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ORIGINAL ARTICLE

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Cannabinoid mimics in chocolate utilized as an argument in court

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Abstract A case is presented involving chocolate cannabinoid mimics which have been utilized in court by the defendant's lawyer in order to clear the accused of smoking and dealing in marijuana after he was found positive for cannabis in a routine urine immunoassay screening test. The argumentation in this case was that the accused had supposedly eaten a massive amount of chocolate which contained anandamide-related lipids. These lipids inhibit anandamide hydrolysis in the brain, act as cannabinoid mimics and, according to the lawyer, were the cause of the positive cannabinoid test. To investigate this in detail, we synthesized *N*-oleoyl- and *N*-linoleoylethanolamide and spiked these compounds together with *N*-arachidonoylethanolamide in urine for immunological investigations. None of the samples were found positive, indicating that no cross-reactivity occurs with cannabinoids. As a result, the lawyer's claim could be refuted and the accused was convicted.

Key words Cannabinoid · Chocolate · Immunoassay · Anandamide · *N*-Acylethanolamide

Introduction

Cannabinoids are C_{21}-containing compounds which can be found in the hemp plant *Cannabis sativa* or marijuana (Mechoulam 1970). It is generally accepted that Δ^9-tetrahydrocannabinol (Δ^9-THC, Fig. 1 A) is the principal psychoactive component which, when used acutely, evokes the desired "high" effect, memory and motor impairment and, when used chronically, is responsible for cardiovascular, pulmonary and reproductive malfunctions (Hollister et al. 1981). When smoking marijuana, the natural cannabinoids are rapidly absorbed from the lungs into the blood and stored together with their metabolites in the fatty tissues of the body (Perez-Reyes et al. 1981). The most prominent Δ^9-THC metabolite is 11-nor-Δ^9-THC-9-carboxylic acid (Δ^9-COOH-THC), which is excreted in the urine even months after the last exposure (Lemberger and Rubin 1975). As a consequence, Δ^9-COOH-THC is the main marker in urine for detecting marijuana use.

Only about 30 years after the discovery of Δ^9-THC, an endogenously occurring counterpart to this plant-derived cannabinoid was discovered. The molecule belonged to a group of modified, eicosanoid-like fatty acids and was named anandamide or *N*-arachidonoylethanolamide (Fig. 1 B) (Devane et al. 1992). Anandamide specifically binds with high affinity to the G protein-coupled cannabinoid receptors CB1 and CB2 (Felder et al. 1995), mimics the pharmacology of natural cannabinoids and is released from neurons in a Ca^{2+}-dependent manner (Di Marzo et al. 1994). Production of anandamide has also been reported to occur via enzymatic condensation of arachidonate and free ethanolamine (Devane and Axelrod 1994; Kruszka and Gross 1994). Not all of the actions of anandamide appear to be mediated via a G protein-dependent mechanism, since the gap junctions of brain astrocytes and brain Kv1.2 K^+ channels can be inhibited in a direct way (Venance et al. 1995; Poling et al. 1996). Finally, it was shown that anandamide is rapidly broken down by amidohydrolase activity (Desarnaud et al. 1995), suggesting that it may be an endogenous cannabinoid neurotransmitter (Matsuda 1997).

Case history

A 46-year-old male prisoner was found positive for cannabinoids in a routine urine screening test carried out in our laboratory with the Abuscreen ONLINE test (Roche Diagnostic Systems, USA) and was accused of smoking and dealing in marijuana. This case is in itself nothing extraordinary, if it were not that the defense lawyer claimed that the accused was a chocolate addict and that based on the publication by di Tomaso et al. (1996), this had caused the positive immunoassay for cannabinoids in urine. In the work by di Tomaso et al. (1996), it was reported that chocolate

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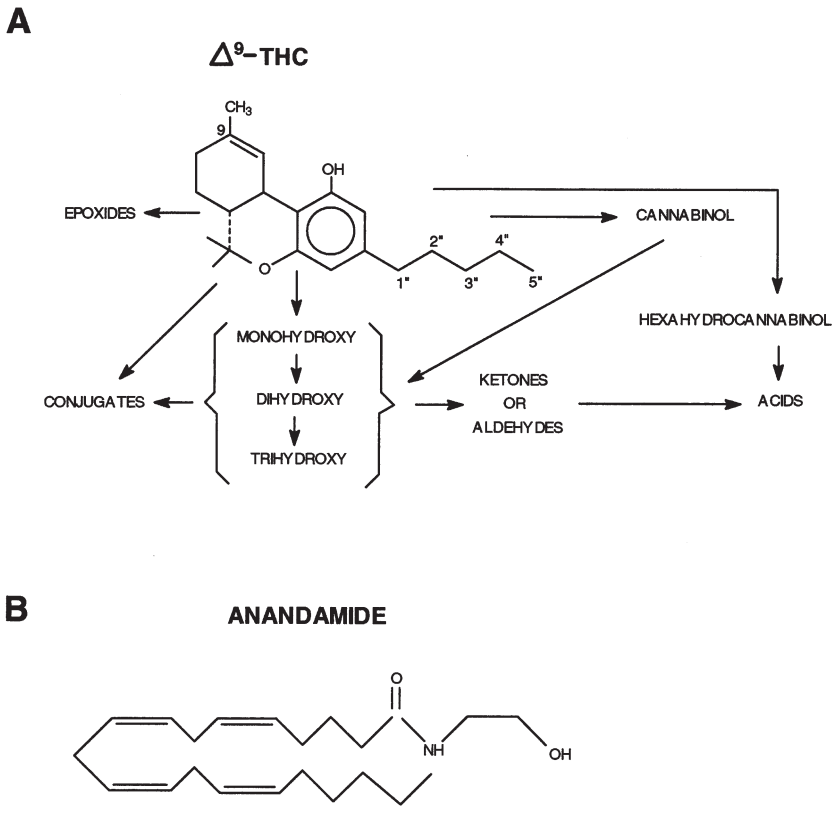

Fig. 1 A, B Molecular structures of cannabinoids and anandamide. **A** It is generally accepted that Δ^9-tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive component of *Cannabis sativa*. Cannabinoids are readily transformed into a large number of metabolites, following the overall pattern shown. **B** Structure of anandamide or *N*-arachidonoylethanolamide, which is a modified, eicosanoid-like fatty acid endogenously occurring in man

contains lipids chemically and pharmacologically related to anandamide, i.e. *N*-oleoylethanolamide, *N*-linoleoylethanolamide and anandamide, in concentrations ranging from 0.5 to 90 μg/g. Most interestingly, it was found that *N*-oleoylethanolamide and *N*-linoleoylethanolamide inhibited anandamide hydrolysis in rat brain microsomes, leading to the conclusion that these *N*-acylethanolamides present in chocolate could act as cannabinoid mimics by increasing endogenous anandamide levels. Exactly on the basis of this publication, the defendant's lawyer argued that it could not be proven that his client had been using marijuana. Instead, he stated that the positive immunoassay was the result of eating large amounts of chocolate.

Since no confirmation analysis, e.g. by GC/MS, was performed on the prisoner's urine, because nowadays in Belgium no systematic confirmation of cannabinoids in urine samples is explicitly demanded due to financial considerations and time constraints, the lawyer's claim was examined by synthesizing *N*-oleoyl- and *N*-linoleoylethanolamide and spiking these compounds together with *N*-arachidonoylethanolamide in human urine for immunological investigations.

Materials and methods

N-Oleoylethanolamide and *N*-linoleoylethanolamide were synthesized by dissolving 2 g oleic acid and 2 g linoleic acid, respectively, in 10 ml dichloromethane. After addition of 500 μl dimethylformamide and 1.15 ml oxalylchloride, the mixture was kept at room temperature for 4 h and the solvent was evaporated under vacuum. The residue obtained was dissolved in 5 ml dichloromethane and cooled to 0 °C. After addition of 2.14 ml ethanolamine the reaction mixture was kept at 0 °C for 1 h and subsequently washed twice with 20 ml water. The dichloromethane fraction was filtered over anhydric sodium sulphate. The solvent was evaporated after adding 4 g silica gel which was placed on top of a silica gel column (3 × 15 cm). Oleoyl- and linoleoylethanolamide were eluted with a mixture of dichloromethane and methanol (98/2 v/v%). *N*-arachidonoylethanolamide was purchased from Sigma (USA). The identity of the three compounds was confirmed with gas chromatography electron-impact mass spectrometry (HP 5890 Series II GC, HP 5971 A mass selective detector, Hewlett Packard, USA).

Furthermore, 20 ml samples of human urine were spiked with the *N*-acylethanolamides to give two final concentrations (300 μM and 1 mM), which completely block the anandamide amidohydrolase activity in rat brain microsomes (di Tomaso et al. 1996). Stock solutions of *N*-acylethanolamides (1 mg/ml) were dissolved in toluene or ethanol. These solvents did not interfere with the immunoassay. The samples were then investigated immunologically for the presence of cannabinoids (Abuscreen ONLINE, Roche Diagnostic Systems, USA). This system is an automated in vitro diagnostic test for the qualitative detection of drugs of abuse in human urine and its principle is based on particle aggregation and absorbance changes depending on the presence or absence of sample drug. The cut-off value for cannabinoids was 50 ng/ml.

Results and discussion

Although from a chemical point of view there is no reason to assume that the unrelated *N*-acylethanolamides would cross-react with the in vitro diagnostic test in use for the qualitative detection of cannabinoids in human urine (Abuscreen ONLINE, Roche Diagnostic Systems, USA), nor with 78 other unrelated drugs mentioned in the manufacturer's technical manual, the aim was to actually prove that chocolate cannabinoid mimics do not cross-react, allowing a scientifically shored testimony in court. No positive reaction, i.e. cross-reaction, was found for human urine spiked with *N*-arachydonoylethanolamide, *N*-oleo-

ylethanolamide and *N*-linoleoylethanolamide at final concentrations of 300 μM and 1 mM. As a consequence, our results could refute the lawyer's claim that the urine of the accused was found positive for cannabinoids because of (excessive) eating of chocolate. The outcome was that the accused was convicted in court.

Although this case might sound exceptional, it illustrates that the increased accessibility of scientific data (e.g. via the Internet), and certainly the correct interpretation thereof by laymen (e.g. by lawyers in this case), represents a new challenge for forensic toxicologists. More importantly, since the greater part of all screening test programs relies solely on diagnostic immunoassays of human urine (e.g. because no GC/MS confirmation technique is available) and since hair samples are not always readily available for confirmation purposes (like in this case where the accused was bald), our findings may be useful for the interpretation of similar cases in the future. Finally, it should also be mentioned that nowadays in Belgium there is a policy of tolerance towards smoking marijuana in private. As a result of this and also because of financial considerations and time constraints, no systematic confirmation of cannabinoids in urine samples is demanded. However, it should be stressed that the evidential value of non-confirmed immunological test results will always remain inconclusive. In order to avoid this situation, it is inevitable that the court of justice and hence the entire society, should consider providing more money and allow more time for the forensic toxicologist to continue guaranteeing quality analyses.

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