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A tobacco extract containing alkaloids induces distinct effects compared to pure nicotine on dopamine release in the rat

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HIGHLIGHTS

- Tobacco alkaloids extract enhances more efficiently striatal dopamine release than nicotine.
- Tobacco alkaloids extract and nicotine similarly alter dopamine release in the nucleus accumbens.
- Tobacco alkaloids extract promotes distinct neurochemical pattern of effects compared to nicotine.
- Alkaloids associated with nicotine may play a role in the psychoactive properties of nicotine.

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ABSTRACT

It has been suggested that minor alkaloids in plants play a role in the biological and neuronal actions of nicotine. We hypothesized that these molecules modulate the effect of nicotine on the activity of central dopamine (DA) neurons, one of the main cellular targets in addiction to drugs. In this study the effect of a single intraperitoneal injection of either nicotine or an alkaloid extract of the tobacco plant (0.5 mg/kg) on the efflux of DA were investigated. DA was measured *in vivo* by intracerebral microdialysis in the nucleus accumbens and the striatum of freely-moving rats. Results show that nicotine enhanced accumbal and striatal DA extracellular levels (+47 and 20% above baseline, respectively). The extract also evoked a significant increase in DA extracellular levels in both regions (+33 and +38% above baseline). However, this effect was significantly higher compared to nicotine in the striatum only. In conclusion, the tobacco extract enhanced the neurochemical effect of nicotine alone in the striatum, a response that could underlie the higher propensity of developing addictive-like behavior using nicotine with tobacco alkaloids.

1. Introduction

Tobacco use remains a major public health concern for many countries due to its high prevalence in nicotine addiction and associated diseases. The psychoactive properties of tobacco have been attributed to nicotine, the main alkaloid fund in the tobacco plant [14,25]. A growing body of preclinical and clinical studies has demonstrated the existence of numerous compounds contained in the plant that could enhance the psychoactive properties of nicotine.

Nicotiana plants are rich in minor alkaloids (cotinine, anabasine, nornicotine, tabagisine, moysmine) in addition to the nicotine that represents 95–97% of total alkaloids. These minor tobacco alkaloids exhibit a similar structure to nicotine and have pharmacological activity, although they are generally less potent than nicotine [26]. Nornicotine and cotinine also play a role as major metabolites of nicotine [5]. It has been reported that the intravenous infusion of nicotine combined with five minor alkaloids found in tobacco smoke (anabasine, nornicotine, anatabine, cotinine and myosmine) increased locomotor activity and behavioral sensitization following self-administration [2]. These results suggest that the minor tobacco alkaloids, particularly anatabine, cotinine and myosmine, could increase the desire for nicotine and thus facilitate smoking behavior.

From a neurochemical point of view, these effects could be associated with an increase in central dopamine (DA) transmission. Indeed, it is widely accepted that the addictive properties of drugs of abuse, such as psychostimulants, opiates and possibly nicotine, are associated with an increase in mesolimbic DA transmission. One symptom of increased DA levels that has been reported in rodents is locomotor hyperactivity [7]. However, the locomotor

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hyperactivity induced by nicotine is not strictly dependent on the DA tone in the nucleus accumbens [1,28] and it has been proposed recently that DA in the dorsal striatum could also be involved [10].

Instead of a cocktail of nicotine supplemented with minor alkaloids, tobacco extracts provide a preparation to investigate the role of minor alkaloids with the relative concentration of the alkaloids representative of those contained in the plant. Previous data have reported that such an extract could induce effects distinct to those of pure nicotine [16,27], and we have postulated that the tobacco extract should enhance central DA transmission. This study compares the effect of pure nicotine with that of a tobacco extract containing alkaloids on DA release using intracerebral microdialysis in the striatum and the nucleus accumbens of freely moving rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Lyon, France), weighing 300–350 g were used. Animals, housed in individual plastic cages, were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12 h light/dark cycle (dark from 7 P.M.) and had free access to water and food. All animals use procedures conformed to International European Ethical Standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drug and alkaloidic extract

Nicotine (nicotine hydrogen tartrate salt, Sigma, France) and an alkaloidic extract (see below) were used throughout the course of the study. To obtain the extract, the Nicotiana humilis Link (Solanacea) plant was first collected from Ktama (Morocco) and the plant's identity was confirmed by Pr. Ouhamou in the faculty of Sciences Semlalia, University Cadi Ayyad (Marrakesh, Morocco). The powdered dry leaves of Nicotiana humilis Link (300 g) were used for extraction with methanol for 5-h in a continuous extraction Soxhlet apparatus. Methanolic extract (88.5 g) was then concentrated and partitioned successively by a series of acidic, basic and dichloro-methane solvents (NaOH, HCl, H_2Cl_2). The obtained fraction was concentrated with a rota evaporator. Pure extract of total alkaloids was 8.47 g. The alkaloidic extract of the tobacco plant contained nicotine (95–97%) and minor alkaloids including cotinine, nornicotine, anabasine, tabagisine, myosmine and anatabine [16].

2.3. Intracerebral microdialysis

Surgery and perfusion procedures were performed as described previously with some modifications [20]. Briefly, a siliconized stainless guide-cannula (Carnegie Medicin, Phymep, Paris, France) was stereotaxically implanted under chloral hydrate (400 mg/kg, i.p.) anesthesia just above the striatum or the nucleus accumbens (coordinates of the lower extremity of the guide, in mm, relative to the interaural point: AP = 9.4 or 10.8, L = 3 or 1.2, V = 6.4 or 2.5, respectively; [22]). The guide cannula was permanently fixed to the skull with stainless steel screws and methylacrylic cement. Five to seven days after surgery, a microdialysis probe (CMA 11, 240 μm outer diameter, 4 or 2 mm length for the striatum or the nucleus accumbens respectively; Carnegie Medicin, Phymep, France) was filled with an artificial cerebrospinal fluid (aCSF), containing (in mM): NaCl: 147, KCl: 2.7, $MgCl_2$: 1, Na_2HPO_4: 2, $CaCl_2$: 1.5, adjusted to pH 7.4 with 2 mM sodium phosphate buffer, and lowered through the guide-cannula. The probe was perfused at a flow rate of 0.5 μl/min with the aCSF by means of a microperfusion pump (CMA111, Carnegie Medicin, Phymep). Dialysates were collected after a 1-h stabilization period.

Dialysate fractions (10 μl) were collected every 20 min. The *in vitro* recoveries of the probes were approximately 10% for DA. At the end of each experiment, the animal was sacrificed by decapitation, and its brain was removed and frozen in isopentane at −30 °C. The location of the probes was determined histologically on serial coronal sections (20 μm) sliced by using a Cryostat (MICROM HM 500M) and stained with cresyl violet.

2.4. Chromatographic analysis

Dialysate samples (10 μl) were collected and placed at −80 °C pending their analysis using reverse-phase high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) [6]. The mobile phase [containing (in mM): 70 NaH_2PO_4, 0.1 EDTA, and 0.1 octylsulfonic acid plus 15% methanol, adjusted to pH 4.5 with orthophosphoric acid] was delivered at 0.25 ml/min flow rate (Beckman pump 116) through an Equisyl-BDS column (C18; 2 × 250 mm; particle size 5 μm; CIL-Cluzeau, Sainte Foy la Grande, France). Detection of DA was performed with an amperometric cell Ag/AgCl (VT-03) coupled to a programmable detector (Decade II Antec, AlphaMos, Toulouse, France). The potential of the electrode was set at +500 mV. Output signals were recorded on a computer (Beckman, system GOLD). Under these conditions, the sensitivity for DA was 0.3 pg/10 μl, with a signal/noise ratio of 3:1.

2.5. Pharmacological treatments

In each experimental group, animals were administered intraperitoneally (i.p.) either drugs or their appropriate vehicle. Alkaloids extract (containing 95–97% of nicotine of total alkaloids; see above) (0.5 mg/kg) and nicotine (0.1 or 0.5 mg/kg) were freshly diluted in physiological saline (NaCl 0.9%). Pharmacological treatments were performed 60 min after the stabilization of DA levels in the perfusate. A stable baseline, defined as three consecutive samples in which DA contents varied by less than 10%, was generally obtained 60 min after the beginning of the perfusion.

2.6. Statistical analysis

DA content in each sample was expressed as the percentage of the average baseline level calculated from the 3 fractions preceding any treatment. Data correspond to the mean ± standard error mean (S.E.M.) values of the percentage obtained in each experimental group. The ability of the tobacco alkaloids and nicotine to modify extracellular levels of DA was studied by a one-way ANOVA with time as repeated measures. A one-way ANOVA using group as the main factor followed by the *post hoc* Tukey's test was performed to determine statistical differences between groups.

3. Results

All measurements were carried out 120 min after the beginning of the perfusion, by which time a steady state was achieved. Absolute basal levels of DA in dialysate did not differ between the different experimental groups throughout the course of the study and were (mean ± S.E.M., without adjusting for probe recovery): 2.4 ± 0.4 (n = 22) and 7 ± 1.2 pg/10 μl (n = 16) rats for the nucleus accumbens and the striatum respectively.

3.1. Effect of nicotine and the extract on basal extracellular DA levels in the nucleus accumbens

In the nucleus accumbens, both nicotine (0.1 and 0.5 mg/kg) and the extract (0.5 mg/kg) enhanced DA extracellular levels across