



Nicotine in the hair of mummies from San Pedro de Atacama (Northern Chile)



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ABSTRACT

The consumption of plant-derived hallucinogenic substances through smoking and snuffing is a long-standing tradition in the south-central Andes. Chemical and archaeobotanical evidence point to the consumption of nicotine and tryptamine alkaloids in Northwestern Argentina and of tryptamine alkaloids in San Pedro de Atacama (SPA), in prehispanic times. In this paper, results are reported of gas chromatography–mass spectrometry (GC/MS) analyses aimed at identifying nicotine and tryptamine alkaloids in the hair of mummies from different cultural periods of SPA. Fifty-six samples were examined. While tryptamines were not found in any of the samples, nicotine was found in 35 samples, assigned to the Late Formative (1 of 1 sample from this period), Late Formative or Middle (1 of 2 samples from either of these periods), Middle (4 of 6 samples from this period) and Late Intermediate periods (8 of 12 samples from this period), or without assignment to period due to lack of contextual information (21 of 35 samples unassigned to a period). These results show a continuous consumption of nicotine from the Late Formative to the Late Intermediate periods of SPA (ca. 100 B.C.–1450 A.D.). No associations were found between presence of nicotine in the hair of mummies and presence of snuffing trays or of other snuffing paraphernalia in the corresponding tomb; furthermore, neither the diversity of the funerary context, measured in terms of the number of types of objects, nor the presence of gemstone necklaces differed between tombs with mummies with or without nicotine in their hair. Overall, these results suggest that consumption of nicotine was performed by members of the society at large, irrespective of their social and wealth status.

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1. Introduction

The consumption of plant-derived hallucinogenic substances through smoking and snuffing is a long-standing tradition in the south-central Andes (Torres, 1986, 1999; Torres and Repke, 2006). While smoking pipes have been found in northwestern Argentina (NWA) in archaeological sites dated as far back as 2100 B.C. (Aschero and Yacobaccio, 1994; Fernández Distel, 1980; Pérez Gollán and Gordillo, 1993, 1994), in San Pedro de Atacama (SPA) in Northern Chile they appear later and are gradually replaced in the archaeological record by snuff trays (Llagostera, 1996; Torres, 1999). Occurrence of snuff trays in SPA is concomitant with the appearance of the first elements associated to the Tiwanaku tradition (Berenguer et al., 1986); a use as vehicles for religious proselytism has been suggested for snuff trays (Berenguer, 1998).

It has been suggested that mummies found with associated snuffing paraphernalia correspond to individuals who performed

shamanic activities (Llagostera et al., 1988), that snuffing implements correspond to status symbols (Berenguer and Dauelsberg, 1989; Llagostera et al., 1988), and that hallucinogen consumption was widespread in the prehispanic SPA society (Thomas et al., 1984). A possible way to distinguish between these possibilities is to verify consumption of hallucinogens in mummies and to explore possible relationships between consumption and presence of snuff trays in the funerary context and also diversity of such context.

Two main sources of hallucinogenic compounds in the south-central Andes are nicotine-containing species of *Nicotiana* (Solanaceae), commonly referred to as tobacco, and tryptamine-containing species of *Anadenanthera* (Fabaceae), commonly referred to as cebil. Thus, chemical analysis of residues in smoking pipes from NWA revealed the presence of tryptamine alkaloids (Fernández Distel, 1980; Aschero and Yacobaccio, 1994) while in other instances, archaeobotanical analyses of pipe residues showed the presence of *Nicotiana* sp. (Capparelli et al., 2006). On the other hand, chemical analysis of the snuff powder contained in leather pouches found in two funerary contexts of SPA showed the presence of several dimethyltryptamines, particularly 5-hydroxy-N,N-dimethyltryptamine (bufotenine), which was taken as evidence for

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the consumption of *Anadenanthera* sp. (Torres et al., 1991); furthermore, thin layer chromatography coupled to a positive spot test for indoles suggested the presence tryptamines in residues associated to a Middle period snuff tray found near the coastal town of Antofagasta, Chile (Gili Hanish et al., 2009). Additionally, archaeobotanical analysis of powdered material in snuffing paraphernalia from NWA showed the presence of *Anadenanthera* sp. (Pochettino et al., 1999). In view of these antecedents, the present analyses were focused on the detection and quantitation of dimethyltryptamines and nicotine.

Consumption of psychotropic substances by modern populations has been widely assessed through analysis of residues in hair (Srogi, 2006; Man et al., 2009). However, such studies have been particularly enlightening in archaeological contexts (Wilson, 2005). Chemical analysis of hair from mummies from the area around Arica in Northern Chile has shown intake of cocaine, most likely from coca leaves (Cartmell et al., 1991a,b; Springfield et al., 1993; Rivera et al., 2005), while a similar study from the same general geographic area suggested the consumption of harmine (Ogalde et al., 2009; but see Trout, 2008), an alkaloid and monoaminooxidase inhibitor (MAO-I) present in the vine *Banisteriopsis* sp. (Malpighiaceae). On the other hand, an analysis of hair of mummies from sites along the Loa valley, also in Northern Chile, showed the absence of tropane and opioid alkaloids (Báez et al., 2000).

In this paper, the results of analyses using gas chromatography mass spectrometry (GC/MS) aimed at identifying nicotine and tryptamine alkaloids in the hair of mummies from different cultural periods of SPA are reported. The relationships between presence or absence of nicotine in mummy's hair with presence or absence of snuffing paraphernalia in the funerary context and the diversity of such context, are also reported.

2. Materials and methods

2.1. Anthropological samples

All mummies housed at the Instituto de Investigaciones y Museo R.P. Gustavo Le Paige de San Pedro de Atacama were examined for the presence of hair. Many of them were wrapped in a complex funerary bundle and their hair was not accessible. Hair samples (one sample from each individual) could be withdrawn from 56 of the ca. 450 existing mummies (Table 1). In addition, one adult hair sample of a current consumer of cebil and tobacco was analyzed as a positive control, and ten contemporary hair samples of adult non-consumers were analyzed as negative controls.

Sex could be determined in only eight mummies (one female and seven male adults); two other mummies corresponded to children and one to an infant (Table 1). Dates for the mummies studied have not been determined directly. In the absence of such direct dates, associated cultural periods were determined from features of contextual elements, mainly style of co-occurring pottery (Berenguer et al., 1986; Tarragó, 1989). Assignments could only be made in 21 cases due to the lack of relevant contextual information for the other mummies (Table 1).

The funerary context of mummies included one or more of the following types of objects: arrows, axes, baskets, bones, bowls, bows, boxes, ceramic objects, chisels, chunks of gemstones, flutes, gemstone beads, hammers, hats, hole punches, metal or gemstone ornaments, needles, necklaces, pieces of raw metals (copper, gold), pigments, squashes, snuffing paraphernalia (bags, mortars, pestles, snails, spatules, trays, tubes), spoons, textiles, threaders, vases, vegetable residues, and urns. A context diversity index was considered a general proxy for social and wealth status of the mummy, and was defined as the number of types of objects among those mentioned above found with the mummy (maximum

possible value of index = 34). Number of types of objects was used instead of number of objects because the latter figure is in many cases ill-defined, only a plural being mentioned in field notes for some objects. Additionally, although the relative cultural value of objects in SPA tombs has not been assessed quantitatively, the funerary context was examined for the presence of necklaces made of gemstones (typically turquoise) or ignimbrite, another proxy that can be assumed to be related to social and wealth status. A list of contextual objects was available for 41 of the mummies studied (Table 1).

2.2. Chemicals

Methanol and chloroform were high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany or JTBaker, USA). Nicotine was purchased from Sigma–Aldrich (St Louis, MO, USA). Bufotenine was generously provided by Prof. Maria L. Dos Santos from Instituto de Química – Universidade de Brasília, Brasil.

2.3. Preparation of hair samples

Hair strands (ca. 100 mg) were cut into 1 mm segments, washed with 3×3 mL of chloroform (15 min each time) and filtered through a composite made with one layer of 0.20 mm steel mesh and five layers of 0.25 mm steel mesh (Millipore Corporation, Billerica, MA, USA) using a 5 mL glass syringe Ultrafit (Henke, Sass Wolf GMBH, Germany). The washed hair was finely pulverized by placing it inside 1.8 mL stainless steel microvials with polyethylene flange caps and containing 25 stainless steel 1-mm diameter beads, and agitating it for 10 min in a bead beater (Mini-Beadbeater-96; Biospec Inc., Bartlesville, OK, USA). The pulverized hair samples were macerated in 500 μ L chloroform with periodical vortexing at ambient temperature for 72 h, and the suspension filtered through cotton wool placed at the tip of a Pasteur pipette using an additional 500 μ L aliquot of chloroform. The remaining hair was dried under nitrogen flow and further macerated in 1 mL of methanol with periodical vortexing at ambient temperature for 72 h. The methanolic extracts were filtered through cotton wool placed at the tip of a Pasteur pipette using an additional 1 mL aliquot of methanol. All extracts were first collected in 2 mL amber vials with teflon-lined screw caps. Aliquots were successively transferred to a 300 μ L glass insert within an amber vial, from which they were taken to dryness by means of a nitrogen flow; this operation minimized the quantity of residue retained in the vessel walls. For gas chromatography/mass spectrometry (GC/MS) analysis in the scan mode (all ions produced in the mass detector are simultaneously detected), the extracts were reconstituted in 20 μ L and for GC/MS analysis in the selective ion monitoring (SIM) mode (only specific ions are detected, thus enhancing the selectivity and sensitivity of analyses), the extracts were reconstituted in 15 μ L.

2.4. GC/MS analysis

GC/MS analysis was performed with a Shimadzu model GCMS-QP 2010 Ultra gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a Rtx-5MS Crossbond 5% diphenyl - 95% dimethyl polysiloxane (Restek, Bellefonte, PA, USA) capillary GC column (30 m length, 0.25 mm I.D., 0.25 μ m film thickness). The GC was operated in the splitless injection mode; injection volume was 1 μ L or 5 μ L for scan and SIM modes, respectively. The column temperature was held at 30 °C for 3 min, raised at 25 °C/min to 230 °C, and maintained for 10 min at 230 °C. The carrier gas was helium at a flow rate of 1.3 mL/min. The mass spectrometer was used in the electron impact ionization mode (70 eV) with an emission current

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