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Quantitative chiral analysis of salsolinol in different brain regions of rats genetically predisposed to alcoholism

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Abstract

A method to determine the catecholamine content in putamen (CPU) and midbrain (MB) regions of the brain of alcohol-preferring rats (P) is presented with a focus on the low-level detection of *S*,*R*-salsolinol, a metabolite of dopamine and a putative alcoholism marker. The developed strategy allows both quantitative profiling of related catecholamines and the enantiomeric separation and quantification of the *S*- and *R*-salsolinol isomers and their ratios. The described LC/MS strategy simplifies the current methodology that typically employs GC–MS by eliminating the need for derivatization. The data also suggest an increase in the non-enzymatic formation of salsolinol as a consequence of ethanol exposure.
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Keywords: Catecholamines; Salsolinol; Alcoholism; Tandem mass spectrometry; Multiple reaction monitoring (MRM); Chiral separations

1. Introduction

The metabolic formation of salsolinol is thought to have a role in alcohol addiction [1–4]. It is assumed that there is a relationship between ethanol intake and the levels of salsolinol in different brain regions [4–6]. While salsolinol can produce reinforcing effects in the mesolimbic dopamine system, its mechanisms of action are largely unknown [7–10]. Salsolinol is postulated to result from the condensation of acetaldehyde, the main metabolite of ethanol, with dopamine in the brain of mammals [3,11,12]. It can be apparently formed *in vitro* through a non-enzymatic condensation involving the Pictet-Spengler mechanism [4,13–15]. Another proposed pathway for the *in vivo* formation of salsolinol is through the condensation of dopamine with pyruvic acid, followed by enzymatic decarboxylation and reduction [16]. *In vivo*, however, the enantiospecific occurrence of *R*-salsolinol in the cerebrospinal fluid [17,18], intraventricular fluid [17,19], and in human brain [17,20–22] suggests that its formation is mediated by an enzyme, *R*-salsolinol synthase [17,21,22]. The presence of optical isomers in the many regions of the brain, where the synthesis of salsolinol is possible, may allude to the different mechanisms of formation being either enzymatic or non-enzymatic (depending on local metabolic conditions). The formation of salsolinol *via* the non-enzymatic mechanism, following ethanol consumption, is thus expected to result in the formation of both enantiomers, which would not be the case through the enzymatic route [4,23]. Conversely, if the formation of one enantiomer preferentially occurs over the other, this indicates that the metabolite formation is regulated through an enzyme which is selective for the geometry of substrate and product [21].

Until recently, isoquinolines had been considered to occur as a racemate in humans, being generated through the non-enzymatic condensation of monoamines [21,22]. However, more accurate chromatographic methods for the analysis of salsolinol enantiomers seem to indicate the predominant occurrence of

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R-salsolinol in mammalian tissues, suggesting that the native salsolinol might be synthesized enzymatically [21,22]. In addition, Li and co-workers were able to measure *R*- and *S*-salsolinol levels in the striatum and adrenal glands of rats selectively bred for varying alcohol preference using gas chromatography [1]. According to this study, the levels of salsolinol in non-preferring rats (NP) were determined to be higher in the striatal regions of the brain relative to that of the preferring (P) rats.

The inbred rats provide excellent animal models for alcoholism [24,25]. In general, such models are invaluable in elucidating normal and abnormal functions in human physiology and behavior. Most animals do not voluntarily consume sufficient amounts of alcohol to produce pharmacologically meaningful blood alcohol levels. However, through selective breeding, the lines of high (P-line) and low alcohol-consuming (NP-line) rats have been produced [24,25], so that such animals would be used to study the influence of genetic factors on the effects of alcohol and on alcohol drinking behavior (for review see Refs. [26,27]).

Differences in the levels of salsolinol found in several brain regions following ethanol administration have been reported in both rats and humans [4,13–15,23]. However, the numbers of available samples in such studies were limited, and the regions chosen for quantitation are not traditionally believed to be involved in the addiction behavior [23,28]. The midbrain (MB) region is commonly targeted in alcoholism studies because it contains two dopamine-rich cell body regions, i.e., the ventral tegmental area (VTA) and the substantia nigra (SN), which project to several brain regions involved in regulating alcohol drinking and the motor impairing effects of alcohol, i.e., the caudate putamen (CPU) [29].

Salsolinol analytical methodologies have substantially benefited from a very extensive set of bioanalytical techniques and tools which have been developed for the detection of catecholamines in biological fluids and tissues. Liquid chromatography (LC) with electrochemical detection is traditionally used for the analysis of this class of compounds due to its excellent detection limits [30–32]. However, a serious limitation of this technique is its inability to positively identify the detected compounds in a complex biological extract. To circumvent these problems, various approaches were developed to convert polar catecholamines to their volatile derivatives for gas-chromatographic/mass-spectrometric (GC/MS) analysis [33,34]. Although the GC/MS methods principally offer the sensitivity and selectivity needed for positive identification together with low-level of quantification, they introduce new challenges of their own, including a need for multiple derivatization steps with multifunctional solutes, variation in sample injection reproducibility, and analyte instability at the elution temperatures [35]. Therefore, it seemed most logical to develop a method where the selectivity of mass spectrometry could be utilized without the need for prior sample derivatization. Recently, we have introduced a new approach which involves a combination of on-line phenyl boronate affinity preconcentration and microcolumn liquid chromatography, followed by mass spectrometry equipped with an atmospheric pressure photoionization (APPI) source [36]. However, this study did not address the chiral separation of salsolinol.

In this paper, a method employing a triple quadrupole mass spectrometer in conjunction with a chiral chromatographic column [37] for the separation of salsolinol enantiomers and chatecholamines has been developed in order to further enhance the detection limits for this class of compounds and the chiral resolution of salsolinol. The LC–MS approach, described here, facilitates accurate and sensitive analysis of salsolinol enantiomers as well as other neurotransmitters.

2. Materials and methods

2.1. Materials

Salsolinol (SAL), dopamine (DA), epinephrine (Epi), norepinephrine (Norepi), and the internal standard, 3,4-dihydroxybenzylamine (ISTD) (Fig. 1), were purchased from Sigma–Aldrich (St. Louis, MO). The solvents used for the LC separations and protein precipitations were purchased from EMD Chemicals (San Diego, CA). Formic acid (98% assay, p.a.) was purchased from Fluka Scientific (Steinheim, Germany). The 96-well plate SPE cartridges were purchased from Varian (Walnut Creek, CA). A Milli-Q water purifying system (Millipore Corp, Bedford, MA) was utilized to generate 18.2-MΩ deionized water.

2.2. Preparation of standards and calibration curves

Standard stock solutions were prepared in a 0.1% formic acid solution to assist dissolution and minimize oxidation of labile samples. These solutions were stored at −20 °C. The standards utilized for the construction of calibration curves were diluted to a proper concentration with the same sample preparation solution. Standard curves were prepared by adding different amounts of salsolinol and catecholamines to a 0.1% formic acid solution to produce a range of concentrations from 0.05 to 5 pg/μL for each salsolinol enantiomer, while 0.1–10 pg/μL was used for other catecholamines. A constant amount of internal standard was added to all standard solutions to produce a fixed concentration (1000 pg/μL). The standard curves were generated through the least-square linear regression.

2.3. Alcohol drinking procedure

Adult male rats from the high-alcohol-drinking rats from a replicate line 2 (HAD-2) were used in this study. Rats ($n = 8$/group) were assigned to three groups: (1) alcohol-naïve (water group); (2) those given a continuous 24-h free-choice access to 15% ethanol and water (CE group), and (3) those granted daily four 1-h access periods to 15% ethanol during the dark cycle with water always available (E4 group). Rats were maintained on these drinking protocols for 8 weeks. HAD-2 rats consume 6–7 g/(kg day) with both drinking protocols. The E4 group consumes their ethanol in discrete bouts of approximately 1.5 g/(kg session), while the CE group consumes most of their ethanol intakes over the 12-h dark

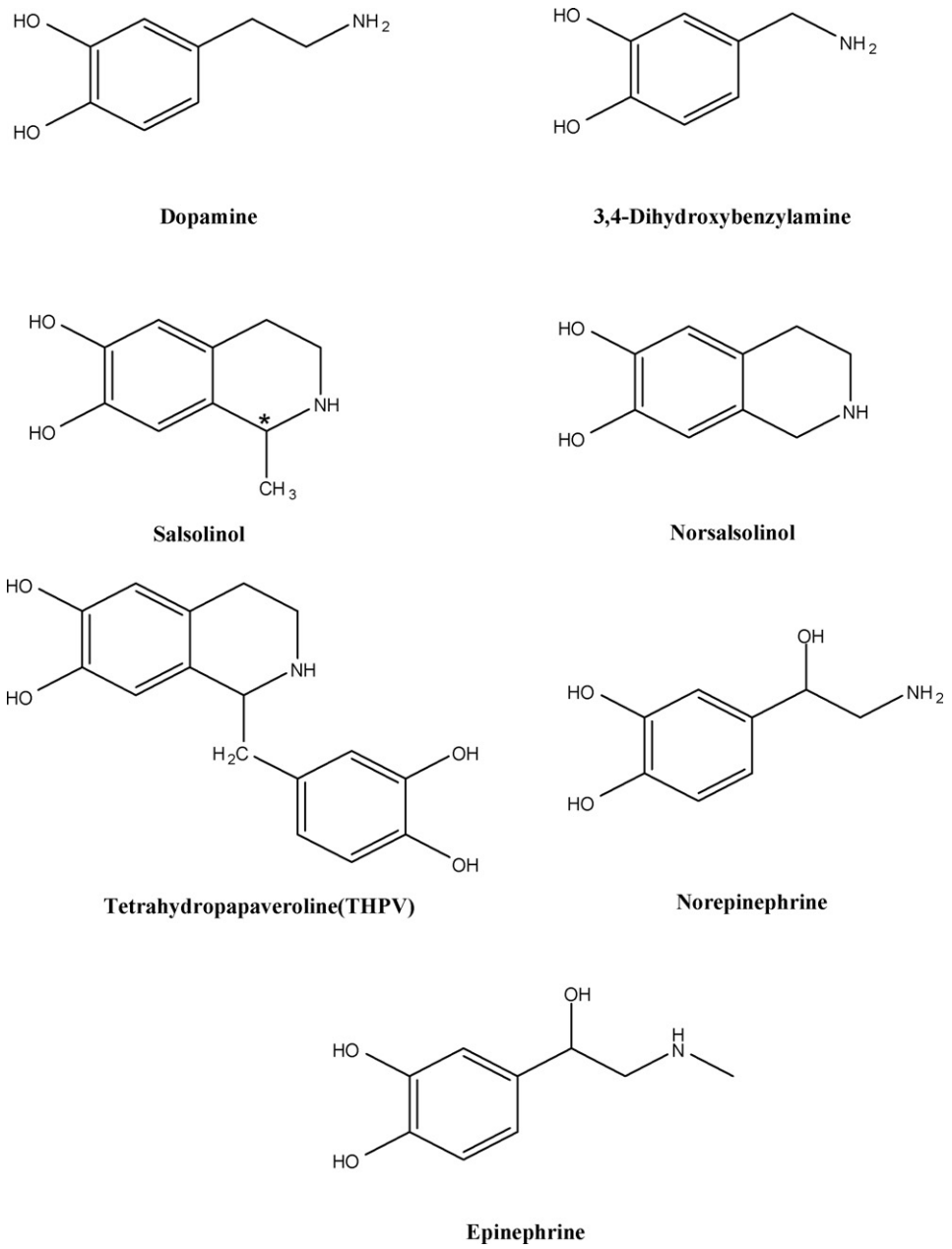


Fig. 1. Chemical structures of salsolinol and catecholamines. The asterisk is donating the chiral center of salsolinol.

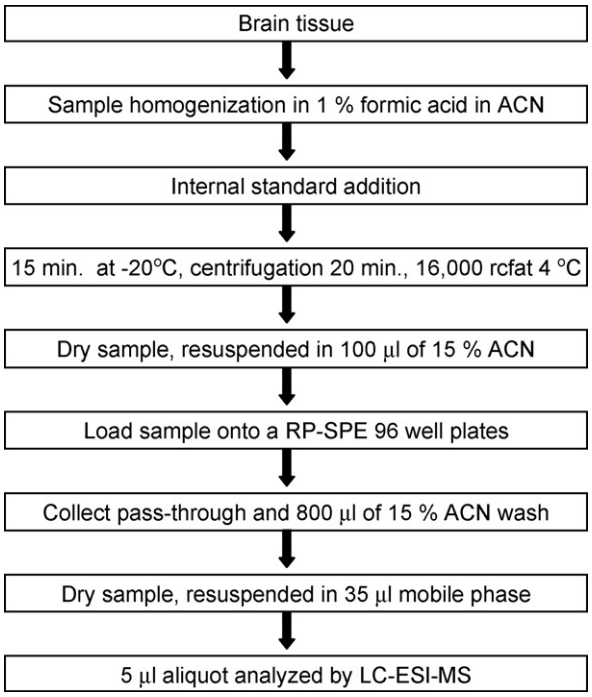
cycle. Accordingly, the E4 group is assured to have the highest blood alcohol concentrations compared to the CE group.

2.4. Sample preparation

Animals were killed by decapitation and the brains were removed. Brain regions were dissected in a cold box at −15 °C. The brain samples were weighed and homogenized with 0.4 mL of a solution of 1% formic acid prepared in cold (−20 °C) acetonitrile using Model 398 tissue Tearor (Biospec Products, Inc., Bartlesville, OK). The internal standard (3,4-dihydroxybenzylamine) was then added. Next, the sample was incubated in the freezer (−20 °C) for 15 min prior to centrifugation for 20 min at 16,000 rcf with the centrifuge held at 4 °C. The supernatant was then collected and dried in a vacuum concentrator. Next, the dried samples were resuspended in 0.1 mL of 15% acetonitrile and loaded onto a pre-conditioned Varian 96-well plates (C18, 25 mg). The flow-through liquid was collected with 0.8 mL column wash of 15% acetonitrile. The final extract was dried and resuspended in 35 μL of mobile phase (Scheme 1).

2.5. Liquid chromatography/tandem mass spectrometry (LC–MS/MS)

A Dionex Ultimate 3000 LC pump (Sunnyvale, CA) was used, consisting of an isocratic pump, a temperature-regulated



Scheme 1. Flow-chart summarizing sample preparation.

autosampler, and a column-cooling compartment. A Nucleodex β-OH column (200 mm × 4 mm, 5 μm particle size, Macherey-Nagel Inc., Easton, PA) with cyclodextrin chiral stationary phase was used for the chiral separations. The mobile phase consisted of 25 mM ammonium formate (pH 3.8) and 10% acetonitrile, at a flow rate of 0.5 mL/min. A 5-μL aliquot of the resuspended sample solution was injected on the column using a full-loop injection mode. The column eluent for the temperature study was coupled to a UV detector set at 280 nm detection wavelength. On the other hand, the LC system, for all other experiments was coupled to a triple–quadrupole/linear ion-trap QTrap 4000 Mass Spectrometer (Applied Biosystems, Framingham MA) that was run in the multiple-reaction-monitoring mode (MRM) where a precursor ion is fragmented in the second quadrupole (Q2), and the transition is isolated in the third quadrupole (Q3). The turbo ion-spray ionization source was heated to 450 °C due to the high LC flow rate, and had a nebulization gas pressure of 50 psi and a vaporization gas flow of 55 psi. The capillary voltage was held at 4800 V, while the entrance potential was set to 30 V. The MRM scans consisted of the following transitions, where the first value is the Q1 ion-selected (precursor ion) and the second is the Q3 ion-selected (product ion): DA (154.3/91.3), SAL (180.1/145.1), Epi (166.0/107.1), Norepi (152.0/107.1), IS (123.0/76.6). The product ions selected for the MRM scans were the most abundant product ions for each of the precursor ions as illustrated in (Fig. 2). For quantification, the peak area ratios of analytes to the IS were calculated as a function of the concentration of the analytes. A calibration report was generated using standard solutions that were treated in a manner identical to tissue samples, thus accounting for any sample losses.

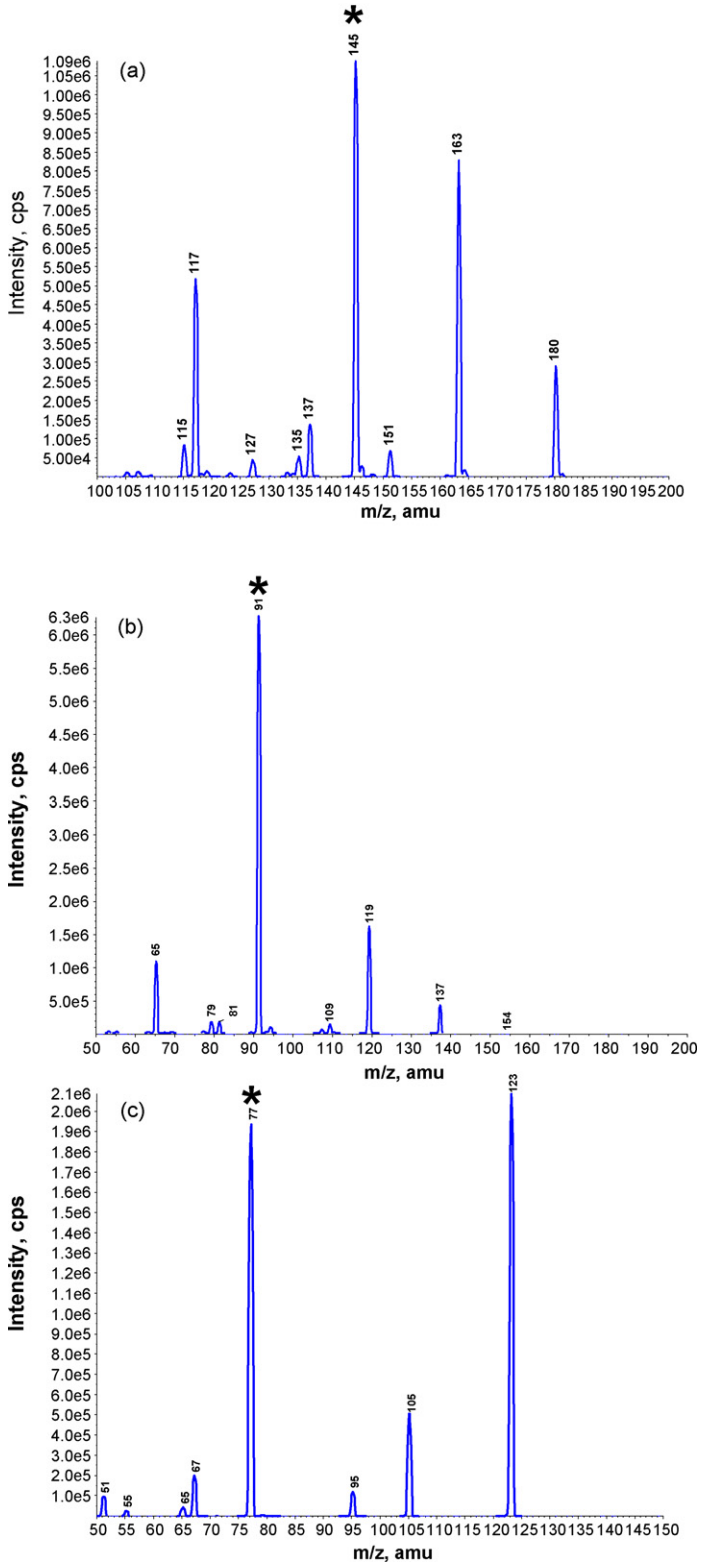


Fig. 2. Tandem mass spectra of salsolinol (a), dopamine (b) and the internal standard (c). In each spectrum, the product ion designated with an asterisk is the one employed for MRM scans.

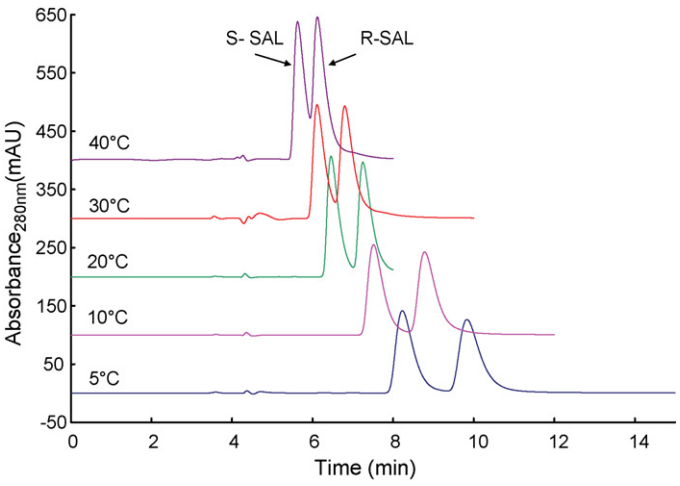


Fig. 3. Chiral LC chromatogram of salsolinol achieved under the chromatographic condition described in experimental under different temperatures.

3. Results and discussion

3.1. Optimized chiral chromatographic conditions for the separation of salsolinol

To achieve the chiral resolution of salsolinol, a β-cyclodextrin-based (CD) column was utilized as previously described [37]. Several parameters influence the chiral separation of compounds using β-CD column, a few of which have been optimized here to achieve the full resolution necessary for a reliable analysis of salsolinol enantiomers. In addition to the column temperature, the flow rate of the mobile phase has a substantial impact on the resolving power for the chiral separation. The column temperature is a vital parameter that can greatly influence the resolution during a chiral separation. Fig. 3 demonstrates the impact of β-CD column temperature on the resolution of salsolinol enantiomers. It is apparent from this figure that the lower column temperature (5 °C) should be used in order to attain a full resolution of the salsolinol enantiomers. At this temperature, the enantiomeric resolution coefficient is 1.8. Generally, *S*-salsolinol exhibits a greater steric hindrance when forming an inclusion complex with β-CD, as compared to *R*-salsolinol, and, therefore, has a shorter retention time. Complexation with the β-CD cavity decreases at higher flow-rates. Lower flow rates prompted a higher resolution of the salsolinol enantiomers over a range of 0.50–0.75 mL/min (data not shown). Here, a flow rate of 0.50 mL/min was selected in order to ensure full resolution of the two enantiomers. In addition, increasing the percentage of the ammonium formate buffer to 90% resulted in an increase in the retention for catecholamines with the β-CD cavity (data not shown). At higher organic mobile-phase content, the acetonitrile enters the hydrophobic cavity of the CD, thereby weakening the interaction of salsolinol with the cavity surface.

3.2. Method validation

The method developed here is more advanced than what have been previously employed to quantify the levels of salsolinol in brain tissue. Unlike GC analysis, there is no need for a prior derivatization to analyze salsolinol proportions. In addition, there is no need for chiral functionalization of salsolinol to get the resolution between the enantiomers. In this method, both the separation of native catecholamines and the chiral separation of the salsolinol enantiomers were simultaneously achieved using a β-CD column. In addition, due to the selective nature of MRM scanning, co-eluting peaks do not interfere with the acquisition of the chromatographic traces, so that a rapid separation can be achieved without full resolution of each component and without detrimental effects on peak integration. The separation is quick, in that it is achieved in less than 8 min, making the analysis of numerous serial samples more practical. In addition, the sensitivity of this method is satisfactory, while the detection limit for salsolinol is approximately 1 pg loaded on-column. On the other hand, the limit of quantitation in this analysis was 5 pg. The observed linear dynamic range extends over four orders of magnitude (5 pg to 50 ng) with a correlation coefficient values (R^2) between 0.98 and 0.99 (Table 1).

Standard samples utilized for the construction of calibration curves were subjected to the same analytical steps endured by the real samples, including the SPE step. The regression analysis data for the analytes are summarized in Table 1. The calibration points were selected to coincide with the concentration of analytes expected in the tissues. The sample preparation procedure utilized in this study is different from the previous procedures, in that a more rudimentary scheme has been used. This is to simplify sample preparation. The mass spectrometer MRM analysis capabilities, allows the selection of specific ions to be measured despite the complexity of sample matrix. The procedure employs protein precipitation in an organic/acidic solution prior to centrifugation. Other hydrophobic compounds are then trapped on a C18 phase, while the polar catecholamines are collected in the pass through liquid.

3.3. Salsolinol and catecholamine concentrations in brain regions of P-rats subjected to ethanol exposure

As discussed above, it is widely known that several regions of the brain are differentiated structurally and chemically [4–6]. For instance, certain regions of the brain are considered a dopamine-rich based upon the presence of dopamine cell bodies and nerve terminals in that region. It is believed that it is more likely in such regions that salsolinol be formed either by the condensation of acetaldehyde with dopamine or through an enzymatic process, yet the predominance of one over the other is not fully understood. The MRM traces for dopamine and salsolinol for the CPU region of rats exposed to ethanol under various conditions are shown in Fig. 4a–d. The chromatographic traces of the analyzed CPU regions of all animals studied here are depicted in each figure, illustrating the biodiversity commonly associated with this type of studies. The data suggest that the CPU is a dopamine-rich region of the brain, whereas the MB does not appear to be (Fig. 5a). This might be due to the fact that the VTA and SN (two regions containing dopamine neurons) are only metabolizing a small part of the MB catecholamines. While the levels of dopa-

Table 1
Figures of merit for the quantification of salsolinol and catecholamines using LC–MS

Analyte	Linear regression (1/x weighting) $y = Ax + B$	R^2	LOD (pg/μL)	Dynamic range (pg/μL)
S-Salsolinol	$y = 0.0296x + 0.0251$	0.98	2.5	5–5,000
R-Salsolinol	$y = 0.0402x + (-0.1910)$	0.98	2.5	5–5,000
Dopamine	$y = 0.0011x + (-0.0064)$	0.99	5.0	10–10,000
Epinephrine	$y = 5.0100x + 0.0286$	0.99	5.0	10–10,000
Norepinephrine	$y = 0.0038x + 0.0323$	0.99	5.0	10–10,000

mine increase under the elevated ethanol exposure conditions (Fig. 5a), the levels of norepinephrine (Fig. 5b) and epinephrine (Fig. 5c) do not significantly change under the two ethanol-drinking conditions. The levels of salsolinol increased under both drinking conditions in the CPU and under continuous access drinking in the MB (Fig. 6a), suggesting the formation of acetaldehyde in both regions. In the alcohol drinking groups, there were no significant changes in the dopamine, norepinephrine, and epinephrine levels in the MB (Table 2).

3.4. Alcoholism and salsolinol

Salsolinol levels in both CPU and MB regions increase as a result of alcohol exposure (Fig. 6a). Moreover, continuous ethanol exposure appears to induce a profound increase in the level of salsolinol relative to that of binge drinking (Fig. 6a). Previously, a racemic mixture of salsolinol was only observed in the hypothalamus brain region of P-rats exposed to alcohol, while the *R*-salsolinol enantiomer was predominantly found in all other anatomical regions of the brain of P-rats [4]. Here, the relative ratios of salsolinol enantiomers in the alcohol naïve and exposed rats displayed a similar pattern of *R*-salsolinol predominance in the brain regions studied here (Fig. 6b). The levels of both enantiomers appear to increase as a result of both a continuous

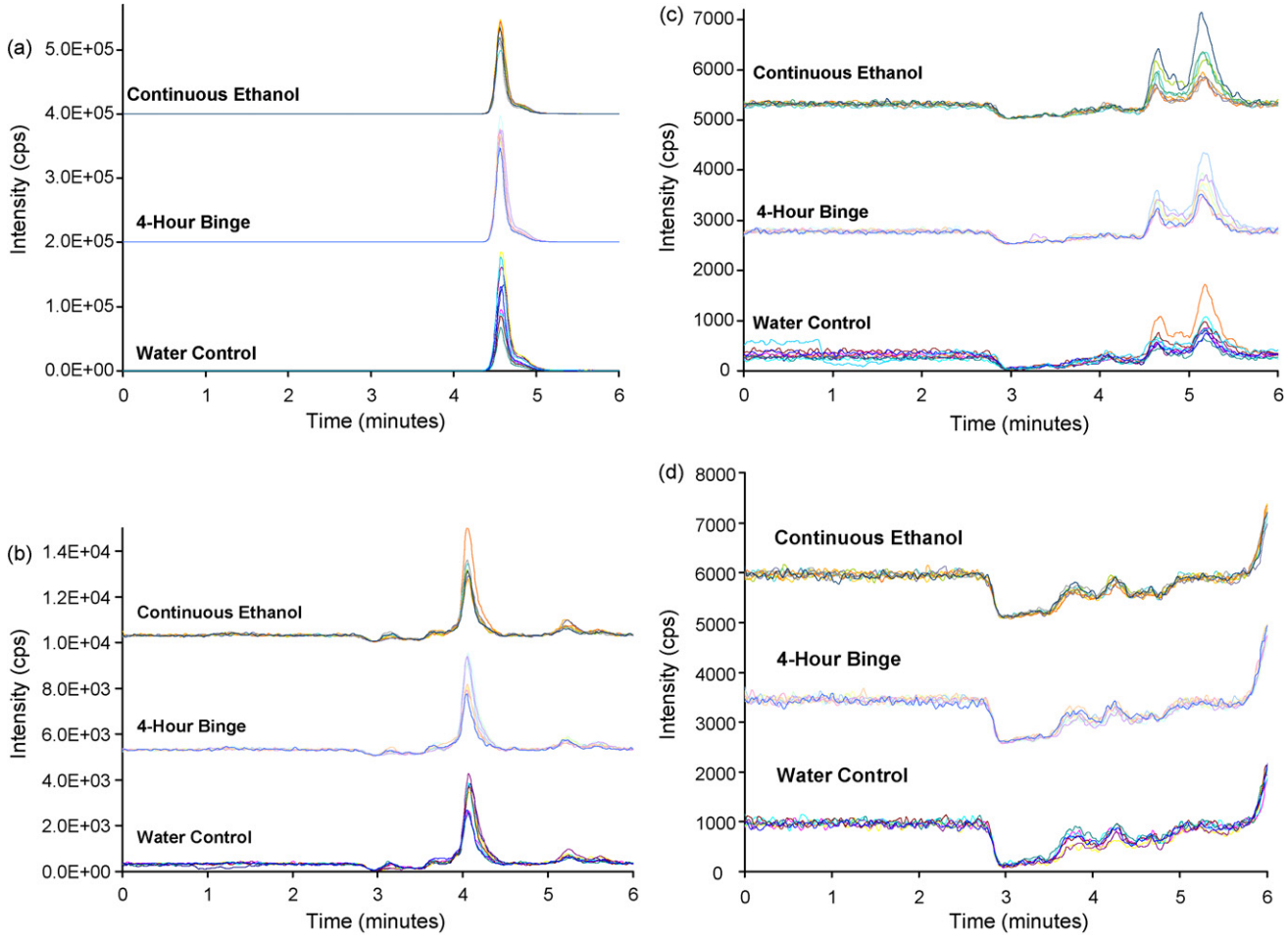


Fig. 4. MRM chromatograms of dopamine, norepinephrine, salsolinol, and epinephrine extracted from the CPU brain region under different ethanol exposure conditions.

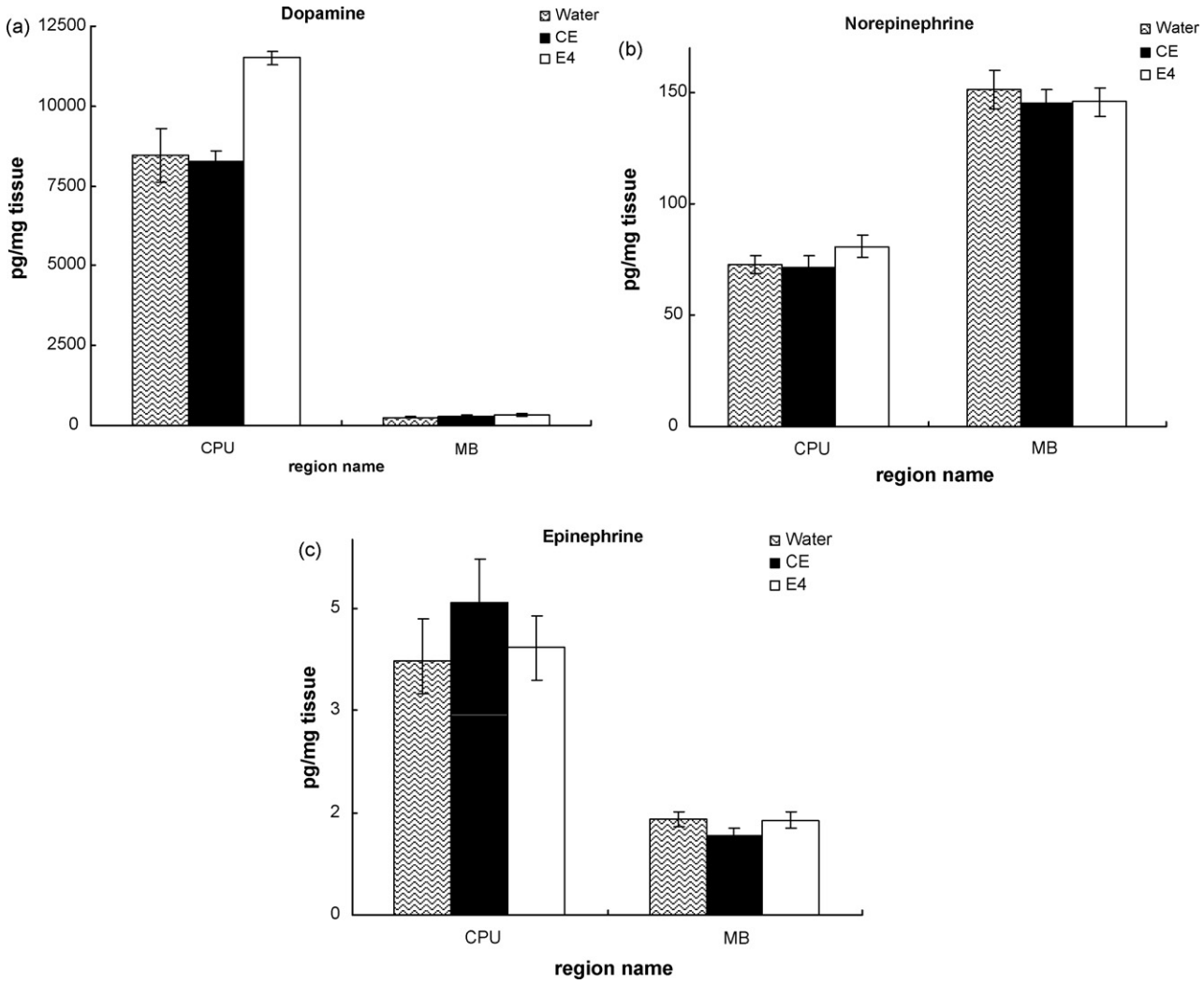


Fig. 5. Bar graphs of the concentrations (pg/mg tissue) of dopamine (a), norapinephrine (b) and epinephrine (c) in the MB and CPU brain regions of rats exposed to water, continuous ethanol drinking (CE) and 4-h binge drinking (E4).

consumption or binge drinking. However, it appears that there is a higher increase in *S*-salsolinol as a result of ethanol exposure. This is deduced from the decrease in the *R*/*S* ratios of the enantiomers upon ethanol exposure (Table 2). A non-enzymatic formation of salsolinol via Pictet-Spengler reaction results in the formation of both *R*- and *S*-salsolinol, while an additional enzymatic formation of just *R*-salsolinol (Fig. 6a and b), especially in the MB, explains the overall high values of *R*-salsolinol in these brain regions. The hypothesis that the overall formation of salsolinol in the MB may be non-enzymatic is partially supported by the data summarized in Table 2. An increase in the levels of *S*-salsolinol upon ethanol exposure originates from the non-enzymatic pathway. Since *R*-salsolinol synthase is specific for the conversion of dopamine to *R*-salsolinol, no increases in *S*-salsolinol would be expected following the ethanol consumption if enzymatic pathway were responsible for this increase. However, as indicated by our results, an increase in both *R*- and *S*-salsolinol is recorded in the CPU and MB brain regions of rats that are chronically exposed to ethanol. The enantiomeric ratio of *R*/*S*-salsolinol decreases as a result of an increased production of both isomers in response to an increased dopamine and acetaldehyde abundance. It appears that salsolinol production in CPU and MB brain regions is due to both enzymatic and non-enzymatic pathways. It also appears that the chemical pathway is favored under alcohol exposure, as suggested by the decrease in the *R*/*S* ratios (Table 2).

The levels of epinephrine and norepinephrine in both CPU and MB brain regions appear not to be influenced by ethanol exposure (Table 2). They all endured some fluctuations as a result of ethanol exposure; however, changes were not significant. On the other hand, dopamine levels did not substantially change as a result of continuous ethanol exposure. A substantial increase was observed in the case of binge drinking. A one-third increase in the level of dopamine was observed in the case of animals subjected to binge drinking.

4. Conclusions

In this paper, the development of a highly sensitive analytical methodology was presented here for measuring the enatiomeric

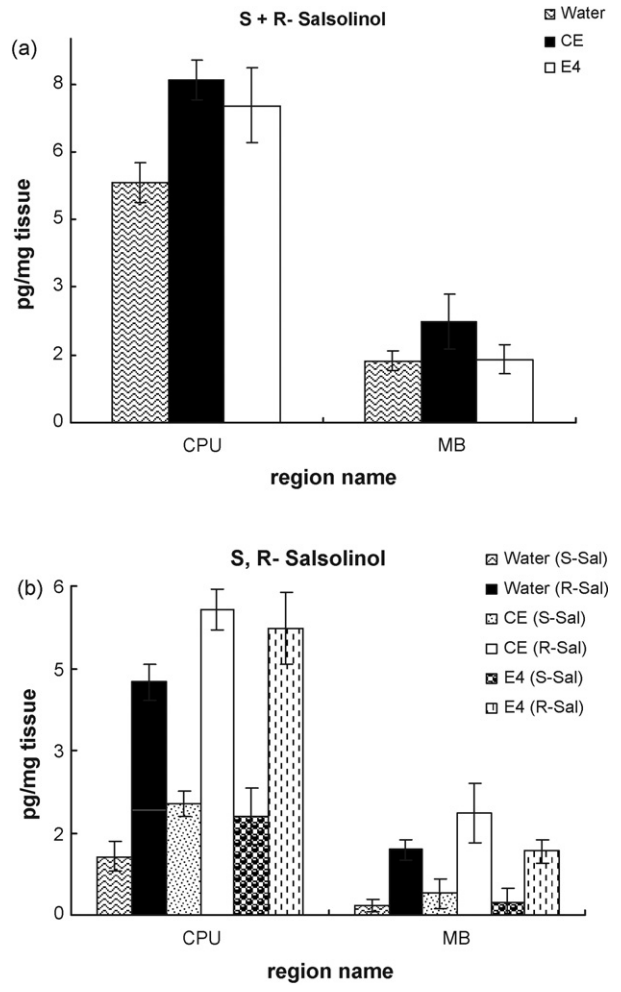


Fig. 6. Bar graphs of the concentration (pg/mg tissue) of salsolinol (total salsolinol (a), individual enantiomers (b)) in the CPU and MB brain regions of rats exposed to water, continuous ethanol drinking (CE) and 4-h binge drinking (E4).

Table 2
Concentrations of salsolinol and catecholamines measured in the CPU or MB of rats exposed to two different ethanol drinking conditions ($n = 8$ animals/group)

	Water	CE	E4
Caudate putamen (CPU)			
S-Salsolinol	1.1 ± 0.3	2.0 ± 0.3	1.8 ± 0.5
R-Salsolinol	4.2 ± 0.3	5.6 ± 0.4	5.2 ± 0.7
Total *S*,*R*-salsolinol	5.32 ± 0.4	7.59 ± 0.4	7.02 ± 0.8
R/*S*-Salsolinol	3.96 ± 1	2.74 ± 0.4	2.91 ± 0.9
Dopamine	8441 ± 830	8250 ± 334	11503 ± 202
Epinephrine	3.7 ± 0.6	4.6 ± 0.6	3.9 ± 0.5
Norepinephrine	72 ± 4	71 ± 5	80 ± 5
Midbrain (MB)			
S-Salsolinol	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.2
R-Salsolinol	1.2 ± 0.2	1.9 ± 0.6	1.2 ± 0.2
Total *S*,*R*-salsolinol	1.36 ± 0.2	2.24 ± 0.6	1.40 ± 0.3
R/*S*-Salsolinol	7.07 ± 5	4.79 ± 4	4.89 ± 5
Dopamine	243 ± 26	259 ± 5	313 ± 56
Epinephrine	1.4 ± 0.1	1.1 ± 0.1	1.4 ± 0.1
Norepinephrine	151 ± 9	145 ± 6	145 ± 6

Measured as pg/mg tissue ± S.E.M.

ratio of salsolinol among catecholamines in regional brain tissue extracts. This method offers low detection limits and the ability to resolve the two enantiomers of salsolinol using LC–MS with a cyclodextrin-based chromatographic column. The approach was utilized to better understand the mechanism of alcohol addiction in rats selectively bred for high alcohol intake. The formation of salsolinol following ethanol consumption is believed to occur through a non-enzymatic process involving the condensation of dopamine with acetaldehyde. From the results presented in this study, it is evident that salsolinol is produced following alcohol consumption. The *R*/*S*-salsolinol ratio observed under these circumstances decreases, suggesting an increase in the non-enzymatic production. While the formation of salsolinol in the brain results from the condensation of dopamine and acetaldehyde, further validation was provided for the biochemical basis of alcoholism. Although other analytical alternatives for catecholamines have been developed over the years, a high-sensitivity definitive method for salsolinol, shown in this study, opens numerous possibilities for assessing the emerging roles of this compound in physiological processes and alcohol abuse. A major advantage of salsolinol measurements through LC/MS is that there is no need for derivatization, compared to the GC-based measurements.

Acknowledgments

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References

[1] H. Rommelspacher, S.S. Baum, P. Dufeu, L.G. Schmidt, Alcohol 12 (1995) 309.
[2] H. Haber, N. Dumaual, D.J. Bare, M.F. Melzig, W.J. McBride, L. Lumeng, T.-K. Li, Addict. Biol. 4 (1999) 181.
[3] H. Haber, A. Winkler, I. Putscher, P. Henklein, I. Baeger, M. Georgi, M.F. Melzig, Alcohol. Clin. Exp. Res. 20 (1996) 87.
[4] F. Musshoff, D. Lachenmeier, W.P. Schmidt, R. Dettmeyer, B. Madea, Alcohol. Clin. Exp. Res. 29 (2005) 46.
[5] P. Dostert, B.M. Strolin, G. Dordain, D. Vernay, J. Neural Transm. Gen. Sect. 85 (1989) 51.
[6] P. Dostert, B.M. Strolin, G. Dordain, J. Neural Transm. 74 (1988) 61.
[7] M. Naoi, W. Maruyama, N. Nakao, T. Ibi, K. Sahashi, M. Strolin Benedetti, Ann. Neurol. 43 (1998) 212.
[8] Z.A. Rodd, R.L. Bell, Y. Zhang, A. Goldstein, A. Zaffaroni, W.J. McBride, T.-K. Li, Alcohol. Clin. Exp. Res. 27 (2003) 440.
[9] Z.A. Rodd-Henricks, R.L. Bell, K.A. Kuc, J.M. Murphy, W.J. McBride, L. Lumeng, T.-K. Li, Alcohol. Clin. Exp. Res. 25 (2001) 1140.
[10] Z.A. Rodd-Henricks, D.L. McKinzie, J.M. Murphy, W.J. McBride, Psychopharmacol 149 (2000) 217.
[11] H. Rommelspacher, S. Saellstroem-Baum, P. Defue, L.G. Schmidt, Alcohol 12 (1996) 309.
[12] B. Sjoquist, A. Erikson, B. Winblad, in: F. Bloom, J. Barchas, M. Sandler, E. Usdin (Eds.), Beta-carbolines and Tetrahydroisoquinolines, Alan R. Liss Publishing, New York, 1982, p. 57.
[13] F. Musshoff, J. Chromatogr. B 781 (2002) 457.
[14] F. Musshoff, in: F.M. Wurst (Ed.), New and Upcoming Markers of Alcohol Consumption, Steinkopff-Springer, Darmstadt, 2001, p. 112.

[15] F. Musshoff, P. Schmidt, R. Dettmeyer, F. Priemer, K. Jachau, B. Madea, Forensic Sci. Int. 113 (2000) 359.
[16] A. Brossi, Prog. Clin. Biol. Res. 90 (1982) 125.
[17] Y. Deng, W. Maruyama, H. Yamamura, M. Kawai, P. Dostert, N. Makoto, Anal. Chem. 68 (1996) 2826.
[18] W. Maruyama, T. Abr, H. Toghi, P. Dostert, M. Naoi, Ann. Neurol. 40 (1996) 119.
[19] W. Maruyama, G. Sobue, K. Matsubara, Y. Hashizume, P. Dostert, M. Naoi, Neurosci. Lett. 223 (1997) 61.
[20] W. Maruyama, H. Narabayashi, P. Dostert, M. Naoi, J. Neural Transm. 103 (1996) 1069.
[21] M. Naoi, W. Maruyama, P. Dostert, K. Kohda, T. Kaiya, Neurosci. Lett. 212 (1996) 183.
[22] M. Naoi, W. Maruyama, G.M. Nagy, Neurotoxicology 25 (2004) 193.
[23] K. Matsubara, S. Fukushima, Y. Fukui, Brain Res. 413 (1987) 336.
[24] T.K. Li, L. Lumeng, D.P. Doolittle, Behav. Gen. 23 (1993) 163.
[25] L. Lumeng, T.D. Hawkins, T.K. Li, Alcohol Aldehyde Metab. Syst. 3 (1977) 537.
[26] W.J. McBride, T.K. Li, Crit. Rev. Neurobiol. 12 (1998) 339.
[27] J.M. Murphy, R.B. Stewart, R.L. Bell, N.E. Badia-Elder, L.G. Carr, W.J. McBride, L. Lumeng, T.-K. Li, Behav. Genet. 32 (2002) 363.
[28] M. Sandler, V. Glover, I. Armando, A. Clow, Prog. Clin. Biol. Res. 90 (1982) 215.
[29] M. Melis, S. Spiga, M. Diana, in: R.J. Bradley, R.A. Harris, P. Jenner (Eds.), International Review of Neurobiology, Elsevier Academic Press, San Diego, 2005, p. 101.
[30] H. He, C.M. Stein, B. Christman, A.J.J. Wood, J. Chromatogr. B 701 (1997) 115.
[31] M.A. Raggi, C. Sabbioni, G. Nicoletta, R. Mandrioli, G. Gerra, J. Sep. Sci. 26 (2003) 1141.
[32] C. Sabbioni, M.A. Saracino, R. Mandrioli, S. Pinzauti, S. Furlanetto, G. Gerra, M.A. Raggi, J. Chromatogr. A 1032 (2004) 65.
[33] H. Haber, H.M. Haber, M.F. Melzig, Anal. Biochem. 224 (1995) 256.
[34] H. Haber, N. Stender, A. Mangholz, H. Ehrenreich, M.F. Melzig, J. Chromatogr. B 735 (1999) 299.
[35] A. Raffaelli, A. Saba, Mass Spectrom. Rev. 22 (2003) 318.
[36] J.A. Starkey, Y. Mechref, J. Muzikar, W.J. McBride, M.V. Novotny, Anal. Chem. 78 (2006) 3342.
[37] S.S. Baum, H. Rommelspacher, J. Chromatogr. B 660 (1994) 235.