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Lipid nanocapsules containing the non-ionic surfactant Solutol HS15 inhibit the transport of calcium through hyperforin-activated channels in neuronal cells

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ABSTRACT

Hyperforin is described as a natural antidepressant inhibiting the reuptake of neurotransmitters and also activating cation channels. However the blood–brain barrier limits the access to the brain of this biomolecule. To circumvent this problem it was envisaged to encapsulate hyperforin into biomimetic lipid nano-carriers like lipid nanocapsules (LNCs). When testing the safety of 25 nm LNCs it appeared that they strongly blocked hyperforin-activated Ca^{2+} channels of cultured cortical neurons. This inhibition was due to one of their main component: solutol HS15 (polyoxyethylene-660-12-hydroxy stearate), a non-ionic soluble surfactant. Solutol HS15 rapidly depresses in a concentration-dependent manner the entry of Ca^{2+} through hyperforin-activated channels without influencing store-operated channels. This effect is mimicked by Brij58 but not by PEG600, indicating that the lipid chain of Solutol HS15 is important in determining its effects on the channels. The inhibition of the Ca^{2+} fluxes depends on the cellular cholesterol content; it is stronger after depleting cholesterol with methyl-β-cyclodextrin and is nearly absent on cells cultured in a cholesterol-rich medium. When chronically applied for 24 h, Solutol HS15 slightly up-regulates the entry of Ca^{2+} through hyperforin-activated channels. Similar observations were made when testing 25 nm lipid nanocapsules containing the surfactant Solutol HS15. Altogether, this study shows that Solutol HS15 perturbs in a cholesterol-dependent manner the activity of some neuronal channels. This is the first demonstration that LNCs containing this surfactant can influence cellular calcium signaling in the brain, a finding that can have important clinical implications.

1. Introduction

Hypericum perforatum (St John's wort, SJW) is a medicinal plant possessing antidepressant properties and total extracts of SJW are currently used to alleviate symptoms of mild to moderate depression (Linde et al., 2005). Hyperforin, a bioactive compound isolated from SJW, is regarded as the main antidepressive agent of total SJW extracts (Muller, 2003). Like synthetic antidepressants, this molecule inhibits the reuptake of neurotransmitters (e.g. dopamine, norepinephrine, serotonin or glutamate) (Roz et al., 2002; Sell et al., 2014; Singer et al., 1999; Wonnemann et al., 2000). In addition, recent data shed new light on this pharmacological agent by revealing three key properties of high physiological interest: i) *In vitro* and *in vivo* experiments showed that a chronic hyperforin treatment activates an intracellular signaling pathway involving a plasma membrane cation channel, the cAMP-dependent protein kinase A as well as the transcription factor CREB (cyclic adenosine monophosphate response element binding protein). This leads to an up-regulation of the expression of TRPC6 channels and TrkB (the receptor of the brain-derived neurotrophic factor, BDNF) (Gibon et al., 2013), ii) *In vitro* experiments conducted on the neuronal cell line PC12, primary hippocampal neurons and CA1 neurons in organotypic slices showed that hyperforin stimulates the growth of

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neurites and influences synaptic plasticity by regulating, in a TRPC6-dependent manner, the morphology and number of dendritic spines (Leuner et al., 2007, 2013), iii) Tetrahydrohyperforin, a hyperforin derivative, enhances the adult hippocampal neurogenesis (Abbott et al., 2013). Altogether, these observations suggest that hyperforin could regulate the production of neurons and synaptic transmission, making this molecule a very attractive therapeutic agent. However, the blood–brain barrier is poorly permeable to hyperforin (Cervo et al., 2002), limiting its access to neural cells of the central nervous system. This feature restrains the wide use of hyperforin as a promising antidepressant drug.

To circumvent the problem of the modest permeability of the blood–brain barrier to hyperforin, we decided to encapsulate the molecule into lipid nanocapsules (LNCs) to facilitate its penetration into the brain with the aim to enhance its antidepressive efficacy.

LNCs are stable lipidic nanocarriers with a size of 20–100 nm. They consist of three main components: an oily phase, an aqueous phase containing NaCl, and a non-ionic surfactant (Tamjidi et al., 2013) (Fig. 1). Schematically, the oily core is composed of medium-chain triglycerides, surrounded by a membrane made of lecithin and solutol (Solutol® HS15, polyoxyethylene-660-12-hydroxy stearate), which critically determines the size of the nanoparticles (Heurtault et al., 2002). One important feature of these LNCs is the presence of Solutol HS15 which is, like Tween 80 and Cremophor EL, a nonionic soluble surfactant. Developed in 1992, Solutol HS15 was chosen due to its physiological compatibility for intravenous applications and is used in injectable-drug products in several countries like Argentina or Canada (Ku and Velagaleti, 2010; Williams et al., 2013). Indeed, a major property of Solutol HS15 is to improve the dissolution and bioavailability of hydrophobic molecules, explaining its use as an important component of various drug delivery systems (e.g. solid lipid nanoparticles, micelles, liposomes, self-nanoemulsifying drug delivery systems, micro- and nano-emulsions). LNCs are regarded as promising tools to deliver therapeutics molecules, including to the brain (Beduneau et al., 2008), but they also offer interesting applications in imaging and radiotherapy (Huynh et al., 2009).

But before testing the antidepressive effects of hyperforin loaded into such lipid nanocarriers, experiments were first conducted to determine the putative biological effects of blank (non-loaded) LNCs. It is particularly important to make sure that these nanocarriers do not perturb the hyperforin-activated cellular responses. Since hyperforin is well-known to promote the entry of cations into cells (Gibon et al., 2010; Leuner et al., 2007; Sell et al., 2014; Treiber et al., 2005), we characterized the impact of blank LNCs on hyperforin-activated channels.

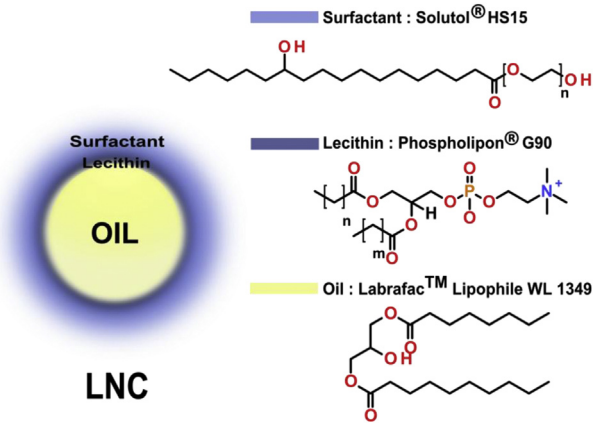

Fig. 1. Solutol HS15-containing LNCs. Schematic representation of the LNCs used in the present study. The structure of the components used to prepare these nanostructured carriers is also given.

2. Experimental procedures

2.1. Materials

Fluo-4/AM and tissue culture media were purchased from Molecular Probes (Invitrogen, France). Hyperforin was a kind gift from Dr. Willmar Schwabe GmbH & Co (Karlsruhe, Germany). Lipid nanocapsules were made of Labrafac™ Lipophile WL 1349 (caprylic/capric triglyceride), Phospholipon® 90G (soybean lecithin at 97.1% of phosphatidylcholine), and Solutol® HS15 (a mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) generously provided by Gattefosse S.A.S. (Saint-Priest, France), Phospholipid GmbH (Köln, Germany), and Laserson (Etampes, France), respectively. Deionized water was obtained from a Milli-Q plus system (Millipore, Paris, France). Brij® 58 (ref. P5884), methanol-d4 (99.8 atom % D, contains 0.05% (v/v), tetramethylsilane (TMS) (ref. 611646), PEG600 (ref. 202401) and unless otherwise indicated all other chemical reagents and solvents were obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France) and used as received.

2.2. Lipid nanocapsules formulation

Lipid nanocapsules (LNCs) were formulated at a nominal size of 25 nm using a phase inversion method of an oil/water system, as described by Heurtault et al. (2002). Briefly, the oil phase containing Labrafac (252 mg) and Phospholipon 90G (37.5 mg) was mixed with the appropriate amounts of Solutol (408 mg), deionized water (540 μL) and NaCl (22 mg), and heated under magnetic stirring up to 85 °C. The mixture was subjected to 3 temperature cycles from 70 to 90 °C under magnetic stirring. Then, the formulation was cooled to 78 °C and cold deionized water (3.3 mL, 0 °C) was added. The suspension (4 mL) was kept under stirring at room temperature for 10 min before further use. LNCs were purified from supernatant using disposable PD-10 desalting columns (Sephadex® G-25 for gel filtration as stationary phase, Amersham Biosciences). A column was stabilized with 25 mL of deionized water and 2.5 mL of LNCs suspensions were deposited on the column. Then 4 mL of deionized water were eluted and the purified LNCs were collected in this eluant (dilution factor of gel filtration = 1.6). In order to estimate the mass concentration, the solution (3 x 100 μL) was freeze-dried and weighed. The mass concentration of purified LNCs suspension was found to be 92.5 ± 0.7 mg/mL.

2.3. Particle size and zeta potential measurements

LNCs were characterized in terms of size and charge. The average diameter and polydispersity index (PI) were determined by dynamic light scattering using a Zetasizer® Nano ZS (Malvern Instruments S.A., Worcestershire, UK). The zeta potential was measured using the electrophoretic mode with the Zetasizer®. All the batches were diluted at 1/100 (v/v) in distilled water (filtered over 0.22 μm) prior to the analysis and analyzed in triplicate.

2.4. ^{1}H NMR for the quantitative analysis of Solutol in LNCs formulation

In order to quantify the Solutol concentration in LNCs formulation, a calibration curve was made using the ratio of the characteristic peak integral of Solutol HS15 (3.75–3.50 ppm) corresponding to PEG moieties and the internal standard (TMS in methanol-d4) as a function of the quantity of known Solutol HS15