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ORIGINALARBEITEN

Acesulfame K, Cyclamate and Saccharin inhibit the anaerobic fermentation of glucose by intestinal bacteria

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Summary

The caecal microflora of Cara rats was incubated in the pH stat with glucose under anaerobic conditions, and the acid production was measured. In the presence of the sweeteners Acesulfame K, Cyclamate and Saccharin, inhibition of the fermentation of glucose was observed with ED_{50} values of 260, 251, and 140 mM, respectively. The nutritional relevance of these observations is probably slight; an interpretation in terms of bacterial physiology leads to the proposal that the sweeteners may act on glucose transport systems at the bacterial cytomembrane.

Zusammenfassung

Die Mikroflora des Zäkums von Cara-Ratten wurde, unter anaeroben Bedingungen, in einem pH-Stat mit Glucose inkubiert, um die Säurebildung zu messen. In Gegenwart der Süßstoffe Acesulfam K, Cyclamat und Saccharin wurde eine Hemmung der Glucosevergärung mit den ED_{50}-Werten von 260, 251 bzw. 140 mM gefunden. Die ernährungsphysiologische Bedeutung dieser Beobachtung ist wahrscheinlich gering; die Interpretation der Versuche führt im Rahmen der Bakterienphysiologie zu dem Vorschlag, daß die Süßstoffe auf Glucose-Transportsysteme in der bakteriellen Zytomembran wirken.

Key words: Acesulfame K, Cyclamate, Saccharin, caecal bacteria, glucose fermentation

Introduction

The anaerobic fermentation of carbohydrates which were not utilized in the small intestine by large bowel microorganisms has received much attention in the last few years (1, 4, 5, 9). Contributions from this laboratory concern microbial adaptation phenomena (2) and a dose-effect relationship between a low-caloric polyol mixture and the amount of colonic H_2 + CH_4 produced (3), both studied in man.

At the same time, the inhibitory action of the sweeteners Acesulfame K, Cyclamate, and Saccharin on the anaerobic fermentation of sucrose, glucose and fructose by *Streptococcus mutans* NCTC 10449 and other oral

microorganisms was discovered (10, 11). Since the site of attack of these sweeteners had apparently nothing to do with the events of the glycolytic chain, it seemed interesting to check whether such inhibitions may also be observed with a very distant population of microbes, namely the caecal flora of the rat. Anaerobic techniques for work with intestinal anaerobic bacteria were developed previously in this laboratory (6). In this paper, we report on an inhibition of glucose fermentation with caecal bacteria by the sweeteners Acesulfame K, Cyclamate, and Saccharin.

Methods

Acesulfame K was a gift from Dr. Rymon von Lipinsky, Hoechst AG, Frankfurt; Cyclamate and Saccharin as their sodium salts were commercial products of food quality. All other reagents were of analytical grade.

Rats of the Cara strain, indigenous to this laboratory, were sacrificed and further processed as described earlier (6). The anaerobically recovered caecal content was, without further washing, suspended in 5 ml/g fresh weight of 75 mM NaCl, 75 mM KCl, 3 mM $MgCl_2$ solution. This bacterial suspension was rapidly transferred to the thermostated incubation vessel of a pH stat as described earlier (8). After addition of glucose to a final concentration of 20–50 mM, acid production was followed by automatic back-titration with 0.02 M NaOH. Sweeteners were added stepwise during the fermentation. Evaluations of their inhibitory activity were made with the aid of a Litchfield-Wilcoxon plot, as described earlier (8).

Statistical calculations followed standard procedures as described (8).

Results

With the aim of checking the incubation system, acid production was followed for 16 hours (Fig. 1) to ensure proportionality with time and to check for the eventual buffering capacity of the caecal content. The range

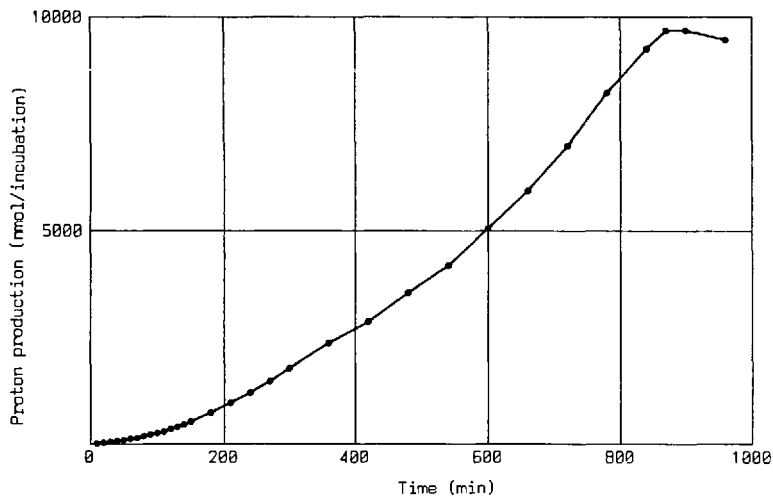

Fig. 1. Acid production by caecal bacteria of the rat from glucose; proportionality with time. 20 mM glucose; 37 °C; anaerobic; final pH 5.2

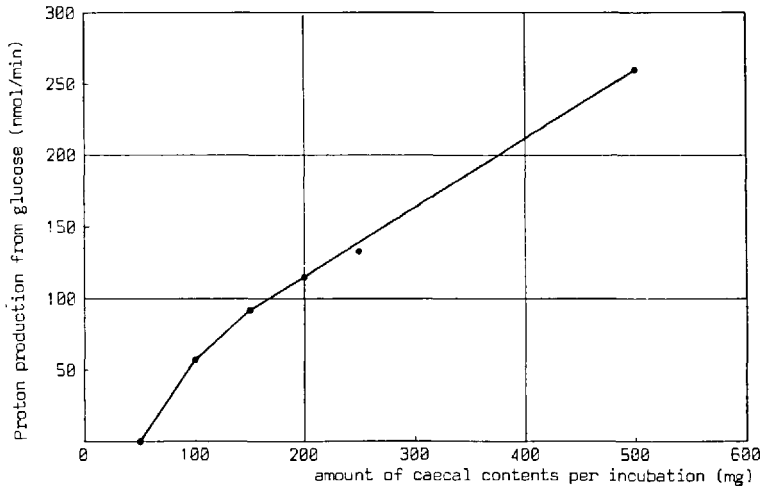

Fig. 2. Proportionality of the proton production with the amount of glucose fermenting caecal bacteria of the rat. 20 mM glucose; 37 °C; anaerobic; back-titration with 0.02 M NaOH to pH 7.0

of proportionality of acid formation with the amount of caecal inoculum is presented in Fig. 2.

The non-nutritive sweeteners Acesulfame, Cyclamate, and Saccharin effectively inhibit anaerobic acid production from glucose by the caecal flora of standard-chow fed rats (Table 1). Interestingly enough, caecal bacteria require 6–8 times higher concentrations of the individual sweeteners than oral microorganism like *Streptococcus mutans* NCTC 10449, *Lactobacillus* LSB 132 and *Actinomyces viscosus* Ny 1 no. 30 (10, 11) to obtain the same inhibitory effect.

Exploratory experiments were made with the caecal contents of rats which were prefed with either 0.5 % Saccharin, or 3 % Acesulfame K, or 5 % Cyclamate for 6 weeks. No obvious deviations of ED_{50} values between control and sweetener fed animals were evident in these experiments (details not shown).

Table 1. Inhibitory action of Acesulfame K, Cyclamate, and Saccharin on anaerobic glucose fermentation by caecal bacteria of the rat. (n = 10 animals; pH stat set at pH 7.0; 37 °C; 50 mM glucose; 250 mg caecal contents per incubation; back-titration with 0.02 M NaOH; inhibitory activity as ED_{50}, i.e. half-inhibitory concentration).

	ED_{50} (mM) $\bar{x} \pm s$
Acesulfame K	260 ± 56
Cyclamate	251 ± 47
Saccharin	140 ± 19

Discussion

The biological relevance of these data has two aspects:

1. Since a mixture of intestinal bacteria as well as oral microorganisms are inhibitable in their glucose fermentation by Acesulfame K, Cyclamate, and Saccharin, the action of these sweeteners most probably represents an event of wide occurrence. The data of this paper support the contention that the site of attack of these sweeteners might be on the microbial cytomembrane (10, 11).

2. The nutritive relevance of these results is probably small: the concentrations for 50 % inhibition of fermentation in intestinal bacteria are 67 times that of Saccharin in soft drinks, 14 times that of Cyclamate in soft drinks, or 76 times that of Acesulfame K, all of them compared on the basis of approximately similar sweetness. Furthermore, taking into account that any food or beverage which contains sweeteners becomes diluted by sialo-gastro-intestinal secretions, it would seem highly improbable that any of the sweeteners after their use in proper concentrations could ever lead to a disturbance of colonic fermentations in man, in spite of the well-known presence (7) of some of the sweeteners in the large intestine.

Acknowledgement

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