Pyrophosphates and Cat Food Palatability

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Introduction

Because domestic cats are extremely sensitive to the palatability of their foods, it is necessary to understand the mechanisms underlying cat preferences. Food preferences may be influenced by several factors including nutritional needs, sensorial characteristics, as well as previous feeding experience. Pyrophosphate salts are well known ingredients used in cat food. Given their importance, we sought to understand their mode of action by two different approaches: the effect of previous feeding experience and the search for specific taste receptors.

Materials and Methods

In order to investigate the influence of cats' exposure to pyrophosphate in terms of taste preference, we bred 3 groups of cats on 3 different diets. The queens of the first group were fed with a control diet, containing no pyrophosphate; the queens of the second group were fed with a diet containing pyrophosphate, and the queens of the third group were fed on a diet containing monosodium glutamate. The cats bred for this study were raised on their specific diets, and trained to perform two pan palatability tests. After a qualification phase for palatability testing, we evaluated the initial preference of the cats for the 3 different diets, using a standard two-pan test. We also evaluated the persistence or modification of this preference over time, and the role of the palatability enhancer associated with the pyrophosphate in its recognition by the animals.

In parallel, other studies have led us to believe that the taste system is involved in cats' perception of pyrophosphate, with a special interest in the feline taste receptor T1R1/T1R3. The molecular and biochemical study has been based on the heterologous expression of the umami feline taste receptor (T1R1/T1R3) transferred into human embryonic kidney cell line (HEK 293). This cell line was transfected with the polynucleotides encoding the feline T1R1/T1R3 receptor and the G protein, G-α15. The cells containing T1R1/T1R3 receptors were loaded with fura-2, a sensitive calcium indicator dye that measures intracellular calcium concentration. We measured the responses of these cells to stimulation using fluorescence ratiometric determination of intracellular calcium. When a receptor was activated, an increased calcium activity was indicated by a dye signal. The activation of the receptors was measured by the visualisation of the calcium flux, in the presence of difference tasty solutions (amino acids, PP alone or association of both). The cells expressed a robust response to isoproterenol, used as a positive control, showing the reliability of the method.

Results

Palatability evaluation

Figure 1 clearly illustrates a strong and immediate preference of the cats for the product containing pyrophosphate, and this, whatever their previous feeding experience. There is no difference of preference between cats bred on diets containing pyrophosphates, and cats for which this test is their first encounter to this ingredient.

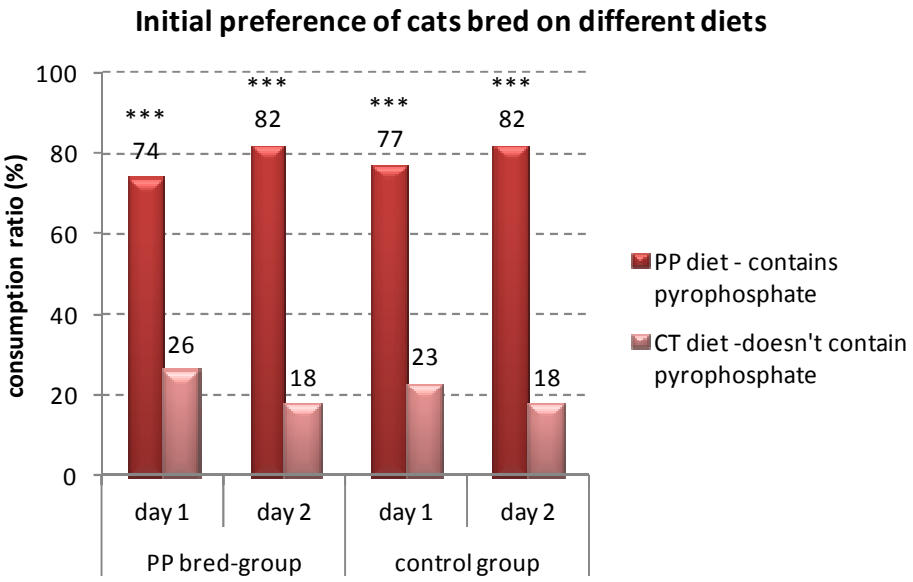

Figure 1 – Palatability results for two groups of cats, comparing 2 diets. The only difference between the two diets is the presence/absence of pyrophosphate in the palatability enhancer.

The second set of palatability tests explores the respective roles and palatability of meat base and pyrophosphate, when applied on separate diets. Figure 2 illustrates how transition periods with certain diets can temporarily modify the preference and thus influence palatability test results. The cats of group A, fed for 21 days with a control diet containing only meat base as a palatability enhancer, show an immediate and significant preference for the diet coated with only pyrophosphate, whereas cats previously fed with the pyrophosphate diet (group B) will show a significant preference for this diet only on the third day of test. In all cases, cats will show the same strong and significant preference for pyrophosphate over meat base at the sixth day of testing.

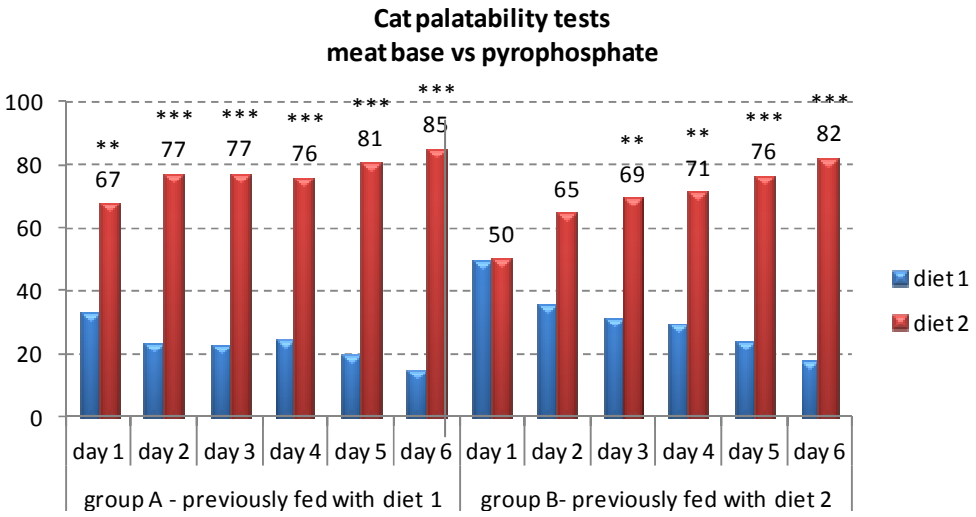

Figure 2 – Patability results for two groups of cats, comparing 2 diets. Diet 1 is coated with meat base only, Diet 2 is coated with pyrophosphate only. Group A is previously fed with diet 1, group B is previously fed with diet 2.

Search for a taste receptor

Different stimuli, such as amino acids and trisodium pyrophosphate, were tested. The expressed feline T1R1/T1R3 receptors showed to be sensitive to various amino acids but they were also stimulated by solutions containing pyrophosphates. The enhancement of the response recorded depended on the nature of the amino acid and the concentration of the compounds tested. Several cells displayed little activation to an amino acid and/or to PP alone but the combination of some amino acids) with PP showed a high degree of enhancement, suggesting a hyperadditivity mechanism or synergism; i.e: a Lys+PP mix was able to induce a more important activation than the arithmetic sum of the responses to the single compounds. Similar results were obtained for L-alanine and L-proline mixed with PP. More cells responded to the mixture of PP plus amino acid than to PP alone or to any amino acid tested, suggesting recruitment of cells that were weakly or non-responsive to either of the compounds alone (figures 3 & 4).

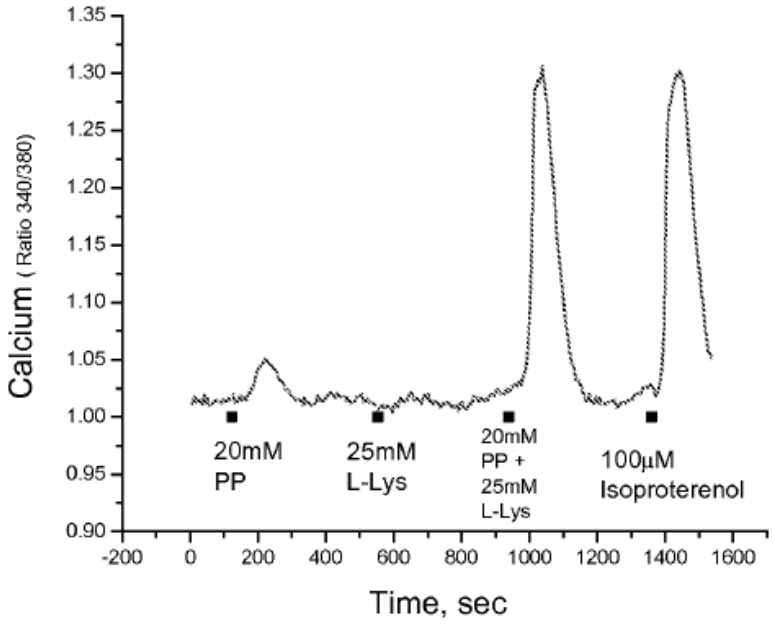

Figure 3 – HEK293 cell expressing feline T1R1/T1R3 receptor stimulated by a mixture of trisodium pyrophosphate and L-Lysine

HEK293 cell expressing feline T1R1/T1R3 receptor and Gα15 gave a weak response to 20mM trisodium pyrophosphate and a null response to 25mM L-lysine alone. However, there was an enhanced response to the mixture of 20mM trisodium pyrophosphate and 25mM L-lysine. 100 µM isoproterenol serves as positive control.

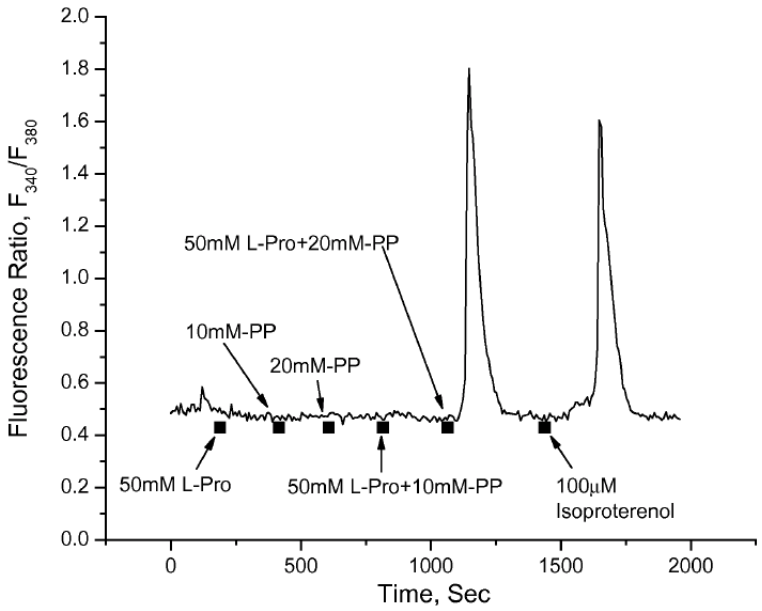

Figure 4 - HEK293 cell expressing feline T1R1/T1R3 receptor stimulated by a mixture of trisodium pyrophosphate and L-Proline

HEK293 cell expressing T1R1-T1R3 receptor and Gα15 gave null responses to 50mM L-proline, 10mM and 20mM trisodium pyrophosphate presented singly but a robust response to the mixture of two compounds 20mM of trisodium pyrophosphate and 50mM of L-proline. 100 µM isoproterenol serves as positive control.

Conclusion

The results of this study allowed us to establish the unique and robust preference of cats for pyrophosphate. Although universal, this preference can be temporarily modified by the cats' current feeding habits.

Our study demonstrated the possibility that one mode of action of pyrophosphate in cat is as a modulator of the activity of the amino acid receptor T1R1-T1R3 toward its many known stimuli. The developed method can allow the screening of different compounds and the determination of their potential as cat palatability agents. This method has been patented (WO 2012/013480): it allows determining if two or more test compounds have an augmenting effect on the T1R1/T1R3 receptor where each test compound elicits a small or moderate response.

References

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