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Responses of the rat foetus to maternal injections of adrenaline and vasopressin

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Summary

1. Injection of 0·5–2·0 units of vasopressin or 25–100 μg of adrenaline into the peritoneal cavity of pregnant rats produced a transient slowing of the foetal heart. The bradycardia could be induced in foetuses after 15–21 days of gestation. Foetal heart rates dropped from normal values of 140–180 beats/min, often to less than 20 beats/minute. The period of bradycardia was dose dependent and ranged from 30 to 65 minutes.

2. Maternal injection of the hormones produced a fall in foetal blood pressure from an average of 54, often to less than 20 mm of water, in 17-day foetuses. Direct injection of the hormones into the pericardial sac of the foetuses had the opposite effect and pressures rose an average of 15 mm of water 1 min after the injection.

3. During the period of bradycardia, the potassium concentrations in foetal serum rose from an average value of 8·9 mequiv/l. to an average of 17·3 mequiv/litre. Concentrations of serum sodium fell from 126·2 to 121·4 mequiv/l. during the bradycardia. No changes were detected in the concentrations of either calcium or chloride. Foetal P_{O2} levels fell from 25 to 15, P_{CO2} rose from 61 to 89 or more, and pH fell from 7·19 to 6·86 during the bradycardia.

4. Maternal death and uterine clamping caused foetal bradycardia and a rise in foetal serum potassium to an average of 20·2 mequiv/litre.

5. It is concluded that interruption of normal uterine blood flow by vasoconstruction (adrenaline or vasopressin) or direct blockage (uterine clamping) results in a transient hypoxia, bradycardia, and serum ion changes in foetuses.

Introduction

Both vasopressin (Robson & Schild, 1938; Carter & Göthlin, 1970) and noradrenaline (Greiss, 1963) may cause a reduction in uterine blood flow, affecting the foetal supply of oxygen and nutrients as well as retarding the removal of waste products.

The short term foetal effects of adrenaline and vasopressin injected into pregnant animals have been observed in several species. A transient reduction of blood pressure was demonstrated in the foetuses of rats (Burlingame, Long & Ogden, 1942), cats (Clark, 1932), and sheep (Reynolds & Mackie, 1962) which had been given injections of adrenaline. A transient bradycardia was demonstrated in the

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foetuses of guinea-pigs (Martin & Young, 1952), monkeys (Adamsons, Mueller-Heubach & Myers, 1971) and man (Beard, 1962; Sani, Giardina & Angiolillo, 1966) after maternal injections of adrenaline. Clark (1932) reported similar results when injections of vasopressin were administered. Most of the above authors used only animals and humans that were close to term in their experiments. This study was initiated to determine the physiological reactions of rat foetuses to these agents and to mechanical interference with uterine blood flow and maternal death.

Methods

Rats used were the Long-Evans strain. They were housed in plastic cages and water and food were fed *ad libitum*. Female rats were mated when they reached weights of approximately 220 g. Females with a positive sperm smear in the morning were considered 1 day pregnant.

Animals were injected under very light ether anaesthesia. Compounds were administered as saline solutions intraperitoneally, intramuscularly (thigh), subcutaneously (epigastric), or intravenously (jugular vein).

The hormones used were as follows: adrenaline (Parke Davis, adrenaline chloride, 1:1,000); vasopressin (Parke Davis, Pitressin, 20 pressor units in 1·0 ml); noradrenaline (Parke Davis, Levarterenol, 1:1,000).

In experiments in which foetuses were examined, the mothers were continuously anaesthetized with ether. A median incision was made in the ventral body wall exposing the uterus. A foetus was carefully removed with amnion and chorion intact through an incision in the uterus. The extra-embryonic membranes were then broken and the foetus placed in a paraffin retaining dish. Foetuses were kept at 37° C by means of a heat source (Sage Microcurtain). Heart rates were obtained visually. During the period of examination of the foetus, the maternal incision was clamped with a haemostat and a heat source placed over it to ensure retention of body warmth.

Foetal blood pressures were obtained directly from the ventricles of the heart using a Landis (1926) blood pressure apparatus as modified by Paff, Boucek & Gutten (1965) and Grabowski, Tsai & Toben (1969). The hearts of 15, 16, and 17-day foetuses were exposed by a median thoracic incision. The hearts of 18-day foetuses were exposed by an incision in the left axillary region.

Foetal blood samples were obtained by heart puncture after opening the thorax upon removal from the uterus. Blood for determination of ions was immediately centrifuged and the serum removed. Concentrations of serum sodium and potassium were measured on an Instrumentation Laboratories Flame Photometer, Model 143.

The calcium and chloride concentrations were determined by spectrophotometric methods on a Beckman/Spinco Model 150 Analytical System. These assays require 5–20 μl of serum each and no pooling was necessary. The whole blood pH, Po_2 and Pco_2 were measured on an Instrumentation Laboratories Ultramicro pH/blood gas Analysing System, Model 113-sl. This last procedure was sensitive to any delay between obtaining the samples and the measurements. Generally, three 17-day foetuses were required to obtain enough blood for a

blood gas determination. The maximum time which elapsed between obtaining the first sample and the reading was 5 minutes.

Uterine clamping was accomplished by the method of Brent & Franklin (1960). Haemostats were placed across the uterus at the ovarian and cervical ends. One horn was left unclamped as a control.

Student's *t* test was used for statistical analysis. Standard deviations given all represent the variation between litter means.

Results

Effect of hormones on the foetal heart rate

Injection of either adrenaline or vasopressin intraperitoneally into the mother resulted in a transient foetal bradycardia which began 5–20 min after the injection and lasted up to 65 min (Fig. 1). Intrajugular injections resulted in similar foetal effects. In experiments in which the mother received intraperitoneal injections of saline or either subcutaneous or intramuscular injections of the hormones, there was no bradycardia and heart rates remained constant up to 80 min after the laparotomy (Fig. 1).

Intraperitoneal injections of 25 μg of adrenaline into 17-day pregnant rats produced a fall in foetal heart rates from normal value of 140–180 beats/min to an average of 35. The duration of the bradycardia ranged from 50 to 65 min

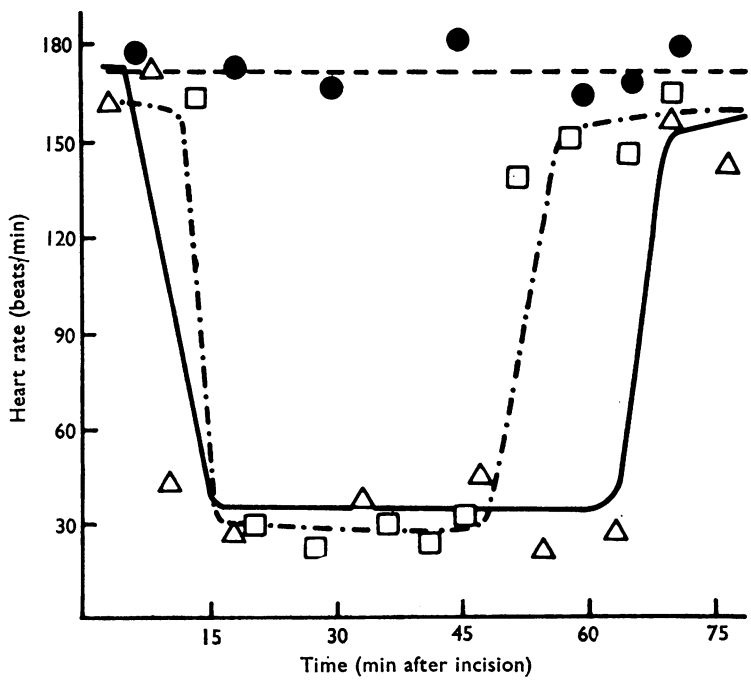

FIG. 1. Typical patterns of foetal bradycardia induced by adrenaline and vasopressin. The curves represent three separate experiments on individual litters of 17 day rats. The two hormone treated animals received intraperitoneal injections 5 min before the incision (0·5 units vasopressin in one (●–●–●), and 25 μg adrenaline (——) in the other. (– – –), Control. Each series of points represents individual foetuses within a single litter. This pattern was found consistently in all experiments.

with an average of 57 minutes. Foetal bradycardia was produced in animals of 16–20 days' gestation.

The pattern of vasopressin-induced bradycardia was similar to that produced by adrenaline. The duration of the bradycardia in 17-day foetuses was related to the dose of vasopressin administered. The average duration of bradycardia was 33 min for 0·5 and 1·0 units of vasopressin. The duration increased to 48 min when 1·5 units were given, and 54 min when 2·0 units were administered (Table 1). The administration of doses greater than 2·0 units resulted in 100% foetal mortality. Foetal bradycardia was produced in animals of 15–21 days' gestation after injection of 1·5 units. Both the inception and the cessation of the foetal bradycardia occurred at the same time in all foetuses in a litter. This was determined by cutting the uterus lengthwise, exposing all the foetuses, and counting their heart rates nearly simultaneously. Two groups of five animals (one group injected with 0·5 units of vasopressin, the other with 25 μg adrenaline) were used.

The intraperitoneal injection of 2·5 μg of adrenaline into seven pregnant mice of 17 days' gestation resulted in a similar pattern of foetal bradycardia and recovery indicating that this response is not specific to the rat.

The direct injection of 1 μg adrenaline (four litters) or 0·02 units vasopressin (five litters) into the pericardial cavities of 17-day rat foetuses did not result in a change in foetal heart rates.

Effects of hormones on foetal blood pressure

Blood pressures were measured in foetuses of 15–18 days of gestation. On day 15 the average blood pressure was 28·3 mm of water, rising to 63·5 mm of water on day 18 (Fig. 2). The foetuses of mothers who received intraperitoneal injections of adrenaline or vasopressin exhibited lower blood pressures whilst in a state of bradycardia. Blood pressures of foetuses were closely related to their heart rates. In all cases, foetuses with bradycardia had lowered blood pressures. Foetuses whose heart rates had fallen from an average of 145 to approximately fifty beats/min exhibited blood pressures that were reduced from an average of 54 to 20–27 mm of water. Once the heart rates dropped below forty-five beats/min, the blood pressures fell below 20 min of water and were too low to register on the equipment. At no time was there any sign of an elevation of foetal blood pressure.

The direct injection of either adrenaline (1 μg in 1 μl saline) or vasopressin (0·02 units in 1 μl of saline) into the pericardial cavity of 17-day foetuses resulted in an increase of blood pressure. In the adrenaline series the average increase was 32% and in the vasopressin series 36%. Experiments in which foetuses were similarly injected with saline did not result in blood pressure increase (Table 2).

TABLE 1. *Relationship between quantity of vasopressin administered and duration of bradycardia in litters of* 17*-day rats*

Number of litters	Dose (units/rat)	Duration of bradycardia (min) Mean	S.D.
9	0·5	31·9	±5·9
8	1·0	34·8	±3·6
10	1·5	48·2	±4·0
6	2·0	54·2	±6·6

Chemical changes in foetal blood during bradycardia

Profound changes in some ionic and gaseous constituents of foetal blood occurred during the period of bradycardia. The constituents were studied in blood obtained from the right ventricles of the foetuses (Table 3).

Sodium and potassium

The normal concentrations of Na^+ and K^+ in 17-day foetal serum are 126·2 mequiv/l. and 8·9 mequiv/l. respectively. Foetuses with bradycardia caused by maternal injection of either adrenaline or vasopressin exhibited a rise in K^+ to an average of 17·3 mequiv/litre. At the same time, the Na^+ was lowered to an average of 121·4 mequiv/l. (Table 3).

A close relationship between the onset and cessation of bradycardia and the value of the serum K^+ was evident. Foetuses with bradycardia always had elevated K^+, whilst normal K^+ concentrations were found in all treated foetuses where the heart had not yet slowed, or where normal heart rates had returned after a period of bradycardia (Fig. 3). The changes in Na^+ did not appear to occur with the same rapidity as those of K^+.

Comparable patterns of ionic change also occurred in foetuses of days 18–20 gestation.

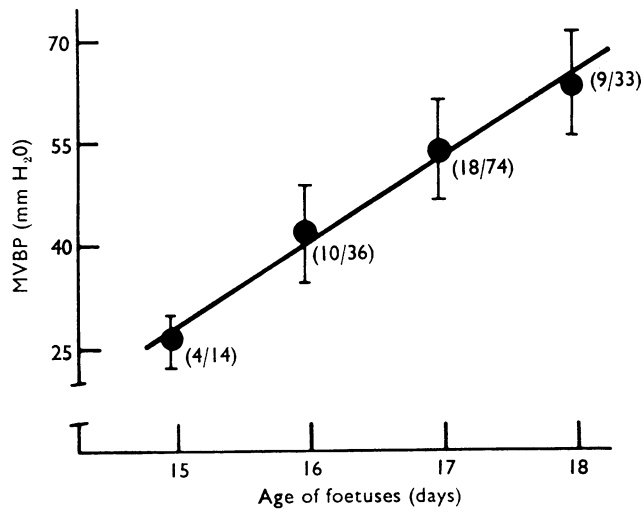

FIG. 2. Normal blood pressures in rat foetuses. MVBP (mean ventricular blood pressure) is the height of the fluid in the water manometer at which the red blood cells in the cannula oscillate (see Grabowski *et al.*, 1969). Figures in the parentheses are the number of litters tested/number of foetuses examined. Because of the time factor, only three to five foetuses were tested in each litter. Vertical lines indicate ± one standard deviation.

TABLE 2. *Effects of injections of adrenaline and vasopressin directly into the pericardial cavities of 17-day rat foetuses*

Treatment	Number of litters	Number of foetuses	Blood pressure of foetus (mm of water) Mean	S.D.
Controls	9	41	46·6	±11·8
Adrenaline	4	20	58·9[1]	±12·9
Vasopressin	5	16	63·5[1]	±12·6

[1] $P<0·05$.

No correlation of the magnitude of ionic change was found with either the type or the quantity of hormone used (0·5 to 2·0 units of vasopressin, and 25 μg to 100 μg of adrenaline).

Calcium

The concentration of calcium in foetal serum was measured in both normal foetuses and those with bradycardia. The Ca^{++} in normal 17-day foetuses averaged 6·0 mequiv/litre. No significant differences were found in sera obtained from foetuses in a state of bradycardia.

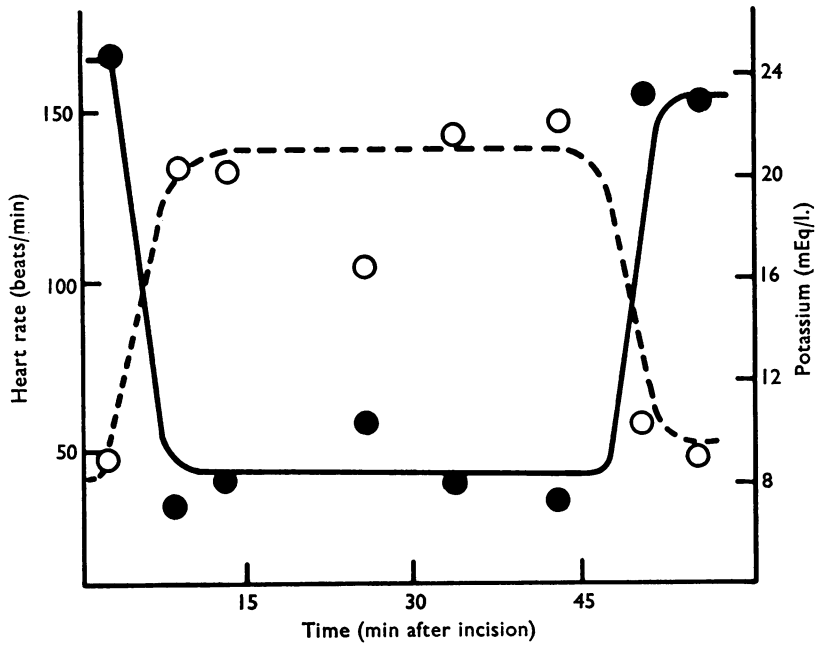

FIG. 3. Typical relationship of bradycardia and serum potassium. The curves represent a 17-day rat which was injected with 0·5 units vasopressin 5 min before the initial incision. Paired points with the same time coordinate represent heart rate (——) and serum potassium (- - -) from an individual foetus. Though only the data from a single experiment are shown here, this was a consistent phenomenon.

TABLE 3. *Blood chemistry of 17-day rat foetus: comparison of normals and those with bradycardia*

	Normal				Bradycardia			
	Number of rats	Number of foetuses	Mean	S.D.	Number of rats	Number of foetuses	Mean	S.D.
Na^+(mequiv/l.)	6	26	126·2	±7·1	14	66	121·4*	±6·6
K^+(mequiv/l.)	6	26	8·9	±1·4	14	66	17·3*	±3·7
Ca^{++}(mequiv/l.)	4	10	6·0	±0·7	4	11	6·4	±0·6
Cl^-(mequiv/l.)	4	23	94·4	±5·2	4	21	98·3	±6·5
Po_2	4	5†	25·0	±8·1	4	11†	15·2†	±3·0
Pco_2	4	5†	61·0	±8·0	4	11†	89+*‡	±11·8
pH	4	5†	7·19	±0·15	4	11†	6·86*	±0·06

(Heart rates below 100 beats/min.) * $P > 0{\cdot}01$. † Pooled samples—from average of three foetuses. ‡ The exact value is not known because several readings were above 100 and therefore off the scale of the machine.

Chloride

Foetal serum chloride concentrations were obtained from both normal foetuses and those with bradycardia. The normal serum concentrations in 17-day foetuses were found to average 94·4 mequiv/litre. No significant differences were found in sera obtained from foetuses during bradycardia.

P_{O_2}, P_{CO_2}, pH

The whole blood values of P_{O_2}, P_{CO_2}, and pH were obtained from both normal foetuses and those with bradycardia. During the period of bradycardia, there was a lowering of the mean P_{O_2} from 25 to 15. The mean P_{CO_2} rose from 61 to an average in excess of 89. The rise in P_{CO_2} was reflected in a lowered mean pH from 7·19 to 6·86.

Effects of maternal death and uterine clamping on foetal heart rates and serum potassium concentrations

The effects of interference with the normal blood supply on the maternal side of the placenta on foetal heart rate and serum potassium concentrations were tested by two methods—maternal death and uterine clamping.

Experiments in which 17-day pregnant rats were killed by excessive ether inhalation resulted in a progressive failure of foetal hearts which began an average of 7 min after cessation of maternal breathing. The serum K^+ of foetuses rose to an average of 20·2 mequiv/l. during the irreversible bradycardia.

The uterine vessels of 17-day rats (eighteen animals) were clamped for periods of up to 45 minutes. All foetuses in the clamped horns developed bradycardia, which began 8–12 min after the clamping. A rise in serum K^+ to an average of $20{\cdot}1 \pm 2{\cdot}3$ mequiv/l. occurred in thirty-six foetuses tested during bradycardia. Upon releasing the clamps, both the heart rates and the serum K^+ returned to normal values within 15 minutes. Foetuses from the unclamped horns exhibited neither bradycardia nor elevated serum K^+ during the same period.

Discussion

The injection of adrenaline or vasopressin into pregnant rats results in foetal bradycardia. In the case of vasopressin, a relationship has been demonstrated between the duration of this bradycardia and the dose of the hormone administered. During this period, blood pressures and serum P_{O_2}, pH, and Na^+ drop whilst serum P_{CO_2} and K^+ increase. When the foetuses resume normal heart rates, their blood pressures and blood chemistry rapidly return to normal.

The foetal bradycardia could be caused by either a direct foetal reaction to the hormones, by a foetal reaction to changes in the maternal system, or by a combination of the two. The injection of these hormones directly into the foetuses did not result in bradycardia but caused elevated blood pressure. Adamsons *et al.* (1971) obtained similar results in monkeys. It follows that the effects of maternal injections on the foetuses were caused by actions these hormones had on the maternal system.

The specific factors which cause the foetal bradycardia should be found in actions common to both adrenaline and vasopressin. An examination of their

physiological effects indicates that there are two basic actions which these otherwise dissimilar hormones have in common. The first is the ability to contract the uterus, and the second is the production of a generalized vasoconstriction in the uterus. Martin & Young (1960) considered that uterine vasoconstriction was the cause of the foetal bradycardia. They based this view in part upon the observation that doses of oxytocin sufficient to result in uterine contraction did not affect foetal heart rate. Adamsons *et al.* (1971) arrived at a similar conclusion since in pregnant monkeys vasoconstriction and foetal bradycardia caused by adrenaline are independent of whether myometrial activity increases or decreases.

The experiments reported here indicate that interference with uterine blood flow by a variety of agents is capable of producing foetal bradycardia and blood chemical changes. When a reversible interruption of blood flow was produced by clamping the uterine arteries, the typical bradycardia and elevation of serum K^+ occurred after the clamps were applied. Both foetal heart rates and serum K^+ returned to normal after removal of the clamps. In addition, the typical hormone-induced pattern of bradycardia and serum K^+ increase began in foetuses soon after cessation of maternal breathing.

The precise aspect of the impairment of uterine blood flow which causes the bradycardia and/or blood chemical changes is unknown. At this time, the foetuses have less maternally supplied oxygen and nutrients. The maternal system absorbs less foetal waste products and carbon dioxide. Any of these factors, or any combination of them, might be causes of the foetal bradycardia, blood chemical changes, or both.

The magnitude of the hormone-induced changes in serum K^+, reduction of blood pressure, and hypoxia are especially significant since similar hormone injections also result in tail amputations and paralysis in litters of treated mothers (Chernoff & Grabowski, 1971). The relationship of blood chemical changes in a foetus and its health is an approach to teratology which needs further exploration.

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REFERENCES

ADAMSONS, K., MUELLER-HEUBACH, E. & MYERS, R. E. (1971). Production of fetal asphyxia in the rhesus monkey by administration of catecholamines to the mother. *Am. J. Obstet. Gynec.*, **109**, 248–262.

BEARD, R. W. (1962). Response of human foetal heart and maternal circulation to adrenaline and noradrenaline. *Br. med. J.*, **1**, 443–446.

BRENT, R. L. & FRANKLIN, J. B. (1960). Uterine vascular clamping: new procedure for the study of congenital malformation. *Science, N.Y.*, **132**, 89–91.

BURLINGAME, P., LONG, J. A. & OGDEN, E. (1942). The blood pressure of the fetal rat and its response to renin and angiotonin. *Am. J. Physiol.*, **137**, 473–484.

CHERNOFF, N. & GRABOWSKI, C. T. (1971). Teratogenic effects of epinephrine and vasopressin on the fetal rat (in the Press).

CLARK, G. A. (1932). Some foetal blood pressure reactions. *J. Physiol., Lond.*, **74**, 391–400.

CARTER, A. M. & GÖTHLIN, J. (1970). Effects of angiotensin, oxytocin and vasopressin on placental circulation. *Invest. Radiol.*, **5**, 86–91.

GRABOWSKI, C. T., TSAI, E. N. & TOBEN, H. R. (1969). The effects of teratogenic doses of hypoxia on the blood pressure of chick embryos. *Teratology*, **2**, 67–76.

GREISS, F. C. (1963). The uterine vascular bed: effect of adrenergic stimulation. *Obstet. Gynec., N.Y.*, **21**, 295–301.

LANDIS, E. M. (1926). The capillary pressure in frog mesentery as determined by micro-injection methods. *Am. J. Physiol.*, **75**, 548–570.

MARTIN, J. D. & YOUNG, I. M. (1960). Influence of gestational age and hormones on experimental foetal bradycardia. *J. Physiol., Lond.*, **152,** 1–13.

PAFF, G. H., BOUCEK, R. J. & GUTTEN, G. S. (1965). Ventricular blood pressures and competency of valves in the early embryonic chick heart. *Anat. Rec.*, **151,** 119–123.

REYNOLDS, S. R. M. & MACKIE, J. D. (1962). Umbilical venous pressure and other cardiovascular responses of fetal lambs to epinephrine. *Am. J. Physiol.*, **203,** 955–960.

ROBSON, J. M. & SCHILD, H. O. (1938). Effect of drugs on the blood flow and activity of the uterus. *J. Physiol., Lond.*, **92,** 9–19.

SANI, G., GIARDINA, L. & ANGIOLILLO, M. (1966). L'azione dell 'adrenalina e della noradrenalina a livello uterino e fetale. *Riv. ital. Ginec.*, **50,** 240–250.

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