Pharmacology Biochemistry & Behavior, Vol 24, pp 429–432, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Caffeine Intensifies Taste of Certain Sweeteners: Role of Adenosine Receptor[1]

S S. SCHIFFMAN, C. DIAZ AND T G. BEEKER

Departments of Psychiatry and Psychology, Duke University, Durham, NC 27710

Received 1 August 1985

SCHIFFMAN, S S , C DIAZ AND T G BEEKER *Caffeine intensifies taste of certain sweeteners Role of adenosine receptor* PHARMACOL BIOCHEM BEHAV 24(3) 429–432, 1986 —Caffeine, a potent antagonist of adenosine receptors potentiates the taste of some but not all sweeteners It significantly enhances the taste of acesulfam-K, neohesperidin dihydrochalcone, d-tryptophan, thaumatin, stevioside, and sodium saccharin Adenosine reverses the enhancement Caffeine has no effect on aspartame, sucrose, fructose, and calcium cyclamate These results suggest that the inhibitory A_1 adenosine receptor plays an important local role in modulating the taste intensity of certain sweeteners and that several transduction mechanisms mediate sweet taste

Caffeine Taste Potentiation Adenosine Receptor Sweeteners

THE sweet taste can be elicited by a wide range of compounds including mono- and disaccharides, diterpene glycosides, polyols, amino acids, dipeptides, proteins, and other nonsugars At present, neither the stereochemical properties of these molecules nor the receptor sites that lead to a sweet sensation are well understood Investigations from a range of disciplines, including organic and medicinal chemistry, biochemistry, neurophysiology, psychophysics, and biophysics, suggest that there are probably a multiplicity of sweet receptor types, each with its own stereochemical and physiocochemical requirements [5, 12, 15]

Recent evidence suggests that the adenosine receptor, which is known to play a significant role in a variety of biological processes, also plays a role in taste perception of sweeteners [14] Adenosine receptors modulate heart rate [4], vasodilation [1], platelet aggregation [7], neural activity [18], steroid production [20], histamine release from mast cells [11], and lipolysis in fat cells [6] Two subtypes of cell surface adenosine receptors, the A_1 (inhibitory) and A_2 (excitatory) receptors, have been described [10,19] The A_1 type are high affinity receptors that show half maximal responses at nanomolar concentrations, half maximal responses for A_2 receptors are found at concentrations 100 to 1000 times higher in the micromolar range [17] Methyl xanthines (MX), including caffeine, theophylline, and theobromine, are potent antagonists of adenosine receptors

Adaptation of the human tongue to methyl xanthines at concentrations ranging from 10^{-5} M to 10^{-2} M has recently been shown to potentiate certain tastes [14] Of the five stimuli tested (NaCl, quinine HCl, KCl, urea, and acesulfam-K), the greatest taste potentiation by methyl xanthines (approximately 100%) was found for the artificial sweetener acesulfam-K, which has a bitter component in addition to sweetness Adenosine reversed this potentiation, presumably by competing with methyl xanthines for the adenosine receptor This increase in perceived intensity occurred after adaption of the tongue to 10^{-5} M MX, a concentration known to inhibit adenosine receptors but lower than that required to inhibit phosphodiesterase [6] Increasing the concentrations of MX as high as 10^{-2} M did not significantly increase the degree of enhancement The results suggested that taste enhancement is predominantly due to antagonizing of the adenosine receptor rather than inhibition of phosphodiesterase

The finding that responses to sweeteners are enhanced by methyl xanthines was further confirmed by electrophysiological data in rat [14] The animal data, however, revealed that neural responses in nucleus tractus solitarius for one sweetener, stevioside, were greatly potentiated by 10^{-5} M caffeine while sucrose was unaffected This suggested that the effect of MX is not uniform across sweeteners.

The purpose of the present experiment was to determine if sweeteners other than acesulfam-K are potentiated by caffeine in humans and to ascertain whether potentiation is uniform across sweeteners or selective as found in rats The results described below indicate that 10^{-5} M caffeine enhanced the taste of some sweeteners including neohesperidin dihydrochalcone, D-tryptophan, thaumatin, stevioside, and sodium saccharin Adenosine reversed this potentiation Four sweeteners, aspartame, sucrose, fructose, and calcium cyclamate, were not enhanced by caffeine

METHOD

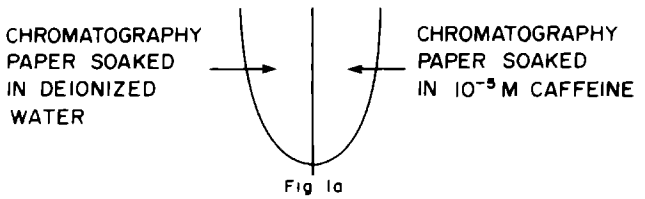
The subjects were 10 Duke University undergraduate students aged 19–22 (2 male, 8 female) who had prior experi-

[1]This research was supported by grant NIA AG 00443 and a grant from General Foods Corporation

TABLE 1

THE CHEMICAL CLASSIFICATIONS OF SWEETENERS AND THE CONCENTRATION OF STANDARDS USED IN THIS STUDY

Acesulfam-K	Oxathiazinone dioxide (methyl derivative) 3,4-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide potassium salt	0 02 M
Aspartame	Dipeptide L-aspartyl-L-phenylalanine methyl ester	8 00 × 10 $^{-3}$ M
Calcium cyclamate	Calcium cyclohexylsulfamate	0 02 M
Fructose	Monosaccharide ketohexose	0 60 M
Neohesperidin dihydrochalcone	Dihydrochalcone glycoside	3 00 × 10^{-4} M
Saccharin (sodium salt)	O-sulfobenzimide 1,2-benzothiazol-3(2H)-one-1,1-dioxide, Na+ salt	1 87 × 10 $^{-3}$ M
Stevioside	Diterpene glycoside	1 17 × 10^{-3} M
Sucrose	Disaccharide	0 80 M
Thaumatin	Several distinct proteins	2 78 × 10 $^{-5}$ M
D-tryptophan	D-amino acid	1 47 × 10 $^{-2}$ M



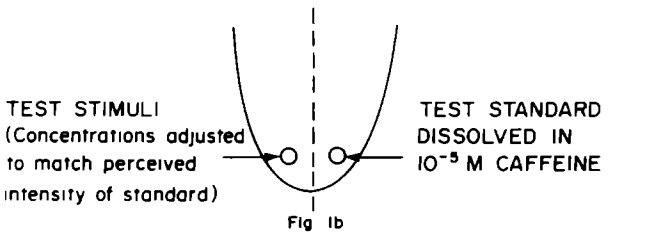


FIG 1 (a) In one set of experiments, one half of the tongue was adapted to 10^{-5} M caffeine for a total of four minutes while the other half of the tongue was adapted to a deionized water control In a second set of experiments, both 10^{-5} M caffeine and 10^{-5} M (or 10^{-4} M) adenosine were simultaneously applied to one half of the tongue during the adaptation procedure with a water control on the other side (b) In one set of experiments, the standard concentration of a sweetener given in Table 1 was dissolved in 10^{-5} M caffeine and placed on the side of the tongue adapted to 10^{-5} M caffeine Test stimuli, dissolved in deionized water, were applied to the noncaffeine-treated side, and the concentrations were adjusted to match the perceived intensity of the standard In the second set of experiments, the standard sweetener was dissolved in a solution containing both caffeine and adenosine

ence in taste experiments Caffeine (1,3,7 trimethylxanthine) was obtained from Sigma Chemical Co and dissolved in deionized water Subjects were required to match the perceived intensity of a standard sweetener concentration presented simultaneously with 10^{-5} M caffeine to a series of intensities of the sweetener presented without caffeine The sweeteners, concentrations used as standards, and chemical classifications are given in Table 1 The concentrations used as standards were found to impart a moderately intense taste both in previous experiments [13] and in pretesting The matching procedure for determining the effect of caffeine on sweeteners has been used previously [13,14] and is summarized as follows Pieces of chromatography paper (Whatman No 1, 0 16-mm thickness) cut in the shape of half tongues were soaked in either 10^{-5} M caffeine or deionized water (control) for 10 minutes Then two pieces of chromatography paper, one impregnated with 10^{-5} M caffeine and a water control, were applied to the tongue for 2 minutes as shown in Fig 1a, this set was removed and replaced by a fresh set for another 2 minutes, this constituted a total application time of 4 minutes

The sweetener standard and test sweeteners were then delivered to the tongue in 1/2-inch circles of chromatography paper as shown in Fig 1b The standard concentration to be matched was dissolved in 10^{-5} M caffeine and placed on the side of the tongue adapted to caffeine The concentration of the test stimulus on the other side was adjusted until a concentration was found that matched the intensity of the standard dissolved in caffeine When the sweetener concentrations applied to the two sides of the tongue were perceived to be equally intense, subjects were also asked which side of the tongue was more bitter and which side was sweeter in order to determine if there was a shift in taste quality Three experiments were performed with each of the following as adapting solutions (1) 10^{-5} M caffeine, (2) 10^{-5} M caffeine and 10^{-5} M adenosine, and (3) 10^{-5} M caffeine and 10^{-4} M adenosine These concentrations of caffeine and adenosine are tasteless, the average recognition thresholds for the bitter taste of caffeine and adenosine for these subjects are 2.5×10^{-3} M and 2×10^{-2} M, respectively

RESULTS

Caffeine (10^{-5} M) was found to potentiate some but not all sweeteners It had its most pronounced effect on artificial sweeteners known to have bitter components (see [16]) as shown in Fig. 2a The striped bar represents the standard concentration, the stippled bar indicates the concentration perceived to match the concentration in the presence of caffeine It can be seen in Fig 2a that caffeine significantly potentiated six sweeteners acesulfam-K (100%), neohesperidin dihydrochalcone (65 4%), d-tryptophan (52 4%), thaumatin (51 4%), stevioside (48 4%), and sodium saccharin

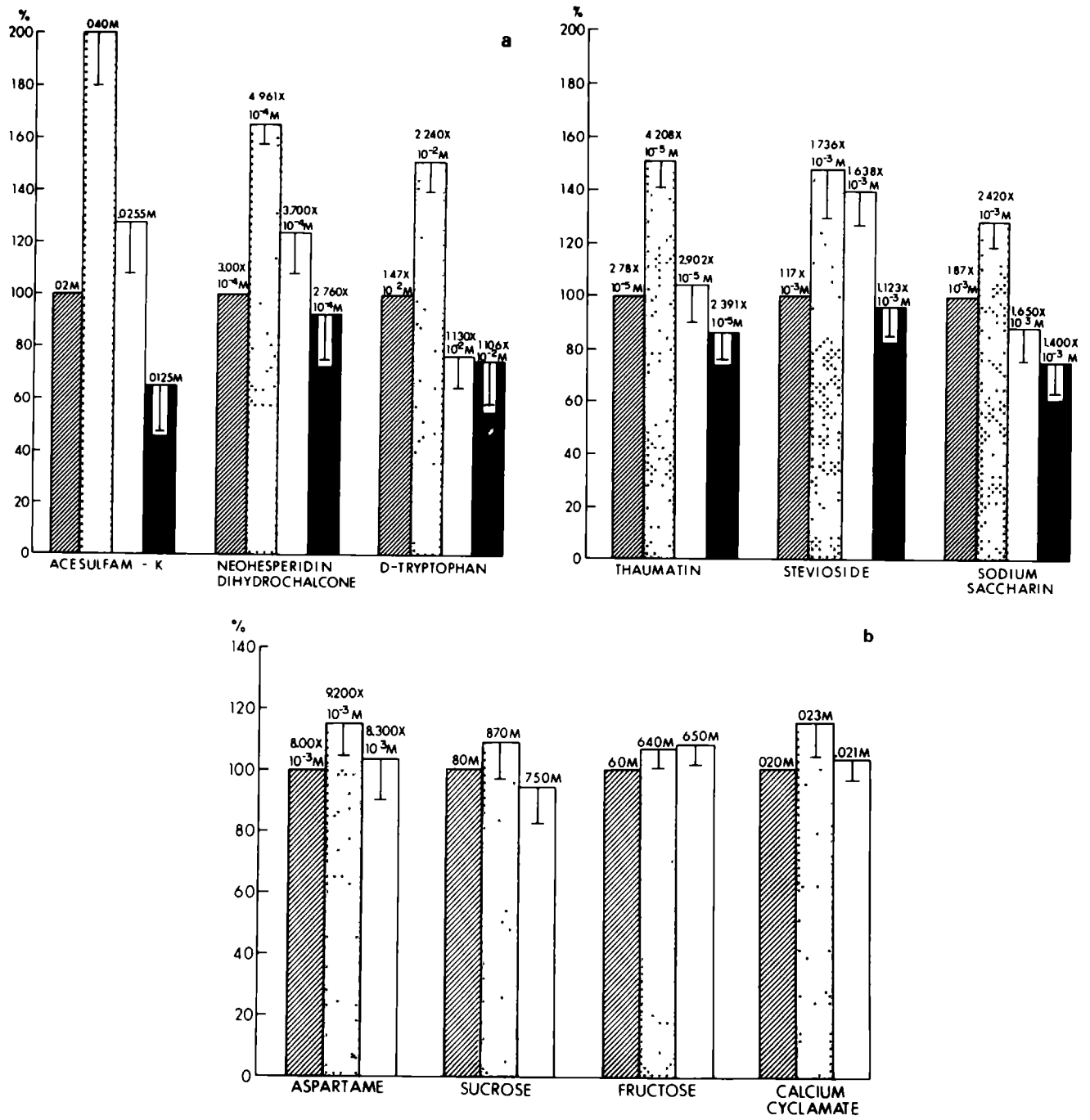


FIG 2 The striped bar represents the standard concentration, the stippled bar indicates the test concentration perceived to match the standard dissolved in caffeine The white bar represents the perceived intensity after simultaneous application of 10^{-5} M caffeine and 10^{-5} M adenosine, the solid bar, after 10^{-5} M caffeine and 10^{-4} M adenosine (a) Six sweeteners enhanced by caffeine (b) Four sweeteners unaffected by caffeine

(29 4%) It had no significant effect on the four sweeteners in Fig 2b that have no pronounced bitter components aspartame, sucrose, fructose, and calcium cyclamate

It is further shown in Fig 2 that adenosine reduced the potentiation achieved by caffeine, 10^{-4} M adenosine was more effective than 10^{-5} M The white bar represents the perceived intensity after simultaneous application of 10^{-5} M caffeine and 10^{-5} M adenosine, the solid bar, the perceived intensity after 10^{-5} M caffeine and 10^{-4} M adenosine

When caffeine was effective in enhancing taste, 78% of the judgments indicated that the total taste quality (both sweet and bitter components) was affected The tastes on the two sides of the tongue were perceived as identical, and thus, for sweeteners with both sweet and bitter tastes, both qualities were apparently enhanced Twelve percent of the responses suggested that only the bitter taste was enhanced and 10%, only sweet taste was increased However, for any given sweetener, these latter two responses were approx-

imately equally frequent, again suggesting no change in overall quality

DISCUSSION

These data strengthen the notion that more than one receptor type mediates sweetness Six of the ten sweeteners tested were enhanced by caffeine, and adenosine reversed the potentiation Four sweeteners were unaffected Thus human data are consistent with previous neural data from rat [14] in which 10^{-5} M caffeine significantly potentiated the neural response to stevioside but did not significantly affect sucrose

While no obvious structural components clearly delineate these two groups of sweeteners, those enhanced by caffeine have been reported to have a bitter component [16] while those that were not potentiated have minimal bitterness For sweeteners modulated by caffeine, both bitter and sweet components appear to have been enhanced It seems plausible that a receptor site of the type previously proposed by Birch and Mylvaganam [2] in which sweet and bitter sites lie proximate one another is involved in the transduction process for caffeine-sensitive sweeteners

Although it is premature to define precisely the biochemical mechanism by which caffeine potentiates the perceived intensity of some sweeteners, it seems possible that increased levels of cAMP are involved Binding of adenosine to the inhibitory A_1 receptor inhibits adenylate cyclase Caffeine antagonizes this response resulting in an increase in cAMP It is possible that increases in cAMP levels produce increases in perceived taste intensity Synthesis of cAMP within a cell takes time, and the fact that enhancement with 10^{-5} M caffeine did not occur instantaneously but rather was time-dependent, requiring preadaptation, is consistent with a role for cAMP On the other hand, it should be noted that cAMP need not be directly involved, since certain cellular effects related to stimulation of adenosine receptors do not appear to be mediated via adenylate cyclase [3, 8, 9]

In conclusion, caffeine potentiated the taste of sweeteners with a bitter component but had no effect on sweeteners lacking bitterness Adenosine reversed the potentiation The results suggest that the A_1 inhibitory adenosine receptor plays an important local role in modulating perception of some sweeteners

REFERENCES

1 Berne, R M , H R Winn, R M Knabb, S W Ely and R Rubio Blood flow regulation by adenosine in heart, brain, and skeletal muscle In *Regulatory Function of Adenosine* edited by R M Berne, T W Rall and R Rubio The Hague Martinus Nijhoff, 1983, pp 293–317

2 Birch, G C and A R Mylvaganam Evidence for the proximity of sweet and bitter receptor sites *Nature* **260:** 632–634, 1976

3 Bohm, M , R Bruckner, I Hackbarth, B Haubitz, R Linhart, W Meyer, B Schmidt, W Schmitz and H Scholz Adenosine inhibition of catecholamine-induced increase in force of contraction in guinea-pig atrial and ventricular heart preparations Evidence against a cyclic AMP- and cyclic GMP dependent effect *J Pharmacol Exp Ther* **230:** 483–492, 1984

4 Drury, A N and A Szent-Gyorgyi The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart *J Physiol (Lond)* **68:** 213–237, 1929

5 Faurion, A and P MacLeod Sweet taste receptor mechanisms In *Nutritive Sweeteners*, edited by G G Birch and K J Parker London Applied Science Publishers, 1982

6 Fredholm, B B Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends Pharmacol Sci* **1.** 129–132, 1980

7 Haslam, R J and G M Rosson Effects of adenosine on levels of adenosine cyclic 3′, 5′-monophosphate in human blood platelets in relation to adenosine incorporation and platelet aggregation *Mol Pharmacol* **11:** 528–544, 1975

8 Huffaker, T , T Corcoran and J A Wagner Adenosine inhibits cell division and promotes neurite extension in PC12 cells *J Cell Physiol* **120:** 188–196, 1984

9 Kuroda, M , R C Honor, C Londos, S W Cushman and I H Simpson cAMP-independent modulation of rat adipocyte glucose transporter intrinsic activity by the adenylate cyclase regulatory system *Fed Proc* **44.** 480, 1985

10 Londos, C D , M F Cooper and J Wolff Subclasses of external adenosine receptors *Proc Natl Acad Sci USA* **77** 2551–2554, 1980

11 Marquardt, D L , C W Parker and T J Sullivan Potentiation of mast cell mediator release by adenosine *J Immunol* **120:** 871–878, 1978

12 Schiffman, S S , H Cahn and M G Lindley Multiple receptor sites mediate sweetness Evidence from cross adaptation *Pharmacol Biochem Behav* **15** 377–388, 1981

13 Schiffman, S S , E Lockhead and F W Maes Amiloride reduces the taste intensity of Na- and Li-salts and sweeteners *Proc Natl Acad Sci USA* **80.** 6136–6140, 1983

14 Schiffman, S S , J M Gill and C Diaz Methyl xanthines enhance taste Evidence for modulation of taste by adenosine receptor *Pharmacol Biochem Behav* **22.** 195–203, 1985

15 Schiffman, S S , A J Hopfinger and R H Mazur The search for receptors that mediate sweetness In *The Receptors* edited by M Conn New York Academic Press, in press

16 Schiffman, S S , D A Reilly and T B Clark Qualitative differences among sweeteners *Physiol Behav* **23** 1–9, 1979

17 Shimizu, H Adenosine receptors associated with the adenylate cyclase system In *Physiology and Pharmacology of Adenosine Derivatives* edited by J W Daly, Y Kuroda, J W Phillis, H Shimizu and M Ui New York Raven Press, 1983, pp 31–40

18 Snyder, S H Drug and neurotransmitter receptors in the brain *Science* **224.** 22–31, 1984

19 Van Calker, D , M Muller and B Hamprecht Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells *J Neurochem* **33:** 999–1005, 1979

20 Wolff, J and G H Cook Activation of steroidogenesis and adenylate cyclase by adenosine in adrenal and Leydig tumor cells *J Biol Chem* **252.** 687–693, 1977