suggested that nicotine may affect behavior via a NE component, but recent research has not supported such claims (Balfour 1982). It has been reported that nicotine releases DA from brain tissue (Westfall, Grant, Perry 1983). Lichtensteiger and colleagues (1982) observed that nicotine releases DA through an acceleration of the firing rate of DA cell bodies located in substantia nigra zona compacta when nicotine is administered via iontophoretic application or s.c. (0.4 to 1.0 mg/kg). This activation was marked by a significant increase in striatal DA turnover; DHBE, but not atropine, attenuated nigrostriatal activation. Evidence that nicotine facilitates the firing of DA cell bodies by stimulating nicotinic cholinergic receptors has recently been reported by Clarke, Hommer, and coworkers (1985), who showed a specific effect of nicotine antagonized by mecamylamine on pars compacta cell bodies. Connelly and Littleton (1983) noted that DA release from synaptosomes lacked stereoselectivity but was blocked by the ganglionic-blocking drug pempidine.

Fuxe and coworkers (1986, 1987) have studied nicotine's effects on central catecholamine neurons in relation to neuroendocrine function. These investigators use quantitative histofluorometric techniques that measure the disappearance of catecholamine stores by administering a tyrosine hydroxylase inhibitor (AMPT) to rats receiving various doses of nicotine or exposed to tobacco smoke. Tissues are then exposed to formaldehyde gas, and histofluorescence in AMPT-treated rats is evaluated in comparison to controls.

Nicotine is a potent activator of both DA and NE neuron systems located primarily in the median eminence and in areas of the hypothalamus. These effects result from a stimulation of nicotinic cholinergic receptors, generally antagonized by mecamylamine. Intermittent nicotine dosing (4 x 2 mg/kg, s.c. every 30 min) or tobacco smoke exposure (rats were exposed to one to four cigarettes with a smoking machine-determined nicotine yield of 2.6 mg; rats received 8 puffs at 10-min intervals) results in a decrease of prolactin, thyroid-stimulating hormone (TSH), and luteinizing hormone (LH) and an increase of plasma corticosterone levels. Nicotine doses of 0.3 mg/kg administered i.v. induce an overall activation of the hypothalamic-pituitary axis, causing an increase of both ACTH and prolactin that subsides within 60 min. Tolerance to the corticosterone response develops after repeated nicotine doses, and there is evidence that it develops after a single dose of nicotine (Sharp and Beyer 1986; Sharp et al. 1987). Restraint stress increases