Altered neurochemical levels in the rat brain following chronic nicotine treatment

Sara Falasca a, Vaclav Ranc a, Filomena Petruzzello a, Abbas Khani a, Robert Kretz a, Xiaozhe Zhang a,*, Gregor Rainer a,b

a Visual Cognition Laboratory, Department of Medicine, University of Fribourg, Chemin du Musée 5, Fribourg CH-1700, Switzerland
b Fribourg Center for Cognition, University of Fribourg, Fribourg CH-1700, Switzerland

ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 21 May 2014
Accepted 25 May 2014
Available online 7 June 2014

Keywords:
Neurochemicals
Chronic nicotine
Prefrontal cortex
Dorsal striatum
Hypothalamus

ABSTRACT

Converging evidence shows that neurochemical systems are crucial mediators of nicotine dependence. Our present study evaluates the effect of 3-month chronic nicotine treatment on the levels of multiple quaternary ammonium compounds as well as glutamate and gamma aminobutyric acid in the rat prefrontal cortex, dorsal striatum and hypothalamus. We observed a marked decrease of acetylcholine levels in the dorsal striatum (22.88%, p < 0.01), reflecting the impact of chronic nicotine in local interneuron circuits. We found decreases of carnitine in the dorsal striatum and prefrontal cortex (19.44%, p < 0.01; 13.58%, p < 0.01, respectively), but robust enhancements of carnitine in the hypothalamus (26.59%, p < 0.01), which may reflect the alterations in food and water intake during chronic nicotine treatment. Finally, we identified an increase of prefrontal cortex glutamate levels (8.05%, p < 0.05), supporting previous studies suggesting enhanced prefrontal activity during chronic drug use. Our study shows that quaternary ammonium compounds are regulated in a highly brain region specific manner during chronic nicotine treatment, and provides novel insights into neurochemical regulation during nicotine use.

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Introduction

Nicotine is the main psychoactive component of tobacco and one of the most widely taken drugs of abuse (Laviolette and van der Kooy, 2004). The use of nicotine has a number of psychoactive effects including euphoria, reduced stress, anti-fatigue and enhanced cognitive functions (Benowitz, 2010). However, similar to other psychoactive drugs such as cocaine and alcohol, the repeated use of nicotine results in physical dependence, which is characterized by diverse aversive effects particularly associated with drug withdrawal. By acting on nicotinic acetylcholine receptors (nAChRs), which are distributed at presynaptic and postsynaptic sites in different types of neurons distributed in various brain areas (Albuquerque et al., 2009), nicotine regulates many pathways such as the reward and stress systems (Watkins et al., 2000). Chronic nicotine differentially affects the function of release-regulating nAChR subtypes (Grilli et al., 2005). So far, a growing body of evidence has demonstrated that chronic nicotine administration alters several neurochemical circuits, for example affecting glutamate and gamma aminobutyric acid (GABA) levels in the ventral tegmental area (VTA) (Changeux, 2010), glutamate levels in the prefrontal cortex (Shameem and Patel, 2012). The modulation of GABA release seems to be very important for the action of nicotine (Barik and Wonnacott, 2006; Zhu and Chiappinelli, 2002). Nicotine application also enhances the levels dopamine and opioid peptides in the striatum (Hadjiconstantinou and Neff, 2011), and the levels of several classes of neuropeptides in the hypothalamus (Chen et al., 2007; Lage et al., 2007; Li et al., 2000; Plaza-Zabala et al., 2012). By comparison, there is still much less known about the impact of chronic nicotine on quaternary ammonium substances, such as acetylcholine, choline, carnitine and acetylcarnitine, which are closely linked to cholinergic neuromodulation.

Acetylcholine and choline are both endogenous agonists of nAChRs. Choline is the precursor for intracellular acetylcholine synthesis and also its degeneration product after extracellular release. By binding to the nAChR, they can both compete with nicotine, thus affecting the activation and desensitization of nAChRs during chronic nicotine treatment (Dani and De Biasi, 2001). For example, previous in vitro studies in a striatal slice preparation showed that acute nicotine application resulted in the increased release of acetylcholine, while this increase could...
be attenuated if the animal was pre-treated with chronic nicotine (Yu and Wecker, 1994). The attenuation of acetylcholine release may be related to nAChR desensitization or the activation of presynaptic nAChRs on GABAergic interneurons that in turn inhibit activity of cholinergic striatum neurons. In addition to regulating nAChR density and sensitivity, the total amount of intra- and extracellular acetylcholine may also be regulated by chronic nicotine treatment, but such data are currently available neither for acetylcholine nor for choline.

Carnitine and acetylcarnitine are two quaternary ammonium substances that have structural similarities to choline and acetylcholine, respectively. Synthesis of acetylcholine and acetyl-L-carnitine is accomplished by coupled enzyme systems choline acetyltransferase and carnitine acetyltransferase (White and Sates, 1990). Due to this structural similarity, carnitine and acetylcarnitine can have an impact on cholinergic neurotransmission, while also exhibiting neuroprotective properties (Imperato et al., 1989; Picconi et al., 2006; Ricny et al., 1992).

Importantly, carnitine and acetylcarnitine are also critical substances involved in fatty acid metabolism (Jones et al., 2010). Fatty acid metabolism is a pathway affected by nicotine treatment, as has been shown in peripheral tissue (Cryer et al., 1976; Hellerstein et al., 1994). Despite the apparent links of carnitine and acetylcarnitine to the cholinergic system, little is known about whether their levels are regulated in the brain during chronic nicotine treatment.

Our study aimed to evaluate the effects of chronic nicotine treatment on the changes of these quaternary neurotransmitters as well as glutamate and GABA as reference, which are fundamental excitatory and inhibitory amino acid neurotransmitter in the brain. Based on the types of cholinergic projections, we selected three representative brain areas, namely prefrontal cortex (PFC), dorsal striatum (DS), and hypothalamus (HT), to study the impact of chronic nicotine on the target neurotransmitters. PFC receives cholinergic project from basal forebrain, HT receives cholinergic projection from brain stem, while DS has its own cholinergic interneurons in the absence of cholinergic innervations from other brain areas (Woolf, 1997). PFC, DS and HT areas are all important brain areas involved in drug dependence (Koob and Volkow, 2010). We used high accuracy mass spectrometry (MS) to monitor the levels of acetylcholine, choline, carnitine, acetylcarnitine, glutamate and GABA. Differential analysis revealed that each neurochemical was altered in at least one brain region. The results revealed the specific decrease of acetylcholine, carnitine and acetylcarnitine in DS, increase of glutamate and decrease of carnitine in PFC, increase of choline, carnitine and GABA in HT. Our study provides novel insights into the involvement of quaternary ammonium compounds in neurochemical distortions caused by chronic nicotine treatment, advancing our understanding of neurochemical mechanisms of drug addiction.

**Materials and methods**

**Reagents**

Choline chloride, acetylcholine chloride, carnitine hydrochloride, glutamic acid, GABA and acetylcarnitine hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Choline-d9 chloride (Sigma–Aldrich), acetylcholine-d4 chloride (Medical Isotopes Inc., Pelham, NH, USA), carnitine-d3 hydrochloride, acetylcarnitine-d9 hydrochloride, GABA-d6 and glutamate-d5 (Cambridge Isotope Laboratories Inc., Andover, MA, USA), were used as internal standards. LC–MS grade formic acid, ammonium hydroxide solution; methanol and acetonitrile were supplied by Sigma–Aldrich. Water was obtained from a GenPure water system (TKA, Niedereubert, Germany).

Animals and sample preparation

Long-Evans rats (Rattus norvegicus) were used in this study (n = 20). The animals were housed under constant temperature and humidity with free access to food and water. The population was divided into a drug and a control group with 10 animals each. The nicotine administration protocol was adapted from previous research performed on mice or monkeys (Oddo et al., 2005; Quik et al., 2006). Drinking solution for the control group consisted of 1% solution of saccharine and was given to the group for 13 weeks. Animals in the drug group received a gradient dose of nicotine in drinking water that also contained 1% saccharine. Animals were started with 10 μg/ml nicotine for the first week, then 25 μg/ml nicotine for the second and third weeks and finally 50 μg/ml for 10 weeks. This profile was used to adapt animals to the drinking water containing nicotine. Water consumption was monitored on a daily basis for both groups. During the final week, the average water consumption was 23 ml for nicotine group rats, and 35 ml for control rats. Average nicotine intake during the last week of administration was thus 1.5 mg/kg/day. The average weight of nicotine rats at the end of the administration period was 393 g, 11 g less than the average weight of control rats (unpaired t-test, p < 0.01). The handling of the animals and the experimental procedures were approved by the veterinary office of Fribourg, Switzerland and in full compliance with applicable European Union veterinary directives. Animals were sacrificed by decapitation after anesthetization with ketamine (100 mg/kg, Streuli Pharma AG, Uznach, Switzerland). The three brain areas of interest were afterwards stored at −80°C prior to dissection. The dissections of HT, DS and PFC were performed carefully from each hemisphere under microscope following their marginal lines according to the adult rat atlas. First the olfactory bulb was removed, then the PFC was cut away by a frontal section about 2 mm from the frontal pole. The rest of the hemisphere was cut in frontal sections using a razor blade. From 1.5 to 2 mm sections, the areas of interest (DS and HT) were trimmed using a miniature scalpel blade. HT including the anterior, tuberal and posterior areas was dissected. The structures were identified on frontal sections based on different colors of the structures (cortex, white matter and nucleus). All the operations were performed on dry-ice cooled glass plate (around −10°C) to protect the decay of tissues.

**Sample pre-treatment**

The tissue of each brain area was collected from the left hemispheres. The extraction method has been described elsewhere (Falasca et al., 2012). Briefly, tissue (21 mg) was spiked with 30 μl of a mixed internal standard spiking solution. The tissues were then homogenized using a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France), with 120 μl ice-cold acetonitrile containing 0.3% formic acid. The homogenates were centrifuged at 22,000 × g for 20 min at 4°C. The supernatants were collected and filtered by 0.20 μm filter membranes (Millex-LG, Millipore, Billerica, MA, USA). 15.6 μl of the filtered supernatants were diluted with 89.4 μl of 85% acetonitrile containing 0.3% formic acid. The concentrations of acetylcholine-d4, choline-d9, carnitine-d3, acetylcarnitine-d9, GABA-d6 and glutamate-d5 in final tissue extracts were 0.3 μM, 4 μM, 1 μM, 1 μM, 15 μM and 50 μM, respectively.

**LC–FT–MS analysis**

In this study, we employed a capillary LC system (Eksigent, Dublin, CA, USA) coupled to a LTQ-Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a nano-electrospray ion source operating in positive
ion mode. For the nanospray ionization, the column was connected to a tip emitter (stainless steel, O.D. 150 μm, I.D. 30 μm, Thermo Fisher Scientific). The ion spray voltage was 2.2 kV, the capillary temperature was set at 250 °C and the capillary voltage was 31 V. To increase the sensitivity of the mass analysis, the acquisition was performed in selected ion monitoring (SIM) mode. For each analyte investigated, a SIM scan event was used in which the scan range was set as M+ ± 1 m/z.

The chromatographic separation of neurochemicals was conducted on custom-made capillary HILIC columns (200 μm ID × 25 cm) using particles of 5 μm (Polyhydroxyethyl Aspartamide, PolyLC, Columbia, MD, USA) (Zhang et al., 2007). In each LC–MS analysis, 1 μL of sample was injected on the column with initial conditions 80% acetonitrile. Separation was then performed using solvents acetonitrile (B) and 50 mM ammonium formate containing 1% formic acid (A) using a profile at a flow rate of 3 μL/min: 0–1 min, 80% (B); 1–10 min, linear gradient 80–40% (B); 10–15 min, 40% (B); 15–25 min, returning linear gradient 40–80% (B); 25–35 min, 80% (B). The mass spectrometer was calibrated using the manufacturer’s calibration standard mixture. The SIM mass spectra were acquired in profile mode with a setting of 30,000 resolutions (FWHM) at m/z 400. The entire LC–MS system and data processing were performed using Xcalibur software (Thermo Fisher Scientific). Each peak area was integrated in its extraction ion chromatogram (EIC) representation with 10 ppm mass tolerance. The LC–FT–MS analysis of each brain sample was repeated two times.

Quantitative analysis

A calibration curve of each neurochemical individual was prepared for quantitative analysis. Standard and internal standard stock solutions were prepared in 20% methanol containing 0.1% formic acid and stored at −20 °C. These stock solutions were diluted to obtain each calibration solution in 85% acetonitrile containing 0.3% formic acid. A 5-point calibration curve was set up for each analyte using its isotopically labeled internal standards. The linear ranges were acquired within an interval of two orders of magnitude for each neurochemical (Li et al., 2012).

The chronic nicotine dependent changes of target neurochemicals were established using relative quantification. Peak area ratio (Rpa) of a neurochemical between its endogenous formation and its internal standard was calculated. Then the peak area ratio of each neurochemical was compared between the control and nicotine group animals. An unpaired t-test was used to evaluate if a neurochemical was significantly altered by chronic nicotine treatment (p < 0.05). The ratio between the content of two paired neurochemicals were calculated by dividing their Rpa values. The calculated ratios of choline-acetylcarnitine, carnitine-acetylcarnitine and GABA-glutamate were Rpa(choline)/Rpa(acetylcarnitine), Rpa(carnitine)/Rpa(acetylcarnitine) and Rpa(GABA)/Rpa(glutamate), respectively. These values were subjected to t-test to examine if the ratio of two paired neurochemicals was significantly altered by chronic nicotine treatment (p < 0.05).

Results

Determination of basal levels of target neurochemicals

The absolute levels of target neurochemicals in each brain region were determined using liquid chromatography (LC)–Fourier Transform (FT)–MS. The levels of neurochemicals in the extracts are shown in the Fig. 1. Acetylcarnitine and carnitine in tissue had very similar levels around 0.06 μmol/g (Fig. 1A). Acetylcholine values were also similar across the three brain regions, but exhibited the lowest abundance with respect to the other neurochemicals monitored with a value of around 0.01 μmol/g. Choline levels in the three brain regions ranged from 0.24 μmol/g to 0.41 μmol/g, with highest levels in DS (paired t-test, p < 0.05). The levels of glutamate and GABA were much higher than those of acetylcarnitine, choline, acetylarnitine and carnitine (Fig. 1B). In particular, glutamate had highest abundance in the three brain areas, with values of 54.3 μmol/g, 34.7 μmol/g and 22.8 μmol/g and in PFC, DS and HT, respectively.

Fig. 1. Absolute basal levels of investigated neurochemicals, evaluated in PFC, DS and HT of control group rats. Each bar represents an average value from 10 animals and the error bars denote the standard error of mean (S.E.M.).
Brain area-specific alteration of neurochemicals by chronic nicotine treatment

Having established the baseline levels of the targeted neurochemicals, we proceeded to examine the change of these neurochemicals following chronic nicotine administration as shown in the Fig. 2. Acetylcholine levels were unchanged in HT and PFC, but showed significant down-regulation in the DS. Choline was significantly up-regulated in HT. Among the six neurochemicals, carnitine was the only neurochemical whose level varied significantly in all the three brain areas, with increases in the HT and decreases in DS and PFC. Along with acetylcholine, carnitine and acetylcarnitine were jointly down-regulated in DS. Modulations of GABA and glutamate levels were relatively modest by comparison to the quaternary ammonium compounds. We did however observe significant increases in GABA in HT and glutamate in PFC.

We examined whether chronic nicotine treatment had any effect on the ratio between several pairs of neurochemicals that are of potential importance for brain and metabolic function, as shown in Fig. 3. The choline/acetylcholine balance was increased—and thus shifted in favor of choline—after chronic nicotine in both DS and HT (p < 0.05). The balance between carnitine and acetylcarnitine was unchanged in DS, reflecting their joint regulation. However, the balance was altered in both PFC and HT, with a shift in favor of acetylcarnitine in PFC and a shift in favor of carnitine in HT. The balance between GABA and glutamate was unaffected by chronic nicotine treatment in all three brain regions.

Comparison of neurochemical changes across brain areas

To assess the overall impact of chronic nicotine treatment on neurochemical regulation between the three targeted brain areas, we performed a correlation analysis on the relative changes of the six targeted neurochemicals between pairs of brain areas as shown in Fig. 4. We observed a weak correlation between neurochemical levels in the DS and HT regions (R = 0.39), and a strong correlation between DS and PFC (R = 0.90). By contrast, neurochemical levels were uncorrelated (R = -0.03) between HT and PFC regions. This analysis supports the notion that neurochemical regulation by
chronic nicotine is brain-region specific, with a high similarity in regulatory mechanisms in DS and PFC.

Discussion

In this study, we measured basal levels of six neurochemicals and then evaluated the effects of chronic nicotine administration on their levels in three brain regions of interest: the hypothalamus (HT), dorsal striatum (DS) and the prefrontal cortex (PFC). We found that the neurochemicals GABA, glutamate, acetylcholine, choline, acetylcarnitine and carnitine changed in a highly brain-region-specific manner. Most prominent among the specific neurochemical changes was an up-regulation of carnitine in HT and a down-regulation of acetylcholine in DS.

We observed that acetylcholine levels were significantly decreased in DS but unchanged in HT and PFC. The DS acts as an important player in the development and maintenance of drug dependence for nicotine and other drugs of abuse (Everitt and Robbins, 2005; Rothemund et al., 2007; Veeneman et al., 2012; Yamamoto and Zahniser, 2012). Our findings are consistent with previous in vitro work demonstrating a decrease in acetylcholine release following acute nicotine delivery to slice preparations of the striatum of rats that had received chronic nicotine treatment (Yu and Wecker, 1994). The decrease of DS acetylcholine levels may be related to characteristics of the local neuronal circuitry. Unlike the PFC and HT, which mostly receive cholinergic projections from other brain areas, the DS contains many cholinergic interneurons and almost no cholinergic projection neurons to or from other brain regions (Zhou et al., 2002). At the same time, the DS also contains many GABAergic interneurons that express nAChRs and exert an inhibitory influence on DS cholinergic neurons. Consequently, the prolonged activation of these GABAergic during chronic nicotine exposure may lead to an inhibition of DS cholinergic networks and thus reduces acetylcholine synthesis and synaptic release. The existence of such a mechanism, which is DS specific and related to its neural architecture, is supported by the relative ratio analysis that demonstrated particularly large choline/acetylcholine ratio changes in DS compared to the other two brain regions. In any case, our results suggest that chronic nicotine treatment distorts cholinergic neurochemical processing in the DS.

Choline is a major precursor necessary for acetylcholine synthesis under the action of the enzyme choline acetyltransferase (ChAT) (Dobransky and Rylett, 2005). It has been reported that ChAT activity is inhibited by chronic nicotine treatment (Fuxe et al., 1994), which might result in elevated choline and reduced acetylcholine levels, as we have observed in the DS. However, choline levels in the HT were also robustly enhanced despite a notable absence of changes in acetylcholine levels, suggesting choline accumulation by a mechanism other than ChAT inhibition. We suggest that membrane structural plasticity associated with chronic nicotine treatment may be largely responsible for HT choline enhancements. Accordingly, phosphatidylcholine, a key component of cell lipid membranes (Li and Vance, 2008), may be degraded by chronic nicotine treatment resulting in the liberation of choline in agreement with reported membrane disturbances (Pettegrew et al., 2001) and reduced brain mass following chronic nicotine treatment (Miller et al., 2001).

Previous studies showed that nicotine could prompt the release of glutamate (Couey et al., 2007; Marchi et al., 2012; Zappettini et al., 2010). Consistent with these findings, we observed that chronic nicotine intake induced a significant increase in PFC glutamate levels. This result is also in line with previous observations based on the entire rat cortex using nuclear magnetic resonance (NMR) spectroscopy (Shameem and Patel, 2012). The regulation of glutamatergic transmission requires the involvement of different nAChR subtypes. In addition, chronically administered nicotine can also regulate glutamatergic neurotransmission functions by modulating the sensitivity of excitatory amino acid receptors (Grilli et al., 2009; Parodi et al., 2006; Risso et al., 2004). Collectively, enhanced PFC glutamatergic activation during chronic nicotine administration can be conceptualized as a consequence of drug-use related modifications in the neural addiction circuitry, particularly concerning circuitry elements that expressing nAChRs (Corrigall, 2009; Laviolette and van der Kooy, 2004). Thus, repeated use of nicotine leads to the desensitization of many subtypes of nAChRs in the brain (Oertels and Arias, 2010), an effect that has been described for example in the ventral tegmental area (VTA) (Laviolette and van der Kooy, 2004). We suggest that in the PFC, chronic nicotine use may also result in the desensitization of nAChRs in GABAergic neurons, reducing the amount of inhibitory influence of these neurons and enhancing glutamate levels (Wang et al., 2008). A role for a nAChR-related mechanism is supported by unchanged PFC GABA levels as well as the unchanged ratio of GABA/glutamate, which suggests that the glutamate enhancements are not related to decreased inhibitory neurotransmitter but rather to reductions of postsynaptic GABAergic efficacy.

A striking aspect of our results is the robust elevation of carnitine in the HT. This enhancement is likely a result of an accelerated HT fatty acid metabolism. It is generally accepted that chronic nicotine application can lead to the loss of weight (Mineur et al., 2011; Sanigorski et al., 2002), as observed in our study. In contrast to the prevailing view that hypothalamic neuropeptides are the main mediators regulating food-intake, an increasing body of evidence indicates that food intake and body weight homeostasis can also be modulated by HT lipogenesis. The fatty acid metabolism can in turn impact peptidergic transmissions and thus synergistically regulate feeding behavior. Carnitine is a critical

![Fig. 4. Correlation of relative changes of targets neurochemicals between (A) HT and DS, (B) PFC and HT, (C) PFC and DS. Triangles indicate the mean relative changes of a neurochemical across two brain areas. The P value indicates the correlation coefficient of the six neurochemical levels across the respective brain areas.](image-url)
substance involved in fatty acid metabolism, where it is conjugated with fatty acids facilitating its transportation into mitochondria (Rebouche and Seim, 1998). An elevation of free carnitine is expected to increase fatty acid metabolism. The enhanced carnitine/acetylcarnitine ratio observed following chronic nicotine administration suggests that the fatty acid metabolic cycle is limited by other factors related to the nicotine treatment. An elevation of free carnitine would thus increase fatty acid entry in the mitochondria and fatty acid oxidation, stimulating ROS production, which has been linked to signaling of anorexigenic signals (Horvath et al., 2009). In this way, elevated carnitine levels might represent a HT mechanism that contributes to down-regulation of food intake and ensuing weight loss that is characteristic of chronic nicotine use. Note that methodological aspects of oral chronic nicotine administration, such as the addition of saccharine to the water to counteract the bitter taste of nicotine and ensuing differences in liquid consumption between chronic nicotine and control group animals, might also potentially play a role in the observed enhanced HT carnitine levels. In addition, nicotine is also known to stimulate other anorexigenic components such as POMC neurons and inhibit orexigenic components such as neuropeptide Y neurons, providing additional mechanisms that lead to decreased food intake (Mineur et al., 2011). In this context, the elevated levels of the inhibitory neurotransmitter GABA in the HT are of interest, because they likely reflect up-regulation of inhibitory drive on orexigenic HT peptide systems involving nAChR-mediated processes. In contrast to the changes in HT, carnitine was decreased in PFC and DS, probably reflecting reduced fatty acid metabolism and increased energetic stress after nicotine treatment (Pettegrew et al., 2001). While the exact causes of this behavior are unknown, this does suggest that the HT may contain specific enzymatic machinery that sets it apart from other brain areas in terms of its response to chronic nicotine.

Compared to the DS–HT and PFC–HT brain area pairs, PFC and DS exhibited similarities in the neurochemical response to chronic nicotine treatment. PFC and DS indeed have a close anatomical relationship, for example DS receiving glutamatergic projections from PFC (Zhou et al., 2002). In line with their anatomical connections, a line of evidence has demonstrated that PFC and DS are both associated with craving and habitual learning (Yalachkov et al., 2009) and are jointly involved in reinstatement of drug seeking (Fuchs et al., 2005), which are critical factors for development and maintenance of drug dependence. These close anatomical and functional associations appear to be responsible for the high correlation between the overall changes of neurochemicals in the two brain areas. In contrast, the DS–HT or the PFC–HT brain area pairs appear to be less closely associated, resulting in lower correlations between the overall changes of neurochemicals.

The changes of a neurochemical might depend on the dosage and exposure length of nicotine applied, as observed in many studies. The chronic administration of nicotine can result in an increase in brain nicotinic acetylcholine receptor density, which is a receptor up-regulation caused by long-term nAChR desensitization in vivo. However, this desensitization relies on many factors, such as brain region, nAChR subtype, nicotine dosage and nicotine exposure period, which can consequently affect the synthesis and release of neurochemicals in the brain. Our study clearly showed that at the present condition used, chronic nicotine generated highly brain region-specific patterns of neurochemical changes. It will be interesting for us to investigate the effects of nicotine dosage and cessation on these neurochemicals in the near future.

The present study focused on the effect of chronic nicotine treatment on targeted neurochemicals, including quaternary ammonium compounds and amino acid neurotransmitters. Our main findings are a selective enhancement of carnitine in the HT, a selective decrease of acetylcholine in the DS and an elevation of glutamate in the PFC. We relate these neurochemical observations to anatomical aspects of the neural circuitry, including the distribution of nAChR receptors. We discuss our results in terms of behavioral, addiction- and metabolism-related aspects of chronic nicotine use. Our results provide novel starting points for advancing the understanding of the neurochemistry of nicotine dependence.

Acknowledgements

We thank Dr. Dulloo Abdul and Dr. Giovanni Solinas for the discussion on the study. This work was supported by the Swiss SNF R’Equip 316000–121308 and a EURYI award to GR.

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