Ingestion Patterns of Food, Water, Saccharin and Sucrose in Streptozotocin-Induced Diabetic Rats

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SMITH, J. C. AND K. S. GANNON. Ingestion patterns of food, water, saccharin and sucrose in streptozotocin-induced diabetic rats. PHYSIOL BEHAV 49(1) 189–199, 1991.—Changes in preference for sweet tasting solutions have previously been reported in diabetic rats. The present study was designed to use detailed analyses of drinking patterns to investigate changes in sweet taste perception in rats made diabetic with a streptozotocin (SZ) injection and tested with saccharin and a broad range of sucrose concentrations. Although meal patterns in diabetic rats have been studied extensively, drinking patterns and their relation to meal patterns have not been described. A second goal of this research was to use the detailed patterns of eating and drinking of the diabetic rat in an effort to understand the resulting hyperphagia. Rats were given IP injection of SZ or saline. Following the SZ injection, the rats showed marked increase in blood glucose levels, an increase in food and fluid intake and they failed to gain weight. The SZ-injected rats showed a loss of preference for saccharin and for high concentrations of sucrose. Patterns of sucrose ingestion that are correlated with taste perception were distorted in the diabetic rats. The detailed pattern analysis of eating showed that the diabetic-induced hyperphagia resulted from a marked increase in length of eating bouts. The increase in fluid intake in the diabetic rats resulted from both an increase in frequency and length of drinking bouts. Circadian patterns of both food and fluid ingestion were maintained in the diabetic rats.

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RATS made diabetic with alloxan injections show a marked reduction in their preference for sodium saccharin solutions (7–9, 19). Sodium saccharin has been characterized as having a dual taste, sweet and bitter. In a conditioned taste aversion study, pairing glucose with a LiCl injection produced a generalized aversion to saccharin in normal rats but not in diabetic rats. In diabetic rats, quinine paired with a LiCl injection resulted in an aversion to sodium saccharin which was not found in normal rats. It was concluded that the generalization from bitter to saccharin in the diabetic rat was because of a reduction in sweet sensitivity, allowing only the bitter component of the saccharin to be tasted (23).

In contrast, a marked increase in intake of lower concentrations of glucose (6,19) and sucrose (18) is exhibited by rats made diabetic with alloxan or streptozotocin. Higher concentrations of glucose (6, 27, 36) and sucrose (18) were not preferred.

It is not clear if changes in preference for saccharin, sucrose and glucose in diabetic rats are due to changes in sweet taste perception or to other factors such as the osmotic effects resulting from ingestion of concentrated sugars. If sweet sensitivity is reduced in the diabetic rat, as previously hypothesized (23), then glucose or sucrose would be perceived as less sweet, but still palatable, and the sweet component of saccharin would be significantly reduced, leaving the predominantly bitter taste. Impairment of sweet taste has been reported in human diabetic patients with both sucrose (1, 17, 20) and glucose (16,28).

Reduction in the perception of sweet tasting compounds in the diabetic could result from one, or combinations, of many factors such as (a) an alteration of taste cells or sensory nerves, (b) a change in rate of taste cell turnover, (c) changes in salivary content or flow rate or (d) the effects of hyperglycemia or hypertonicity in the blood on taste receptors. Alteration in taste cells, sensory nerves or turnover rate would likely be reflected in electrophysiological recordings to taste stimuli, but such recordings from the chorda tympani nerve to sucrose solutions in diabetic rats are normal (18). Although the chorda tympani nerve is responsive to sweet compounds, recent electrophysiological evidence has demonstrated that the greater superficial petrosal nerve is important in mediating sweet taste for the rat (24,37). The GSP nerve innervates the palate, where a rich supply of taste receptors are found in the region of the nasoincisor ducts, in the Geschmackstreifen and in the posterior palatine field (21,24). It is possible that there would be a decrement in the response to sucrose or saccharin from the greater superficial petrosal nerve in the diabetic rat, but this has not been tested.

Increased salivary glucose levels in diabetic rats could result

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in a decreased sensitivity to sucrose (38). Hiji (18) found a 40-
fold increase in salivary glucose levels in diabetic rats which could
have a masking effect on the receptors, reducing the effective
sweet taste in the rat’s oral cavity. There is recent evidence that
diabetes in rats causes neuroaxonal abnormalities in both the
parasympathetic and sympathetic systems innervating the parotid
glands resulting in reduced salivary flow (2). No data are availa-
ble for similar abnormalities in other salivary glands. It is possi-
ble that this reduced flow in diabetics could contribute to the
excessively high salivary glucose level reported by Hiji (18).

It is also possible that high levels of glucose in the blood
could have some effect on the sensitivity of the taste receptors.
Bradley (5) has shown that adding compounds possessing known
taste qualities to the blood in a rat influenced the response of the
chorda tympani nerve to similar taste compounds topically placed
on the tongue. Giza and Scott (14) have recently reported that
blood glucose level affects the perceived sweet intensity of glu-
cose in the rat’s mouth. Rats with elevated blood glucose showed
a 46% reduction in perceived glucose intensity of a 1.0 M
solution.

The behavioral measures reported in the literature showing
changes in sweet preference in diabetic rats have been based only
on the amount of fluid consumed in taste tests (6–9, 18, 19, 23, 36).
Better measurements of the taste of sweeteners in the rat re-
sult from observing the patterns of ingestion rather than relying
solely on the mere amount ingested (29, 30, 33). It has been
shown in normal rats that the “rate” of licking within daily
drinking bouts is a direct function of the concentration of the
sugar. Using Biedler’s Taste Equation (3), these “rate data” cor-
respond to electrophysiological recordings from the greater su-
perficial petrosal nerve in response to a broad range of sucrose
concentrations (24,29). Other variables obtained from a pattern
analysis of sugar ingestion by the rat, such as the proportion of
drinking bouts that occur in daylight hours, are also highly corre-
lated with the electrophysiological recordings to sucrose (29).

In addition, prior research has focused on only day-night meal
patterns and little is known about the 24-h distribution of drink-
ing and its relation to meals in the diabetic rat. There is some
conflict in the literature regarding meal patterns in the diabetic
rat. Booth (4) found that the increase in food intake of streptozot-
tocin-diabetic rats was the result of an increase in the number of
meals and not the result of an increase in meal length. In contrast,
other investigators (11, 26, 35) found that the increase in food
intake in diabetic rats was the result of an increase in meal size
and not in meal number. This difference in ingestive pattern may
result from the quality and form of the food (pellets, powder,
blocks or liquid) or in the operant task required for food acquisi-
tion. In most of the previous meal pattern studies with diabetic
rats the food was in pellet form, delivered one at a time after
some required movement or manipulation by the rat (4, 11, 26).

In the present experiments we utilized detailed ingestive mea-
sures of saccharin and sucrose to investigate the possible changes
in taste sensitivity to saccharin and sucrose solutions in streptozot-
tocin-diabetic rats. The moment-by-moment ingestion of the
sweetened solutions over the normal day-night cycle was quanti-
ﬁed by measuring eating and drinking activity every six seconds.
Therefore, a second outcome of these experiments will be to pro-
vide a detailed description of the patterns of ingestion of food,
water and sweetened solutions in diabetic rats.

METHOD

Subjects

The subjects were 11 male Sprague-Dawley rats (Harlan) which
were 40 days of age at the start of testing. The rats were housed
in a temperature- and humidity-controlled environment. The rats
always had access to tap water which was contained in glass bot-
tles ﬁtted with stainless steel drinking tubes. Powdered Purina
Chow (5012) was also available except where noted in the proce-
dure. The animals were on a 12-h light:12-h dark schedule with
the onset of lighting at 0700 h.

Apparatus

The rats were housed individually and given all of their inges-
tive tests in Hoelteg 11B cages. The cages were modiﬁed by
making a 4 × 4 cm opening in the lower right front of the cage
and attaching a housing arrangement for holding a 4-oz food jar.
The water bottles were hung beside the food jar on the front of
the cage. After the streptozotocin (SZ) or saline injections, eight
of the rats were moved into Hoelteg 11B cages that were further
modiﬁed as previously described by Spector and Smith (33). In
these cages, special adapters were attached to the rear of the cage
to hold two drinking spouts. Photo-transistor and infrared light-
emitting diodes were mounted in front of each lick spout and
above the food jar mouth so that licking or eating by the rat in-
interrupted the infrared beam. Beam breaks were transmitted to
a microprocessor. Eating and drinking were monitored for 23 hours
each day, allowing one hour for maintenance of the animals, cage
cleaning and measurement of daily food and solution consump-
tion. The number of licks on each of the two spouts and the
number of seconds during which the rat’s head broke the beam
above the food jar were recorded in consecutive six-second time
bins. There were 13,800 such six-second bins in each 23-h ses-
sion. Daily strip charts were plotted for each animal, giving an
overall pattern of eating and drinking across the 23-hour period.
An analysis program allowed for the establishment of bout crite-
ria for both food and fluid ingestive activity. Criteria for feeding
and drinking bouts have been previously described (29, 30, 32,
33). Ingestive activity within these deﬁned bouts accounts for
over 98% of the total ingestive activity. These bout criteria and
other ingestive related behaviors were deﬁned as follows.

Feeding. (a) If the rat placed its head in the feeding jar for
two seconds a potential bout was initiated, and (b) this bout
ended when there was no beam break for 50 consecutive bins
(five minutes). (c) Bouts of less than 30-s duration were
discounted. These feeding bout criteria result in accounting for
over 99% of all feeding activity.

Drinking. (a) If the rat licked three times on one of the tubes
a potential bout was initiated, and (b) this bout ended when there
were no licks on that tube for 50 consecutive bins (ﬁve minutes).
(c) Bouts of less than 30 licks were discarded.

Switching. It was of interest to know how frequently the rats
switched from eating to a drinking bout and vice versa. Since
the criterion above for termination of an ingestive session is five
minutes of no activity, the same criterion was applied to switch-
ing from food to water or water to food. For example, if the rat
initiated a drinking bout within ﬁve minutes after an eating bout,
this was subsequently referred to in this report as a “switch.”

Eating efficiency. This measure reﬂected the proportion of
time during eating bout that the rat’s head was actually breaking
the infrared photo beam. In previous research we have found that
this “rate of eating” measure was remarkably consistent from
day to day for a rat, but was quite sensitive to insults such as
damage to the chorda tympani nerve or loss of salivary flow (31).

With these criteria, quantiﬁcation of the food and liquid inges-
tion for day and night periods could be established. The number of
bouts, bout duration, interbout interval and the rate of eating
or drinking within a bout were recorded daily for each rat.
For blood glucose measurements each rat was restrained and blood was drawn from a vein in the tail. A drop of blood was placed on a Chemstrip 6G Reagent Strip and read by an Accu-Chek II blood glucose monitor (Boehringer-Mannheim Diagnostics). These measurements were made two days before and two days after the rats were injected with SZ and on three other days throughout the five weeks of testing. Body weight measurements were taken before injection and periodically during the postinjection testing period.

Procedure

Daily food and water intakes were measured for all of the rats for the first 11 days after arrival in the laboratory. On the 11th day in the laboratory nonfasting blood glucose level was measured for all rats. On the 12th day the rats were placed on food deprivation for 24 hours and then divided into two groups for the streptozotocin (SZ) and saline (SHAM) injections. Immediately prior to injection, the SZ (Upjohn) was dissolved in 0.1 M citrate buffer (pH 4.3–4.5). The rats in the SZ Group (N = 7) received a single injection (IP) of SZ at a dose of 75 mg/kg (22). One of these rats died forty-eight hours following injection. The rats in SHAM Group (N = 4) served as saline-injected controls.

For both groups of rats, food, water and a 0.28 M glucose solution were placed on the rats’ cages immediately following injection and left there for 48 hours. The glucose was available to insure survival during the hyperinsulinaemic period resulting from SZ-induced beta cell lysis (22).

On the fourth day following the injections, the six surviving rats from the SZ group and two of the SHAM rats were placed in the eight special cages described above. They remained in these cages for the duration of the experiment where the patterns of ingestive behavior as well as total consumption could be obtained. The other two SHAM rats were kept in their home cages for all subsequent testing where only daily consumption could be measured. For the next seven days the rats were allowed to stabilize their eating and drinking. During the last five days of this seven-day period the rats were given access to a 0.004 M saccharin solution by adding a second drinking bottle to the cages. Only daily intakes were measured during this time. Therefore, when the rats were subsequently given saccharin-water or sucrose-water preference tests (described below) they were familiar with both nutritive and nonnutritive sweeteners.

Postinjection testing was started 10 days after the SZ or SHAM treatments. Throughout this testing period the rats always had access to the powdered Purina Chow. From Days 19–19 the only solution available was water. From Days 20–24 the rats were given five days of preference testing with 0.004 M sodium saccharin solution vs. water. On Days 25 and 26 only water was available. From Days 27 to 30, the rats were given two-bottle preference tests with an ascending series of sucrose solutions. They received two days each with 0.0025 M, 0.125 M, 0.250 M, 0.50 M and 1.00 M sucrose. Finally, on Days 37 and 38 only water was available.

RESULTS

Two days before the SZ injection, the blood glucose levels for the rats ranged from 45 mg/dl to 95 mg/dl with an average of 68 mg/dl. As can be seen in Fig. 1, the mean blood glucose level for the SZ group rose to 437 mg/dl on the second day following injection and remained between 385 mg/dl and 416 mg/dl for the duration of the experiment. No individual rat’s blood glucose level dropped below 362 mg/dl for any of the measurements taken after SZ injection. The mean blood glucose levels for the SHAM-injected rats varied between 80 mg/dl and 102 mg/dl during the remainder of the experiment.

The mean body weights are illustrated in Fig. 2. The mean body weight for the six SZ rats was 133 g and for the four SHAM rats was 131 g on the day of injection. The SHAM rats continued to grow normally, weighing an average of 312 g 42 days after injection when the experiment was terminated. In contrast, the SZ rats gained only a few grams over the same time period. Forty-two days after injection the mean body weight for the six SZ rats was only 134 g. The rats displayed the typical diabetic hyperphagia as can be seen in the excess food consumption illustrated in Fig. 3. The
average daily food intake for SZ rats was 17.01 g and for the SHAM rats was 15.81 g prior to injection. A repeated sampling of the same subjects ANOVA (13) was run over the four preinjection days and the group difference was not significant, $F(1,8) = 1.58$, n.s. Following the injections, the food intake gradually increased for the SZ group, while remaining relatively constant for the SHAM group. An ANOVA was run for the last three days shown in Fig. 3 and the SZ group was significantly different from the SHAM group, $F(1,8) = 15.19$, $p<0.01$. The average daily food intake for the two groups remained relatively constant for the remainder of the experiment.

Fluid intake for the three days prior to and five days after injection can be seen in Fig. 4. Water intake was not different for the SZ and the SHAM groups for the three days prior to injection, $F(1,8) = 1.58$, n.s. The increase in fluid intake following SHAM injections was attributed to the intake of the 0.28 M glucose, which the rats drank almost exclusively. The intake of glucose solution by the SHAM rats averaged 44.7 ml as compared to a mean water intake of 6.6 ml. Fluid intake for the SZ-injected rats increased markedly, averaging 150.9 ml over the last three days shown. This difference was statistically significant with the ANOVA, $F(1,8) = 143.28$, $p<0.01$. Daily water intake by the SZ rats remained quite high over the next thirty days of testing and averaged 179.2 ml on the last two days of testing.

**ANALYSIS OF SACCHARIN TESTS**

The saccharin preference tests were conducted on Days 20-24. Comparisons between the last day of food and water testing (Day 19) and five days of saccharin testing for both the SZ and the SHAM groups were made. On the first day of saccharin tests the total fluid intake for the SHAM rats more than doubled. As would be expected, this was because the rats drank an average of 68.6 ml of the saccharin solution, compared to normal daily water intake of 33 ml.

In contrast, the total fluid intake for the SZ group averaged 220.4 ml on this first day, which did not differ significantly from the water intake mean of 213.5 ml for the last day of testing before saccharin was introduced. As can be seen in Fig. 5, the total fluid intake for SZ rats remained very consistent across the five days of testing, averaging 214.3 ml daily. The mean saccharin intake decreased and the mean water intake increased across the five days of testing, but this trend was not statistically significant. The amount of saccharin ingested varied markedly from day to day and from rat to rat. The lowest daily consumption was 17.0 ml and the highest was 301.4 ml. As a result of the noted variability in saccharin intake, the proportion of the total fluid intake which was saccharin (preference score) varied both between and within the five days of testing from 0.06 to 0.96. One rat consistently preferred saccharin, one rat was consistently indifferent and the other four varied their intake from day to day. The repeated sampling of the same subjects ANOVA on these daily preference scores across the five days of testing yielded neither a significant difference across subjects nor across days, $F(4,20) = 2.44$, n.s., $F(5,20) = 2.00$, n.s. The mean preference score for the six rats on the last day of testing was 0.62, with a range from 0.36 to 0.95.

This amount of variability in saccharin drinking by the SZ rats is quite uncommon in normal rats. Saccharin preference scores for a 0.004 M sodium saccharin solution in Sprague-Dawley rats are normally exceedingly high. For example, in a comparison group of eight Sprague-Dawley rats run in our laboratory prior to this experiment, the mean preference score was 0.97 with a range from 0.95 to 0.98 (unpublished data).

The pattern of ingestion of both saccharin and water during these tests was not unlike the pattern when only water was present. On the last day of saccharin testing, the rats showed a similar pattern of switching from food to fluid and from fluid to food as they did on the last water baseline day before saccharin testing began. The rats were indifferent to the saccharin solution and it appeared that the strong motivation to drink any fluid overshadowed the normal taste preference for the sweetened substance.

Both the amount of food ingested and the patterns of ingestion (number of bouts, bout duration, eating efficiency and switches from food to fluid) did not change for the SZ-treated rats during the saccharin testing. Matched t-tests on these variables between Day 19 and Day 24 (5th day of saccharin testing) were not significantly different.
SWEET TASTE IN DIABETIC RATS

FIG. 5. Fluid intake is plotted for the five days of saccharin preference testing for the SZ group.

FIG. 7. Mean sucrose consumption on the second day for sucrose concentrations of 0.0625 M, 0.125 M, 0.25 M, 0.5 M and 1.0 M are shown for the diabetic rats.

ANALYSIS OF SUCROSE TESTS

Each sucrose concentration was presented for 2 consecutive days and the analyses were performed on the data from the second day. The mean sucrose intake for the SZ and SHAM groups for the five concentrations are plotted in Fig. 6. The typical inverted U-shaped curve for sucrose over a variety of concentrations is seen for the SHAM group only. The SZ rats drank excessive amounts of sucrose at the two lowest concentrations and less than the SHAM rats at the higher two concentrations.

Fig. 7 the sucrose intake for the SZ group is replotted along with the water intake and the total fluid intake. Unlike the results seen above for saccharin intake, the total fluid intake when the three lowest sucrose concentrations were available significantly exceeded the water intake on Day 26. Among the SZ rats, as the concentration of the sucrose increased, the amount of sucrose ingested decreased and the water intake increased. In Fig. 8 the water intake for the SZ rats was replotted and compared with water intake from the two SHAM rats across the five sucrose testing sessions. As indicated here, normal rats drank very little water when these particular concentrations of sucrose were available. Mean intakes of food consumed during each of the sucrose presentations for both the SZ and SHAM rats are seen in Fig. 9. As caloric intake from the sucrose rises with increasing concentrations, the caloric intake from food is reduced. As has been shown elsewhere (33) total caloric intake increases as rats are given higher and higher sucrose concentrations up to at least 0.5 M. However, the caloric intake for the SZ group is already quite high at the lowest sucrose concentration and decreases as the concentration of the sucrose is increased. These functions can be seen in Fig. 10.

Changes in sucrose and water intake by the two groups at different sucrose concentrations could be accounted for by changes in bout number, bout length, rate of drinking or some combination of these factors. The numbers of drinking bouts for the SZ and SHAM rats at each of the sucrose concentrations are plotted in Fig. 11 and the lengths of these bouts are plotted in Fig. 12. In the SZ group, the decrease in sucrose intake across the higher concentrations is the result of both a decrease in number and length of the bouts. The length of the bouts at night contribute more to this than those occurring in the daytime hours. The contrasting increase in water intake while drinking these concentrations of sucrose is due more to the number of drinking bouts than to the bout duration. This is true for both day and night bouts.

The percentage of sucrose bouts occurring in the daytime and the rate of sucrose drinking, previously described as measures of sweet taste perception, appear to be distorted in the SZ group. It
can be seen in Fig. 13 that nearly half of the drinking bouts occurred in the daytime for all of the sucrose concentrations for the SZ group. The two SHAM rats show the characteristic increase in proportion of daytime bouts up to 0.25 M. The rate of drinking for the SHAM rats (see Fig. 14) increases as a function of sucrose concentration. The SZ rats show no comparable rate increase and actually show a decrease at the two higher concentrations.

The general pattern of ingesting food, sucrose and water in the SZ group is quite different from that in the SHAM animals. SHAM rats in the special cages spend about three hours each day in eating and drinking. When the lower concentrations of sucrose were available, the SZ rats spent as much as 9.5 hours ingesting food, water and sucrose solution. This value decreased to a mean of 6 hours when 1.0 M sucrose was available. Regardless of the sucrose concentration, the SZ rats had a total mean number of 55 daily eating and drinking bouts. Because of the large number of

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**FIG. 8.** Mean water consumption on the second day of preference testing for each sucrose concentration is plotted for the diabetic and control rats.

**FIG. 9.** Mean daily food consumption for the diabetic and control rats is illustrated for the periods when the indicated sucrose concentrations were available.

**FIG. 10.** Mean daily caloric intake (from both food and sucrose) is plotted for the diabetic and control rats when each of the indicated sucrose concentrations were available.

**FIG. 11.** Mean number of daily sucrose and water drinking bouts are plotted for the second day for each of the indicated sucrose concentrations.
FIG. 12. Mean daily sucrose and water bout lengths are plotted for the second day for each of the indicated sucrose concentrations.

These ingestive bouts in the SZ rats, almost every eating bout was followed by drinking. The number of switches from food to sucrose for the SZ and SHAM groups are shown in Fig. 15. There are large numbers of switches for the SZ group at the lower sucrose concentrations, but this number rapidly drops as the concentration increases. As one would expect this decrease in switches is correlated \((r = .97)\) with the number of sucrose drinking bouts.

FIG. 13. The mean percent of total sucrose drinking bouts which occurred in the daylight hours are plotted as a function of the sucrose concentrations available for the diabetic and control rats. These data are based on the average percentage scores for the two days of testing at each concentration.

The number of switches for the SHAM group drops in the typical manner. The number of switches from food to water is plotted in Fig. 16. Since in the SHAM group there is little water drinking at any concentration of sucrose, it is not surprising to see so few switches. The switches from food to water for the SZ group are highly correlated with the number of water drinking bouts for that group \((r = .97)\).

FIG. 14. Mean drinking rate is plotted for the diabetic and control rats at each sucrose concentration. Drinking rate was calculated by dividing total number of licks by the product of bout number and bout length. These scores were averaged over the two days of testing at each concentration.

FIG. 15. The mean number of daily switches from food to sucrose that occurred during the presentation of each of the indicated sucrose concentrations. To count as a switch, the period of time from termination of eating to initiation of sucrose drinking had to be less than five minutes.
FIG. 16. The mean number of daily switches from food to water that occurred during the presentation of each of the indicated sucrose concentrations. To count as a switch, the period of time from termination of eating to initiation of water drinking had to be less than five minutes.

In the SHAM rats, since there is almost no water drinking, sucrose bouts are never followed by water bouts at any sucrose concentration. In contrast, in the SZ rats sucrose bouts are followed by water bouts at the higher concentrations. At the 0.06 M level only 9% of the sucrose bouts are followed by water drinking, but this value increases to 60% by the 1.0 M level.

PATTERNS OF EATING AND DRINKING IN SZ RATS

A detailed analysis of the pattern of food and water intake was conducted from Days 10–19 (prior to saccharin and sucrose tests) as well as for Days 25, 26 (following saccharin tests) and 37, 38 (after sucrose tests). These detailed observations were made on the six SZ and two SHAM rats that were housed in the special cages. During the first ten-day testing period the SZ rats averaged 199 ml of daily water intake and 32.4 g of powdered Purina Chow compared to the SHAM rats which drank an average of 37.8 ml of water and ate an average of 20 g of chow.

A comparison of the ingestive patterns of one SZ-treated rat with one SHAM rat for the last day of this food and water testing period can be seen in Fig. 17. Data for the SHAM rat are plotted in the upper panel, and data for the SZ-treated rat are plotted in the lower panel. The horizontal bars in each panel indicate the portion of the 23-h test period when the lights were off. By the criteria described above, the SHAM rat had 15 feeding bouts, five of which were in the lighted period. The average duration of the day bouts was 3.9 min and of night bouts was 6.7 min. The eating efficiency score was 0.81. The SHAM rat had 29 drinking bouts, three of which were in the lighted period. The mean duration of day drinking bouts was 2.9 min and the mean night bout duration was 1.5 min. The rat switched from food to water 12 times indicating that 80% of the feeding bouts were followed within five minutes by a drinking bout. Only 23% of the 29 drinking bouts were followed within five minutes by eating. These values are well within the range of normal eating and drinking behavior previously found in this laboratory (29, 30, 33).

FIG. 17. In the upper panel water bouts and meals for one day are illustrated for a control rat. The horizontal bar indicates the 12-h period when the lights were off. In the lower panel a similar record is shown for a streptozotocin injected rat. These records were taken from the last day of food and water baseline before the initiation of saccharin preference testing.

As can be seen in the lower panel of Fig. 17, the food and water ingestive data for the SZ-treated rat are markedly altered. This rat had a total 18 feeding bouts. Four of these were in the lighted period. The fourth food bout was initiated in the lighted period and did not terminate until after the lights went off. The durations of the feeding bouts were much longer, averaging 13 min in the light and 16.3 min in the dark. The eating efficiency score was 0.94. There were 32 total drinking bouts with 11 of these occurring during the lighted period. They averaged 6.3 and 5.9 min, respectively, during the light and dark periods. This rat switched from food to water following 100% of the feeding bouts and from water to food following 47% of the drinking bouts.

Feeding and drinking pattern data for all eight rats are presented in Tables 1 and 2, respectively. It can be seen from Table 1 that the SZ injections resulted in a significant increase in amount of food consumed. This increase was due to longer food bouts rather than an increase in the number of bouts. The eating efficiency score for the SZ rats also increased. Finally, almost all of the feeding bouts were followed by drinking in the SZ rats.

It can be seen from Table 2 that there was a marked increase in amount of fluid consumed by the SZ-injected rats. Since these rats weighed only about 130 g, they consumed over one and a half times their body weight in daily water intake. This increase in water intake was accomplished in two ways. First, the number of daytime drinking bouts increased significantly. Second, the duration of both day and night drinking bouts more than doubled. Since the SZ rats are eating and drinking so much more than the SHAM rats, one would expect them to exhibit more switching behavior, which they did.

In order to show that the atypical patterns of food and water intake were stable during the postinjection testing, matched t-tests were run between Days 19 and 26, comparing food intake, water intake, number of eating and drinking bouts, bout length, eating efficiency and proportion of switches from food to liquid. None of these tests resulted in a statistically significant difference. The
same eating and drinking patterns were still present on Days 37 and 38, the last two days of testing.

DISCUSSION

Three days after a single injection of streptozotocin the rats showed clear signs of the diabetic symptoms. Food and water intake increased markedly, the rats failed to gain body weight and blood glucose levels were increased to values in excess of 350 mg/dl. These factors remained fairly constant for 36 days of subsequent testing.

The measurements of taste made in this experiment would indicate that the sweet taste in the rat was altered by the SZ injection and the subsequent diabetic condition. After five days of testing, the rats displayed a complete indifference to saccharin, a conclusion that is similar to the findings of Brookshire (7). However, the rats in the present experiment did not avoid saccharin, since they consumed over 100 ml of it. Mean preference scores were low due to elevated water intake. In fact, their water intake increased daily as testing with saccharin proceeded. Brookshire (7) postulated two mechanisms to be involved in this indifference or aversion to saccharin. (a) If the first encounter with saccharin occurred close in time to when the alloxan was administered, a conditioned aversion would be formed to the saccharin solution. (b) If the first encounter with the saccharin solution happened some days after the alloxan injection, a more modest reduction in saccharin palatability occurred, which was associated with the diabetes condition and not the trauma of the drug injection. The latter conclusion would be reached for the present experiment since the saccharin solution was first presented several days after the streptozotocin injection.

The pattern of saccharin ingestion, in terms of bout frequency, rate of drinking, interbout intervals and amount of fluid ingested, could not be distinguished from previous ingestion of water prior to the beginning of the saccharin test. It was apparent that the saccharin did not taste bitter enough to make the rats avoid it. The only way that one could distinguish saccharin bouts from water bouts was that the nighttime saccharin drinking bouts were longer than water drinking bouts. The indifference to saccharin was also shown by the fact that on some occasions following a food bout the rats would switch to drinking saccharin and on other times switch to water. There seemed to be no pattern for this switching from food to fluid behavior.

The behavior of the SZ rats toward the five sucrose concentrations also indicated a loss of sweet taste sensitivity. At the lower concentrations of sucrose the consumption by the SZ rats was over twice the amount that normal rats drink. This increase was the result of longer drinking bouts and an increase in bout number. Normally rats don’t drink much water when it is paired with sucrose, but in the SZ rats the average water intake was over 50 ml, which was considerably higher than their normal daily water intake when sucrose was not present. This increase in water intake was also the result of much longer drinking bouts and not the result of an increase in the number of bouts. The sucrose and water intake for the SZ rats was quite similar to that reported earlier (18,34) where there was a strong sucrose preference at the lower concentrations, but a significant drop in preference at concentrations above 0.2 M. Water intake was moderate when lower sucrose concentrations were available, but it increased markedly when paired with the higher level of sucrose. The apparent aversion that these SZ-treated rats demonstrated for the higher concentrations of sucrose seems to also be true for strong glucose solutions (6, 27, 34, 36).

In normal rats the number of sucrose drinking bouts typically declines as the concentration is increased. A similar decline was seen in the present study with the SZ-treated rats (Fig. 11). The most striking difference between the SZ and SHAM rats during the sucrose ingestion period was the increase in water bouts as the sucrose concentration was increased. At the 1.0 M level the SHAM rats had almost no water bouts as compared to over 30 bouts for the SZ rats. Normal rats showed a gradual increase in sucrose bout duration up to about 0.5 M and then showed a decline. In the SZ rats the bout length peaked at the 0.125 M level and then declined as the concentration increased. The bout length function for SZ rats correlated with the preference data for sucrose seen in this and other studies (18,34). The length of a drinking episode, much more than the frequency, has been shown to be related to the taste of the sweet compound (30,33).

The two measures of the “taste” of sucrose (proportion of

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### TABLE 1

<table>
<thead>
<tr>
<th>Feeding Behavior</th>
<th>SZ Rats</th>
<th>SHAM Rats</th>
<th>t-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount consumed</td>
<td>33.17 g</td>
<td>20.45 g</td>
<td>4.43*</td>
</tr>
<tr>
<td>Number of daytime bouts</td>
<td>5.57</td>
<td>4.50</td>
<td>1.66</td>
</tr>
<tr>
<td>Number of nighttime bouts</td>
<td>13.50</td>
<td>10.50</td>
<td>1.24</td>
</tr>
<tr>
<td>Duration of daytime bouts</td>
<td>15.10 min</td>
<td>3.91 min</td>
<td>8.04*</td>
</tr>
<tr>
<td>Duration of nighttime bouts</td>
<td>16.71 min</td>
<td>7.78 min</td>
<td>4.46*</td>
</tr>
<tr>
<td>Eating efficiency</td>
<td>0.91</td>
<td>0.65</td>
<td>2.51*</td>
</tr>
<tr>
<td>Proportion of switches</td>
<td>0.94</td>
<td>0.80</td>
<td>2.53*</td>
</tr>
<tr>
<td>from food to water‡</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant beyond 0.01 level. †Significant beyond 0.05 level. All independent t-tests based on df = 6.
‡This is the proportion of the total food bouts followed within five min by a water bout.

### TABLE 2

<table>
<thead>
<tr>
<th>Drinking Behavior</th>
<th>SZ Rats</th>
<th>SHAM Rats</th>
<th>t-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount consumed</td>
<td>213.52 ml</td>
<td>33.08 ml</td>
<td>6.37*</td>
</tr>
<tr>
<td>Number of daytime bouts</td>
<td>10.50</td>
<td>2.50</td>
<td>8.14*</td>
</tr>
<tr>
<td>Number of nighttime bouts</td>
<td>22.00</td>
<td>27.50</td>
<td>1.62</td>
</tr>
<tr>
<td>Duration of daytime bouts</td>
<td>6.61 min</td>
<td>2.43 min</td>
<td>8.33*</td>
</tr>
<tr>
<td>Duration of nighttime bouts</td>
<td>6.69 min</td>
<td>1.78 min</td>
<td>10.72*</td>
</tr>
<tr>
<td>Proportion of switches</td>
<td>0.44</td>
<td>0.20</td>
<td>4.81*</td>
</tr>
<tr>
<td>from water to food†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant beyond 0.01 level. All independent t-tests based on df = 6.
†This is the proportion of the total water bouts followed within five min by a food bout.
Daytime sucrose drinking bouts and rate of licking within a bout that previously have been shown to correlate with electrophysiological recordings from the greater superficial petrosal nerve were markedly distorted in the SZ rats. In the SZ rats about 40% of the sucrose bouts occurred in the daytime regardless of the sucrose concentration. Normally, rats do not show this level of daytime drinking except with 1.0 M sucrose concentrations. In most of our previous work with normal rats this proportional measurement can be plotted as a sigmoidal curve that fits Nejad's (24) electrophysiological curve quite well (29). When these measures were determined for the SZ-treated rats it was obvious that they did not show functions that fit the electrophysiological measure of sweet taste in the normal rat.

Rate of drinking sucrose within a bout in normal rats also increased with an increase in sucrose concentration. This function was highly correlated with taste as measured by electrophysiological recordings from the greater superficial petrosal nerve (29). In the SZ rats the rate decreased with increasing concentrations, providing further evidence that the sweet taste of the diabetic rat was distorted. The data are clear that the preference for saccharin and higher concentrations of sucrose and glucose seen in normal rats in daily 2-bottle preference tests was lost in the diabetic rat (6, 14, 18, 27, 36). Tepper and Friedman (34) have shown this same loss of preference in a 30-min 2-bottle preference test, where long term post-ingestional feedback would at least be reduced from that evident in the 24-h test.

It is quite reasonable that factors other than taste contribute to this reduction in sweet taste preference. Although the SZ treatment resulted in reduced body weights, it is not likely that it was the lower body weights of the SZ rats that caused the preference changes for saccharin and sucrose. Tepper and Friedman (34) have shown that diabetic rats which are fed a high-fat diet do not show the changes in sugar preference seen in those fed a low-fat chow diet, even though they also have reduced body weight.

However, as Tepper and Friedman indicate, it is possible that this reduction in preference for the concentrated sugars may be due to the osmotic effects of the sugar loads (34). In their study and the present one, the reduced preference for sucrose at higher concentrations is largely a result of the increase in water intake. In the pattern analysis of the present data it was found that the SZ rats increasingly switched from sucrose bouts to water bouts as the sucrose concentration was increased, giving evidence for an osmotic effect. When 1.0 M sucrose was available, 58% of the sucrose bouts were followed by water bouts. Switching from sucrose to water in the SHAM rats was almost totally absent at all concentrations. This would not explain the reduction in preference for saccharin. In only rare instances did any of the SZ rats have a water bout following a saccharin bout. A further difficulty in explaining the present data on the basis of taste alone comes from the report that feeding high-fat diets to SZ-treated rats normalized their responses to a variety of sugar solutions including sucrose. It remains to be explained how the high-fat diet could alter mechanisms of taste affecting the perception of sweet.

Finally, the larger increase in overall ingestive behavior of the SZ rats may have masked the subtle "sweet taste" measure reported in this study. Further investigation using short-term sweet taste tests (30) would be justified.

As has been shown before (11, 26, 35), the chronic hyperphagia in the SZ rats used in the present study was clearly the result of an increase in food bout length with little change in the number of bouts. The eating efficiency, which is normally around 0.80 for Sprague Dawley rats, increased significantly in the SZ rats as compared to the SHAM rats. Therefore, the SZ rats not only had longer feeding bouts but they ate at a higher rate within a bout. In normal rats the nighttime feeding bouts are usually significantly longer than daytime bouts. In the SZ rats the lengths of the daytime bouts were equal to the nighttime ones. Gastric emptying is known to be accelerated in SZ-treated rats (12, 15). It has been suggested that this accelerated clearance of food from the gastrointestinal tract in SZ rats contributes to the induced hyperphagia (15). This more rapid clearance would lead to a more rapid decrease in gastric distention, which would seem to initiate more meals rather than sustaining longer ones. It would be of interest to observe changes in meal size and frequency in SZ-treated rats given high-fat diets which significantly reduce the hyperphagia.

Both the frequency and duration of water bouts was significantly greater for the SZ than for the SHAM rats. The increase in frequency of the water bouts was solely due to the increase in daytime bouts. The duration of day and night bouts increased equally across the SHAM and SZ treatment groups.

Switching from food to water in normal rats occurs reliably after nighttime meals and less reliably after daytime meals with an overall occurrence of about 80%. In these normal rats it is not uncommon for a food bout to be followed by a food bout during the daylight hours. In the SZ rats 94% of the meals were followed by drinking. This significant increase is the result of more daytime meals being followed by water bouts. In no instance was a daytime meal not followed by a drinking bout. The SZ rats seem to be coping with the osmotic effects of food by more reliably drinking after each feeding bout.

In normal rats it is a rare event for rats to immediately start eating after a drinking bout. In the SZ rats 44% of the drinking bouts were followed within 5 minutes by eating. Since both the feeding and drinking bout durations are increased so much in the SZ rats, the interbout intervals are decreased, leading to a higher probability that water bouts would be followed by eating. When only food and water are present, the normal rat spends about 99 minutes eating and 55 minutes drinking each day. The SZ rats spent 310 minutes eating and 217 minutes drinking each day. This marked increase in ingestive activity is clearly shown by the data from a single rat presented in Fig. 17. As can be seen in the lower portion of the figure, food and water bouts are so long it is difficult to find portions of time (especially during the nighttime) when food and water bouts are not interrelated.

Although the SZ rats became more active in eating and drinking during the daytime period, they continued to show the normal circadian patterns, eating and drinking predominantly at night. Christensen and Agner (10) claimed a complete obliteration of all circadian variations in hereditary diabetes insipidus in Brattleboro rats. However, the resolution of their measurements was only four hours, making it impossible to conclude that all circadian variations were missing.

Evidence from the present paper indicates that at least some of the loss of preference is due to loss of taste sensitivity for the sweetened solutions. It is important to gain a clear understanding about potential changes in sweet perception since the taste of sweet substances is responsible for the preabsorptive insulin response (25). The problems of the diabetic patient are compounded if the sweet taste response is indeed decreased.

Acknowledgements

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34. Tepper, B.; Friedman, M. I. Altered acceptability of and preference for sweet solutions by diabetic rats is normalized by high-fat diet. Appetite, in press; 1990.


