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Review

Capillary liquid chromatography combined with tandem mass spectrometry for the study of neurosteroids and oxysterols in brain

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Abstract

Neurosteroids and neurosterols are found in brain at low levels (ng/g–μg/g) against a high background of cholesterol (mg/g). As such their analysis can be challenging. Traditionally, these molecules have been analysed by gas chromatography (GC)–mass spectrometry (MS), however, the absence of molecular ions in GC–MS spectra, even from derivatised molecules, can make the discovery and identification of novel neurosteroids/sterols difficult. To avoid this scenario, liquid chromatography (LC) combined with desorption ionisation methods are employed. In this review we discuss the application of LC–MS and LC–tandem mass spectrometry (MS/MS) for the identification of neurosteroids/sterols, paying particular attention to the use of low-flow-rate LC to maximise chromatographic and mass spectrometric performance.

Keywords: Sterol; Steroid; Cholesterol; Pregnanolone; Hydroxycholesterol; Alzheimer's disease

1. Introduction

The term neurosteroid was coined by Baulieu in Paris to describe steroids which are synthesised in the nervous system (Baulieu, 1997, 1998). Neurosteroids can act as allosteric modulators of neurotransmitter receptors such as gamma aminobutyric acid A ($GABA_A$), *N*-methyl-D-aspartate (NMDA), and sigma receptors; they include 3β-hydroxy-Δ^5 steroids such as dehydroepiandrosterone (DHEA, A^5-3β-ol-17-one) and pregnenolone (P^5-3β-ol-20-one), their reduced metabolites, and supposedly their sulphates (Baulieu, 1997, 1998). Progesterone (P^4-3,20-dione) is also a neurosteroid and can interact with the progesterone receptor which is expressed in brain. Steroids which have activity in the nervous system, but are not necessarily synthesised by nerve cells are called neuroactive steroids, these include synthetic steroids and endogenous steroids produced in the nervous system and peripheral glands. Oxysterols are oxidised forms of cholesterol, they are biologically active molecules, and when synthesised in brain, or when active in brain, we term these molecules neurosterols. Oxysterols act as ligands to the liver X receptor (Janowski et al., 1999), the β form of which is expressed in brain (Teboul et al., 1995), and have also been proposed to interact (indirectly) with steroid regulatory element binding proteins (SREBP), transcription factors which regulate the expression of enzymes involved in cholesterol synthesis (Adams et al., 2004; Goldstein et al., 2006).

The levels of both neurosteroids and neurosterols in brain are low (ng/g–μg/g, Tables 1 and 2), and they are present against a background of cholesterol (mg/g) and its precursor sterols. Further, the levels of neurosteroids/sterols vary temporally and spatially, making their analysis challenging. A neurosteroid biosynthetic pathway has been proposed (Mellon and Griffin, 2002), Fig. 1, but the presence in brain of many of the enzymes involved in the pathway have yet to be determined on the protein level. As such the challenge is to perform proteomic and steroidomic studies to identify the steroidogenic enzymes in brain and the complimentary neurosteroid/sterol metabolites, and to investigate how their levels vary according to location in brain, with age, and in healthy and diseased states.

2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS has been (Corpéchot et al., 1981, 1983, 1993), and is (Vallée et al., 2000, 2004; Liere et al., 2004) extensively used for neurosteroid analysis. The advantage of this method is the

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Table 1
Neurosteroids and neuroactive steroids present in the nervous system

Neurosteroid	Chemical formula/ mass (Da)	Human CSF	Human plasma	Rat brain	Rat plasma
Allopregnanolone, 5α-P-3α-ol-20-one	$C_{21}H_{34}O_2$/318.26	0.05 ng/mL[a]	0.07 ng/mL[a]	≤2.5 ng/g[b], 0.5 ng/g[c], 2.1–11 ng/g[d], 0.6 ng/g[e]	0.12 ng/mL[a], 0.15 ng/mL[b]
Epipregnanolone, 5β-P-3β-ol-20-one	$C_{21}H_{34}O_2$/318.26			0.05–0.14 ng/g[c,f], 1.1–2.5 ng/g[d,f]	0.11 ng/mL[a]
Epiallopregnanolone, 5α-P-3β-ol-20-one	$C_{21}H_{34}O_2$/318.26			≤2.5 ng/g[b], 0.05–0.14 ng/g[c,f], 1.1–2.5 ng/g[d,f]	
Pregnanolone, 5β-P-3α-ol-20-one	$C_{21}H_{34}O_2$/318.26		0.11 ng/mL[a]	0.1–0.2 ng/g[c]	
5α-Pregnane-3,20-dione, 5α-P-3,20-dione	$C_{21}H_{32}O_2$/316.24			1.9 ng/g[g], 0.8 ng/g[e]	
Pregnenolone, P^5-3β-ol-20-one	$C_{21}H_{32}O_2$/316.24	0.04 ng/mL[a]	4.18 ng/mL[a], 0.1–2.3 ng/mL[h]	8.9 ng/g[g], ≤2.5 ng/g[b], 7 ng/g[b,i], 0.6–1.2 ng/g[c], 2.7–3.9 ng/g[d], 2.8 ng/g[e], 7 ng/g[j], 61 ng/g[j,k]	1.2 ng/mL[g], 0.15 ng/mL[a]
Pregnenolone sulphate, P^5-3β-ol-20-one 3-sulphate	$C_{21}H_{32}O_5S$/396.20		87 ng/mL[l]	14.2 ng/g[g], <0.1 ng/g[m], <0.3 ng/g[n], 0.5 ng/g[o], 0.05–0.5 ng/g[p]	2.1 ng/mL[g]
Progesterone, P^4-3,20-dione	$C_{21}H_{30}O_2$/314.22		0.1–2.5 ng/mL[h]	2.2 ng/g[g], 1.0–3.4 ng/g[c], 4.4–21.0 ng/g[d], 1.9 ng/g[e], <0.5 ng/g[j], 13 ng/g[j,k]	1.9 ng/mL[g]
5α-Androstane-3α,17β-diol, 5α-A-3α,17β-diol	$C_{19}H_{32}O_2$/292.24			0.2 ng/g[q]	
Androsterone, 5α-A-3α-ol-17-one	$C_{19}H_{30}O_2$/290.22	0.050 ng/mL[a]	35 ng/mL[a]		0.24 ng/mL[a]
DHEA, A^5-3βol-17-one	$C_{19}H_{28}O_2$/288.21		1.3–12.5 ng/mL[h]	0.24 ng/g[g], ≤2.5 ng/g[b], 0.05–0.11 ng/g[c], 0.04–0.08 ng/g[d]	0.06 ng/mL[g]
DHEA sulphate, A^5-3β-ol-17-one 3-sulphate	$C_{19}H_{28}O_5S$/368.17		1500 ng/mL[l], 1000–2800 ng/mL[h]	1.7 ng/g[g], <0.1 ng/g[m], <0.3 ng/g[n]	0.20 ng/mL[g]
Testosterone, A^4-17β-ol-3-one	$C_{19}H_{28}O_2$/288.21	0.20 ng/mL[a]	2.7 ng/mL[a], 1.2–11.1 ng/mL[h,r], 0.03–0.46 ng/mL[h,s]	<2.5 ng/g[b], 0.4–0.5 ng/g[c], 0.04–0.11 ng/g[d], 1.3 ng/g[q]	2.64 ng/mL[a], 2.5 ng/mL[b], 2.7 ng/mL[q]

[a] Data for male human and male rats, from Kim et al. (2000).
[b] Data for male rat, frontal cortex, from Vallée et al. (2000).
[c] Data for male rats, from Liu et al. (2003b).
[d] Data for female rats, from Liu et al. (2003b).
[e] Data for adrenalectomised/castrated male rats, from Uzunov et al. (1996).
[f] Epipregnanolone or epiallopregnanolone.
[g] Data for male rats, from Baulieu (1997, 1998) and Corpéchot et al. (1981, 1983, 1993).
[h] Data from Shackleton (2007).
[i] Data for male rats, frontal cortex after swim stress, from Vallée et al. (2000).
[j] Data for male rats, from Higashi et al. (2005a).
[k] Data for male rats after fixation stress, from Higashi et al. (2005a).
[l] Data for male adult, from Liu et al. (2003a).
[m] Data for male rats, from Liere et al. (2004).
[n] Data for male and female rats, from Liu et al. (2003b).
[o] Data for mate rats, from Mitamura et al. (1999).
[p] Data for male rats, from Higashi et al. (2003).
[q] Data for male rats, from Higashi et al. (2006).
[r] Data for human adult male, from Shackleton (2007).
[s] Data for female adult female, from Shackleton (2007).

Table 2
Oxysterols present in the brain, CSF and plasma

Sterol[a]	Chemical formula/mass	Human plasma	Human CSF	Human brain	Rodent brain
Desmosterol[b] $C^{5,24}$-3β-ol	$C_{27}H_{44}O$/384.34				Rat 0.1% of total sterol[d], mouse 50 ng/mg[e]
Cholesterol[b], C^5-3β-ol	$C_{27}H_{46}O$/386.35	2 mg/mL[c]	3 μg/mL[c]	7–8 μg/mg[c]	Mouse 20 μg/mg[e]
Cholesterol sulphate[f], C^5-3β-ol 3-sulphate	$C_{27}H_{46}O_4S$/466.31	50–300 ng/mL[g]			Rat 1.2 ng/mg[f]
7-Oxocholesterol, C^5-3β-ol-7-one	$C_{27}H_{44}O_2$/400.33		0.9 ng/mL[h]	170–640 ng/mg[i]	
24-Oxocholesterol, C^5-3βol-24-one	$C_{27}H_{44}O_2$/400.33				Rat ∼0.5 ng/mg[j]
7α-Hydroxycholesterol, C^5-3β,7α-diol	$C_{27}H_{46}O_2$/402.35	40 ng/mL[k]			
7β-Hydroxycholesterol, C^5-3β,7β-diol	$C_{27}H_{46}O_2$/402.35	5 ng/mL[k]			
7-Hydroxycholesterol, C^5-3β,7-diol	$C_{27}H_{46}O_2$/402.35			234–745 ng/mg[i]	
20*S*-Hydroxycholesterol, C^5-3β,20*S*-diol	$C_{27}H_{46}O_2$/402.35				Rat 50 pg/mg rat[l]
22*R*-Hydroxycholesterol, C^5-3β,22*R*-diol	$C_{27}H_{46}O_2$/402.35			45–90 pg/mg[m]	