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***Drosophila melanogaster* as a model system to study long-chain fatty acid amide metabolism**

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Abstract

Long-chain fatty acid amides are cell-signaling lipids identified in mammals and, recently, in invertebrates, as well. Many details regarding fatty acid amide metabolism remain unclear. Herein, we demonstrate that *Drosophila melanogaster* is an excellent model system for the study long-chain fatty acid amide metabolism as we have quantified the endogenous levels of *N*-acylglycines, *N*-acyldopamines, *N*-acylethanolamines, and primary fatty acid amides by LC/QTOF-MS. Growth of *Drosophila melanogaster* on media supplemented with [1-¹³C]-palmitate lead to a family of ¹³C-palmitate-labeled fatty acid amides in the fly heads. The [1-¹³C]-palmitate feeding studies provide insight into the biosynthesis of the fatty acid amides.

Keywords

long-chain fatty acid amide; endogenous quantification; *Drosophila melanogaster*; biosynthetic pathway; model system; heavy-labeled precursor

1. Introduction

The long-chain fatty acid amides are a family of bioactive lipids. Members of this family include the *N*-acylethanolamines, the *N*-acyl amino acids, the *N*-acylarylalkylamides, the *N*-monoacylpolyamines, and the primary fatty acid amides. Fatty acid amides have long been important biologically, dating back to 1820's with the identification of hippurate (*N*-benzoylglycine) as a mammalian metabolite of benzoate [1]. The first long-chain fatty acid amide identified from mammals was *N*-arachidonylethanolamine (anandamide), determined to function as the endogenous ligand for the cannabinoid receptors in the brain [2]. The discovery of anandamide and other long-chain *N*-acylethanolamides (NAEs) [3] led to an interest in the fatty acid amides which increased significantly upon the demonstration that oleamide is an endogenous sleep-inducing lipid found in the mammalian brain [4]. The application of modern lipidomic approaches has added to our knowledge about an ever

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growing family of mammalian fatty acid amides [5,6] and a few reports show that these lipids are found in invertebrates, as well [7,8]. Much remains to be uncovered about the pathways leading to the biosynthesis and degradation of the different fatty acid amides [9]. Also, it is not clear if the biosynthetic and degradative pathways of fatty acid amide metabolism are similar or different between vertebrates and invertebrates.

Drosophila melanogaster is an invaluable invertebrate model organism for biomedical research because its genome has been sequenced and it can be genetically manipulated with relative ease. The finding of fatty acid amides in third instar larvae of *D. melanogaster* [8] suggests that *D. melanogaster* (a) could serve as a model system for the investigation of fatty acid amide metabolism and (b) must, at least, possess the enzymatic machinery for fatty acid amide production. Note that orthologs for a few of the mammalian enzymes proposed to serve in fatty acid amide biosynthesis (Fig. 1) have been identified in *Drosophila* [10–13].

The life cycle of *D. melanogaster* progresses through distinct stages: egg, larva, pupa, and adult. We report herein on the identification and quantification of a panel of endogenous long-chain fatty acid amides in the heads and abdomen-thorax of adult *D. melanogaster* by LC/QTOF-MS. Growth of *D. melanogaster* on media supplemented with [1-¹³C]-palmitate leads to the production of ¹³C-palmitamide, ¹³C-palmitoylglycine, and ¹³C-palmitoyldopamine. These data are consistent with the formation of palmitoyl-CoA as a palmitoyl donor to other fatty acid amides. Relevant to this suggestion is our recent description of an *N*-acyltransferase catalyzing long-chain *N*-acylserotonin and *N*-acyldopamine production in *D. melanogaster* [14], acyl-CoA + serotonin (or dopamine) → *N*-acylserotonin (or *N*-acyldopamine) + CoA-SH. In sum, these findings reported herein establish that *D. melanogaster* is a useful and intriguing invertebrate model for the study of long-chain fatty acid amide metabolism.

2. Materials and Methods

2.1. Materials

N-Oleoylglycine, *N*-palmitoylglycine, *N*-linoleoylglycine, *N*-arachidonoylglycine-d₈, *N*-arachidonylethanolamine, *N*-oleylethanolamine, *N*-palmitoyldopamine, *N*-oleoyldopamine, and *N*-arachidonoyldopamine were from Cayman Chemical Company. [1-¹³C]-Palmitic acid, HPLC grade methanol, and HPLC grade acetonitrile were from Sigma-Aldrich. *Drosophila melanogaster* (Oregon R) and 4-24 Instant Medium were from Carolina Biological. Silica was from Suppelco. All other reagents were of the highest quality available from commercial sources.

2.2 Primary fatty acid amide synthesis as standards

The PFAM standards were synthesized as described in Farrell *et al.* [15]. Oleoyl chloride, palmitoyl chloride, palmitoleoyl chloride, or linoleoyl chloride were each added drop-wise to ice-cold concentrated NH₄OH at a ratio of 1:6 (v/v) acyl chloride:NH₄OH. The resulting primary fatty acid amide crystallized from solution, the crystals were collected, and then were washed with H₂O to remove excess NH₄OH.

2.3 *Drosophila melanogaster* culture

D. melanogaster were maintained on 4-24 Instant Medium at room temperature. During the [1-¹³C]-palmitic acid feeding study, *D. melanogaster* were reared in plastic tubes with 2 mL of 4-24 Instant Medium supplemented with an equal volume of 1.0 mg/mL [1-¹³C]-palmitic acid (dissolved in H₂O). After 5 days, *D. melanogaster* were collected by immobilizing them with ice, flash frozen, and shaken vigorously to detach the head from the thorax-abdomen. The heads were separated from thorax-abdomens by sifting them through a wire mesh.

2.4 Extraction of long-chain fatty acid amides

The long-chain fatty acid amides were extracted using a method slightly modified from that described by Farrell *et al.* [15]. *D. melanogaster* heads or thorax-abdomen in 1.0 g batches were ground in a mortar with 30 mL of methanol and the resulting paste was sonicated for 15 minutes on ice. Cellular debris was removed by centrifugation and the supernatant was dried under N₂ at 40°C. The pellet was re-extracted with 30 mL of 1:1:0.1 (v/v/v) chloroform:methanol:water followed by sonication for 10 minutes on ice. The supernatant was collected by centrifugation and added to the dried supernatant from the first extraction. The resulting combination dried under N₂ at 40°C. The pellet was re-extracted for a third time with a 41 mL solution prepared by mixing together 36 mL of 2:1 (v/v) chloroform:methanol and 5 mL of 0.5 M KCl/0.8 M H₃PO₄, followed by sonication for 2 minutes on ice. After vigorously mixing the pellet with this solution for 2 minutes using a vortex, the mixture centrifuged to create a phase separation, the lower lipid phase removed, and then added to the dried mixture of first and second extractions. The combination of the 3 extractions was dried under N₂ at 40°C.

The solid-phase extraction method of Farrell *et al.* [15] was used to further purify the fatty acid amides from the dried lipid extracts. The dried lipid extract was dissolved in 200 µL of *n*-hexane and dissolved lipids were loaded onto 0.5 g of *n*-hexane-washed silica. The mobile phase was run as follows: 4 mL of *n*-hexane, 1 mL of 99:1 (v/v) hexane:acetic acid, 1 mL of 90:10 (v/v) hexane:ethyl acetate, 1 mL of 80:20 (v/v) hexane:ethyl acetate, 1 mL of 70:30 (v/v) hexane:ethyl acetate, 1.5 mL of 2:1 (v/v) chloroform:isopropanol, and ending with 1 mL of methanol. Fractions were collected from each mobile phase solution. The fatty acid amides were contained in three fractions: the 70:30 (v/v) hexane:ethyl acetate fraction, 2:1 (v/v) chloroform:isopropanol fraction, and methanol fraction. These three fatty acid amide-containing fractions were combined, dried under N₂ at 40°C, and then stored at -20°C until analyzed in more detail. The lipids from 1.0 g of *Drosophila* media were extracted as described above to ensure that the *Drosophila* were not being exposed to arachidonic acid via the media.

2.6 Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry

The dried extracts were reconstituted in HPLC grade methanol and spiked with an internal standard, 10 pmoles of *N*-arachidonoylglycine-d₈. An Agilent 1260 liquid chromatography system with a Kinetex™ 2.6 µm C18 100 Å (50 × 2.1 mm) reverse phase column was used for the separation. Mobile phase A consisted of water with 0.1% formic acid and mobile phase B consisted of acetonitrile with 0.1% formic acid. The gradient started at 10% B and

was linearly increased to 100% B over the course of 5 minutes followed by a hold of 3 minutes at 100% B. Equilibrium was achieved by holding at 10% B for 8 minutes. The flow rate was 0.6 mL/min and each injection was 10 μ L. The liquid chromatography system was connected to an Agilent 6540 Quadrupole Time-of-Flight Mass Spectrometer with a Dual Agilent Jetstream ESI source in positive ion mode; data were collected at an acquisition rate of 2 spectra/s in the extended dynamic range mode (2 GHz). The capillary voltage was set to 3.5 kV; the drying gas temperature was set to 300 °C with a gas flow rate of 8 L/min; the sheath gas temperature was set to 350 °C with a gas flow rate of 11 L/min; and the nebulizer pressure was set to 35 psig. For the LC-MS/MS analysis of anandamide, collision energy was set to 20 eV.

2.7 Data analysis

The extractions for both *D. melanogaster* heads and thorax-abdomens were run in triplicate on LC/QTOF-MS, with 3 total ion chromatograms (TICs) for each determination. Extractions from heads of 5 separate *D. melanogaster* cultures (5 determinations), extractions from thorax-abdomens of 4 separate cultures (4 determinations), and [1-¹³C]-palmitic acid incubation extractions from heads (3 determinations) were analyzed. Extracted ion chromatograms (EICs) were obtained from the TICs for each of the long-chain fatty acid amides using Agilent MassHunter Qualitative Analysis B.04.00. The amides in each extraction were identified by comparison to synthetic standards by molecular ion *m/z* and retention time. Retention times for all metabolites varied by ± 0.1 minutes from run to run. For validation of anandamide in head or thorax-abdomen extracts, 100–200 pmoles of synthetic anandamide was spiked into the extract samples and reanalyzed.

Quantification of the identified fatty acid amides was performed by integrating the area under the chromatographic peak and comparing that value against standard curves constructed using the same fatty acid amide. The amount of each fatty acid amide in the sample was quantified along with the internal standard, *N*-arachidonoylglycine-d₈. Standard curves were in the linear range of 1 – 200 pmoles on the column ($r^2 > 0.99$). *N*-Arachidonoylglycine-d₈, 1 pmole per 10 μ L injection, was spiked into each extraction to measure instrument performance and for data normalization. Solvent and slip additive blanks were run to evaluate background PFAM levels and the background levels were subtracted from the analyte levels. For palmitamide, background levels were 13–20% of the endogenous levels in *D. melanogaster* heads. For oleamide, background levels were 7–11% of the endogenous levels in *D. melanogaster* heads. For palmitamide and oleamide, background levels were 100% of the endogenous levels in *D. melanogaster* thorax-abdomen, indicating that these primary fatty acid amides were not present endogenously in the thorax-abdomen. The amounts of endogenous fatty acid amides in *D. melanogaster* heads and thorax-abdomens were reported as the average of the determinations for each anatomical location along with the standard deviation. The levels of the *N*-acyldopamine reported herein represent the sum total of the amount of the *N*-acyldopamine and the amount of the *N*-acyldopamine quinone. The *N*-acyldopamine quinones form spontaneously by the air oxidation of the *N*-acyldopamines [16]. The amounts of fatty acid amides in the heads after [1-¹³C]-palmitic acid feeding were reported as the average and standard deviation for 3 determinations

3. Results and Discussion

3.1 Identification and quantification of endogenous long-chain fatty acid amides in *D. melanogaster*

A panel of endogenous long-chain fatty acid amides was identified in *D. melanogaster* heads and thorax-abdomens by the comparison of molecular ion m/z values and retention times against synthetic standards (Table 1). The chromatographic peaks and mass spectra of a synthetic standard, palmitamide, matched those of endogenous palmitamide identified in the *D. melanogaster* head extract (Fig. 2). These data are representative of the data collected for each fatty acid amide identified in the *D. melanogaster* extracts. Endogenous *N*-acylglycines and *N*-acylethanolamines were identified in both the heads and the thorax-abdomens while the primary fatty acid amides were identified only in the fly heads. These data are consistent with reports of the primary fatty acid amides from either the mammalian brain [4,17–19] or cultured neuroblastoma cells [15,20]. The *N*-acyldopamines were identified by the accurate masses of the *N*-acyldopamines and their oxidized form, the *N*-acyldopamine quinones, as the *N*-acyldopamines can be oxidized during the extraction process. The anatomical localization of the *N*-acyldopamines was dependent on the acyl chain, as *N*-palmitoyldopamine was identified in both the heads and thorax-abdomens, *N*-oleoyldopamine was in the thorax-abdomen, and *N*-arachidonoyldopamine was in the head. Differences in the panel of endogenous fatty acid amides between the head and the thorax-abdomen (Table 2) likely result from variations in biosynthesis, degradation, transport, or some combination of these factors between these anatomical regions in the flies.

The endogenous levels of fatty acid amides in *D. melanogaster* heads are on the same order of magnitude as endogenous levels of fatty acid amides quantified from mammalian systems [17,21,22]. The endogenous levels of fatty acid amides in *D. melanogaster* heads range from 7.3 ± 4.9 pmoles g^{-1} head to 500 ± 300 pmoles g^{-1} head (Table 2). The levels in *D. melanogaster* thorax-abdomens are 10-fold lower than what we measured in the heads, ranging from 0.75 ± 0.45 pmoles g^{-1} thorax-abdomen for *N*-palmitoyldopamine to 19 ± 11 pmoles g^{-1} thorax-abdomen for anandamide. Consistent with these data, Tortoriello *et al.* [8] reported the quantification of *N*-linoleoylglycine in *D. melanogaster* third instar larvae to be ~ 13 pmoles g^{-1} tissue.

3.2 Eicosanoids and Other Long-chain (> C_{18}) Fatty Acids in *Drosophila*

Arachidonoyl-conjugates, anandamide and *N*-arachidonoyldopamine, were identified in *D. melanogaster* extracts (Table 1). Anandamide was identified in the head and thorax-abdomen extracts (supplementary material, Fig. S1) while *N*-arachidonoyldopamine was only identified in the head extracts. The head and thorax-abdomen extract samples were spiked with synthetic anandamide and reanalyzed. An increase in the integration area of the identified anandamide peak provides additional validation for the identification of anandamide in the head and thorax-abdomen (supplementary material, Table S1). LC-MS/MS analysis of the anandamide parent ion from a synthetic standard and the thorax-abdomen extract yielded product ion scans with the expected fragment ion for *N*-acylethanolamines, 62.1 (supplementary material, Fig. S2). This result provides additional validation to our identification of anandamide in the *D. melanogaster* extracts. The

identification of these eicosanoids is of note since anandamide was not identified in *D. melanogaster* third instar larvae [8] and arachidonic acid and related metabolites were not identified in the *Drosophila* media (data not shown).

The presence of the fatty acids (or their metabolites) with acyl chains >18 carbon atoms is controversial in the literature. There are reports of trace levels of C₂₀ and longer acyl chain-fatty acids in *Drosophila* [23–27] and there are reports that arachidonates cannot be identified in *Drosophila* [8,27–31]. A reasonable assessment of the reported data on arachidonates, other long-chain fatty acids, and their related metabolites is the following: (a) long-chain (>18 carbon atoms in the acyl chain) are present in *Drosophila* at low levels and are, thus, difficult to detect, (b) arachidonates, other long-chain fatty acids, and their related metabolites cannot be produced endogenously in *Drosophila* from shorter-chain fatty acids [28,31], (c) arachidonates and other long-chain fatty acids can be assimilated into *Drosophila* lipids directly from dietary sources, and (d) arachidonates, other long-chain fatty acids, and their related metabolites found in *Drosophila* can be derived by the metabolism of even longer-chain (>22 carbon atoms) fatty acids [31]. Another consideration regarding the presence of long-chain fatty acids in *Drosophila* is potential variations in the cellular levels of these compounds in different tissues [Table 2 and ref. 14], at different stages of development [24,32,33], and due to differences in their diet [29,31,34], further complicating reports of these trace metabolites. Similar conclusions regarding presence of C₂₀ fatty acids and their metabolites were reached by Vrablik and Watts in a recent review [35]. Our identification of anandamide and *N*-arachidonoyldopamine coupled to previous reports of trace levels of C₂₀-, C₂₂-, and C₂₄-containing ceramides [25,27], *N*-arachidonoylserotonin [14], *N*-arachidonoylglycerol [26] in *Drosophila* indicate that these lipids probably result from the enzymatic machinery of *Drosophila* incorporating dietary long-chain fatty acids into the normal lipid flux of the flies. Additional research is necessary to determine if such long-chain acyl-containing compounds serve an important functional role in *Drosophila*.

3.3 Analysis of long-chain fatty acid amides from 1-¹³C-palmitic acid

D. melanogaster were reared on media supplemented with [1-¹³C]-palmitic acid to investigate if heavy-labeled precursor feeding is feasible in this model system, as this would aid in future studies of fatty acid amide metabolic flow. After a 5-day exposure to [1-¹³C]-palmitic acid, the lipids were extracted from *D. melanogaster* heads to analyze for fatty acid amides that contain the ¹³C-*N*-palmitoyl chain as well as the other unlabeled metabolites. Excitingly, ¹³C-*N*-palmitoylglycine, ¹³C-*N*-palmitamide, and ¹³C-*N*-palmitoyldopamine were identified in the head extracts, showing that these metabolites are synthesized by the flies from the exogenous [1-¹³C]-palmitic acid (Table 3). Similar to the endogenous analysis of the *N*-acyldopamines, the levels of ¹³C-*N*-palmitoyldopamine and ¹³C-*N*-palmitoyldopamine quinone were combined and represented as a total amount for ¹³C-palmitoyldopamine. The total palmitamide (¹³C-labeled and unlabeled) in the *D. melanogaster* heads that were grown in the presence of [1-¹³C]-palmitic acid was ~150 pmoles g⁻¹, consistent with the endogenous amount of palmitamide reported, 130 ± 80 pmoles g⁻¹. A similar trend was also observed for *N*-palmitoyldopamine.

4. Conclusion

D. melanogaster is an innovative model system to study long-chain fatty acid amide biosynthesis as the most of the previous work has been investigated in mammalian systems. A panel of long-chain fatty acid amides was identified and quantified in two anatomical regions of *D. melanogaster*, the head and thorax-abdomen. The higher steady-state concentrations of fatty acid amides in the *D. melanogaster* heads are consistent with the known biological functions of these lipids in mammalian systems; however, the biological relevance of these molecules in insects has yet to be determined. Based on homology studies, *D. melanogaster* do not express the cannabinoid receptors [26], leading to an intriguing question about the role these fatty acid amides, especially anandamide, serve in this insect. One possibility could be that *Drosophila* and other insects possess cannabinoid-like receptors that have little homology to their mammalian counterparts.

The presence of endogenous long-chain fatty acid amides in *D. melanogaster* indicates that the enzymatic machinery for their biosynthesis and degradation must be expressed in these organisms. In addition, these data further point towards *Drosophila* as a model system for fatty acid amide metabolism orthogonal to the mouse neuroblastoma N₁₈TG₂ cells. Mouse neuroblastoma N₁₈TG₂ cells produce a number of different fatty acid amides and proven valuable in study of these lipid amides [15,36,37]. Our demonstration that flies fed a diet supplemented with [1-¹³C]-palmitic acid led to the formation of ¹³C-*N*-palmitoyldopamine, ¹³C-*N*-palmitoylglycine, and ¹³C-palmitamide is consistent with, but does not prove, that palmitoyl-CoA is a central palmitoyl donor in the biosynthesis of the different classes of palmitoylated fatty acid amides. We have recently identified and characterized an enzyme from *D. melanogaster* that catalyzes the formation of long-chain *N*-acylalkylamides, notably the *N*-acylserotonins and the *N*-acyldopamines, from the corresponding long-chain acyl-CoA thioesters and alkylamine: acyl-CoA + serotonin (or dopamine) → *N*-acylserotonin (or *N*-acyldopamine) + CoA [14]. Importantly, we also found that the expression pattern of this enzyme in *D. melanogaster* co-localized to the tissues containing measurable levels of the endogenous long-chain *N*-acylserotonins. These data provide evidence for the central role served by the acyl-CoA thioesters in fatty acid amide biosynthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATP adenosine triphosphate

CoA	coenzyme A
EIC	extracted ion chromatogram
FAAH	fatty acid amide hydrolase
HPLC	high performance liquid chromatography
LC/QTOF-MS	liquid chromatography/quadrupole time-of-flight mass spectrometry
NAE	<i>N</i> -acylethanolamine
PFAM	primary fatty acid amide
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
PAL	peptidyl- α -hydroxyglycine- α -amidating lyase
PAM	peptidylglycine α -amidating monooxygenase
PHM	peptidylglycine- α -hydroxylating monooxygenase
TIC	total ion chromatogram

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Highlights

- Endogenous long-chain fatty acid amides were identified in *D. melanogaster*.
- Long-chain fatty acid amides were quantified in *D. melanogaster* using LC/QTOF-MS.
- Heavy-labeled precursors were fed to *D. melanogaster*.
- A model system for studying the biosynthesis of fatty acid amides was established.

Fig. 1.

Proposed pathways for the biosynthesis of the *N*-acylglycines, primary fatty acid amides, and *N*-acyldopamines. Other possible biosynthetic reactions are discussed in the review by Farrell and Merkler [9]. The putative enzymes are in blue boxes. The red carbons represent the metabolic flow the 1-¹³C atom based on data reported herein.

Fig. 2.

Identification of palmitamide in *D. melanogaster* heads by LC/QTOF-MS. The EIC peak and mass spectrum of a synthetic standard, palmitamide, (Panel A) matched those of endogenous palmitamide identified in the *D. melanogaster* head extract (Panel B). These data are representative of the data collected for each long-chain fatty acid amide identified in *D. melanogaster* head and thorax-abdomen extracts.

Table 1
Identification of endogenous long-chain fatty acid amides in *D. melanogaster* heads and thorax-abdomen by LC/QTOF-MS

Fatty Acid Amide	Standard		<i>D. melanogaster</i> head		<i>D. melanogaster</i> thorax-abdomen	
	[M+H] ⁺ m/z	Retention Time (minutes)	[M+H] ⁺ m/z	Retention Time (minutes)	[M+H] ⁺ m/z	Retention Time (minutes)
<i>N</i> -Acylglycines						
<i>N</i> -Palmitoylglycine	314.2704	5.839	314.2704	5.857	314.2668	5.842
<i>N</i> -Oleoylglycine	340.2859	6.040	340.2808	5.858	340.2863	6.099
<i>N</i> -Linoleoylglycine	338.2697	5.814	338.2697	5.865	338.3030	5.766
<i>N</i> -Acylethanolamines						
<i>N</i> -Oleylethanolamine	326.3065	6.017	326.3066	6.099	326.3053	5.957
<i>N</i> -Arachidonylethanolamine	348.2903	5.659	348.2904	5.611	348.2527	5.580
Primary Fatty Acid Amides						
Palmitamide	256.2635	6.033	256.2634	6.050	n.d. ^a	n.d. ^a
Palmitoleamide	254.2487	5.802	254.2487	5.856	n.d. ^a	n.d. ^a
Oleamide	282.2795	6.089	282.2796	6.102	n.d. ^a	n.d. ^a
Linoleamide	280.2637	5.922	280.2636	5.990	n.d. ^a	n.d. ^a
<i>N</i> -Acyl dopamines ^c						
<i>N</i> -Palmitoyldopamine	392.3161	5.984	392.3161	6.066	392.3139	5.922
<i>N</i> -Palmitoyldopamine quinone	390.3003	6.129	390.3057	6.108	390.2779	6.080
<i>N</i> -Oleoyldopamine	418.3527	6.101	n.d. ^b	n.d. ^b	418.3063	6.138
<i>N</i> -Oleoyldopamine quinone	416.2676	6.209	n.d. ^b	n.d. ^b	416.3062	6.221
<i>N</i> -Arachidonoyldopamine	440.3171	5.934	440.3565	6.110	n.d. ^a	n.d. ^a
<i>N</i> -Arachidonoyldopamine quinone	438.3010	6.017	438.3803	6.160	n.d. ^a	n.d. ^a

^a n.d. indicates “not detected” in one gram of *D. melanogaster* thorax-abdomen.
^b n.d. indicates “not detected” in one gram of *D. melanogaster* head.

Table 2Quantification of endogenous long-chain fatty acid amides in *D. melanogaster* heads and thorax-abdomen^a

Fatty Acid Amide	Endogenous Amount	
	pmoles g ⁻¹ head ^a	pmoles g ⁻¹ thorax-abdomen ^b
<i>N</i> -Palmitoylglycine	70 ± 43	5.4 ± 3.5
<i>N</i> -Oleoylglycine	500 ± 300	14 ± 8.3
<i>N</i> -Linoleoylglycine	180 ± 160	3.1 ± 2.4
<i>N</i> -Oleylethanolamine	49 ± 29	1.5 ± 0.48
<i>N</i> -Arachidonylethanolamine	100 ± 97	19 ± 11
Palmitamide	130 ± 79	n.d. ^c
Palmitoleamide	7.3 ± 4.9	n.d. ^c
Oleamide	130 ± 38	n.d. ^c
Linoleamide	20 ± 12	n.d. ^c
<i>N</i> -Palmitoyldopamine	15 ± 13	0.75 ± 0.45
<i>N</i> -Oleoyldopamine	n.d. ^d	1.1 ± 0.47
<i>N</i> -Arachidonoyldopamine	96 ± 36	n.d. ^c

^a Amounts reported as average ± SD from 5 determinations.^b Amounts reported as average ± SD from 4 determinations.^c n.d. indicates “not detected” in one gram of *D. melanogaster* thorax-abdomen.^d n.d. indicates “not detected” in one gram of *D. melanogaster* head.

Table 3Amounts of long-chain fatty acid amides in *D. melanogaster* heads after 1-¹³C-Palmitic acid feeding

Fatty Acid Amide	Amount ^a (pmoles g ⁻¹ head)
<i>N</i> -Palmitoylglycine	130 ± 84
¹³ C- <i>N</i> -Palmitoylglycine	240 ± 13
<i>N</i> -Oleoylglycine	420 ± 340
<i>N</i> -Linoleoylglycine	120 ± 91
<i>N</i> -Oleoylethanolamine	16 ± 4.6
<i>N</i> -Arachidonoylethanolamine	38 ± 30
Palmitamide	140 ± 110
¹³ C-Palmitamide	16 ± 0.84
Palmitoleamide	29 ± 24
Oleamide	160 ± 90
Linoleamide	8.9 ± 8.7
<i>N</i> -Palmitoyldopamine	4.7 ± 3.0
¹³ C- <i>N</i> -Palmitoyldopamine	22 ± 19
<i>N</i> -Oleoyldopamine	n.d. ^b
<i>N</i> -Arachidonoyldopamine	24 ± 21
Total <i>N</i> -Palmitoylglycine ^c	370 ± 85
Total Palmitamide ^c	160 ± 110
Total <i>N</i> -Palmitoyldopamine ^c	27 ± 19

^a Amounts reported as average ± SD from 3 determinations.^b n.d. indicates “not detected” in one gram of *D. melanogaster* head.^c Amounts reported as the total of the ¹³C-labeled plus the unlabeled amount of the *N*-palmitoylamides.