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Methyl Xanthines Enhance Taste: Evidence for Modulation of Taste By Adenosine Receptor[1]

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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(2) 195–203, 1985.—The methyl xanthines (MX), theophylline, caffeine, 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 was found to potentiate taste. The artificial sweetener acesulfam-K, which has a bitter component, was potentiated the most by MX, i.e., approximately 100%. This increase in perceived intensity for acesulfam-K occurred at 10^{-5} M MX, a concentration known to inhibit adenosine receptors but below that required to inhibit phosphodiesterase. Increasing the concentration of MX as high as 10^{-2} M did not increase the degree of enhancement appreciably. Taste enhancement was found for NaCl and quinine hydrochloride as well. When 10^{-5} M adenosine was added to the MX, the potentiation was reversed. The human results were confirmed by animal studies in which single unit extracellular recordings were made from the nucleus of the solitary tract. These results suggest that the inhibitory A_1 adenosine receptor plays an important local role in taste perception.

Taste Caffeine Methyl xanthines Adenosine Receptors

ADENOSINE plays a significant role in the regulation and control of a variety of biological processes. The regulatory effects of adenosine were first recognized by Drury and Szent-Györgyi in 1929 [7], who found that intravenous injection of adenosine had profound effects on the cardiovascular system, including slowed sinus rate, atrioventricular block, vasodilation, and hypotension. More recently, adenosine has been found to inhibit lipolysis in fat cells [8,17], inhibit platelet aggregration [13,23], increase steroid production [40], potentiate histamine release from mast cells [21], cause vasodilation in heart [2, 24, 25], brain, and skeletal muscle [2], produce renal constriction [26,38], depress neural activity [28,37], and modulate synaptic activity [34], with subsequent behavioral effects [27, 28, 37].

Two major subtypes of cell surface adenosine receptors have been described [18, 20, 39], the so-called A_1 (or R_i) type that is a high affinity receptor inhibitory to adenylate cyclase and the A_2 (or R_s) type that is a low affinity receptor and stimulatory to adenylate cyclase. A_1 receptors have nanomolar affinities for adenosine while A_2 receptors have affinities 100 to 1000 times higher in concentration in the micromolar range [36]. Methyl xanthines (e.g., caffeine) have the capacity to antagonize both types of adenosine receptors, although there are subclasses of A_2 receptors that are relatively insensitive to methyl xanthines as antagonists [36]. In addition, adenosine receptors may exist that are independent of the adenylate cyclase system.

In the present experiments, we investigated the effects of methyl xanthines on taste. Adaptation of the tongue to caffeine (and other methyl xanthines) was found to potentiate certain tastes presumably by blocking adenosine receptors; and adenosine reversed this potentiation presumably by competing with methyl xanthines for the adenosine receptor. It seems likely that the A_1 adenosine receptor plays an important local role in modulating taste.

METHOD

Human Psychophysical Studies

Subjects. The subjects were ten Duke University undergraduates who ranged in age from 19–22 years. Two were male and eight were female. Most of the subjects had previous experience in taste experiments.

Stimuli. The methyl xanthines, theophylline (1,3 dimethylxanthine), caffeine (1,3,7 trimethylxanthine), and theobromine (3,7 dimethylxanthine) were obtained from Sigma Chemical Co. and diluted in deionized water. The concentrations of methyl xanthines employed were: 10^{-5} M, 10^{-3} M, and 10^{-2} M caffeine; 10^{-5} M, 10^{-3} M, and 10^{-2} M theophylline; and 10^{-5} M and 10^{-4} M theobromine. Higher concentrations of theobromine were not used in the human studies because they were difficult to keep in solution. In addition, either 10^{-5} M or 10^{-4} M adenosine (obtained from Sigma) was applied simultaneously with 10^{-5} M caffeine to determine if adenosine antagonized the effects of caffeine.

A range of concentrations of methyl xanthines was used

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because the actions of this class of compounds appears to depend on the intensity employed. In most systems the bulk of the biological activity for methyl xanthines occurs between 10^{-6} M and 10^{-3} M. Methyl xanthines, in addition to their role as adenosine antagonists, have been used widely as cyclic nucleotide phosphodiesterase inhibitors. The concentrations of methyl xanthines required to inhibit phosphodiesterase are higher than those that block the adenosine receptor [19]. For example, in fat cells [9], theophylline inhibits the actions of adenosine at 10^{-6}–10^{-4} M; concentrations of 10^{-4} M and above are required to block phosphodiesterase and hence the breakdown of cyclic AMP (cyclic adenosine monophosphate).

Subjects were required to match standard stimulus concentrations presented with methyl xanthines to a range of stimuli presented without methyl xanthines. The taste stimuli and concentrations used as standards were: 0.20 M and 0.40 M NaCl, 0.30 M KCl, 0.002 M quinine hydrochloride (QHCl), 1.5 M urea, and 0.02 M acesulfam-K. The standard concentrations are also given at the top of the striped bars in Fig. 2, 5, and 7. The salts NaCl and KCl were both used because they have been shown to have different transduction mechanisms [6, 14, 32]. Two bitter stimuli, urea and quinine HCl, were both employed because previous studies have suggested they are mediated by separate receptor types [22]. Acesulfam-K (methyl derivative of oxathiazinone dioxide: 3,4-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide potassium salt) has a sweet, bitter taste [31]. Most of the standard concentrations have been determined to impart a moderately intense taste in previous experiments [32]. The test stimuli were dilutions both higher and lower than a given standard concentration (e.g., 0.20 M NaCl) that differed from one another by a factor of 2 (e.g., 0.05 M, 0.10 M, 0.20 M, and 0.40 M NaCl). Intermediate concentrations between two successive dilutions were included as well (e.g., 0.30 M NaCl). The standard stimuli were dissolved in methyl xanthine solutions to permit simultaneous presentation of the standard and MX because in pretesting it was found that methyl xanthines were easily washed away with water rinses. The test stimuli were dissolved in deionized water.

Procedure. Pieces of chromatography paper (Whatman No. 1, 0.16 mm thickness) cut in the shape of half tongues were soaked in a methyl xanthine solution or deionized water (control) for 10 minutes. Then, two pieces of the chromatography paper, one impregnated with a methyl xanthine and one water control were placed on the tongue for 2 minutes (see Fig. 1a) and removed; a fresh set was immediately placed on the tongue for another 2 minutes and removed for a total application time of 4 minutes.

The test standard and test stimuli were delivered to the tongue in 1/2 inch circles of chromatography paper as shown in Fig. 1b. The standard concentration to be matched was placed on the side of the tongue adapted to a methyl xanthine such as 10^{-5} M caffeine; the standard was dissolved in the same concentration as the methyl xanthine solution used for adaptation (e.g., 10^{-5} M caffeine). The concentration of the test stimulus on the other side was adjusted to determine that concentration which matched the perceived intensity of the standard placed on the side of the tongue adapted to the methyl xanthine. At each trial, a pair of 1/2 inch circles (one standard and one test stimulus) was applied simultaneously to the two sides of the tongue with forceps. Ten trials (pairs of circles) were presented on each of two consecutive days for a total of 20 trials per standard. The concentration at which a subject judged the test stimulus to be higher 50% of the time and lower 50% of the time was considered to match the intensity on the side of the tongue adapted to a methyl xanthine. A brief deionized water rinse (10 ml) was used after each pair.

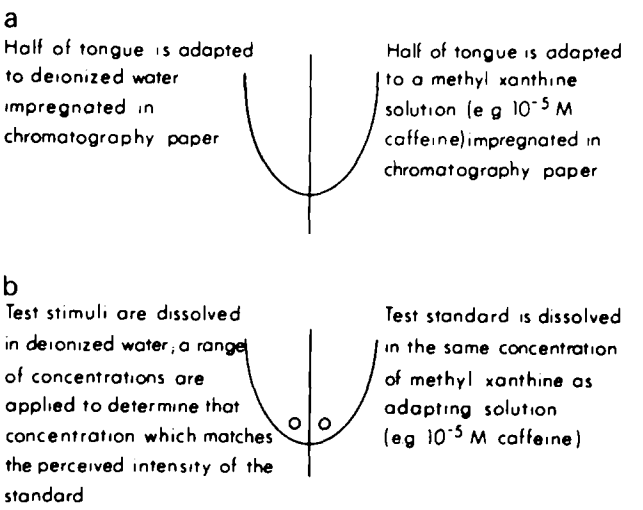

FIG. 1a. In one set of experiments, half of the tongue was adapted to a methyl xanthine impregnated in filter paper and the other half to a filter paper soaked in a deionized water control. In another set of experiments, both caffeine and adenosine were applied simultaneously to one half of the tongue during the adaptation procedure with a water control on the other side. b. In one set of experiments, a standard concentration of a taste stimulus dissolved in the same methyl xanthine solution used for adaptation was placed on the side of the tongue to which a methyl xanthine had been applied. Test stimuli dissolved in deionized water were applied to the nonxanthine-treated side, and the concentrations were adjusted to match the perceived intensity of the standard. In another set of experiments, the standard stimulus was dissolved in a solution containing both caffeine and adenosine.

Ten human psychophysical experiments were performed with each of the following as adapting solutions: caffeine (10^{-5} M, 10^{-3} M, and 10^{-2} M), caffeine (10^{-5} M) plus adenosine (10^{-5} M or 10^{-4} M), theophylline (10^{-5} M, 10^{-3} M, and 10^{-2} M), and theobromine (10^{-5} M and 10^{-4} M).

Neurophysiolgical Studies

Single unit extracellular recordings (see Wollston and Erickson [41] for method) were made from 45 neurons in the nucleus tractus solitarius (NTS) of 25 female Sprague Dawley rats. The animals were anesthetized with an intraperitoneal injection of Nembutol (60 mg/kg). Supplemental doses of Nembutol were administered when needed.

Recordings were made through glass micropipettes filled with 3 M NaCl. The taste area of NTS was generally localized by moving 3 mm rostral, 2 mm lateral to the obex and between 1 mm and 2 mm below the surface of the medulla. Once a single cell was isolated, a stimulus train consisting of the stimuli shown in Figs. 3, 6, 8, and 9 were delivered to the entire oral cavity by a gravity flow tube inserted into the mouth. The stimuli included sucrose, stevioside (a diterpene glycoside that has a sweet-bitter taste in humans [31]) and citric acid in addition to many of the stimuli used in the human studies. Approximately 1 minute was allowed between each stimulus; a brief water rinse followed each stimulus.

At the end of the first presentation of the stimulus train, a methyl xanthine dissolved in deionized water was applied to the tongue of the rat. The methyl xanthines used were: caffeine (10^{-5} M, 10^{-3} M, 10^{-1} M), theophylline (10^{-3} M), theobromine (10^{-3} M), and 3-isobutyl-l-methyl xanthine—IBMX (10^{-3} M). Caffeine, theophylline, and theobromine were obtained from Sigma; IBMX was obtained from Aldrich. The responses from at least 10 neurons were recorded for each methyl xanthine. The methyl xanthines were allowed to flow freely into the oral cavity for 1 minute and then dripped slowly for 4 minutes. At the end of the fourth minute, another dose was allowed to flow onto the tongue for 30 sec. Following the presentation of the methyl xanthine, the stimulus train was repeated in the same order as that prior to methyl xanthine application. For all the methyl xanthines except caffeine, there was an additional application of the drug after the 6th stimulus. Reapplication was helpful because the effect of methyl xanthines tended to disappear after 10 minutes.

The spike train was analyzed by passing it through a window discriminator and the time of occurrence of each action potential was recorded by a microcomputer. The number of spikes for a 5 second interval that occurred after the first noticeable change in background activity was counted. The background rate was calculated by determining the activity in the unit for the 500 msec prior to the change in activity resulting from the stimulus presentation. The background was then normalized to the value expected for 5 seconds, and this number was subtracted from the 5 sec stimulus totals. The data were expressed as the mean response rate per second.

RESULTS

The results indicate that methyl xanthines enhance some tastes but have no effect on others. When taste potentiation occurred, it was not limited to a narrow subclass of tastes but was found for stimuli with a wide range of sensory properties including sweetness, bitterness, and saltiness. In addition, stimuli with similar qualities were differentially effected. Methyl xanthines potentiated the taste of the artificial sweeteners acesulfam-K and stevioside but had no effect on the natural sweetener sucrose. Methyl xanthines were also found to strongly potentiate one bitter taste QHCl with much less effect on another, urea.

Caffeine

The results from the human psychophysical studies are shown in Fig. 2a, b, and c. The striped bar indicates the standard concentration applied to the caffeine-adapted side of the tongue. The dotted bar indicates the concentration perceived to match the standard. The results from the neurophysiological studies are given in Fig. 3a, b, and c. The standard errors are shown as well.

At all three concentrations of caffeine used in the psychophysical studies, the perceived intensity of acesulfam-K was greatly enhanced. The increases were approximately equal for all caffeine concentrations, that is 100%, 95%, and 105% for 10^{-5} M, 10^{-3} M, and 10^{-2} M, respectively. The tastes of NaCl and KCl were also significantly enhanced at all three concentrations although the degree of the effect was much less than acesulfam-K. The taste of QHCl was significantly potentiated at concentrations of 10^{-5} and 10^{-3} M while urea showed little change when all three concentrations are considered together. The average enhancements over all six stimuli were similar: 47.1%, 40.7%, and 41.1% for 10^{-5} M, 10^{-3} M, and 10^{-2} M respectively, in spite of the fact that the two lowest concentrations of caffeine were tasteless and the highest had a bitter taste. The bitterness recognition threshold for caffeine on the front of the tongue was found to be 2.5×10^{-3} M in pretesting.

The neural data indicate significant increases in firing rate for stevioside at all three caffeine concentrations. The percentage increase is given on the ordinate. Caffeine would often promote a neural response when there was none prior to application of the methyl xanthine. At 10^{-5} M, caffeine significantly enhanced responses to low levels of NaCl, specifically 0.10 M NaCl which was not used in the human studies. It should be noted, however, that the decreased potentiation found at high levels of NaCl (0.40 M) may be due to the fact that the maximum neural firing rate had been achieved and no further stimulation was possible. Like the psychophysical data, the potentiation for neural data was greatest at the lowest concentration of caffeine, i.e., 10^{-5} M.

Caffeine Plus Adenosine

When the tongue was adapted to both caffeine and adenosine simultaneously, the potentiation of acesulfam-K and QHCl was greatly reduced as shown in Fig. 4. The striped bar represents the standard concentration; the dotted bar, the perceived intensity resulting from adaptation by 10^{-5} M caffeine alone; the white bar represents the perceived intensity after simultaneous application of 10^{-5} M caffeine and 10^{-5} M adenosine; the black bar, the perceived intensity after application of 10^{-5} M caffeine and 10^{-4} M adenosine. While caffeine applied alone potentiates the tastes of acesulfam-K and QHCl, adenosine reduces the effect. Application of 10^{-4} M adenosine is more effective than 10^{-5} M.

Theophylline

The results from the human psychophysical studies are given in Fig. 5a, b, and c. Those from the neurophysiological studies are given in Fig. 6. Like caffeine, the human studies show the greatest enhancement for acesulfam-K, i.e., 80%, 100%, and 120% at 10^{-5} M, 10^{-3} M, and 10^{-2} M, respectively. The taste of quinine HCl was also considerably enhanced at all theophylline concentrations, i.e., 65%, 55%, and 80% at 10^{-5} M, 10^{-3} M, and 10^{-2} M respectively. NaCl and KCl are significantly enhanced but to a lesser degree. The potentiation of urea is minor overall. The neural data, however, are equivocal showing possible enhancements for low levels of NaCl and QHCl.

Theobromine

The results from the human psychophysical studues are given in Fig. 7a and b. Like caffeine and theophylline, the artificial sweetener acesulfam-K was enhanced the most at both 10^{-5} M and 10^{-4} M theobromine. At 10^{-5} M theobromine, quinine HCl was also greatly potentiated. Enhancements for the remaining stimuli were found at both concentrations. The neural data shown in Fig. 8 indicate a definite enhancement of 0.10 M NaCl after application of 10^{-3} M theobromine with smaller effects for NaCl and KCl as well. The human studies show no clear differences in relative potency for caffeine, theophylline, or theobromine.

Isobutyl Methyl Xanthine

The neural data in Fig. 9 suggest enhancement by IBMX of quinine HCl, stevioside, and low levels of NaCl.

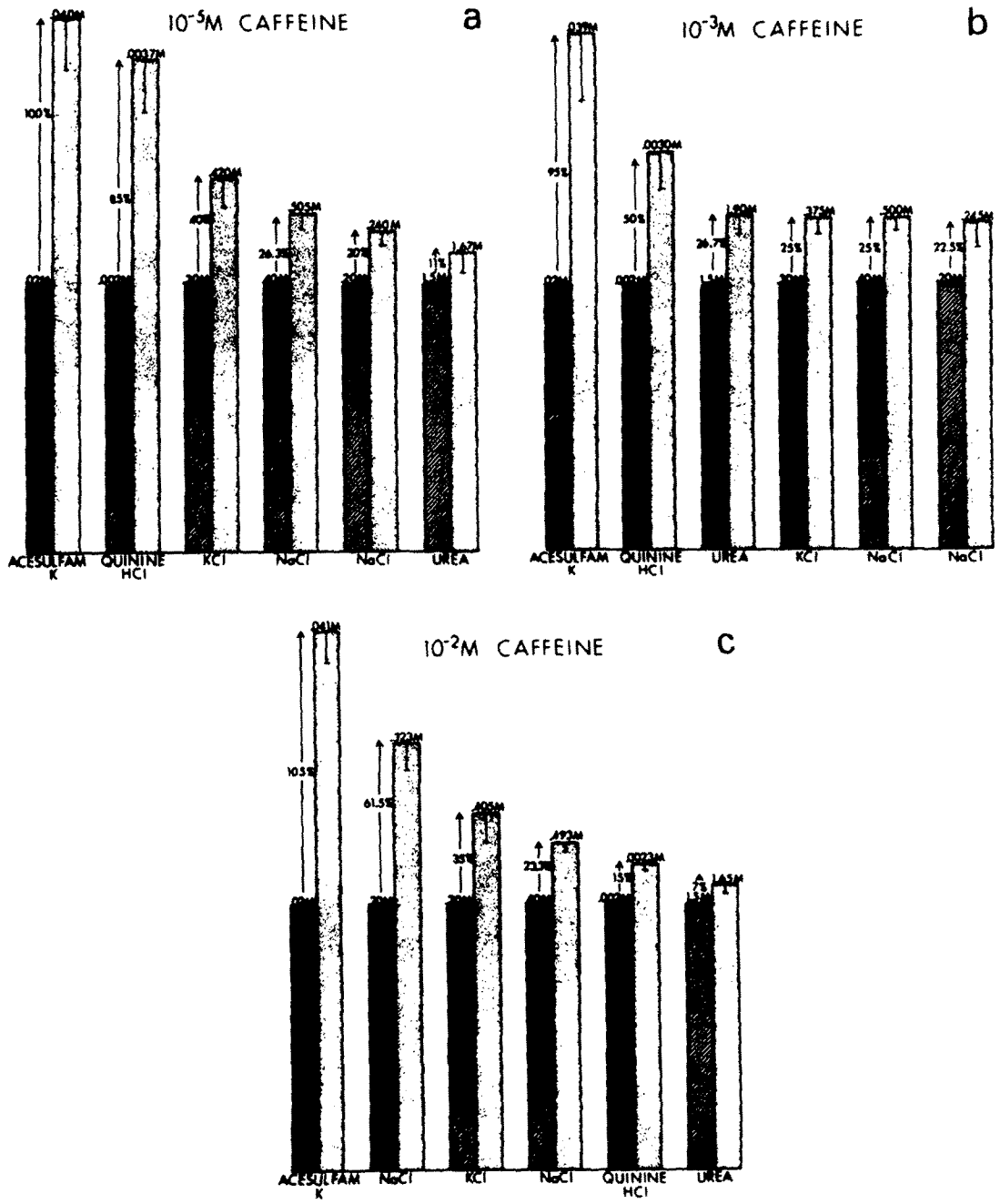

FIG. 2a. Human studies. The striped bar indicates the standard concentration applied to the side of the tongue adapted to 10^{-5} M caffeine. The dotted bar indicates the concentration perceived to match the standard. Standard errors are shown as well. The percentage is the percent potentiation achieved by 10^{-5} M caffeine. b. Percent potentiation achieved by 10^{-3} M caffeine in humans. c. Percent potentiation achieved by 10^{-2} M caffeine in humans.

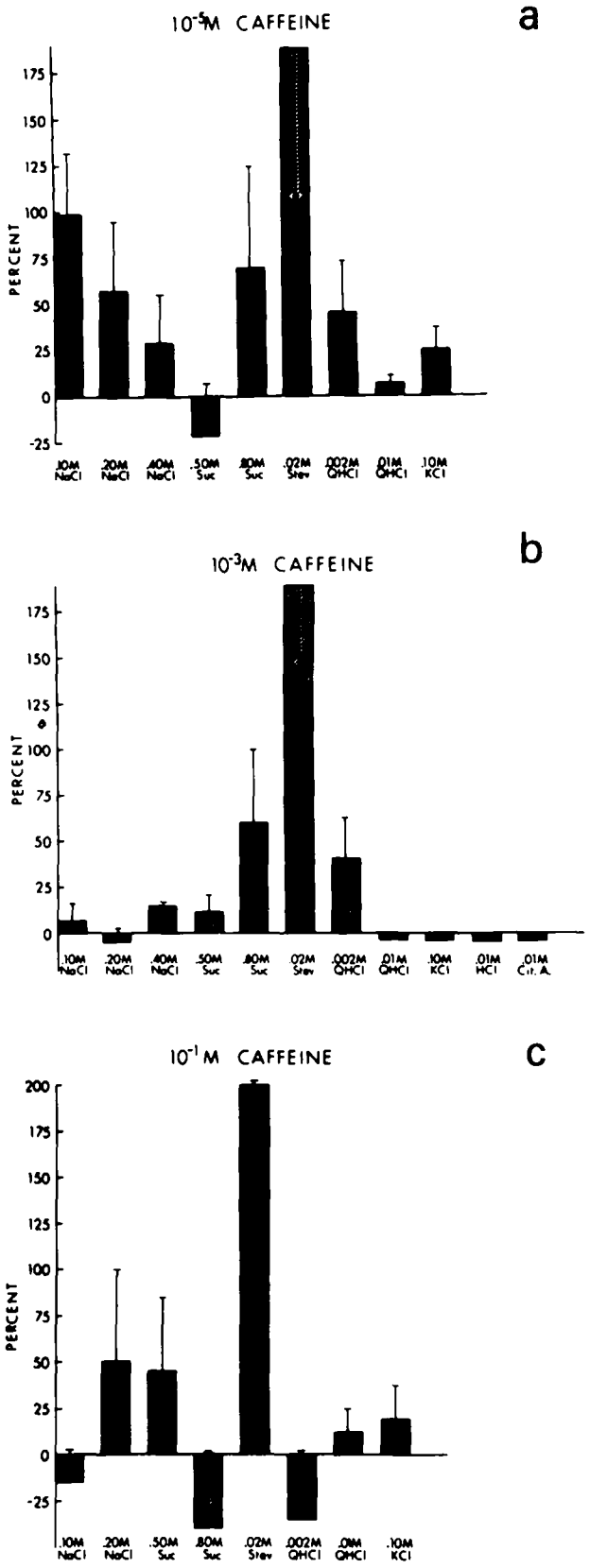

FIG. 3a. Percent potentiation achieved by 10^{-5} M caffeine in rats. b. Percent potentiation achieved by 10^{-3} M caffeine in rats. c. Percent potentiation achieved by 10^{-1} M caffeine in rats.

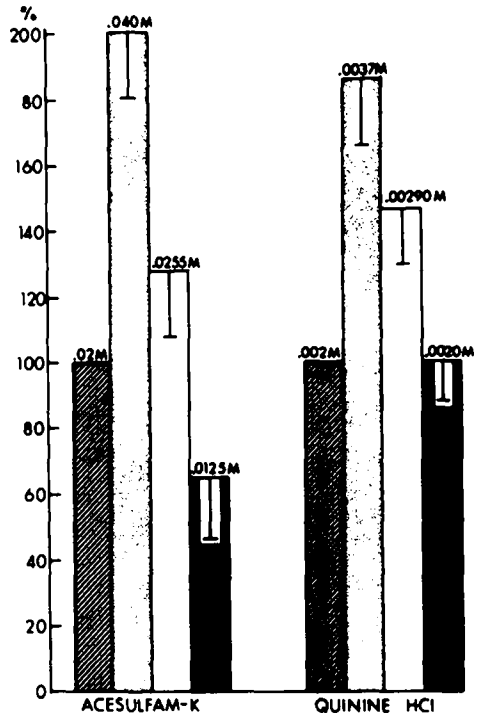

FIG. 4. Human studies. The striped bars indicate the standard concentrations of acesulfam-K and QHCl applied to the side of the tongue adapted to 10^{-5} M caffeine. The dotted bar represents the concentrations perceived to match the standards after adaptation to 10^{-3} M caffeine. Acesulfam-K was potentiated by 100%, and QHCl by 85% by 10^{-3} M caffeine. The white bar represents the perceived intensity after application of 10^{-3} M caffeine and 10^{-5} M adenosine. This adaptation mixture resulted in less potentiation, 27.5% for acesulfam-K and 45.0% for QHCl, than 10^{-5} M caffeine alone. A mixture of 10^{-3} M caffeine and 10^{-4} M adenosine actually depressed the response for acesulfam-K, leaving the response to QHCl unchanged, as shown by the solid bar. These results indicate that adenosine reverses the potentiation found by caffeine.

DISCUSSION

The data reported here indicate that the presence of methyl xanthines on the anterior tongue can potentiate taste, and that adenosine reverses this potentiation. These findings suggest that the physiologic effects of methyl xanthines on taste are due to inhibition of the A_1 adenosine receptor. The taste enhancement by methyl xanthines probably results from inhibition of endogenous adenosine, made locally, that may well saturate the high affinity A_1 receptors on the tongue.

The data also suggest that the taste enhancement is predominantly due to antagonism of the A_1 adenosine receptor rather than inhibition of phosphodiesterase. This conclusion is drawn from the observation that the potentiation of acesulfam-K is approximately the same at lower concentrations of caffeine (10^{-5} M) as at higher ones (10^{-2} M). An enhancement of 100% for acesulfam-K was found at 10^{-5} M caffeine (Fig. 2a), a concentration below that known to prevent inactivation of cAMP by phosphodiesterase. An enhancement of 105% for acesulfam-K (Fig. 2c) was found at 10^{-2} M caffeine, a concentration known to block phosphodiesterase [9,19].

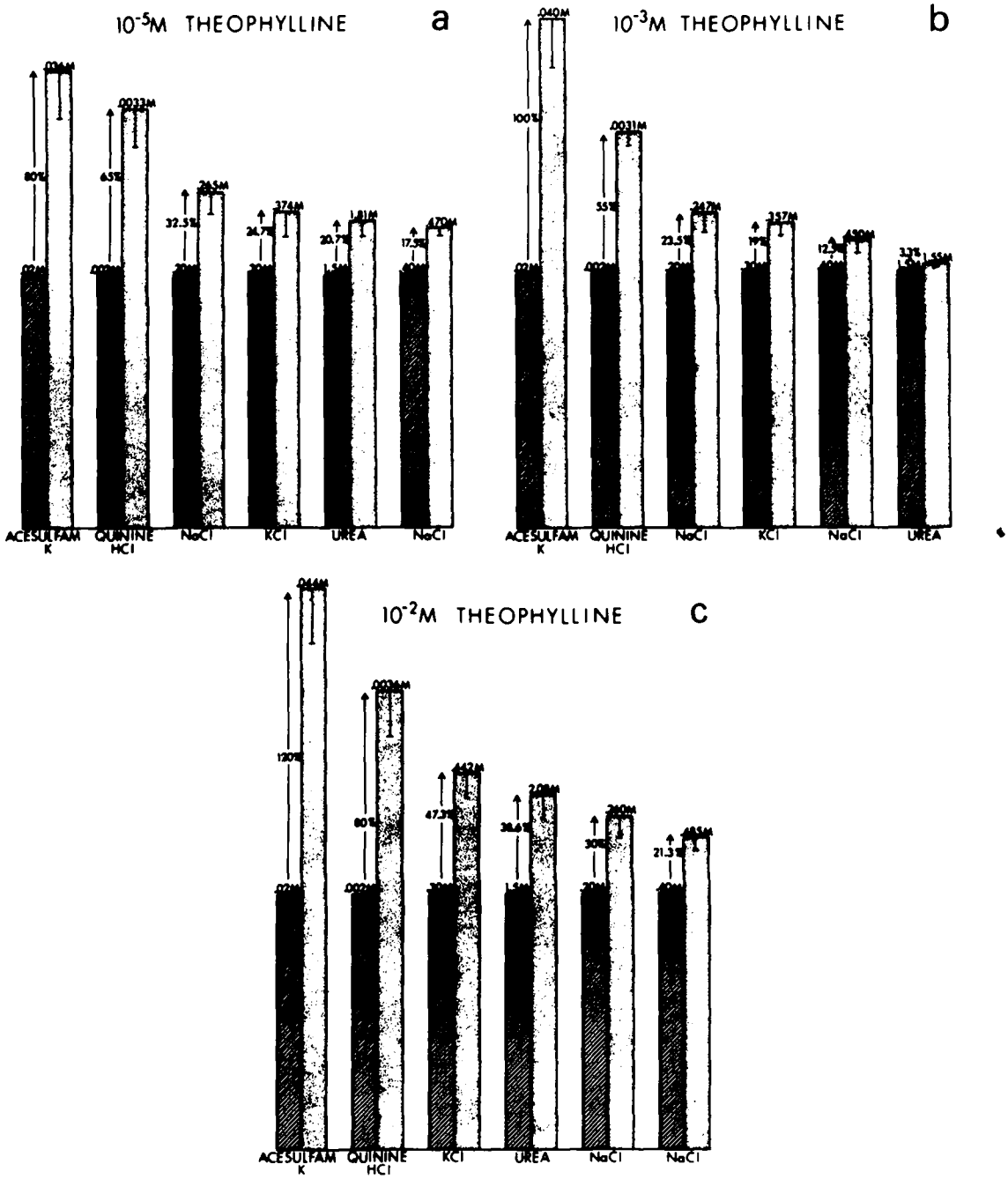

FIG. 5a. Percent potentiation achieved by 10^{-5} M theophylline in humans. b. Percent potentiation achieved by 10^{-3} M theophylline in humans. c. Percent potentiation achieved by 10^{-2} M theophylline in humans.

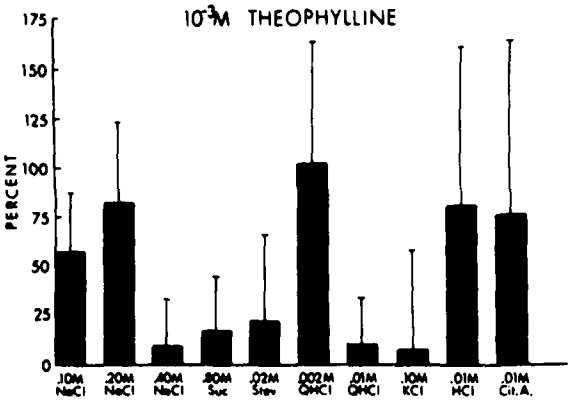

FIG. 6. Percent potentiation achieved by 10^{-3} M theophylline in rats.

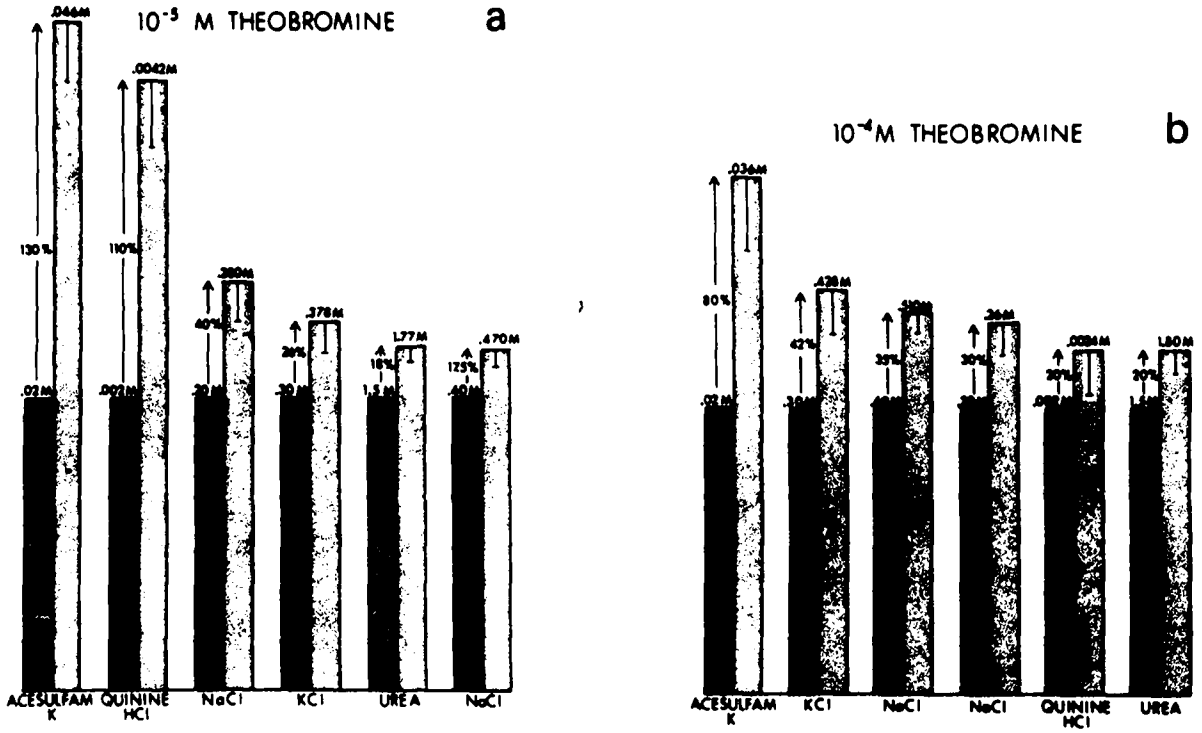

FIG. 7a. Percent potentiation achieved by 10^{-5} M theobromine in humans. b. Percent potentiation achieved by 10^{-4} M theobromine in humans.

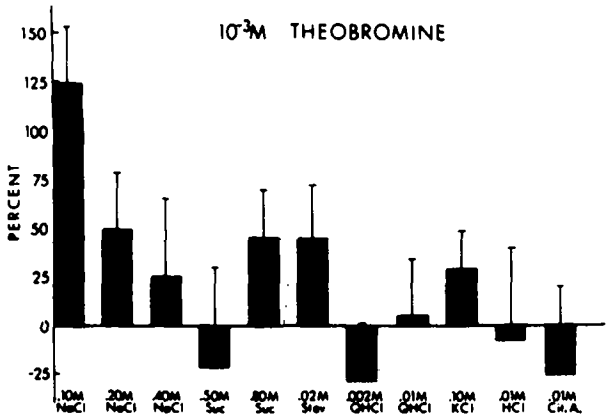

FIG. 8. Percent potentiation achieved by 10^{-3} M theobromine in rats.

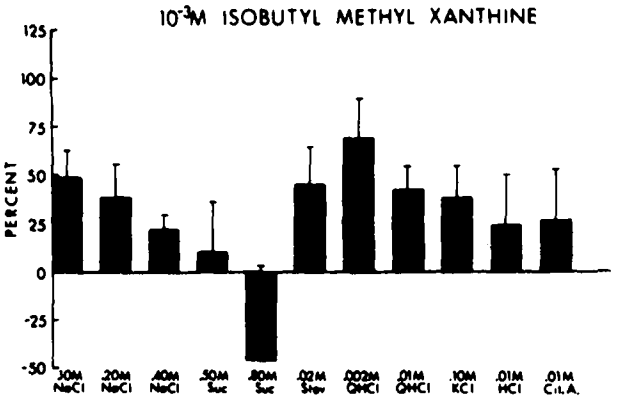

FIG. 9. Percent potentiation achieved by 10^{-3} M IBMX in rats.

Although adenosine appears to play a role in the modulating of taste perception, it cannot be confirmed from these studies whether cAMP is indeed involved as it is in other systems. Biochemical data do reveal that adenylate cyclase, the cyclic AMP forming enzyme, does exist in taste cells [1,16]; however, a precise function for cAMP in gustation has not been fully clarified [3, 4, 5, 15, 29].

While it is clear that caffeine and other methyl xanthines have many central effects, the taste potentiation found here is probably due to a local modulation at the level of the tongue rather than a central mechanism. The amount of caffeine applied to the tongue never exceeded 0.3 mg, far below the average of 120 mg in a single cup of coffee or the 55 mg found in a single cup of tea [11,12]. Also, in an unpublished study, Schiffman *et al.* [33] found that taste recognition thresholds for a range of compounds were not altered by intake of a single cup of tea in ten subjects who had been caffeine-free for 18 hours. Recognition thresholds were determined 15 min after the intake of tea. The precise location of the local effect of methyl xanthines cannot be specified at this time but may involve the taste cells themselves or the nerve endings and fibers of the chorda typmpani associated with taste cells on the anterior tongue.

Other studies show that chronic administration of methyl xanthines may improve taste perception. Fregly [10] found that rats who were fed theophylline (2.5 gm/kg) could detect the difference between NaCl and water at 0.010 M NaCl while control rats required a concentration of 0.030 M NaCl. In our laboratory, we have observed that some subjects taking theophylline and aminophylline have increased sensitivity to both tastes and odors that is reversed when the drugs are discontinued. Increased taste and smell sensitivity may be one of the factors that leads the elderly to consume greater amounts of coffee and tea than their younger counterparts [30]. Caffeine may be excreted in the saliva, inhibiting the A_1 adenosine receptors and increasing sensation.

In conclusion, the data presented here suggest that caffeine and other methyl xanthines potentiate certain tastes owing to their inhibition of the A_1 receptor. Further confirmation of a role for adenosine in taste might be obtained by radioactive ligand binding studies [35] to identify adenosine receptors on the tongue.

REFERENCES

1. Asanuma, N. and H. Nomura. Histochemical localization of adenylate cyclase and phosphodiesterase activities in the foliate papillae of the rabbit: II. Electron microscopic observations. *Chem Senses* **7**: 1–9, 1982.
2. 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.
3. Cagan, R. H. Cyclic AMP in bovine taste papillae: Regulation by sweet and bitter taste stimuli. *Proc Ninth Internat Congr Biochem*, Stockholm Abstr. Sect. 8, p. 380, 1973.
4. Cagan, R. H. Biochemistry of sweet sensation. In: *Sugars and Nutrition*, edited by H. L. Sipple and K. W. McNutt. New York: Academic Press, 1974, pp. 19–36.
5. Cagan, R. H. Biochemical studies of taste sensation: II. Labeling of cyclic AMP of bovine taste papillae in response to sweet and bitter stimuli. *J Neurosci Res* **2**: 363–371, 1976.
6. DeSimone, J. A., G. L. Heck, S. Mierson and S. K. DeSimone. The active ion transport properties of canine lingual epithelium in vitro. *J Gen Physiol* **83**: 633–656, 1984.
7. Drury, A. N. and A. Szent-Györgyi. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J Physiol (Lond)* **68**: 213–237, 1929.
8. Fredholm, B. B. Effect of adenosine, adenosine analogues and drugs inhibiting adenosine inactivation on lipolysis in rat fat cells. *Acta Physiol Scand* **102**: 191–198, 1978.
9. Fredholm, B. B. Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends Pharmacol Sci* **1**: 129–132, 1980.
10. Fregly, M. J., O. E. Lockley and K. A. Carlberg. Effect of methylxanthines on the spontaneous appetite for NaCl solutions in rats. *Proc Soc Exp Biol Med* **170**: 182–187, 1982.
11. Graham, D. M. Caffeine—Its identity, dietary sources, intake and biological effects. *Nutr Rev* **36**: 97–102, 1978.
12. Groisser, D. S. A study of caffeine in tea. I. A new spectrophotometric micromethod. II. Concentration of caffeine in various strengths, brands, blends, and types of teas. *Am J clin Nutr* **31**: 1727–1731, 1978.
13. 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.
14. Heck, G. L., S. Mierson and J. A. DeSimone. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science* **223**: 403–404, 1984.
15. Kurihara, K. Inhibition of cyclic 3′,5′-nucleotide phosphodiesterase in bovine taste papillae by bitter taste stimuli. *FEBS Lett* **27**: 279–281, 1972.
16. Kurihara, K. and N. Koyama. High activity of adenyl cyclase in olfactory and gustatory organs. *Biochem Biophys Res Commun* **48**: 30–34, 1972.
17. Londos, C., D. M. F. Cooper, W. Schlegel and M. Rodbell. Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: Basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. *Proc Natl Acad Sci USA* **75**: 5362–5366, 1978.
18. Londos, C., D. M. F. Cooper and J. Wolff. Subclasses of external adenosine receptors. *Proc Natl Acad Sci USA* **77**: 2551–2554, 1980.
19. Londos, C., J. Wolff and D. M. F. Cooper. Adenosine as a regulator of adenylate cyclase. In: *Purinergic Receptors*, edited by G. Burnstock. London: Chapman and Hall, 1981, pp. 287–323.
20. Londos, C., J. Wolff and D. M. F. Cooper. Adenosine receptors and adenylate cyclase interactions. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 17–32.
21. Marquardt, D. L., C. W. Parker and T. J. Sullivan. Potentiation of mast cell mediator release by adenosine. *J Immunol* **120**: 871–878, 1978.
22. McBurney, D. H., D. V. Smith and T. R. Shick. Gustatory cross adaptation: Sourness and bitterness. *Percept Psychophys* **11**: 228–232, 1972.
23. Mills, D. C. B., D. E. MacFarlane, B. W. G. Lemmex and R. J. Haslam. Receptors for nucleosides and nucleotides on blood platelets. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 277–289.
24. Olsson, R. A. Adenosine receptors on vascular smooth muscle. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 33–47.
25. Olsson, R. A., J. A. Snow, M. K. Gentry and G. P. Frick. Adenosine uptake by canine heart. *Circ Res* **31**: 767–778, 1972.

26. Osswald, H. Adenosine and renal function. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 399–415.
27. Phillis, J. W. and P. H. Wu. Roles of adenosine and adenine nucleotides in the CNS. In: *Physiology and Phamacology of Adenosine Derivatives*, edited by J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu and M. Ui. New York: Raven Press, 1983, pp. 219–236.
28. Phillis, J. W. and P. H. Wu. The role of adenosine in central neuromodulation. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 419–437.
29. Price, S. Phosphodiesterase in tongue epithelium: Activation by bitter taste stimuli. *Nature* **241:** 54–55, 1973.
30. Schiffman, S. S. and E. Covey. Changes in taste and smell with age: Nutritional aspects. In: *Nutrition in Gerontology*, edited by J. M. Ordy, D. Harman and R. Alfin-Slater. New York: Raven Press, 1984, pp. 43–64.
31. Schiffman, S. S., D. A. Reilly and T. B. Clark. Qualitative differences among sweeteners. *Physiol Behav* **23:** 1–9, 1979.
32. 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.
33. Schiffman, S. S., M. Schwartz and H. Soriano. Unpublished data, 1983.
34. Schubert, P., K. Lee, M. Reddington and G. Kreutzberg. Synaptic modulation by adenosine: Electrophysiological and biochemical characteristics. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 439–454.
35. Schwabe, U. General aspects of binding of ligands to adenosine receptors. In: *Regulatory Function of Adenosine*, edited by R. M. Berne, T. W. Rall and R. Rubio. The Hague: Martinus Nijhoff, 1983, pp. 77–96.
36. 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.
37. Snyder, S. H. Drug and neurotransmitter receptors in the brain. *Science* **224:** 22–31, 1984.
38. Thurau, K. Renal hemodynamics. *Am J Med* **36:** 698–719, 1964.
39. Van Calker, D., M. Müller 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.
40. 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.
41. Woolston, D. C. and R. P. Erickson. Concept of neuron types in gustation in the rat. *J Neurophysiol* **42:** 1390–1409, 1979.