POSTINGESTIVE CONTROLS OF CARBOHYDRATE-BASED FOOD PREFERENCES: EFFECT OF SITE AND DELAY OF NUTRIENT INFUSIONS

by

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Abstract

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Advisor: Professor Anthony Sclafani

The physiological controls which mediate the acquisition of food preferences are not well understood. This dissertation research investigated how the gastrointestinal site and delay of carbohydrate delivery influence the development of flavor preferences in rats. The conditioned flavor preference procedure was used in all experiments. On alternate training days, rats consumed nonnutritive CS+ and CS- cue flavors which were paired with carbohydrate and water infusions, respectively. On test days, rats were given a choice between the two cue flavors without infusions, and preferences were assessed. In Experiment 1, the preferences conditioned by intragastric (IG) and intraduodenal (ID) glucose infusions were compared in two groups of rats. Strong and comparable flavor preferences were conditioned by IG and ID glucose infusions. Experiment 2A examined whether the actions of glucose in the stomach alone are sufficient to condition flavor preferences. When IG glucose infusions were restricted to the stomach during training by inflation of a pyloric cuff, preferences were not obtained. Preferences were observed in a control group that was able to experience the post-gastric effects of the IG glucose infusions. Experiment 2B demonstrated that pyloric cuff inflation during conditioning does not prevent conditioning when the delayed post-gastric

effects of the glucose were permitted following each conditioning session. If preferences are mediated by post-gastric effects, conditioning must be able to withstand delays. Experiment 3 examined preference conditioning with delays of 2.5, 10, 30 and 60 minutes between cue flavor consumption and IG 8% glucose infusions. The strength of the preferences decreased as the delay interval increased; with a 60 minute delay preferences were not exhibited. To determine whether increasing the magnitude of reinforcement promotes conditioning with a 60 minute delay, Experiment 4 compared the preferences conditioned by IG infusions of 8% and 16% Polycose. Preferences were conditioned by both concentrations of Polycose, although, 16% Polycose tended to condition stronger and more reliable preferences. Taken together, the findings suggest that preference conditioning is mediated by the post-gastric actions of carbohydrates which may occur following a delay. Possible mechanisms for long-delay learning and communication between gut and brain were discussed.

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INTRODUCTION

There is evidence from both animal and human research that individuals learn to prefer nutritious foods and avoid toxic ones (Booth, Mather, and Fuller, 1982; Birch, McPhee, Steinberg, and Sullivan, 1990; Sclafani, 1990; Tordoff, 1991; Rozin and Kalat, 1971; Barker, Best, and Domjan, 1977). This suggests that information regarding a food's value is detected and processed in a manner which promotes adaptive food selection. It is believed that an association is formed between the flavor of foods and their postingestive effects. Where and how nutritional information is detected and by what pathways it is conveyed to the brain to modulate food preferences are not well understood. This dissertation research investigated two aspects of the postingestive actions of carbohydrates and their influence on the development of food preferences.

The first issue that was addressed was the site of nutrient actions. Specifically, the contribution of the stomach and post-gastric sites in postingestive reinforcement by carbohydrates was examined. This was accomplished in two experiments. Experiment 1 evaluated the development of preference when gastric stimulation by nutrients was eliminated by infusing nutrients directly to the intestine as rats consumed a cue flavor. Experiment 2 assessed the development of preference when nutrients were restricted to the stomach and not permitted to stimulate post-gastric sites.

The second issue that was addressed in this dissertation was the effectiveness of carbohydrates in reinforcing flavor preferences when their postingestive actions were delayed. This relates to the site of action issue because if the reinforcing effects occur beyond the stomach there will invariably

be a delay between the flavor and postingestive reinforcer. Two experiments examined the extent to which flavor preferences can be acquired with increasing delays between cue flavor consumption and postingestive nutritive effects. Both sets of experiments added important new information regarding the site and temporal aspects of nutrient reinforcement.

The Physiology of Digestion

The ingestion of food elicits a number of events which could provide a stimulus for reinforcement. The sight, smell and taste of food produce "cephalic phase" preparatory responses including gastric and pancreatic secretions and receptive gastric relaxation. These responses prepare the body for arrival of food in the gut and promote digestive efficiency (Berthoud and Jeanrenaud, 1982; Powley, 1977; Tordoff and Friedman, 1989). When food is ingested, receptors in the mouth and nose respond to its taste, odor, temperature and texture. A substantial amount of mechanical breakdown of foods occurs in the mouth and chemical breakdown of carbohydrates and fats by salivary amylase and lipase begins there.

Mechanical and chemical digestion continues in the stomach. Foods of varying composition are broken down physically and chemically by acid and enzymes and by the churning action of the stomach. Foods are transformed into chyme, a semi-liquid state, in preparation for the absorptive surface of the intestine.

The pyloric sphincter lies between the stomach and duodenum, the first segment of the small intestine, and it either constricts or relaxes allowing for

controlled gastro-duodenal transit. When liquid nutrients first enter the stomach there is initially a rapid rate of emptying into the duodenum. But, within minutes, this rapid emptying phase is interrupted by pyloric constriction, and the rate of gastric emptying then demonstrates a linear calorie-constant function (McHugh & Moran, 1985; Kalogeris, Reidelberger, and Mendel, 1983; Maerz, Sankaran, Scharpf, and Deveney, 1994). It has been proposed that this control is provided by the intestinal hormone cholecystokinin (CCK) (McHugh & Moran, 1985). Specifically, the entry of nutrient into the duodenum causes the release of CCK which inhibits further gastric emptying by causing constriction of the pylorus. As nutrients are absorbed from the intestine, CCK secretion declines, and the pylorus relaxes, leading to the further delivery of nutrients to the duodenum and further secretion of the hormone. Other hormones, including glucagon and insulin, are released in response to the presence of nutrients in the gastrointestinal (GI) tract as well.

Nutrients absorbed from the intestine are transported to the circulatory and lymphatic systems. The lymphatic system carries most of the products of fat digestion to the blood; products of carbohydrate and protein digestion are absorbed from the capillaries surrounding the intestines and travel via the hepatic portal vein to the liver and then to the general circulation.

Microelectrode recording has established that the GI tract is studded with receptors which gives rise to a wide range of neural signals, including information regarding the chemico-physical properties of the chyme (including its chemical composition, osmotic pressure, temperature and pH) and the physical state produced by it (degree of distension or contraction of the GI

tract); this information is carried to the brain mainly by afferent vagal and splanchnic nerves (Mei and Lucchini, 1992; Mei, 1993). The hepatic branch of the vagus nerve carries information from the liver and also from the intestine (Ritter, Brenner & Yox, 1992; Niijima, 1983). Nutrient information from the periphery may also be carried to the brain by the blood. Information received by the brain from one or more of these signals may contribute to food's reinforcing effects.

Learning and Food Preference

It is generally believed that the vast majority of food preferences and aversions are not innate, but rather are acquired and molded by experience. Pavlovian conditioning has been offered as a major explanation for acquired food likes and dislikes (e.g.,Rozin and Zellner, 1985). According to this paradigm, the preference for a novel flavor, referred to as a conditioned stimulus (CS) can be altered by the value of an unconditioned stimulus (US) (e.g. nutrient or poison) with which it is paired. Through conditioning, preference for the CS may increase or decrease. If the US is positive, then the preference for the CS increases; if the US is negative, then the preference for the CS decreases. Two different forms of Pavlovian food conditioning have been identified: flavor-flavor and flavor-postingestive conditioning.

In the flavor-flavor conditioning paradigm, the simultaneous presentation of a novel flavor (CS), with an already liked (or disliked) flavor (US) will bring about an increase (or decrease) in preference for the originally novel flavor. For instance, Fanselow and Birk (1982) observed that rats increased their

preference for a novel flavor that was mixed into a sweet saccharin solution and decreased their preference for a flavor that was mixed into a bitter quinine solution. The preference shifts for these CSs are attributed to their association with the flavor of the USs.

In flavor-postingestive conditioning, the preference for the flavor CS is modified by its association with the postingestive consequences of the US. The best known example of this is conditioned flavor aversions (CFAs) in which a CS flavor that is followed by a toxin US is subsequently avoided. CFAs have been demonstrated in numerous animal studies (see Riley and Tuck, 1985) and have also been observed in cancer patients receiving nausea-producing chemotherapy (Bernstein & Meachum, 1990).

The majority of research examining the role of postingestive consequences in food selection has focused on flavor aversions. However, in nature the number of food aversions developed is relatively rare compared to the vast number of food preferences that individuals display. Recent studies have demonstrated that flavor preferences can be conditioned by the positive postingestive consequences of nutrients (Booth, 1985; Sclafani, 1990; Tordoff, 1991). Although flavor preferences have been conditioned in humans (Booth, Mather, and Fuller, 1982; Birch, McPhee, Steinberg, and Sullivan, 1990; Zellner, Rozin, Aron, and Kulish, 1983; Hammer, Shide, and Rolls, 1994), experimental techniques used in rats has provided more definitive evidence for the postingestive modulation of food preferences.

The acquisition of food preferences has been studied experimentally using the conditioned flavor preference paradigm. Consumption of a novel cue

flavor, the CS +, is paired with a nutrient US; another novel cue flavor, the CS-, is paired with something nonnutritive, less nutritive, or nothing at all. After pairings with each, animals are given two-bottle choice tests with the CS + and the CS-. Preference is determined by calculating the CS + intake with respect to total consumption in the choice test.

In some of the earliest flavor preference conditioning studies, CS flavors were mixed directly into powdered diets differing in energy density during training (Booth, 1972; Bolles, Hayward, and Crandall, 1981; Hayward, 1983). The establishment of flavor preferences was assessed by presenting rats with a choice between the two CS flavors mixed into a mid-energy mixture of the two diets. Other studies mixed CS flavors into nutritive and non-nutritive solutions during training (Booth, Lovett, and McSherry, 1972; Capaldi, Campbell, Sheffer, and Bradford, 1987; Fedorchak and Bolles, 1987; Holman, 1975; Mehiel and Bolles, 1984; Mehiel and Bolles, 1988; Simbayi, Boakes, and Burton, 1986; Sclafani, 1990). Demonstrations of CS + preferences were generally attributed to the greater caloric consequences of the US. However, because the flavor of nutrients such as sugar and starch typically used in these conditioning studies are very palatable to rats (Sclafani, 1987) the observed preferences may have been due to flavor-flavor rather than, or in addition to, flavor-postingestive conditioning. Though these initial experiments established that flavors associated with energy-rich foods become preferred, they did little to elucidate whether it is food's flavor, its postingestive effects, or both that produce learned preferences.

The strongest evidence for learning based on the postingestive effects

of nutrients comes from studies that paired consumption of a nonnutritive flavor with intragastric (IG) nutrient infusions. Because the animals never taste the IG nutrients, learning must be attributable to its postingestive consequences. One of the first demonstrations of the ability of IG nutrients to condition a flavor preference was made by Holman in 1968. Since that time several studies have shown that rats acquire preferences for flavors paired with IG infusions of various nutritive substances including milk, glucose, ethanol, sucrose, casein, polysaccharides (e.g. Polycose), corn oil, and fructose (Puerto, Deutsch, Molina, and Roll, 1976; Puerto, Deutsch, Molina, and Roll, 1976; Deutsch and Wang, 1977; Sherman, Hickis, Rice, Rusiniak, and Garcia, 1983; Baker, Booth, Duggan, and Gibson, 1987; Sclafani and Nissenbaum, 1988; Lucas and Sclafani, 1989; Tordoff, Ulrich, and Sandler, 1990). However, some attempts to condition preferences for flavors paired with IG nutrients have failed (Mather, Nicolaidis, and Booth, 1978; Koopmans and Maggio, 1978; Puerto, Deutsch, Molina, and Roll, 1976; Puerto, Deutsch, Molina, and Roll, 1976; Ramirez, 1984; Gonzalez and Deutsch, 1985; Sclafani, Cardieri, Tucker, Blusk, and Ackroff, 1993) and others have resulted in conditioned aversions (Deutsch, Molina, and Puerto, 1976; Ramirez, 1984; Koopmans and Maggio, 1978; Sherman, Hickis, Rice, Rusiniak, and Garcia, 1983).

These discrepant findings may be attributable to procedural differences such as salience and volume of the CSs and USs; differences in the infusion parameters (e.g., concentration, rate and volume); and differences in the reinforcing potencies of different classes of nutrients. Negative or null results may be merely artifacts of the unnatural route of nutrient delivery and should

not detract from the positive findings. For instance, bypassing oral processing of nutrients can interfere with digestion and alter metabolism (Molina, Thiel, Deutsch, and Puerto, 1977; Ramirez, 1986; Ramirez, 1985), and concentrated glucose infusions can have aversive osmotic effects that counteract their positive reinforcing effects (Booth, 1985).

Robust flavor preferences have been conditioned by IG infusions by Sclafani and his colleagues (Sclafani and Nissenbaum, 1988; Elizalde and Sclafani, 1990b; Drucker, Ackroff, and Sclafani, 1993; Sclafani, Cardieri, Tucker, Blusk, and Ackroff, 1993; Pérez, Ackroff, and Sclafani, 1995). The strongest have been produced by IG infusions of the carbohydrate, Polycose (Sclafani and Nissenbaum, 1988). Rats displayed a near total preference (96%) for a CS+ flavor paired with IG Polycose over a CS- flavor paired with IG water after only four training days. The effectiveness of this conditioning procedure was attributed to the use of long-term (23-hr/day) training procedures which allowed multiple CS-US pairings, the rats' ability to control the size, duration and frequency of the infusions, and the use of Polycose. Polycose is a corn starch hydrolyzate made up of glucose with chains lengths mainly greater than 3 units which yield a relatively low osmolarity which lessens the potential for osmotic disturbances (Ross Laboratories, 1984). The observed preferences for flavors paired with IG Polycose cannot be attributed to a CS- aversion due to its pairing with water infusions, instead, because rats show indifference when subsequently allowed to choose between the CS- and water (Elizalde and Sclafani, 1990b).

Preferences conditioned by IG nutrient infusions clearly demonstrate the

capacity for learning based on the postingestive consequences of nutrients. However, the specific postingestive event(s) involved in this learning still remains unclear.

Identifying the Mechanism for Nutrient Conditioning

Satiety and Food Preference

It has often been assumed that the satiating actions of foods reinforce preferences (LeMagnen, 1955; Booth, 1985; Rozin and Vollmeche, 1986; Bolles, Hayward, and Crandall, 1981). LeMagnen (1955) argued that animals learn to associate the (postabsorptive) satiating qualities of foods with their flavor, resulting in an increase in palatability, but did not demonstrate that the intake of a flavor paired with intraperitoneal (ip) glucose infusions was associated with an increased preference for that flavor. Bedard and Weingarten (1989) also failed to condition a preference for a flavor paired with ip glucose. These negative results may have been due to some aversive component of the ip infusions that were not addressed, however.

As discussed above, multiple signals are produced by the digestion of food. Several investigators have attempted to determine whether satiety signals originate in the digestive tract. This has typically been accomplished by examining changes in food intake when nutrients are delivered to various segments of the digestive tract. For instance, the finding that food intake is grossly exaggerated when food deprived rats sham-feed suggests that feedback from the mouth does not contribute substantially to satiety under these conditions. Other studies have shown that feedback from the stomach (Kraly

and Smith, 1978; Deutsch, Young, and Kalogeris, 1978), intestine (Liebling, Eisner, Gibbs, and Smith, 1975; Vanderweele, Novin, Rezek, and Sanderson, 1974), and the liver (Novin, 1993; Tordoff and Friedman, 1986; Russek, 1970) can all contribute to the control of meal size because nutrients delivered to each of these sites reduces voluntary food consumption. Thus, information received from multiple sites in the digestive tract and the liver contributes to the satiating actions of foods.

Although only a few experiments have simultaneously examined both satiation and reinforcement by nutrients, the existing evidence suggests that it may not be the satiating actions of nutrients which reinforce flavor preferences. First, by definition, the satiating effects of foods decrease food intake. However, preferred foods may be consumed in greater quantity than unpreferred foods (Elizalde and Sclafani, 1988). Second, nutrient-based flavor preferences are learned in the absence of satiety (Deutsch and Wang, 1977; Van Vort and Smith, 1983) and the satiating effects of nutrients do not always reinforce flavor preferences (Van Vort and Smith, 1983; Sclafani, Cardieri, Tucker, Blusk, and Ackroff, 1993; Sclafani, Nissenbaum, and Ackroff, 1994). Furthermore, experimental treatments (e.g., CCK, capsaicin, and vagal nerve transection) which affect meal satiety do not affect the development of food preferences in a corresponding fashion (Pérez and Sclafani, 1991; Horn, Mitchell, and Martinson, 1993; Sclafani and Lucas, 1995). These dissociations of satiety and reinforcement suggest that the physiological mechanisms responsible for them may differ. Although there is likely to be some overlap in these processes, it can not be assumed that the satiating quality of foods is

what makes them reinforcing.

Although many studies have attempted to identify the peripheral sites that generate satiety signals, relatively few studies have examined the contribution of different sites to the reinforcing actions of nutrients. Some early studies examined whether the reinforcing effect of foods can be mediated by increases in blood glucose (Coppock and Chambers, 1954; Revusky, Smith, and Chalmers, 1971; Mather, Nicolaidis, and Booth, 1978). More recently, Tordoff (1991) has suggested that reinforcement is derived from the state of fuel oxidation in the liver which is conveyed to the brain via hepatic vagal afferents. Alternatively, Deutsch and Wang (1977) suggested that preabsorptive afferent feedback from the stomach signals reinforcement. The relevant findings pertaining to each of these hypotheses are reviewed below.

Postabsorptive Conditioning

In an attempt to determine whether postabsorptive blood-borne factors can modulate food preferences one can examine the reinforcing effect of intravenous (IV) nutrient infusions, thereby bypassing the GI tract. Only a few studies have paired consumption of flavored food or nonnutritive solutions with IV infusions (Mather, Nicolaidis, and Booth, 1978; Revusky, Smith, Jr., and Chalmers, 1971; Coppock and Chambers, 1954; Gowans and Weingarten, 1991; Tordoff and Friedman, 1986). Infusions have been delivered to the jugular and hepatic-portal veins.

Intrajugular Conditioning. Only one study has been successful in conditioning preferences for a flavor paired with intrajugular glucose infusions

(Mather, Nicolaidis, and Booth, 1978). Other attempts have been ineffective (Coppock and Chambers, 1954; Revusky, Smith, Jr., and Chalmers, 1971; Tordoff and Friedman, 1986; Gowans and Weingarten, 1991). Mather et al. argued that the negative findings may have been due to the failure to mimic the normal context of nutrient utilization (e.g. adequate secretion of insulin). They presumed that their success was based on the use of meal-contingent glucose infusions which evoked the appropriate physiological conditions for nutrient use. However, Tordoff and Friedman (1986) also paired meal-contingent flavor cues with jugular glucose infusions, but did not obtain CS+ preferences.

Hepatic-Portal Conditioning. Tordoff and Friedman (1986) obtained preferences for a flavor paired with meal-contingent hepatic-portal glucose infusions, but did not obtain preferences with meal-contingent jugular infusions. Based on this finding and other less direct evidence (Tordoff, Tepper, and Friedman, 1987; Tordoff and Friedman, 1988), these investigators suggested that fuel oxidation in the liver is the postingestive event that conditions flavor preferences. Furthermore, because they found that preferences conditioned by oral or IG fructose were blocked by hepatic vagotomy (Tordoff, Ulrich, and Sandler, 1990) they argued that this information is conveyed to the brain via the hepatic branch of the vagus nerve . However, both Horn et al. (1993) and Sclafani and Lucas (1995) have recently found that an intact hepatic vagus is not required for carbohydrate-conditioned flavor preferences. Nevertheless, Tordoff and Friedman's (1986) finding that preferences can be conditioned by hepatic-portal glucose infusions during meals indicates that additional nutrients delivered to the liver are sufficient to produce preference conditioning.

Interestingly, it has not been demonstrated whether postabsorptive increases in nutrient levels (via jugular or hepatic-portal infusions) in the absence of preabsorptive nutrient actions are sufficient to condition preferences. The two studies discussed above in which preferences were conditioned by jugular and hepatic-portal vein infusions mixed the cue flavors into a nutrient-rich chow vehicle which was orally consumed. Thus the infused nutrients were processed in conjunction with orally consumed nutrient. The finding that conditioning based on postabsorptive nutrients has only been observed under these conditions raises the possibility that preabsorptive nutrient stimulation may be required for postingestive conditioning. In support of this concept, Gowans (1992) found that hepatic-portal glucose infusions supported conditioning only when the cue flavors were mixed into a nutritive sugar solution, but not when they were mixed into a saccharin solution. In another study, Gowans and Weingarten (1991) failed to condition preferences for a flavor of sham-fed sucrose that was paired with jugular glucose infusions. Thus, reinforcement based on postabsorptive glucose infusions may be possible only in the presence of nutrients stimuli in the stomach or intestine. Even if postabsorptive nutrient effects are sufficient to support conditioning in isolation, it is conceivable that preabsorptive nutrient actions may be sufficient as well.

Preabsorptive Conditioning

Of all the studies involving nutrient infusions, IG infusions have been, by far, the most successful in demonstrating postingestive conditioning. This suggests that gastric or intestinal stimuli may contribute substantially to the

reinforcing effects mediating conditioned food preferences. However, as discussed below, the roles of the stomach and intestine in mediating nutrient reinforcement have not been adequately explored.

Intestinal Conditioning: There have been no published studies employing the conditioned preference paradigm with intestinal infusions. Deutsch, Molina and Puerto (1976) examined the effect of intraduodenal (ID) glucose infusions on one-bottle consumption of a paired nonnutritive flavor. Over 8 training days, 6 food and water deprived rats were given 15 minute alternate-day access to one of two nonnutritive flavored solutions. On odd days, consumption of one flavored solution was followed by 3 ml ID infusions of 18% glucose solution; on even days consumption of another flavor was followed by 3 ml ID infusions of physiological (0.9%) saline solution. A control group of rats received saline infusion or no infusion paired with the two flavors. Over the 8 days rats that were infused with glucose decreased their intake of the associated flavor and increased their intake of the saline-associated flavor. In contrast, the control group consumed equivalent amounts of the two flavored solutions. Although two-bottle preference tests were not conducted, the authors concluded that ID infusions may be aversive. They maintained that their findings cast doubt on the role of the duodenum as a potential site of nutrient detection (in satiety) as advocated by others (Snowdon, 1975; Ehman, Albert, and Jamieson, 1971; Gibbs, Young, and Smith, 1973).

As already discussed, negative results must be interpreted cautiously because inappropriate infusion parameters may produce aversive effects. In fact, the rate at which Deutsch et al. (1976) delivered the glucose solution to

the duodenum was more than twice the observed rate of gastric emptying in rats (Kalogeris, Reidelberger, and Mendel, 1983). In addition, the use of a hypertonic (18%) glucose solution may have produced osmotic bloating, which has been shown to decrease consumption and produce behavioral elements of aversion in rats (Bardos, 1989; Deutsch and Gonzalez, 1978). A 5% glucose solution is approximately isotonic to body fluids. Thus, upper gastrointestinal discomfort produced by the 18% glucose infusions may have counteracted its positive nutritional effects resulting in decreasing intakes of the associated flavor.

Other interpretations are also possible in explaining the decreased intake of the glucose-associated flavor observed by Deutsch et al. (1976). Conditioned satiety is one. Booth (1972) has observed that rats learn to consume less of a flavor associated with an energy-dense food and more of a flavor associated with an energy-dilute food, a process that he labelled conditioned satiety. Although rats will consume less of a flavor in a one-choice test that was paired with an energy-dense food than they do of a flavor that was paired with a less energy-dense food, when given a choice between the two flavors, the one associated with the food of higher energy density is preferred (Booth, 1972). The animals in the Deutsch et al. (1976) study may also have consumed less of the glucose-paired flavor due to conditioned satiety. It is also worth noting that Liebling, Eisner, Gibbs, and Smith (1975) have observed the stereotypical *behavioral sequence of satiety* (rather than that of aversion) following ID nutrient infusions in rats. And, in the same study, a flavor aversion was not formed to saccharin solution when it was paired with

similar ID infusions. These observations demonstrate that ID nutrient infusions are not necessarily aversive. Whether or not ID nutrient infusions are sufficient to mediate preference conditioning was addressed in this dissertation research.

Gastric Conditioning: Deutsch and colleagues proposed that information from the stomach alone is capable of controlling meal size and conditioning food preferences (Puerto, Deutsch, Molina, and Roll, 1976; Puerto, Deutsch, Molina, and Roll, 1976; Deutsch and Wang, 1977; Deutsch, Young, and Kalogeris, 1978; Deutsch, 1978; Deutsch, 1983; Deutsch, Gonzalez, and Young, 1980; Deutsch, 1987). According to their hypothesis, nutrients stimulate receptors in the gastric wall, and this information is rapidly conveyed to the brain where it becomes associated with the flavor of the food currently being consumed.

Deutsch and colleagues further proposed that the stomach produces two types of signals: one which conveys information about the degree of gastric distension via the vagal nerve and one which conveys information about gastric chemical content via the splanchnic nerve. This was based on two findings. First, they found that severing the vagus nerve abolished control of meal size by distension, though control by content was left intact (Gonzalez and Deutsch, 1981). Second, severing the splanchnic nerve abolished control of feeding by nutrient content even though control by distension was preserved (Deutsch and Jang Ahn, 1986).

The ability of the stomach to detect chemical food stimuli has not been thoroughly investigated. One study done in the cat stomach demonstrated the existence of receptors which respond specifically to carbohydrates (El Ouazzani and Mei, 1981). Such gastric nutrient receptors may signal the arrival of

nutrient in the stomach and consequently mediate food preferences.

Deutsch and colleagues offered the results of several conditioning studies to support this gastric model of nutrient reinforcement. In one experiment, food and water deprived rats were allowed to choose for 10 minutes each day between a CS + and a CS- flavor paired with IG nutrient and saline infusions, respectively (Puerto et al., 1976). The nutrient used for infusions was a preingested milk mixture which was pumped out of the stomachs of donor rats; the CS- paired infusion was a physiological saline solution. When the rats drank the CS + or the CS-, equivalent volumes of the paired solutions were infused. Within the first 10-minute session, 7 of 8 rats drank more CS + than CS-. The authors concluded that the preferences emerged too quickly to be a result of postabsorptive nutrient actions and must therefore have been mediated by nutrient detectors in the upper gastrointestinal tract.

Because animals acquire flavor-nutrient through a process of learning, the preferences observed by Puerto et al. (1976) in the first 10-minute conditioning session are somewhat surprising. These findings suggest that rapid, one-trial, flavor-postingestive learning is possible. This effect is not reliable, however. In the same report, another group of rats that were trained similarly (except that no infusion was paired with consumption of the CS- flavor) did not demonstrate a CS + preference until the third test. The authors argued that the apparent indifference on the first two days was actually a partial avoidance that was due to the novelty of the stomach infusion that was paired with the CS + flavor. If this were the case, then CS- preferences rather

than indifference should have been initially observed. In the same study, other groups of rats failed to display a CS+ preference in the first training session (and sometimes not at all) when other nutritive USs, such as preingested glucose or fresh milk were infused.

In an attempt to explain the discrepant findings with preingested versus undigested milk, Deutsch et al. (1977) obtained some evidence to suggest that undigested nutrients infused directly into the stomach may produce aversive consequences due to premature emptying into the duodenum. These aversive consequences could offset the rewarding consequences of the nutrient and, thereby, interfere with preference conditioning. Although this explanation may address the lack of conditioning with fresh milk, it does not explain why they failed to get conditioning with preingested glucose. Another problem with their explanation is that, as indicated above, several investigators have obtained preferences for flavors paired with IG nutrient infusions, despite the absence of their prior digestive processing. However, it is also worth noting that few studies have examined and obtained preference conditioning with milk as the nutritive US (Holman, 1968).

In a subsequent experiment, Deutsch and Wang (1977) attempted to more clearly determine whether the rapid discrimination observed by Puerto et al. (1976) was due to gastric or post-gastric nutrient actions. To this end, rats were surgically implanted with two gastric catheters and an inflatable cuff that surrounded the pylorus. When the cuff was temporarily inflated by filling it with fluid, the gastric contents were restricted to the stomach. As was done in the previous study, the rats were given concurrent 10 minute/day access to both

the CS+ and CS- flavors paired with IG preingested milk or saline infusions, respectively. During each 10-minute session the cuff was inflated. Following each 10-minute session, the pyloric cuff was deflated allowing the gastric contents to empty into the intestine.

The rats demonstrated a slight, nonsignificant CS+ preference on the first day and significant preferences on subsequent days. The authors concluded that neural information from the stomach alone can produce rapid reinforcement of flavor preferences. They claimed that the rewarding effect is not due to absorption because each daily session lasts only 10 minutes and most rats sampled both flavors in each session. They argued that the reinforcement must be tied to immediate detection in the stomach, since they would not otherwise be able to discriminate between the mixture of infusates. As described in the next section, however, more recent data call this assertion into question. Therefore, the present dissertation includes experiments to determine whether IG nutrient stimuli are sufficient to condition flavor preferences.

Delay Conditioning

Baker and Booth (1989) questioned Deutsch and Wang's (1977) gastric model of reinforcement. Instead, they proposed that the delayed emptying of the nutrient/saline mixture into the intestine (following deflation of the cuff) could have reinforced the CS+ preferences. If the flavor of food does indeed become associated with actions mediated by post-gastric sites, preference conditioning must be able to withstand CS-US delays because it takes some

time for nutrients to be processed by intestinal and post-intestinal sites. Baker and Booth reasoned that if rats can learn to discriminate between a mixture of two infusates delivered 10 minutes after consumption of proportional amounts of associated CSs, the preferences observed by Deutsch and Wang need not have been due to gastric actions. They tested this possibility by giving rats concurrent access to two cue flavors for 10 minutes. After a 10-minute delay, amounts of associated high or low energy density solutions based on the amount of CS+ and CS- ingested were delivered intragastrically. If a rat consumed 60% or more of one CS, they were infused with 100% of the associated infusate. Preferences were obtained under these conditions in two separate groups of rats where the CS+ paired infusion was either a 20% carbohydrate solution or a 10% protein solution. Moreover, the preferences did not reach significance until the fifth training trial suggesting that flavor-nutrient preferences are not formed by an immediate association between the flavor and gastric feedback. Rather, they appear to be due to a more gradual learning process which may be mediated by the delayed effects of the nutrients. Although there have been no attempts to demonstrate the rapid reinforcement effect observed by Puerto et al. (1976), several studies have demonstrated conditioning based on delayed nutrient actions.

Holman (1975) was the first to demonstrate that animals are capable of associating a flavor with delayed nutritional reinforcement. The series of experiments conducted by Holman originally set out to determine whether reinforcement depended upon need reduction. For this reason both saccharin and sugar solutions were used as USs in food deprived rats. Rats were given

alternate day access to cinnamon or wintergreen flavored nonnutritive CSs to drink, and only one of them was followed by access to a rewarding US solution. In two-bottle choice tests between cinnamon and wintergreen, Holman found that the flavor that had been followed by immediate access to nonnutritive sweet saccharin came to be preferred over the non-reinforced flavor, whereas if the saccharin was delayed by 30 minutes during training, preferences were not acquired. However, if a flavor was followed after 30 minutes by a nutritive glucose solution, significant preferences for the paired cue flavor were acquired.

Using this "oral-delay" conditioning procedure, Elizalde and Sclafani (1990a) obtained the same pattern of results with oils as USs. That is, preferences were acquired for a flavor paired with access to corn oil emulsion that was delayed 10 minutes, but not for a flavor paired with delayed mineral oil emulsion. Finally, Lavin (1976) found that rats failed to demonstrate an association between two nonnutritive flavors when there was a delay greater than a few seconds between them. The conclusion from these three studies is that preferences based on flavor-flavor associations can not be formed with reinforcement delays whereas preferences based on flavor-nutrient associations can. In actuality, however, while preferences have been conditioned with delays between 5 and 60 minutes between CS and US (Simbayi, 1987; Capaldi, Campbell, Sheffer, and Bradford, 1987; Elizalde and Sclafani, 1988; Elizalde and Sclafani, 1990a; Pérez, Lucas, and Sclafani, 1995), the preferences have been relatively weak or inconsistent.

Several explanations of the weak results obtained with the delay conditioning procedure are possible. First, it is conceivable that the

postingestive reinforcing effect peaks when the experience of the flavor of the food temporally overlaps with rapidly occurring pre- or post-absorptive nutrient actions. Accordingly, preferences are weakened by temporally dissociating the experience of the CS and the US in the delay conditioning paradigm. If this prediction were true, it could partially explain the finding that IG conditioning by fats is substantially weaker than IG conditioning by carbohydrates (Lucas and Sclafani, 1989; Sclafani and Nissenbaum, 1988) because the digestion of fats takes substantially longer than that of carbohydrates (Greenberg, Smith, and Gibbs, 1993; Greenberg, Kava, Lewis, Greenwood, and Smith, 1995). However, this CS-US "temporal overlap" or "contiguity" hypothesis does not explain why relatively strong preferences were obtained in one study with a delay as long as one hour between the CS and US (Elizalde & Sclafani, 1988).

Procedural problems related to the oral-delay training procedure have been offered as a major explanation of the weak and inconsistent results reported with CS-US delays. According to Revusky's (1971) concurrent interference theory of delay conditioning, associations will be formed between two events over a long delay provided that other non-target stimuli do not occur in the delay interval. Several investigators have suggested that in the oral-delay conditioning paradigm the flavor of the nutrient, a non-target stimulus, may interfere with the association between the CS and postingestive consequences of the nutrient US (Simbayi, Boakes, and Burton, 1986; Sclafani, 1990; Capaldi, 1993). This potential drawback of the oral-delay procedure has limited its use in examining conditioning with CS-US delays. By delivering the nutrient post-orally one can eliminate the influence of the nutrient's flavor and examine delay

conditioning more clearly. To date, only one study has used IG infusions to examine the ability of preferences to be conditioned with delays (Baker and Booth, 1989). Although preferences were obtained with a 10 minute CS-US delay, the procedures were more complicated than those typically used in flavor conditioning experiments. As discussed above, the rats were given concurrent 10 minute access to the two cue flavors and, following the delay interval, were infused with a mixture of the two infusates whose proportion depended upon the amount of the cue flavors that were previously consumed. Thus, the rats were faced with a seemingly difficult learning task. Preference conditioning based on delayed IG nutrients may be enhanced when the rats are provided with separate training sessions with each CS-US pair. The extent to which strong preferences can be conditioned with even longer delays will support the idea that reinforcement could be mediated by the post-gastric or postabsorptive actions of nutrients.

Goals of Present Research

There is still much to be learned about the postingestive controls underlying the development of flavor preferences. The present research investigated the site and temporal aspects which mediate carbohydrate-based flavor preferences in rats. To eliminate the influence of the nutrient's flavor, post-oral infusions were used in all experiments. Two carbohydrates, glucose and Polycose, were used as USs. Although natural foods are more complex and require greater processing time, these two carbohydrates were chosen because they are rapidly absorbed. Therefore, the delay in nutrient actions could be

better manipulated by the experimental procedures employed. In addition, these carbohydrates have been successfully used in previous infusion studies.

The conditioned flavor preference procedure was used in all experiments in which consumption of flavored CS+ and CS- solutions were paired with carbohydrate and water infusions, respectively, during training. Subsequent two-bottle choice tests between the CS+ and CS- solutions without infusions were administered to evaluate the development of preference. Although it could be argued that factors other than nutrient detection (e.g., osmolarity) may contribute to the development of flavor preferences when water is used as the CS- paired infusate, nutrient specificity has been demonstrated by the finding that equiosmolar glucose and fructose infusions do not condition equivalent preferences (Sclafani, Cardieri, Tucker, Blusk, and Ackroff, 1993). It is also worth noting that CS+ preferences were observed in some studies when IG saline infusion was paired with the CS- (e.g., Puerto, Deutsch, Molina and Roll, 1976; Deutsch and Wang, 1977).

In order to determine whether actions produced by nutrients in the stomach are necessary for reinforcement, Experiment 1 examined whether ID nutrient infusions are sufficient to condition flavor preferences. The strength of preferences conditioned by ID infusions were compared with the preferences conditioned by IG infusions in another group of rats.

Experiment 2A examined whether the action of nutrients in the stomach alone can condition flavor preferences in the absence of post-gastric nutrient processing. This was done by employing Deutsch and Wang's (1977) pyloric cuff manipulation with IG infusions with the modification that the solutions

were removed from the stomach after each conditioning session to eliminate their delayed post-gastric actions. The preferences obtained under these conditions were compared with the preferences obtained in another group of rats that experienced pyloric cuff compression following conditioning sessions. This was employed as a control to determine whether compression by the pyloric cuff was capable of blocking conditioning.

Preferences were not conditioned in experiment 2A when the post-gastric effects of the nutrient were eliminated. Experiment 2B was run as an additional control for Experiment 2A to evaluated whether stomach distension with pyloric compression during conditioning preclude the development of flavor preferences. Rats were trained to associate cue flavors with IG infusions that were restricted to the stomach by pyloric cuff inflation during conditioning. Following each session, the pyloric cuffs were deflated and the nutrients were permitted to stimulate post-gastric sites.

The results of Experiment 2B indicated that the delayed actions of nutrients can reinforce strong flavor preferences. Experiments 3 and 4 more thoroughly evaluated the extent to which preference conditioning is influenced by delays between CS consumption and IG infusion of a nutritive US. Experiment 3 examined the preferences conditioned by delay intervals between 2.5 and 60 minutes. Preferences were not displayed with a 1 hr delay in Experiment 3. Experiment 4 determined whether the amount of nutrient delivered in the US affects preference conditioning with a one hour delay.

GENERAL METHODS

Subjects

The subjects were adult female Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) or bred in the laboratory from Charles River stock. Rats were between 12-16 weeks of age at the beginning of experimentation. The rats were individually housed in stainless steel cages in a temperature-controlled vivarium maintained at 21° C and under a 12:12 hr light:dark cycle. Unless otherwise noted, the rats were maintained at 85% to 90% of their free-feeding body weight on Purina pelleted (#5001) or powdered lab chow. Food rations were adjusted daily as body weight fluctuated from the target weight and were provided about two hours after each daily test session. Except during test sessions, tap water was freely available.

Surgery

Rats were implanted with indwelling Silastic catheters for nutrient infusions. Some rats were also equipped with pyloric cuffs to isolate nutrient infusions to the stomach. Catheter and pyloric cuff construction and implantation are described in more detail in the individual Method sections. Rats were anesthetized with a mixture of Ketamine HCl (63 mg/kg) and Xylazine (9.4 mg/kg). Except where noted, all catheters were routed under the skin to the back of the head where they were connected to 20-gauge L-shaped stainless steel tubing which was fixed onto the skull with screws and dental cement.

Apparatus

The apparatus consisted of eight Plexiglas cages (22.5 cm long x 22.5

cm wide x 26.5 cm high) kept in an isolated room maintained at 21° C. The front wall of each cage had two 1.8 cm holes (8 cm apart) through which stainless steel sipper tubes attached to 50-ml graduated cylinders could be mounted on the outside of the cage. A slot (1 cm wide x 16 cm long) in the top of the cage allowed passage of the infusion tubes. Tygon microbore plastic tubing (0.030" I.D. x 0.090" O.D.) attached to 30 ml syringes in variable-rate syringe pumps (Razel, model A-99) were connected to the input port of a swivel (Instech Laboratories, Horsham, PA) on a counterbalanced lever. A 50 cm length of the same tubing, protected by a stainless-steel spring, connected the swivels' output port to the rats cannula.

The infusion pumps were automatically operated by the rats' licking from a sipper tube at the front of the cage. Each spout was connected to an electronic lickometer interfaced with a microcomputer which controlled the infusion pumps. Thus, whenever the rat drank from a sipper tube, the pumps were turned on. The computer program allowed experimenter control over the number of licks necessary to activate the infusion pumps and the infusion volume.

Conditioning Procedure

Rats were trained according to the conditioned flavor preference procedure. On alternating days, the CS+ cue flavor was paired with nutrient infusions and the CS- cue flavor was paired with water infusions. Water was used on CS- days to provide a nonnutritive volume control. Following 4 or 6 days of these single-bottle training trials, rats were given two-bottle preference tests without infusions for two sessions. During training and testing the left-

right position of the CS+ and CS- were counterbalanced. Two or three training-test cycles were conducted.

Test Solutions

Solutions were prepared on a weight/volume basis. The conditioned stimuli (CSs) consisted of 0.2% sodium saccharin (Sigma Chemical Company, St. Louis, MO) solutions flavored with either 0.05% cherry or grape Kool-Aid (unsweetened mix; General Foods Corp., White Plains, NY). Previous work has shown that these Kool-Aid flavors are isohedonic with each other and equally unpreferred to water by naive rats (Elizalde & Sclafani, unpublished data). For half the rats in each group, the CS+ was cherry and the CS- was grape; for the other half the CS flavors were reversed. In some cases, strawberry and orange Kool-Aid flavors were also used. The infusion solutions consisted of 8% or 16% glucose or Polycose (Ross Laboratories, Columbus OH) dissolved in tap water. Polycose is a corn starch hydrolysate extensively used in flavor preference conditioning studies (Sclafani, 1990). Infusions were delivered at room temperature. The 8% and 16% solutions delivered 0.32 kcal/ml and 0.64 kcal/ml, respectively. Oral and infusion intakes were measured to the nearest 0.5 ml.

Statistical Analysis

The primary data included the amount of fluid orally consumed during training (one-bottle) and testing (two-bottle) sessions. The data were averaged over two-day or three-day periods and were analyzed using repeated measures analysis of variance followed by simple main effects tests and Newman-Keuls tests, where appropriate. The amount of fluid consumed in two-bottle tests

was also expressed as percent CS+ preference ((CS+ intake/total intake) x 100).

EXPERIMENT 1: IG AND ID CONDITIONING

Preferences for nonnutritive cue flavors paired with IG nutrient infusions have been demonstrated (e.g.,Sclafani, 1990). These preferences may be attributable to feedback from the stomach, duodenum, the liver, or other postabsorptive sites. This experiment determined whether nutrient detection by the stomach is necessary to support preference conditioning by infusing nutrients into the duodenum. To date, there have been no published reports of the ability of ID nutrient infusions to condition flavor preferences. In one study, however, Deutsch et al. (1976) reported an apparent aversion to a flavor paired with ID infusions of 18% glucose. It is likely that the infusion parameters exceeded the normal rate of gastric emptying which may have counteracted the positive effects of the nutrient. Furthermore, two-bottle preference tests were not conducted so a decrease in consumption of the glucose-paired flavor could have been due to its satiating effects rather than an aversion per se.

Experiment 1 examined the ability of ID glucose infusions to support conditioned preferences using infusion parameters that more closely approximated the observed rate of gastric emptying of 0.2 kcal/min in rats (Kalogeris, Reidelberger, and Mendel, 1983). In addition, the total volume infused was controlled by the rats' oral consumption of the associated cue flavor; the amount infused was approximately equivalent to the amount consumed orally. Thus, the rats were able to control the infusions they

received by their consumption of the associated cue flavors.

To determine whether feedback from the stomach contributes to conditioning, the preferences conditioned by ID glucose infusions were directly compared to preferences conditioned by IG glucose infusions in a separate group of rats run concurrently. If IG and ID glucose infusions condition equivalent preferences, this would indicate that feedback from the stomach is not necessary. If IG infusions produce stronger preferences than ID infusions this would indicate that the stomach plays an important role in preference conditioning.

Method

Subjects

Eleven rats implanted with IG catheters and 9 rats implanted with ID catheters completed the study. Eight rats were omitted from the study due to technical problems (e.g., detached headpieces, leaking or clogged cannulas), and 7 rats' data were discarded because it was not possible to verify the proper placement of the catheter at autopsy.

Surgery

Gastric Catherization: Gastric catheters were constructed of 15 cm lengths of medical grade silicone tubing (0.04" I.D. x 0.085" O.D.; Baxter, Scientific Products Division, McGaw Park, IL). The outer circumference of the gastric end was coated with a ring of Silastic medical adhesive (Dow Corning, Midland, Michigan) to round sharp edges and to serve as a hub to prevent dislodging from stomach. A 2-cm square piece of marlex mesh was fixed to the

cannulas with silicone adhesive at a distance of 1 cm from the tip. This mesh was sewn to the outer gastric wall which served to fix the position of the cannula within the stomach permanently and prevent it from being dislodged from the stomach.

A 3-cm incision was made just lateral to the midline. The stomach was located and brought to rest outside of the incision. An area devoid of large blood vessels in the fundus of the stomach along the greater curvature was identified. A purse-string suture was placed in this area and a stab wound was made in the center. The cannula was inserted with some pressure into the incision until the Silastic ridge was inside the gastric lumen. The suture was then tightened around the cannula tubing. The marlex mesh was then sutured at its edges to the stomach. The abdominal muscular and skin incisions were then separately sutured.

Duodenal Catherization: Duodenal catheters were constructed and implanted according to a technique adapted from Davis and Campbell (1975). Briefly, a Silastic tube (0.25" I.D. x 0.047" O.D., Baxter Scientific) was inserted approximately 1 cm into the duodenum through an entry point in the antrum of the stomach. The tubing was fixed in place by suturing an attached 2 cm square piece of marlex mesh to the outer stomach wall.

Procedure

Pre-Surgery Training: The rats were familiarized with the taste of 0.2% saccharin solution and drinking in the infusion cages for several days prior to surgery. The rats were first given ad libitum access to the saccharin solution in their home cages (2 days). Next, the rats were placed in the infusion cages

with access to saccharin solution and water for 1-hr/day, first under fluid restriction (10 ml of saccharin solution, 2 days) and then under food restriction (2-4 days) until their saccharin solution intakes stabilized. Rats were returned to ad libitum food consumption for at least two days. Rats were then divided into two groups, matched for saccharin solution intakes and body weight, and had gastric or duodenal catheters surgically implanted.

Post-Surgery Training: Rats were allowed four to seven days of recovery and were then food restricted to maintain them at 85% of their post-recovery body weights. Prior to flavor conditioning, the rats were acclimated to being attached to the infusion system and infusion itself; for four days they had 1-hr access to saccharin solutions paired with simultaneous water infusions.

Training and testing occurred over three 6-day cycles. On days 1 and 3 the rats were given 1-hr access to the CS- solution paired with water infusions, and on days 2 and 4 they were given 1-hr access to the CS+ solution paired with 8% glucose infusions. On days 5 and 6, the rats were given a two-bottle preference test with the CS+ and CS- solutions without infusions. Cycle 2 differed only in that the CS+ was presented on days 1 and 3 and the CS- was presented on days 2 and 4. Cycle 3 was the same as cycle 1. The left/right position of the cue flavors was counterbalanced throughout training and testing. Rats in the IG group were infused at a rate of 1.2 ml/min as they consumed the CS flavors while the ID group was infused at a rate of 0.6 ml/min. These rates were used to approximate the rates of intake and gastric emptying, respectively (Maerz, Sankaran, Scharpf, and Deveney, 1994; Kaplan, Spector, and Grill, 1992; Kalogeris, Reidelberger, and Mendel, 1983).

The rats' licking at the CS solution bottle activated the pumps and the amount infused was approximately equivalent to the amount of CS flavor consumed. The infusions were longer for the ID group since the rate of infusion was slower. That is, for the IG group each set of 20 licks activated 3 seconds of infusion pumping, whereas 20 licks activated 6 seconds of infusion pumping for the ID group.

At the conclusion of flavor conditioning, a final one-bottle session was conducted in which the rats were infused with glucose containing green food coloring as they drank the CS+ solution. Rats were then anesthetized and physical placement of the catheters was assessed. Rats in the ID group that had green solution inside the stomach were excluded (n = 4).

Results

One-Bottle Training: The oral intakes during one-bottle training and two-bottle tests for the IG and ID groups are displayed in Figure 1. Overall, the CS intakes increased over cycles [$F(2,36) = 39.1$, $p < .0001$], with the rats consuming more during cycles 2 and 3 than during cycle 1 ($p < .0001$). Furthermore, the rats consumed more CS+ than CS- during training overall [$F(1,18) = 29.9$, $p < .0001$] and the IG group consumed marginally more CS+ than the ID group [*group x flavor interaction*: $F(1,18) = 4.4$, $p = 0.051$].

Within group comparisons of the one-bottle training intakes revealed that the IG group consumed significantly more of the CS+ than the CS- during cycles 1, 2, and 3 [$F(1,25) = 9.4$, $p < .01$; $F(1,25) = 10.0$, $p < .01$; and $F(1,25) = 11.9$, $p < .01$] whereas the ID group consumed significantly more of

the CS+ than the CS- during cycles 1 and 3 [$F(1,18) = 5.2$, $p < .05$; $F(1,18) = 7.2$, $p < .05$] but not during cycle 2 ($p > .05$).

Two-Bottle Preference Tests: After only one training cycle, the IG and ID groups acquired similar CS+ flavor preferences of 76% and 79% respectively. Further training cycles increased the magnitude of preference reached by both groups. The IG and ID groups displayed 94% and 87% CS+ preferences by the third cycle. Analysis of the two-bottle intakes revealed that the rats consumed more CS+ than CS- overall [$F(1,18) = 82.8$, $p < .0001$] and this difference increased over cycles (cycle x flavor interaction [$F(2,36) = 14.17$, $p < .0001$]). The two-bottle intakes of the two groups did not reliably differ in any respect.

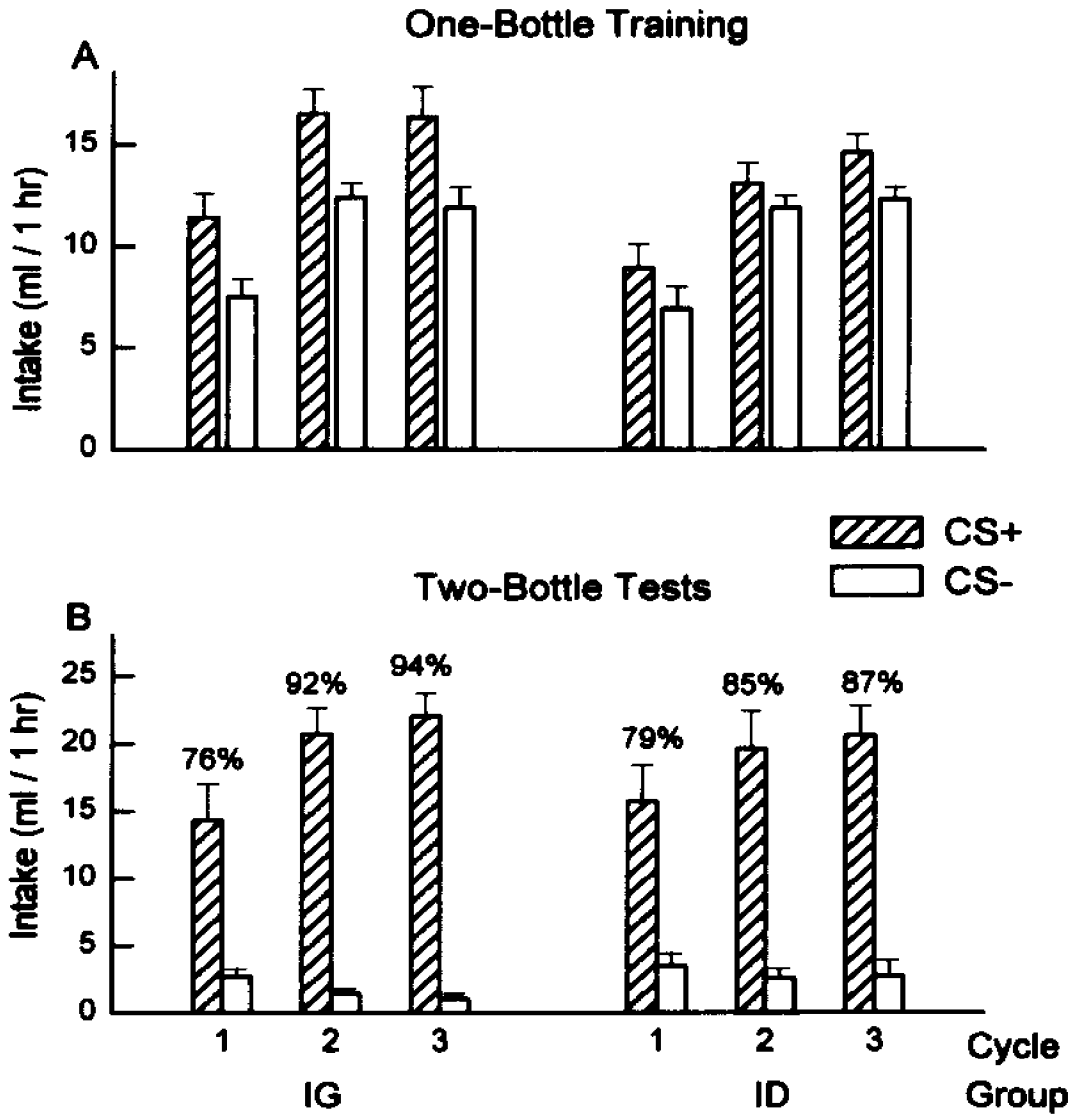

Figure 1. Experiment 1: Mean (+SE) oral intake of CS+ and CS- during cycles 1, 2, and 3 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of two days. The CS+ and CS- were saccharin-sweetened cherry- or grape-flavored water and were paired with 8% glucose and water infusions, respectively, during one-bottle training. No infusions were given during two-bottle preference tests.

Discussion

The present results confirm previous reports that strong preferences can be conditioned by IG carbohydrate infusions (see Sclafani, 1990). The new finding is that strong flavor preferences were also produced by ID glucose infusions. Furthermore, the preferences conditioned by ID glucose were similar to the preferences conditioned by IG glucose, which suggests that gastric nutrient actions are not necessary to establish preferences. The IG group displayed a slightly stronger CS + preferences overall. This may have been due to their increased exposure to the CS + and glucose because they consumed significantly more of the CS + during one-bottle training and therefore got more glucose infused.

Subsequent to the completion of experiment, two additional reports have confirmed that preferences can be conditioned by ID infusions of Polycose (Lucas and Sclafani, 1995b; Simons, Martinson, Watson, Horn, and Mitchell, 1994). Simons et al. (1994) have also shown that ID carbohydrate infusions are as effective as IG infusions in conditioning flavor preferences. It may be argued that the strong preferences conditioned in the ID group could be due, at least in part, to the infusion parameters employed. It is conceivable that the ID rate of infusion delivered nutrient more rapidly than the intestine normally sees under natural circumstances which could have enhanced conditioning. However, the rate was based on observed gastric emptying rates (Kalogeris et al., 1983). And, related to this suggestion, strong (85%) preferences were recently obtained with ID carbohydrate infusion delivered at half the rate that was used in the present study (Lucas and Sclafani, unpublished findings).

The greater intakes of the CS + during one-bottle training suggests that the glucose infusions served to increase the acceptance of the associated CS + flavor with respect to the CS- flavor. It is conceivable that these intakes may have been limited by a ceiling effect. By cycle 3 the rats were receiving approximately 30 ml of fluid during the 1-hr session (≈ 15 ml of CS + and 15 ml of 8% glucose). The greater CS + intakes of IG group during one-bottle training may represent the greater capacity of the stomach, than the intestine, to handle large loads at one time.

The observed increase in one-bottle intake of the CS + paired with ID glucose contrasts with the results of Deutsch et al. (1976) who observed decreases in intake of a flavor paired with ID glucose. The two studies differed in several ways (e.g., glucose concentration and amount, deprivation level) which may account for the discrepant results. Deutsch et al. (1976) suggested that decreases in voluntary food intake associated with ID nutrient infusion may be due to the development of aversions, rather than due to satiety as is commonly assumed. In contrast, the increasing CS + intakes during one-bottle training in the present study demonstrates that ID infusions can also serve to increase the acceptance of an associated flavor.

The equivalent preferences conditioned by IG and ID glucose in the present study raise considerable doubt concerning a critical role for the stomach in the reinforcement of glucose-mediated flavor preferences. The possibility that the preferences observed in the ID group were due to reflux of glucose into the stomach was addressed and eliminated by the verification procedure described in the method section. In addition, subsequent work in this laboratory

using a dilution method has also shown that minimal or no gastric reflux occurs with ID nutrient infusions of similar parameters (Lucas, unpublished data).

The results of this experiment demonstrate that gastric feedback is not necessary to obtain glucose-conditioned flavor preferences. It is conceivable, however, that redundant information is provided from the stomach and post-gastric sites and information from the stomach alone may be sufficient to mediate conditioning. This possibility was examined in Experiment 2.

EXPERIMENT 2A: CONDITIONING BY GASTRIC NUTRIENT ACTIONS?

The results from Experiment 1 indicate that the actions of glucose in the stomach are not necessary for the acquisition of flavor preferences. However, Deutsch et al. (1977) proposed that feedback from the stomach alone is capable of mediating preference conditioning. This claim was based primarily on the finding that IG nutrient infusions conditioned flavor preferences in rats with an inflated pyloric cuffs which prevented the infused nutrients from passing into the duodenum. Importantly, though, the pyloric cuff was deflated after each daily 10-min training session, allowing the infused nutrients to be processed by post-gastric sites. As described above, the results of Baker and Booth (1989) suggest that the preferences observed by Deutsch and Wang may have been due to the post-gastric actions of the nutrients which occurred following deflation of the pyloric cuff.

Experiment 2A reevaluated whether feedback from nutrients in the stomach alone is capable of reinforcing flavor preferences. The rats in the present experiment were fitted with an inflatable pyloric cuff and IG catheter as

employed by Deutsch and Wang (1977). However, post-gastric processing of the infused nutrient was prevented in this experiment. At the end of each daily training session with the pyloric cuff inflated, the gastric contents were withdrawn through the IG catheter and the stomach was rinsed clean with warm water before the cuff was deflated. In addition, the rats in the present study were given alternating one-bottle training sessions with the CS + paired with IG glucose and CS- paired with IG water. The training sessions were extended to 30 minutes, which is the longest duration reported for pyloric compression (Rauhoffer, Smith, and Gibbs, 1993). These procedures were designed to eliminate post-gastric nutrient actions and to optimize the likelihood of reinforcement by gastric chemoreceptors. Note, that although the same procedures were employed on both CS + and CS- days, it is possible that 30 minute pyloric compression followed by gastric withdrawal and rinsing may have a nonspecific disruptive effect on conditioning. Therefore, another group of rats was tested as a control to determine if these procedures block conditioning when the post-gastric nutrient actions are permitted. This group had 30-min/day training sessions with the pyloric cuff uninflated allowing the infusions to empty into the duodenum. Then, at the end of each session, the pyloric cuff was inflated for 30 minutes which was followed by withdrawal of the remaining gastric contents and stomach rinsing. The two groups were referred to as the "gastric-only" and the "gastric-plus" groups, respectively. Note, that the gastric-plus was not a complete control because it differed from the gastric-only group with respect to when the pyloric cuff was inflated. Experiment 2B was run as additional control to address this temporal aspect.

Method

Subjects

Following pre-training, 24 rats were surgically implanted with IG catheters and pyloric cuffs. Four rats were excluded during the course of the experiment due to illness, leaving 9 rats in the gastric-only group and 11 in the gastric-plus group. For one week prior to surgery and during recovery, rats were given ad libitum access to a sweetened milk diet. Following recovery from surgery, the volume of milk diet provided was individually adjusted daily in order to keep rats at approximately 90% of their post-recovery free-feeding body weight. The milk diet was prepared by mixing 360 grams of evaporated milk (Pathmark) with 530 grams of water, 177 grams of sucrose and 3.5 grams of AIN-76 vitamin mix (Bio-Serve F8000). This diet provides 22.2% of kcal from fat, 7.9% from protein, and 69.9% from carbohydrate. This liquid diet was used to facilitate withdrawal of gastric contents.

Surgery

Rats had a Silastic gastric catheter and a pyloric cuff implanted. The catheters were constructed and implanted according to the procedure described in Experiment 1. To permit withdrawal of gastric contents, a larger diameter tubing (0.078" I.D. x 0.125" O.D) was used.

Pyloric cuff fabrication and surgical implantation were adapted from the procedures used by Rauhoffer et al. (1993) and Young and Deutsch (1981). The pyloric cuff was constructed using a 20 X 40 mm piece of Silastic sheeting (Dow Corning #500-1) 0.005" thick, a 6 X 40 mm piece of Silastic sheeting (Dow Corning #500-3) 0.010" thick and a 15 cm length of Silastic tubing

0.030" I.D. X 0.065" O.D. (Dow Corning #602-175). The edges of the 20 X 40 mm piece of Silastic sheeting were covered with silicon medical adhesive. The Silastic tubing was placed in the center of the 20 X 40 mm piece. The 6 X 40 mm piece was placed directly over the tube and lightly pressed into the glued edges of the underlying larger sheet. Each edge of the larger piece was folded on top of the smaller piece, creating an water-tight pocket. Adhesive was placed over the seam where the larger sheeting overlapped and at both ends of the cuff.

After the gastric catheter was implanted the pylorus was located. A curved hemostat was used to separate the pylorus from the underlying tissue and blood vessels. Extreme care was taken to avoid damaging blood vessels or nerves. Once the hemostat was directly under the pylorus, it was used to pull the pyloric cuff under the pylorus. The ends of the pyloric cuff were then sutured (Ethicon, tapercut V-5 needle with 4-0 silk suture) together at the outer edges taking care not to puncture the tubing or water-tight pocket region. Warm water from a 1 ml syringe was infused through the end of the pyloric cuff tube to determine the amount of fluid needed to occlude the pylorus. The volume was recorded for each rat and that volume or more was used during testing. Cuff fill volumes ranged between 0.2 to 0.5 ml.

Once the duodenum and stomach were returned to the abdominal cavity the tubing from the gastric cannula and pyloric cuff were brought through separate small incisions in the abdominal musculature and routed to a 3 cm incision at the back of the neck. The ends of the two tubes were passed through a 2 cm length of Silastic tubing (0.132" I.D. x 0.183" O.D). This 2 cm

tubing was previously attached through the center of 3 cm square piece of marlex mesh with Silastic adhesive. The marlex mesh was sutured to the muscle inside the neck incision and the skin incision was then sutured closed around the tubing. The abdominal musculature and overlying skin incision were closed separately by interrupted 4-0 silk sutures.

Infusion Parameters

During 1-bottle training with the pyloric cuffs inflated, rats were permitted to consume a maximum of 8 ml of the CSs and receive a maximum volume of 8 ml intragastrically. These maximum volumes were set because pilot work indicated that rats trained with the pyloric cuffs inflated may drink less than rats with the pyloric cuffs uninflated. Thus, these measures were taken as an attempt to equate the CS and US exposure of the two groups.

A 16% glucose solution was infused on CS+ training days. The concentration of glucose used was greater than Experiment 1 because the volume was limited. Note, however, that the maximum volume of 8 ml of 16% glucose infusion contains approximately the same amount of nutrient that successfully conditioned preferences in Experiment 1 (about 16 ml of 8% glucose).

Procedure

Pre-Surgery Training: Prior to surgery, the rats were pre-trained to drink saccharin in the infusion cages under fluid- and food-restricted conditions as described above. Rats were trained to drink for 60-min/day (4 days) then for 30-min/day (6 days).

Post-Surgery Baseline Training: Following 8 to 12 days of recovery from

surgery, access to the maintenance milk diet was restricted in order to reduce and maintain the rats at 90% of their recovery body weights. The rats' saccharin solution intakes were measured for six baseline days (30-min/day). On the third and fourth day, the rats were connected to the infusion system and received water infusions as they drank the saccharin solution; on the fifth and sixth day they received oral and intragastric solutions with the pyloric cuffs inflated. Two groups matched on total intakes (oral + IG) during the two cuff inflated sessions were formed and flavor conditioning commenced.

Flavor Conditioning: During conditioning, each rat in the gastric-only group was removed from its home cage and a 20-gauge luer stub adapter connected to a 1-cc syringe filled with warm tap water was attached to the external end of the pyloric cuff tubing. The pyloric cuff was inflated by delivering the amount of fluid necessary to occlude the pylorus based on the volume predetermined at the time of surgery. If this predetermined amount did not produce any backward resistance on the plunger of the syringe, additional fluid was slowly infused until resistance was noted. In such cases, the new cuff fill volume was recorded and used in subsequent tests. The tubing was gently clamped while the syringe was removed and the pyloric cuff tubing was sealed with a plug. Then, the plug from the gastric catheter was removed and mild pressure was exerted on the rat's abdomen to allow any fluid remaining in the stomach to be expelled prior to each daily session. The infusion tube was pressure-fit into the gastric catheter tubing and the rat was placed in the infusion cage.

The rats were provided 30-minute access to the CS solutions paired with

their respective IG infusions. At the end of the session, the rats had their gastric contents withdrawn. This was accomplished by attaching an infant feeding tube (Becton Dickson Co., NJ) connected to a 10 ml syringe to the end of gastric catheter and slowly retracting the plunger. During withdrawal, the animal was able to move freely within the infusion cage. When the syringe was filled with gastric fluid it was disconnected and emptied into a 100-ml graduated cylinder. When gastric fluid could no longer be removed, the rats stomach was rinsed with 30 ml of warm water which was infused and withdrawn in six 5-ml aliquots; if necessary, additional aliquots were used until the contents withdrawn were clear. All fluid removed from the stomach was emptied into the graduated cylinder. When gastric rinsing was completed, the amount of water used for rinsing was subtracted from the total volume collected and the remaining volume was recorded to the nearest 0.5 ml. After the gastric contents were withdrawn and the stomach was rinsed, the pyloric cuff was deflated by withdrawing the amount of fluid infused at the beginning of the session. The rat was then returned to the infusion cage for an additional 30 minutes before it was returned to its home cage. This was done to equate the time that the rats in the two groups spent in the infusion cages because the procedures for the gastric-plus group required 60 minutes in the infusion cage (see below). The order in which rats were selected for withdrawal of stomach contents and rinsing was counterbalanced over days because the gastric withdrawal and rinsing took approximately an additional 2-5 minutes per rat. Note, however, that for each group, squads of only four rats were run at one time, thereby reducing the waiting time for rats to have gastric contents

withdrawn, rinsed and pyloric cuff deflated.

The rats in the gastric-plus group did not have their pyloric cuffs inflated during the 30-minute training session so that infusions could empty from the stomach into the duodenum. At the end of the session, the pyloric cuffs were inflated for 30 minutes which was followed by withdrawal of the remaining gastric contents and stomach rinsing.

The rats were trained and tested over two 6-day cycles as described in Experiment 1. On two-bottle preference test days, the rats had unlimited access to the CS+ and CS- without infusions during 30-min sessions; the pyloric cuffs remained deflated.

At the end of the experiment, the body weights of 5 rats were beginning to decrease and they were showing some signs of illness. These rats were infused with glucose with green food coloring as they drank the CS+ with the pyloric cuff inflated. They were then anesthetized and a laparotomy was performed and proper functioning and placement of the pyloric cuffs was verified. The remaining rats were tested with this green glucose method after participating in Experiment 2B.

Results

Baseline Intakes: The baseline saccharin solution intakes paired with IG water infusions were compared during cuff-deflated and cuff-inflated conditions. The 2-day means for cuff-deflated and cuff-inflated were 23.3 ml (12.5 ml oral/10.7 ml IG) and 15.3 ml (8.3 ml oral/7.1 ml IG), respectively; yielding a significant difference between the two conditions [$t(19) = 7.18$, $p < .0001$].

One-Bottle Training: CS Intakes The oral intakes during one-bottle training and two-bottle preferences tests for both gastric-only and gastric-plus groups are displayed in Figure 2. Although the rats were able to consume up to 8 ml of the CSs during one-bottle training, the average intakes collapsed across CS solution and cycles were 5.7 ml and 6.6 ml for the gastric-only and gastric-plus groups respectively. Analysis of the data revealed no group, cycle, or CS flavor differences ($p > .05$). Thus, the rats in the two groups did not reliably differ in their CS intakes during one-bottle training.

Infused Glucose: The volume of IG glucose infused during one-bottle training was compared for the two groups. The IG glucose intakes (collapsed across cycles) were 5.9 ml and 6.7 ml for the gastric-only and gastric-plus groups respectively. A separate analysis of these data revealed no group differences ($p > .05$). Thus, although the sites of glucose exposure differed between the two groups, they did not differ on the total amount of nutrient infused during training.

Gastric Content Withdrawal: The volumes recovered from the stomachs of the rats during one-bottle training were recorded and the percentage of the total volume received was computed. The percent recovered collapsed across all training sessions was 156% and 58% for the gastric-only and gastric-plus groups respectively. The volume recovered from the gastric-only group was significantly greater than the volume recovered from the gastric-plus group [$t(18) = 6.9$, $p < .0001$] (see explanation below).

Two-Bottle Preference Tests: After the first training cycle, the gastric-

only and gastric-plus groups demonstrated CS+ preferences of 54% and 81% respectively (see Figure 2). After the second training cycle, the CS+ preferences were 63% and 88%. Analysis of variance performed on the two-bottle intakes indicated that the two groups consumed comparable total volumes of the CSs. However, the gastric-plus group consumed significantly more of the CS+ than the gastric-only group [group x flavor interaction: $F(1,18) = 6.5$, $p < .05$]. Within-group comparisons revealed that the gastric-plus group consumed reliably more of the CS+ than the CS- during test 1 and test 2 [$F(1,17) = 19.2$, $p < .001$; $F(1,17) = 33.4$, $p < .0001$] whereas the gastric-only group did not reliably consume more of the CS+ than the CS- during either test. Thus, the gastric-only group failed to develop a reliable CS+ preference.

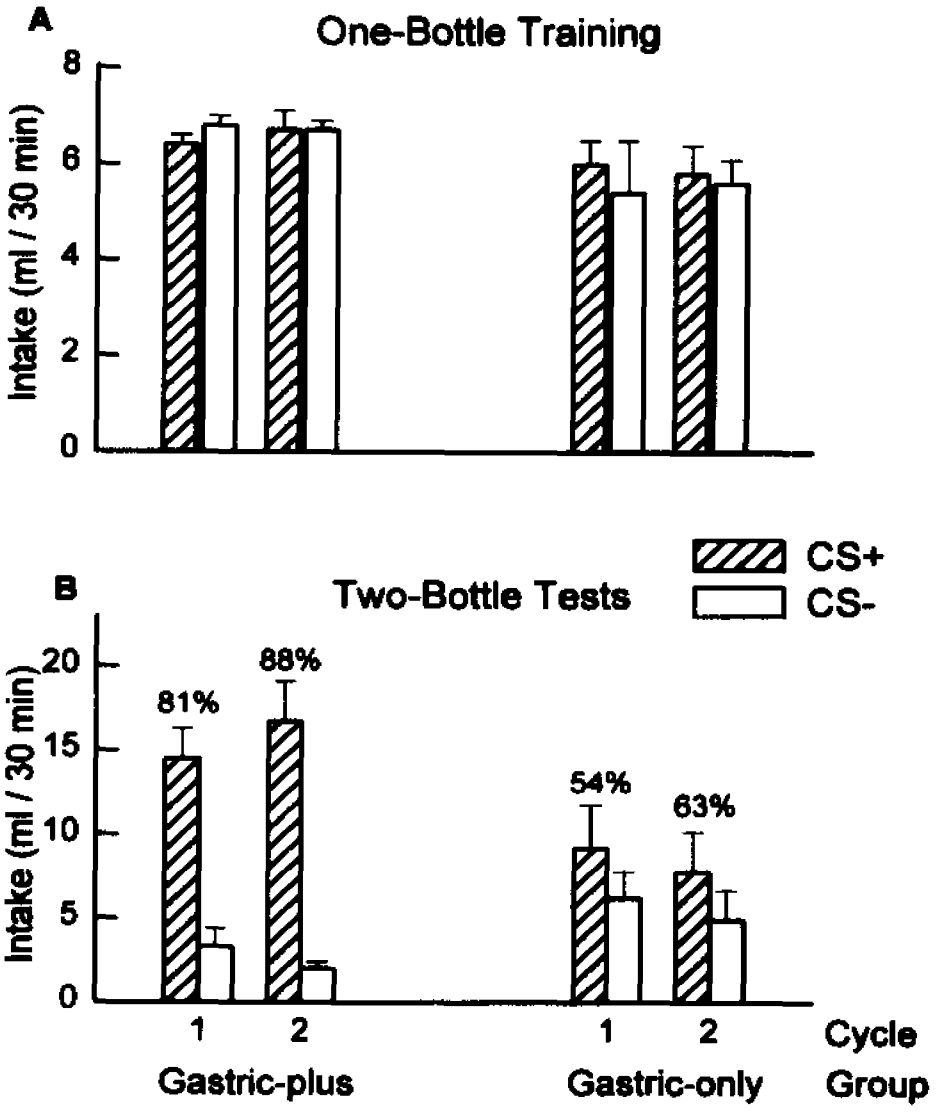

Figure 2. Experiment 2A: Mean (+SE) oral intake of CS+ and CS- during cycles 1 and 2 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of two days. The CS+ and CS- were saccharin-sweetened cherry- or grape-flavored water and were paired with 16% glucose and water infusions, respectively, during one-bottle training. The gastric-only group had the pyloric cuff inflated during one-bottle training sessions; the gastric-plus group had the pyloric cuff inflated after each one-bottle training session. No infusions were given during two-bottle preference tests.

Discussion

These results are the first to indicate that glucose in the stomach alone is not sufficient to mediate flavor preference conditioning. In Experiment 1 and in the gastric-plus group of the present experiment, IG glucose infusions conditioned strong flavor preferences. Thus, when glucose is prevented from emptying into the duodenum and stimulating post-gastric sites, its reinforcing action was prevented. This result indicates that gastric feedback is neither necessary nor sufficient for establishing flavor preferences.

It is important to point out that although the mean preference of the gastric-only group demonstrated a tendency to prefer the CS+, there was a large degree of variability among the individual rats in the group. While some rats exhibited CS+ preferences (n = 4), some displayed CS- preferences (n = 2) and others did not reliably prefer one flavor to the other (n = 3). Wide variability such as this is not unlike the intakes of these flavors in naive rats consuming them for the first time without infusions (unpublished data). Thus, although some rats preferred the CS+, the preferences may have been due to unconditioned flavor effects rather than any postingestive effect of the nutrient infusion.

To date, there has been only one other report using the pyloric cuff preparation in a preference conditioning experiment (Deutsch and Wang, 1977). Although preferences were obtained for a flavor paired with IG infusion of a preingested milk mixture, the interpretation of these results are complicated by the fact that the nutrient was allowed to empty into the duodenum when the cuff was deflated after each 10-minute training session. The outcome of the

present experiment and Experiment 1 suggests that the preferences observed by Deutsch and Wang (1977) were more likely to have been mediated by the post-gastric actions of the nutrients rather than from the stomach. On the other hand, procedural differences may have produced the different results because the nutrient used for infusion and the deprivation level of the rats differed in the two studies.

The baseline intakes observed in this experiment demonstrated that the rats consume less when the pylorus is occluded. This finding contrasts with reports that food deprived rats consume the same amount under pylorus occluded and unoccluded conditions (Deutsch and Gonzalez, 1980; Kraly and Smith, 1978; Rauhoffer, Smith, and Gibbs, 1993). This discrepancy may be due to the use of highly palatable, nutrient-rich solutions in the previous experiment compared to the saccharin solution used in the present experiment. Because of this observation we imposed a maximum total volume of 16 ml during training so that rats in the gastric-plus group with the pyloric cuff deflated would not receive greater total volumes of the CSs and US. The possible impact of this on flavor preference conditioning was not assessed.

There was a great disparity in the amount of fluid recovered from the stomachs of the gastric-plus and gastric-only groups during one-bottle training (60% vs. 155% of oral and infusion intake, respectively). This large difference obviously reflects the volume which emptied from the stomach during the 30-min session in the gastric-plus group. Since the experimental procedures (chronic food restriction and gastric fluid expulsion prior to daily testing) were designed to minimize food in the stomach prior to daily conditioning sessions,

it is presumed that the volume removed exceeding 100% in the gastric-only group was primarily due to gastric secretions. Other investigators using the pyloric cuff technique have also noted a substantially greater amount withdrawn than the amount consumed and have attributed this to gastric secretions (Smith & Gibbs, 1979; Seeley, Kaplan, and Grill, 1995; Rauhoffer, Smith, and Gibbs, 1993).

EXPERIMENT 2B: PYLORIC OCCLUSION AND IG CONDITIONING

The gastric-only group of Experiment 2A failed to reliably prefer the CS+. It is conceivable that these rats experienced gastric distension beyond normal limits which may have produced mild discomfort which, in turn, could have reduced the postingestive reinforcing effect of the glucose. Whether visceral discomfort produced by distension with the pylorus occluded could have impacted on preference conditioning was not assessed in the gastric-plus group since the amount of fluid in the stomach during pyloric occlusion was markedly less because gastric contents could empty into the duodenum for 30 minutes prior to pyloric occlusion. The present experiment was conducted to address how gastric distension with the pyloric cuff inflated during training affects the development of flavor preferences when the post-gastric effects of the nutrient infusions are experienced.

The rats from Experiment 2A were re-trained during 30-min/day sessions in which new Kool-Aid flavors were paired with IG glucose or water infusions with the pyloric cuff inflated. The daily procedures were the same as the gastric-only group of Experiment 2A, except that after each one-bottle training

session the stomachs were not emptied before the pyloric cuff was deflated; that is the infused glucose was permitted to empty into the duodenum and stimulate post-gastric sites following each session.

Method

The subjects were 15 rats from Experiment 2A (n = 10 from the former gastric-plus group and n = 5 from the gastric-only group) the remaining subjects (n = 5) were not included because they were beginning to lose weight. One day after the end of Experiment 2A the rats were re-trained to associate new CS flavors with 16% glucose and water infusions. The CS solutions were saccharin-sweetened strawberry and orange Kool-Aid flavors. Training and testing were carried out over two 6-day cycles. The rats received 30-min/day conditioning sessions with the pyloric cuff inflated; they could consume a maximum of 8 ml of each CS and receive a maximum of 8 ml of the associated infusion. After each session the pyloric cuff was deflated allowing the gastric contents to empty into the duodenum. At the conclusion of flavor conditioning, a final one-bottle session was conducted in which the rats were infused with glucose containing green food coloring as they drank the CS+ solution. Rats were then anesthetized at the end of the session and laparotomy was performed in which proper placement and functioning of the pyloric cuffs were assessed.

Results

<u>One-Bottle Training:</u> The one-bottle training and two-bottle test data are

displayed in Figure 3. Overall, the rats consumed more CS solutions in cycle 1 than cycle 2 [5.8 vs 6.5 ml; $F(1,14) = 10.8$, $p < .01$]. No other reliable differences were obtained.

Two-Bottle Tests: The CS+ preferences were 65% and 86% for cycle 1 and cycle 2 respectively (see Figure 3). The rats consumed more of the CS+ than the CS- overall [$F(1,14) = 14.9$, $p < .01$] and this difference increased from cycle 1 to 2 [cycle x flavor interaction: $F(1,14) = 11.9$, $p < .01$]. Tests of simple main effects revealed that the difference between CS+ and CS- did not reach significance during test 1, but was highly significant during test 2 [$F(1,20) = 17.9$, $p < .001$].

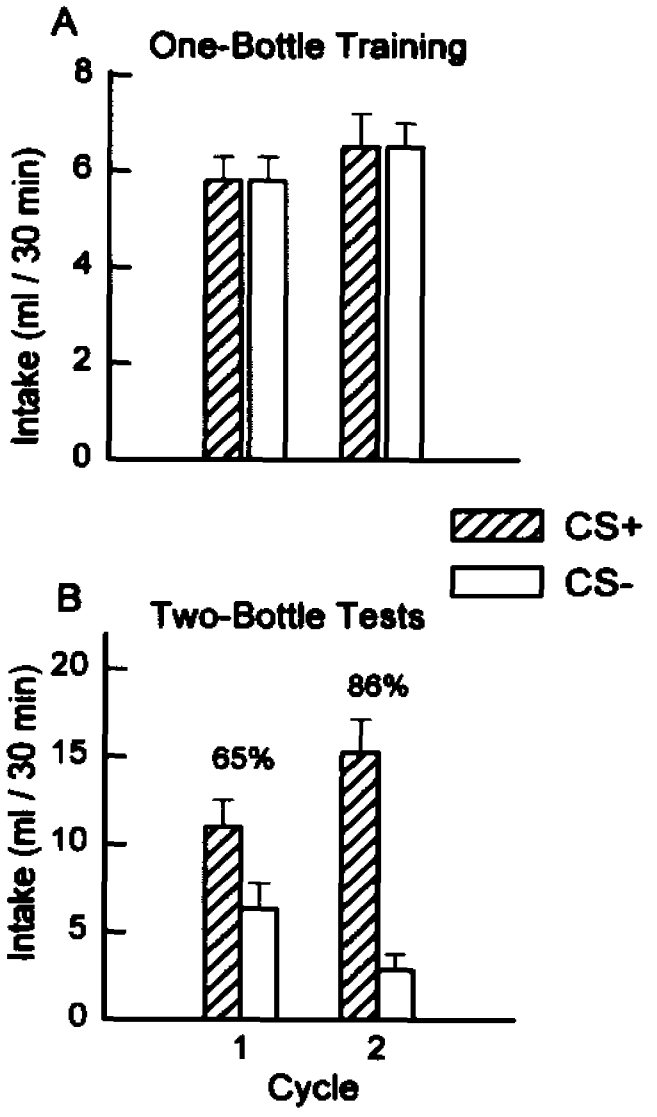

Figure 3. Experiment 2B: Mean (+SE) oral intake of CS+ and CS- during cycles 1 and 2 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of two days. The CS+ and CS- were saccharin-sweetened orange- or strawberry-flavored water and were paired with IG 16% glucose and water infusions, respectively, which were retained in the stomach for 30 minutes by pyloric cuff inflation during one-bottle training. No infusions or pyloric cuff inflation occurred during two-bottle preference tests.

Discussion

The results of the present experiment indicate that gastric distension with pyloric occlusion does not preclude glucose conditioned flavor preferences. In fact, the voluntary increase in one-bottle intake from cycle 1 to cycle 2 suggests that the amount of gastric distension induced by the oral and IG intake with the pyloric cuff inflated was not aversive. This result suggest that the weak nonsignificant CS + preferences demonstrated by the gastric-only group of Experiment 2A was due to the insufficiency of gastric feedback, rather than any potential aversive effect of the experimental manipulations per se.

Although the final preferences conditioned by the rats of Experiment 2B were equivalent to those of the gastric-plus group of Experiment 2A (86% and 88% for the two groups, respectively), in Experiment 2B two cycles were required to condition significant CS + preferences whereas reliable preferences were obtained after only one cycle in the gastric-plus group of Experiment 2A. Some differences existed which may account for these results.

First, the rate of acquisition in the present experiment may have been influenced by the rats' prior experience in Experiment 2A. Conceivably, prior flavor training may either diminish or enhance subsequent training. However, this has not been systematically explored.

Second, and probably more importantly, while the purpose of this experiment was to reveal whether conditioning was blocked by gastric distension with the pyloric cuff inflated, one feature of the methodology may have attenuated conditioning. That is, the infusions were retained in the stomach for 30 minutes during each one-bottle training session. Consequently,

a 30 minute delay was imposed between onset of CS + consumption and post-gastric processing of the nutrient US. This delay in nutrient processing could account for the slower acquisition of flavor preferences as compared to the gastric-plus group of Experiment 2A. When a delay is imposed between the CS and US with the oral-delay procedure, the rate of learning is generally slower and the preferences that are obtained are often substantially weaker than those conditioned without delays (Simbayi, Boakes, and Burton, 1986; Elizalde and Sclafani, 1990a; Sclafani and Ackroff, 1994; Elizalde and Sclafani, 1988). The affect of delays in nutrient processing was examined further in Experiments 3 and 4.

EXPERIMENT 3: IG-DELAY CONDITIONING

Taken together, the results of Experiments 1 and 2 indicate that the reinforcing effects of glucose are mediated by post-gastric actions which may occur following a delay. These actions may be generated pre- or postabsorptively. If it is the postabsorptive actions of foods which are reinforcing then conditioning must be able to withstand delays at least as long as 30 minutes because for some foods (i.e., fat) there is an interval of 30 to 45 minutes between the onset of consumption and postabsorptive actions (Greenberg, Smith, and Gibbs, 1990). Although Experiment 2B demonstrated that strong conditioning is possible when onset of the post-gastric nutrient actions was delayed, the interval between the CS and the US was not manipulated. Therefore, Experiment 3 systematically evaluated the extent to which preferences are conditioned with delays of 2.5 to 60 minutes between

CS consumption and onset of IG glucose infusions.

Flavor preference conditioning has been studied with delays between consumption of *nonnutritive* CSs and *nutritive* USs using the *oral-delay* procedure (Holman, 1975; Simbayi, 1987; Capaldi, Campbell, Sheffer, and Bradford, 1987; Elizalde and Sclafani, 1988; Elizalde and Sclafani, 1990a; Pérez, Lucas, and Sclafani, 1995). However, the preferences observed were often weak or unreliable, particularly when compared to the preferences conditioned by the simultaneous presentation of the CS and US. Holman (1975) observed a 63% preference for a cue flavor paired with the consumption of a 20% glucose solution after a 30 minute delay. In three subsequent studies weaker, stronger and no preferences were obtained with a 30 minute CS-US delay (Simbayi et al., 1986; Capaldi et al., 1987; Elizalde & Sclafani, 1988).

Conditioning with delays shorter than 30 minutes has also been examined. Simbayi et al. (1986) obtained a significant 56% preference for a CS + flavor which was followed after 20 minutes with a 20% glucose solution. However, other groups of rats identically trained failed to display CS + preferences when 20% maltodextrin solution or food pellets were used as the nutrient US. And, in a follow-up study, Boakes, Rossi-Arnaud, and Garcia-Hoz (1987) failed to obtain preferences with a 20 minute delay between a CS + and a 20% glucose solution.

Conditioning with delays between 5 and 10 minutes has also yielded varying results. Experiments by Capaldi et al. (1987) and Capaldi and Sheffer (1992) have resulted in preferences ranging from 48% to 76% for a CS + flavor followed after five minutes by sucrose, Polycose, high- and low-fat mash, or

chocolate milk. The strongest of these preferences (76%) was based on delayed (5 min) access to chocolate milk and was obtained after 50 days of training which is substantially greater than the amount commonly used in flavor conditioning studies.

Despite all the varied results, more recent 10 minute oral-delay studies by Sclafani and colleagues have been successful in conditioning preferences. CS+ preferences in the range of 74% to 81% have been reported with carbohydrates (Polycose and glucose), protein, and fat as nutrient USs (Elizalde and Sclafani, 1988; Elizalde and Sclafani, 1990a; Sclafani and Ackroff, 1994; Pérez, Lucas, and Sclafani, 1995). However, in a few cases weaker or null effects have also been obtained (Sclafani and Lucas, 1995; Pérez & Sclafani, unpublished data). The relative success of the studies by Sclafani and colleagues may be partially attributable to the cue flavors used as CSs. Unlike most of the previous work which used cinnamon and wintergreen as cue flavors, Sclafani and colleagues used grape and cherry Kool-Aid flavors. Elizalde and Sclafani (1988) obtained preferences with the delayed presentation of Polycose when grape and cherry Kool-Aid were used as CSs, but failed to obtain preferences when cinnamon and wintergreen were used.

As previously addressed, several investigators have also speculated that a potential problem with the oral-delay procedure is that the nutrient's flavor interferes with conditioning. It may do so in two ways. First, because of closer temporal proximity, the taste of the nutrient, rather than the cue flavor, may become associated with the postingestive nutritive effects. Second, since the nutrient flavor is substantially more palatable than the nonnutritive CS+ cue

flavor, it may overshadow the CS+ flavor and thereby weaken conditioning. Thus, the flavor of the US may be a more salient stimulus for association than the cue flavor. Pavlov as well as many others have demonstrated that the salience or intensity of the stimuli impacts on conditioning. When two stimuli (CSs) are presented prior to a US, the more intense or more salient of the two will become more strongly associated with the US. In the case of oral-delay preference conditioning, the nutrient flavor, because of its greater salience, may become more strongly associated with the nutrient's postingestive effects than the less salient CS+ flavor.

Elizalde and Sclafani (1988) minimized overshadowing by the flavor of the carbohydrate, Polycose by pre-exposing rats to a Polycose plus acarbose solution. The drug acarbose retards the digestion and absorption of carbohydrates (Puls, Keup, Krause, Muller, Schmidt, and Thomas, 1980) without altering its palatability (Sclafani, Nissenbaum, and Vigorito, 1987). This pre-exposure presumably reduced the ability of Polycose to interfere with conditioning to the US by teaching the rats that its flavor is not associated with rapid nutritive effects. The rats were subsequently trained to associate a CS+ flavor with the delayed (10 min) consumption of Polycose (without acarbose) and a CS- flavor not followed by solution. Rats that received Polycose plus acarbose before training acquired stronger preferences than rats that were pre-trained without acarbose (83% versus 73% CS+ preferences, respectively).

Using this Polycose plus acarbose preexposure procedure, preferences were conditioned with delays of 10, 30, and 60 minutes between presentation of the CS and US (Elizalde and Sclafani, 1988). This study provided the first

demonstration that preferences can be conditioned with a delay of one hour between CS and US. However, Capaldi and Sheffer (1992) reported a significant, but relatively weak (58%) CS+ preferences with a 5 hour CS-US delay following 50 days of training.

Preference conditioning with the acarbose pre-exposure procedure supports the idea that overshadowing by the nutrient's flavor interferes with the intended association between the CS+ and the postingestive consequences of the nutrient in delay conditioning. Thus, the oral-delay procedure is less than optimal for examining the impact of postingestive feedback delays on preference conditioning.

One can more clearly evaluate the effects of CS-US delays without the influence of the nutrient's flavor by delivering the nutrient post-orally. Only one study has used IG nutrient infusions to evaluate preference conditioning with a delay (Baker & Booth, 1989). Baker and Booth assessed rats' ability to acquire preferences with a 10 minute delay between CS consumption and the onset of IG nutrient infusions. Two separate groups were tested with a 20% carbohydrate solution US and a 10% protein solution as USs. The training procedure was quite complicated compared to the commonly used one-bottle training procedure. The rats had concurrent 10-minute/day access to the CS+ and the CS- solutions. Depending upon the volume of each CS consumed, a "proportional" volume of each associated infusate (20% vs. 2% carbohydrate or 10% protein vs 0% protein) was infused following the delay interval. If, however, a rat consumed more than 60% of a particular CS, only the associated infusate was given, rather than a mixture of the two. Thus, the rats

had to learn which of the two CSs provided greater nutritional consequences. With this seemingly difficult discrimination procedure, the carbohydrate and protein groups exhibited 73% and 79% CS+ preferences after only six sessions.

Experiment 3 further examined the extent to which preferences can be conditioned by the delayed effect of carbohydrates using IG infusions. This experiment employed the more conventional one-bottle training followed by two-bottle preference test procedure. The present study extended previous work by systematically evaluating the impact of increasing CS-US delay intervals of 2.5, 10, 30 and 60 minutes. These delay intervals were chosen because prior studies have not established clearly whether or not conditioned flavor preferences can be established through this range. Delays longer than 60 minutes were not examined because under normal circumstances it is unlikely that the onset of feedback from ingested foods would occur after 60 minutes.

Method

Subjects

Thirty-two rats participated in the experiment. There were four separate groups named for the delay interval examined: 2.5-min group (n = 7), 10-min group (n = 8), 30-min group (n = 10) and 60-min group (n = 7). Following pre-training, all rats were implanted with indwelling gastric catheters as described in Experiment 1.

Infusion Parameters

The CS + was paired with 10 ml of IG 8% glucose, which was delivered continuously at a rate of 1.29 ml/min over 7.7 minutes; on CS- days 10 ml of water was infused at the same rate. Fixed infusion volumes were used in order to equate the groups for the amount of the nutrient US delivered. Eight percent glucose was used because it was effective in conditioning flavor preferences in Experiment 1. The infusion volume was limited to 10 ml since it was not under the rats' control nor was it tied to oral consumption which would normally cause gastric reflex relaxation. Preliminary pilot work demonstrated that this fixed volume of IG glucose conditioned strong (91%) flavor preferences when there was no delay between CS consumption and IG infusion (unpublished data).

Procedure

Pre-Surgery Training. The rats were familiarized and trained to drink saccharin solutions in the manner described in Experiment 1. In this experiment, however, due to the number of animals to be run and the limited number of infusion cages, the rats were trained to drink the saccharin (and later the CSs) solution in their home cages, which were brought into a quiet test room. To enhance consumption during the daily sessions the rats were first trained to drink 2% sucrose-0.2% saccharin solution (2 days) during 30-minute sessions, followed by 30-minute access to plain saccharin solution (2 days); then 10-minute access to plain saccharin solution (1 to 3 days). Drinking sessions were limited to 10 minutes in order to allow greater experimental control over the delay interval between offset of the CS and onset of the US. Most rats that are provided access to a saccharin solution will still be drinking

after 10 minutes has elapsed. In addition, 10 minute CS consumption intervals has been used in previous oral-delay studies in this laboratory.

Post-Surgery Training. Rats were given approximately one week to recover from surgery before food restriction and training recommenced. Rats were given some additional post-surgery pre-training to re-acclimate them to drinking saccharin during 10-minute sessions (2-3 days) and to adapt them to being connected to the infusion system and infused with 10 ml of water following their specified delay interval (3 days).

Flavor conditioning occurred over two 8-day cycles. On days 1,3 and 5 the rats were given 10-min access to the CS+; on days 2,4, and 6 they were given 10-min access to the CS-. The rats in the 2.5-min delay group were immediately transferred to the infusion cages to receive the associated IG infusions. For the 2.5-min group, the nominal delay interval of 2.5 minutes was actually based on the average delay interval which varied from 1 to 5 minutes. Specifically, as soon as the 10-min CS drinking period ended, the rats were transferred, one at a time, into the infusion cages and as soon as all rats in the group were transferred, the infusions were started. The rats in the longer delay groups waited in their home cages until approximately five minutes before the specified delay interval elapsed at which time cage transferring began. When the appropriate time interval elapsed, the pumps were activated and infusions occurred. Although the 10 ml infusions were completed in 7.7 minutes, the rats were left in the infusion cages undisturbed for a total of 20 minutes to allow some gastric emptying to occur before being detached from the infusion system. No fluids were available during the delay interval. On days, 7 and 8

of each cycle, the rats were given 30-minute two-bottle preferences tests in their home cages without infusions. The experimental procedures took place in a quiet test room.

Results

One-Bottle Training: The results of the one-bottle training and two-bottle tests for cycle 1 and cycle 2 are displayed in Figures 4 and 5, respectively. Between-groups analysis of the one-bottle training data revealed that there were no overall group differences. However, the rats consumed more of the CS solutions during cycle 2 than they did during cycle 1 [10.1 ml vs 6.7 ml, cycle 2 & 1, respectively: $F(1,28) = 231.5, p < .0001$].

Two-Bottle Preference Tests: Between-groups analysis of the two-bottle test intakes revealed that the rats consumed significantly more of the CSs in cycle 2 than they consumed in cycle 1 [9.0 ml versus 8.1 ml for cycle 2 and 1, respectively: $F(1,28) = 16.0, p = 0.001$]. The rats consumed more of the CS+ than the CS- overall [$F(1,28) = 40.6, p < .0001$]. However, the relative consumption of the CS+ and CS- differed among the groups [group by CS interaction: $F(3,28) = 4.3, p < .05$].

The two-bottle test intakes for cycle 1 and cycle 2 were submitted to separate between-group analyses of variance followed by tests of simple main effects. During cycle 1, the rats in the 2.5, 10, and 30-min groups consumed reliably more of the CS+ than the CS- [2.5, 10, and 30-min groups respectively: $F(1,28) = 6.4, p < .05$; $F(1,28) = 4.5, p < .05$; and $F(1,28) = 14.6, p < .001$]. The 60-min group did not prefer one CS flavor to the other

($p > .05$). Overall, a similar result was demonstrated during cycle 2 [2.5-, 10, and 30-min groups respectively: $F(1,28) = 19.7$, $p < .0001$; $F(1,28) = 15.3$, $p < .001$; and $F(1,28) = 9.0$, $p = 0.01$].

The mean percent CS+ preferences obtained during cycle 2 were submitted to a nonparametric Kruskal-Wallis H-test. Overall, the preferences of the four groups reliably differed [$H(3) = 13.0$, $p < .005$]. Pairwise comparisons revealed that the preferences of the rats in the 2.5-min group were significantly greater than the preferences of the rats in the 30-min group [$t(15) = 2.2$, $p < .05$] and the 60-min group [$t(12) = 4.4$, $p < .0001$] and the preferences of the rats in the 10-min group and the 30-min group were significantly greater than the preferences of the 60-min group [$t(13) = 3.0$, $p < .01$; $t(15) = 2.6$, $p < .05$].

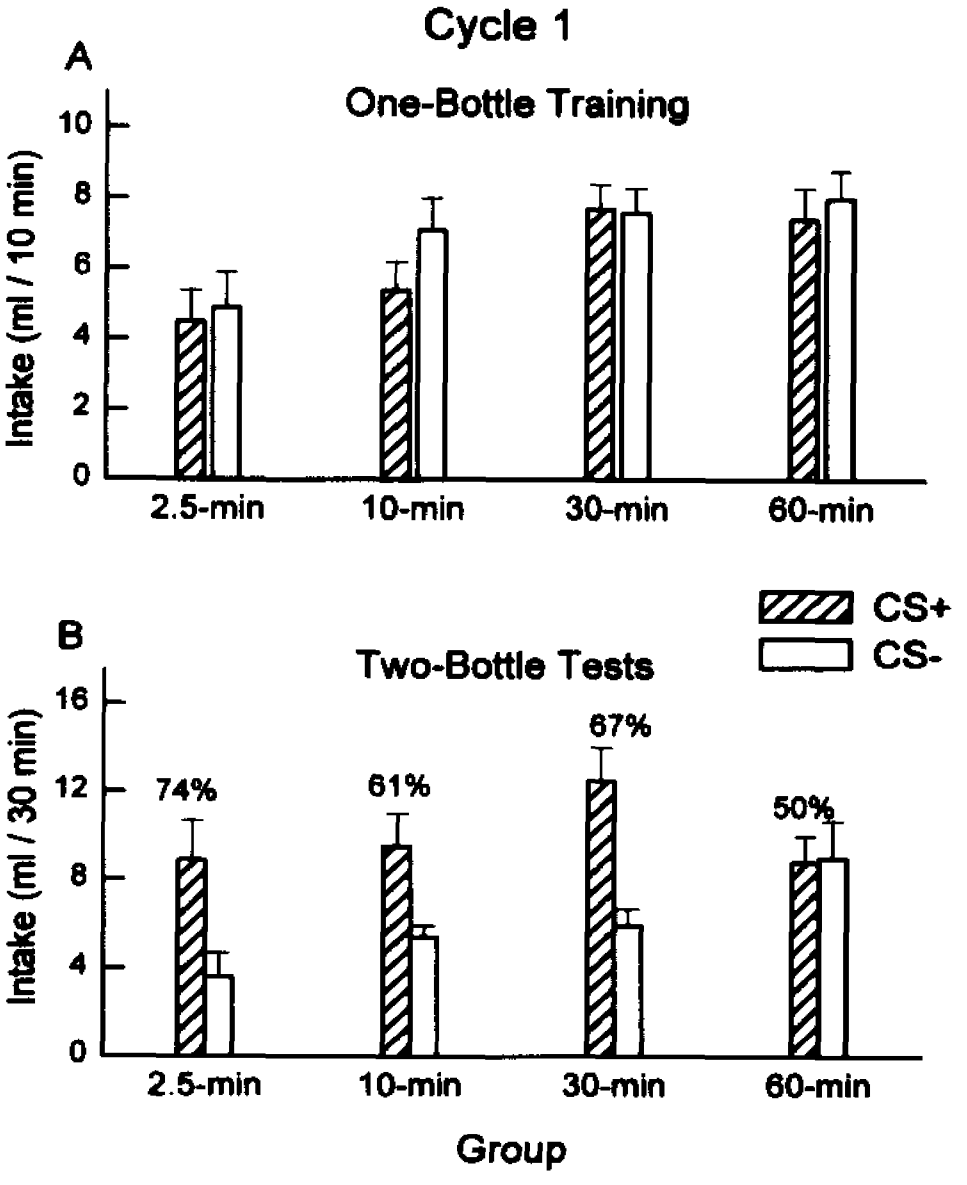

Figure 4. Experiment 3: Mean (+ SE) oral intake of CS + and CS- during cycle 1 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of three days during training and two days during preference tests. The CS + and CS- were saccharin-sweetened cherry- or grape-flavored water and were paired with 10 ml IG infusions of 8% glucose and water, respectively, during one-bottle training. No infusions were given during two-bottle preference tests.

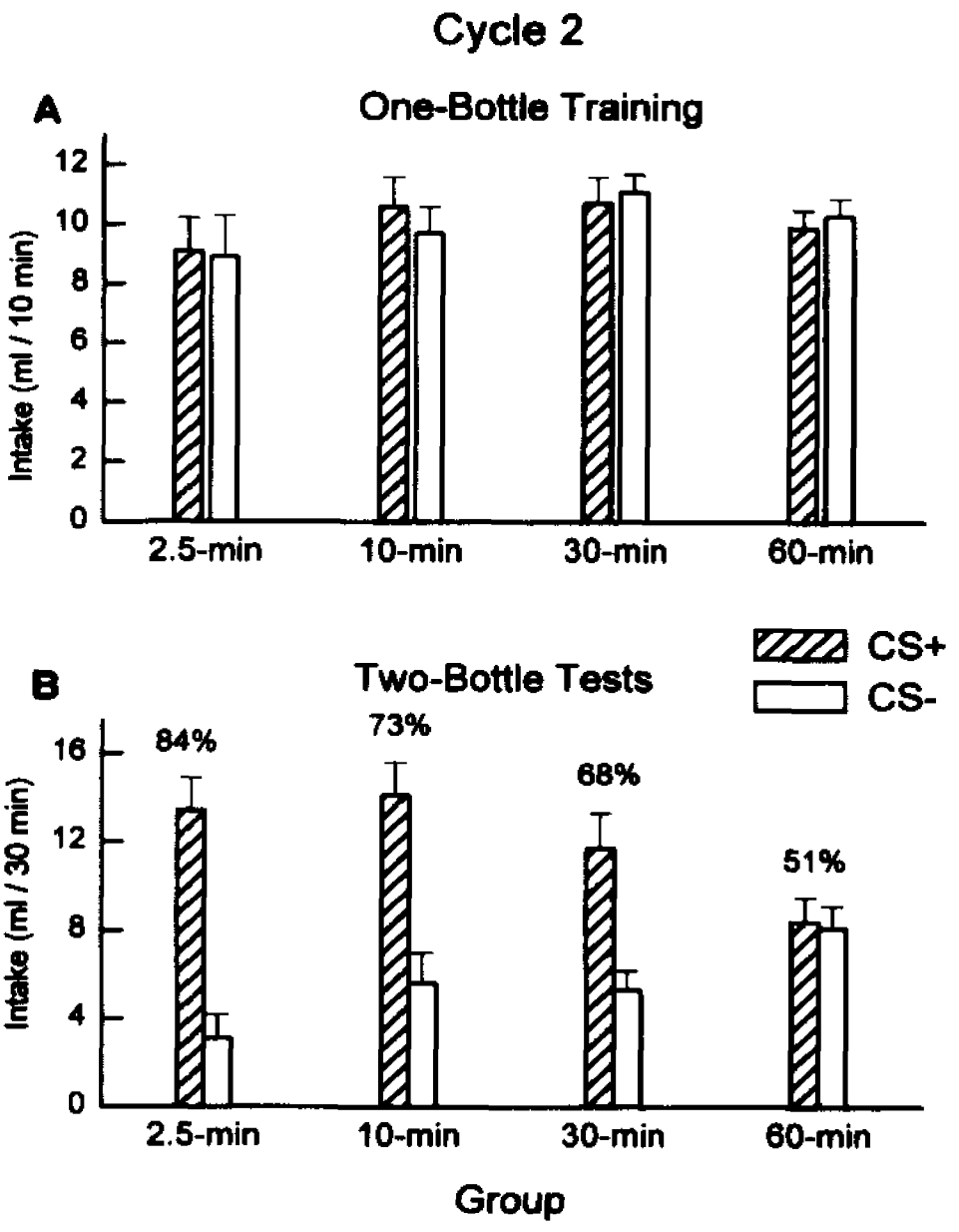

Figure 5. Experiment 3: Mean (+SE) oral intake of CS+ and CS- during cycle 2 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of three days during training and two days during preference tests. The CS+ and CS- were saccharin-sweetened cherry- or grape-flavored water and were paired with 10 ml IG infusions of 8% glucose and water, respectively, during one-bottle training. No infusions were given during two-bottle preference tests.

Discussion

The present findings demonstrated that IG glucose conditions flavor preferences with delays of 2.5, 10, and 30 minutes, but not with 60 minutes, between the CS and US. Although step-wise differences in the magnitude of the preferences were not significant, overall, CS + preferences declined as the delay interval between CS and US increased.

Only one other study compared the preference conditioned by different delays (Elizalde and Sclafani, 1988). In that study, three separate groups of rats that were trained to associate a CS + with consumption of a Polycose solution after delays of 10, 30 or 60 minutes acquired equivalent preferences of 77%, 79%, and 78% respectively. In contrast, the final preferences of the 10, 30, and 60-min groups of the present study were 73%, 68%, and 51%. These discrepant findings were unexpected based on the prediction that overshadowing of the CS flavor may occur with the oral-delay procedure as previously addressed. The incongruous findings may be due to procedural differences such as the route of nutrient delivery (oral versus IG), the particular carbohydrate used (Polycose versus glucose), and/or differences in the amount of nutrient in the US. The rats in the Elizalde and Sclafani study received more than four times the amount of nutrient as the rats in the present study ($\approx$ 20 ml of a 16% Polycose solution versus 10 ml of an 8% glucose solution).

The prediction that a larger US may be capable of sustaining conditioning over long delays has been borne out in the conditioned taste aversion paradigm (Revusky, 1968; Nachman and Ashe, 1973; Martin and Timmins, 1980; Andrews and Braveman, 1975). That is, aversions were noted at longer delays

when the dose (i.e., magnitude) of the illness-inducing US was increased. Capaldi et al. (1987) also obtained some evidence to suggest that a substantial number of calories is required for preference conditioning with delays. Flavor preferences were conditioned by 8% carbohydrate solutions following a 5 minute delay but were not conditioned by 1% carbohydrate solutions using the same delay. Greater nutrient loads condition stronger preferences without delays as well (Bolles, Hayward, and Crandall, 1981; Hayward, 1983; Ackroff and Sclafani, 1994; Lucas and Sclafani, 1995a).

It is worth noting that the 68% preferences acquired by the 30-min group in the present study contrast with the 86% preferences obtained in Experiment 2B. The two experiments differed in several important ways which may also account for these apparently divergent results. First, while the nominal delay interval was the same for the two experiments, the delay between offset of the CS and onset of the US was likely to be substantially less in Experiment 2A. If a rat took its last drink of the CS+ at the end of the 30 minute session, the actual delay interval could have been as little as one minute while the minimum delay interval in the present experiment was 30 minutes. The magnitude of preferences conditioned by the rats in Experiment 2A are similar to the 2.5-min delay group (see Figure 5). Perhaps, then, the CS-US delay in Experiment 2A was in the range of zero to five minutes as it was for the 2.5-min group of the present experiment. Temporal differences of 0 to 30 minutes can clearly impact on the strength of conditioning as evidenced by the decreasing preferences with increasing delays in the present experiment. The dissimilar glucose concentrations of the two studies (16% vs 8%) may also

contribute to the different results.

Based on the results of the present study and those of Elizalde and Sclafani (1988) it is reasonable to predict that a greater amount of nutrient or greater nutrient density may be necessary to sustain preference conditioning with extended delays. This issue was further addressed in Experiment 4.

EXPERIMENT 4: 60-MINUTE IG-DELAY: 8% AND 16% POLYCOSE

The results of Experiment 3 indicated that significant preferences can be conditioned with delays up to 30 minutes between the offset of cue flavor consumption and onset of IG delivery of 8% glucose. Preferences were not acquired by the 60-min delay group, however. As already indicated, Elizalde and Sclafani (1988) obtained preferences with a 60 minute delay between consumption of a CS+ and a 16% Polycose solution. The ability of 16% Polycose to condition preferences with a 60 minute delay, but not 8% IG glucose, may be attributable to the amount of nutrient, rather than the carbohydrate form or route of delivery. To assess whether conditioning with extended delays is directly influenced by the amount of nutrient, the preferences conditioned by 8% and 16% IG Polycose were compared in two groups of rats. Polycose was used instead of glucose to more closely parallel the procedures in which preferences were obtained with a 60 minute delay and because it has a lower osmolarity than glucose (per calorie) which lessens the potential for aversive consequences at higher concentrations.

Method

Eighteen rats received IG infusion catheters and were trained and tested according to the same procedures described for Experiment 3. Following post-surgery pre-training, the rats were divided into two groups matched on 10-minute saccharin intake. One group received 10 ml infusions of 8% Polycose while the other group received 10 ml infusions of 16% Polycose on CS+ days following a 60 minute delay. Three 8-day cycles were conducted.

Results

One-Bottle Training: The one-bottle training and two-bottle test data for the two groups are displayed in Figure 6. During one-bottle training the two groups did not differ in any respect. Overall, the rats increased their intake of the CS solutions over cycles [$F(2,32) = 103.5, p < .0001$]. Newman-Keuls pairwise comparisons revealed a reliable increase in CS solution intake from each cycle to the next ($p < .0001$).

Two-Bottle Preference Tests: The intakes during two-bottle preference tests for the 8% and 16% groups were submitted to a between-groups analysis of variance. Overall, the rats consumed more of the CS+ than the CS- [$F(1,16) = 21.1, p < .001$] and their CS solution intakes increased over cycles [$F(2,32) = 7.3, p < .005$]. No significant group effects were obtained ($p > .05$).

Separate within group comparisons were performed on the intakes of the two groups. The rats in the 16% group consumed significantly more of the CS+ than the CS- during cycle 2 and 3 [$F(1,11) = 7.4, p < .05$; $F(1,11) = 6.5,$

$p < .05$] but not during cycle 1. The rats in the 8% group consumed significantly more of the CS+ than the CS- only during cycle 2 [$F(1,12) = 5.1$, $p < .05$].

Separate analyses were also performed in which the CS intakes of the two groups were compared at each cycle. No group by CS interactions were obtained during cycles 1, 2, or 3 ($p > .05$).

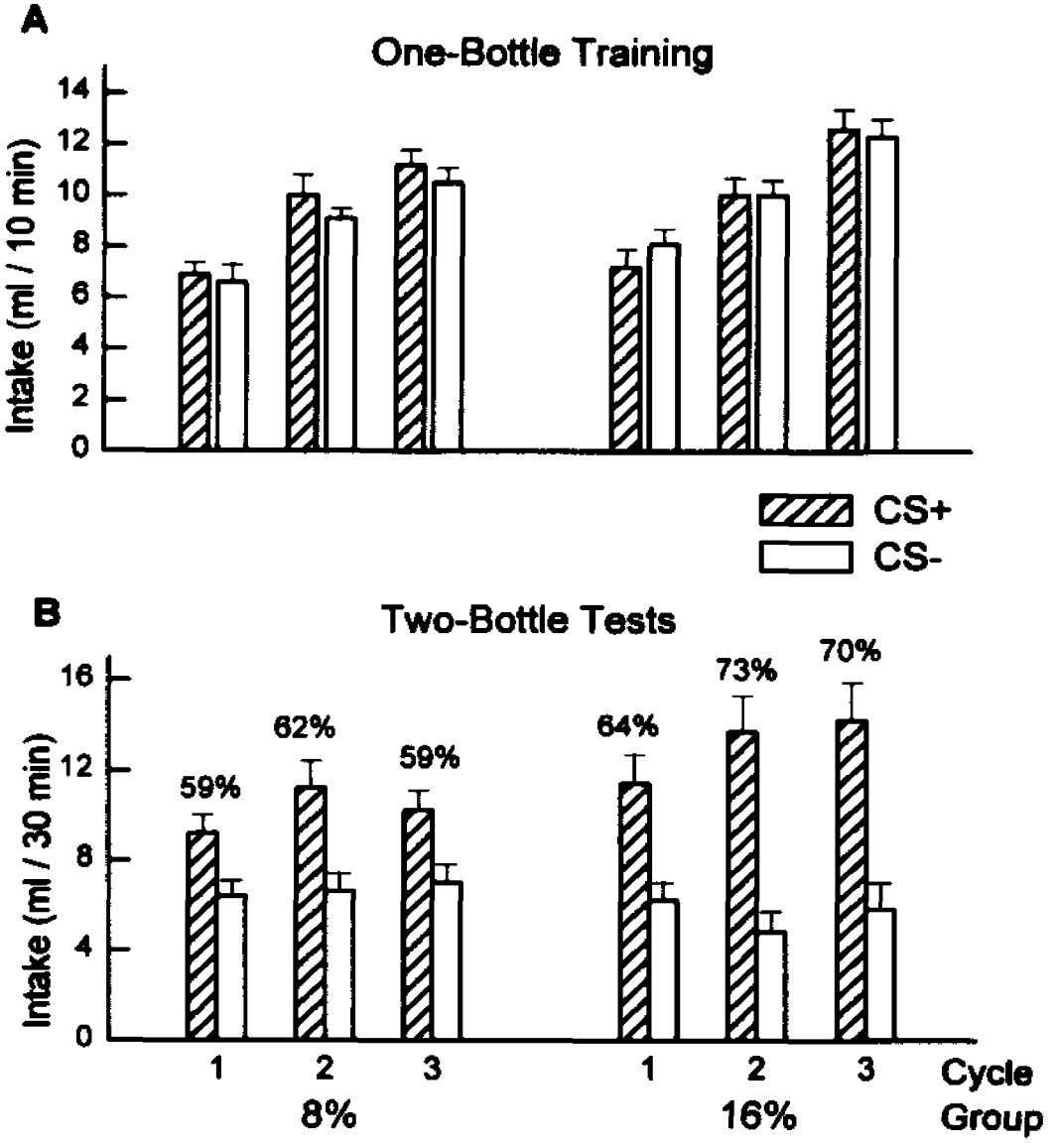

Figure 6. Experiment 4: Mean (+ SE) oral intake of CS + and CS- during cycles 1, 2, and 3 of the one-bottle training (A) and two-bottle preference tests (B); each bar represents a mean of three days during training and two days during preference tests. The CS + and CS- were saccharin-sweetened cherry- or grape-flavored water and were paired with IG Polycose and water infusions, respectively, following a 60 minute delay during one-bottle training. No infusions were given during two-bottle preference tests.

Discussion

The present findings confirm that preferences can be conditioned with a 60 minute delay between the flavor and postingestive consequences of nutrients (Elizalde and Sclafani, 1988). The preferences conditioned by 16% Polycose tended to be stronger than the preferences conditioned by 8% Polycose, though no reliable group differences were obtained. Perhaps, when greater differences in the nutrient concentration are used, significant results would emerge.

An intriguing result of the present study is the preferences conditioned by 8% Polycose. The significant 62% preference exhibited in the second cycle contrasts with the complete lack of preferences using 8% glucose as the US in the 60-min group of Experiment 3. However, when the two-bottle CS intakes for cycle 1 and 2 of the 60-min delay group of Experiment 3 and the 8%-Polycose group of the present experiment were submitted to a between-groups analysis of variance, the group by flavor interaction did not quite reach significance [$F(1,14) = 4.5$, $p = .0534$] indicating that the two groups did not reliably differ in their preferences.

Because Polycose is digested to glucose in the intestine there is no obvious reason why Polycose should be a more potent reinforcer than glucose. In addition, when hydrolysis of Polycose to glucose is impaired by the addition of acarbose, preferences are not obtained (Elizalde & Sclafani, 1988). This indicates that it is the glucose which is liberated from Polycose which mediates reinforcement.

One major difference between equicaloric concentrations of Polycose and

glucose which may indirectly contribute to their potentially different reinforcing effects, particularly at high concentrations, is the osmolarity. As previously indicated, Polycose has a much lower osmolarity than free glucose which lessens the likelihood for intestinal bloating and aversive effects. In addition, one report has suggested that infused Polycose produces an enhanced absorption of glucose at the intestinal mucosal cells compared to infused glucose (Daum, Cohen, McNamara, and Finberg, 1978). Thus, the glucose that is liberated from Polycose may be absorbed more efficiently than free glucose. Perhaps, under certain circumstances, these differences give Polycose an advantage over glucose.

The potential superiority of Polycose to glucose in postingestive conditioning has recently been investigated in a follow-up study in this laboratory using a more sensitive within-subjects design (Azzara & Sclafani, unpublished data). Azzara and Sclafani compared, in separate phases, the preferences conditioned by 8% and 32% glucose and Polycose using both the IG-delay and simultaneous IG conditioning procedures. Rats were trained with three cue flavors; one was paired with IG Polycose, another was paired with IG glucose, and the third was paired with IG water. In phase 1, the rats did not demonstrate a preference for a flavor paired with the delayed (60 min) delivery of IG 8% Polycose to another flavor paired with delayed IG 8% glucose. However, unlike the results of Experiment 3, when the rats were given the choice between the glucose-paired flavor and the water paired flavor, preferences for the glucose-paired flavor were observed (68%). The lack of preference between the two carbohydrate-paired flavors suggests that the

postingestive effects of 8% Polycose and 8% glucose are equally reinforcing.

In phases 2 and 3, the rats were given 23-hr/day training without a delay between CS consumption and IG infusions of 32% and 8% carbohydrates, respectively. When 32% carbohydrates were infused, the rats preferred the Polycose-paired flavor to the glucose-paired flavor (≈71%). When 8% carbohydrates were infused, the rats did not develop preferences for one carbohydrate-paired flavor over the other. These results demonstrate that at a higher concentration, Polycose conditions stronger preferences than glucose, but does not do so at a lower concentration. This supports the interpretation that the lower osmolarity of Polycose contributes to its potentially greater reinforcing effects.

The preferences observed by Azzara and Sclafani for the flavor paired with the delayed (60 min) delivery of 8% glucose to the water-paired flavor stand in contrast to the lack of preference in the 60-min delay group of Experiment 3 and a replication of that result not reported here (Drucker & Sclafani, unpublished pilot data). A number of procedural differences existed which probably account for the incompatible results. For instance, while the rats in both studies received six pairing of each flavor, the rats in the Azzara and Sclafani study received all their one-bottle pairings prior to the two-bottle unreinforced choice tests whereas the rats in Experiment 3 had unreinforced choice tests interspersed between each 6-day one-bottle training cycle. These imposed "extinction" tests could potentially have attenuated conditioning to some extent. In addition, the rats in Experiment 3 consumed the CS solutions in their home cages and were later transferred into different cages to receive

infusions; Azzara and Sclafani's rats were trained and tested entirely in their home cages. Interstimulus handling and the different contexts in which the rats experienced the CS and US in Experiment 3 may have also offset conditioning to some extent.

In conclusion, the results of the present experiment indicate that conditioning is possible with a 60 minute delay between CS and IG infusion. As the foregoing discussion indicates, this effect appear to be sensitive to variations in the training procedures employed, however. The finding that relatively strong (70%) preferences were obtained with a delay of one hour between flavor consumption and IG nutrient infusion suggests that even longer delays are possible. Capaldi and Sheffer (1992) have even observed mild flavor preferences ranging between 52% and 62% with a delay of five hours between a CS+ cue flavor and consumption of chocolate milk. In general, preference conditioning is possible with long delays between the flavor and postingestive effects of nutrients. However, the results appear to be highly sensitive to variations in the training procedures employed.

GENERAL DISCUSSION

While several studies have demonstrated the capacity for learning based on the postingestive effects of nutrients (Booth, 1985; Sclafani, 1990; Tordoff, 1991), relatively few have attempted to isolate the specific controls underlying their development. This dissertation research investigated how the site and temporal aspects of carbohydrate actions influence the acquisition of postingestively-based flavor preferences.

In Experiment 1 the preferences conditioned by gastric and duodenal glucose infusions were compared in two groups of rats. Similar preferences were conditioned in the two groups which indicates that gastric stimulation is not necessary for carbohydrate conditioning. Although many studies have demonstrated flavor preferences based on IG carbohydrate infusions, this was the first experiment to demonstrate conditioning by ID infusions. This finding has subsequently been replicated by other investigators (Lucas and Sclafani, 1995b; Simons, Martinson, Watson, Horn, and Mitchell, 1994).

Experiments 2A and 2B assessed whether the actions produced by glucose in the stomach could reinforce flavor preferences. This was done by restricting glucose infusions to the stomach with a pyloric cuff during flavor conditioning. When the post-gastric effects of glucose were eliminated in the gastric-only group of Experiment 2A, the rats failed to develop reliable flavor preferences. However, when the glucose was permitted to empty into the intestine during conditioning in the gastric-plus group of Experiment 2A and after a 30 minute delay in the post-gastric delay group of Experiment 2B, strong flavor preferences were obtained. These results indicate that the actions produced by glucose in the stomach are not sufficient to mediate conditioning, at least not under the experimental procedures employed in this study. These results do not exclude the possibility that the stomach can detect and mediate preference conditioning with other nutrients (i.e., milk) or with other experimental procedures, however. Nevertheless, these results suggest that it is post-gastric events which provide the stimulus for the preferences conditioned by glucose.

The "gastric model" of carbohydrate-based conditioning is also not supported by the finding that gastric vagotomy does not the development of preferences (Sclafani and Lucas, 1995; Booth and Davis, 1973). Deutsch and Ahn (1986) proposed that chemosensory information from the stomach is transmitted via splanchnic afferents instead. Preference conditioning with splanchnicectomy has not been performed. However, nonspecific visceral deafferentation by the neurotoxin capsaicin also does not block preference conditioning (Lucas and Sclafani, 1995b). Taken together, these results raise doubt concerning the role of neural feedback for preference conditioning in general and from the stomach in particular.

Because previous studies have indicated that the actions produced by nutrients in the stomach alone are capable of mediating normal satiety (Deutsch, Young, and Kalogeris, 1978; Kraly and Smith, 1978; Rauhoffer, Smith, and Gibbs, 1993; but see Seeley, Kaplan, and Grill, 1995 for an alternative explanation), the present findings establish yet another distinction between these two processes and suggest that satiety is not necessarily a determining factor in food preference conditioning.

Preference conditioning may be mediated by events stimulated by nutrients in the intestine. This possibility has not been directly addressed since it is difficult to restrict nutrients to the intestinal tract and prevent their absorption. It has been suggested, however, that the intestinal hormone, CCK, mediates conditioning (Mehiel and Bolles, 1988). In support of this possibility, preferences have been conditioned for flavors paired with exogenous administration of CCK (Mehiel, 1989; Weller and Blass, 1990; Pérez and

Sclafani, 1991); however other attempts have either not produced preferences or have even produced aversions (Deutsch and Hardy, 1977; Deupree and Hsiao, 1987). Other investigators have examined the possibility that the hormone, insulin, contributes to reinforcement (Vanderweele and Oetting Deems, 1989; Vanderweele, Deems, and Kanarek, 1990; Ackroff, Sclafani, and Axen, 1995). The data do not argue strongly in favor of this prospect, however.

The postabsorptive hepatic effects of nutrients may contribute to preference conditioning as well. Tordoff (1991) has argued that the state of nutrient oxidation in the liver provides the stimulus for nutrient reinforcement and this information is communicated to the brain by the hepatic vagus nerve. Although preferences have been conditioned by hepatic-portal glucose infusions (Tordoff & Friedman, 1989), an intact hepatic vagus is not required for carbohydrate conditioning (Horn, Mitchell, and Martinson, 1993; Sclafani and Lucas, 1995). Additional evidence for a postabsorptive site of action is the report of preference conditioning with jugular glucose infusions (Mather, Nicolaidis, and Booth, 1978).

The finding that flavor preferences occur with delays between the CS flavor and US supports the possibility that the postabsorptive effects may mediate conditioning. This is physiologically relevant since the postabsorptive effects of some foods may not occur until several minutes after consumption. The strength of the preferences conditioned by the delayed post-gastric actions of glucose in Experiment 2B were equivalent to the preferences conditioned without a delay in post-gastric processing (Experiment 2A, gastric-plus group).

This differs from the generally weaker conditioning effects that have been obtained in other experiments using the oral-delay procedure (Capaldi, Campbell, Sheffer, and Bradford, 1987; Simbayi, Boakes, and Burton, 1986; Holman, 1975). This result raised the possibility that conditioning with delays can be as strong as conditioning without delays when interference by the nutrient's flavor is eliminated.

Experiments 3 and 4 further assessed conditioning with delays between the CS and US and eliminated the potential confounding influence of the nutrient's flavor by infusing the nutrient intragastrically. In Experiment 3, conditioning with delays of 2.5, 10, 30 and 60 minutes was examined. As the delay interval between flavor consumption and IG nutrient infusion increased from 2.5 to 60 minutes, the strength of flavor preferences tended to decrease. With a 60 minute delay preferences were not obtained.

Delay gradients have been obtained in many conditioning paradigms (Mackintosh, 1983). However, in most paradigms, conditioning is possible with delays of only a few seconds whereas flavor-postingestive conditioning can withstand quite long CS-US delays. In the flavor aversion paradigm conditioning has been observed with delays of 1, 6, 12 and even 24 hours between the CS and delivery of the US (Garcia, Ervin, and Koelling, 1966; Revusky, 1968; Smith and Roll, 1967; Nachman, 1970; Etscorn and Stephens, 1973). The present study showed that preference conditioning is possible with delays up to one hour between CS and US. Although the physiological relevance of nutritional information occurring several hours after consumption is questionable, it is worth noting that weak preferences were reported with a

delay of five hours between CS and US (Capaldi and Sheffer, 1992). Whether preference conditioning is possible with longer delays remains to be determined.

Several explanations for the mechanisms responsible for long-delay learning in the taste system have been offered (e.g., Logue, 1979; Seligman, 1970; Rozin and Kalat, 1971). Many early accounts incorporated the concept of 'preparedness' (also 'belongingness' or 'relevance') in associating orosensory and viscerosensory cues as first advanced by Garcia and Koelling (1966). Accordingly, learning with long delays is enhanced because there would be few potential sources of interference during the delay interval (Revusky, 1971). Other explanations have conceived long-delay learning as a special case of association by contiguity of stimuli by assuming a lingering 'after-taste' or decaying neural trace of the CS which overlaps temporally with the occurrence of the US. Consequently, weaker associations are formed with increasing delays as the representation of the CS degrades (Barker, Best, and Domjan, 1977). Nonassociative accounts have also been offered (see DeCola and Fanselow, 1995). One of the most recent accounts suggests that taste stimuli are stored in a specialized memory buffer for long periods. Events that occur later act as retrieval cues that initiate a retrospective scan for recent ingestion-relevant experiences (Bitler and Riley, 1992). Different stimuli (e.g., tastes, shock, illness) are differentially effective as retrieval cues (Barker, Best, and Domjan, 1977). The latter account may explain the finding that preference conditioning is possible with a 60 minute delay with IG 8% glucose when concurrent orosensory stimulation by saccharin was provided (Drucker & Sclafani, pilot data) but not by the infusions alone in Experiment 3. The

saccharin solution in combination with the IG glucose infusion may have been a better retrieval cue than the IG glucose infusion alone, perhaps by initiating the cephalic response that normally accompanies the arrival of food. However, it is not clear how the retrieval account would explain the decrement in learning with increasing delays.

The CS-US delay effect obtained in Experiment 3 differed from a previous study by Elizalde and Sclafani (1988). Elizalde and Sclafani observed preferences with a 60 minute delay and the 60 minute preferences were equivalent to the preferences conditioned with delays of 10 and 30 minutes. There were several procedural differences which may account for these discrepant findings. Unlike the oral-delay procedure, the IG-delay procedure used in Experiment 3 allowed for complete dissociation of orosensory stimulation at the time of nutrient arrival. This is desirable from an associative learning perspective because it eliminates potential interference by the nutrient's flavor. However, the lack of oral processing may also diminish the full metabolic effects of the nutrient. When nutrients are placed directly into the stomach in the absence of orosensory stimulation, biochemical responses which occur as a result of the sight, smell and taste of food are eliminated. The absence of these cephalic phase preparatory responses have been shown to alter digestion and metabolism in rats (Molina, Thiel, Deutsch, and Puerto, 1977; Ramirez, 1985) and may also serve to diminish the postingestive reinforcing effect.

There is some indication from early operant conditioning studies that IG nutrient infusions do not reinforce lever pressing responses unless an oral stimulus is also provided (Holman, 1968; Berkun, Kessen, and Miller, 1952).

Generalizing from these findings, it is quite conceivable that in the IG-delay conditioning procedure, the absence of concurrent orosensory stimulation at the time of gastric nutrient arrival may have dampened the reinforcing effect of the nutrient. Hence, as the delay between cue flavor consumption and delivery of the nutrient increased, the potential for the maximum reinforcing effect may have decreased due to a diminution of the cephalic phase reflexive responses. In fact, the observation that conditioning by IG 8% glucose is possible when concurrent oral saccharin consumption is provided supports this hypothesis (Drucker & Sclafani, unpublished data). This could also account for the finding of Elizalde and Sclafani (1988) that orally consumed Polycose maintained its associative strength over increasing delays whereas a decrement in conditioning with delays was observed in the present IG-delay experiment.

The rats in the Elizalde and Sclafani (1988) study also received four times the amount of carbohydrate than the rats in Experiment 3. Therefore, Experiment 4 examined whether the magnitude of the nutrient US plays a role in conditioning with extended delays by comparing the preferences conditioned with a 60 minute delay when 16% Polycose was infused versus when 8% Polycose was infused. Both concentrations of Polycose were effective, however, the preferences conditioned by 16% Polycose tended to be stronger. Perhaps, reliable group effects would show up with more power or greater differences in the nutrient concentrations. Magnitude effects have been demonstrated in the flavor aversion paradigm whereby conditioning was extended when the toxin dosage was increased (Revusky, 1968; Nachman and Ashe, 1973; Martin and Timmins, 1980; Andrews and Braveman, 1975).

The 8% Polycose following a 60 minute delay produced reliable conditioned preferences in Experiment 4 (59% to 62%), whereas in Experiment 3, 8% glucose with a 60 minute delay did not. This suggested that Polycose may be a more potent reinforcer than equicaloric glucose. While there is reason to suspect differences in their reinforcing effects at high concentrations due the osmolarity, differences at concentrations of 8% are somewhat unexpected. A follow-up study using a more sensitive within-subjects design found that rats do not learn to prefer a flavor paired with IG infusions of 8% glucose to a flavor paired with IG infusions of 8% Polycose. However, when 32% carbohydrate concentrations were infused, preferences for the Polycose-paired flavor where observed (Azzara & Sclafani, unpublished data). This result suggests that any differentially reinforcing effects are due to osmolarity or due to differential absorption rates (Daum et al., 1978).

Taken together, the results of past and present work indicate that preference conditioning is influenced by delays in nutrient processing. Seemingly subtle differences in nutrient form, osmolarity, stimulus magnitude, and training procedures affect the development of these preference. Inasmuch as the findings clearly demonstrate the capacity for learning based on delayed nutritional reinforcement, it is important to point out that the experimental procedures produce an artificial state because under normal feeding conditions nutritional information would become available soon after consumption. The artificial nature of the procedure may also weaken the effects obtained with the delay conditioning paradigm. However, laboratory-induced artificial states are essential if we are to provide evidence for cause-effect relationships.

Another issue of digestion not specifically investigated here which may influence conditioning is the rate of GI processing of foods. Several indirect lines of evidence support this possibility. Carbohydrate diets presented in a highly soluble liquid form are preferred to the same carbohydrate in a solid or gel form which may be more slowly digested (Sclafani, 1987). When the rate of Polycose digestion is delayed by the actions of acarbose, preference conditioning is attenuated (Elizalde & Sclafani, 1988). Booth and Davis (1973) observed more rapid conditioning in rats with gastric vagotomy, which is known to accelerate the rate of gastric emptying of liquids. Finally, though not a conditioning effect, Geiselman and Novin (1982) found that rabbits consume more of a food associated with rapid ID nutrient infusions than food associated with slow ID infusions. Whether the rate of nutrient delivery influences the development of preference has yet to be directly explored.

In conclusion, although there are likely to be redundant signals produced by the detection of nutrients at different pre- and postabsorptive sites which contribute to conditioned flavor preferences, the present findings revealed no role of the stomach in the preferences produced by glucose. This, together with previous findings indicating that preferences are not conditioned by the postabsorptive actions of carbohydrates alone (Gowans, 1992; Gowans & Weingarten, 1991) suggests that feedback from the intestine is necessary.

Postingestive reinforcement is likely to be influenced by a combination of many factors. Presumably, this information is summated centrally and modulates the extent to which preferences (or aversions) are formed. Different classes, types, or amounts of nutrients as well as different conditioning

procedures can cause subtle or gross changes in postingestive processing which would cause adjustments to the information received by the brain and the resulting preference. There is still much to be learned about the mechanisms controlling food choice. Although socio-cultural influences undoubtedly play an important role in the food choices of humans, continued experimental work with animals will uncover the underlying physiological mechanisms mediating the acquisition of food preferences.

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