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COMMENTARY

DOPAMINERGIC MECHANISMS IN THE LOCOMOTOR STIMULANT EFFECTS OF NICOTINE

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Nicotine is one of the most widely consumed psychoactive drugs. Although cigarettes represent the most common source of the drug, pharmacologically important levels of the drug are also obtained through polacrilex gum ("Nicorette™"), pipe smoke, nasal snuff, cigars, and chewing tobacco. The initiation and maintenace of the tobacco "habit" are affected by a host of non-pharmacological as well as pharmacological factors. However, the present consensus is that people consume tobacco products on a long-term basis principally in order to self-administer nicotine for its central effects [1].

Nicotine exerts several psychopharmacological actions which may help to explain why the drug is sought after so avidly [2]. In particular, cigarette smokers commonly refer to a stimulant or arousing effect which accompanies smoking. In animals, nicotine exerts depressant and stimulant effects on a variety of conditioned and unconditioned behaviours, with stimulant effects predominating in chronically-treated subjects (see Ref. 2 for review).

Several drugs, particularly *d*-amphetamine and cocaine, appear to exert their reinforcing and/or stimulant effects through their abilities to enhance dopaminergic tone within the mesolimbocortical system [3, 4]. Both the reinforcing and stimulant effects of *d*-amphetamine appear to be mediated via the nucleus accumbens (NACC†) with little if any contribution from dopaminergic afferents to olfactory tubercle (OT) or medial prefrontal cortex [5–7]. In the case of cocaine, the precise mesolimbocortical terminal fields are less clearly defined (see Ref. 4 for review).

Recent evidence, reviewed below, suggests that nicotine also activates the mesolimbic dopamine system and that such an activating effect underlies the reinforcing and stimulant effects of this drug.

Nicotine and locomotor activity—Behavioural studies

In drug-naive rats, systemic administration of nicotine produces ataxia and typically depresses locomotor activity for several minutes, after which a locomotor stimulant effect may emerge, lasting 1 hr or more [8]. With repeated drug testing, the depressant effect wanes and a more pronounced stimulant effect manifests itself; little, if any, tolerance develops to this stimulant effect with daily administration of nicotine [8, 9].

Some authors have viewed the emergence of the locomotor stimulant effect as a sensitization phenomenon, perhaps by analogy with cocaine, *d*-amphetamine and opiates. There is evidence both for and against the suggestion that the locomotor stimulant effect of nicotine increases as a result of environmental conditioning, i.e. that cues in the test environment come to have a stimulant effect through association with the drug [10–12].

Nicotine is a lipophilic compound which rapidly enters and accumulates in the central nervous system after systemic injection. Both the depressant and the stimulant actions of nicotine are produced by central actions of nicotine [8, 10]. The ataxia induced by nicotine may result from a depression of spinal reflexes as well as through actions at supraspinal sites. In contrast, the stimulant effect of nicotine, as reviewed below, most probably reflects enhanced release of dopamine (DA) from terminals in the nucleus accumbens.

Receptor localization studies

Receptor binding studies have identified two anatomically distinct populations of nicotinic site in rodent brain [13–15]. One population is labelled by [^{125}I]α-bungarotoxin, but its pharmacological and physiological relevance is unclear. The other population is labelled with high (nanomolar) affinity by [^{3}H]nicotine, [^{3}H]acetylcholine and other nicotinic agonists, and appears to represent the predominant receptor subtype activated by "smoking" doses of nicotine [16].

Autoradiographic studies of rat brain show that these high-affinity agonist binding sites are densely represented in the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) [13], which contain the dopaminergic cell bodies of the nigrostriatal and mesolimbic systems respectively. A moderate density of sites occurs in the terminal regions of these two systems, i.e. in the caudate-putamen (CP), and in the NACC and OT. Intraventricular administration of 6-hydroxydopamine led to partial DA depletion and a modest reduction of nicotinic [^{3}H]acetylcholine binding in microdissected neostriatal tissue [17]. In a subsequent autoradiographic

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† Abbreviations: ACh, acetylcholine; CP, caudate-putamen; DA, dopamine; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; NACC, nucleus accumbens; OT, olfactory tubercle; SNC, substantia nigra pars compacta; and VTA, ventral tegmental area.

study, unilateral administration of 6-hydroxydopamine into the medial forebrain bundle achieved a near-total destruction of dopaminergic neurons and resulted in a concomitant reduction of [^{3}H]nicotine binding in all these regions [18]. These findings suggested that nicotinic receptors are associated with nigrostriatal and mesolimbic dopaminergic neurons at the level of cell bodies and terminals.

Consistent with this conclusion, nicotinic acetylcholine receptor-like immunoreactivity has been demonstrated in SNC and VTA neurons and within associated dopaminergic terminal structures [19, 20]. Recently, there has been identified a family of genes which are homologous to those encoding individual subunits of known subtypes of nicotinic acetylcholine receptor. *In situ* hybridization histochemistry has suggested that nigrostriatal and mesolimbic dopaminergic neurons express the mRNA (and hence presumably the protein) corresponding to some of these genes [21]. Thus, several neuroanatomical approaches suggest that dopaminergic neurons possess nicotinic receptors, and that these receptors are located on cell bodies and/or dendrites and on axon terminals.

Electrophysiological studies

Single unit extracellular recording studies have shown that nicotine excites DA neurons in the SNC and in the VTA. In rats anaesthetized with urethane, nicotine (1 mg/kg, s.c.) induced a brief decrease in firing rate, followed by a sustained increase in firing of SNC neurons [22]. However, the responsive cells were not definitively identified as dopaminergic, and the mean firing rate observed after nicotine administration (12 Hz) was out of the range typically reported for SNC DA neurons. Nevertheless, in a subsequent study [23], these investigators showed that identified SNC DA neurons were excited by microiontophoretic administration of nicotine with no initial depression. Subsequent investigators confirmed the excitatory effect of nicotine (1 mg/kg, s.c.) on identified SNC DA neurons in chloral hydrate anaesthetized rats, but noted that lower doses were without significant effect; the initial depressant effect of nicotine was not observed [24]. The excitatory effect of nicotine appeared to be centrally-mediated, since it was prevented by the centrally-active antagonist mecamylamine but not by the quaternary blocker chlorisondamine.

Grenhoff and coworkers [25] reported that a lower dose of nicotine (0.16 mg/kg base, i.p.) increased the firing rate of SNC DA neurons but not of VTA DA neurons in rats anaesthetized with chloral hydrate. However, nicotine promoted burst firing of DA neurons in both SNC and VTA, affecting in particular those DA cells which were susceptible to burst firing under baseline conditions. Mereu and coworkers [26] have shown that anaesthesia can greatly affect nicotinic responses. Under chloral hydrate anaesthesia, intravenous nicotine stimulated SNC cells but depressed VTA cell firing. However, in paralysed, locally-anaesthetized rats, nicotine excited both cell types, and VTA neurons were considerably more responsive.

In summary, electrophysiological studies suggest that nicotine can excite both nigrostriatal and mesolimbic DA neurons in rats, but that in the absence of anaesthesia, moderate doses of nicotine preferentially stimulate the mesolimbic system.

In vitro *studies of DA release*

Numerous studies have shown that stimulation of nicotinic acetylcholine receptors promotes DA release in the CP *in vitro*. In rat CP slices superfused with [^{3}H]tyrosine, acetylcholine, nicotine and carbachol all enhanced release of newly-synthesized [^{3}H]DA; these effects were reduced or blocked by nicotinic antagonists such as hexamethonium and mecamylamine [27, 28]. Nicotine, when applied at a relatively low concentration (10^{-6} M), stimulated the release of [^{3}H]DA in a Ca^{2+}-dependent manner [28–30]. This DA-releasing action of nicotine was reduced to some extent by tetrodotoxin in two studies [28, 29], but in a third study, it was unchanged by a concentration of tetrodotoxin (5×10^{-7} M) sufficient to abolish veratridine-induced DA release [30]. Despite these different results, nicotine continued to release [^{3}H]DA in the presence of tetrodotoxin in all three studies, suggesting the involvement of presynaptically-located nicotinic receptors.

The concentrations of nicotine used in these studies are behaviourally relevant, since dose-dependent behavioural effects of nicotine are typically achieved at brain concentrations in the low micromolar range. At concentrations of 1–100 μM, nicotine appears to release DA from a newly-synthesized cytoplasmic pool of transmitter, and not from the reserpine-sensitive vesicular pool [29]. At higher concentrations probably only achieved at near-lethal doses, nicotine releases DA from CP slices by a different mechanism which may not be receptor mediated [29].

Nicotine also stimulates *in vitro* DA release from the NACC. Nicotine (10^{-7} M and above) produced a concentration-dependent increase in [^{3}H]DA release from rat NACC slices preloaded with [^{3}H]DA [31]. This effect was almost completely Ca^{2+} dependent, was mimicked by nicotinic agonists, and was partially blocked by nicotinic antagonists. Fung [32] has reported increased release of newly-synthesized [^{3}H]DA from rat NACC slices in response to 10 μM (but not 1 μM) nicotine.

Thus, *in vitro* studies consistently indicate that nicotine can promote the release of newly-synthesized DA through a direct action on terminals of the nigrostriatal and mesolimbic systems.

Acute effects of nicotine on DA release or utilization in vivo

Although the foregoing *in vitro* studies suggest that behaviourally relevant doses of nicotine should increase DA outflow in both nigrostriatal and mesolimbic terminal fields, *in vivo* experiments have shown that the mesolimbic system is more sensitive.

Imperato and coworkers [33] used intracerebral dialysis to measure extracellular levels of DA and its major metabolites in the dorsal caudate and NACC of freely moving rats. Subcutaneous administration of nicotine increased DA release in both structures in a dose-related manner. However, nicotine acted more potently in the NACC than in the caudate

nucleus (ED_{50} 0.32 and 1.85 mg/kg respectively). Nicotine, at a dose (0.6 mg/kg) which is subconvulsive but at the high end of previously reported behavioural dose–response curves, significantly increased extracellular DA in both structures; the peak effect was considerably greater in NACC than in dorsal caudate. Curiously, nicotine produced its maximal effect on NACC DA within the first 20 min of systemic injection, and the drug effect declined steadily over the next hour, whereas in the caudate, extracellular DA increased more slowly after nicotine injection, and declined only marginally over the following 2 hr. Nicotine-induced DA release was prevented by systemic administration of mecamylamine (a nicotinic antagonist which penetrates centrally) but not by the quaternary compound hexamethonium. This is weak evidence for a central action of nicotine, since it is doubtful whether hexamethonium, at the dose administered, would have blocked the peripheral actions of nicotine [10].

Mifsud and colleagues [34] have suggested that nicotine can release NACC DA via a local action in freely moving rats. Infused directly into the posterior NACC via a microdialysis probe, nicotine increased extracellular DA in a dose-related manner, and this action was blocked by systemically administered mecamylamine.

In this study, nicotine, infused at a concentration (2.4 μM) which gave a near maximal effect, increased DA release 5-fold, yet did not alter extracellular concentrations of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). In contrast, Imperato and colleagues [33] observed a marked rise in extracellular DOPAC and HVA in this structure following subcutaneous administration of nicotine. This difference may reflect the routes of administration used. In particular, when nicotine is applied directly to the NACC *in vitro*, the drug appears to enhance the release of newly-synthesized DA (see above). This pool of DA is metabolized intracellularly and appears to comprise the major source of extracellular DOPAC [35]; thus, if nicotine were to deplete this pool, extracellular DOPAC levels would not necessarily increase. On the other hand, systemic administration of nicotine would tend additionally to increase the impulse flow of mesolimbic neurons, and perhaps this accounts for the elevated DA metabolite levels observed.

Several groups, using less direct measures, have also obtained evidence suggesting that nicotine increases DA utilization in terminal regions in freely-moving drug-naive rats. Andersson *et al.* [36] reported reductions in DA stores and increases in DA utilization (DA disappearance after α-methyl-*p*-tyrosine) restricted to medial caudate, NACC and OT, following intraperitoneal injection of nicotine. However, given by this route, nicotine appears painful, and it seems possible that the alterations observed were peripheral in origin. Lapin *et al.* [37] reported no significant alterations in DA/metabolite ratios in microdissected tissue from various DA areas including CP, NACC and OT, following acute injection of nicotine (0.8 mg/kg, s.c.). However, in a subsequent study [38], these authors noted a significant increase in the DOPAC/DA ratio, restricted to the NACC. Possibly, these largely negative findings reflect the short interval (10 min) selected between injection of nicotine and killing.

Acute exposure to cigarette smoke also increases DA utilization (assessed by quantitative histofluorimetry), an effect largely restricted to the "diffuse" (as opposed to "dotted") types of DA terminal systems in the anterior NACC and the lateral posterior OT in rats [39]. Most effects were observed after exposure to one or two cigarettes, which resulted in serum nicotine levels comparable to those found in human habitual smokers. DA utilization was not altered in CP or in medial posterior OT. DA stores were reduced slightly in NACC but unaffected elsewhere. These smoke-induced neurochemical alterations were due to nicotine, since they were blocked by mecamylamine administration and were not observed when the smoke was passed through filters which removed the alkaloid.

These *in vivo* studies, taken together, show that acute administration of nicotine can potently stimulate the release of mesolimbic DA, whereas higher concentrations of the drug are required in order to increase nigrostriatal DA release.

Chronic effects of nicotine on DA function

Repeated dosing with nicotine can result in two forms of tolerance. *Acute* tolerance, or tachyphylaxis, can appear rapidly and persists for minutes or hours after a single dose administration. It reflects receptor desensitization and/or other factors. Acute tolerance can develop to most if not all central actions of nicotine, and has been reported, for example, in studies of nicotine-induced DA release *in vitro* [30]. *Chronic* tolerance persists for weeks or months and occurs to some effects of nicotine but not, apparently, to others (see Ref. 2); its neurobiological basis is unknown. Habitual cigarette smokers experience pharmacologically significant levels of nicotine on a 24-hr basis, and there is evidence for some degree of both acute and chronic tolerance [1]. Although acute tolerance to nicotine has typically been noted when two administrations of the drug are given a short time apart, it may also be present when continuous chronic infusion of the drug is given. For this reason, studies employing chronic *intermittent* dosing are discussed separately from those using *continuous* administration.

Chronic intermittent administration of nicotine. The possibility of *acute* tolerance can be minimized by giving nicotine intermittently. Lapin *et al.* [38] examined the effects of ten injections of nicotine (0.8 mg/kg, s.c.), given over a 2-week period, on the response to a subsequent *in vivo* nicotine challenge. Following chronic nicotine treatment, a nicotine challenge still increased DOPAC/DA ratios, suggestive of increased DA turnover. Although it was claimed that this response was blunted by chronic nicotine treatment, the data presented do not appear to support this conclusion. Indeed, two other recent reports suggest that chronic daily injections of nicotine do *not* alter subsequent responses to nicotine [40, 41].

In another study [42], the effects of nicotine on locomotor activity and DA utilization were investigated in rats that had all been preexposed to daily

nicotine injections for 2 weeks in order to emphasize the locomotor stimulant effect of the drug. Each rat was tested subsequently with saline and L-nicotine to demonstrate an acute locomotor stimulant effect of nicotine. After a few days to permit drug clearance, subjects received a single subcutaneous injection of saline or nicotine and were killed 30 min later for biochemical assay of OT, NACC, and CP tissue. In the first experiment, L-nicotine (0.2 to 0.8 mg/kg, s.c.) increased HVA/DA ratios in the OT, but this was not dose-related; a similar trend occurred in the NACC. DOPAC/DA ratios were not altered significantly. In subsequent experiments, subjects were treated with the L-aromatic acid decarboxylase inhibitor NSD-1015 in order to inhibit the conversion of dihydroxyphenylalanine (DOPA) to DA, and DA utilization was measured by DOPA/DA ratios. L-Nicotine stimulated locomotor activity and increased DOPA/DA ratios in OT and NACC but not in CP. Both the behavioural and neurochemical effects were significantly dose-related (0.1 to 0.4 mg/kg) and stereoselective in the expected direction (L > D isomer). In a final experiment, the locomotor stimulant effect of L-nicotine (0.4 mg/kg, s.c.) was examined before and after depletion of mesolimbic DA. Dopamine depletion was achieved by intra-accumbens infusion of 6-hydroxydopamine; control subjects received vehicle infusions. The lesion, which resulted in a substantial (89%) depletion of DA in NACC and OT, abolished the locomotor stimulant effect of L-nicotine when tested 2 weeks later. This study suggests that in rats which have been chronically but intermittently preexposed to the drug, the acute administration of nicotine produces a locomotor stimulant action which is not only accompanied by, but is *dependent* upon, an increase in DA utilization in mesolimbic terminal regions.

Chronic continuous administration of nicotine. Several animal studies suggest that chronic continuous administration of nicotine can have effects on behaviour and DA function that are different and even opposite from those seen after multiple intermittent exposure.

In behavioural tests, chronic (2 week) infusion of nicotine via osmotic minipumps *reduced* locomotor activity compared to saline-treated control rats [43]. The plasma nicotine levels attained (77 ng/mL) were approximately twice those seen in habitual smokers. This result contrasts sharply with those obtained by giving nicotine on a long-term but intermittent basis, where a robust stimulant effect is seen (see above).

In a neurochemical study, acute administration of nicotine (0.1 mg/kg, s.c.) to drug-naive animals clearly increased *in vitro* tyrosine hydroxylase activity in NACC tissue punches [44]. However, chronic (2 week) nicotine infusion produced appreciable plasma nicotine levels (48 ng/mL) but did not alter significantly the activity of this enzyme. In another study [45], nicotine infusion at the same or at a higher dose for 3 weeks resulted in a significant *reduction* in DA turnover in striatum, as measured by tissue (DOPAC + HVA)/DA ratios; the NACC was not examined.

The issue of tolerance has also been investigated by examining the effects of an acute nicotinic challenge after chronic *in vivo* treatment with a nicotinic agonist. Thus, Fung [32] reported that chronic infusion of a low dose of nicotine (1.5 mg/kg/day) for 2 weeks increased the release of newly synthesized DA in NACC tissue slices in response to bath-applied nicotine. In contrast, Westfall and Perry [46] found partial tolerance to the DA-releasing effect of the nicotinic agonist dimethylphenylpiperazinium (DMPP) in neostriatal slices after rats had been chronically treated with this compound; it was not ascertained whether DMPP, a quaternary compound, was acting centrally *in vivo*.

Taken together, these studies indicate that chronic continuous infusion of nicotine can result in either tolerance or reverse-tolerance. Clearly, the conditions under which acute and chronic forms of tolerance are seen are poorly understood.

Sites of action

As reviewed above, dopaminergic neurons appear to express nicotinic receptors at the level of cell bodies and terminals. In animals that are not receiving continuous infusion of nicotine, there is electrophysiological evidence for a direct effect on the soma or dendrites, and evidence from transmitter release studies pointing to a local action of the drug on DA terminals. To determine which site of action may be more important in the behaving animal, Pert and Clarke [47] investigated the locomotor responses of rats microinfused with a nicotinic agonist at selected brain sites. Cytisine was employed for this purpose because it is less lipophilic than nicotine. Intraventricular administration of cytisine significantly increased locomotor activity, and this effect was prevented by systemic injection of mecamylamine and by 6-hydroxydopamine lesions of the NACC. Cytisine significantly increased locomotor activity when injected in the VTA, but not in the NACC, SNC or CP. Using similar methods, Reavill and Stolerman have similarly observed locomotor stimulant effects resulting from intra-VTA infusion of cytisine or nicotine; these effects were blocked by systemic administration of mecamylamine [48]. No locomotor stimulation was observed following infusions of nicotine or cytisine into NACC, CP, dorsal hippocampus or motor thalamus. Taken together, these studies, although not definitive, indicate that the acute locomotor stimulant effect of nicotine may be mediated principally via an action within the VTA.

Future directions

The acute effects of nicotine on mesolimbic DA function appear straightforward. The neurons possess nicotinic receptors, and nicotine can enhance the release of DA by increasing neuronal firing and via a direct presynaptic action on terminals. Although the receptor mechanisms are in place to permit nicotine to increase DA release, it is by no means certain that elevated DA release occurs in animals infused at a constant rate with nicotine. It is also unclear whether the pharmacokinetics of nicotine in human cigarette smokers is better modelled by constant infusion as opposed to multiple intermittent dosing.

Human subjects and laboratory animals will voluntarily self-administer intravenous doses of nicotine

[2]. Animal studies suggest that these rewarding effects are due to a central action of the drug [49]. Recently, we found that responding for nicotine was reduced greatly by 6-hydroxydopamine lesions which resulted in a marked depletion of NACC DA with little depletion in CP (Corrigall WA, Franklin KJB and Clarke PBS, unpublished observations).

Animal studies, therefore, indicate that nicotine can directly activate mesolimbic dopaminergic neurons and that this action may contribute importantly to both the acute stimulant and reinforcing effects of the drug. In this respect, nicotine resembles several other drugs of abuse which are psychostimulants. Whether nicotine exerts an analogous neuropharmacological effect in cigarette smokers is impossible to answer with current techniques.

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