Drinking in the Rhesus Monkey: Peripheral Factors

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Rhesus monkeys prepared with chronic cannulae implanted in the stomach and duodenum were water deprived for 22.5 hr. With both cannulae closed, a mean of 137 ml was drunk within a 60-min period. When ingested water drained freely from an open gastric cannula, continuous drinking far in excess (878 ml mean intake) of normal occurred. Sham drinking also exceeded (634 ml mean intake) normal when ingested water drained through an open duodenal cannula. This pattern of continuous sham drinking indicates that oropharyngeal stimulation is not sufficient alone, or together with the passage of water through the stomach and the initial part of the duodenum, to terminate drinking. Duodenal infusions of water (25–100 ml) slowed or stopped gastric sham drinking in a dose-dependent fashion, but equivalent infusions of isotonic saline were without effect. Thus, pre- or postabsorptive signals at or beyond the level of the intestine distal to the site of the duodenal cannula are probably important for the termination of drinking in the rhesus monkey.

Although there have been extensive investigations of the physiology of thirst in a variety of species, little is known about thirst in nonhuman primates. Because of this and because of its special relevance for the understanding of the control of drinking in humans, we are now studying the physiological controls of drinking in the rhesus monkey. The investigation reported here concerns the peripheral controls of drinking. One question considered is what factors maintain, i.e., provide the incentive for, drinking? Does the monkey drink to obtain oropharyngeal sensations, such as the taste of water, or does gastric or intestinal stimulation or the reduction of fluid deficits by absorption provide the incentive for drinking? In the latter case, animals should not drink if water consumed by mouth is lost through a gastric cannula. A second question is, once drinking is in progress, what mechanism acts to terminate drinking when an appropriate quantity of water has been drunk? This could also rely on the attenuation or removal of fluid deficits in the two main fluid compartments following absorption, or on “feed forward” mechanisms, such as the oropharyngeal metering, gastric distension, or the activation of receptors in the gut or the hepatic portal circulation.

In the present study, the time course of normal drinking due to water deprivation was established. To investigate the function of oropharyngeal factors in determining this pattern of ingestion, we equipped monkeys with gastric cannulae to allow drinking to be measured in the absence of gastric distension, intestinal stimulation by water, and absorption. To investigate the role of postgastric stimulation by water in the pattern of drinking termination, we also equipped the monkeys with duodenal cannulae.

The results of the present study indicate that in this species, oropharyngeal factors are not sufficient to terminate drinking but do maintain drinking in the thirsty animal. In contrast, signals arising at or beyond the level of the duodenum are sufficient to elicit an apparent satiety even in the absence of significant gastric distension.
General Method

Subjects

Five male rhesus monkeys (Macaca mulatta), 3.1-4.2 kg in body weight at the time of surgery, served as subjects in all experiments.

Apparatus

Monkeys were tested daily in primate chairs to which they were accustomed prior to surgery. The animals were chaired only during the daily drinking tests and were returned to the home cage at the conclusion of testing.

For testing, the primate chairs were placed inside sound-proofed cubicles (1.7 x 6 x 0.6 m). The cubicles were ventilated by means of a fan in the roof, the noise generated by the fan serving to mask extraneous noises, and were illuminated by a strip light positioned above and behind the monkey. The animals were observed during testing by means of a one-way glass panel (58 x 27 cm) in the door of the cubic.

Water was provided by means of a measuring cylinder suspended outside the cubic. A flexible polyethylene tube attached to the cylinder and terminating in a drinking spout was fed through a small hole in the cubicle wall. During testing, the drinking spout was clamped in front of the monkey in such a position as to allow ready access. Drainage tubes, when in use, were led out through appropriately located holes in the cubic walls.

Surgery

Under sodium thiopental anesthesia (5 mg/kg initial dose) each monkey was equipped with a gastric and a duodenal cannula. One nylon and stainless steel cannula was sutured into the most dependent portion of the greater curvature of the stomach and the other into the duodenal wall 2 cm distal to the pylorus. The cannulae were externalized through the abdominal musculature and closed with removable stainless steel screw caps. The animals were given 4-7 days for recovery from surgery, during which water and solid food (Dixons diet FP1) were available ad lib. The cannulae were inspected daily and cleaned when necessary.

Procedure

Following recovery from surgery the animals were adapted to a 22.5 hr/day water deprivation schedule. Water was available only during a daily test session of 1-hr duration and a subsequent 30-min rehydration period. Solid food, made up as 200 g of chow mixed with 100 ml of water, was available daily on returning to the home cage.

During the test period the procedure was as follows: The animal was placed in the primate chair, and the caps were removed from the cannulae. The stomach and duodenum were flushed with 20-30 ml of warm (38 °C) isotonic saline, and flexible polyethylene drainage tubes were attached to the cannulae. The tubes were initially clamped closed. The animal was prevented from manipulating the drainage tubes by means of a Plexiglas tray attached to the primate chair at the level of the diaphragm. The primate chair was then placed in the testing cubicle, and the drainage tubes were passed through the holes in the cubicule wall. One or both clamps were then removed from the drainage tubes, according to the requirements of the particular experiment. In control experiments the tubes remained clamped throughout. The drinking spout was then clamped in position and the animal was allowed to drink. During the 60-min test, the volume consumed and the volume of drainage were noted at 1-min intervals.

At the conclusion of the test, the drinking spout was removed, the drainage tubes were disconnected, and the caps were replaced on the cannulae. The animal was then given access to the drinking spout for a further 30 min after which it was returned to the home cage where food was available.

The order in which the experiments were performed varied among animals. Experiments 1 and 2 always preceded Experiment 3, but three of the five monkeys performed Experiment 1 (gastric cannula open) before Experiment 2 (duodenal cannula open), and two performed these experiments in the reverse order. This permitted observation of the effect on drinking of having either cannula open on the first occasion on which sham drinking was tested.

Behavioral Observations

During each test, the behavior of the animal was noted, especially any abnormal behavior such as vocalizing and unusual restlessness, which might indicate discomfort or malaise as a consequence of the experimental treatment.

Experiment 1

In the first experiment, drinking in the 22.5-hr water-deprived monkey was compared between a control condition in which ingested water was retained, to determine the normal time course of drinking in the monkey, and a sham-drinking condition in which ingested water was continuously drained from the gastric cannula, to determine the role of oropharyngeal stimulation in the regulation of drinking.

Method

Two or three days of control testing, i.e., drinking with both drainage tubes remaining closed throughout, preceded the start of the experiment. Stability of intake from day to day was thus ascertained before the first “sham” drinking experience.

On subsequent test days either both cannula drainage...
tubes remained closed throughout, or the clamp was removed from the drainage tube attached to the gastric cannula immediately before the animal was given access to the drinking spout, with the duodenal tube remaining closed. This situation is referred to below as gastric sham drinking. Throughout the test, gastric drainage was collected in a 1-l measuring cylinder.

Each animal was given at least two control and two sham-drinking tests, the order of these being randomized among animals. In sham-drinking tests, only those tests in which drainage equaled or exceeded intake were included in the results analysis. Rejection of test results on this basis was necessary on few occasions only.

Results

The mean cumulative intakes in the control and gastric sham-drinking conditions are shown in Figure 1.

The characteristic pattern for rehydrational drinking in this species was observed in the control condition. Water intake was rapid in the first 5–7 min but decreased sharply in rate between 7 and 10 min, drinking being virtually complete at this time. A few small drinks, usually less than 10 ml, occurred from time to time after this point but not with any regularity within or between animals. Mean control intake over the test period was 137 ml, 87% of which was consumed in the first 10 min.

With the gastric cannula open, all animals drank several times their control intake. This was observed in the first gastric sham-drinking test, even when this was the first test in which either cannula was open, so the effect is not a result of learning. Drinking under these conditions was vigorous and sustained at a high rate throughout the 60-min test period, pauses being brief and infrequent in all tests. Mean gastric sham intake at 60 min was 878 ml. A paired t-test comparison of 60-min intakes shows a highly significant difference, \( t(4) = 7.82, p < .01 \). It can also be seen that mean gastric sham intake exceeded mean control intake by the 5-min point. A brief report of these data has been published previously (Maddison, Rolls, Rolls, & Wood, 1977).

Discussion

Normal drinking in response to water deprivation in the monkey is initially rapid and is virtually complete within 10 min. Although the monkey is a relatively rapid drinker, it is less rapid than the dog in which after a period of water deprivation, drinking is usually complete within 2 to 3 min. It has been shown in the dog that the termination of this drinking occurs before there has been significant plasma dilution (Ramsay, Rolls, & Wood, 1977). In contrast, the rat is a relatively slow drinker, and significant plasma dilution has occurred by the termination of drinking (Hatton & Bennett, 1970). Given that the time course of drinking in the monkey is intermediate between the dog and the rat, plasma dilution may have started to occur by the termination of drinking in the monkey but was not probably sufficient to account for the termination of drinking.

When the gastric cannula was open, which allowed ingested water to drain and prevented it from reaching the intestine, the drinking (gastric sham drinking) was prolonged and almost continuous. This shows that oropharyngeal stimulation by water is
not sufficient to terminate drinking. The fact that the monkeys drank water when the gastric cannula was open suggests that oropharyngeal stimulation by water provides a reward for drinking in the thirsty animal.

Experiment 2

The occurrence of vigorous and continuous gastric sham drinking in Experiment 1 indicates that signals important for the termination of drinking occur beyond the oropharynx. In the present preparation, the implantation of a second cannula in the duodenum permitted the examination of another type of sham drinking in which water was allowed to accumulate temporarily in the stomach and also to stimulate the first few centimeters of the duodenum, in which osmoreceptors, which appear to contribute to the regulation of gastric emptying, have been demonstrated (Hunt & Knox, 1971; Sharma, 1967). Consequently, drinking in the control condition (i.e., both cannulae closed) was compared in the present experiment with drinking when the duodenal cannula remained open and ingested water drained continuously as it reached the duodenum.

Method

The procedure in Experiment 2 was essentially the same as in Experiment 1 except that the clamp was removed from the duodenal drainage tube immediately before the animal was given access to water and the gastric tube remained clamped in sham-drinking tests. This is referred to below as duodenal sham drinking. As in Experiment 1, only those sham-drinking tests in which drainage was equal to or exceeded intake were included in the analysis of results. As there was an appreciable transit time between ingestion and drainage of water in this experiment (see below), satisfaction of the drainage-based rejection criterion was ascertained 2–3 min after the end of the test, by which time the lagging drainage was complete. Again, rejection of test results was rarely necessary.

Results

Drinking in control tests was, in all aspects, as described in Experiment 1. Drinking with the duodenal cannula open was again vigorous and sustained, though pauses in drinking were more frequent and longer than during gastric sham drinking.

Discussion

The continuous sham drinking that occurred when ingested water was drained through the duodenal cannula indicates that stimulation of receptors signaling the presence of water in the oropharynx, stomach, or the initial part of the duodenum (2–3 cm) is
not sufficient to terminate drinking due to water deprivation in the absence of significant gastric distension. It is clear that water must accumulate in the small intestine in order for drinking to terminate normally. Important signals for satiety may originate at some lower point in the intestine, or postabsorptively.

It is not clear why the total volume sham drunk with the duodenal cannula open was less than that sham drunk with the gastric cannula open (Experiment 1). However, it is possible that in the case of duodenal sham drinking, the temporary accumulation of small amounts of water in the stomach may have provided some of the signals associated with satiety and therefore reduced sham drinking.

Experiment 3

If preabsorptive factors play a role in the control of water ingestion in the rhesus monkey, other than the maintenance of drinking already observed, the results of Experiments 1 and 2 indicate that the origin of such factors is probably located distal to the position of the duodenal cannula in the present preparation. This implies that it should be possible to terminate drinking by stimulation with water below this point. In this experiment, therefore, intestinal infusions of water were made through the duodenal cannula during gastric sham drinking. If intestinal receptors do mediate the termination of intake, inhibition of sham drinking at short latency would be expected. Furthermore, this procedure also allows some indirect assessment of the significance of gastric distension by observations of the efficacy of intestinal stimulation in producing satiety in the total absence of gastric distension.

Method

Before starting the experiment the animals were given 3–4 days of control testing in which to establish baseline levels of water intake. In subsequent tests, infusions of water or isotonic saline, maintained at 38 °C, were made into the duodenum during gastric sham drinking. Infusions were made at a rate of 5 ml/min by means of a peristaltic pump (Watson Marlow flow inducer) connected to the duodenal drainage tube. The tube was filled with warm (38 °C) water or isotonic saline prior to the start of the test, and infusions began simultaneously with access to water. At the end of the infusion the pump was switched off, and the duodenal drainage tube was clamped closed. A few drops of food coloring were added to the infusate to determine whether reflux through the pylorus and drainage through the gastric tube occurred. A number of pilot experiments indicated that 5 ml/min was the optimum rate at which water could be infused into the duodenum without gastric reflux.

Infusions of 25, 50, 75, and 100 ml of tap water and 100 ml of isotonic saline were made in random order, each infusion volume being given twice and the order of infusions being randomly varied between animals. Each test was followed by a 30-min rehydration period as in Experiments 1 and 2.

Results

Infusions of water or isotonic saline into the duodenum were generally accomplished without significant reflux of the infusate into the stomach. In a few experiments reflux was indicated by discoloration of gastric drainage. In these cases the experiment was terminated and the results were discarded.

Mean sham intakes during the infusion tests are shown in Figure 3. It can be seen

Figure 3 Cumulative water intake in five monkeys after 22.5-hr water deprivation, with the gastric cannula open, when different volumes of water or isotonic saline were infused through the duodenal cannula. (Significant differences from the control intake at 60 min are indicated by p values. The duration of the infusions is indicated by the horizontal bars beside the corresponding infusion volumes at the top of the figure.)
that infusion of 100 ml of isotonic saline into the duodenum had little suppressant effect on gastric sham drinking, which continued vigorously and with few pauses throughout the test period and resembled the sham drinking observed in Experiment 1. The animals did not appear to be distressed by this or any other infusions; characteristic signs of distress (restlessness, vocalizing, etc.) were not observed.

Total sham intakes over the 60-min test period were depressed by duodenal infusions of water, relative to control saline infusions. A repeated measures analysis of variance (Winer, 1962) applied to these data indicated a highly significant effect of infusion on sham intakes, $F(4, 16) = 13.18$, $p < .001$. Furthermore, a trend analysis of 60-min total intakes indicated a significant linear trend. Multiple comparisons of the total sham-intake data indicated that a duodenal infusion of 25 ml of water did not significantly depress sham drinking relative to the saline control. However, 50 ml of water produced a significant suppression of intake relative to saline ($p < .05$), and the level of significance was increased for the two larger infusions.

The decrease in slope of the curves in Figure 3 with increasing volume of water infused was evident behaviorally as an increase in both the frequency and duration of pauses in sham drinking. Furthermore, with increasing volume of water, a distinct pattern of response became apparent in individual intake patterns. In addition to the increased frequency and duration of pauses, there was a tendency for one extended pause to appear in the cumulative intake curve, the duration of the pause increasing in proportion to the volume of water infused. During isotonic saline infusions the mean longest pause was only 1.4 min. This was not greatly extended in the 25-ml water (2.6 min) or 50-ml water (3.3 min) infusions. However, the pause was much more clearly defined in the 75-ml water (7.4 min) and 100-ml water (22.5 min) conditions. The latency of onset of these extended pauses in sham drinking showed some variability, but a distinct pattern of drinking, pausing, and resumed drinking was seen in the data of individual animals.

The pattern of pauses in drinking behavior is obscured in the mean intake curves in Figure 3, but it may be seen clearly in an example from one animal (see Figure 4). It can be seen that during the infusion of 100
ml of isotonic saline, pauses in sham drinking were brief throughout the test period during which a high rate of sham intake was maintained. However, when 50 ml of water was infused into the duodenum, there was a marked decrease in the rate of sham intake at about 10 min from the start of the test, which coincided in this case with the point at which the infusion was terminated. The decreased rate appeared as a sudden cessation of drinking followed by a period of some 8–12 min duration in which occasional small drinks were interspersed with periods of visual exploration of the test chamber. Toward the end of this period the animal again began to return to the spout more frequently and at approximately 21–22 min resumed sham drinking quite vigorously, though at a lower rate than initially. Sham drinking was then maintained until the end of the test.

A duodenal infusion of 100 ml of water produced a considerably greater effect on sham intake. The monkey again showed a sharp decrease in the rate of sham drinking at 10 to 12 min. In this case, the infusion was still in progress at this point, and the abrupt cessation of drinking noted above was followed by a period in which there was no further intake, extending beyond the point at which the infusion was terminated (i.e., 20 min). During this period the animal gave no indication of illness or discomfort but remained quiescent and apparently resting. Animals were occasionally observed to close their eyes and become drowsy in this period. In this example, when drinking resumed, only small drafts were taken at frequent intervals for the remainder of the test period. A similar pattern of response to duodenal infusions in sham drinking was shown by all animals in the experiment.

**Discussion**

Duodenal stimulation with water has a direct effect on drinking due to water deprivation. In the absence of gastric distension, relatively small infusions of water into the duodenum at low rates decrease the rate of sham drinking, whereas larger infusions elicit extended pauses in drinking, which is suggestive of satiety. The relatively long latency with which the effect occurs after the onset of the infusions suggests that stimulation of receptors in the upper duodenum is not responsible for the termination of drinking. Rather, the presence of water farther down the alimentary tract or the postabsorptive effects of the infused water may be the important factor.

The fact that saline infusions did not attenuate drinking suggests that the cessation of drinking was not produced by duodenal distension or by any discomfort accompanying infusions of the fluid volumes used in these tests. The behavioral observations of the animals during the duodenal infusions also indicate that the effects on drinking were not due to debilitation or malaise. Thus, when the animals began to pause in drinking during the infusions, they would typically sit quietly in the testing chair, without any undue vocalization. When drinking ceased, the animals would sometimes appear drowsy and close their eyes for several minutes. Also, if at the end of the test, peanuts were offered, the animal would readily eat. Furthermore, there is no reason to suppose that the rate of infusion was unphysiologically large since in preliminary experiments in two monkeys we observed gastric emptying rates (ascertained from draining gastric contents at different times after drinking) during normal drinking to be comparable with the 5 ml/min infusion rate used. These observations, then, are consistent with the conclusion that a relatively normal temporary satiety could be elicited by the experimental procedures.

One further point should be mentioned. It can be seen in Figure 3 that the total volume sham drunk in the saline infusion condition greatly exceeded that sham drunk in Experiment 1 in which there was no infusion during gastric sham drinking. This was probably a consequence of the temporal sequence of the experiments for two reasons. First, sham drinking may increase with repeated tests for the same deprivation conditions, as appears to be the case with sham feeding (Gibbs & Falasco, 1978), and the animals had had considerable sham drinking experience by the time Experiment 3 was conducted. Second, increases in body weight over the period between Experiment
2 and Experiment 3 may have augmented the fluid requirements of the monkeys. It should be noted, however, that although these conditions may have served to increase intakes between experiments, stable baseline intakes were established prior to the start of each experiment, and within-test variability in the performance of individual animals was small.

General Discussion

The finding that drinking is prolonged and much greater than normal when water is allowed to drain from a gastric cannula shows that oropharyngeal factors are not sufficient to terminate drinking in the monkey. Also, as duodenal sham drinking is prolonged and much greater than normal, oropharyngeal factors combined with stimulation of a part of the duodenum are not sufficient to terminate drinking. For comparison, in the dog, when ingested water is drained through an esophageal or a gastric cannula, water intake is in excess of normal (200%-250%), but the animal pauses after a relatively short time (Adolph, 1939; Adolph, Barker, & Hoy, 1954; Bellows, 1939; Towbin, 1949). Sham drinking is then resumed after a period of up to 15 min, and the pattern of temporary cessation of drinking is repeated. This indicates that in the dog, oropharyngeal stimulation is not sufficient to provide normal satiety, although it is sufficient after a greater than normal intake to temporarily stop drinking. A similar pattern of drinking cessation after sham drinking in excess of normal (250%-900%) has been reported in the sheep, another species that consumes water rapidly when rehydrating (Bott, Denton, & Weller, 1965). Therefore, in the dog and sheep, which drink rapidly so that the termination of drinking may normally occur before substantial absorption of water, oropharyngeal factors may be relatively more important in terminating drinking than in the monkey.

The rat, which is a relatively slow drinker, also shows sham drinking (Blass & Hall, 1976; Blass, Jobaris, & Hall, 1976). It should be noted, however, that because of its small size, the rat is not as suitable as the dog, sheep, or monkey for this type of experiment. We have observed that the standard-size cannula readily blocks with mucus from the stomach. Any blockage confounds the results since some trapped water may be absorbed. In individual rats in which the cannula appeared to be draining freely and on inspection was found to be clear, drinking was continuous over 3 hr. During this period these animals consumed more than nine times their normal intake, with no indication that oral factors alone were sufficient to terminate drinking (Rolls, Rowe, Gibbs, Rolls, & Maddison, Note 1). Thus we conclude that sham drinking in the rat is similar to that in the monkey. A further discussion of comparative aspects of drinking may be found in Rolls, Wood, and Rolls (1980).

When water was being drained through the gastric or duodenal cannula, the monkey still drank water from the drinking tube in a vigorous and prolonged fashion. It is not necessary for the water to reach the intestine or to be absorbed for the drinking to occur. Thus intestinal stimulation by water, or removal of fluid deficits, is not essential for water to provide an incentive to drink. Rather, the monkey's drinking is guided and maintained by oropharyngeal sensations, such as the taste of water, and in this respect the sensations provide the incentive (or reward) for drinking. This situation presumably arises, whether as a result of evolution or learning, because oropharyngeal stimulation produced by the consumption of water normally results in the reversal of body fluid deficits in the thirsty monkey. This must be true for other species in which, as noted above, sham drinking also occurs. It is also consistent with the observations that prolonged training is necessary for rats to learn to regulate their water intake in the absence of oropharyngeal stimulation (Epstein, 1960; Nicolaids & Rowland, 1974).

Although oropharyngeal factors alone are not sufficient to produce termination of drinking, Experiment 3 shows that post-gastric stimulation with water is sufficient to reduce or terminate sham drinking. Gastric distension during the duodenal infusion procedure is not significant, so it may be concluded that distension signals are not essential for drinking termination. Nevertheless, we have repeatedly observed
that draining the stomach contents soon after normal drinking has ceased leads to an immediate recurrence of drinking in the monkey. This suggests that during and for a short time after the period when drinking terminates in the monkey, gastric distension, together with passage of water through the intestine, may contribute to the inhibition of drinking.

The present experimental procedure differs from that previously employed to show inhibition of sham drinking by water loads. In the present experiments, oropharyngeal and gastric sensations accompanying gastric sham drinking occurred simultaneously with duodenal sensations caused by the continuous infusion of water, as would normally occur during drinking. This, together with the dose-dependent suppression of drinking by infusion volumes that fell within the normal range of intake and the lack of behavioral side effects of the infusions, suggests that the flow of water from the stomach to the duodenum contributes to satiety in the monkey.

The reduction of drinking that is produced by water in the duodenum could be explained by postulating the action of receptors within the intestine or of receptors in the hepatic-portal circulation receiving water by absorption from the intestine, or by plasma dilution following absorption. The latency with which drinking terminated during duodenal infusions (between 10 and 20 min) makes it unlikely that termination was due only to stimulation of receptors previously demonstrated in the duodenum (Sharma, 1967) unless a considerable length of the intestine is required. The involvement of hormonal or hepatic-portal mechanisms is a possibility. Hepatic-portal osmoreceptors have been demonstrated electrophysiologically in the rat (Adachi, Nijima, & Jacobs, 1976), and there is evidence that portal infusions of water increase the osmotic threshold to thirst in the dog (Bellows, 1973). The termination of drinking may depend to some extent on the ability of the animal to sense changes in systemic fluid balance due to absorption. We are currently investigating the relation between the termination of normal and sham drinking and changes in systemic plasma composition in the rhesus monkey. Preliminary results indicate that in the monkey, systemic changes alone are not sufficient to account for the normal pattern of drinking because intravenous infusions of water are usually less effective in terminating intake compared with water loads ingested orally or given by duodenal infusions. Further investigation of how presystemic factors activated by the flow of water in the duodenum may contribute as one of a number of factors to the termination of drinking in the primate, as suggested by the present study and the preliminary results noted above, is now in progress.

Reference Note


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Received March 31, 1979