MEMBRANE ASSOCIATION OF N-OLEOYL-DOPAMINE IN RAT BRAIN

1Department of Respiratory Research, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland; 2Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; 3Faculty of Chemistry, Warsaw University, Warsaw, Poland

N-oleoyl-dopamine (OLDA) belongs to a novel class of bioactive amides of fatty acids. The compound, a lipid derivative of dopamine, holds promise as a potential prodrug or carrier of dopamine into the brain. In this context, a key issue concerning OLDA is the integrity of the compound once it enters the brain. We addressed this issue in the current study by assessing the propensity of OLDA for hydrolysis in rat brain tissue in vitro. The brains were dissected from surgically anesthetized rats after they had been sacrificed by perfusion with physiological saline through the heart. Membrane fractions of brain tissue were isolated and incubated with 1 mmol/l OLDA. Stability of the OLDA molecule was assessed from the spectrophotometric recordings of OLDA spectra in membrane fractions at hourly time points for up to 24 hours. The methodological assumption was that any major change in the shape of the OLDA spectrum would point to a structural, and thus also possibly functional, alteration of the molecule. We found that the OLDA spectrum remained unchanged in the assays for up to 17 h of incubation. We conclude that OLDA strongly resists hydrolysis in brain membrane fractions. The results suggest that dopamine-like biological effects of OLDA might have to do with the interaction of the integral OLDA compound, rather than a dissociated-off dopamine moiety, with the dopaminergic system.

Key words: brain tissue, membrane fraction, N-oleoyl-dopamine, prodrug, rat

INTRODUCTION

N-oleoyl-dopamine (OLDA) is an amide of dopamine and oleic acid (Fig. 1). OLDA is a member of the family of N-acyl-dopamines that are now recognized as novel biologically active lipid derivatives of dopamine. The endogenous presence of these lipid compounds was first predicted by Pokorski and Matysiak
in 1997 (1), and recently confirmed in mammalian brain (2, 3). The in vivo pathways of OLDA synthesis are unsettled. The most probable pathway seems N-acylation of tyrosine by a fatty acid, with tyrosine entering then the normal pathway of dopamine synthesis consisting of hydroxylation by tyrosine hydroxylase and decarboxylation by the aromatic amino acid decarboxylase to form N-acyl-dopamine (2).

A number of biological properties of OLDA have lately been unraveled. The compound is an agonist of the vanilloid type 1 transient receptor potential channel receptor (TRPV1). These receptors are cationic, notably Ca$^{2+}$, channels and thus are engaged in cellular Ca$^{2+}$ influx upon sensory stimulation by heat, protons, and pain (2, 3). That points to the probable role of OLDA in mediating hyperalgesia and inflammatory reactions (4). There are, however, other biological actions of OLDA. It enhances motor behavior in freely moving rats, the action being counteracted by haloperidol, and thus being likely mediated by central dopamine D2-like receptors (5). A central action of OLDA is not incongruous in view of its ability to permeate through the blood-brain barrier in a manner manifold greater than dopamine does (6). Such actions of OLDA alongside its lipophilicity make it an attractive and novel candidate for a prodrug or carrier of dopamine into the brain. In this context, an issue of the stability of the OLDA compound in brain tissue arises. The basic question is of whether and for how long OLDA would stay in brain tissue as an integral compound or whether the dopamine moiety would dissociate off to act on dopamine receptors. We addressed this issue in the current study by assessing the propensity of OLDA for hydrolysis in the membrane fractions of brain homogenates in vitro for up to 24 hours.

**MATERIAL AND METHODS**

The study was approved by a local Ethics Committee. The experiments were performed on brain tissue obtained from 4 male Wistar rats weighing 300-310 g, anesthetized with $\alpha$-chloralose and urethane (35 and 800 mg/kg, ip, respectively). The rats were sacrificed by perfusion with ice-cold 0.9% NaCl through the left heart and the brains were enucleated from the skulls. The basis of the study was in vitro incubation of brain tissue with OLDA and recording changes in its spectrophotometric spectra in the membrane fraction at hourly time points, for up to 24 h (see

![Molecule of N-oleoyl-dopamine.](image)
Membrane fraction preparation

The membrane fraction was prepared according to the method of Xu et al. (7). Briefly, brain tissue was homogenized in the membrane isolation buffer containing 2.5 mM MgCl$_2$, 2 mM Tris x HCl pH 7.4, 3 mM EDTA, 100 µM benzamidine, and 100 µM PMSF. The sample was centrifuged at 2000 x g for 10 min at 4°C. The supernatant was collected and re-centrifuged at 40000 x g at 10°C for 18 min in an L7 Ultracentrifuge (Beckman Instruments, Palo Alto, CA). The pellet, representing the membrane fraction, was washed two times with the isolation buffer. The membrane fraction was dissolved in 1 ml of the isolation buffer. The membrane fractions prepared were kept at -80°C until use.

Stability experiments

At the start of the experiment, membrane fractions were diluted to a volume of 24 ml in Krebs solution, containing 118 mM NaCl, 6 mM KCl, 0.6 mM NaH$_2$PO$_4$, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 25 mM NaHCO$_3$, pH 7.4 adjusted with carbogen, and divided into two equal parts. To one part, OLDA suspended in 2-3 drops of Tween 80 was added, whereas the other part, which served as a control, was supplemented only with the Tween 80. The starting concentration of OLDA was 1 mmol/l. Both solutions were kept stirred at 37°C during the whole experiment.

Half a milliliter sample was taken from both OLDA-loaded and control stock solutions every hour (hours 0-7 and 12-24). The OLDA-loaded sample contained 0.5 µmol of OLDA. Then, 100 µl of 8% trichloroacetic acid was added to pellet proteins and the whole was centrifuged at 2000 x g for 4 min at 4°C. The supernatants were extracted 4 times with 0.25 ml chloroform, the organic phases collected and dried under nitrogen. The aqueous phases were frozen-dried and both phases were analyzed with a Cintra 10e UV/VIS Spectrophotometer equipped with Spectral 1.70 software (GBC Scientific Equipment Pty Ltd., Victoria, Australia). In addition, spectrophotometric measurements of DA alone were performed in the aqueous solution. No trace of DA spectra was detected in these measurements (data not shown).

Thin-Layered Chromatography

The nature of the OLDA compound in the membrane fraction of rat brain tissue also was verified using thin-layered chromatography (TLC). After incubation with OLDA, organic phases of membrane fractions were dissolved in 0.2 ml of chloroform and 50 µl samples, containing now 0.125 µmol of OLDA, were applied alongside the 25 µl OLDA standard, corresponding to 0.006 µmol of OLDA, (dissolved in chloroform, 100 µg/ml) on silica gel 60 Â plates (Merck, Darmstadt, Germany, using chloroform:methanol (95:5, v/v) as solvents. The plates were developed with iodine vapor and the resolved spots were photocopied.

RESULTS

Fig. 2 demonstrates an example of spectrophotometric spectra recorded from membrane fraction solutions incubated with OLDA across several time points ranging from minutes to hours. A typical OLDA spectrum, recorded right after its addition to the membrane fraction (1 min), consisted of an asymmetrical shoulder...
with the nadir and peak invariably at 262 nm and 281 nm, respectively. This shape persisted in an unchanged form for up to 17 h of OLDA incubation with the membrane fraction. Afterward, the OLDA spectrum underwent changes consisting of a gradual leveling off the difference between its maximum and minimum. The OLDA spectrum entirely lost its characteristic shape at 20 h of incubation, which raised the possibility that the molecule changed structurally at this time and had no longer its original functional status. For comparison, a reference spectrum recorded from a membrane fraction solution with no OLDA added showed no presence of the OLDA shoulder.

The 17-h time span of the OLDA compound integrity was confirmed by TLC screening of membrane fractions obtained from rat brain extracts. The R<sub>f</sub> of the OLDA-loaded samples was 0.29, directly corresponded to that of the OLDA standard, and stayed constant for up to 20 h (not shown). Afterward, the OLDA spots were no longer present. Control samples, containing no OLDA, did not show any spots at the level of OLDA bands.

**DISCUSSION**

The main finding of this study was that OLDA, an amide of dopamine and oleic acid, remains stable in the membrane fraction of rat brain tissue for up to 17 hours. The integrity of the OLDA compound was ascertained by its unchanged spectrophotometric UV/VIS absorption spectra in the aforementioned time frame, which was confirmed in a qualitative manner by thin-layered chromatography. The methodological assumption for the spectrophotometric estimation of OLDA integrity was that any major change in the shape of the OLDA spectrum would
point to a structural, and thus possibly functional, alteration of the molecule. Thus, the results of the study indicate that OLDA is capable of entering biomembranes, where it resists hydrolysis for a substantial length of time.

OLDA is a member of the family of N-acyl-dopamines that constitute a novel class of biologically active lipids, called dopamides, a condensation product of long-chain, unsaturated free fatty acids and dopamine at the amino terminal. A number of dopamides have recently been identified as native compounds in mammalian brain (3). Biological properties of different dopamides vary. OLDA, apart from being a potent agonist of TRPV1 receptors, shows apparent dopamine-like activities, such as stimulation of locomotor activity in rats (5). The latter action is most pronounced during the first hour after intraperitoneal administration of OLDA (5). The point of major research interest has arisen of whether dopamine-like actions of OLDA are exerted by the whole integral compound or, may be, dopamine dissociates off from the compound before interacting further with a dopaminergic pathway. Addressing this issue seemed even more warranted in the face of the capability of OLDA to cross the blood-brain barrier (6), which could make it a potential carrier of a hydrophilic dopamine into the brain; the action that might be desirable, but remains still out of reach, in some neurodegenerative brain conditions.

The rate of decomposition of OLDA in tissue has not yet been explored. There are several possible ways in which OLDA could be metabolized after it has penetrated into biomembranes. Fatty acid amide hydrolases (FAAH) are endogenous enzyme proteins that could promptly hydrolyze OLDA. However, dopamides, in general, are known to be pretty resistant to hydrolysis by FAAH. The underlying reason for this resistance may be that dopamides have, in fact, inherent inhibitory activity directed toward FAAH (2). OLDA hydrolysis by FAAH should yield a hydrophilic dopamine as a product. In the present study we could not detect any traces of dopamine in the aqueous cellular fraction (data not shown) either spectrophotometrically or by TLC, which makes OLDA hydrolysis by FAAH unlikely in the time frame studied.

Two other possibly ways of OLDA metabolism are oxidation of the dopamine ring or formation of a complex between the dopamine ring and cations present in the isolation and Krebs solutions. Dopamine is known for its propensity for oxidation giving rise to dopamine quinone (8). If the oxidation reaction of OLDA took place, one or two hydroxyl groups could be oxidized to ketone group(s) yielding N-oleoyl-dopamine semiquinone or quinone, respectively. The reaction between the dopamine ring and cations, such as calcium ions, present in the solution would modify the hydrophobic properties of OLDA by forming a salt and allowing it to shift to the aqueous phase of the extraction procedure, but we failed to detect OLDA in this phase. These chemical reactions are usually fairly rapid, assuredly not of the order of hours, in the case of dopamine proper (8), and had they taken place with OLDA in the present study, their initiation would immediately have produced spectral changes. Therefore, the OLDA molecule is
more stable and resistant to structural chemical alterations than that of dopamine, due either to the presence of the amide bond or being built in the membrane lipid bilayers. The chemical nature of decomposition of the OLDA molecule is unsettled at present and alternative study designs are required to further explore the exact determinants of OLDA stability.

In conclusion, the results of the present study give a consistent impression that OLDA is a stable compound that stays for hours in biomembranes. Therefore, biological effects of OLDA observed shortly after its administration, such as dopamine-like stimulation of motor behaviors (5), would rather depend on the engagement of the integral OLDA compound, and not just its off-dissociated dopamine moiety, in the central dopaminergic pathway.

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REFERENCES


Author’s address: M. Pokorski, Medical Research Center, Polish Academy of Sciences, Pawińskiego 5 St., 02-106 Warsaw, Poland; phone/fax: +48 22 6685416.
E-mail: mpokorski@cmdik.pan.pl