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Evidence for the contribution of multiple mechanisms in the feeding pattern of rats exposed to *p*-chloro-diphenyl diselenide-supplemented diets

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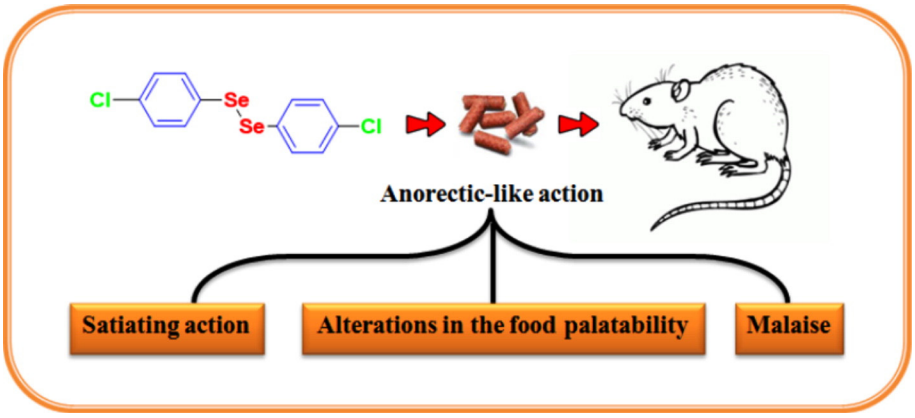
HIGHLIGHTS

- $(p\text{-ClPhSe})_2$ induces an anorexigenic action in rats through multiple mechanisms.
- At first contact, $(p\text{-ClPhSe})_2$ causes a satiating action.
- Rats re-exposed to $(p\text{-ClPhSe})_2$ develop aversive reactions, indicative of malaise.
- A specific flavor of $(p\text{-ClPhSe})_2$ also contributes to its anorexigenic action.

GRAPHICAL ABSTRACT



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ABSTRACT

Preliminary findings suggest that food intake reduction induced by *p*-chloro-diphenyl diselenide [$(p\text{-ClPhSe})_2$] in rats is mediated by a satiating action; however, additional experiments are necessary to clarify its actions. The purpose of this study was to investigate the effects of diets supplemented with $(p\text{-ClPhSe})_2$ on feeding behavior of rats as well as the $(p\text{-ClPhSe})_2$ effectiveness in producing aversive reactions or specific flavor. The results demonstrated that behavioral satiety sequence (BSS) was preserved in animals exposed to $(p\text{-ClPhSe})_2$-supplemented diets (0.01 and 0.1%) and associated with a shift of the onset of resting to the left indicating a satiating action at the first contact. In addition, the frequency, the mean duration and the mean size of meals were decreased in rats exposed to a 0.1% $(p\text{-ClPhSe})_2$ diet. Alternatively, a second contact with a 0.01% $(p\text{-ClPhSe})_2$ diet caused disruption of BSS and pronounced changes in the meal pattern, suggesting that it produces aversiveness. In fact, rats developed a significant taste aversion to the saccharin solution after receiving the administration of $(p\text{-ClPhSe})_2$ (1 and 10 mg/kg; i.p.). Lastly, a diet containing 0.1% of $(p\text{-ClPhSe})_2$ seems to alter the palatability of food given that rats had a preference for the control diet. The findings of the present

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study suggest that $(p\text{-ClPhSe})_2$ reduced the food intake of rats by inducing a satiating action at the first contact, but it also produced aversive reactions when rats were re-exposed to it. A specific flavor seems also to contribute to $(p\text{-ClPhSe})_2$ suppressant effects on feeding.

1. Introduction

The increasing prevalence of obesity is a worldwide phenomenon, affecting people from diverse cultural and economic background [1]. Unfortunately, many new agents that were heralded as the answer to the obesity problem were hastily withdrawn owing to unacceptable side-effect burden [2]. Since existing pharmaceuticals fail to come up with long-term solutions to address the obesity and obesity-related complications, there is an ever-pressing need to find and develop new drugs and alternatives [3]. In this way, selenium (Se) is an important element for maintenance of overall health, especially for the thyroid, immunity and homeostasis and its multifaceted aspects have attracted worldwide clinical and research interest [4]. The thyroid hormone and energy metabolism can be modulated by dietary Se intake with consequent body weight loss in humans [5]. However, the biological narrative of Se has been marked by a contrast between its toxic and its beneficial effects [6]. In general, organoselenium compounds have substantially greater bioavailability and are usually found to be less toxic than that of inorganic Se [7].

It has been demonstrated that intraperitoneal (i.p.) administration of the organoselenium compounds diphenyl diselenide [$(PhSe)_2$] and *p*-chloro-diphenyl diselenide [$(p\text{-ClPhSe})_2$] plays anorectic-like actions in rats resulting in body weight loss. The food intake suppressant effects displayed by diselenides are linked to changes in the microstructure of the feeding pattern as well as the preservation of the behavioral satiety sequence (BSS) and an earlier-than-normal onset of satiety, without major apparent toxic effects [8]. Despite that, the role of the diselenides in appetite has not been fully determined and thus, further investigations about the action mechanisms for diselenides are essential to elucidate their anorexigenic property in rats. Moreover, additional toxicological studies are necessary to establish these compounds as potential anorexigenic drugs given that their toxic properties have also been cited [6,9].

The suppression of eating can occur by activation of the physiological process (satiety and satiation), or by producing physiologic disturbances which induce illness (pain or discomfort), or by altering the palatability of food [10]. The anorexigenic effects of the i.p. administration of diselenides in rats seem to be closely related to a satiating action that could be partially explained by the inhibition of hypothalamic serotonin uptake [8]. In fact, serotonin has been known to play an important role in appetite and food intake regulation [11]. However, the use of a different route of administration as well as experiments designed to investigate if the diselenides produce aversive reactions or alter the palatability of food are suitable to obtain more comprehension about their anorectic-like actions.

Therefore, the aim of the present study was to investigate the effects of diets supplemented with different concentrations of $(p\text{-ClPhSe})_2$ on feeding behavior of rats as well as the $(p\text{-ClPhSe})_2$ effectiveness in producing aversive reactions or specific flavor.

2. Material and methods

2.1. Animals

The experiments were carried out using male adult Wistar rats (200–250 g) from our own breeding colony. The animals were housed in cages (41 × 34 × 16 cm, 5 rats per cage) with free access to food and water. They were kept in a separate animal room, on a 12-h light/12-h dark cycle; the lights were turned on every day at 7:00 a.m., in a controlled temperature environment (22 ± 2 °C). A commercial diet (Guaiba, RS, Brazil) and tap water were supplied *ad libitum*. The present experimental study was approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria, RS, Brazil and registered under the number 050/2012. The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. The rats were acclimated to the experimental room before the procedures.

2.2. Chemicals

$(PhSe)_2$ and $(p\text{-ClPhSe})_2$ were prepared and characterized based on a previous study carried out by Paulmier [12]. ^1H and ^{13}C Nuclear Magnetic Resonance Spectroscopy analysis showed analytical and spectroscopic agreement with the assigned structures. The chemical purity of these organoselenium compounds (99.9%) was determined by gas chromatography–mass spectrometry (Shimadzu QP2010PLUS GC/MS combination). Quinine was obtained from Sigma (St. Louis, MO, USA). For experimental studies, $(PhSe)_2$ and $(p\text{-ClPhSe})_2$ were mixed with the commercial diet or dissolved in mineral oil (1 ml/kg).

2.3. Repeated exposure to a diet supplemented with $(PhSe)_2$ or $(p\text{-ClPhSe})_2$

During 3 days before starting the test, animals were placed in individual home cages (30 × 19 × 13 cm) and baseline measures of food intake and body weight were carried out in order to designate rats to experimental groups.

The commercial diet was powdered in a blender to obtain a powdered diet. Each diselenide was weighed and gradually incorporated to the powdered diet by constant mixing to obtain a homogeneous mixture. Then, water was added to this mixture in order to obtain a mash. The mash was manually shaped in pellets which were dried with laboratory oven. Regarding the control diet, the same procedure was carried out but in the absence of the compounds. The experiments were started by testing the concentration of 0.1% of diselenides and given that this concentration caused an expressive food intake inhibition, lower concentrations were also selected for testing. For the test, sated rats (n = 8/group) were exposed to a diet supplemented with $(PhSe)_2$ or $(p\text{-ClPhSe})_2$ (0.001–0.1%) for 7 consecutive days. The food intake during the dark phase (when rats search for food) and body weight were daily checked. After, the animals were decapitated under isoflurane anesthesia and the epididymal fat content was removed and weighed.

Taking into account that the effects of both diselenides were similar and studies have shown that the introduction of a functional group (chloro) in the aromatic ring of $(PhSe)_2$ did not change [13] or reduced [14,15] the toxicity of this compound, $(p\text{-ClPhSe})_2$ at effective concentrations was chosen for subsequent experiments.

2.4. Food intake pattern: microstructural analysis of feeding behavior and behavioral satiety sequence (BSS)

Feeding behavior analysis was carried out in an acrylic cage (40 × 35 × 25 cm) with a feeder connected at the center of the cage. A hydrated mash was chosen as the diet source and prepared freshly each morning from the commercial diet (1 g of powdered diet in 2 ml of water). Experiments were performed between 9:00 and 11:00 a.m. for four consecutive days. During the first day rats were adapted to the observation cage and to the new type of food. For the next two days, animals were