



## Mineral intake independent from gastric irritation or pica by cell-dehydrated rats

Juliana Constancio, Daniela T.B. Pereira-Derderian, José V. Menani, Laurival A. De Luca Jr. \*

Department of Physiology and Pathology, School of Dentistry, São Paulo State University, UNESP, Araraquara 14801-903, São Paulo, Brazil

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### ABSTRACT

Gavage of 2 M NaCl (IG 2 M NaCl), a procedure to induce cell-dehydration—and water and 0.15 M NaCl intake in a two-bottle choice test—is also a potential gastric irritant. In this study, we assessed whether mineral intake induced by IG 2 M NaCl is associated with gastric irritation or production of pica in the rat. We first determined the amount of mineral solution (0.15 M NaCl, 0.15 M NaHCO<sub>3</sub>, 0.01 M KCl and 0.05 mM CaCl<sub>2</sub>) and water ingested in response to IG 2 M NaCl in a five-bottle test. Then, we used mineral solutions (0.01 M KCl and 0.15 M NaHCO<sub>3</sub>), whose intakes were significantly increased compared to controls, and water in three-bottle tests to test the gastric irritation hypothesis. The IG 2 M NaCl induced KCl and NaHCO<sub>3</sub> intake that was not inhibited by gavage with gastric protectors Al(OH)<sub>3</sub> or NaHCO<sub>3</sub>. IG 2 M NaCl or gavage of 0.6 N acetic acid induced mild irritation, hyperemia, of the glandular part of the stomach. A gavage of 50% ethanol induced strong irritation seen as pinpoint ulcerations. Neither ethanol nor acetic acid induced any fluid intake. Neither IG 2 M NaCl nor acetic acid induced kaolin intake, a marker of pica in laboratory rats. Ethanol did induce kaolin intake. These results suggest that IG 2 M NaCl induced a mineral fluid intake not selective for sodium and independent from gastric irritation or pica.

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### 1. Introduction

Hypertonic loads of NaCl given to a rat by different routes of administration, whether subcutaneous, intravenous or intragastric, increase blood osmolality and produce cell-dehydration and water intake [1]. Hypertonic loads of NaCl given by same routes also induce both water and 0.15 M NaCl intake in two-bottle, free-choice tests [2–4]. Thus, cell dehydration is a common cause of water and isotonic NaCl intake in response to different routes of hypertonic NaCl administration. However, the ingestion of a fluid such as NaCl, which contains solutes with potential osmotic effects, even at the isotonic concentration preferred by some rat strains [5], does not match what is expected from behaviors that should contribute towards a strict regulation of blood osmolality.

Rats may also increase their sodium consumption in response to social stress [6]. Therefore, not only dehydration but also other factors associated with the route of administration of hypertonic NaCl, could contribute to induce isotonic NaCl intake. For example, hypertonic NaCl given intragastrically induces mild gastric irritation [7], which may cause the ingestion of isotonic NaCl as an attempt to relieve the animal from a collateral symptom and not from cell-dehydration.

We showed in a previous article that an intragastric load (IG) of hypertonic (2 M) NaCl given to a rat induces both water and 0.15 M NaCl intake in a two-bottle test [4]. Cell dehydration certainly produces the water intake induced in this test, but it might also produce some, if not all, of the 0.15 M NaCl intake. However, the isotonic NaCl intake could also mask a drive to ingest a mineral that helps to alleviate gastric irritation produced by hypertonicity acting on the gastric mucosa [7]. If this is correct, IG 2 M NaCl should also induce the ingestion of another mineral more likely than isotonic NaCl to help against gastric discomfort.

Wild animals access salt or mineral licks to consume carbonates and kaolinites, which have been hypothesized to relieve symptoms produced by irritation of the gut [8–10]. Humans and laboratory rats display similar ingestive behaviors. Humans ingest minerals [10–12] that protect the gastric mucosa by either reducing stomach acidity, as NaHCO<sub>3</sub> does, or forming a barrier against irritants, as Al(OH)<sub>3</sub> does. Rats ingest kaolin, aluminum clay used in the laboratory as a marker of pica [13–15]. Kaolin intake apparently alleviates the nausea caused by signals originating in the gut from ingested toxins [13–15]. It is possible that rats ingest kaolin in response to any kind of signal associated with malaise. Thus, stomach irritation could also induce kaolin intake.

Therefore, if IG hypertonic NaCl produces stomach irritation then it should also induce, for example, the ingestion of NaHCO<sub>3</sub> solution and kaolin. Moreover, the rat should prefer NaHCO<sub>3</sub> over NaCl. In addition, other stomach irritants, such as ethanol or acetic acid [16,17], should also have the same effects. We may also predict that the stronger the irritation, the stronger the ingestion of NaHCO<sub>3</sub> or kaolin.

\* Corresponding author at: Departamento de Fisiologia e Patologia, Faculdade de Odontologia, Universidade Estadual Paulista, UNESP, Rua Humaitá, 1680, 14801-903, Araraquara, São Paulo, Brazil. Tel.: +55 16 3301 6484; fax: +55 16 3301 6488.

E-mail address: [lucajr@foar.unesp.br](mailto:lucajr@foar.unesp.br) (L.A. De Luca).

The present study was conducted to find out whether IG 2 M NaCl, given to normovolemic rats, induces mineral intake in association with gastric irritation. There are a variety of reasons to look for such association. First, this association provides a direct test of the hypothesis that stomach irritation induces mineral intake in animals. The results of such test may expand the information available about how a laboratory rat behaves in response to malaise and how it develops pica. Second, this study has methodological and conceptual implications about the gavage of hypertonic NaCl as a procedure for inducing cell-dehydration and thirst. As a result, we first established the types of mineral solution ingested in a five-bottle test, in response to the IG 2 M NaCl. Next, using the two solutions ingested in this test, 0.15 M NaHCO<sub>3</sub> and 0.01 M KCl, we tried either to prevent the mineral intake, using gastric protective agents, or to produce mineral fluid intake, by giving two other irritants, ethanol or acetic acid, by gavage. Finally, we tested if stomach irritants produce kaolin intake.

## 2. Methods

### 2.1. Animals

Adult male Holtzman rats (280–320 g) were individually housed in stainless steel cages in a room with a controlled 12:12 h light–dark cycle at 23 ± 2 °C and 55 ± 10% humidity. Three or five polypropylene bottles (100 ml capacity with divisions to the nearest ml) with stainless steel spouts, one containing deionized water and each of the others containing a different palatable mineral solution (see below), were freely available unless otherwise noted. Guabi rat chow (Paulínia, Brazil; 0.5–1.0% sodium) was available in a container placed at the side of each cage. Every experiment began at least seven days after the animals were housed with all fluids available. All tests began between 12:00 noon and 2:00 p.m. In all experiments, the animals were tested only once, except in the five-bottle test. The procedures were approved by the Institutional Ethical Committee for Animal Care (School of Dentistry, Araraquara, UNESP) and followed the recommendations of the Brazilian College of Animal Experimentation (COBEA).

### 2.2. Reagents

A palatable concentration of each mineral solution (0.15 M NaCl, 0.15 M NaHCO<sub>3</sub>, 0.05 mM CaCl<sub>2</sub> and 0.01 M KCl) and deionized water was utilized to test the mineral preference in accordance with previous works [18,19]. Each salt (LABSYNTH, Brazil) was dissolved in deionized water.

Kaolin (hydrated aluminum silicate—LABSYNTH, Brazil) was mixed with 1% arabic gum in deionized water and completely dried at room temperature to form pellets similar in size to chow pellets, as described previously [13].

Aluminum hydroxide (VETEC, Brazil), sodium chloride and acetic acid (LABSYNTH, Brazil), and ethanol (QHEMIS, Brazil) were dissolved in deionized water for the intragastric load (2 ml). Deionized water was used as the vehicle control in all experiments.

### 2.3. Screening test, intake test and intragastric (IG) hypertonic NaCl load

The animals were removed from their cages and gently trained to receive an IG load or gavage by infusing deionized water through polypropylene tubing (PE-10) connected to a syringe. The volume of water infused for training was 1 ml/2 s and the volume of different kinds of solutions infused for all tests was 2 ml/5 s (see screening test and experiments). The length of tubing was enough to reach the stomach, as determined by previous studies [4]. The training began after two days of adaptation to the cage and was given once a day for five days (training period).

About 25% of the animals that entered the five-bottle tests (Experiment 1) ingested negligible amounts, if any, of 0.15 M NaHCO<sub>3</sub> in response to the IG 2 M NaCl. Therefore, based on the minimum amount ingested in the five-bottle test, only the animals that ingested at least 2 ml of NaHCO<sub>3</sub> in the screening test were used in the subsequent tests labeled Experiment 2 to Experiment 5.

The screening test was performed the day after the training period was over. Food and every fluid were removed from the cage, and then the animals received an IG administration of 2 ml of 2 M hypertonic NaCl by gavage (IG 2 M NaCl). The IG 2 M NaCl induces hypernatremia, hyperosmolemia and reduction in plasma renin activity, but no alteration in total plasma protein concentration or total hematocrit, up to 1 h after the gavage [4]. One hour after the IG 2 M NaCl, the animals had access to water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub>; fluid intake was recorded for 60 min (intake test). After the test, food, water and palatable mineral solutions were made available to the animals until the next test. The intake tests were performed three days after the screening test. The fluids or kaolin were returned to the cages 1 h after the gavage (see experiments). All fluids were offered in 0.1 ml graduated glass burettes fitted with stainless steel spouts.

### 2.4. Statistical analysis

Two-way ANOVA were used to compare groups or to compare animals ingesting different fluids within a group for the five-bottle test or three-bottle test. Two-way repeated measures ANOVA was used to compare fluid intake in five bottle-tests following a counterbalanced design (Experiment 1) and daily fluid intake in a three-bottle choice test (Experiment 2). One-way ANOVA was used to compare water or kaolin intake. A non-paired *t*-test was used where appropriate. Data are reported as means ± standard error of the mean and all ANOVA tests were followed by Student–Newman–Keuls post hoc test for comparisons.

A chi-squared test was used for the macroscopic analysis of the gastric mucosa. Because there was a positive association between the two variable treatments and gastric mucosa coloration, a residual analysis was made to determine how each result contributed to the final outcome of the chi-squared test. The results are reported as the number of stomachs in each class and the percentage relative to the total number of examined stomachs.

The level of significance was set at  $p < 0.05$  in all tests.

### Experiment 1. Mineral solution preference in a five-bottle test.

The objective of this experiment was to find out if IG 2 M NaCl induces selective 0.15 M NaCl intake. The test was performed in a counterbalanced design, with each animal being tested twice, on different days, for each treatment, at a three-day interval.

On the first test after the training period was over, 8 rats were separated into two groups: one that would receive 2 ml of water by gavage and the other to receive IG 2 M NaCl. One hour after the gavage, all rats had access to water, 0.01 M KCl, 0.05 mM CaCl<sub>2</sub>, 0.15 M NaHCO<sub>3</sub> and 0.15 M NaCl for a five-bottle intake test. Upon completion of the test, food was returned and the polypropylene bottles replaced the glass burettes. Three days later the animals were tested again in a counterbalanced design.

Experiment 1 served as a pilot to show that from the four mineral solutions offered for ingestion, IG 2 M NaCl induced only 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> intake in the five-bottle test (see Results). Therefore, the subsequent experiments (from Experiment 2 to Experiment 5) involving mineral solution intake were conducted with only these two solutions and water in three-bottle tests.

### Experiment 2. Daily fluid intake.

Water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> intake was recorded daily prior to the screening test from 12 animals assigned to enter the group

of H<sub>2</sub>O/NaCl in Experiment 3b. Those animals passed the screening test; therefore, their daily data was considered for statistical analysis.

### Experiment 3. Mineral intake and gastric irritation.

#### Experiment 3a. Stereoscopic analysis of gastric mucosal response to hypertonic NaCl and other potential irritants.

Because hypertonic NaCl is considered a gastric irritant [7], we first checked for signs of irritation, such as macroscopic hyperemia or bleeding of the gastric mucosa, in response to the IG 2 M NaCl. We also tested the capacity of other irritants, such as ethanol or acetic acid [16,17], to induce similar irritation.

The animals (n = 22) were assigned to four groups that received 2 ml gavage of water (n = 4), 2 M NaCl (n = 6), 50% ethanol (n = 6) or 0.6 N acetic acid (n = 6). One hour after the gavage they were killed by intraperitoneal injection of Thiopental (80 mg/kg of body weight; Cristália, Brazil). Next, the stomach was excised, cut longitudinally in half along the convex surface through its major curvature and spread onto absorbent paper for analysis under a stereoscopic surgical microscope at 10× magnification (D. F. Vasconcelos, M900, Brazil). Two people not aware of the treatments classified separately the degree of irritation of the gastric mucosa into categories according to the following visual scale: A (rosy), B (reddish), C (deep reddish) and D (presence of petechial hemorrhage or pinpoint ulcers). This classification is based on the assumption that an irritant such as hypertonic NaCl or acetic acid increases mucosal blood flow [7] leading to hyperemia and a change in color of the mucosa.

#### Experiment 3b. Combination of gastric protectors with IG 2 M NaCl: effect on mineral intake.

Aluminum hydroxide given 1 h prior to 100% ethanol is an effective mechanical protector of the gastric mucosa [12]. Therefore, it was given 1 h prior to hypertonic NaCl. Sodium bicarbonate acts as an antacid and was mixed and given with the hypertonic NaCl in an attempt to provide immediate protection against irritation [11].

Animals (n = 44) were assigned to four groups that received the following fluids by gavage: 1) 2 ml of water 1 h prior to a second load of 2 ml of deionized water (H<sub>2</sub>O/H<sub>2</sub>O, n = 8); 2) 2 ml gavage of water 1 h prior to 2 ml of 2 M NaCl (H<sub>2</sub>O/NaCl, n = 12); 3) 2 ml of Al(OH)<sub>3</sub> 1 h prior to 2 ml of 2 M NaCl (Al/NaCl, n = 8); 4) 2 ml of 2 M NaCl plus the average amount of NaHCO<sub>3</sub> (0.05 g/2 ml or 0.3 M) that was ingested in the five-bottle test (Na/Na; n = 16).

One hour after the IG 2 M NaCl, with or without NaHCO<sub>3</sub>, the animals had access to water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> (intake test).

#### Experiment 4. Irritants and mineral solution intake.

This experiment was conducted to find out if the other two irritants, 50% ethanol and 0.6 N acetic acid, also induced 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> intake.

#### Experiment 4a. Effect of an IG load of either ethanol or acetic acid on mineral solution intake.

All fluids and food were removed from the cages of thirteen rats that received 2 ml gavage of water. They were then separated into three sub-groups: one received 2 ml of 2 M NaCl (H<sub>2</sub>O/NaCl, n = 4), another received 2 ml of 50% ethanol (H<sub>2</sub>O/ethanol, n = 5) and a third group received 2 ml of 0.6 N acetic acid (H<sub>2</sub>O/Acetic, n = 4), all by gavage. One hour later, they were given access to water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> (intake test).

#### Experiment 4b. Effect of ethanol or acetic acid on fluid intake.

The two irritants failed to induce mineral solution intake in Experiment 4a. As a result, it became necessary to check whether the

irritants also inhibited ingestive behavior because the absence of fluid intake could have resulted from a general inhibition of behavior.

Twenty-two rats received IG 2 M NaCl. One hour later they were separated into three groups that received a 2 ml gavage of: water (NaCl/H<sub>2</sub>O, n = 5), 50% ethanol (NaCl/ethanol, n = 9) or 2 M NaCl (NaCl/NaCl, n = 8). One hour after the second gavage, they were given access to water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> (intake test).

Another group of nineteen rats also received IG 2 M NaCl. One hour after the IG 2 M NaCl, they were separated into two groups that received either 2 ml of water (NaCl/H<sub>2</sub>O, n = 9) or 0.6 N acetic acid (NaCl/Acetic, n = 10). One hour after the second gavage, they were given access to water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> (intake test). There was an apparent reduction in fluid intake in group NaCl/Acetic compared to group NaCl/H<sub>2</sub>O. As this raised the possibility that the former was also not different from another control group, we included the H<sub>2</sub>O/H<sub>2</sub>O group from Experiment 4a in the statistical analysis (see Results).

#### Experiment 5. Effect of irritants on kaolin intake.

Thirty-eight rats, selected from the screening test, were housed for 24 h with stainless steel containers containing kaolin pellets in addition to food, water and the palatable mineral solutions that had been available since the beginning of the experiment. The screening test was necessary to have animals comparable to the previous experiments.

On the test day, all food, fluids and kaolin were removed from the cages. All animals received 2 ml gavage of water. One hour later, they were separated into four groups to receive a 2 ml gavage of: water (n = 11), 2 M NaCl (n = 11), 0.6 N acetic acid (n = 8) or 50% ethanol (n = 8). One hour after the second gavage, they had access to kaolin pellets in a pre-weighed container placed inside the cage and water in a 0.1 ml-graduated glass burette fitted with a stainless steel spout. Ingestion of water was recorded at 120 min and then the containers were weighed again. The amount of kaolin consumed was calculated from the difference in container weight.

## 3. Results

### Experiment 1. Mineral fluid and water intake in a five-bottle test.

Gavage of 2 M NaCl induced 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> intake, but not water, 0.15 M NaCl or 0.05 mM CaCl<sub>2</sub> intake, compared to gavage of water (Fig. 1). There was a significant effect of treatment between IG H<sub>2</sub>O and NaCl [ $F(1, 7) = 43.0$   $p < 0.05$ ]. There was also a significant effect of fluid [ $F(4, 28) = 5.2$   $p < 0.05$ ] and an interaction between treatment and fluid [ $F(4, 28) = 5.4$   $p < 0.05$ ].

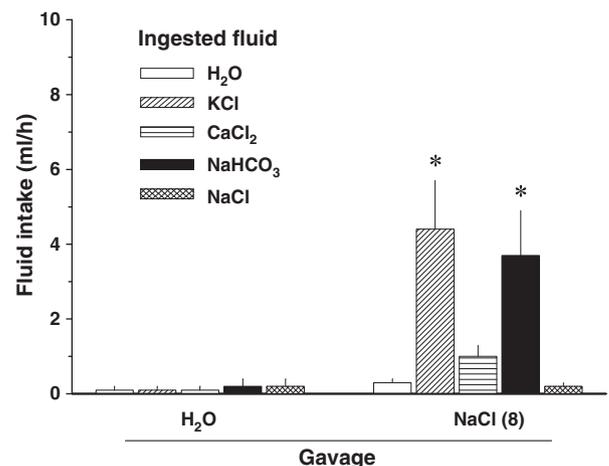
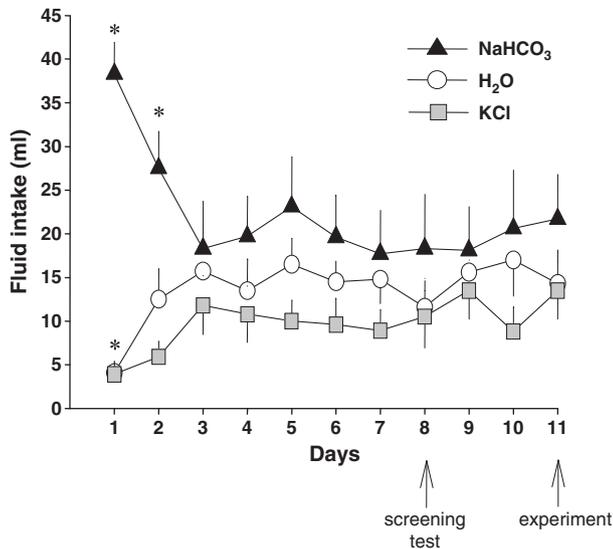


Fig. 1. Ingestion of deionized water (H<sub>2</sub>O) and palatable mineral solutions (0.01 M KCl, 0.05 mM CaCl<sub>2</sub>, 0.15 M NaHCO<sub>3</sub> and 0.15 M NaCl) in a five-bottle test by rats that received either gavage of H<sub>2</sub>O or 2 M NaCl (2 ml). \* $p < 0.05$  vs. respective fluid of control group (H<sub>2</sub>O). The number of rats is given within brackets.



**Fig. 2.** Daily deionized water (H<sub>2</sub>O) and palatable mineral solution (0.01 M KCl and 0.15 M NaHCO<sub>3</sub>) intake of rats screened for mineral solution intake and entered in the experiments (arrows). \**p* < 0.05 vs. all other days of the same fluid. *N* = 12.

### Experiment 2. Daily water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> intake.

NaHCO<sub>3</sub> intake decreased and water intake increased from the first to the third day of recording, but there was no statistical difference among fluids from the third to the last day of recordings (Fig. 2). There was no significant effect of fluid among water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub> [*F*(2, 22) = 2.6, *p* > 0.05]. There was, however, a significant effect of days [*F*(10, 110) = 2.9, *p* < 0.05] and an interaction between fluid and days [*F*(20, 220) = 3.9, *p* < 0.05].

### Experiment 3. Mineral intake and gastric irritation.

#### Experiment 3a. Stereoscopic analysis of gastric mucosa in response to hypertonic NaCl and other potential irritants.

Gavage of 50% ethanol or water were the only treatments associated with specific degrees of irritation in the gastric mucosa [Chi-squared = 35.1, *p* < 0.05]: categories D (most irritation) and A (least irritation), respectively (Table 1). The stomachs of all animals treated with ethanol had petechiae and pinpoint ulcers. The stomach of all animals treated with water had a rosy coloration. Gavage of 2 M NaCl or 0.6 N acetic acid produced a mucosa varying from rosy to reddish (categories from A to C), but neither fluid was significantly associated with a specific degree of irritation.

#### Experiment 3b. Combination of gastric protectors with IG 2 M NaCl: effect on mineral intake.

The IG H<sub>2</sub>O/NaCl (same animals from Experiment 2), Al/NaCl and Na/Na increased 0.15 M NaHCO<sub>3</sub> compared to IG H<sub>2</sub>O/H<sub>2</sub>O, but Na/Na

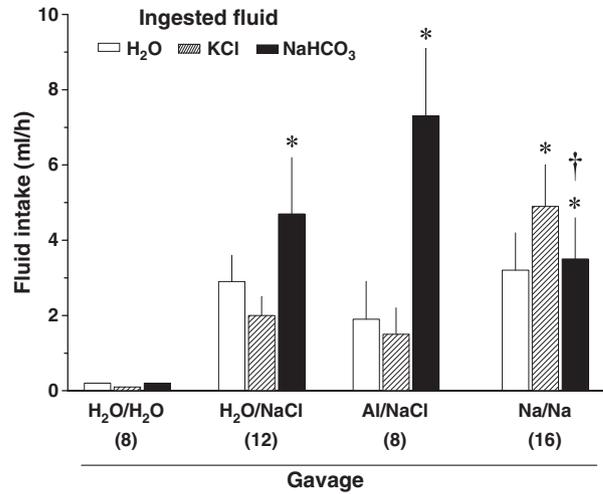
**Table 1**

Category (A–D) distribution of the gastric mucosa coloration of rats that received gavage of: deionized water, 2 M NaCl, 0.6 N acetic acid or 50% ethanol.

Gavage	A	B	C	D	N (100%)
Water	4 (100%)*	0 (0%)	0 (0%)	0 (0%)	4
NaCl	3 (50%)	3 (50%)	0 (0%)	0 (0%)	6
Acetic ac.	2 (33.3%)	3 (50%)	1 (16.6%)	0 (0%)	6
Ethanol	0 (0%)	0 (0%)	0 (0%)	6 (100%)*	6
Total	9	6	1	6	22

A: rosy; B: reddish; C: deep reddish; D: petechiae and pinpoint ulcers. N: number of animals per group.

\* Significant at *p* < 0.05.



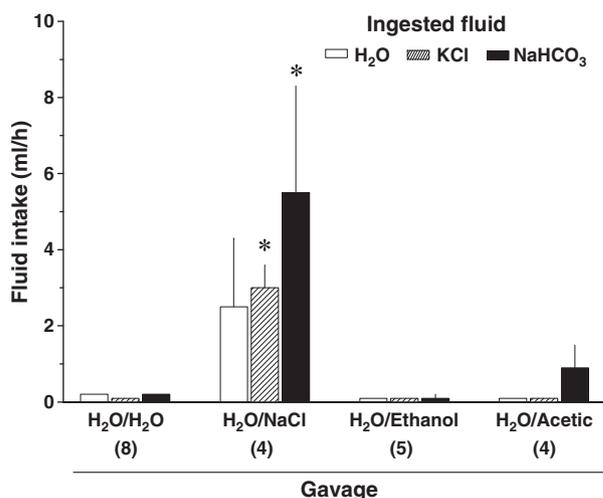
**Fig. 3.** Ingestion of deionized water (H<sub>2</sub>O) and palatable mineral solutions (0.01 M KCl and 0.15 M NaHCO<sub>3</sub>) in a three-bottle test by rats that received a gavage (2 ml) of 2 M NaCl preceded by a gavage of either H<sub>2</sub>O or Al(OH)<sub>3</sub> (H<sub>2</sub>O/NaCl and Al/NaCl). Two other groups received a gavage of either H<sub>2</sub>O preceded by a gavage of H<sub>2</sub>O (H<sub>2</sub>O/H<sub>2</sub>O) or a single gavage of 2 M NaCl mixed with NaHCO<sub>3</sub> (Na/Na). \**p* < 0.05 vs. same fluid drank by H<sub>2</sub>O/H<sub>2</sub>O. †*p* < 0.05 vs. same fluid drank by Al/NaCl. The number of rats is given within brackets.

ingested less than Al/NaCl (Fig. 3). There was a significant effect of treatment among IG H<sub>2</sub>O/NaCl, Al/NaCl, Na/Na and H<sub>2</sub>O/H<sub>2</sub>O [*F*(3, 120) = 6.3, *p* < 0.05]. There was also a significant effect of fluid [*F*(2, 120) = 3.6, *p* < 0.05] and an interaction between treatment and fluid [*F*(6, 120) = 2.4, *p* < 0.05].

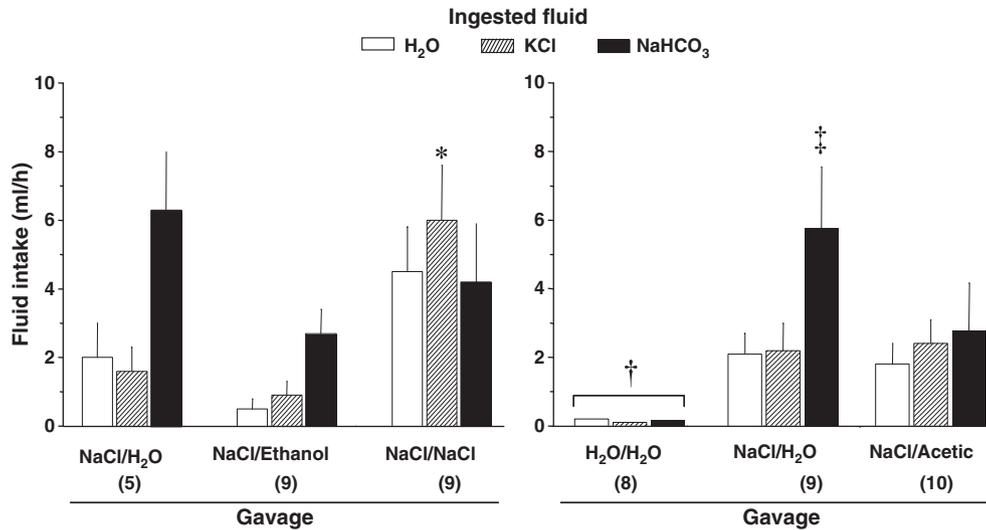
### Experiment 4. Irritants and mineral fluid intake.

#### Experiment 4a. Effects of gavage of either ethanol or acetic acid on mineral fluid intake.

The IG H<sub>2</sub>O/NaCl induced KCl and NaHCO<sub>3</sub> intake compared to IG H<sub>2</sub>O/H<sub>2</sub>O, but IG H<sub>2</sub>O/ethanol or acetic acid produced no fluid intake compared to IG H<sub>2</sub>O/H<sub>2</sub>O (Fig. 4). There was a significant effect of treatment among IG H<sub>2</sub>O/NaCl, H<sub>2</sub>O/ethanol, H<sub>2</sub>O/Acetic and H<sub>2</sub>O/H<sub>2</sub>O [*F*(3, 51) = 13.8, *p* < 0.05]. There was no significant effect of fluid [*F*(2,



**Fig. 4.** Ingestion of deionized water (H<sub>2</sub>O) and palatable mineral solutions (0.01 M KCl and 0.15 M NaHCO<sub>3</sub>) in a three-bottle test by rats that received a gavage (2 ml) of H<sub>2</sub>O, 2 M NaCl, 50% ethanol or 0.6 N acetic acid preceded by a gavage of H<sub>2</sub>O. \**p* < 0.05 vs. same fluid drank by other groups. The number of rats is given within brackets.



**Fig. 5.** Ingestion of deionized water (H<sub>2</sub>O) and palatable mineral solutions (0.01 M KCl and 0.15 M NaHCO<sub>3</sub>) in a three-bottle test by rats that received a gavage (2 ml) of H<sub>2</sub>O, 50% ethanol or 2 M NaCl (left) and of H<sub>2</sub>O or 0.6 N acetic acid (right) preceded by a gavage of 2 M NaCl. Left: \*p<0.05 vs. KCl drank by NaCl/ethanol. Right: †p<0.05 vs. other groups; ‡p<0.05 vs. NaHCO<sub>3</sub> drank by H<sub>2</sub>O/H<sub>2</sub>O. The number of rats is given within brackets.

51) = 2.0, p>0.05] and no interaction between treatment and fluid [F(6, 51) = 0.8, p>0.05].

**Experiment 4b.** Effect of ethanol or acetic acid on fluid intake.

The IG NaCl/NaCl increased KCl intake compared to NaCl/ethanol, but produced no effect compared to NaCl/H<sub>2</sub>O; the treatments produced no significant difference in H<sub>2</sub>O or NaHCO<sub>3</sub> intake (Fig. 5, left). There was a significant effect of treatment among IG NaCl/H<sub>2</sub>O, NaCl/ethanol and NaCl/NaCl [F(2, 57) = 8.5, p<0.05]. There was no significant effect of fluid [F(2, 57) = 2.6, p>0.05] and no interaction between treatment and fluid [F(4, 57) = 1.9, p>0.05].

The IG NaCl/H<sub>2</sub>O or NaCl/Acetic increased H<sub>2</sub>O, KCl and NaHCO<sub>3</sub> intake compared to H<sub>2</sub>O/H<sub>2</sub>O, but IG NaCl/Acetic reduced NaHCO<sub>3</sub> intake compared to NaCl/H<sub>2</sub>O (Fig. 5, right). The overall fluid intake of group H<sub>2</sub>O/H<sub>2</sub>O was different from NaCl/H<sub>2</sub>O and NaCl/Acetic, but only NaCl/H<sub>2</sub>O ingested significantly more NaHCO<sub>3</sub> than H<sub>2</sub>O/H<sub>2</sub>O.

There was a significant effect of treatment among IG H<sub>2</sub>O/H<sub>2</sub>O, NaCl/H<sub>2</sub>O and NaCl/acetic acid [F(2, 72) = 8.7, p<0.05]. There was no significant effect of fluid [F(2, 72) = 2.5; p>0.05] and no interaction between treatment and fluid [F(4, 72) = 1.4; p>0.05].

**Experiment 5.** Effect of irritants on kaolin intake.

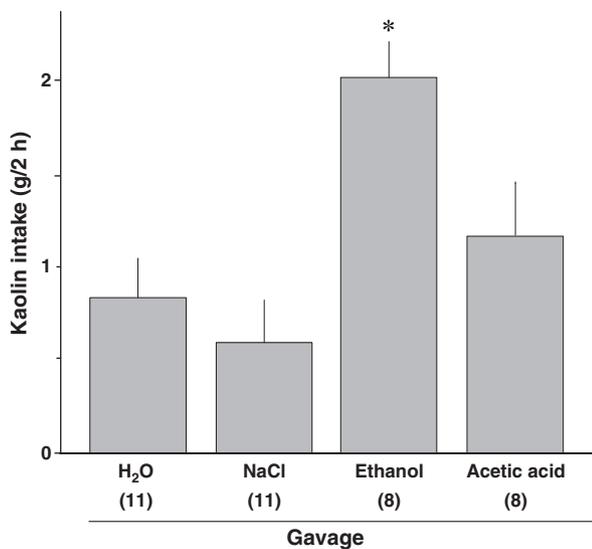
The gavage with ethanol increased kaolin intake compared to the gavage with water, whereas gavage with NaCl or acetic acid produced no change on kaolin intake (Fig. 6). There was a significant effect of treatment among IG H<sub>2</sub>O, NaCl, ethanol and acetic acid [F(3, 34) = 7.3, p<0.05, 1-way ANOVA].

Gavage of NaCl induced water intake (7.8 ± 1.0 ml/120 min) compared to gavage of only water (1.3 ± 0.6 ml/120 min, p<0.05). Water intake after gavage of ethanol or acetic acid was negligible (<0.6 ml).

**4. Discussion**

In tests involving rats with a choice among five mineral solutions, gavage of 2 ml water did not elicit intake of any solution, but gavage of 2 ml 2 M NaCl (IG 2 M NaCl) induced intake of 0.01 M KCl and 0.15 M NaHCO<sub>3</sub>, but not of water, 0.05 mM CaCl<sub>2</sub> or 0.15 M NaCl. In three-bottle (water, 0.01 M KCl and 0.15 M NaHCO<sub>3</sub>) tests performed in subsequent experiments, NaHCO<sub>3</sub> intake induced by IG 2 M NaCl was inhibited by neither prior gavage of Al(OH)<sub>3</sub> nor the addition of NaHCO<sub>3</sub> to the IG 2 M NaCl. The gavage of either 50% ethanol or 0.6 N acetic acid did not induce any fluid intake. The gavage of either ethanol or acetic acid may have produced some inhibition of fluid intake induced by IG 2 M NaCl, but this did not seem to be a strong effect that disabled the animal. In addition, ethanol induced kaolin intake, but neither 2 M NaCl nor acetic acid induced kaolin intake. The gavage of 2 M NaCl or acetic acid may have produced some stomach irritation ranging from category A (lowest level) to the intermediate categories B and C, but it was not possible to associate any of these two treatments to any specific category. Whereas IG water was associated with category A (rosy stomach mucosa), IG 50% ethanol was associated with category D (petechiae and pinpoint ulcerations), the maximum level of irritation.

Taken together, these results indicate that the effect of IG 2 M NaCl on mineral intake is not selective for isotonic NaCl intake, or associated with stomach irritation or pica.



**Fig. 6.** Kaolin intake by rats that received a gavage (2 ml) of deionized water (H<sub>2</sub>O), 2 M NaCl, 50% ethanol or 0.6 N acetic acid. \*p<0.05 vs. kaolin ingested by other groups. The number of rats is given within brackets.

The results show that, when given the option, a rat that received IG 2 M NaCl chose KCl or NaHCO<sub>3</sub> instead of isotonic NaCl. This is consistent with the rejection of isotonic NaCl obtained with intravenous infusion of hypertonic NaCl in a previous study [18]. Thus, significant ingestion of isotonic NaCl occurs when it is the only alternative to water in two-bottle tests [2–4]. The ingestion of NaHCO<sub>3</sub>, but perhaps not of KCl, is also consistent with the irritation hypothesis. However, different from predicted in the Introduction and for reasons further discussed, the preference for NaHCO<sub>3</sub> or KCl over NaCl was not associated with stomach irritation. These results suggest that in the two-bottle test, the animal looks for and finds some kind of mineral taste in the NaCl solution that is associated with cell-dehydration. However, such taste is apparently more appealing when the cell-dehydrated rat has NaHCO<sub>3</sub> or KCl as options.

Sodium appetite is expressed by selective ingestion of sodium salts, particularly those that have a “salty” flavor like NaCl, and a hedonic shift, characterized by an increased acceptance of hypertonic NaCl [18–23]. It is also expressed several hours after sodium deficiency or when extracellular dehydration is produced experimentally [20,21]. Although the mineral intake by a cell-dehydrated animal contradicts the osmometric theory of thirst [1,24], it is not a behavioral expression of something similar to sodium appetite. First, this is due to the rejection of NaCl in the five-bottle test [18, present work]. Second, the concentration of the ingested NaCl, when such ingestion occurs [2–4], is isotonic at most. Finally, KCl and NaHCO<sub>3</sub> fall into a category of minerals with little or no salty flavor [22,23].

However, the present results reinforce what has been shown repeatedly in the literature: that cell dehydration may produce mineral fluid intake similarly to that produced by early extracellular dehydration, be it in two- or five-bottle tests. Rats also ingest isotonic NaCl in two-bottle tests [2–4] or other palatable mineral solutions, NaHCO<sub>3</sub> among them, showing no preference for NaCl in five-bottle tests in the first hours of extracellular dehydration [18]. In addition, exogenous administration of angiotensin II, a hormone that mediates several important responses to extracellular dehydration including sodium appetite, also induces selective 0.15 M NaHCO<sub>3</sub> intake in five-bottle tests [19]. Moreover, the potassium intake in response to a load of hypertonic NaCl is not related to potassium deficit [18]. Water containing several types of minerals, including sodium and carbonates, is found in natural mineral licks and consumed by several species, including rodents [8,9,25]. Thus, it is possible that brain circuits that control thirst in the cell-dehydrated rat are adapted to command the ingestion of water containing minerals, similar to brain circuits that control thirst in the extracellular-dehydrated rat.

We do not know the reason for the initial transitory preference we may sometimes see for daily NaHCO<sub>3</sub> intake compared to other fluids [18, Fig. 2]. The absence of statistical difference in the remaining days suggests that the animals were tested when they had no spontaneous preference for NaHCO<sub>3</sub>. However, the trend to ingest more of NaHCO<sub>3</sub> solution is intriguing and it could be related to either the concentration of the solutions or to some natural preference for NaHCO<sub>3</sub>. One may speculate that this preference for NaHCO<sub>3</sub> is related to an adaptation to ingest water mixed with sodium and carbonates, similar to those found at mineral licks [8,9,25].

These results also show that, in spite of producing a similar mild irritation of the stomach, IG 2 M NaCl and IG acetic acid produced two fundamentally different effects on behavior. Whereas the former induced the ingestion of two different mineral solutions—including one made of the potential gastric protector NaHCO<sub>3</sub>—the latter produced no significant fluid intake. Acetic acid also produced no significant alteration on fluid intake induced by IG 2 M NaCl, but only IG NaCl/H<sub>2</sub>O, not IG NaCl/Acetic, produced a significant increase in NaHCO<sub>3</sub> intake compared to IG H<sub>2</sub>O/H<sub>2</sub>O. Although we cannot rule out that some sort of behavioral inhibition was produced by acetic acid, the fluid intake induced by IG NaCl/Acetic suggests that the animals are still competent to ingest fluids. Therefore, if the irritation caused by acetic acid was

important to produce mineral fluid intake we would expect at least some significant mineral intake or an intermediate result for IG H<sub>2</sub>O/Acetic when compared to IG H<sub>2</sub>O/H<sub>2</sub>O or H<sub>2</sub>O/NaCl (Fig. 4). Finally, both IG H<sub>2</sub>O/Acetic and H<sub>2</sub>O/NaCl failed to induce kaolin intake, a marker of pica [13–15]. This last result suggests that the mild irritation of the stomach was not associated with malaise. It also suggests that the mineral intake induced by IG 2 M NaCl is not pica.

The gavage with 50% ethanol was more irritating to the stomach than the other two treatments and produced signs of micro-hemorrhage significantly associated with category D irritation (petechiae and pinpoint ulcerations). It also produced kaolin intake. Kaolin intake provides health benefits for an animal intoxicated with chemotherapy drugs [15]. These drugs seem to activate vagal afferents from the duodenum, which, in turn, activate a circuit analogous to the emetic pathways and result in pica [14]. The present results show that ethanol also produces pica, but the mechanisms remain to be elucidated. A candidate mechanism is the aggression to the stomach mucosa. Note that kaolin has aluminum, an active component of formulas for gastric protection used by humans [10,12]. Thus, the ingestion of kaolin in response to stomach irritation might relate to another potential therapeutic property of kaolin and to the possibility that the rat, like other animal species [26,27], also expresses a self-medicative behavior.

Ethanol (IG NaCl/ethanol) ingested less KCl than rats treated IG NaCl/NaCl (Fig. 5). It is possible that, in this case, ethanol induced some inhibition of fluid intake because IG NaCl/H<sub>2</sub>O was not different from IG NaCl/NaCl. However, treatment with ethanol doubled the kaolin intake compared to controls. This suggests that animals that received IG 2 M NaCl plus ethanol, or ethanol alone, were behaviorally competent to respond to gastric irritation. If gastric irritation were a major determinant of mineral fluid intake induction, then ethanol alone should have produced some NaHCO<sub>3</sub> or KCl intake; this did not happen. Thus, contrary to what was predicted in the Introduction, there is hardly any association between stomach irritation and NaHCO<sub>3</sub> intake or kaolin and NaHCO<sub>3</sub> intake. This contradicts what has been suggested about the role for bicarbonate in the wild [8,9,25]. Further work accounting for species, environment and behavioral factors (learning, for example) is necessary for a definitive conclusion.

The osmometric theory of thirst predicts that a cell-dehydrated animal ingests the exact amount of water necessary to correct the increased tonicity of the extracellular fluid [1,24]. The animal with functional kidneys ingests less water than necessary to compensate for the osmotic load because part of this load is eliminated in the urine, revealed by a conspicuous natriuresis [1,4,24]. However, the behavior deviates slightly from what is predicted by the osmometric theory when the rat also has the choice to ingest isotonic NaCl [2–4] or other palatable mineral solutions [18] because the reported behavior is opposite the prediction based on the ingestion of only water [1,24]. It is possible that, when available to be ingested by a cell-dehydrated rat, NaCl works as a marker for the presence of minerals, similar to what it might do for mineral-deficient animals [28]. However, the result is the ingestion of a hypotonic mixture that does not compromise cell volume. Thus, as suggested previously [18,19,29], cell-dehydration, similar to extracellular dehydration, activates mineral intake, in addition to water intake. It might help to rehydrate an animal that has selective dehydration or—when water deprived—double dehydration of the body-fluid compartments [18,19,29].

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