Taste and acceptance of pyrophosphates by rats and mice

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McCaughey SA, Giza BK, Tordoff MG. Taste and acceptance of pyrophosphates by rats and mice. Am J Physiol Regul Integr Comp Physiol 292: R2159–R2167, 2007. First published March 1, 2007; doi:10.1152/ajpregu.00886.2006.—The palatability and taste quality of pyrophosphates were evaluated in a series of behavioral and electrophysiological experiments. In two-bottle choice tests with water, rats strongly preferred some concentrations of Na3HP2O7 and Na4P2O7, moderately preferred some concentrations of K4P2O7 and Fe4(P2O7)3, and were indifferent to or avoided all concentrations of Ca3P2O7 and Na2H2P2O7. The contribution of sodium to the preference for sodium pyrophosphates was ascertained: 1) Rats with a choice between Na3P2O7 and NaCl preferred 1 mM Na3P2O7 to 4 mM NaCl but preferred 40 or 150 mM NaCl to 10 mM Na3P2O7, 2) blocking salt taste transduction by mixing Na3P2O7 with amiloride reduced preferences but did not eliminate them, and 3) three mouse strains (FVB/J, C57BL/6J, and CBA/J) known to differ in sodium preference had the same rank order of preferences for Na3P2O7 and NaCl, but peak preferences were higher for Na3P2O7 than for NaCl. The taste qualities of pyrophosphates were determined by measuring taste-evoked responses of neurons in the nucleus of the solitary tract of rats. Across-neuron patterns of activity for sodium pyrophosphates were similar to that of NaCl but the pattern of Na3HP2O7 plus amiloride was unique from those of sweet, salty, sour, bitter, and umami stimuli. Taken together, the results indicate that the high palatability of some concentrations of Na3HP2O7 and Na4P2O7 is due partially to their salty taste, but there must also be another cause, which may include a novel orosensory component distinct from the five major taste qualities.

two-bottle preference test; gustatory electrophysiology; sodium; taste quality

PYROPHOSPHATES ARE COMPOUNDS with a P2O7 anion. They are available as di-, tri- and tetra-sodium salts (Na2H2P2O7, Na3HP2O7, and Na4P2O7, respectively), as well as several non-sodium forms, and they have several uses. They are used ubiquitously as cat food palatability agents (e.g., Ref. 8) and often as flavor enhancers in meat products for human consumption. Pyrophosphate infusions or marinades increase the moisture content and perceived tenderness, juiciness and/or saltiness of hot-boned pork, sausages, and chicken filets (2, 33, 34). Despite considerable commercial interest in these compounds there has been little effort to understand their gustatory properties. Boudreau et al. (4, 5) found that 50 mM Na3P2O7 increased the firing rates of taste-responsive cells in the petrosal and geniculate ganglia of rats, with larger responses in salt- than acid-sensitive neurons in the geniculate ganglion. These data are consistent with Na3P2O7 tasting salty to the animals, bearing in mind that perceptions of taste quality must be inferred in nonhuman species, and a salty component could be responsible for some or all of the palatability of sodium pyrophosphates (12, 14, 32). On the other hand, gustatory responses can be influenced by the particular anion associated with sodium (23, 35) and the tuning of the neurons described by Boudreau et al. (4, 5) was broad enough to allow for nonsodium components. Thus, sodium pyrophosphates may also have a substantial nonsalty component to their taste.

We conducted a series of experiments to determine the palatability of pyrophosphate solutions in rats and mice and to investigate the mechanisms involved. We first determined the concentration-response functions of rats for three sodium pyrophosphates and three nonsodium pyrophosphates dissolved in water. The results showed that the rats avidly drank two of the sodium pyrophosphates (Na3HP2O7 and Na4P2O7) but were generally indifferent to the other compounds or avoided them, depending on concentration. Next, we examined the contribution of sodium to the response by 1) providing rats with a choice between Na3P2O7 and NaCl, to see whether the animals are capable of discriminating between the two compounds, 2) determining whether rats’ preferences for Na3P2O7 were influenced by amiloride, a sodium-channel blocker thought to reduce the perception of saltiness (17, 26), and 3) comparing the concentration-response functions for Na3HP2O7 in three strains of mice known to differ in NaCl preference. To determine the taste quality perceptions evoked by pyrophosphate compounds, we measured the taste-evoked responses in the nucleus of the solitary tract (NST) in rats. Stimuli thought to evoke similar taste quality perceptions generally evoke similar patterns of activity across NST cells. Therefore, these across-neuron patterns can be correlated with each other and compared using multidimensional scaling to determine how stimuli are perceived by rats (13).

METHODS

General Procedures

Subjects were male Sprague-Dawley rats [Crl:CD(SD)ICSBR] purchased from Charles River Laboratories (Wilmington, MA) or male mice from the FVB/J, C57BL/6J, and CBA/J strains purchased from The Jackson Laboratory (Bar Harbor, ME). Rats and mice were maintained in separate vivariums, each at 23°C with a 12:12-h light-dark cycle. Each rat was housed individually in a 18 × 24 × 18 cm stainless-steel cage with a grid front and floor. Deionized water was available from an inverted 300-ml glass bottle with a neoprene stopper and a stainless-steel drinking spout. The bottle was attached to the front wall of the cage with a steel spring. Rats were given ad libitum access to powdered AIN-76A diet, a semisynthetic diet formulated by the American Institute of Nutrition, which was prepared by Dyets, (catalog no. 100,000, Bethlehem, PA). The food was available from a glass jar (Qorpak, 6-cm diameter × 7.5 cm high), which was held upright by a steel spring. Each mouse was housed in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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individually in a plastic tub cage with pelleted AIN-76A diet to eat and deionized water to drink [see Ref. 27 for details]. At the start of two-bottle tests in rats, a weighed bottle of deionized water and a weighed bottle of pyrophosphate solution were placed on the front of the animal’s cage. The drinking spout tips extended into the cage 5 cm and rested 1 cm above the cage floor and 3 cm apart. The bottle containing pyrophosphate solution was presented on the rat’s left, and the bottle containing water was presented on the rat’s right. At 24 h, the bottles were removed, reweighed, and returned in the opposite position. At 48 h, the bottles were removed and weighed again. All measurements were made to the nearest 0.1 g, and intakes were converted from weights to volumes assuming 1 g was equivalent to 1 ml.

For all two-bottle tests, water and solution intakes for each of the two days of each test were averaged and used in subsequent analyses. Occasionally, the daily intakes of a rat were lost because a bottle was spilled. When this happened, intakes on the other day were used for analyses.

Total fluid intake was calculated from the sum of water intake and solution intake. Solution preference was derived using the formula, Solution preference = 100 × solution intake/total fluid intake. For each pyrophosphate, the “behavioral detection threshold” was calculated based on the lowest concentration at which the animals preferred the pyrophosphate significantly more than 50%, according to two-tailed, one-sample t-tests. Regions of indifference (not different from 50%) and avoidance (significantly less than 50%) were also determined using the same method.

The criterion for statistical significance of all tests was P < 0.05, with Bonferroni corrections for multiple comparisons where appropriate, as described below. Calculations were performed using the software package Statistica 6.1 (Statsoft, Tulsa, OK). Values given in the text are means ± SE.

Preferences for Pyrophosphate Compounds in Rats

This experiment was conducted in two replications involving a total of 84 rats, weighing 368 ± 4 g at the start of the experiment. One replication involved three sodium pyrophosphates, and the other involved a replication of the test with Na4P2O7 (as a control for consistency) and three nonsodium pyrophosphates.

Groups of 11 or 12 rats, were given a choice between water and one of the six pyrophosphate compounds listed in Table 1. The concentrations tested ranged from 0.1 to 100 mM in ½-log steps, except that 0.18 mM sodium pyrophosphates were not tested in the first replication, and the highest concentration of Ca3P2O7 tested was 1.78 mM due to solubility limits. Concentrations of Na2P2O7 of 10 mM and above were hard to dissolve and had to be stirred for several minutes. All of the solutions were clear except for high concentrations of Na4P2O7, which were slightly cloudy. All pyrophosphates were obtained from Sigma-Aldrich (St. Louis, MO) or the SPF-Diana (Elven, France). Fresh pyrophosphate solutions were made before each test using deionized water. The pH of the solutions was measured before each test using deionized water. The pH of the solutions was measured (Table 1).

After 8 days to allow adaptation to vivarium conditions, each rat received a 48-h test with two bottles of deionized water, which was considered to be a 0 mM concentration of the pyrophosphate compound. The animal then received a choice between deionized water and ascending concentrations of one of the pyrophosphates. When the test of one concentration was complete, the test of the next concentration in the series began, using fresh bottles of both pyrophosphate solution and water.

To compare mean intakes or preferences for different pyrophosphates, we used two-way mixed-design ANOVAs, with factors of pyrophosphate type and concentration. Because not all concentrations of Ca3P2O7 were tested, this group was not included in the omnibus ANOVA, and a separate ANOVA involving just the concentrations given to this group was conducted. Individual pairs of means were compared using Tukey’s post hoc tests.

Choice Between Na3P2O7 and NaCl

Subjects were 16 rats used previously in the test of Na3P2O7 and nonsodium pyrophosphates (four from each group). The animals weighed 565 ± 16 g at the start of the experiment. Each rat received three separate 48-h three-bottle choice tests, in which the middle bottle contained water. Initially, the left bottle contained Na3P2O7 solution and the right bottle contained NaCl solution; their positions were switched after 24 h. The three tests involved a choice between: 1) 1 mM Na3P2O7, 4 mM NaCl, and water; 2) 10 mM Na3P2O7, 40 mM NaCl, and water; or 3) 10 mM Na3P2O7, 150 mM NaCl, and water. The concentrations for tests 1 and 2 were chosen to match sodium content of the two compounds, and the concentrations for test 3 are near those that are maximally preferred by rats (see below for Na3P2O7, Refs. 10 and 32 for NaCl). All rats received all three combinations of taste solutions in a counterbalanced order. There were 1–3 days between tests, during which water was the only fluid available.

Solution intakes were compared within each test using one-way ANOVAs. Preferences were calculated for each solution as a proportion of total solution intake excluding water.

Addition of Amiloride to Na3P2O7

First, we determined a concentration of amiloride that would reduce intake of Na3P2O7. Subjects were 12 rats used previously to test sodium pyrophosphates and now weighing 467 ± 9 g. They received three counterbalanced 48-h two-bottle tests in which there was a choice between water and 10 mM Na3P2O7 mixed with 0, 10, or 100 μM amiloride. The results indicated that the addition of 100 μM amiloride reduced Na3P2O7 intake and preference so additional

Table 1. Compounds tested, their chemical formula, pH, and the concentrations at which they are detected, most preferred, and avoided

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>pH of 1 mM Solution*</th>
<th>Behavioral Detection Threshold, mM</th>
<th>Peak Preference, mM</th>
<th>Avoidance Threshold, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium pyrophosphate</td>
<td>Na2H3P2O7</td>
<td>4.9</td>
<td>31.6</td>
<td>none</td>
<td>31.6</td>
</tr>
<tr>
<td>Trisodium pyrophosphate</td>
<td>Na3HP2O7</td>
<td>7.3</td>
<td>0.10</td>
<td>17.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Tetrasodium pyrophosphate</td>
<td>Na4P2O7</td>
<td>9.9</td>
<td>0.10†</td>
<td>17.8†</td>
<td>75.0‡</td>
</tr>
<tr>
<td>Tetaferic pyrophosphate</td>
<td>Fe2(HP2O7)</td>
<td>6.8</td>
<td>5.62</td>
<td>5.62</td>
<td>56.2</td>
</tr>
<tr>
<td>Tetrapotassium pyrophosphate</td>
<td>K3P2O7</td>
<td>9.9</td>
<td>0.10</td>
<td>0.10</td>
<td>31.6</td>
</tr>
<tr>
<td>Dicalcium pyrophosphate</td>
<td>Ca2P2O7</td>
<td>1.8</td>
<td>0.18</td>
<td>none</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*pH was measured both before and after each 48-h test and was stable. At higher concentrations, pH varied little from the values obtained with 1-mM solutions.
†Same value in both experiments.
‡Average of logarithms of values from two experiments (56.2 mM in experiment 1, 100 mM in experiment 2). Behavioral detection threshold is the lowest concentration at which pyrophosphate preference differed significantly from 50% (i.e., indifference). Peak preference is the concentration at which the highest pyrophosphate preference was recorded. Avoidance threshold is the lowest concentration at which pyrophosphate preference was significantly lower than 50%.
tests were conducted using 12 naive rats, weighing 298 ± 4 g. These animals received four counterbalanced 48-h two-bottle tests with a choice between water and 100 μM amiloride mixed with 0, 1, 10, or 100 mM Na₃P₂O₇.

Intakes and preferences were analyzed using one-way within-subjects ANOVAs.

**Mouse concentration-intake functions for Na₃HP₂O₇ and NaCl.** In this experiment, we compared the concentration-intake functions of FVB/J, C57BL/6J, and CBA/J mice given Na₃HP₂O₇ and NaCl. We used these three strains of mice because they differ markedly in their avidity for NaCl (3, 28). Ten male mice from each strain arrived in the lab at 6 wk of age. After ~7 days to adapt to vivarium conditions, all mice began two series of 48-h two-bottle choice tests. In the first series, they received a choice between two drinking tubes of water, followed by water and ascending concentrations of Na₃HP₂O₇ from 0.1 to 100 mM in 1/2-log steps. In the second series, they received a choice between two drinking tubes of water, followed by a choice of water and ascending concentrations of NaCl, from 1.0 to 562 mM NaCl in 1/2-log steps. There was a 4-day period between the two series in which the mice received water alone.

Details of drinking tube construction, cage layout, and daily test procedures are outlined in detail elsewhere (3, 28). Every 24 h, fluid intakes were measured volumetrically (± 0.1 ml), and the position of drinking tubes was switched to control for side preferences. Data were analyzed in a similar manner as for the rat experiments described earlier, except that strain was included as a between-subject factor in ANOVAs, and Tukey’s tests were conducted post hoc to determine which stimulus concentrations were responsible for significant ANOVAs.

**Taste-evoked responses of neurons in the nucleus of the solitary tract.** Twenty male Sprague-Dawley rats that weighed 469 ± 18 g were used for neural recording. They were anesthetized using urethane (1.5 g/kg ip, with further doses as necessary). The surgical procedure has been described in detail elsewhere (21). Briefly, a tracheotomy was performed to prevent suffocation, and a fistula was inserted into the esophagus to prohibit ingestion of stimuli. The head was secured in a nontraumatic head holder, and the surface of the medulla was exposed. The activity of single units in the rostral NST was isolated using glass microelectrodes filled with 1.6 M potassium citrate. The signal was amplified, filtered, displayed on an oscilloscope, and stored on videotape for off-line analysis. Body temperature was maintained at 35–37°C, and subcutaneous electrodes were used to monitor heart rate.

After the activity of a single neuron was isolated, responses were recorded to a broad stimulus array that included the compounds listed in Table 5. In 11 of these cells, plus in one additional neuron, at least part of a concentration series of Na₃HP₂O₇ ranging from 0.033 to 100 mM in half-log molar steps was presented in ascending order. All compounds were mixed in distilled water, with the exception of sucrose and glucose, to which 10% tap water was added to ensure activation of an automatic stimulus onset marker.

The stimulus delivery procedure followed that of Chang and Scott (9). Five milliliters of each solution was presented as a room-temperature spray that contacted the entire tongue and oral cavity at a rate of 1 ml/s. Stimulus presentations were separated by at least a minute and were followed by at least 20 ml of deionized water as a rinse. Additional time and rinse were used after application of stimuli mixed in amiloride. Solutions were given in a semirandom order, in which compounds likely to share taste qualities were not presented consecutively to avoid adaptation effects. Stimuli were sometimes presented more than once, in which cases responses were averaged across all applications.

Action potentials were counted with a window discrimination program created using the DasyLab software package (Dasytec, Amherst, NH). Interspike intervals were calculated to ensure that there was a clear refractory period, indicating the presence of only one neuron at a time. Activity was monitored for 3 s before stimulus onset to determine spontaneous firing rate and for 5 s afterward to determine evoked firing. Responses were then expressed as net spikes/s (evoked minus spontaneous). A detailed analysis of the temporal patterns of responding did not reveal any important information, so they are not presented.

The responses to some compounds were compared against each other using paired t-tests, with a Bonferroni correction made for the number of comparisons (α = 0.008, two tailed). The threshold response for Na₃HP₂O₇ was determined by calculating a 95% confidence interval for each concentration that was used, and threshold was defined as the lowest concentration for which the mean response was larger than this confidence interval. Across-neuron profiles of responding to different stimuli were compared with each other by calculating Pearson correlation coefficients. On the basis of the resulting correlation matrix, a multidimensional space was generated in which stimuli with similar across-neuron profiles were located close to each other. Two dimensions were used for the space because adding a third dimension resulted in only a small reduction in statistical stress.

**RESULTS**

Preferences of rats for pyrophosphate compounds. The concentration at which each pyrophosphate first elicited a change in preference ranged from 0.1 to 31.6 mM, depending on the pyrophosphate tested (Table 1). For Na₃HP₂O₇, Na₄P₂O₇, Fe₄(P₂O₇)₃, and K₄P₂O₇, at least one concentration was preferred significantly above indifference (Fig. 1, Table 2). Na₂H₂P₂O₇ and Ca₃P₂O₇ were never preferred. High concentrations of all pyrophosphates were avoided relative to water, with a very low threshold for avoidance of Ca₃P₂O₇ (0.18 mM) and more moderate values for the other pyrophosphates (31.6–100 mM).

In the tests of the three sodium pyrophosphates, rats drank significantly more Na₂P₂O₇ and Na₃HP₂O₇ than Na₂H₂P₂O₇ at all concentrations above 1 mM and below 100 mM, and they drank significantly more 10, 17.8, and 31.6 mM Na₂H₂P₂O₇ than equivalent concentrations of Na₃HP₂O₇. Water intakes showed an almost reciprocal pattern so that, in most cases, total fluid intake was similar and more-or-less constant among the three groups (see Fig. 1). However, for the 10, 17.8, and 31.6 mM concentrations, rats given Na₃HP₂O₇ had significantly higher total fluid intakes than for other concentrations, and significantly higher total fluid intakes than did rats given Na₂H₂P₂O₇ (at 10 and 17.8 mM) or Na₄P₂O₇ (at 10 and 31.6 mM).

In the second replication, preferences for Na₄P₂O₇ were similar to those obtained in the first replication (Fig. 1). The rats drank statistically indistinguishable volumes of Na₂P₂O₇ and Fe₄(P₂O₇)₃ at all concentrations, and more of 5.62–56.2 mM concentrations of these two pyrophosphates than corresponding concentrations of K₄P₂O₇. They drank less Ca₃P₂O₇ than the other solutions at all concentrations tested except 0.1 mM. Water intakes generally showed a reciprocal pattern to pyrophosphate intakes. However, water intakes of rats with access to Na₂P₂O₇ were significantly lower than those with access to all concentrations of Fe₄(P₂O₇)₃ and those with access to 0.56–31.6 mM K₄P₂O₇. There were no significant differences among the groups in total fluid intake with the exception that total fluid intakes were significantly higher in the group given Fe₄(P₂O₇)₃ than the group given K₄P₂O₇. The following significant differences were present in preference scores: The group given Na₄P₂O₇ had higher preferences than Na₃HP₂O₇.
the other three groups at all concentrations between 0.56 and 56.2 mM. The group given Ca$_2$P$_2$O$_7$ had significantly lower preferences than the other three groups at all concentrations it was tested with, except 0.1 mM. The groups receiving Fe$_4$(P$_2$O$_7$)$_3$ and K$_4$P$_2$O$_7$ had similar preferences at all concentrations except for 31.6 mM, at which preferences for Fe$_4$(P$_2$O$_7$)$_3$ were significantly higher.

Choice between Na$_4$P$_2$O$_7$ and NaCl. In the test with a choice between water, 1 mM Na$_4$P$_2$O$_7$ and 4 mM NaCl, the rats drank significantly more Na$_4$P$_2$O$_7$ than NaCl, and significantly more NaCl than water [Fig. 2; $F(2,30) = 190.2$, $P < 0.001$]. The preference ratio for Na$_4$P$_2$O$_7$ relative to NaCl was 67%, which was significantly greater than indifference (50%). In both tests with 10 mM Na$_4$P$_2$O$_7$ and NaCl, the rats preferred the NaCl. They drank significantly more 40 mM NaCl than 10 mM Na$_4$P$_2$O$_7$, $F(1,30) = 24.4$, $P < 0.001$, and significantly more 150 mM NaCl than Na$_4$P$_2$O$_7$, $F(2,30) = 20.3$, $P < 0.001$. The preference ratio for 10 mM Na$_4$P$_2$O$_7$ relative to 40 mM NaCl was 37%, and it was 35% for 10 mM Na$_4$P$_2$O$_7$ relative to 150 mM NaCl, both of which were significantly less than indifference.

Addition of amiloride to Na$_4$P$_2$O$_7$. Intake of 10 mM Na$_4$P$_2$O$_7$ + 100 μM amiloride was not significantly different than intake of 10 mM Na$_4$P$_2$O$_7$ alone. In contrast, intake of 10 mM Na$_4$P$_2$O$_7$ + 100 μM amiloride was significantly reduced (Fig. 3), $F(2,22) = 3.48$, $P = 0.04$. Preference scores were significantly lower for the solution containing 100 μM amiloride (48 ± 7%) than for the solution without amiloride (68 ± 7%), $F(2,22) = 6.27$, $P = 0.007$. The preference score for the intermediate concentration (55 ± 7%) did not differ significantly from either of the other two scores.

In tests with 100 μM amiloride mixed with Na$_4$P$_2$O$_7$, there were significant effects of the concentration of Na$_4$P$_2$O$_7$ on intake, $F(3,33) = 3.05$, $P = 0.04$, and preference, $F(3,33) = 3.65$, $P = 0.02$. Intake of the 10 mM Na$_4$P$_2$O$_7$ + 100 μM amiloride mixture was significantly greater than intake of any of the other mixtures or amiloride alone (Fig. 3). Similarly, preference for this mixture (39 ± 6%) was significantly higher than for the other taste solutions (in ascending concentration, 24 ± 4%, 24 ± 5%, and 21 ± 4%). Thus, 10 mM Na$_4$P$_2$O$_7$ increased solution intake and preference even when mixed with amiloride. It should be noted that 100 μM amiloride presented...
alone was strongly avoided by our rats (Fig. 3, right), even though rats are supposedly unable to taste this concentration (17, 20). The reason for this discrepancy is unclear, although we suspect it may be due to differences in test procedures. Earlier studies have involved brief exposures to amiloride, whereas our result is based on a 48-h preference test. The preference test is arguably a more sensitive measure. It also allows more opportunity for postingestive and learned effects to influence intake, including the effects of amiloride on renal function. Other potential causes for the difference between studies include differences in the genetic makeup of the animals, the type of diet they were fed, and the prior environmental conditions to which they were exposed.

Comparison of $\text{Na}_3\text{HP}_2\text{O}_7$ and $\text{NaCl}$ consumption between strains was complicated by differences in total fluid intake, with the C57BL/6J strain generally drinking more than the FVB/J and CBA/J strains, except where intake of $\text{Na}_3\text{HP}_2\text{O}_7$ was high in the FVB/J strain (Table 4 and Fig. 4). Therefore,

![Figure 3. Intake of water and $\text{Na}_3\text{P}_2\text{O}_7$ solutions mixed with amiloride in two-bottle choice tests. Left: intake of water (○) and various concentrations of amiloride mixed with 10 mM $\text{Na}_3\text{P}_2\text{O}_7$ (•). Right: intake of water (○) and 100 μM amiloride mixed with various concentrations of $\text{Na}_3\text{P}_2\text{O}_7$ (•).]

![Figure 4. Intakes and preferences of FVB/J (○), C57BL/6J (•), and CBA/J (▲) mice for various concentrations of trisodium pyrophosphate ($\text{Na}_3\text{HP}_2\text{O}_7$; left) and $\text{NaCl}$ (right).]

Table 3. Concentrations of $\text{Na}_3\text{HP}_2\text{O}_7$ and $\text{NaCl}$ that mice detected, found most preferred, and avoided

<table>
<thead>
<tr>
<th>Strain</th>
<th>Taste Solution</th>
<th>Behavioral Detection Threshold, mM</th>
<th>Peak Preference, mM</th>
<th>Avoidance Threshold, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA/J</td>
<td>$\text{Na}_3\text{HP}_2\text{O}_7$</td>
<td>0.56</td>
<td>17.8</td>
<td>56.2</td>
</tr>
<tr>
<td></td>
<td>$\text{NaCl}$</td>
<td>56.2</td>
<td>none</td>
<td>56.2</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>$\text{Na}_3\text{HP}_2\text{O}_7$</td>
<td>5.62</td>
<td>17.8</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>$\text{NaCl}$</td>
<td>100.0</td>
<td>100.0</td>
<td>316.0</td>
</tr>
<tr>
<td>FVB/J</td>
<td>$\text{Na}_3\text{HP}_2\text{O}_7$</td>
<td>0.31</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>$\text{NaCl}$</td>
<td>17.8</td>
<td>178.0</td>
<td>562.0</td>
</tr>
</tbody>
</table>

Behavioral detection threshold is the lowest concentration at which pyrophosphate preference differed significantly from 50% (i.e., indifference). Peak preference is the concentration at which the highest pyrophosphate preference was recorded. Avoidance threshold is the lowest concentration at which pyrophosphate preference was significantly lower than 50%.
only the preference results are described here. Relative to the other two strains, the FVB/J strain had significantly higher Na3HP2O7 preferences for all concentrations between 0.32 and 56.2 mM, except for the 0.56 mM concentration. This strain also had significantly higher NaCl preferences for all concentrations of NaCl between 10 and 316 mM except for 56.2 mM, for which the FVB/J and C57BL/6J strains did not differ. Relative to the CBA/J group, the C57BL/6J group had higher preferences for 31.6 and 56.2 mM Na3HP2O7, 31.6 mM NaCl, and 100–316 mM NaCl. At other concentrations, preference scores of the three strains did not differ.

Peak Na3HP2O7 intakes and preferences were substantially higher than peak NaCl intakes and preferences for two of the strains. Specifically, the FVB/J strain drank almost twice as much 10 mM Na3HP2O7 than 178 mM NaCl (6.09 ± 0.51 ml/day vs. 3.21 ± 0.33 ml/day; preferences, 96 ± 1% vs. 78 ± 5%). The CBA/J strain drank 3.32 ± 0.32 ml/day of 17.8 mM Na3HP2O7 but only 2.17 ± 0.19 ml/day of 3.16 mM NaCl (preferences, 79 ± 7% vs. 59 ± 5%). The difference in the C57BL/6J strain was in the same direction but less pronounced (maximum intakes, 3.82 ± 0.22 ml/day of 17.8 mM Na3HP2O7 vs. 3.15 ± 0.16 ml/day of 100 mM NaCl, preferences, 78 ± 2% vs. 62 ± 4%).

Taste-evoked responses of neurons in the nucleus of the solitary tract. Table 5 shows the mean responses across the 30 NST cells in which the array of 15 stimuli was applied. The mean response evoked by NaCl was largest and differed significantly from responses to Na3HP2O7, t(29) = 4.4, P < 0.001 but not to Na4P2O7 or NaH2P2O7. In addition, the mean response to KCl was significantly larger than the response to K4P2O7, t(29) = 3.4, P = 0.002. The response to the concentration series of Na3HP2O7 is shown in Fig. 5. The threshold for a neural response was 3.3 mM.

Figure 6 shows a two-dimensional space in which stimuli were compared with each other based on their across-neuron patterns of activity. The sodium pyrophosphate compounds mixed in water were grouped with stimuli that are thought to taste salty, and K2P2O7 was located close to a grouping of sour and bitter stimuli that included KCl. MSG mixed in amiloride was not located near any of the sweet, salty, sour, or bitter compounds, as is appropriate for an umami-tasting stimulus. The location of Na3HP2O7 mixed in amiloride was distinct from all of the other compounds.

DISCUSSION

Preference tests. The results indicate that some pyrophosphate solutions are highly palatable to rats and mice. Of the three sodium pyrophosphates tested, two, Na3HP2O7 and Na3P2O7, were highly preferred by rats at some concentrations. We note that the preferences were sufficiently strong to drive total fluid intake, which is rare in preference tests except for those involving NaCl or sweet compounds. These findings suggest that Na2HP2O7 and Na3P2O7 have a strong positive hedonic impact to rats. They are good-tasting in their own right, in addition to their previously characterized ability to

Table 5. Taste stimuli that were applied in the gustatory electrophysiology experiment, their abbreviations, and means ± SE net responses across all 30 cells

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Abbreviation</th>
<th>Mean Net Response, spikes/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mM NaCl</td>
<td>NA</td>
<td>35±5</td>
</tr>
<tr>
<td>500 mM sucrose</td>
<td>SUC</td>
<td>14±3</td>
</tr>
<tr>
<td>10 mM HCl</td>
<td>HCL</td>
<td>26±5</td>
</tr>
<tr>
<td>10 mM quinine HCl</td>
<td>Q</td>
<td>10±2</td>
</tr>
<tr>
<td>10 mM disodium inosine 5° monophosphate</td>
<td>IMP</td>
<td>16±2</td>
</tr>
<tr>
<td>100 mM monosodium glutamate</td>
<td>MSG</td>
<td>30±5</td>
</tr>
<tr>
<td>100 mM monosodium glutamate + 100 μM amiloride</td>
<td>MSGAM</td>
<td>12±2</td>
</tr>
<tr>
<td>1 M glucose</td>
<td>GLU</td>
<td>14±2</td>
</tr>
<tr>
<td>10 mM citric acid</td>
<td>CI</td>
<td>29±6</td>
</tr>
<tr>
<td>100 mM KCl</td>
<td>KCL</td>
<td>16±3</td>
</tr>
<tr>
<td>50 mM Na2H2P2O7</td>
<td>NA2P</td>
<td>33±4</td>
</tr>
<tr>
<td>33 mM Na3HP2O7</td>
<td>NA3P</td>
<td>24±4</td>
</tr>
<tr>
<td>33 mM Na3HP2O7 + 100 μM amiloride</td>
<td>NA3AM</td>
<td>10±1</td>
</tr>
<tr>
<td>25 mM Na3P2O7</td>
<td>NA4P</td>
<td>28±4</td>
</tr>
<tr>
<td>25 mM K2P2O7</td>
<td>KP</td>
<td>9±1</td>
</tr>
</tbody>
</table>

(Milliamiloride) 2009 4%)

Table 4. Results of omnibus analyses of variance for the experiment comparing concentration-intake functions of three strains of mice

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Strain</th>
<th>Concentration</th>
<th>Strain × Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na3HP2O7</td>
<td>(df)</td>
<td>(2.27)</td>
<td>(13.351)</td>
</tr>
<tr>
<td>Water</td>
<td>10.65†</td>
<td>79.10†</td>
<td>4.01†</td>
</tr>
<tr>
<td>Solution</td>
<td>9.36†</td>
<td>22.08†</td>
<td>6.29†</td>
</tr>
<tr>
<td>Total fluid intake</td>
<td>2.57</td>
<td>43.15†</td>
<td>2.49†</td>
</tr>
<tr>
<td>Preference</td>
<td>13.03†</td>
<td>55.10†</td>
<td>5.15†</td>
</tr>
<tr>
<td>NaCl</td>
<td>(df)</td>
<td>(2.27)</td>
<td>(13.324)</td>
</tr>
<tr>
<td>Water</td>
<td>3.48*</td>
<td>12.61†</td>
<td>5.96†</td>
</tr>
<tr>
<td>Solution</td>
<td>14.68†</td>
<td>15.22†</td>
<td>2.58†</td>
</tr>
<tr>
<td>Total fluid intake</td>
<td>7.59†</td>
<td>6.83†</td>
<td>2.59†</td>
</tr>
<tr>
<td>Preference</td>
<td>12.59†</td>
<td>17.72†</td>
<td>4.40†</td>
</tr>
</tbody>
</table>

Numbers in the body of the table are F values. *P < 0.05; †P < 0.002.
improve the physical attributes and flavor of food (see introduction).

A major interpretative issue was whether the preferences for Na₅H₂P₂O₇ and Na₄P₂O₇ were due to the inherent preference of rats for sodium or salty taste, to a preference for the pyrophosphate anion, or some interaction between cation and anion. Several results suggest that these preferences were not due simply to the sodium. First, the most preferred concentration of sodium is ~150 mM (10, 32), but the most preferred concentration of both Na₃HP₂O₇ and Na₄P₂O₇ was 17.8 mM, which contains only 53 or 71 mM Na⁺, respectively. Second, rats did not prefer Na₃H₂P₂O₇ at any concentration. Third, at least two nonsodium pyrophosphates, Fe₅(P₂O₇)₃ and K₄P₂O₇, were preferred significantly over water at some concentrations. The strongest avoidances were for Ca₃P₂O₇ and Na₃H₂P₂O₇, which unlike the other compounds were acidic. It is thus possible that acidity (or a related cause of sourness) was responsible for this avoidance to some extent, although determination of the compounds’ titratable acidity, in addition to their pH, would be important to resolve the issue.

To assess the relative palatability of sodium paired with pyrophosphate and chloride, we allowed rats to choose between them. Intake of the two compounds appeared to be independent, and the concentrations used determined which one was preferred. Intriguingly, rats preferred a low concentration (4 mM Na⁺) of Na₃P₂O₇ to NaCl but a high concentration (40 mM Na⁺) of NaCl to Na₃P₂O₇. This was not simply because the rats could not taste 4 mM NaCl because they drank significantly more of this solution than water. Instead, the finding that the rats preferred 1 mM Na₄P₂O₇ to 4 mM NaCl suggests that either low concentrations of Na₄P₂O₇ have a taste (or other positive attribute) that is more preferred than sodium, or that the pyrophosphate somehow enhances the saltiness (or other attribute) of sodium.

In contrast to the results with low concentrations, rats preferred high concentrations of NaCl to equisodium concentrations of Na₄P₂O₇. It is unlikely that this was because 10 mM Na₄P₂O₇ was too intense because this concentration and higher ones were preferred to water (Table 1). Perhaps high concentrations of Na₄P₂O₇ have negative secondary taste qualities that are observed only in comparisons with NaCl. Alternatively, NaCl may taste more palatable at higher concentrations. Whatever the explanation, these data suggest that rats do not choose to drink Na₅P₂O₇ based solely on its sodium content.

Amiloride at 100 μM lowered preferences for 10 mM Na₅P₂O₇ relative to the value for the solution presented without amiloride. On the basis of prior work, this may have occurred because of amiloride eliminating the salty taste of the compound (17, 26), which would suggest that saltiness is one of the factors that drives intake of Na₅P₂O₇ by rats. Alternatively, it may have occurred, in part, because of amiloride bitter taste, given that we observed that rats avoided 100 μM amiloride when it was presented with water. Our finding that rats preferred a mixture of 10 mM Na₅P₂O₇ with 100 μM amiloride to 100 μM amiloride alone supports the possibility that Na₄P₂O₇ has a nonsalty component to its taste that enhances its palatability. However, we cannot rule out the possibility that a small amount of saltiness could remain in the amiloride-Na₅P₂O₇ mixture.

All three mouse strains tested preferred some concentrations of Na₃HP₂O₇ to water, and in FVB/J mice preferences were over 95%. The rank order of preferences across the strains was similar for Na₃HP₂O₇ and NaCl for most concentrations. This is consistent with salty taste being one of the factors that influences the palatability of Na₃HP₂O₇ for mice, although stronger evidence would be provided by testing a larger number of strains and finding a significant correlation between preferences for Na₃HP₂O₇ and NaCl. It also suggests that saltiness was not the only factor that determined how Na₄HP₂O₇ was preferred, since the peak preference for all of the three strains was higher for Na₄HP₂O₇ than for NaCl. Most notably, the CBA/J mice preferred Na₃HP₂O₇ at some concentrations despite showing only indifference or avoidance to NaCl. Furthermore, all of the three strains had much lower behavioral response thresholds to Na₃HP₂O₇ than NaCl, even if concentrations were expressed in terms of sodium content rather than molarity.

Given that our preference tests were conducted over 48 h, postigestive effects must have contributed to the results. Na₃HP₂O₇ and Na₄P₂O₇ may have been highly preferred because of positive effects after they were ingested, although we cannot specify their nature. Pyrophosphates decrease intestinal transit times in humans (1) and influence the absorption of roughage by cattle (16). They have a chelating action that can result in hypocalcemia in rats (30) and play a critical role in bone formation (22). One intriguing possibility is that the phosphate contained in the pyrophosphate anion acted to increase production of ATP, which provided energy to the animals and served to reinforce further ingestion, much in the way that gastric infusion of carbohydrates is able to condition preferences for noncaloric solutions (24). However, such an explanation does not explain why Na₃H₂P₂O₇ was preferred to a lesser extent than matched concentrations of the other two sodium pyrophosphates, since all three contained equal amounts of phosphate.

**Gustatory electrophysiology.** The responses of NST cells to pyrophosphate compounds were determined predominantly by the cation that was associated with them. The sodium pyrophosphates evoked across-neuron patterns that were similar to those of other sodium-containing compounds, whereas the pattern for K₃P₂O₇ was similar to that of KCl. The pattern for Na₃H₂P₂O₇ was not especially similar to those of HCl and citric acid, despite its low pH. These data suggest that sodium...
pyrophosphates taste primarily salty and $K_2P_2O_7$ tastes primarily sour and/or bitter to rats. The across-neuron profiles of Na$_3$HP$_2$O$_7$ and Na$_4$P$_2$O$_7$ differed from those of other highly preferred stimuli (e.g., the sugars and MSG + amiloride).

Na$_3$HP$_2$O$_7$ appeared to be dominated by a salty taste when it was mixed in water. However, when this stimulus was mixed with amiloride to block its saltiness (17, 25), the result was a taste that was distinct from those of sweet, salty, sour, bitter, and umami stimuli. If the nonsalty component of its taste had been due primarily to amiloride-insensitive transduction of sodium, such as passage through VR-1 channels (19), then the across-neuron profile of Na$_3$HP$_2$O$_7$ plus amiloride should have been similar to those of sour and bitter compounds [e.g., KCl (6, 25)]. However, this was not the case. Presumably, the unique taste of Na$_3$HP$_2$O$_7$ plus amiloride arose from the nonsodium component of the molecule, suggesting that P$_2$O$_7^{4-}$ acts on a separate gustatory transduction mechanism than those associated with the five prototypical taste qualities. It is unclear, though, what impact transduction mechanisms activated by P$_2$O$_7^{4-}$ would have had in the behavioral studies in which amiloride was not applied, given that Na$_3$HP$_2$O$_7$ presented without amiloride appeared to taste primarily salty, rather than to have both salty and nonsalty tastes.

Although the taste quality evoked by pyrophosphate compounds appeared to be dominated by their cation, there was an influence of the anion on the magnitude of the neural response. NST responses to Na$_3$HP$_2$O$_7$ were significantly smaller than those to NaCl, and responses to $K_2P_2O_7$ were significantly smaller than those to KCl, despite the compounds being matched for cation concentration. These data are consistent with previous work in which larger anions resulted in smaller taste-evoked responses for sodium-containing compounds (31, 35), possibly due to a poorer penetration through tight junctions between taste receptor cells as anion size increases (11). The use of P$_2$O$_7^{4-}$ or H$_2$P$_2$O$_7^{2-}$ paired with sodium (Na$_2$P$_2$O$_7$ or Na$_4$H$_2$P$_2$O$_7$, respectively), though, did not result in smaller responses compared with those to Cl$^-$ paired with sodium (NaCl). These data suggest that Na$_3$HP$_2$O$_7$ tastes less intense than NaCl, whereas Na$_3$P$_2$O$_7$ and Na$_4$H$_2$P$_2$O$_7$ do not. Perceived intensity can sometimes affect the palatability of solutions, but intensity differences do not provide a good explanation for why Na$_3$HP$_2$O$_7$ is more highly preferred than NaCl and Na$_3$HP$_2$O$_7$, given the wide range of concentrations over which differences between the compounds are observed.

**Perspectives**

Despite their common use in human and animal foods, there is virtually nothing known about the chemosensory properties of pyrophosphates. The results presented here are a first step toward their characterization. Overall, there were several indications that the saltiness of the compounds contributed to their palatability. First, none of the nonsodium pyrophosphates tested were preferred to the same extent as Na$_3$HP$_2$O$_7$ and Na$_4$P$_2$O$_7$. Second, preferences for Na$_4$P$_2$O$_7$ were reduced when it was mixed with amiloride. Third, mouse strains that had higher preferences for NaCl also preferred Na$_3$HP$_2$O$_7$. Fourth, the pattern of activity across NST cells was similar for NaCl, Na$_3$HP$_2$O$_7$, and Na$_4$P$_2$O$_7$, indicating that the taste quality evoked by all three is primarily salty.

However, there were also signs that the animals did not respond solely to the sodium cation. First, Na$_3$H$_2$P$_2$O$_7$ was not preferred by rats at any concentration. Second, when rats were given a choice between Na$_4$P$_2$O$_7$ and NaCl, they consumed different volumes of the solutions, even when they were matched for sodium content. Third, preferences for Na$_4$P$_2$O$_7$ mixed in amiloride, which presumably lacked salty taste, depended on concentration. Fourth, mice preferred Na$_3$HP$_2$O$_7$ more avidly than they did NaCl, with the CBA/J strain showing preferences for Na$_3$HP$_2$O$_7$ but never for NaCl. Moreover, the peak preferences for Na$_3$HP$_2$O$_7$ were far below the expected 150-mM peak preference for NaCl.

It remains to be determined what nonsalty quality of Na$_3$HP$_2$O$_7$ and Na$_4$P$_2$O$_7$ was responsible for them being preferred to such a large extent. The responses of NST cells to Na$_3$HP$_2$O$_7$ mixed in amiloride indicate that the nonsalty component to its taste was not sweet or umami, but rather a taste quality that was distinct from the five prototypical tastes.

We note that pyrophosphates interfere with the metabolism of inositol-1,4,5-trisphosphate in catfish taste cells (18), and this is a second messenger for taste transduction. Thus, it is feasible that oral pyrophosphates may interfere with chemosensory transduction, although there is no evidence to our knowledge that they can gain entry into taste cells.

It is important to remember that the taste properties of mixtures of compounds are sometimes not equal to the sum of their individual components’ properties (15). Thus, it is difficult to predict how the addition of pyrophosphates to complex foods would affect their taste quality. Sodium salts with chloride, acetate, and gluconate anions block perceived bitterness, thereby increasing palatability, in human subjects (7), and NaCl also blocks bitter taste in hamsters (15). It is possible that sodium pyrophosphates will do the same. The taste-modifying effects, moisture-retaining effects, and other physical characteristics of pyrophosphates (1, 33, 34) presumably act in concert with their high palatability to increase food liking, at least in rodents.

**GRANTS**

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**REFERENCES**


