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Afferent Control of Pressor Responses to Feeding in Young Rats

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SHAIR, H. N. AND M. A. HOFER. *Afferent control of pressor responses to feeding in young rats.* PHYSIOL BEHAV **53**(3) 565–576, 1993.—A series of experiments investigated the eliciting stimuli and physiological systems involved in triggering the blood pressure (BP) response to feeding in the young rat. The studies included 1) tongue cannula (TC) infusions of milk to nipple-attached and unattached pups; 2) TC infusions of saline, sucrose, and water to nipple-attached pups; 3) maternal milk ejections to 3-week-old pups that rarely have stretch responses; 4) gastric cannula infusions of milk to nipple-attached pups; and 5) maternal milk ejections and TC infusions to pups whose mouths were topically anesthetized. The results implicate the contact of fluid with oral and/or lingual surfaces as the critical stimulus primarily responsible for the BP rise. Although nipple attachment appears to be an important precondition for the typical pressor response, the cooccurrence of a behavioral stretch response is not essential.

Blood pressure Milk ejection Tongue cannula Feeding Suckling Development Stretch response

INCREASES in blood pressure during feeding and drinking have been described in a number of species, at a variety of ages (1,2,6,8,16). In our laboratory, we have investigated this phenomenon in 2-week-old rats both in an outbred Wistar strain (28) and in the inbred Kyoto spontaneously hypertensive rats (23,26). In both studies, when young rats receive a milk letdown from their dams, they respond with the vigorous extension of their bodies known as the stretch response and a rapid increase in blood pressure (BP) of 30–50% over baseline, which lasts an average of 8–9 s. The size of the increase depends both on the deprivational state and the genetic heritage of the animal. No other BP rise during normal behavior of the pups is of the same magnitude.

Whether the transient BP increase to feeding has any function in immediate nutrient processing is still speculative. It is hard to imagine that a response present in so many species is without purpose. Independent of immediate function, there are data from our laboratories that suggest a link between the frequency of food delivery prior to weaning in rat pups and later BP in adults (21,22). Thus, studies of this physiological and behavioral response may provide insight into how mother/infant interactions can alter and direct the course of ontogeny.

In this paper, we consider the possible sources for the afferent signal that elicits the BP increase. The possible external triggers include

1. some cue from the dam that is not intrinsic to the milk; e.g., changes in the olfactory, thermal, or tactile cues from the dam coincident with milk delivery;
2. the transfer of any fluid from the dam; and
3. some specific factor in the dam's milk.

The possible internal sources of the signal include

1. feedback from the stretch response;
2. the presence of milk in the stomach and/or intestines; and
3. the passage of milk through the mouth and/or throat.

This final possibility could work through olfactory cues, taste, and/or orosensory cues, or feedback from sucking and swallowing. In the experiments reported here, we have eliminated many of these possible pathways, leaving contact of fluid with orosensory nerve fields as the most likely candidate.

GENERAL METHOD

Rats of the Wistar strain (*Rattus norvegicus*: stock from Hilltop Lab Animals, Inc.) were bred, born, and raised in our laboratory. Dams were housed individually with their litter in clear polycarbonate cages (46 × 24 × 20 cm). Hardwood shavings were supplied as bedding. The cages were kept in a colony room maintained at 25 to 27°C, 50% humidity, and on a reversed 12:12 light:dark cycle. Food and water were available to the animals ad lib. Litters were culled to eight to nine pups 2–3 days after birth and were not further disturbed until the procedures detailed below were begun.

Two to 3 days before the test procedures, pups from each litter to be used were implanted with silver wire heart rate (HR) electrodes using Metofane anesthesia as described elsewhere (10).

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Body weights were taken, and the pups were returned to their home cage and dam. The age, sex, and numbers of pups will be described in the Method section for each individual experiment. No differences in responses between male and female pups were ever noted. On the day prior to testing, mothers were removed from the litters, resting heart rates were recorded for 1 min, rectal temperatures and body weights were taken. Pups that were abnormal on any of these measures were excluded from further study. Then the pups from each litter underwent surgery of various types as described below.

Surgery

The procedure for implanting cannulae in the common carotid in the 2-week-old rat pups has been described in great detail recently (27). Very briefly, pups were anesthetized with inhaled Metofane and the left common carotid artery was exposed and ligated just caudal to its bifurcation. A cannula made of 2–3 cm of Silastic tubing (#602-105, Dow Corning) with a 10 cm length of PE50 (Clay Adams) tubing attached, was passed subcutaneously from the back of the animal forward under the angle of the jaw to the anterior neck. The cannula was filled with heparin (1,000 USP units/ml, Elkins-Sinn, Inc.), and the distal end plugged with a small Amphenol connector. The artery was clamped and a small hole made between clamp and ligature. The Silastic end of the cannula was inserted approximately 5 mm into the artery. The Silastic cannula was anchored with ligatures and the wound closed. The joint between the Silastic and the PE50 tubing was secured at the point of exit from the skin with collodion and a ligature.

In Experiments 1, 2, and 5, all test pups were also implanted with posterior tongue cannulae at the same time [for details see (24)]. Following the experiments, autopsies were performed on all animals, and only those animals whose tongue cannulas were found to be in the proper position by the intermolar eminence of the tongue were used in data analysis.

In Experiment 4, pups were implanted with gastric cannulae at the same time as their carotid cannulae. Gastric cannulae were fashioned from polyethylene tubing (PE10, Clay Adams) by techniques modified from Messer et al. (19) and described previously (9). Briefly, the PE tubing was shaped by heating it in hot water so that it could be curved to fit the form of the body of the young pup. The tubing passed through the ventral surface of the stomach, through a purse string suture, curved laterally and dorsally underneath the skin around the back to the spine. At this point it curved abruptly in a caudal direction after surfacing in the middle of the back, and lay along the fur toward the lower back. Approximately 5 to 6 cm was exposed outside the animals. Sutures distal to the beads in the wall of the polyethylene tubing at the stomach wall and the abdominal wall as well as the ligature and a patch of collodion at the exit point of the skin prevented displacement of the cannula.

In all experiments, on the day of testing, the dams were implanted with jugular cannulae using methods modified from previous research (25). The dam was anesthetized with urethane (2 mg/kg) and the left external jugular vein exposed. A cannula made of Silastic tubing (#602-105) was inserted into the jugular vein and secured using ligatures. The skin of the dam was closed using continuous suture, and a bandage was placed over the wound. The cannula was filled with 10% heparin diluted in 5% dextrose in water.

Procedure

Operated animals were kept separated overnight from their mother. They were housed in small cages (10 × 15 × 15 cm) containing home-cage bedding and kept on a regulated heating pad (36°C). Each operated animal was housed with an anesthetized littermate (urethane, 2 mg/kg). This companion gave the operated pup social contact without the danger of having the various cannulae destroyed. The remaining nonoperated pups, at least three, were returned to the mother. In Experiment 3 the procedure for housing and testing were different as will be described later.

On the next morning, dams were anesthetized with urethane and a jugular cannula implanted as described above. Each test pup, in its turn, was connected via leads to the polygraph for recording of physiological variables (Model 7, Grass Instrument Co.). The BP was recorded using the Statham P23 pressure transducer; HR and respiration were measured through the EKG wires from the animal's back. Then the pup was placed in the home cage with the anesthetized dam. Note that in Experiment 1 some recording was done in the home cage without the dam present. This will be described below. A second nonimplanted pup was placed in with the test pup and its reactions to the mother were used for behavioral verification of milk letdown.

If the pup was not attached to a nipple after about 5 min, it was nudged towards the mother's nipple and was encouraged to attach. After recording BP and HR for about 5–10 min the pup would receive either milk ejections elicited from the mother via infusion of oxytocin through a jugular cannula (Pitocin, 0.012 units/kg), or small infusions of fluid through the pup's tongue cannula. The details are described below and in the specific experiments. The infusions and milk ejections were spaced at least 3 min apart and the order in which the various infusions and milk ejections were done was counterbalanced. Some data from milk ejection elicitations and tongue cannula infusions are not reported due to problems that occurred, e.g., the BP cannula clogged briefly, the control littermate showed no behavioral response after oxytocin infusion to the dam, etc.

To our knowledge, no one has accurately measured the amount of milk available to a suckling pup at a given teat. Estimates have been made by weighing litters of animals before and after milk ejections, both during normal nursing and with the anesthetized dam/oxytocin preparation. The average amounts ingested by any pup appear to depend on the recency with which the dam has nursed [e.g., (5)], the deprivation state of the pup [e.g., (18)], the order of the milk ejection within a series [e.g. (4,17)], and the size of the oxytocin infusion [e.g., (14)]. From these various sources and our own pilot work, the estimated amount of milk ingested by a pup per milk ejection ranges from as little as 0.01 cc or less to as high as 0.3 cc.

The duration of the milk ejection is also uncertain. It can be estimated both by the rise in intramammary pressure [e.g., (17,33)] and by the stretch and BP responses of the pups (28). There is considerable variation in the durations seen, ranging from 2 to 10 s. Further, no one knows if milk flow is necessarily coincident with these indirect measures. On one hand, Hall and Williams (7) report that attached pups will maintain stretches for 1–2 min as long as milk flow (rate not specified) continues through a tongue cannula. On the other hand, it has been reported that stretch responses can be elicited in nipple-attached pups without any fluid transfer (3,15).

Due to the lack of precise information concerning the natural milk ejection, we determined the size and durations of the tongue cannula infusions on pragmatic grounds (within the estimated ranges given above). The amounts given (generally from 0.03 to 0.07 cc), and the duration of infusions (generally from 2 to 8 s) would reliably elicit BP and stretch responses in nipple-attached, 24-h maternally deprived rat pups. Within each experiment, unless otherwise stated, the amounts and durations

were matched across experimental groups. Occasionally, in experimental groups that showed no response to infusions, the range of amounts and times were increased in an attempt to elicit a response (described in the individual experimental Method section).

Behavioral Observations

In all experiments the behavior of each test pup was observed carefully, especially in the period during which milk ejections or tongue cannula infusions were performed. Notes on the pup's behavior and the behavior of a nipple-attached littermate were written on the polygraph record in relation to those physiologic events recorded. When pups were recorded while not nipple-attached, frequent behaviors included mouthing, head movements, locomotion, digging in the shavings, rising up against the side of the test cage, nosing the mother's ventrum, self-grooming behavior, etc. During attachment, the behaviors noted included treadling against the mother's ventrum (i.e., pushing it with an alternating motion with forelimbs), nipple switching, and the stretch response. The stretch response has been shown to be associated with milk intake (3) and is characterized by rigid body extension of the pups with forelimbs pressed against the mother's ventrum and the hindlimbs pressed against the cage floor. The behavioral observations confirmed that no non-feeding behaviors were coincident with BP increases of similar magnitude as those seen during ingestion (23,28).

In the first experiment we merely noted the presence or absence of a stretch response in relation to tongue cannula infusions and induced milk ejections. The time of onset and offset of the stretch response was recorded as well. In all later experiments each stretch response was rated as well as timed. The ratings were: 0 = no visible change in behavior to milk ejection or tongue cannula infusion; 1 = a slight tensing of the body without extension, possibly some treadling behavior of the forepaws; 2 = body extension with the limbs pressed against the ventrum; and 3 = a more rigid body extension with fully arched back held without movement for at least 2 s.

Polygraph Scoring

The methods for polygraph scoring have been previously described (28). Briefly, BP and HR were sampled once each second for 5 s prior to the start of a milk ejection or tongue cannula infusion. The prevalue for each milk ejection response was the median of those five samples. The peak value was the highest BP and HR readings that occurred before the tracings returned to at least 5 consecutive seconds of prevalue levels. The change value was the difference between pre and peak.

The start of the response was defined differently depending up on whether a milk ejection or tongue cannula infusion was performed. For the milk ejection, the beginning was marked on the polygraph tracing using behavioral notes and by locating the first evidence of activity shown by muscle movement artifact in the EKG channel. For tongue cannula infusions we were aware to the precise fraction of a second when the infusion started and when it stopped, so those times were noted on the polygraph tracing.

A control pre-to-peak value was calculated for comparison. Two minutes prior to each tongue cannula infusion, an artificial start point was selected on the polygraph tracing. That start value was used to collect data of pre, peak, and change as if this were a true milk ejection. The mean change value in this situation was 3.6 ± 0.7 mmHg. Change values within the experiments were compared to this control change figure. All statistical comparisons with the control value are evaluated as one-tailed tests, because there is no reason to expect decreases in BP to feeding. BP values are reported in millimeters of mercury (mmHg); HR in beats per minute (BPM). In this paper we will concentrate on BP responses; the HR changes will be reported only where they are of special interest.

Each BP reading was taken from the polygraph tracing at the midpoint of the pulse wave. This may overestimate true mean arterial pressure due to a difference in the amount of time spent in systole among different animals, but this approximation is the best measure in these young animals with small diameter cannulae. No animal was used whose pulse pressure was smaller than 5 mmHg. (We have informally compared mean pressures of animals with large and small pulse pressures and found that the mean arterial pressure does not vary as long as the pulse pressure is not smaller than 5 mmHg with the Grass Driver Amplifier set at its highest frequency response. Data were often then recorded at lower frequency settings which reduced pulse pressure size and facilitated scoring of the mean value.)

Data Analysis

Statistical comparisons of two groups were made by *t*-test, either independent or dependent as appropriate. Analysis of variance (with repeated measures as appropriate) were used when more than two groups were compared. Dependent or independent *t*-tests were performed for post hoc analysis using Bonferroni adjusted probabilities. Pearson product-moment correlations were performed to compare the effects of changes of one variable upon another.

Due to the occasional unequal number of milk ejection or tongue cannula infusion trials among pups within and between test groups, analyses were generally performed on the mean value for each pup under each condition. For the trials used as data in each experiment, correlations were run between test order and the size of BP change. No such correlations reached significance. Thus, in these experiments, which were not designed specifically to test these phenomena, there was no evidence for habituation or sensitization.

EXPERIMENT 1

Tongue cannula infusions of milk to nipple-attached and unattached rat pups were used to investigate whether such attachment was necessary for elicitation of the full BP response to feeding. Blood pressure rises to feeding and drinking in various animals not being nursed have been found by others (13,16), including Myers and Scalzo (23), in two inbred strains of young rats. However, the large magnitude of the BP response while nipple attached and its coincidence with the stretch response suggested that the question should be studied.

Any differences in the patterns of BP change between nipple-attached and unattached rat pups will be informative as to necessary stimuli for that change. For example, we know that rat pups do not stretch to tongue cannula infusions unless attached to the teat. Thus, if there is a significant and equal BP rise under the two conditions, then we will have ruled out the stretch response as a necessary component to the BP rise. Also olfactory, tactile and thermal properties of the dam, as well as some specific factor in the rat's milk, will be eliminated as signals for the BP response in our strain, as suggested for SHR and WKY rats (23).

Method

Nine animals (four male, five female) from eight different litters were tested in response to infusions of milk both on and off the mother's ventrum. The pups were 11 to 14 days of age

at the time of testing. Age within this range had no influence on any results. Pups were implanted and tested as described in the General Method section with these specific alterations. Each pup was tested in response to tongue cannula infusions of 0.07 ml of Carnation evaporated milk diluted with one-third water, both while attached to the mother's teat and while quietly resting in the home cage not in the presence of the mother. Each pup received at least three infusions in both conditions. Mean values for the three or more infusions were taken on each pup, and these mean values were used for further analyses. Both the size (0.07 ml) and the duration (3 to 6 s) of the infusion were based on pilot work aimed at finding the parameters of infusion that reliably reproduced normal milk ejection responses in nursing pups. We used a greater variety in rates of infusion of the 0.07 ml in the unattached condition as we attempted to find an infusion duration which would reliably elicit a BP rise.

Results

Although there were occasional BP increases to tongue cannula infusions of milk while off the teat, nipple-attached pups had significantly more consistent increases and of larger magnitude. The range of pre-to-peak change was from 5 to 28 mmHg when attached and only 0 to 11 mmHg when unattached. While suckling the teat, the form of the BP response (see Fig. 1A for an example) approximated that seen during normal milk ejections much more closely (28): rapid increase, high peak, and abrupt return to baseline within 8–9 s. When off the teat, there was often little increase in BP (see Fig. 1B for an example). These differing results were probably not due to differences in baseline (pre) BP. Although the low baseline of the unattached is associated with little response (see Table 1), there was no evidence for a positive correlation between pre BP and the size of the rise to milk infusion in either condition [$r(7) = 0.164$, unattached; $r(7) = -0.612$, attached; NS, for each]. And the high baseline of the attached seems less likely to be an explanatory factor because its correlation (noted above) approaches significance as a negative factor. When an increase did occur in unattached pups, there was usually a more gradual rise and fall (see Fig. 1C for an example). When attached, most cases in which there was no high BP peak were accompanied by some unusual events which might interrupt the blood pressure rise, e.g., large bradycardias or respiratory difficulty (see Fig. 1D for an example). Summary statistics characterizing these results can be seen in Table 1.

Behaviorally, the two conditions were quite distinct. No pup infused off the nipple showed any behavior which even approximated a stretch response (Table 1). Mouthing and other movements of the head were their most common responses. And when these pups engaged in very vigorous activities like burrowing, they did not have BP increases comparable to those in nipple-attached pups during the stretch response. Swallowing movements could be observed only very rarely. It was our impression that these evident swallows were often accompanied by bradycardias of the kind seen in Fig. 1C. This impression remains to be tested. In contrast, when attached to the teat, the usual behavioral response to milk infusion was a pattern consisting of a brief treadle, a stretch, and a nipple switch. In those few cases in which no stretch response occurred, the BP increase tended to be low, ranging from 5 to 16 mmHg. However, that maximum increase of 16 mmHg was greater than many of those occurring during stretches.

Discussion

These results suggest that in Wistar rats nipple attachment is an important precondition for the regular occurrence of a BP increase to milk infusion that is comparable in size and form to the milk ejection response. BP responses to milk were observed in nonattached pups, but these responses were less consistent and had more gradual rise and fall times than the typical attached pattern. Thus, attachment to a teat may not be absolutely necessary for all aspects of the BP response. It is possible that nipple attachment influences the likelihood of occurrence of some third factor (e.g., proper state of the pup, optimal pattern of motor behaviors, rapidity of ingestion, area of the mouth stimulated by the milk) which, in turn, influences the BP response.

The most obvious difference between the pups when attached to the teat and unattached was the lack of a stretch response in the latter condition. Yet, as described just above, some pups off the teat did have BP increases. Similarly, in nipple-attached pups, good size BP rises did occur (rarely) without any stretching behavior. As with attachment itself, the stretch response seems unlikely to be a necessary component of all aspects of the BP rise. This result confirms in Wistar pups what was found by Myers and Scalzo (23) in inbred strains (SHR and WKY). Their pups tested in isolation showed BP increases of considerable magnitude with no trace of a stretch.

The fact that pups do show a BP rise when nipple attached but receiving tongue cannula infusions of bovine milk, demonstrates that there is no special ingredient in the dam's milk necessary for a response to occur. Also, it demonstrates that changes in the temperature, configuration, or olfactory properties of the dam or teat are not essential for the response. However, the magnitude of the response may be influenced by one or more of these factors because the average response of 14 mmHg was smaller than the response to maternal milk ejections seen in our previous work (28). In the present experiment, five pups were also measured during oxytocin-induced milk ejections from the dam. The average BP rise in this reduced number of pups was 26.0 mmHg, significantly higher than the rise to tongue cannula infusions [dependent $t(4) = 3.723$, $p < 0.05$]. Other explanations for the reduced response to tongue infusion exist. For example, there are changes at the teat which precede each milk ejection (15). The 11–14-day-old pups tested here have experienced such changes and accompanying milk ejections hundreds of times during their short lives. These stimuli, which are absent during tongue cannula infusions, may begin to arouse the pup and optimize the feeding response. The lack of this positive alerting signal may also explain the high frequency of bradycardia responses seen to tongue cannula infusions.

EXPERIMENT 2

In Experiment 1, attachment was found to be an important variable in determining the size and rapidity of the BP response to milk delivery. However, the contribution of other factors remained to be tested further. In this experiment, we tested the possibility that the composition of the fluid infused through the tongue cannula could influence the size of the BP response. The fluids used were commercial milk, water, and several concentrations of sucrose and saline. For comparison, milk ejections were induced from each anesthetized dam as well.

Method

Twelve pups (six male, six female) from six different litters underwent a series of tongue cannula infusions. Each pup was tested attached to the teat of its anesthetized dam. Each pup received at least one infusion of 0.03 ml of each of the following solutions: milk (commercial half and half), sucrose in dilutions of 1, 5, and 20%, and tap water. Saline infusions in dilutions of 0.9% and 5% were done with some pups. Each pup also received

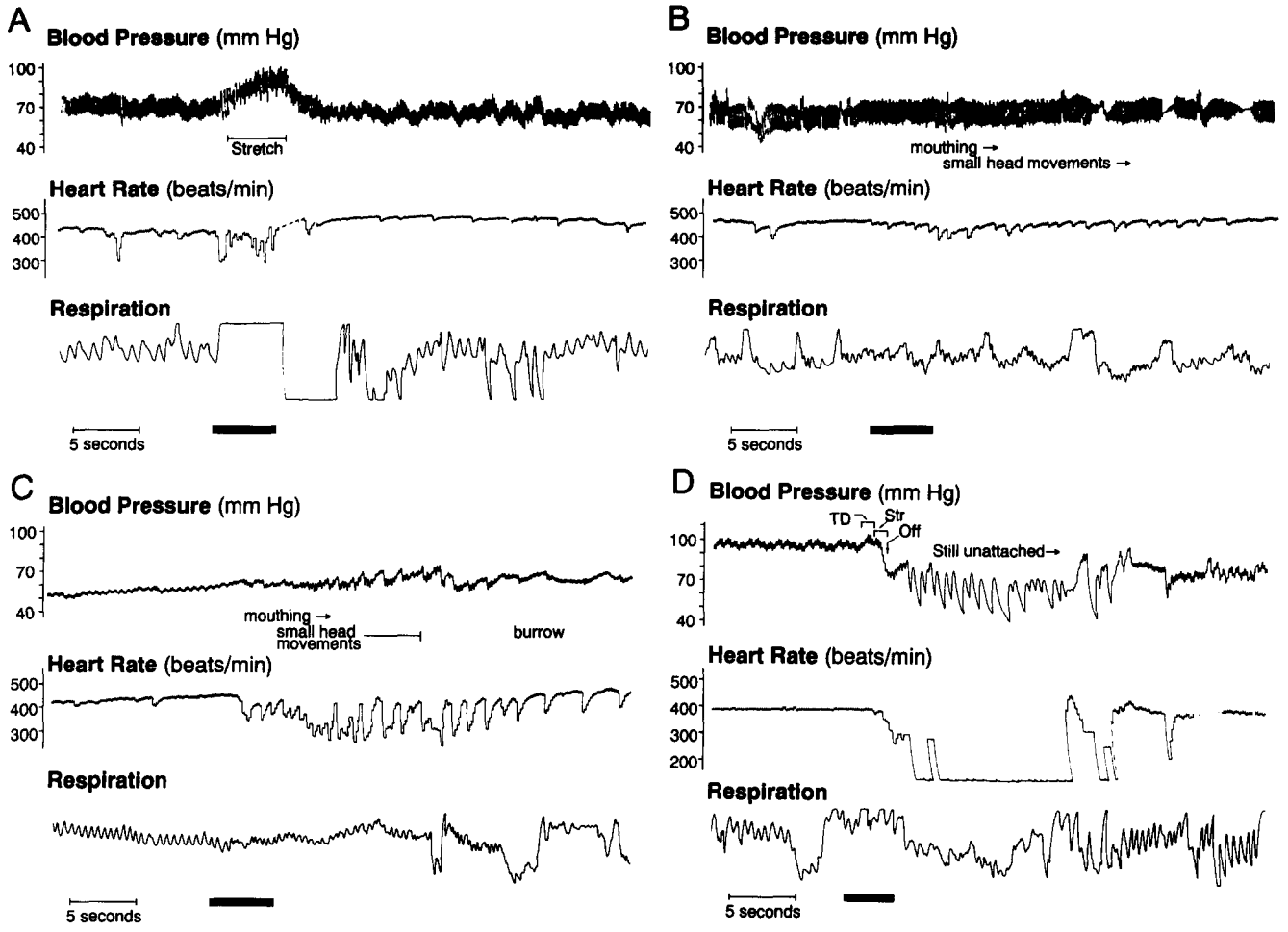


FIG. 1. Examples of heart rate (HR) and blood pressure (BP) recordings before, during, and after infusion of milk through a posterior tongue cannula (TC). Pups were either quietly attached to the teat of an anesthetized dam (A,D) or lying quietly next to several anesthetized littermates (B,C) when the infusion was started. The time and amount of infusion and behavioral responses observed are noted on each tracing. Str = stretch. TD = treadle.

at least one milk ejection induced by oxytocin infusion to the anesthetized dam. If pups received more than one infusion of any test solution, a mean value was taken. The order of presentation was counterbalanced. Although a small infusion size, 0.03 cc., was chosen to reduce the possibility that gastric distention might influence the results over the course of the experiment, this amount was adequate to induce a BP rise and stretch response in pilot tests. The time of the infusion ranged from 2 to 5 s, with rates matched across fluid conditions.

Animals' polygraph tracings were analyzed and behavior noted as described in the General Method section. In this experiment, rating of the stretch response was begun. This rating was continued in all further experiments. The control pre-to-peak values from Experiment 1 were used in this experiment as well. Sampling of records in this experiment showed no difference in control pre-to-peak values, justifying the use of these data from Experiment 1. Thus the control pre-to-peak significance t-tests were independent in this and all further experiments.

Results

The saline infusion condition had to be dropped from the experiment. Pups infused with normal or 5% saline very frequently exhibited massive bradycardias, interruptions of respirations, and prolonged periods of gasping up to 15–20 s similar to the result shown in Fig. 1D. Although all pups quickly returned to normal, these initial responses interrupted their fluid intake and any BP rise. Once the high frequency of this response was determined (8 of 11 trials), further infusions of saline were discontinued.

As shown in Fig. 2A, all other fluids tested caused a BP rise when the pups were attached to the teat. These BP rises were comparable to those received when milk ejections were induced [one-way ANOVA with repeated measures, $F(5, 55) = 1.75$, NS]. However, when the tongue cannula infusions alone were considered, there was a significant effect of the type of fluid infused, $F(4, 44) = 3.420$, $p < 0.05$, and were significantly greater than the control pre-to-peak values described in the General Method section (independent t-tests with $p = 0.01$ or better, one tailed). Post hoc analyses were not able to pinpoint which fluid or fluids were responsible for the significant ANOVA result.

The rated intensity of the stretch response showed similar differences in magnitude, $F(4, 44) = 9.972$, $p < 0.01$, with 5% sucrose producing a response significantly greater than all other fluids except 20% sucrose (see Fig. 2B). In addition, the size of the BP rise and the intensity of stretch were significantly positively correlated across all feeding conditions [$r(70) = 0.564$, p

< 0.001]. Even within each fluid group, the correlations were quite positive (ranging from 0.31 to 0.88) although only MEs, H_2O, and 20% sucrose reached 0.05 or greater significance, probably due to the small n of 12.

The number of pups in each group showing bradycardias was examined to see if differential responses would help explain the significant effects on BP change. The number of bradycardias exhibited did differentiate between the various groups, with the H_2O group having bradycardias in 8 out of 12 animals, and the 20% sucrose animals having bradycardias in only 2 out of 12 pups [Cochran's one-way ANOVA with repeated measures for dichotomous data, $F(5) = 12.2$, $p < 0.05$]. However, these bradycardias were almost always of small magnitude, unaccompanied by signs of distress. Also, the group with the second highest number of bradycardias was the 5% sucrose infusion with 6 out of 12 pups showing bradycardias. Thus bradycardias by themselves are unlikely to explain the differential BP rise seen with the various fluids.

Discussion

These results demonstrate that fluid infusion to the mouth of a 2-week-old rat pup when it is attached to the teat is a sufficient stimulus for producing both a BP rise and a stretch response. It is not necessary to hypothesize some special ingredient in the milk of the rat dam which produces this BP rise. The properties of the fluid that act to elicit the most intense response remain to be determined. For example, it is possible that rat milk is the optimal signal. The experiment of milking mother rats, then delivering that milk through a tongue cannula, is yet to be done. The differential effects of the fluid types found in this experiment suggest that it may be a worthwhile experiment.

Comparison of the present results with the findings on the elicitation of the fetal stretch response (30,31) may be instructive. Under very different conditions from our test, fetuses on postconception days 20 and 21 show a reliable stretch response to the first oral infusion of milk. A variety of other fluids including saline and sucrose do not elicit stretching behavior. Taking a leap of faith across the different studies and test conditions, it is possible that our nipple-attached older pups stretch to a variety of fluids due to a generalization of their experience with milk during normal mother–infant interactions.

The perinatal cardiac response to fluid infusion has an intriguing similarity with our results. As with our older animals, saline (and lemon) infusions produce bradycardia responses in the very young pups (32). But there are differences between their results and ours. In perinatal pups, the magnitude of bradycardia is less intense than in our pups. Also, the effect diminishes over the first few postnatal days. We have no way to know as yet whether the reappearance of bradycardia to saline at postnatal days 11–13 is due to some developmental process or to differences in test conditions.

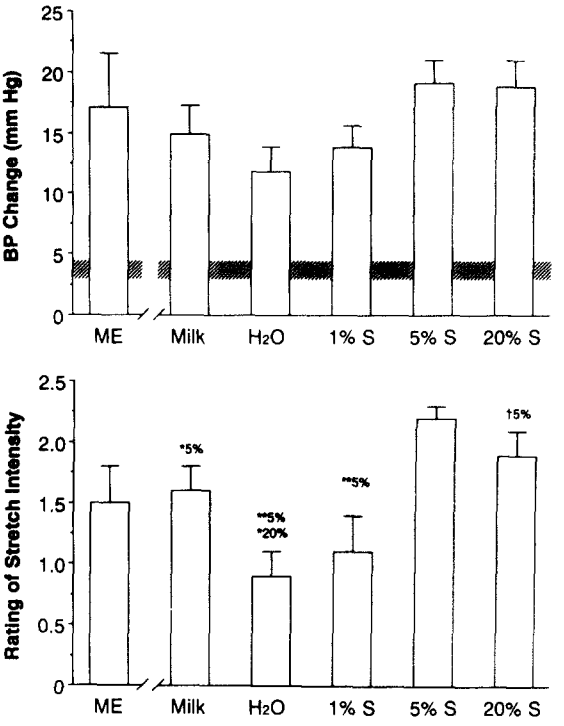


FIG. 2. Average (±SEM) responses of 12 pups to milk ejections (ME) from an anesthetized dam (on the left) and to TC infusions of various fluids while nipple attached. Each pup received at least one stimulation in each condition. If a pup received more than one stimulation in any condition, a mean response was used for analysis. Top, The increase in BP from baseline to the peak response value. The shaded area represents the mean (±SEM) control pre-to-peak values. See the Method section for details on scoring. Bottom, The rating of stretch response intensity to the same stimuli. Dependent t-tests with Bonferroni-adjusted probabilities were used for post hoc comparisons between conditions. Any significant differences are listed over each bar, such that *20% means that the bar differs from the infusion of 20% sucrose at $p < 0.05$. **$p < 0.01$. †$p < 0.10$.

TABLE 1

BLOOD PRESSURE AND STRETCH RESPONSES TO TONGUE CANNULA INFUSION OF MILK IN NIPPLE-ATTACHED AND UNATTACHED PUPS

	Mean Baseline BP (mmHg)	Mean BP Change (mmHg)	Mean Time to Peak Value (s)	Mean Time to Return to Baseline (s)	Percent Pups Showing Stretch Response
Attached	71.6	14.0*	4.5	21	95
Unattached	64.9	6.4†	8.9	42	0
Dependent t-test	2.850	2.470	2.622	1.579	—
	$df = 8$	$df = 8$	$df = 7$	$df = 7$	
	$p < 0.05$	$p < 0.05$	$p < 0.05$	NS	

See the General Method section for details concerning how these values were obtained.

* Significantly greater than control pre to peak value (dependent t-test = 3.813, $df = 8$, $p < 0.01$, one tailed).

† Significantly greater than control pre to peak value (dependent t-test = 1.848, $df = 8$, $p < 0.05$, one tailed).

One cautionary note must be made in this experiment. The magnitude of BP rise to the oxytocin-induced milk ejections was quite low. Perhaps the tongue cannula implant may interfere with that response in some manner. Yet, in the first experiment, that subset of five cannula-implanted pups that received milk ejections had very robust responses to milk from the dam. Thus, it is clear that tongue cannulae do not always cause interference.

EXPERIMENT 3

In the first two experiments and in a previous study (28), a consistent BP increase to oral fluid was seen only in those situations that also produced a consistent stretch response. In fact, a significant positive correlation between the magnitude of the BP rise and the stretch response was found (Experiment 2). These findings raised the question of how closely coupled the two responses are and the nature of this coupling. Experiments using hexamethonium, an autonomic ganglionic blocker, demonstrated that pups could show normal stretch responses to milk ejection with no accompanying BP rise (23,26,28). These data ruled out the possibility that the stretch response caused the BP increase by some form of hydraulic feedback and showed dependence of the BP rise on autonomic pathways. However, it was not yet known if it was possible consistently to elicit BP increases to milk ejections without stretch responses. Feedback from the stretch response through the central nervous system (CNS) might be a necessary trigger for the autonomically mediated BP rise.

We used a developmental change in behavior of the rat pups to test whether the BP increase and stretch response are dissociable. Three-week-old rat pups continue to receive milk from their mother through occasional milk ejections. However, at three weeks of age nondeprived rat pups rarely show stretch responses during normal milk let downs. To ascertain whether BP responses also would be diminished in these older pups, we studied both responses in 3 week olds as they received oxytocin-induced milk ejections from their anesthetized dams. These pups were studied again after being separated from their dam overnight to see if there were any deprivation-induced changes in the response to milk ejection comparable to those seen in 2 week olds (28).

Method

Ten pups (four male, six female) from seven litters were tested before a period of deprivation in this experiment. The age at the day of testing ranged from 20 to 22 days. The time of surgery of this experiment differed slightly from the other experiments. In this case, pups underwent surgery 2 days prior to the day of testing. The carotid cannula was tucked back under the skin so that it would not be bitten off by dam or littermate. The pup was then returned to the dam and allowed to undergo normal mother–infant interactions for the following 2 days. All pups used for analysis gained weight during the period following surgery and prior to testing.

On the day of testing the litter was placed in the polygraph room, the dam was anesthetized and implanted with a jugular cannula as described in the General Method section. All pups had a resting heart rate taken. At that point, the BP cannulas were taken out from underneath the skin. This involved lightly anesthetizing the pups with ether, then cutting the silk ligature that held the cannula in place, and gently exposing several centimeters. A drop of collodion was placed over the exit hole again. Pups were returned to their holding cage and allowed to recover for over an hour prior to commencing testing with the anesthetized dam. The procedure on the anesthetized dam was similar to the procedure described before except that most pups received a series of three milk ejections from the dam. One pup received only two milk ejections before its BP cannula malfunctioned. One pup received six as it alternated between responses with a stretch and those without. There were no tongue cannula infusions. Behavior was observed closely in order to rate any stretch responses, as described in the General Method section. Because not all pups showed both types of milk ejection response (with and without stretch), the data was analyzed for individual trials in order to avoid losing considerable data.

One or two younger pups (ages 8–12 days) were kept nipple attached during the test procedure. The stretch responses of these pups were used to verify the fact that a milk letdown had occurred.

Following the tests, the littermates of the pups tested on the first day were separated overnight. During this time of separation overnight they were housed in a group, on a regulated heating pad (36°C). The dam was maintained under urethane anesthesia overnight. On the second day of testing a similar series of milk ejections were elicited from the dams. Only four pups had patent BP cannulae on the second day; thus, the results reported are only from those four animals. Comparisons between littermate mean responses on the first and second day were done on this smaller group.

Results

The 10 nondeprived pups received a total of 32 milk ejections from their anesthetized dams. In 17 cases, the pups showed no observable stretch response. There was a stretch response in 15 cases. Two pups never stretched in response to a milk ejection; one pup stretched at each trial; the other pups exhibited both types of response. The intensity of the stretch responses ranged from very low to medium (rated from 0.5 to 2). There were no stretch responses rated higher than 2.

Three-week-old pups had robust BP increases to feeding comparable to those of 2-week-old pups. The presence or absence of a stretch response did not appear to influence the BP response. In both cases (see Fig. 3 and Table 2) there was a significant BP increase. There was no significant difference in the magnitude of BP increase based on whether the pups stretched or not. Base-

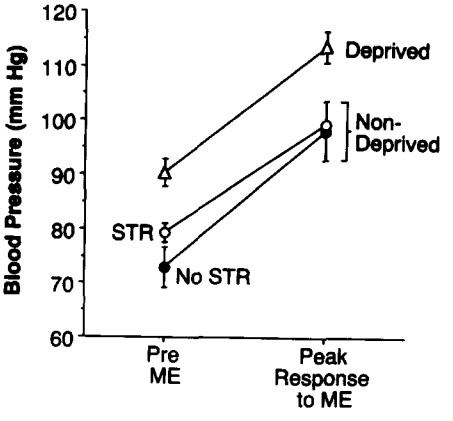


FIG. 3. The average baseline and peak BP response values of 3-week-old pups receiving MEs induced by oxytocin infusions to their anesthetized dams. The values of 10 nondeprived pups are divided into cases in which there was no stretch response (No STR) and cases in which the pups did stretch (STR). Four littermates of these pups were returned to their anesthetized dam following 22 h of maternal deprivation. All deprived pups stretched in response to every milk ejection.

TABLE 2

BLOOD PRESSURE AND NIPPLE SWITCH RESPONSES OF NONDEPRIVED 3-WEEK-OLD PUPS TO OXYTOCIN INDUCED MILK EJECTIONS FROM THEIR ANESTHETIZED DAMS

	Mean Baseline BP (mmHg)	Mean BP Change (mmHg)	Mean Time to Peak Value (s)	Mean Time to Return to Baseline (s)	Percent of Cases With Nipple Switch
No STR $n = 17$	72.9	24.9*	7.1	50	82
STR $n = 15$	79.3	20.0†	5.7	42	93
Independent t-test	1.496	1.030	1.049	0.451	—
$df = 30$	n.s.	n.s.	n.s.	n.s.	

* Significantly greater than control pre to peak value (independent t-test = 4.449, $df = 24$, $p < 0.001$, one tailed).
† Significantly greater than control, pre to peak value (independent t-test = 3.960, $df = 22$, $p < 0.001$, one tailed).

line BP (pre) prior to milk ejections did not differ in the two groups. Somewhat to our surprise, pups were very likely to nipple switch following a milk ejection, even without the activation of the stretch response.

As a further test of the relationship between the stretch response and the BP rise magnitudes, a correlation was done across all 32 cases and showed no effect [$r(30) = -0.044$, NS]. If only the pups that stretched were examined, the correlation was positive although not significant [$r(13) = 0.30$].

Four pups were tested during reunion after a 24-h separation from the dam. In this case, all pups stretched vigorously in response to each milk ejection. The stretch ratings ranged from 1.5 to 3, significantly more intense than prior to deprivation [dependent $t(3) = 29.007$, $p = 0.000$]. Although the baseline BP was higher after separation [dependent $t(3) = 3.685$, $p < 0.05$, see Fig. 3], the BP rise to milk ejection was not significantly greater than that seen in the preseparated pups [dependent $t(3) = -0.113$, NS]. The correlation of BP rise and stretch rating was positive [$r(6) = 0.55$] although not statistically significant.

Discussion

It is clear that in the 3-week-old pup the stretch response is not a necessary part of the BP rise. Milk ejections with no stretch response in this experiment elicited BP rises just as high and just as rapid as those that elicited clearcut stretches. There is no compelling reason to believe that the underlying physiology of BP rise and stretch response differs in the 2-week-old rat. Two-week-old rat pups have been observed to have BP increases without stretches, although the form of the rise is often very different [(23,26), Experiment 1]. BP increases to feeding are common in many species without anything comparable to the stretch response (1,2,6,8,16). It remains possible that in rat pups feedback from muscle tension through the CNS may contribute to the BP rise. This muscle feedback could be from some specific set of muscles including those involved in swallowing, or from general muscle contractions in the body. Although the lack of a stretch response was easy to observe, the test pups may have had a tensing of the body that was not visible to us. However, because the muscular tension that occurs during the pups' rising and locomotion or treadling and nipple switching (see examples, Figs. 1 and 4) does not produce comparable BP increases, this possibility seems unlikely. Thus, stretch response and BP increase do appear to be correlated under most conditions, but the one does not appear to be dependent on the other. Perhaps they are both reflections of some third central process reflecting a more general intensity of responding.

Although not a focus of this particular paper, one additional difference between the 3-week-old pups and the 2-week-old pups should be mentioned. In 2-week-old animals the baseline BP is not affected by a 24-h period of deprivation (29), but the response to a milk ejection is increased (28). In 3-week-old animals, the reverse appears true. The BP response to milk ejection is not increased by a period of deprivation, but the baseline BP level is significantly higher.

EXPERIMENT 4

Other possible sites for the stimulus for BP increase to fluid are the stomach and intestines. Due to the very rapid response time of the BP rise, gastric hormone release seemed an unlikely trigger. However, stimulation along the vagal or sympathetic pathways as fluid reached the gut seemed quite possible.

We addressed this issue by infusing milk directly into the stomach through a gastric cannula while the pups were lying quietly attached to the teat of their anesthetized dams. Control milk ejections were induced from the anesthetized dams to ascertain whether gastric surgery altered BP responses.

Method

Five pups (four male, one female) from five different litters served as subjects for this experiment. Pups ranged in age from 11 to 13 days on the day of testing. In this experiment all pups were implanted with intragastric cannulae on the day prior to testing as described in the General Method section. On the day of testing, each pup was encouraged to attach to the teat of its anesthetized dam and a series of three to five infusions through the intragastric cannula were begun. Because we had no idea of the time course at which fluid from a milk ejection entered the stomach, we gave a wide range of size and rates of fluid intragastrically. The infusions ranged in volume from 0.03 ml to 0.1 ml. The time of infusion ranged from 2 s to 7 s. In a few pilot animals, even larger infusions were given. After these infusions each pup was given at least one milk ejection induced from its anesthetized dam.

Results

Pups receiving intragastric milk infusions showed no behavioral or physiological change. In a typical example (see Fig. 4A) there was no change in BP, HR, or respiratory tracing during or after the time of milk infusion to the gut. The pup was, however, capable of responding to a milk ejection (see Fig. 4B). Both BP and behavior showed the normal pattern of response. Note in this example again that neither treadling nor nipple switching produces a comparable increase in BP.

For the group as a whole, there was no BP increase to these stomach infusions (see Fig. 5). Change values were not signifi-

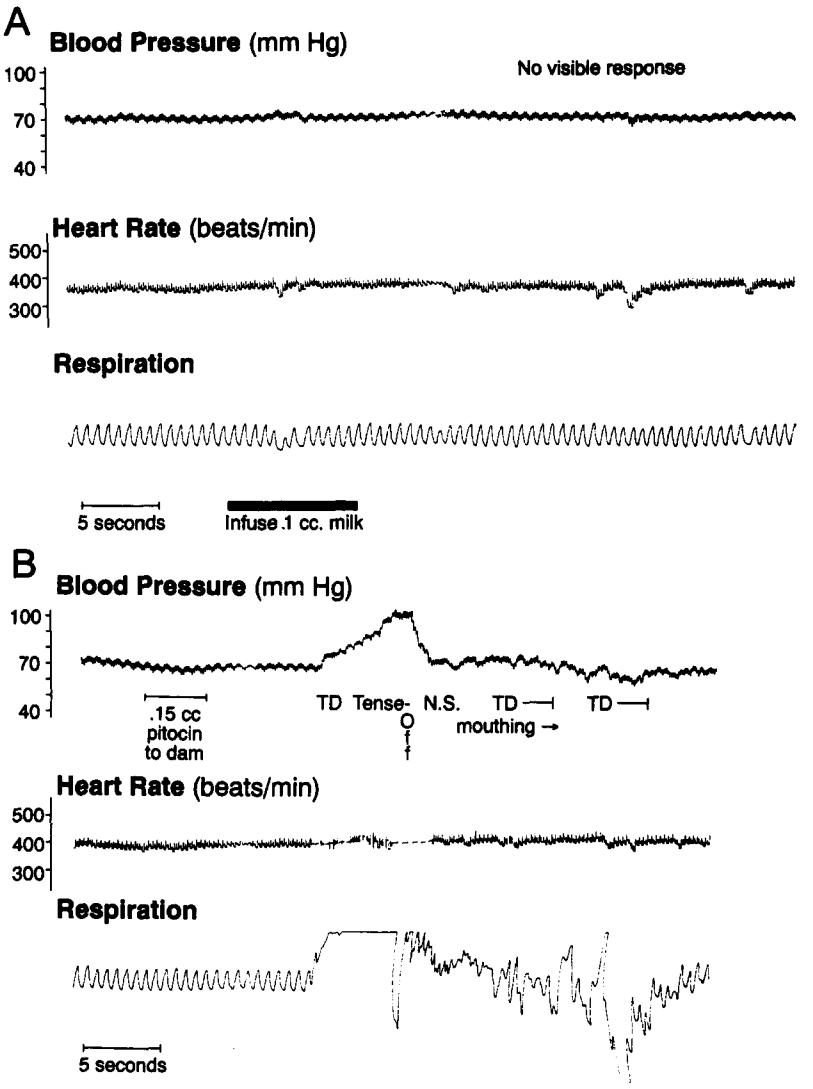


FIG. 4. Two examples of HR and BP recordings from the same 2-week-old pup while attached to the teat of its anesthetized dam. (A) The pup receives an infusion of 0.1 cc of milk through its stomach cannula. (B) Its response to an oxytocin-induced milk ejection is shown. TD = treadle; N.S. = nipple switch.

cantly higher than the control pre-to-peak scoring value [independent $t(27) = 0.389$, NS]. Yet, there was a large increase averaging 28 mmHg to the oxytocin-induced milk ejections. This was significantly higher than the intragastric milk change [dependent $t(4) = 6.321$, $p < 0.01$]. No pups receiving stomach infusion showed a stretch response. Each pup did stretch when receiving a milk ejection from the anesthetized dam.

Discussion

The lack of behavioral and BP response to the intragastric milk infusion demonstrates that neither gut nor stomach receptors are sufficient cues for the BP increase in the natural situation. This experiment has not ruled out the possibility that some sort of stomach or gut receptor signals are necessary in combination with oral or esophageal cues.

EXPERIMENT 5

Having eliminated the gut as a sufficient trigger for the BP response, we turned to the mouth and throat. If the trigger is localized in the oral cavity, one might expect that the gustatory nerves would be involved. The results of Experiment 2 showing differential response to various fluids in the mouth would support this hypothesis. However, another set of nerves innervates the oral cavity as well: the trigeminal orosensory nerves (11,12). Both sets of nerves have close anatomical connections with the nucleus tractus solitarius, which is highly involved in the regulation of the cardiovascular system. Thus, either or both types of nerves might well be involved in the BP response. There are other possible triggers connected with the oral cavity as well. The vomeronasal organ (accessory olfactory organ) is one such. The nasoincisor ducts may also permit the involvement of the olfactory system itself (20). Another route might be feedback from the muscles involved in swallowing.

As a first approach to this issue we bathed the pup's oral cavity with a mild topical anesthetic. We reasoned that if we were able to get pups to reattach to the teat after anesthetic we might interrupt any signal from the oral cavity. Because a lack of response to an oxytocin-induced milk ejection might occur

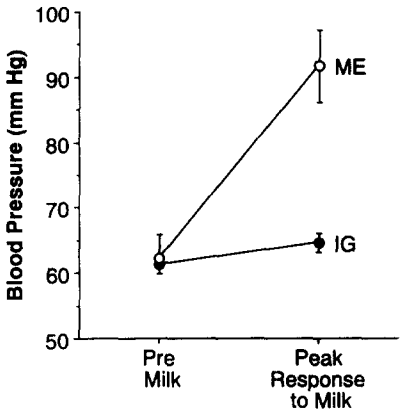


FIG. 5. The average baseline and peak BP response values of five 2-week-old pups receiving either intragastric (IG) milk (● — ●) or MEs (○ — ○) while attached to the teat of an anesthetized dam.

because the pups did not sense the presence of milk in the teat and did not receive fluid into the mouth, we also infused bovine milk into the mouth through a tongue cannula. If pups receiving milk through a tongue cannula did not spill any out of their mouths, they must be ingesting. Therefore, the feedback from the passage of fluid down the throat would be tested in this experiment as well.

Method

In this experiment, six pups (four male, two female) from six different litters ranging in age from 11 to 13 days postnatal were used for data analysis. Pups were implanted with carotid and tongue cannulae as described in the General Method section. On the day of testing, the pups' responses to a tongue cannula infusion of half and half and to a milk ejection from the mother were measured. Following these, each pup was picked up, its mouth was bathed with from 0.05 to 0.1 ml of a 0.4% solution of dibucaine. The dibucaine was applied using a syringe with a tube on the end so as to coat both the upper and lower portions of the mouth while rotating the animal. A dye analysis showed that all surfaces of the mouth and tongue were coated. The pup was then replaced on the ventrum of its anesthetized dam and encouraged to attach again. Due to the difficulty of getting treated pups to reattach, it was not possible to get each pup to satisfy all conditions of the experiment. Ten pups had to be dropped totally from the analysis because they would not reattach. In all, five of the six pups got tongue cannula infusions; four of the six pups received milk ejections from the dam. Thus, in the analysis, a two-way ANOVA without repeated measures was used. And, in the post hoc analyses, we used independent *t*-tests with Bonferroni-adjusted probabilities. As described in the General Method section, at least 3 min was allowed between each infusion, and the order of tongue cannula infusions and milk ejections were counterbalanced. With one exception, the time of infusion ranged from 2 to 8 s, and the amount infused was either 0.03 or 0.04 cc. In the first animal tested, we tried two longer and larger infusions (0.05 cc, 5 s; 0.07 cc, 20 s) following dibucaine treatment in an (unsuccessful) attempt to elicit a BP response. Infusion times and amounts did not differ significantly before and after dibucaine treatment. Pups receiving milk ejections in the postdibucaine condition had littermates attached to the teat so that we could be sure that milk ejections occurred. All data is reported from instances in which the littermates showed a stretch response. In the tongue cannula infusion situation, both before and after dibucaine, no fluid leaked out of the mouth and none remained in the mouth after the test. Thus, all fluid must have been ingested.

After comments from a reviewer, we ran some pilot observations on four dibucaine-treated pups. These pups showed an almost complete insensitivity to a light prick to any surface in the anterior part of their mouths. Deep pressure evoked tongue movements and head turning. Normal littermates jerked away even to a light prick. It was not possible to test the posterior portion of the oral cavity, as all pups struggled as we attempted to hold open their mouths. Dibucaine-treated pups showed as marked behavioral activation as normals when placed on the ventrum of an anesthetized dam, suggesting that their olfactory sense was not substantially impaired. Small amounts of bovine milk infused slowly into the mouths of the treated pups elicited mouthing behaviors and considerable ingestion. Thus, these unattached pups appeared to be capable of sensing and swallowing milk. However, it is not clear whether pups swallow in the same manner when nipple attached as when unattached.

Results

The ANOVA showed no difference between responses to tongue cannula infusions and milk ejections, but a very significant effect of dibucaine treatment, $F(1, 29) = 68.137$, $p < 0.001$. Prior to the dibucaine infusion, pups showed brisk BP and stretch responses to both oxytocin-induced milk ejections and to tongue cannula infusions of milk. After dibucaine anesthetic was applied, six pups successfully reattached to their dams' teats. There was little, if any, BP response to oxytocin-induced milk ejections, significantly less than their predibucaine response [independent $t(12) = 7.969$, $p < 0.05$], and not significantly different from the control pre-to-peak value [independent $t(16) = 0.199$, NS]. No pup showed a stretch in any of the cases; there were no bradycardias. Control littermates nipple attached at the same time showed quite evident stretch responses to the milk ejections. Thus, the test pups were not responding with either BP change or behavior to the milk ejections.

However, as noted above, the pups may not have actually gotten any milk in their mouths because sucking on their part is necessary to withdraw milk from the teat. Yet, we found that even when milk was infused directly into the mouth through the tongue cannula there was only a minimal change in BP (see Fig. 6). This small response was of significantly greater magnitude than the control pre-to-peak value [independent $t(17) = 4.226$, $p < 0.05$, one tailed], but significantly less than their predibucaine response [independent $t(17) = 4.262$, $p = 0.05$]. The average stretch rating was virtually zero. One pup did show a stretch response to one of three tongue cannula infusions. In 10 of 12 cases where milk was infused through tongue cannulas, the pups did have bradycardia responses. These bradycardias were rarely so massive that they led pups to detach from the teat. Although it is possible that the bradycardias interfered with a milk ejection response, this seems less likely because there was no significant correlation between the size of the bradycardia and the size of the BP response. Further, one pup that had no bradycardias was exactly average in the size of its minimal BP response.

Discussion

The results of this experiment suggest that fluid in the oral cavity is the stimulus that elicits BP response to milk ejections. It must be admitted that this was a crude experiment. Although the pilot observations described in the Method section suggest

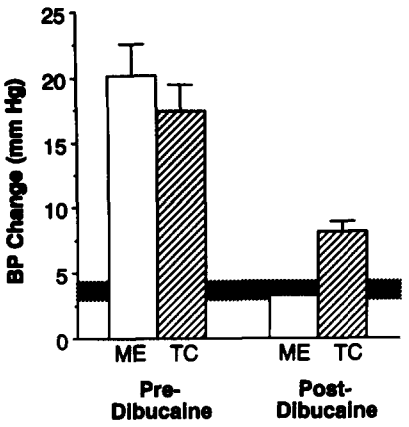


FIG. 6. The average BP response to either milk ejection (ME) or tongue cannula (TC) infusion of six 2-week-old pups while attached to the teat of their anesthetized dam. Pups were tested prior to treatment (PRE-DIBUCAINE) and following the bathing of the oral cavity with a dilute solution of local anesthetic (POSTDIBUCAINE). The shaded area represents the mean (±SEM) control pre-to-peak value. Due to the difficulty of the experiment, not all pups were represented in each condition (see Methods and text).

that the dibucaine has its major effect on surface sensation in the mouth, there was no measure for the completeness of anesthesia in the experimental pups except the marked diminution of behavioral and physiological responses. Thus, the small response to tongue cannula infusions needs further clarification. For example, the infused milk may have stimulated oral or pharyngeal areas where the anesthesia was incomplete. Further, although oral nerves may be one pathway that stimulates the response, other additive pathways (olfaction, swallowing feedback) may exist.

We also do not know precisely which sensory nerve fields the topical anesthetic may have blocked. Trigeminal, glossopharyngeal, and facial nerve distributions are prime candidates, but we have not ruled out olfactory or vomeronasal responses as contributory. It remains a possibility that some precise pattern of swallowing, with which dibucaine may have interfered, is critical. Finally, this experiment does not eliminate the possibility that a coordinated response from many areas is necessary for the BP response to occur, and that interrupting any one of these signals blocks the BP rise.

GENERAL DISCUSSION

The function of the BP response to feeding, if any, is yet unknown. However, the results of this series of experiments have tentatively localized the source of the afferent stimulus for that BP response to the oral cavity. The oral cavity appears also to be the source of stimulus for the stretch response, but it is clear from the 3-week-old experiment that BP and stretch response are not causally related. Attachment to the teat appears to play an important role in determining some aspects of the BP rise in pups of our strain and age, notably the magnitude and rapidity. The reasons for the influence of the teat remain to be determined.

The oral cavity is innervated by both taste and orosensory nerves, branches of the glossopharyngeal, vagal, facial, and trigeminal nerves. All of these nerves have connections to the nucleus tractus solitarius, which also is involved in controlling the cardiovascular system. It is tempting to believe that one or more of these nerves are involved in controlling the BP response. In the next series of experiments we plan to lesion these nerves, one at a time and in combination, and test these pups for BP and behavioral responses to feeding. If we can localize the afferent pathway, it may be possible to examine the long-term effect of stimulation along the oral nerves on the development of the cardiovascular system and adult BP in particular.

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