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Salsolinol and Norsalsolinol in Human Urine Samples

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MUSSHOFF, F., T. DALDRUP, W. BONTE, A. LEITNER AND O. M. LESCH. *Salsolinol and norsalsolinol in human urine samples.* PHARMACOL BIOCHEM BEHAV **58**(2) 545–550, 1997.—The tetrahydroisoquinoline alkaloids salsolinol and norsalsolinol were found in human urine samples in concentrations ranging from 0.1 to 29.5 ng/ml. Great interindividual variation was found in urine levels of these alkaloids in a collection of chronic alcoholics and in a group of nonalcoholics. Thus, levels of the individual alkaloids are insufficient markers for distinguishing between alcoholics and nonalcoholics. However, by using the concentration ratio of norsalsolinol and salsolinol, the so-called dopamine–aldehyde adduct ratio (DAAR), significant differences between alcoholics (median 1.3) and nonalcoholics (median 0.6) were detected. This concentration ratio could serve as a marker for the processor state of the dopaminergic system. © 1997 Elsevier Science Inc.

Tetrahydroisoquinolines   Human urine samples   Salsolinol   Norsalsolinol   Dopamine   Alcoholism   Dopamine–aldehyde adduct ratio

DURING the last two decades, interest has focused on the molecular basis of alcoholism and its aetiology. Endogenously formed tetrahydroisoquinoline (TIQ) alkaloids were discussed as neurochemical factors contributing to psychotic effects observed in alcoholism (9,10,13,16,17,23,50) and other diseases such as schizophrenia or Parkinson's disease (5,38–40). Acute and chronic administration of selected TIQ compounds to rats has been reported to alter alcohol consumption significantly (29,30,35,36). TIQ alkaloids produced via the Pictet–Spengler reaction of phenylethylamines or indole ethylamines with aldehydes or α-keto acids were seen as connecting links between alcoholism and opioid mania. Formation occurs readily under physiological conditions (45,51) and results in substances that can function as neurotransmitters or neuromodulators. The pharmacological, neuroanatomical, and physiological attributes of TIQ alkaloids in specific regions of the brain were investigated. Endogenous formation of condensation products and a connection to chronic alcoholism were discussed controversially (11,12,17,23,33,34,37). Possible dietary sources must be taken into account, because TIQs have also been detected in a variety of foods (27,41).

In particular, connections between processor states of the dopaminergic system and the in vivo formation of dopamine aldehyde condensation products have been multiply documented. In the cerebrospinal fluid of Parkinson's disease patients with a dopamine deficit, lower concentrations of salsolinol were found compared with levels in normal persons (18). After L-dopa therapy, dopamine aldehyde condensation products increased [(15,44) and others]. Faraj et al. (21) confirmed the connection between the processor state of the dopaminergic system and the formation of dopamine aldehyde condensation products by activating the system in Parkinson patients through transplantation of dopamine-producing adrenal gland; simultaneous increases in salsolinol concentrations in the cerebrospinal fluid were demonstrated. Sjöquist et al. (48) showed a significant correlation between salsolinol concentrations in putamen and nucleus caudatus and dopamine concentration. Camp et al. (7) and Adachi et al. (2) found

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simultaneous increases in salsolinol concentrations and dopamine concentrations in the urine of alcoholics. These results were confirmed by Faraj et al. (19) in plasma samples from alcoholics; the availability of dopamine and of acetaldehyde [which is affected by mitochondrial monoamine oxidase (MAO) and aldehyde dehydrogenase (AlDH) activity] was believed responsible for the formation of salsolinol.

Increased salsolinol excretion rates in the urine have been noted in patients with lowered MAO activity (7) and in patients with AlDH I deficiency (2). In the opinion of Faraj et al. (19), measurement of plasma salsolinol and dopamine in conjunction with platelet MAO function should provide a specific biochemical profile that could be helpful in screening subgroups of alcoholics and possibly in identifying individuals at high risk before exposure to alcohol.

For the most part, the ethanol oxidation product acetaldehyde has been regarded as the reaction partner in forming TIQ compounds, of which the condensation product with dopamine—salsolinol—is the most discussed. Recent investigations have proved that alcoholic beverages contain not only ethanol but also congener alcohols, especially methanol (3). This alcohol is used as an alcoholism marker and has been discussed as a decisive factor in the aetiology of chronic alcoholism. It is regarded as an exogenous formaldehyde source (4,43,49). This oxidation product is a more potent reaction partner for TIQ formation than is acetaldehyde (26). Additionally, there are some known metabolic pathways of formaldehyde generation that may account for in vivo TIQ formation in tissues (24,25,31,42).

In our opinion, chronic consumption of alcoholic beverages coupled with methanol accumulation may influence the C_1 metabolism and cause an induction of TIQ-forming mechanisms. Such formaldehyde-derived compounds could be involved in the aetiology of alcoholism.

We recently reported on a qualitative gas chromatographic–mass spectrometric (GC-MS) screening procedure for measurement of formaldehyde-derived TIQ compounds (32) and demonstrated the following substances in human urine samples: 1,2,3,4-tetrahydroisoquinoline, 6-methoxy-7-hydroxy-1,2,3,4-tetrahydoisoquinoline, 4,6,7-trihydroxy-1,2,3,4-tetrahydro isoquinoline, *N*-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (norsalsolinol), and salsolinol. Conspicuous differences in the norsalsolinol–salsolinol ratios were found. Based on these observations, we developed a quantitative method, including an internal standard, for determination of these dopamine condensation products.

METHODS

Materials

Methanol, ethyl acetate (Uvasol, Merck, Darmstadt, Germany), water (HPLC grade, Baker, Gross-Gerau, Germany), 3,4-dihydroxybenzylamine, salsolinol, and β-glucuronidase type H-1 (all from Sigma, Deisenhofen, Germany) were used as obtained from the suppliers. Norsalsolinol was synthesized according to Buck (6). Other chemicals used were ammonia (25%), boric acid, sodium tetraborate, sodium hydroxide, orthophosphoric acid, hydrochloric acid, acetic acid, pyridine, propionic anhydride, and semicarbazide (all p.a., Merck). Phenylboron acid (PBA) extraction columns were purchased from Analytichem International (ICT-Handelsgeselschaft, Frankfurt a.M., Germany).

Borate buffer (pH 9.0) consisted of 835 ml of solution A (12.37 g of boric acid + 100 ml of 1 M sodium hydroxide with 0.05 M sodium tetraborate made up to 1 liter) and 165 ml of solution B (0.1 M hydrochloric acid).

Instrumentation

A model 5890A gas chromatograph with a model 5970A mass selective detector (Hewlett-Packard) and an OV1 fused silica capillary column (12 m × 0.2 mm internal diameter, film thickness 0.33 μm) were used.

Sample Preparation and Data Analysis

The sample consisted of 2 ml of urine with 0.01 ml of standard solution and 0.05 ml of a solution of semicarbazide (1 M) adjusted to pH 4.5 with acetic acid, to which 2,500 U β-glucuronidase was added. For enzymic hydrolysis, the sample was incubated for 2 h at 55°C. Then the mixture was adjusted to pH 9.0 with 0.2 ml of ammonia and 2 ml of borate buffer.

Before application of a sample, the PBA extraction columns were conditioned with 2 ml of methanol followed by 1 ml of HCl, 1 ml of NaOH, 1 ml of water, and 1 ml of borate buffer. Prepared samples were applied to the columns under vacuum at a flow rate of approximately 1 ml/min. The columns were washed with 2 ml of water followed by 4 ml of methanol. The TIQ compounds were eluted with two 0.75-ml volumes of 1 M acetic acid in methanol and collected in a vial. For derivatization, the eluate was reconstituted in 0.05 ml of propionic anhydride/pyridine (1:1) and incubated for 30 min at 80°C. The solution was evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 20 μl of ethyl acetate, and a 2-μl aliquot was subjected to GC-MS.

The temperature of the GC-MS system was programmed from an initial value of 60°C, held for 2 min, followed by a linear increase to 210°C at 30°C/min and then by a linear increase to 300°C at 10°C/min. The final temperature was held for 5 min. The split–splitless injector (2 min split off) was maintained at 270°C.

Electron impact mass spectra of the compounds were recorded by total ion monitoring (Fig. 1). Characteristic mass fragments of the derivatives were chosen for acquisition in the selected-ion monitoring (SIM) mode. The following mass fragments were chosen to monitor the presence of the internal standard and the TIQ compounds: m/z 164, 220, 276, 332, and 347 for salsolinol; m/z 57, 221, and 333 for norsalsolinol; m/z 57, 195, and 250 for 3,4-dihydroxybenzylamine.

Experiments with spiked urine samples revealed that linearity was obtained over the range of 0.1–50 ng TIQ compound/ml, with coefficients of correlation of 0.999 for salsolinol and 0.998 for norsalsolinol (Table 1). Recoveries (without calculation over the internal standard) determined with spiked samples (5 ng/ml) were 78.3 ± 4.5 and 76.9 ± 5.6%, respectively (n = 10). Using the routine method described above, the minimum detectable concentrations of salsolinol and norsalsolinol were 50–100 pg/ml (signal–noise ratio 3:1). In Fig. 2, the identification of salsolinol and norsalsolinol in an authentic urine sample of a chronic alcoholic is demonstrated.

Statistics

For statistical analyses, the Wilcoxon signed-rank sum test was used.

Sample Material

Urine samples of alcoholics were taken from patients who had been admitted in an alcoholized state for alcohol withdrawal. Samples were collected in three different states of al-

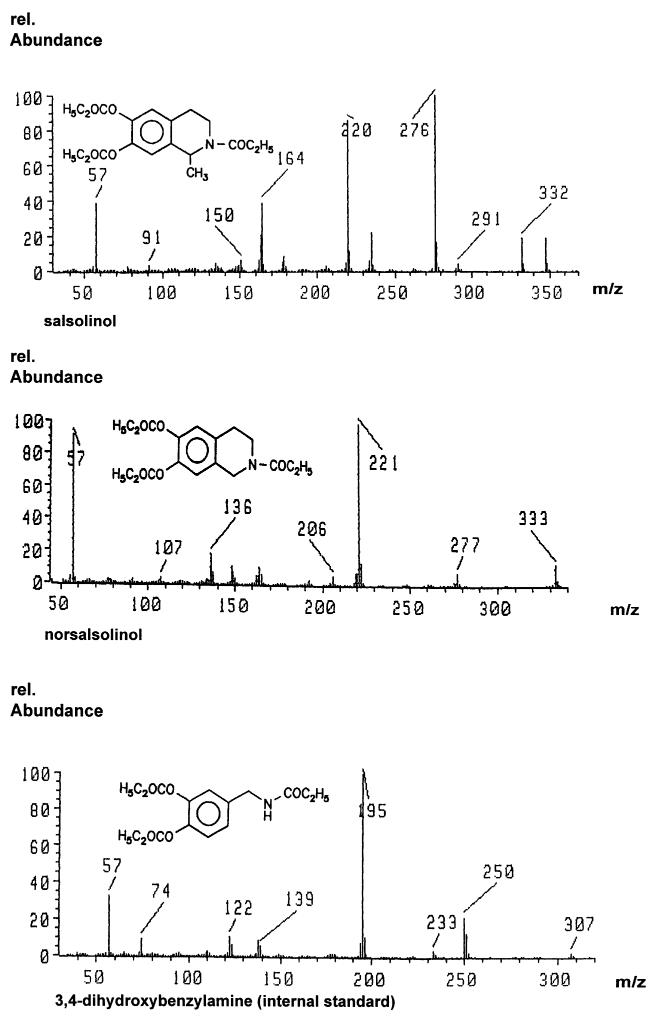

FIG. 1. Mass spectra of salsolinol, norsalsolinol, and 3,4-dihydroxybenzylamine (propionyl derivatives).

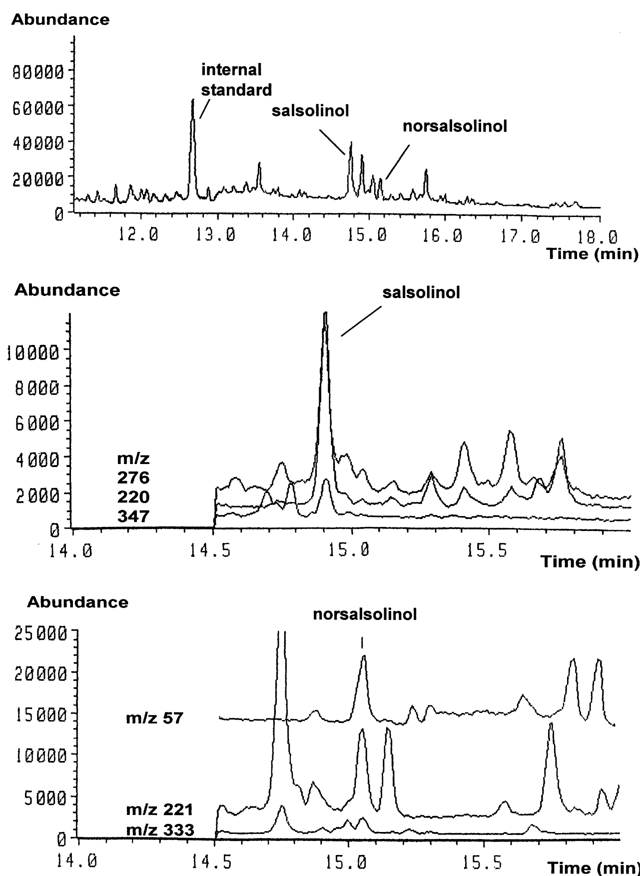

FIG. 2. Identification of salsolinol and norsalsolinol in an authentic urine sample of a chronic alcoholic.

coholization: breath alcohol concentration higher than 0.5 g/kg (64 samples) and lower than 0.5 g/kg (59 samples) and on the next morning (65 samples). A second sample population consisted of healthy volunteers with no alcohol consumption for the previous 24 h (50 samples).

RESULTS

Positive results for the condensation products of dopamine with acetaldehyde and formaldehyde could be obtained for alcoholics in all states of alcoholization. In 170 positive cases, concentrations of salsolinol ranged from 0.1 to 13.3 ng/ml (Table 2). The amounts of norsalsolinol ranged from 0.2 to 26.8 ng/ml in 160 positive samples. No correlation between the states of alcoholization and the concentrations of salsolinol or norsalsolinol were observed.

Investigation of urine samples of nonalcoholics proved that salsolinol (in 49 samples, with concentrations between 0.3 and 25.3 ng/ml) and norsalsolinol (in 40 samples, with concentrations between 0.2 and 9.5 ng/ml) could be detected (Table 3).

No significant differences in the salsolinol concentrations of alcoholics and nonalcoholics were determined, but there was a significant difference in the norsalsolinol concentrations ($p = 0.0101$; Wilcoxon signed-rank sum at 5% level). Conspicuous results are given for the formation of concentration ratios of norsalsolinol and salsolinol, the so-called dopamine–aldehyde adduct ratio (DAAR). Significantly higher ratios were observed in alcoholics (median 1.3) compared with nonalcoholics (median 0.6) ($p = 0.0001$; Wilcoxon signed-rank sum at 5% level).

DISCUSSION

Salsolinol has been discussed controversially as an alcoholism marker. Our results correspond to those of authors who

TABLE 1

PRECISION DATA FOR THE ASSAY METHOD DESCRIBED

	Salsolinol	Norsalsolinol
Spiked (ng/ml)	5.0	5.0
Recovered (ng/ml)	5.36 ± 0.43	5.51 ± 0.48
Coefficient of variation (%)	8.02	8.71
Recovery (%)	78.32 ± 4.55	76.93 ± 5.63
r (calibration curve)	0.999	0.998

Amount recovered and recovery percentage values (without calculation over the internal standard) are mean ± SD.

TABLE 2

URINE SALSOLINOL AND NORSALSOLINOL CONCENTRATIONS AND DOPAMINE–ALDEHYDE ADDUCT RATIOS FOR ALCOHOLICS

Salsolinol	Norsalsolinol	DAAR	Salsolinol	Norsalsolinol	DAAR	Salsolinol	Norsalsolinol	DAAR	Salsolinol	Norsalsolinol	DAAR	
0.1	1.4	13.1	0.6	1.0	1.6	3.2	3.7	1.2	6.8	3.6	0.5	
3.1	26.8	8.8	0.3	0.6	1.6	6.8	7.9	1.2	6.5	3.5	0.5	
0.6	3.8	6.9	0.6	0.9	1.6	0.4	0.5	1.1	7.2	3.8	0.5	
0.3	1.5	5.9	2.7	4.0	1.5	6.8	7.7	1.1	7.9	4.1	0.5	
2.9	15.0	5.2	0.8	1.2	1.5	11.8	13.3	1.1	1.2	0.6	0.5	
0.1	0.3	5.2	1.5	2.2	1.5	4.0	4.5	1.1	0.8	0.4	0.5	
0.5	2.4	4.9	0.7	1.0	1.5	1.0	1.1	1.1	5.7	2.6	0.5	
0.8	3.5	4.4	2.4	3.6	1.5	1.9	2.1	1.1	0.5	0.2	0.4	
0.9	4.0	4.4	2.3	3.3	1.4	10.2	11.2	1.1	2.8	1.1	0.4	
0.7	3.0	4.4	0.6	0.9	1.4	0.7	0.8	1.1	9.4	3.1	0.3	
0.4	1.7	4.3	0.6	0.8	1.4	0.7	0.8	1.1	4.0	1.3	0.3	
0.6	2.3	4.1	1.8	2.6	1.4	7.6	8.3	1.1	5.5	1.6	0.3	
0.7	2.8	3.9	0.3	0.4	1.4	0.3	0.4	1.1	13.2	3.8	0.3	
0.7	2.6	3.9	0.6	0.9	1.4	1.7	1.8	1.1	7.7	1.7	0.2	
2.4	9.3	3.9	3.4	4.7	1.4	1.7	1.8	1.1	7.3	1.6	0.2	
2.4	7.4	3.1	0.5	0.6	1.4	2.9	3.2	1.1	13.3	2.6	0.2	
1.3	4.0	3.1	0.5	0.6	1.4	4.8	5.1	1.1	6.7			
0.9	2.8	3.0	3.5	4.8	1.4	1.3	1.4	1.1	5.5			
0.3	1.0	3.0	1.9	2.6	1.4	2.5	2.6	1.1	4.4			
0.6	1.6	2.9	8.1	10.8	1.3	3.2	3.4	1.1	2.5			
0.5	1.5	2.9	0.5	0.7	1.3	1.4	1.5	1.1	1.6			
0.7	1.9	2.8	2.9	3.9	1.3	5.1	4.5	0.9	1.1			
1.2	3.3	2.7	10.3	13.6	1.3	0.6	0.6	0.9	0.9			
3.1	8.2	2.6	9.3	12.2	1.3	6.7	5.9	0.9	0.8			
7.8	20.3	2.6	2.5	3.3	1.3	4.1	3.5	0.9	0.7			
0.5	1.4	2.6	2.4	3.1	1.3	0.8	0.6	0.9	0.6			
1.2	3.1	2.6	1.2	1.6	1.3	0.7	0.6	0.9	0.5			
0.7	1.6	2.3	1.1	1.4	1.3	4.5	3.6	0.8	0.5			
0.8	1.9	2.3	0.8	1.0	1.3	5.2	4.1	0.8	0.4			
2.1	4.7	2.2	3.1	3.9	1.3	0.6	0.5	0.8	0.1			
2.2	4.6	2.1	1.8	2.3	1.3	1.1	0.8	0.8	0.1			
1.6	3.3	2.1	1.1	1.4	1.3	6.5	4.9	0.8	0.1			
0.5	1.0	2.1	2.4	3.0	1.3	7.7	5.7	0.7		0.7		
2.3	4.7	2.0	0.7	0.8	1.2	4.1	3.0	0.7		0.9		
1.0	2.0	2.0	0.3	0.3	1.2	10.4	7.5	0.7		1.2		
0.3	0.6	2.0	5.3	6.6	1.2	2.9	2.1	0.7		1.3		
1.5	2.8	1.9	5.3	6.6	1.2	3.8	2.7	0.7		1.8		
3.5	6.7	1.9	0.9	1.1	1.2	6.4	4.3	0.7		1.9		
3.0	5.7	1.9	1.9	2.4	1.2	7.3	4.9	0.7				
1.6	2.9	1.9	2.1	2.6	1.2	1.0	0.7	0.7				
0.8	1.5	1.8	1.2	1.4	1.2	7.9	5.2	0.7	170	160	154	*n*
1.1	1.9	1.8	2.9	3.5	1.2	3.6	2.4	0.7	2.8	3.3	1.7	Mean
0.5	0.8	1.7	1.9	2.3	1.2	10.3	6.0	0.6	2.9	3.5	1.6	SD
2.1	3.7	1.7	1.5	1.8	1.2	4.6	2.6	0.6	13.3	26.8	13.1	Maximum
3.2	5.5	1.7	0.5	0.6	1.2	5.9	3.3	0.6	0.1	0.2	0.2	Minimum
0.4	0.6	1.7	3.4	4.0	1.2	3.7	2.1	0.6	1.7	2.6	1.3	Median

Salsolinol and norsalsolinol concentrations are expressed in ng/ml, and dopamine–aldehyde adduct ratios (DAAR) are calculated as norsalsolinol/salsolinol.

described salsolinol as an insufficient marker for distinguishing between alcoholics and nonalcoholics. In contrast to the findings of Faraj et al. (19–21), Matsubara et al. (28), Adachi et al. (1,2), Camp et al. (7), Sjöquist et al. (46,47), and Collins et al. (14) and in agreement with the results of Feest et al. (22), Clow et al. (8), and Dordain et al. (18), no significant differences in salsolinol concentrations between alcoholics and nonalcoholics could be observed. In both groups, great interindividual variation of concentrations could be demonstrated. It was not possible to fix a standard value of salsolinol for diagnostic use as an alcoholism marker to distinguish between alcoholics and nonalcoholics. In addition, great variation in norsalsolinol concentrations was observed in both groups.

Our results suggest that the formation of dopamine aldehyde condensation products has a physiological rather than pathologic origin. Conspicuous differences are noted upon formation of a concentration ratio of norsalsolinol and salsolinol, the DAAR: higher ratios are observed among alcoholics

TABLE 3

URINE SALSOLINOL AND NORSALSOLINOL CONCENTRATIONS AND DOPAMINE-ALDEHYDE ADDUCT RATIOS FOR NONALCOHOLICS

Salsolinol	Norsalsolinol	DAAR	Salsolinol	Norsalsolinol	DAAR	Salsolinol	Norsalsolinol	DAAR	
0.3	2.0	6.3	3.9	2.2	0.6	2.1	0.2	0.1	
0.4	1.6	4.0	5.3	2.9	0.6	25.3	2.3	0.1	
0.8	3.0	3.7	2.2	1.2	0.5	5.9			
0.5	1.8	3.6	1.9	0.9	0.4	4.2			
1.2	3.8	3.1	4.3	1.9	0.4	3.4			
1.0	2.9	3.0	10.1	4.2	0.4	3.4			
0.5	1.3	2.7	0.9	0.4	0.4	2.0			
0.6	1.6	2.4	4.4	1.7	0.4	1.5			
0.6	1.4	2.3	2.4	0.9	0.4	1.0			
4.2	9.5	2.2	2.5	0.8	0.3	0.6			
2.5	3.2	1.3	5.6	1.2	0.2	0.5			
1.5	1.6	1.1	8.0	1.6	0.2				
3.1	3.4	1.1	2.2	0.4	0.2				
3.3	3.3	1.0	2.8	0.5	0.2	49	40	40	*n*
3.4	3.3	1.0	2.7	0.4	0.2	3.4	2.0	1.2	Mean
2.3	2.0	0.9	3.4	0.5	0.2	3.8	1.7	1.4	SD
7.4	5.2	0.7	4.3	0.6	0.1	25.3	9.5	6.3	Maximum
4.2	2.8	0.7	5.1	0.7	0.1	0.3	0.2	0.1	Minimum
1.6	1.0	0.6	5.1	0.7	0.1	2.5	1.6	0.6	Median

Salsolinol and norsalsolinol concentrations are expressed in ng/ml, and dopamine–aldehyde adduct ratios (DAAR) are calculated as norsalsolinol/salsolinol.

than among nonalcoholics. In our opinion, the DAAR could be an indicator of metabolic stress or metabolic derailment. A displacement toward norsalsolinol can be seen under the influence of ingested methanol on the C_1 metabolism, producing increased formation of formaldehyde condensation products. The DAAR could also indicate an increased dopamine level in manifestly sick persons. Dopamine and the dopaminergic reward system have been proven to be of concern in the nascency or maintenance of various addiction processes. All drugs with addiction potential activate the reward system in direct or indirect ways through maintenance of high dopamine concentrations in certain brain regions. Higher dopamine concentrations could result in increased formation of condensation products. Because in the competition reaction formaldehyde possesses a higher reactivity than acetaldehyde in statu nascendi, a displacement toward norsalsolinol can be expected based on the higher availability of the educt dopamine.

In our study, an arbitrarily set DAAR value of 1 as a possible diagnostic indication for alcoholism produced a sensitivity of 73.4% and specificity of 67.5%. These results could form the basis for discussion of the possible use of this concentration ratio as a marker for the processor state of the dopaminergic system. Other diseases, such as Parkinson's disease or schizophrenia, should also be involved in further investigation. Easily available urine samples were the first material for study; future analyses should include other relevant materials, such as plasma, cerebrospinal fluid, or postmortem brain samples (especially from selected regions such as the nucleus caudatus or putamen).

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