Histamine H_3 receptor gene variants associated to drug abuse in patients with cocaine use disorder

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Abstract

Background: Preclinical work revealed significant interactions between ligands of the histamine H_3 receptor and different drugs of abuse. In the case of psychostimulants the results reported are somewhat controversial and human data are still scarce, despite the fact that an inverse agonist of the H_3 receptor (pitolisant) has reached the market after approval for the treatment of narcolepsy.

Aims: We have studied associations between histamine H_3 receptor gene variants and cocaine use disorder in order to increase the knowledge of the possible involvement of histamine H_3 receptor in drug abuse.

Methods: Seven single nucleotide polymorphisms (SNPs) of the histamine H3 receptor gene were genotyped by using a multiplexing assay in 248 samples of subjects with cocaine use disorder and 500 randomized samples of subjects representative of the Spanish population.

Results: The study of the epidemiological information associated to the samples revealed that subjects with cocaine use disorder broadly abused alcohol, tobacco and cannabinoids. Two SNPs (rs3787430 and rs74627870) were found significantly associated with the occurrence of addiction and one more (rs13042865) was specifically related to the severity of cocaine dependence within drug abusers.

Conclusions: The associations found in this study further extend the hypothesis that histamine H_3 receptor function could be relevant in drug abuse in general and cocaine addiction in particular.

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Keywords: histamine H_3 receptor, cocaine use disorder, genetic polymorphisms

Introduction

Histaminergic pathways arising from the tuberomammillary nucleus of hypothalamus act upon different key structures in addictive disorders such as prefrontal cortex and the striatum, and have been proposed to play an important role on the rewarding effects of drugs of abuse (Brabant et al., 2010). The effects of histamine are mediated by four main receptors in the brain and one of them, the histamine H_3 receptor (H_3R), has been suggested to be a potential drug target for the treatment of addiction and other diseases of the central nervous system such as Alzheimer and Parkinson diseases, Gilles de la Tourette syndrome, and disorders related to sleep and wakefulness (Alguacil and Pérez-García, 2003; Nieto-Alamilla et al., 2016). Within the brain reward system, H_3Rs are mainly located postsynaptically on medium sized spiny output neurons of the striatum but also presynaptically on key sites such as dopaminergic nerve terminals, where they can control neurotransmitter release; Ellenbroek (2013) reviewed the literature on the role of these receptors and their ligands on dopamine function and drug addiction and showed complex interactions and apparent discrepancies between studies. Our research team was the first to describe a limitation of opioid addiction by H_3R blockade, since the antagonist thioperamide was able to prevent morphine-induced place preference in rats (Pérez-García et al., 1999). H_3R antagonists and/or inverse agonists were also shown to reduce alcohol consumption, alcohol-induced place preference and cue-induced reinstatement of alcohol seeking behaviours in a very consistent manner (Panula, 2020). However, the effects H_3R ligands on psychostimulant abuse are much more controversial. H_3R antagonists and/or inverse agonists such are thioperamide and clobenpropit were found to potentiate methamphetamine self-administration (Munzar et al., 2004),

and thioperamide was also found to increase cocaine-induced hyperlocomotion and place preference (Brabant et al., 2009). These findings suggested that H_3R blockade could increase the risk of psychostimulant abuse. Later work did not confirm these findings since the H_3R inverse agonist pitolisant, already marketed for the treatment of narcolepsy, did not seem to exhibit addictive potential by itself in animals and humans, neither modified the reinforcing effects of cocaine in several experimental models (Huyts et al., 2019; Setnik et al., 2019). It has been argued that thioperamide, unlike pitolisant, could potentiate the effects of cocaine by increasing plasma concentration of this drug after blocking cytochrome P_{450} activity (Brabant et al., 2016), hence pharmacokinetic interactions may represent a confounding factor in this kind of studies.

Up to our knowledge, genetic studies have not been conducted to investigate possible relationships between polymorphisms of the H_3R gene (HRH3) and psychostimulant abuse by humans. In this work we have applied this approach to provide further knowledge of H_3R involvement on drug addiction.

Method

Seven SNPs of the HRH3 gene were studied in subjects with cocaine use disorder (CUD) and control individuals. DNA samples from 248 subjects diagnosed of CUD as main addiction were provided by the Spanish Addiction Disease Network Biobank (Biobanco RTA), integrated in the Valencian Biobanking Network. There was a disproportion between males and females in the sample that fits with the higher number of male addicts attending health care facilities previously described (European Monitoring Centre for Drugs and Drug Addiction, 2019). Samples from 250 male and 250 female control subjects were provided by the Spanish DNA National Bank Carlos

III (BNADN) after random selection; the sex, ages and BMIs of the donors were similar to those reported for the general Spanish population (Instituto Nacional de Estadística, 2017). Bearing in mind the dissimilar distribution of males and females in both samples, the comparative study of SNPs was first performed with the complete dataset of individuals and then confirmed with datasets matched by age and sex, thus trying to avoid any confounding effect of sex unbalance. Table 1 collects the main characteristics of the groups studied. DNA samples were collected with the written informed consent of the participants and processed and stored following standard operating procedures approved by the Ethical and Scientific Committees of the two biobanks involved. The full study was finally approved by the San Pablo CEU University Ethics Committee (USP 191-17).

Seven SNPs of HRH3 with a minor allele frequency greater than 5% were selected for this study from the dbSNP database (NCBI). All of them were shown to be in Hardy-Weinberg equilibrium (Table 2). Genotyping was performed in the facilities of the Spanish National Center for Genotyping (CEGEN-PRB3-ISCIII). Briefly, samples received from the biobanks were normalized to 20 ng DNA/µl with Milli Q water in a Evo Freedom liquid handling robot (Tecan, Männedorf, Switzlerand), then analysed by using a multiplexing platform (Sequenom iPLEX Gold, Agena Bioscience, San Diego CA, USA) based on a simple single-base primer extension assay and Matrix-Assisted Laser Desorption/Ionisation, Time-of-Flight mass spectrometry (MALDI-TOF) for allelic discrimination. In this assay, polymerase chain reaction (PCR) primers were designed in a region of approximately 100 base pairs around each SNP of interest, and an extension primer was designed immediately adjacent to the SNP; PCR products were single-nucleotide-extended using dideoxynucleotide triphosphates (ddNTPs) with some of their atoms substituted in order to

generate weight differences between alleles. Mass spectrometry was then used for allele differentiation by weight.

Two separate data analysis were performed (SPSS® V24 software) with each dataset: first, the genotype of cocaine users was compared to that of controls to investigate SNPs associated with the presence of CUD; second, the genotype of cocaine users was studied for associations between SNPs and severity of cocaine dependence according to the Substance Dependence Severity Scale of the Disease and Statistical Manual IV (SDSS/DSM-IV, with grouped values of 0/1/2 for absent/intermediate/high dependence). Binary (BLR) and ordinal (OLR) logistic regressions were applied for the first and second analysis, respectively; both models fitted with the data, as assessed by Log Likelihood ratio test, R^2 Nagelkerke, Hosmer – Lemeshow (BLR), correct classification percentage of the model, parallel test (OLR), and Pearson deviation (OLR). The level of statistical significance was always set at $p < 0.05$.

Results

The information associated with biological samples showed that most subjects with CUD also abused other legal and illegal drugs beyond cocaine (Figure 1). Table 3 shows the HRH3 polymorphisms that were found differentially distributed in the groups of study. Two of them, rs3787430 and rs74627870, were found to be differentially distributed in drug abusers and control subjects, either in the complete and the matched datasets. In the case of rs13042865 an association was obtained with the degree of cocaine dependence among drug abusers.

Discussion

The study of the data associated to our biological samples shows that subjects with CUD must be better considered polydrug abusers. The strong association of cocaine and alcohol abuse is specially relevant and fits very well with many studies from the literature, even beyond self-reported data: thus, for instance, the determination of the cocaethylene/benzoylecgonine ratio in raw wastewater from a Spanish town quantified in 58% the co-consumption of cocaine and alcohol during weekends (Rodríguez-Álvarez et al., 2015). This finding provides epidemiological validity to our sample but makes it difficult to interpret the exact meaning of genetic differences between drug abusers and controls concerning vulnerability to cocaine addiction. Accordingly, we interpret that the two HRH3 polymorphisms showing differences between both populations (rs3787430 and rs74627870) could be properly associated to drug abuse in general, rather than cocaine addiction in particular. By contrast, the association between rs13042865 genotypes and severity of cocaine dependence within drug abusers suggests a closer relationship between this SNP and cocaine addiction in particular, even though the study of “pure” cocaine addicts is mandatory to confirm this point.

The observed associations further extend the hypothesis that H_3R could be relevant in drug addiction, as previously commented (Munzar et al., 2004; Brabant et al., 2009). A convincing biological interpretation of these results cannot be yet provided, bearing in mind that the functional

consequences of the genetic variants detected are largely unknown and thus require additional, specific work to be fully understood. In fact, functional data is still missing for most HRH3 polymorphisms (Micallef et al., 2013; Panula et al., 2015). The SNP rs3787430 is located in exon 3 and therefore could affect receptor function since this region encodes T4-T7 transmembrane domains and C-terminal, which are essential for receptor signaling (Bongers et al., 2007). Interestingly, this SNP has been reported to be of predictive value on the antipsychotic effect of the D_2R receptor antagonist risperidone (Wei et al., 2012). Taken together, these findings suggest that genetic differences affecting H3R-modulation of the dopaminergic system could be relevant to determine dissimilar sensitivities to those agents that mainly act on these pathways, i.e. drugs for psychosis and drugs of abuse. This is consistent with the reported influence of the histaminergic tone on dopamine function and the rewarding effect of addictive drugs, which are mediated, at least partially, through H_3Rs (Brabant et al., 2010; Ellenbroek, 2013). H3HR genotypes associated with different functional states of H_3Rs could then determine addiction vulnerability and this potentially applies to exonic variants (i.e. rs3787430) as well as intronic polymorphisms that could influence the generation of different receptor isoforms through RNA splicing (i.e. rs74627870). In fact, several H_3R isoforms with different signaling efficacy have been described in human brain areas closely related to addiction such as striatum, prefrontal cortex or amygdala (Nieto-Alamilla et al., 2016).

Polymorphisms of 3'UTR regions are known to affect mRNA stability and the initiation or inhibition of protein translation. Thus, the rs13042865 genotype could influence the actual density of H_3Rs in the brain. How this translates into differences in the severity of cocaine dependence remains to be seen, but probably involves the dopaminergic system as well. One possible mechanism arises from the suggested control exerted by H_3Rs on D_1 receptor overstimulation (Moreno et al., 2014): H_3Rs heterodimerize with D_1 receptors to prevent the consequences of excessive

activation, i.e. cocaine-induced cell death; by turn, cocaine recruits σ_1R chaperones to interact with D_1/H_3R complexes and remove the H_3R break. In this context, a higher density of H_3Rs could be expected to better counteract the effects of cocaine, i.e. the ability of the drug to trigger dependence. Clearly, all these mechanisms are very speculative at this stage of research and thus the functional consequences of the HRH3 variants described in our work must be specifically addressed to confirm the hypothesis advanced. Even more, additional mechanisms beyond dopaminergic control could also play a significant role in the putative associations between H_3Rs and addiction, such as modulation of noradrenergic, GABAergic, cholinergic or glutamatergic transmission by presynaptic H3Rs in different areas of the brain (Panula et al., 2015).

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Availability of data and material

The datasets generated during the current study are available from the authors.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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Table 1. Main characteristics of the subjects included in the study. Numbers represent means ± SEM.

		Cocaine users	**Controls**
Complete dataset N = 748	Sex (females : males)	46:202	250:250
	Age (years)	38.0 ± 8.7	40.2 ± 3.9
	BMI (kg/m^2)	25.0 ± 4.3	26.2 ± 4.4
	Length of cocaine consumption (years)	14.5 ± 8.4	
	Cocaine dependence (SDSS / DSM-IV score)	7.0 ± 14.3	
Matched dataset N = 336	Sex (females : males)	39 : 129	39 : 129
	Age (years)	37.3 ± 6.2	38.2 ± 5.4
	BMI (kg/m^2)	25.3 ± 4.4	26.6 ± 4.9
	Length of cocaine consumption (years)	13.8 ± 7.3	
	Cocaine dependence (SDSS / DSM-IV score)	7.2 ± 3.6	

Table 2. SNPs included in the study. MAF, minor allelic frequencies observed in the sample / reported in 1000Genome project population – dbSNP, NCBI. XX, XY, YY represent observed frequencies of wild type, homozygous, and mutant genotypes used for Hardy-Weinberg equilibrium analysis.

SNP	MAF (%)	XX	XY	YY	X^2	p-value
rs3787430	12 / 18	580	155	13	0.50	0.48
rs74627870	10 / 12	606	133	7	0.01	0.92
rs13042865	22 / 7	456	259	33	0.25	0.62
rs6062153	16 / 21	537	188	22	1.24	0.27
rs6587298	35 / 30	310	338	91	0.01	0.94
rs1739583	18 / 26	507	214	27	0.55	0.46
rs7344029	11 / 11	587	151	10	0.01	0.93

Table 3. HRH3 gene variants significantly associated with the presence of cocaine use disorder (CUD) and the severity of cocaine dependence. OR = odds ratio (95% confidence interval) obtained in the confirmatory analysis of the matched datasets.

SNP	Locus	Alleles	Genotype	Association	OR
rs3787430	Exon 3, codding for intracellular domains	C > T	C / _	Higher risk of CUD	1.69 (1.02 – 2.81)
			C / C	Higher risk of CUD	3.38 (2.04 – 5.62)
rs74627870	Intron	G > C	C / _	Higher risk of CUD	1.89 (1.01 – 3.55)
			C / C	Higher risk of CUD	3.78 (2.02 – 7.09)
rs13042865	UTR	G > C	G / G	Lower severity of cocaine dependence	0.34 (0.12 – 0.93)

FIGURE LEGENDS

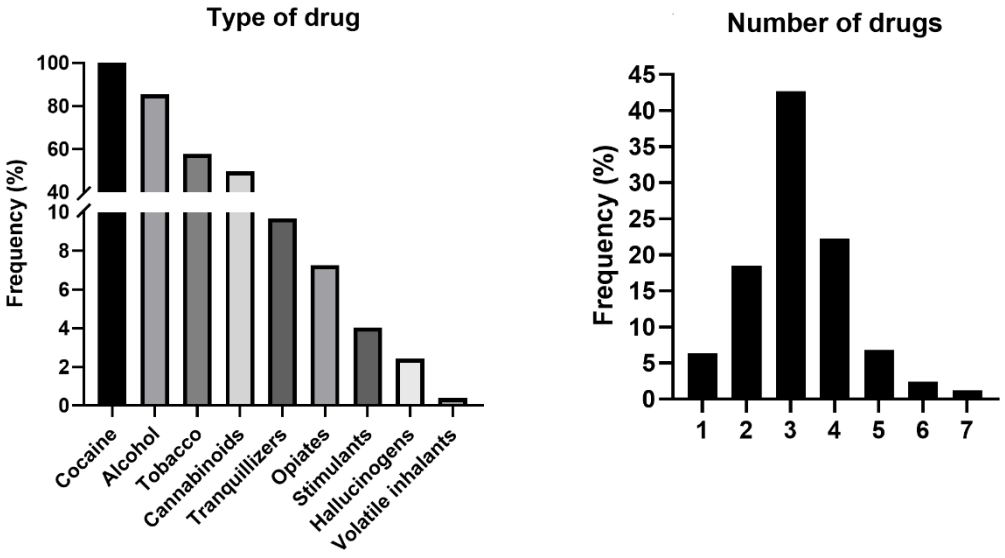

Figure 1. Drugs abused by CUD-diagnosed patients. The left graph shows frequency of abuse by type of drug and the right graph frequency of abuse by number of drugs.