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Effect of early exposure to sucralose on fluid intake in rats

Efecto de la exposición temprana a la sucralosa sobre el consumo de líquidos en ratas

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Abstract

Experimental evidence has demonstrated the effect of exposure history on feeding behavior in organisms. However, it is reported that sucralose is not adequately consumed by the rats.The aim of this study was to evaluate effect of early exposure to sucralose in subsequent fluid intake in Wistar rats. Twenty rats were assigned into two groups, A and S. The S group was exposed to sucralose from nursing, since her mother drank a solution with sucralose during this stage. Group A was exposed to water. At 90 days of age both groups received a solution with sucralose for 10 days, followed by 10 days in which they returned to initial conditions (A, water and S, sucralose). Later both groups received water for 10 days, ending the experiment with a return to initial conditions for 10 days. The results showed a differential effect in sucralose and water consumption with respect to sex. In particular females consumed more sucralose than males. However, experimental group exposed to it early sucralose showed a higher consumption compared with the group exposed to water. These results showed that exposure to sucralose flavor during lactation influences the fluids habitually consumed by females, also showed differential effects with respect to sex. Animals exposed to sucralose from nursing showed an increase in sucralose consumption after water intake. This evidence demonstrates the role that early exposure to specific flavors in the pattern of consumption in adulthood. Additionally, it is possible to point out the importance of learning at an early age as a tool to prevent the development of food pathologies.

Resumen

La evidencia experimental ha demostrado el efecto de la historia de exposición sobre el comportamiento de alimentación en los organismos. Sin embargo, se ha reportado que la sucralosa no es consumida por las ratas. El objetivo de este trabajo fue evaluar el efecto de la exposición temprana a la sucralosa en el consumo de líquidos de ratas de la cepa Wistar. Veinte ratas de la cepa Wistar se dividieron en dos grupos: A y S. El grupo S, estuvo expuesto a la sucralosa desde la lactancia, ya que su madre tomó una solución con sucralosa en esa etapa. El grupo A estuvo expuesto a agua. A los 90 días de edad ambos grupos recibieron como bebida una solución con sucralosa por 10 días, seguido de 10 días en los que retornaron a condiciones iniciales (A, agua y S, sucralosa). Posteriormente recibieron como bebida agua por 10 días, finalizando el experimento con un retorno a condiciones iniciales por 10 días. Los resultados mostraron un efecto diferencial en el consumo de sucralosa y agua con respecto al género. En particular, las hembras consumieron más sucralosa que los machos. Sin embargo, el grupo experimental expuesto a la sucralosa mostró un consumo más alto en comparación con el grupo expuesto al agua. Estos resultados mostraron que la exposición al sabor de la sucralosa desde la lactancia, influyó en el consumo habitual de líquidos en las hembras. Los animales expuestos a la sucralosa desde la lactancia mostraron un aumento en el consumo de sucralosa después de la ingesta de agua. Esta evidencia demuestra el papel que tiene la exposición temprana a sabores específicos en los patrones de consumo en la edad adulta. Además, es posible señalar la importancia del aprendizaje a una edad temprana como una herramienta para prevenir el desarrollo de patologías alimentarias.

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Introduction

Characteristics of foods such as flavor, smell and texture provide the animals with important information. From these information organisms determines if a food should be consumed or avoided, responding to taste elements based on innate and learned preferences, and also, animals learn to avoid foods with toxic effects and prefer those that provide nutritional benefits (Sclafani, 1990). Many species are genetically predisposed to prefer sweet flavors, since sweet foods are a safe source of energy and nutrients. Therefore, the adaptive evolutionary development has resulted in a preference for sweet foods (Keskitalo et al., 2007). However, even when there is an innate preference to sweet flavor, the contextualization of it occurs and begins to develop as soon as sweet flavor is provided and is experienced (Booth, 1994).

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Several authors have studied the effect of different sweeteners in food and water intake in animal models. Martínez, López-Espinoza, Díaz and Valdés (2009) reported a gradual increase on glucose sweetened solution intake, whereas sucralose solution intake remained stable. Conversely, Kenney and Collier (1976) reported an increase in saccharine consumption in comparison to water intake. When presented simultaneously with a sucrose solution, a saccharine solution, and water, rats consumed twice as much liquid and saccharine solution intake represented 60% of total consumption, sucrose solution 35.3%, and water 4.5%. The authors concluded that saccharine and sucrose solutions are independent to each other and that sucrose solution consumption does not depend only of sweet flavor.

Torres-González et al. (2009) evaluated the effect of stress on water, sucralose solution and glucose solution consumption. As in the water consumption condition, authors did not find any changes in sucralose solution during the exposure to stress compared to no-stress phases, while an increase in glucose solution intake was reported during exposure to stress. Bello and Hajnal (2005) analyzed the effect of water or sucralose consumption on eating behavior of male rats, varying sweetener concentration. Authors reported that at low quantities the rats did not prefer sucralose, whereas at high concentrations they avoided it. On the other hand, Sclafani y Clare (2004) showed bimodal differences in the preference and aversion for sucralose in rats, their reports indicate that about half of the rats preferred sucralose in comparison to water, whereas the rest of them avoided sucralose. These authors pointed out that sucralose seem to be less palatable in comparison to other sweeteners. However, there are modifications in sucralose preference depending on the purity of this sweetener. It has been reported that sucralose combined with maltodextrine (Splenda) increases the palatability of solution and decreases aversion to taste of sweetener (Dess, Chapman y Monroe, 2009).

A common trait among these experimental findings is the use of rats that were not previously exposed to sucralose. However, influence of prior exposure, preferences and aversions to sucralose sweetened drinks has not been sufficiently explored. García and Bach (1999) pointed out that the exposure is one of the most powerful influences to develop most food preferences. To illustrate this connection between exposure and food preference, Beauchamp and Moran (1984) pointed out that two years old children who were fed with sweetened water during the first months of their lives showed a higher preference towards sweetened water when compared to those which had little or no exposure to it.

Nevertheless, two aspects which should be considered in the procedures to exposing the sweetener are age and gender of subjects. Provenza (1994) indicated that age at moment to exposure to foods influences in development of dietary habits in ruminants, there is a sensitive period for learning and development of food preferences which corresponds to weaning. This seems to be biologically reasonable because milk production decreases in this phase, so, milk deprivation can provide knowledge to develop a preference for certain foods. Moreover, contradictory evidence exists of differential effect of sex of organism on sucralose intake, on one hand it has been reported that females tend to consume greater quantities of glucose or saccharine sweetened solutions in comparison to males (Valenstein, Cox y Kakolewski, 1967), and on the other hand, there is evidence that sex does not change sucralose intake (Loney, Torregrosa, Smith, Sclafani y Eckel, 2011).

Study on sweet flavor preference results relevant because it is the only definite example of innate preference to sense of taste. However, also of interest the study of interaction of this innate preference with the learned preference, such is case of sucralose sweet flavor preference as an effect from exposure. Nevertheless, also is important to observe influence that exposure to taste has on fluids intake in adult life. At the moment, it is considered that early exposure to sweet foods and beverages intake may be an important factor to obesity development in childhood (Institute of Medicine of the National Academies, 2004). Capaldi (1996) pointed out that repeated consumption of a specific food, increases preference for

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that food. Furthermore, it has been observed that the early experience with a certain food modifies its later consumption increasing its preference (Beauchamp y Moran, 1984). This could suggest existence of a sensitive phase for acquirement of flavors preference. Currently, great availability to sweet beverages such as, soft-drinks and bottled juices exists, and it was estimated that in Mexico increased calories consumption resulting from beverages from 1999 to 2006, and it was reported that Mexican in preschool and elementary school children have an intake of sweet beverages which corresponds to 27.8% and 20.7% of total caloric intake, respectively (Barquera et al., 2010). Additionally, it has been found an association between sweetened beverages consumption before six years of age and an the increase in body weight and body mass index in following ages (Pérez-Morales, Bacardí-Gascón y Jiménez-Cruz, 2013) and it has been reported that the increase of obesity prevalence simultaneously increased with sweetened beverage consumption on general population (Woodward-López, Kao y Ritchie, 2010). Accordingly the purpose of this experiment was to evaluate the effect of early exposure to sucralose in subsequent fluid intake in Wistar rats. From the available evidence, we hypothesized that animals that are early exposed to specific flavor subsequently will modify their intake of that specific flavor.

Method

Subjects

Twenty Wistar stock rats from vivarium at Behavioral Feeding and Nutrition Research Center (CICAN) from South Campus of University of Guadalajara, México were used. They were divided into two groups, A and S, each one with five males and five females, which received a code (MA, males exposed to water; MS, males exposed to sucralose; HA, females exposed to water; and HS, females exposed to sucralose) and number (from 1 to 5). Subjects began the experiment from the first day of their birth to three months and ten days of age. At the end of the experiment female rats weighed an average of 230.7±14.75g and male rats weighed 387±16.79g (means ± SEM).

Materials and Equipment

Twenty transparent plastic home boxes were used, measuring 18 x 28 x 15 cm, equipped with a metal grille on the top, with two dividers used as a food and a drinking trough. Boxes had a floor covering of sawdust, which was removed every five days. An electronic precision weight scale was used to register food intake and body weight of each subject. The pellets provided as food were from the commercial brand *Nutricubos*, which contain the necessary standard nutrients for laboratory animals its nutritional formula is as follows: 23% protein, 3% fats, 49% NFE (nitrogen-free extract), 7% ash, 1% calcium, 6% fiber, 0.6% phosphorus, and 12% humidity, plus it provides 3.06 kcal/g of food. The beverages used were distilled water and a sucralose solution at 0.5%, which were provided in 240ml graduated drinking troughs. Temperature was maintained at an average of 20 ^{o}C during the day and at 18 degrees ^{o}C by night. The light-dark cycle was regulated by the natural cycle.

Procedure

Two groups were formed: A and S. From the birth of offspring, mothers received water (group A) or a sucralose solution at 0.5% (group S). This way, during nursing stage the offspring were either exposed to sucralose solution or to water. From weaning, offspring were classified into groups A or S depending on the exposure to sucralose or water during the nursing stage and continued under the same feeding conditions. Their body weight and food and water intake was registered daily at 12:00pm.

Experimental Design

Experiment was divided into six phases. Phase 1 consisted of nursing stage in which offspring remai-

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ned with their mother. Phase 2 began from weaning (from 29 days of age) until they were 90 days old, in this phase group S continued consuming the sucralose solution, while group A consumed water. In phase 3 both groups were provided with the sucralose solution. During phase 4, groups returned to conditions of phase 2. In phase 5 they were provided with water. Finally, during phase 6 they were returned to the conditions of phase 2.

Table 1.
Experimental Design

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Group A	Water	Water	Sucralose	Water	Water	Water
Group S	Sucralose	Sucralose	Sucralose	Sucralose	Water	Sucralose
Days	29	69	10	10	10	10

Results

Figure 1 shows the mean and standard deviation for fluid intake of both groups. The top row corresponds to intake of group A and the lower row to intake of group S, left column corresponds to males and the right to females. The white circles show water intake and black circles sucralose solution intake.

For statistical analysis of data a student t test was applied for independent samples, which produced results with significant differences when comparing the fluid intake between female groups during phase 2, S group had the highest intake (A: 31.47±5.18 ml vs. S: 32.76±4.66 ml), among males a significant difference was not found during this phase. In phase 3 there were no significant differences when the intake of both groups was compared. In phase 4, there was a significant difference in case of males (A: 40.20±3.06 ml vs. S: 42.62±4.04 ml), S group had the highest intake. On the other hand, when both groups were exposed to water, significant differences were detected in females (A: 32.40±6.52 ml vs. S: 35.58±6.07 ml) as well as in males (A: 44.24±5.70 ml vs. S: 41.48±3.69 ml). Finally, in phase 6, fluid intake of females in group S was significantly greater in comparison to intake of group A (A: 33.44±6.38 ml vs. S: 39.02±6.51 ml), in regards to males no significant difference was found.

Additionally, data was analyzed by means of the ANOVA test to compare fluid intake by phases (f). In regards to MA group, no significant difference was found in intake of phases 2, 3, and 4, however, intake during phase 3 was lower than the rest (f2: 40.46 ml vs f3: 39.70 ml vs f4: 40.20 ml), on the other hand, fluid intake in phases 5 and 6 differed from previous phases (f5: 44.24 ml, f6: 45.44 ml).

Regarding fluid intake of the HA group, no significant differences were found when comparing phases, however, as in males, fluid intake during exposure to sucralose was lower than the rest (f2: 31.47 ml, f3: 30.82 ml, f4: 32.22 ml, f5: 32.40 ml, f6: 33.40 ml). Nevertheless, intake of the MS group had a significant difference in phase 6 when returned to sucralose intake after water consumption during phase 5 (f2: 40.99 ml, f3: 40.00 ml, f4: 42.62 ml, f5: 41.48 ml, f6: 45.60 ml). Finally, HS group had significant differences in consumption in phase 5 and phase 6 regarding previous phases (f2: 32.76 ml, f3: 32.08 ml, f4: 32.42 ml, f5: 35.58 ml, f6: 39.02 ml).

Particularly in case of MS group when comparing the average intake of fluids from phase 2 with the first day of water access, a decrease of 5.07±3.62 ml is observed, and when compared to intake of first day of phase 6, that is, the first day with access to sucralose after consuming water for 10 days, there is an increase in consumption of 13.32±5.95 ml.

It was different in the case of the HS group, since when comparing the intake of phase 2 with the first day of water access, only 3 subjects decreased their intake (HS2: -3.15 ml, HS3: -6.42 ml, HE4: -3.42 ml), while the remaining two subjects at increased (HS1: +1.87ml, HS5: +1.69 ml), and regarding the first day of sucralose intake in phase 6 their intake increased an average of 12.79 ml.

Differences in consumption of the MA group were less evident, when average intake of phase 2 with the first day of sucralose intake was compared, three subjects increased their consumption (MA2: +5.7 ml, MA3: +2.23 ml and MA4: +0.96 ml) and two subjects decreased it (MA1: -2 ml and MA5: -2.42 ml). On the other hand, when fluid intake was compared with the first day of water access, after sucralose intake, two subjects increased their consump-

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tion (MA2: +1.7 ml and MA4: +0.96 ml), while the remaining three subjects decreased it (MA1: -1 ml, MA3: -5.53 ml and MA5: -2.42 ml).

Finally, regarding the HA group, when the fluid intake of phase 2 was compared with the first day of sucralose consumption, four of the subjects increased their intake (HA1: +1.09 ml, HA2: +2.35 ml, HA4: +3.03 ml and HA5: +1.64 ml) and only one decreased it (HA3: -5.42 ml). When the comparison of water consumption from the first day after the exposure to sucralose, four subjects decreased their intake (HA1: -3.91 ml, HA2: -3.65 ml, HA3: -5.42 ml and HA5: -7.36 ml) and only one of them increased it (HA4: +8.03 ml).

Discussion

The obtained results showed the following: 1) fluids intake was higher in females that consumed sucralose solution in comparison to females that only consumed water; 2) when exposed to water as a drink, the females in group S consumed more water in comparison to females in group A, whereas in males, it was those from group A that registered a higher consumption; 3) fluid intake in phase of sucralose solution exposure was lower in group A (males and females) in relation to rest phases; 4) when exposed to sucralose, after having water intake for ten days, group S (males and females) had a significantly higher intake to that of rest of phases. These results showed that exposure to the taste of **sweeteners** from lactation influence the fluids customarily consumed by females, also showed differential effects with respect to sex (Sedova et al., 2007). There is existing literature that points out those females tend to consume higher quantities of sweetened solutions. Valenstein et al. (1967) pointed out that female rats prefer glucose or saccharine solutions in procedures with two drinking troughs in 24 hour periods in comparison to consumption of males. However, it cannot be affirmed that increase in regular intake of sucralose in the present study is a preference for sweet taste of the sweetener since subjects in this study were only exposed to one beverage. In contrast with this evidence, Loney et al. (2011) reported results that do not show differences in sucralose intake regarding sex. Authors indicated a possible connection with the range of concentrations used or possibility that

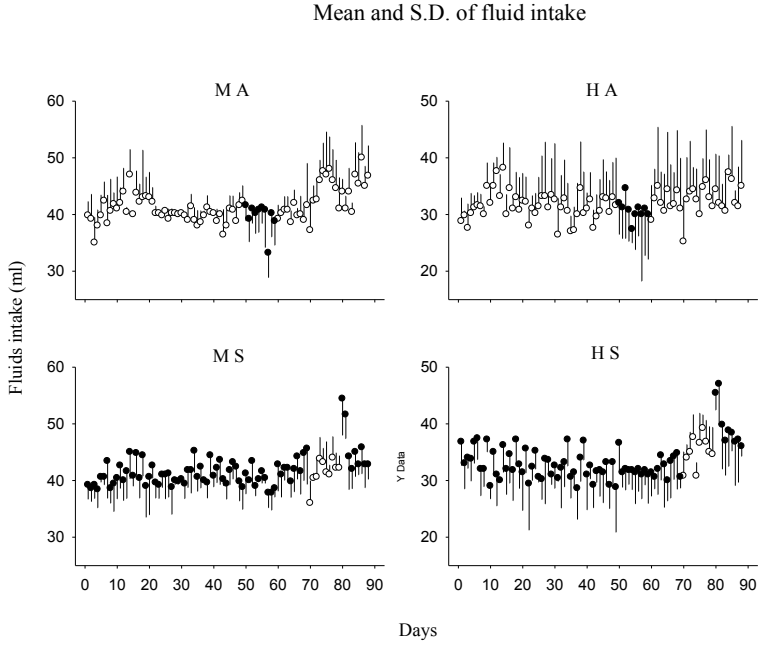

Fig. 1 Mean and standard deviation of fluid intake. Top row corresponds to intake of group A and lower row to intake of group S, left column corresponds to males and right column to females. White circles show water intake and black circles show sucralose solution intake.

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sucralose has an aversive component which could have impeded observation of differences between genders.

On the other hand, it is important to point out that even though no significant differences were found in sucralose intake of group A, a lower intake was registered regarding the rest of the phases, indicating indifference for sweet taste of sucralose when subjects are exposed to it for first time. This is consistent with the findings of various researchers whom show that the fluid intake does not increase when sweet taste of sucralose is added under various experimental procedures (Martínez et al., 2009; Torres-González et al., 2009). Furthermore, it reinforces the finding that flavor is not a strong enough to produce and maintain an increase on consumption responses.

Then again, it seems that previous exposure to taste of sucralose established an increase in the intake of sweetened solution following water consumption. Although it is not an excessive fluid intake as has been reported in other experimental manipulations, such as water and/or food deprivation (López-Espinoza y Martínez, 2001, 2004) or when exposed to sweetened glucose solutions (Martínez et al., 2009). It does represent an increase regarding its regular fluid intake. This contrasts with literature that confirms little preference or aversion to the taste of sucralose. To explain this increase in intake different arguments emerge.

First, existing evidence shows that short-term restriction to a specific food increases its subsequent consumption, in food-restricted conditions in animals as well as humans. DiBattista (1991) carried out an experiment in which he evaluated the effect of specific restriction of protein and carbohydrates in rats and mice regarding protein and carbohydrate consumption. Results indicated that both rats and mice consumed more protein at the end of restriction, though carbohydrate intake was not as strong as protein. In turn, Polivy, Coleman and Herman (2005) carried out a study where restricted and unrestricted women were assigned to chocolate deprivation, vanilla deprivation or without any deprivation for a week. Results showed that participants with a food restriction when deprived of chocolate consumed more chocolate than the rest of the participants when they had access to it. The authors pointed out that it is probable that energy deficit or loss of weight may have influenced in chocolate consumption.

Secondly, when preference to taste in childhood is established, an interaction between innate and learned responses exists. Experimental evidence demonstrates that it is possible to develop a preference to a determined taste during childhood. However, this tendency may be modified by pre- and postnatal experiences, which are important in establishing eating habits throughout life (Beauchamp y Mennella, 2009). Oostindjer, Bolhuis, Van den Brand, Roura and Kemp (2010) carried out an experiment where they exposed pigs in gestation to specific flavors. After giving birth some of piglets were exposed during their nursing to certain flavors and others were not. Results showed that piglets exposed to flavors during gestation and nursing demonstrated a better conditions of survival, were more active, and they achieved better health. However, during their growth when being exposed to other types of food and tastes their initial preference was modified. Meanwhile, Beauchamp and Moran (1984) pointed out that two year old children whom were fed with sweetened water during their first months of life showed a greater preference to sweetened water when compared to those that had little or no exposure to sweetened water. Accordingly, Pepino and Mennella (2005) reported that children between 6 and 10 years old that had consumed water with sugar during their infancy preferred significantly higher levels of sucrose when compared to children that had little exposure to sugar. Additionally, it has been pointed out that ingestive expression for innate preference for sweet substances may be subject to modification in postnatal life (Beauchamp y Cowart, 1985).

Although, the objective of this study consisted in comparing fluid intake among groups with different exposure to sucralose, it is relevant to point out the importance of carrying out new experiments with different procedures. These studies must allow the in depth study of possible preference for taste of

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sucralose caused by exposure to this sweetener from a young age. In evaluating results, questions arise regarding the procedure used and results obtained, what would happen if intermittent exposure of water and sucralose solution continued? Would there be a similar result if the exposure had not initiated from nursing? Is nursing a sensitive period to acquiring a preference to flavors such as that of sucralose? Would habitual sucralose consumers show greater intakes of glucose sweetened solutions? Like all experimental studies, this work has generated new question which without a doubt reinforce this line of investigation. However, it is important to point out the importance of continuing with study of the variables evaluated in this study. Data obtained will provide elements to characterize and prevent obesity development in children, associated to early exposure to sweetened foods and beverages.

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