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Octopamine and biogenic amines

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Abstract

Octopamine is prohibited as stimulant according to “The 2010 Prohibited List” of the World Anti-Doping Agency (WADA). Synefrine is enclosed in the WADA monitoring program.

Both, octopamine and synefrine, are neurotransmitters in invertebrates and supposed to be co-transmitters for neurotransmitters (noradrenaline, serotonine, dopamine) in the mammalian brain and the sympathic nervous system. Since *Ephedra* was banned from the market in 2004, *Citrus aurantium*, a bitter orange extract containing synefrine and octopamine in minor amounts, is promoted as alternative in nutritional supplements and fatburning products.

Octopamine is synthesized endogenously from tyramine and metabolized to *p*-hydroxy-mandelic acid and in minor amounts to synefrine.

The aim of this study was to find out to what extent octopamine and synefrine are detectable in the urine after ingestion of nutritional supplements containing *Citrus aurantium* and tyramine-containing food. In an excretion study it was shown that after a single dose of different nutritional supplements or after food intake with high tyramine-content, octopamine was not traceable in collected urine samples. Indeed after a p.o. dose of a pharmaceutical containing 150 mg of octopamine the active compound was verified in urine. However, conclusions about cumulative effects of repeated intake of the nutritional supplements used in our study are not possible on the basis of these data.

Introduction

Octopamine is prohibited as stimulant acording to “The 2010 Prohibited List” of the World Anti-Doping Agency (WADA). Synefrine is enclosed in the WADA monitoring program.[1]

Both, octopmine and synefrine, are neurotransmitters in invertebrates and supposed to be co-transmitters for neurotransmitters (noradrenaline, serotonine, dopamine) in the mammalian brain and the sympathic nervous system.[4] Since *Ephedra* was banned from the

market in 2004, *Citrus aurantium*, a bitter orange extract containing synefrine and octopamine in minor amounts, is promoted as alternativ in nutritional supplements and fatburning products.[5]

Octopamine is synthesized endogenously from tyramine and metabolized to *p*-hydroxy-mandelic acid and in minor amounts to synefrine (Fig. 1).[3]

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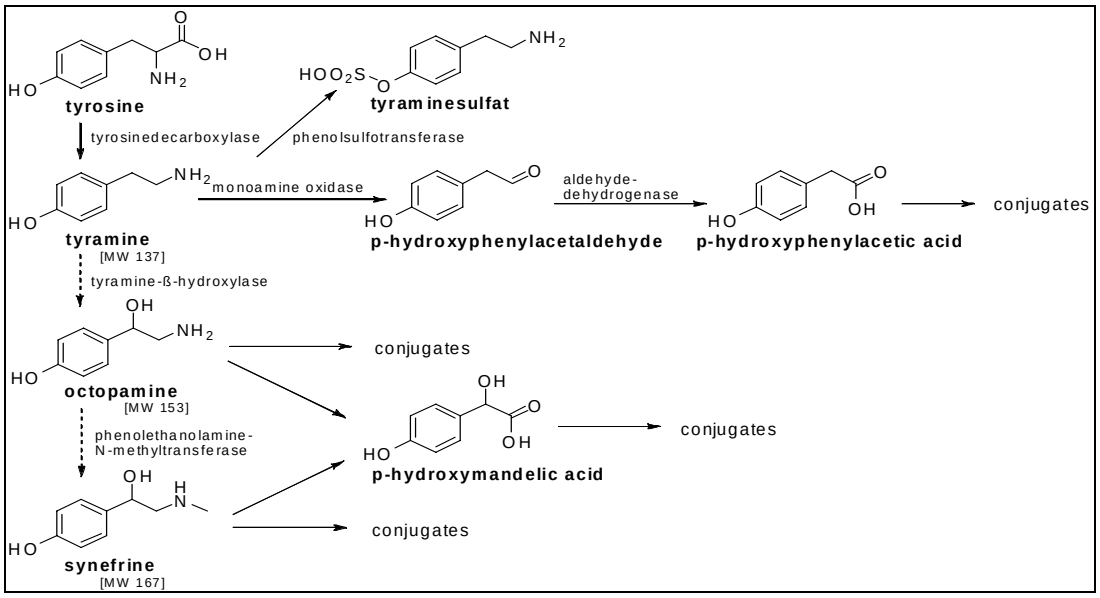

Figure 1: In vivo pathways of biogenic amines [3]

Materials and Methods

The present study included several excretion studies with synefrine (Sympatol®, 150mg) and octopamine (Norphen retard®, 150mg), 3 different nutritional supplements containing *Citrus aurantium (Peak Thermodyn®, Ultra Ripped®, Animal Cuts®)* and food with high tyramine-content (200g cheese or soy). The nutritional supplements were ordered in the internet and were labelled with different concentrations of synefrine and octopamine.

After a single oral dose the urine was collected for 2 days.

Sample preparation for LC-MS/MS

- 2 mL of urine + 5 µg mL^{-1} of $^2H_3$-ephedrine
- hydrolysis with hydrochloric acid at pH 1.0 at 80°C for 45 min

- SPE-extraction with Strata-X-CW 33µm at pH 6.5
- elution with 350 µL of methanol/formic acid (5% v/v) and evaporation to dryness
- reconstitution with 200 µL of water/acetonitrile (9/1)
- injection volume: 5 µL

LC-MS/MS analysis

LC-MS/MS analyses were performed on an Applied Biosystems API 2000 mass spectrometer utilising atmospheric pressure chemical ionisation (APCI).

- Phenomenex Gemini C_6-phenyl column (4.6x150 mm, particle size 3 µm).
- flow rate 800 µL min^{-1}, eluents A: 5 mM ammonium acetat containing 0.1% acetic acid and B: acetonitrile.
- gradient: 98% A for 2 min, increased to 10% B in 6 min, equilibrate at 98% A for 2.5 min
- total run time: 10.5 min employing a post-column split of 1:10

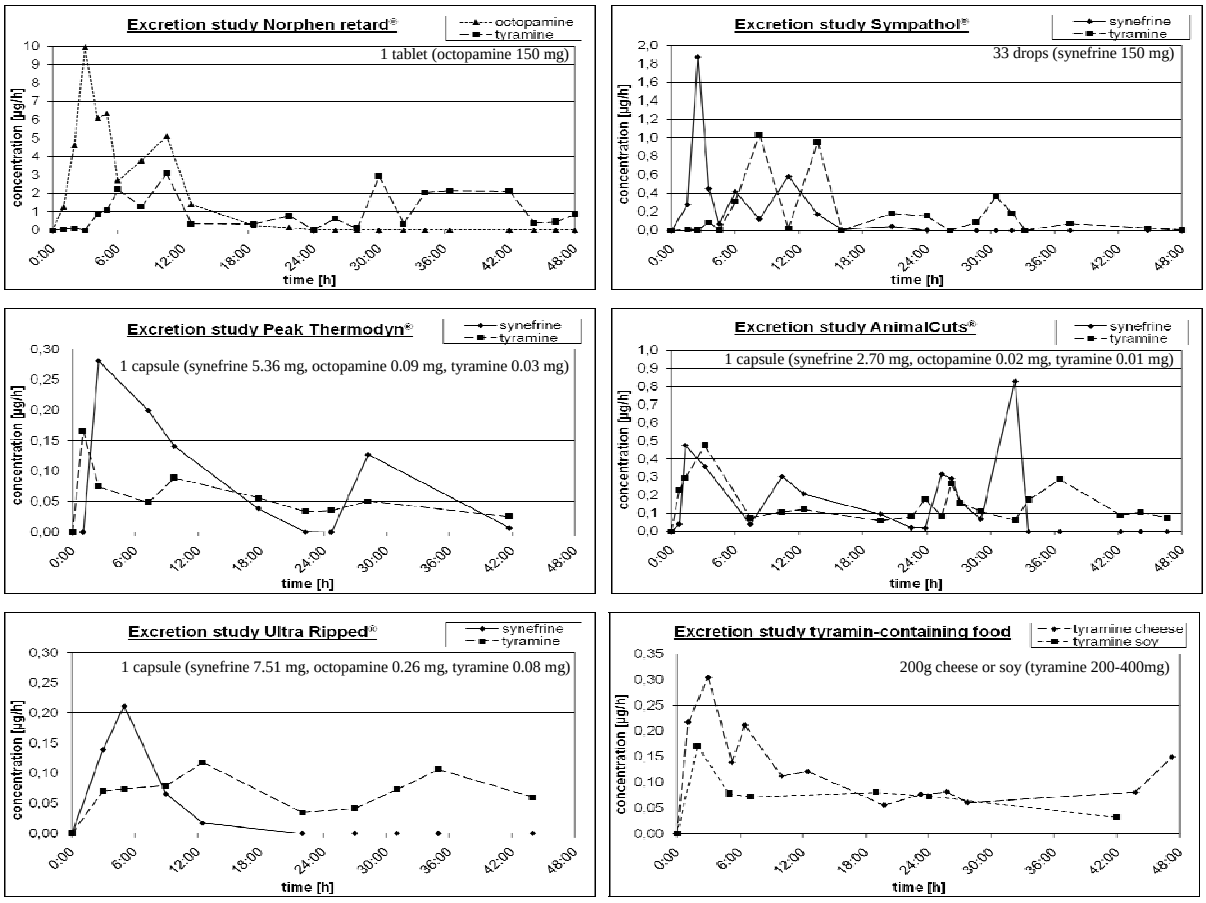

Figure 2: excretion rates of the biogenic amines evaluated from the excretion studies

Results and Discussion

The samples were analysed with an existing confirmation procedure for octopamine using a

LC-MS/MS system.[2] Although *Citrus aurantium* is supposed to contain synefrine and octopamine only synefrine was detected in the excretion studies with the nutritional supplements and Sympatol®. Octopamine was verified only in the excretion study with Norphen retard®. (Fig. 2) In all excretion studies a fast rise and decline of the concentration of the biogenic amines was observed. The main dose was excreted in 12 hours.
However tyramine is metabolized *in vivo* to octopamine in trace amounts, after the intake of tyramine-containing food octopamine was not verified in the urine. Indeed the variability of synefrine and tyramine concentrations in the urine hours after the ingestion could be caused by food intake.

Conclusion

The method is capable to verify biogenic amines in the urine. In our studies octopamine, prohibited according to "The 2010 Prohibited List", was only detected after p.o. ingestion of octopamine itself and not after ingestion of nutritional supplements or food intake.
However conclusions about cumulative effects of repeated intake of the nutritional supplements used in our study are not possible on the basis of these data.

References

[1] World Anti-Doping Agency. The 2010 Prohibited List. International Standard, Montreal (2010) http://www.wada-ama.org/ Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN .pdf (access date 03.01.2010)

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