Role of adrenoceptors in the hypertensive response to feeding in the conscious calf


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Bowman, E. C. J., G. P. Roderick, S. R. Bloom, and A. V. Edwards. Role of adrenoceptors in the hypertensive response to feeding in the conscious calf. Am. J. Physiol. 272 (Regulatory Integrative Comp. Physiol. 41): R607-R614, 1997.—Ingestion of milk during suckling causes hypertension and tachycardia in young, unweaned animals of many species, but these responses are most pronounced in the calf. The present study was undertaken to assess the extent to which this phenomenon depends on activation of adrenoceptors in these animals. Mean basal heart rate was 100 ± 8 beats/min and mean basal aortic blood pressure was 92 ± 5 mmHg. The rise in heart rate during feeding was almost completely suppressed after propranolol (2-4 mg/kg iv), which also significantly reduced the rise in blood pressure from 67 ± 4 to 44 ± 3 mmHg (P < 0.005). Additional pretreatment with phentolamine (1.0 mg/kg and <0.1 mg·min⁻¹·kg⁻¹ iv) virtually eliminated the rise in blood pressure during feeding; it rose by only 8 ± 4 mmHg (P < 0.001). Section of both splanchnic nerves also significantly reduced the rise in blood pressure during feeding, especially after pretreatment with propranolol. Neither section of the splanchnic nerves nor the administration of the blocking agents significantly affected the rises in plasma insulin and pancreatic polypeptide that occurred after feeding. There was no detectable rise in plasma neuropeptide Y concentration in response to feeding. The hypertensive response to direct electrical stimulation of the peripheral end of a splanchnic nerve and to intra-arterial injections of norepinephrine were completely abolished after combined pretreatment with atropine, propranolol, and phentolamine after the ipsilateral adrenal vein had been tied off. It is concluded that the cardiovascular changes during feeding in these animals are attributable very largely, if not entirely, to activation of adrenoceptors.

METHODS

Animals. A total of 15 pedigree Jersey calves was used. They were obtained from local farms and kept in the laboratory animal house, where they were habituated to drinking milk from a bucket for at least 1 wk before experiments were performed. The animals were all healthy and were normally fed 8 or 9 pints of milk per day in three meals. Preliminary surgical operations were performed on calves aged between 13 and 37 days, and experiments were done between 25 and 47 days.

Surgical procedures. Two groups of calves were used in feeding experiments. In the first group (n = 5), after overnight starvation, anesthesia was induced with Saffan (0.9% alphaxalone, 0.3% alphadalone acetate, Pitman-Moore, 0.3 ml/kg) and maintained with halothane (May & Baker) and chloroform (Fisons). Polytetrafluoroethylene catheters (inside diameter 0.97 mm, wall width 0.3 mm) were inserted into both saphenous arteries and positioned so that the tips lay in the lower thoracic aorta. The free ends of the catheters were threaded under the skin to emerge on the flanks, where they were secured in polyvinyl chloride bags sewn to the calf’s flanks. One catheter was subsequently used for blood pressure recording and the other for collection of arterial blood samples and infusion of drugs. In the second group (n = 5), an identical procedure was followed, but, in addition, paravertebral incisions were made on each flank and both splanchnic nerves were sectioned immediately below the diaphragm. Recovery from anesthesia was rapid, and all calves were left to recover for at least 2 days before experiments.

Feeding procedures. All calves were fasted for at least 4 h before feeding and heparinized (700 units/kg iv Multiparin; ...
Calves in which the preparatory surgery was carried out under halothane anesthesia (~2% in O₂; May & Baker) mounted at the level of the heart and recorded on a Lectromed M19 physiological recorder, along with heart rate, which was derived from the phasic blood pressure.

A bucket containing 3 pints of warm cows' milk was placed in front of the animal without warning, to avoid anticipatory responses, and drinking always began unhesitatingly. Consumption of milk took between 15 and 90 s; the bucket was then immediately removed from sight. Samples of arterial blood for glucose and peptide analyses and hematocrit estimations were collected at intervals before and after feeding as required. Propranolol (propranolol hydrochloride; Sigma Chemical; 1–4 mg/kg iv) was administered 10 min before feeding to block β-adrenoceptors, phentolamine (phentolamine mesylate; CIBA Laboratories; 1 mg/kg iv followed by an infusion at 0.05–0.10 mg·min⁻¹·kg⁻¹ iv) was given to block α-adrenoceptors, again starting 10 min before feeding. Norepinephrine ([D]-norepinephrine acid tartrate; NBS Biologicals), phenylephrine ([D]-phenylephrine hydrochloride; Sigma Chemical), atropine (atropine methyl nitrate; Sigma Chemical) and angiotensin (angiotensin II, human octapeptide; Bachem UK) were used as described in RESULTS.

All animals were eventually killed with an intravenous injection of 20% sodium pentobarbitone (Pentoject, Animalcare). Section of splanchnic nerves was verified by macroscopic examination at postmortem when appropriate.

Analytic procedures. Arterial blood samples were kept on ice after collection. Samples of 0.5 ml were centrifuged in an Eppendorf 5415 C centrifuge at 13,000 revolutions/min (rpm) for 3 min, and plasma glucose concentrations were estimated with a Beckman Glucose Analyzer 2. Samples of 4.5 ml were collected into heparinized tubes containing aprotinin (1,000 kIU/ml blood; Trasylol, Bayer) and centrifuged at 3,500 rpm for 3 min, and plasma glucose concentrations were estimated at 4°C in a Beckman Koolspin refrigerated centrifuge. The plasma was removed and stored at −20°C for hormonal assays. Hematocrits were measured with the use of a microhematocrit centrifuge (Hawksley). Pancreatic glucagon was measured by a radioimmunoassay using antisemur relatively specific for pancreatic glucagon, which was COOH-terminal reacting (6). This assay cross-reacted <5% with glucagon-like peptide-1. Insulin, pancreatic polypeptide (PP), NPY, and glucagon-like peptide-1 were measured by conventional immunoassays based on established methods (1, 2, 5, 12).

Splanchnic nerve stimulation in anesthetized calves. Five animals with intact splanchnic nerves were anesthetized with pentobarbitone (6% sodium pentobarbitone; Sagatal, Rhone Merieux; 30–40 mg/kg iv), and the vein from the right adrenal gland was ligated. The ipsilateral splanchnic nerve was sectioned, and the peripheral end was mounted in a fluid electrode. Arterial blood pressure and heart rate were constantly monitored, blood pressure being maintained at basal level with an intra-arterial infusion of nitroprusside (sodium nitroferriyanide; Sigma) following the administration of propranolol (2 mg/kg, followed by 1 mg/kg as necessary) and atropine (0.2 mg/kg, followed by further doses as necessary) intravenously. The splanchnic nerve was stimulated at 20 V (0.5 ms) and different frequencies for a time equaling that taken by the calf to feed after propranolol.

Splanchnic nerve stimulation in conscious calves. The above procedures were also carried out in three conscious calves in which the preparatory surgery was carried out under halothane anesthesia (~2% in O₂; May & Baker) after induction with Saffan (0.3 ml/kg iv). These animals rapidly recovered from anesthesia, and the experiments were carried out below behavioral threshold and without the need to infuse sodium nitroprusside.

Results are expressed as the mean ± SE. Values in normal calves and calves with cut splanchnic nerves were compared using Student’s t test; values from different feeding runs in the same calves were analyzed with Student’s paired t-test. Differences were considered to be statistically significant when P < 0.05.

RESULTS

Cardiovascular responses to feeding under normal (control) conditions. Mean resting aortic blood pressure in these animals was 92 ± 5 mmHg, and heart rate was 100 ± 8 beats/min (n = 5). Ingestion of milk invariably caused an abrupt rise in both parameters, mean aortic blood pressure rising by 67 ± 4 mmHg (75 ± 9%) and heart rate by 54 ± 13 beats/min (59 ± 19%; see Figs. 1 and 2). Both effects were highly significant (P < 0.01), and there was also a rise in packed cell volume (2.5 ± 1.0%; an increase of 9% over basal). Twenty minutes after feeding, mean plasma glucose concentration had risen by 2.2 ± 0.4 mmol/l, from an initial value of 4.7 ± 0.2 mmol/l (Fig. 3). Feeds were followed by a steady rise in mean plasma insulin concentration, which had increased by 198 ± 36 pmol/l at 20 min (Fig. 3; P < 0.001). There was also a substantial rise in mean plasma PP concentration to a peak incremental value of 29 ± 2 pmol/l 10 min after feeding had ended (Fig. 3; P < 0.001). There was no significant change in the mean plasma concentrations of pancreatic glucagon or glucagon-like peptide-1 (data not shown), nor any detectable rise in mean plasma NPY concentration (Fig. 4).

The effect of β-adrenoceptor blockade. Propranolol (2–4 mg/kg iv) had no significant effect on mean basal aortic blood pressure in these animals (recorded at 105 ± 8 mmHg), on heart rate (103 ± 3 beats/min), or on drinking behavior. However, it reduced very substantially the rise in mean heart rate that occurred during feeding (3 ± 2 beats/min; +3 ± 2%; P < 0.001; Figs. 1 and 2) and also significantly reduced the rise in mean aortic blood pressure (44 ± 3 mmHg; 42 ± 4%; P < 0.005; Figs. 1 and 2). Neither the rises in packed cell volume (2.0 ± 1.0%) nor the rise in mean plasma glucose concentration at 20 min (1.4 ± 0.4 mmol/l) was
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Fig. 2. Changes in heart rate and aortic blood pressure in a conscious 4-wk-old calf during feeding in the absence of adrenoceptor blockade (A), after propranolol (4 mg/kg iv; B), and after combined pretreatment with propranolol and phentolamine (1 mg/kg and 0.07 mg min⁻¹ kg⁻¹ iv; C). Horizontal bars indicate duration of feeds. Filled rectangles on event marker indicate periods of feeding.

significantly affected by pretreatment with propranolol. The rises in mean plasma insulin and PP concentrations were not significantly different from controls after pretreatment with propranolol.

The effect of combined α- and β-adrenoceptor blockade. Effective α-adrenoceptor blockade was difficult to achieve, and the dose of phentolamine required was determined as follows. In animals pretreated with propranolol, the peak rise in aortic blood pressure that occurred during feeding was noted, and propranolol (2-4 mg/kg iv) was given again, together with atropine (0.2 mg/kg iv), to prevent parasympathetic reflex bradycardia. Calves were then given intra-aortic infusions of norepinephrine and phenylephrine for precisely the same period as occupied by the feed with propranolol; the doses that most closely mimicked the peak rise in aortic blood pressure during that feed were thereby established. Typically these amounted to 1 µg min⁻¹ kg⁻¹ norepinephrine and 10 µg min⁻¹ kg⁻¹ phenylephrine. The dose of phentolamine required to block the response to norepinephrine under these same conditions was then determined and invariably exceeded the dose required to block the response to phenylephrine.

After administration of phentolamine (1.0 mg/kg iv followed by a continuous intravenous infusion ≤0.1 mg min⁻¹ kg⁻¹), following pretreatment with propranolol (2-4 mg/kg iv), mean resting aortic pressure (88 ± 3 mmHg before feeding) was not significantly lower than that recorded under control conditions; neither was the mean resting heart rate (96 ± 3 beats/min). However, the rise in mean aortic blood pressure was reduced very

Fig. 3. Changes in mean plasma insulin, glucose, and pancreatic polypeptide (PP) concentrations in response to feeding (filled rectangle) in 5 3- to 7-wk-old calves. ○, Normal control animals; †, cut splanchnic nerves. Vertical bars show SE of each mean value where this exceeds the size of the symbol.

Fig. 4. Changes in mean plasma NPY concentration in response to feeding (filled rectangle) in 5 3- to 7-wk-old calves. Vertical bars show SE of each mean value where this exceeds the size of the symbol.
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Substantially, to a mean maximum value of 8 ± 4 mmHg (9 ± 5% increase; P < 0.001), and the rise in mean heart rate was effectively eliminated (2 ± 1 beats/min; +2 ± 1%; Figs. 2 and 5). The additional administration of phentolamine had no statistically significant effect on packed cell volume, mean plasma glucose concentration, or the changes in mean plasma insulin and PP concentration, although the animals invariably consumed the whole of the feed offered to them, as in the absence of pharmacological blockade.

The effect of splanchnic nerve section. In five calves with cut splanchnic nerves, neither mean resting aortic blood pressure (101 ± 9 mmHg) nor mean resting heart rate (107 ± 12 beats/min) differed significantly from the values in normal animals. The rise in mean aortic pressure that occurred during feeding (46 ± 6 mmHg) was significantly less than that in the control animals (P < 0.05; Fig. 6A), despite a more pronounced tachycardia (mean increase in heart rate 89 ± 17 beats/min; Fig. 6A). When this was eliminated by pretreatment with propranolol (4 mg/kg iv), the rise in mean aortic blood pressure was further reduced (25 ± 5 mmHg; Fig. 6B), and the difference from the rise (44 ± 3 mmHg) in propranolol-pretreated animals with intact splanchnic nerves achieved a higher level of statistical significance (P < 0.02). The result of one such experiment is illustrated in Fig. 7.

Neither the rises in mean packed cell volume nor in mean plasma glucose concentration that occurred in response to feeding were significantly reduced in calves with cut splanchnic nerves, whether or not they were given propranolol. The reduced rise in plasma glucose was associated with a reduced rise in mean plasma insulin, and mean plasma PP concentrations were consistently higher than in the control group (Fig. 3). However, neither of these differences were statistically significant.

Splanchnic nerve stimulation in anesthetized calves. The results described above provide compelling evidence that vasoconstriction in the splanchnic vascular bed makes a substantial contribution to the hypertension that occurs during feeding in these animals. Accordingly, the possible role of nonadrenergic agonists in the response was investigated acutely in five calves under barbiturate anesthesia; in these the peripheral end of a splanchnic nerve was stimulated electrically after functional unilateral adrenalectomy. Each animal was atropinized (≥0.2 mg/kg iv) to prevent any reflex bradycardia, which, by reducing cardiac output, would minimize the rise in aortic blood pressure when peripheral resistance increased.

In each of these experiments, the animals were pretreated with propranolol (2–4 mg/kg iv). The peripheral end of the right splanchnic nerve was then stimulated for the same period as the animal had previously taken to consume 3 pints of milk after propranolol, at various frequencies (20–40 Hz for 1 s at 10-s intervals and 4–15 Hz continuously) until a rise in aortic blood pressure was obtained that fell roughly within the range encountered during that feed. Norepinephrine was then infused intra-aortically, and the dose needed to produce the same hypertensive effect was determined (1.5–4.0 μg.min⁻¹.kg⁻¹). Finally, each animal was given an intra-aortic infusion of angiotensin II (3.3 μg.min⁻¹.kg⁻¹). The mean peak rise in aortic blood pressure was 48 ± 5 mmHg in response to splanchnic nerve stimulation, 51 ± 3 mmHg during norepinephrine infusions, and 50 ± 11 mmHg during angiotensin infusions (n = 5). The corresponding values after phentolamine (1 mg/kg and 0.1 mg.min⁻¹.kg⁻¹ iv) were 3 ± 1 (P < 0.01), 2 ± 1 (P < 0.01), and 22 ± 5 mmHg (P < 0.01). Two hours later, each response had partially recovered and the corresponding values were 21 ± 4.
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Fig. 7. Changes in heart rate and aortic blood pressure in a 4-wk-old calf with cut splanchnic nerves during feeding in the absence of adrenergic blockade (A) and after propranolol (2–4 mg/kg iv; B).

(P < 0.05), 25 ± 4 (P < 0.001), and 39 ± 6 mmHg (NS). The record from an individual animal in this series is illustrated in Fig. 8.

Splanchnic nerve stimulation in conscious calves. Closely similar responses were obtained in three conscious calves in which a splanchnic nerve was stimulated below behavioral threshold as described previously (10). The rise in aortic blood pressure was effectively abolished after phentolamine, as was the hypertensive response to an intra-aortic infusion of norepinephrine, and both responses exhibited a closely similar rate of recovery. The record from one of these experiments is illustrated in Fig. 9.

DISCUSSION

This study shows that the remarkable rises in aortic blood pressure and heart rate that occur when unweaned calves drink milk from a bucket are virtually eliminated after the administration of fully effective doses of propranolol and phentolamine: thus they are attributable largely, if not entirely, to adrenergic receptor activation. This must be accomplished by the release of norepinephrine from postganglionic sympathetic nerve terminals because feeding is not associated with any increase in the release of catecholamines from the adrenal medullas (7). The finding that prior section of the splanchnic nerves significantly reduced the hypertensive response to feeding showed that sympathetic splanchnic vasoconstriction normally contributes to it. When these nerves are stimulated electrically there is a substantial rise in the concentration of NPY in the circulating plasma (3). Because exogenous NPY produces a rise in blood pressure in these animals when injected in a sufficient dose (1 nmol/kg iv; Edwards, unpublished observations), it would be the most likely candidate neuropeptide to exert a hypertensive effect during feeding. However, there was no detectable release of NPY during feeding. Furthermore, the hyper-

Fig. 8. Changes in heart rate and aortic blood pressure in response to stimulation of the peripheral end of a splanchnic nerve (Spl; 20 Hz for 1 s at 10-s intervals for 1 min), an intra-arterial infusion of norepinephrine (NE; 1.5 µg min⁻¹ kg⁻¹ for 1 min), and an intra-arterial infusion of angiotensin II (ANG II; 3.3 µg/kg) in a 3-wk-old adrenalectomized calf pretreated with atropine (0.2 mg/kg iv) and propranolol (2 mg/kg) before (A) and after (B) phentolamine (1.0 mg/kg and 0.1 µg·min⁻¹·kg⁻¹ iv; arrow) and then again 2 h later (C). Filled rectangles on event marker indicate the duration of each test.
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A

EVENT MARKER

BLOOD PRESSURE (mm Hg)

HEART RATE (beats/min)

EVENT MARKER

B

SPL 20 1:10

SPL 2

NE

ANG II 0.2

ANG II 1.0

C

D

TIME (minutes)
tensive response to exogenous NPY can be blocked by the nonspecific NPY antagonist 1229U91 (Burroughs Wellcome) at a dose that is ineffective against the hypertensive response to stimulation of the splanchnic nerve, at a frequency known to release endogenous NPY (40 Hz for 1 s at 10-s intervals; Ref. 3, A. V. Edwards and A. J. Daniels, unpublished observations). There is no reason to suppose that NPY would contribute to the tachycardia and any associated increase in cardiac output. In isolated perfused hearts, NPY causes a dose-dependent negative inotropic effect, without significantly changing heart rate (4). The available data suggests that the sympathetic activation during feeding is rather selective in that it does not involve either release of catecholamines from the adrenal medullas (7) or release of NPY from splanchnic sympathetic nerve terminals (present results).

On the basis of all this evidence, it is difficult to suppose that NPY contributes significantly to the sympathetic cardiovascular changes during feeding in the calf. It is also clear that in the calf nonadrenergic mechanisms play a much less important part in producing hypertension during feeding than they apparently do in the lamb (13). This may be due simply to a species difference. The two species do differ in their response to feeding in that the rise in heart rate, which persists throughout the period of feeding in calves (Ref. 7 and present study), is more transient in lambs (7, 13, 14). It is also noteworthy that adrenoceptor blockade significantly reduces the basal blood pressure in lambs (13, 14), unlike the calves in the present study. However, the dose of phentolamine employed in the lambs (0.32 mg/min) may have been lower than that used in the present experiments (body weights and species of lamb not stated) and was validated only by the fact that it blocked the hypertensive response to phenylephrine. Norepinephrine is the amine that is actually released from the postganglionic sympathetic nerve terminals and requires a substantially higher dose of phentolamine for effective blockade, at least in the calf. One cannot therefore be certain that the residual hypertensive response to feeding in the lamb was not due to residual activation of α-adrenoceptors rather than an effect of some neuropeptide, as the authors speculated. On the other hand, chemical sympathetic with 6-hydroxydopamine, at a dose that abolished the hypertensive response to tyramine, merely reduced the rise in blood pressure during feeding by ~40% (14). Whereas the doses of both propranolol and phentolamine used in the present study were undeniably large, we are confident that inhibition was not due to a nonspecific action because neither significantly affected the resting blood pressures or heart rates, appetite, or postprandial changes in plasma hormone concentrations (insulin, pancreatic glucagon, glucagon-like peptide-1, PP, and NPY).

The extent of the reduction of the hypertensive response to feeding after splanchnic nerve section in calves pretreated with propranolol shows that the splanchnic vasculature makes a substantial contribution to the response in intact animals. That being so, the conclusion that the hypertensive response to feeding in calves can be accounted for very largely, if not entirely, by activation of α-adrenoceptors was further substantiated by the results of the acute experiments. The hypertensive responses to direct electrical stimulation of the peripheral end of a splanchnic nerve (after functional elimination of the ipsilateral adrenal gland) and to intra-aortic infusions of norepinephrine were effectively eliminated by α-adrenoceptor blockade with phentolamine in anesthetized calves. This was achieved despite the fact that compensatory changes in cardiac output had been prevented by prior treatment with atropine and propranolol; normally this treatment greatly enhances the hypertensive effect of α-adrenoceptor activation. These same procedures produced closely similar effects in conscious animals, in which splanchnic nerve stimulation was carried out below behavioral threshold. Phentolamine also caused a significant reduction in the hypertensive response to angiotensin II in these experiments (P < 0.01). Recovery from angiotensin II followed a very similar time course to that exhibited by the responses to both splanchnic nerve stimulation and exogenous norepinephrine. It therefore seems most likely that phentolamine blocked the hypertensive effect of norepinephrine released by angiotensin from postganglionic sympathetic nerve terminals, as first reported by Schuman and Guthier (21); angiotensin II has been shown to exert this effect at a concentration that has no direct effect on vascular smooth muscle (21). However, it could also be that a direct effect of angiotensin on α-adrenoceptors was blocked in the presence of phenolamine (17). These two possibilities are not mutually exclusive. Angiotensin II has also been reported to evoke the release of catecholamines from cultured bovine chromaffin cells at quite low concentrations (8); thus it might conceivably have exerted such an effect on the contralateral adrenal gland in the present experiments. It has, however, been reported that angiotensin II binding sites are present in higher density over epinephrine-secreting cells than over norepinephrine-secreting cells in this species (16).

It is concluded that the rise in heart rate that occurs during feeding in the calf is mediated largely by activation of β-adrenoceptors. When these are blocked, the residual rise in blood pressure is mediated largely by activation of α-adrenoceptors, without an important

Fig. 9. Changes in heart rate and aortic blood pressure in response to stimulation of the peripheral end of a splanchnic nerve at 2 Hz continuously (Sp1 2) or at 20 Hz for 1 s at 10-s intervals for 1 min (Sp1 20 1:10), an intra-arterial infusion of norepinephrine (1.5 μg·min⁻¹·kg⁻¹ for 1 min), and an intra-arterial injection of angiotensin II at a dose of 0.2 μg/kg (ANG II 0.2) or 1.0 μg/kg (ANG II 1.0) in a 3-wk-old anesthetized, adrenalecetomized calf pretreated with atropine (0.2 mg/kg iv) and propranolol (2 mg/kg iv) before (A) and after (B) phenolamine (1.0 mg/kg and 0.1 mg·min⁻¹·kg⁻¹ iv) and then again 2 (C) and 3 h (D) after infusion of phenolamine had been terminated. Filled rectangles on event marker indicate the duration of each test.
contribution from NPY or any other sympathetic neuropeptide.

Perspectives

Although the cardiovascular changes that occur during suckling are more pronounced in the calf than in other species, they do occur widely and, presumably, confer some biological advantage. What this might be remains a matter for speculation. One possibility is that they reflect a state of excitement associated with feeding that arouses the mother at a time of maximum vulnerability (in the wild). Excited behavior on the part of suckling young, such as head-butting of the udder, which is a prominent feature of the process in the bovine, is also likely to play an important role in achieving effective let-down of milk. A particularly interesting feature of the sympathetic discharge that produces these changes is its relative specificity, reflected in the fact that there is no significant release of NPY, as occurs when the peripheral ends of the splanchnic nerves are stimulated electrically (3), nor any detectable involvement of the adrenal medullas (7). The latter point is important because catecholamines strongly inhibit contractions of the esophageal (reticular) groove (10) and so would interfere with normal digestion. Because they do so via β-adrenoceptors (Edwards, unpublished observations), the effect is produced primarily when the adrenal medullas are activated and so not during the sympathetic discharge that occurs during suckling.

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