Effects of morphine and naloxone on fetal heart rate and movement in the pig

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¹Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, 3584 CL Utrecht; ²Department of Obstetrics, Neonatology and Gynecology, University Medical Center, 3584 EA Utrecht, The Netherlands; and ³Department of Physiology, Institute for Animal Science and Animal Behavior, FAL, 31535 Neustadt/Rhge, Germany

Received 16 February 2000; accepted in final form 16 October 2000

Cohen, S., N. Parvizi, E. J. H. Mulder, H. A. Van Oord, F. H. Jonker, G. C. Van Der Weijden, and M. A. M. Taverne. Effects of morphine and naloxone on fetal heart rate and movement in the pig. J Appl Physiol 90: 1577–1583, 2001.—To test the hypothesis that an increasing opioid tonus in control of cardiovascular function and locomotion has been a subject of research in adults for many years (26, 27). In general a suppressive action on heart rate (22, 26) and stimulatory effects on locomotion (27) have been reported. By contrast, only a few fetal studies have focused on cardiovascular and behavioral effects of opioids and opioid antagonists. These studies are difficult to compare, and their results are rather controversial. For example, administration of opiate agonists to the fetal lamb increases FHR (36, 38), body and eye movements (39), and electrocortical and respiratory activities (6, 17, 18, 35) and disturbs sleep-wake organization (37). Interestingly, the administration of naloxone to fetal sheep caused an increase in FHR as well (1). Smotherman and Robinson (34) found that morphine increased fetal motility (arousal) in the rat, whereas naloxone had no effect.

To explore the involvement of the opioidergic system in the control of cardiovascular and kinetic mechanisms in the fetal pig, we characterized and quantified the effects of a nonspecific opioid-receptor agonist (morphine) and antagonist (naloxone) on basal FHR, FHR variation, and FM.

METHODS

Animals. Twenty-one German Landrace sows (11–24 mo old) were kept in individual pens under controlled environmental conditions (temperature 20–22°C; lights on from 0600 to 1800). They received a standard diet twice daily (at 0630 and 1130) and had access to water ad libitum. Food was withheld from 12 h before to 12 h after surgery. Between 90 and 104 days of gestation (median: 100 days; term in this breed = 113 ± 1 days), one fetus per sow was chronically catheterized (n = 21) and 11 of them were also instrumented with electrocardiograph (ECG) electrodes.

Surgical procedures. Surgical procedures were followed as previously described (4). Sows were sedated with azaperone (2 mg/kg; Stresnil, Janssen) 30 min before the induction of general anesthesia by means of ketamine-hydrochloride (12 ml via an ear vein, ketamine; Atarost). A Silastic catheter

Changes in fetal heart rate (FHR) and fetal movement (FM) are expressions of the development of the fetal nervous system. The mechanisms involved in the control of these vital fetal functions are of ample interest from both a scientific and clinical point of view. It has recently been reported that noninvasive monitoring of FHR (7) and FM (8) is feasible in the pig during late gestation. These studies revealed that basal FHR and the incidence of FM (percentage of time) decrease in the pig fetus during the last stage of gestation. A decrease in FHR and FM during late gestation has also been reported to occur in ovine (5, 13, 14), bovine (11, 15), equine (12, 16), and human (10, 25) fetuses. However, the physiological background of this phenomenon remains to be elucidated.

The role of the endogenous opioid system in the control of cardiovascular function and locomotion has been a subject of research in adults for many years (26, 27). In general a suppressive action on heart rate (22, 26) and stimulatory effects on locomotion (27) have been reported. By contrast, only a few fetal studies have focused on cardiovascular and behavioral effects of opioids and opioid antagonists. These studies are difficult to compare, and their results are rather controversial. For example, administration of opiate agonists to the fetal lamb increases FHR (36, 38), body and eye movements (39), and electrocortical and respiratory activities (6, 17, 18, 35) and disturbs sleep-wake organization (37). Interestingly, the administration of naloxone to fetal sheep caused an increase in FHR as well (1). Smotherman and Robinson (34) found that morphine increased fetal motility (arousal) in the rat, whereas naloxone had no effect.

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was placed into the maternal external jugular vein and was only used for maintenance of anesthesia with ketamine hydrochloride. Lateral laparotomy was performed, and one fetal compartment of the uterus, just ventral to the incision, was exteriorized and opened. A Silastic catheter (Silastic Medical Grade, no. 602-155, 0.51 mm ID and 0.94 mm OD, Dow Corning, Midland, MI) was placed into the fetal jugular vein and tunneled to the neck of the fetus. In 11 fetuses in addition to the vascular catheter, ECG electrodes (silver bars soldered to copper wires) were placed subcutaneously on both sides of the thorax and fixed to the fetal skin. After intraamniotic administration of 500 mg kanamycin (Albrecht, Aulendorf, Germany), the fetal membranes and uterine wall were closed with one suture and the uterus was placed back into the abdominal cavity. The fetal catheter and ECG wires were tunneled subcutaneously and exteriorized at the back of the sow. On the day of surgery and the 2 following days, all sows received a standard antibiotic therapy (2 x 10⁶ IU penicillin and 2.5 g dihydrostreptomycin im; Tardomyocel, Bayer, Munich, Germany). Fetuses and dams were allowed to recover for 48 h before the experiments were started. Fetal catheters were flushed daily with 1 ml of saline and filled with 0.6 ml of saline containing 50 IU heparin (Kettelhack Riker, Borken, Germany) to keep them patent.

Experimental protocol. Fetuses were randomly assigned to a morphine or naloxone treatment group. The days of gestation on which FHR and/or FM recordings were made in individual fetuses are presented in Table 1. FM and FHR could be recorded simultaneously (i.e., around the same injection on which FHR and/or FM recordings were made in each catheterized pig fetuses in each period was analyzed separately. During the Doppler-FHR recordings, FM recordings, FMs felt by the observer’s hand and movements of the sow were encoded with an event marker on the paper tracing, but they were not analyzed afterward.

Recording and analysis of FM. FM recording and analysis were performed as described previously (8). In short, the catheterized fetus was localized by means of transabdominal ultrasonography (Scanner 200, Pie Medical, Maastricht, The Netherlands), using a 3.5-MHz linear-array back fat probe with a scanning field of 18 cm (width) by 16 cm (depth). Then, a 1.5-MHz Doppler transducer connected to a cardiograph (Meridian 800, Oxford Sonicaid, Abingdon, UK; paper speed 3 cm/min) was placed on the maternal abdominal wall over the fetal heart. In case of a fetus with ECG electrodes, the electrodes were connected to the same cardiograph. FHR was recorded using Doppler ultrasound if the ECG electrodes were nonfunctional. Once a good-quality FHR signal was obtained, a handheld computer (Psion Organizer LZ II, Psion PLC, London, UK) was programmed to acquire data for 60 min. Off-line, the stored data were fed into a personal computer running a software package developed for human FHR analysis (System 8002, Oxford Sonicaid, Abingdon, UK; paper speed 3 cm/min) was placed on the maternal abdominal wall over the fetal heart. In case of a fetus with ECG electrodes, the electrodes were connected to the same cardiograph. FHR was recorded using Doppler ultrasound if the ECG electrodes were nonfunctional. Once a good-quality FHR signal was obtained, a handheld computer (Psion Organizer LZ II, Psion PLC, London, UK) was programmed to acquire data for 60 min. Off-line, the stored data were fed into a personal computer running a software package developed for human FHR analysis (System 8002, Oxford Sonicaid, Abingdon, UK; paper speed 3 cm/min) was placed on the maternal abdominal wall over the fetal heart. In case of a fetus with ECG electrodes, the electrodes were connected to the same cardiograph. FHR was recorded using Doppler ultrasound if the ECG electrodes were nonfunctional. Once a good-quality FHR signal was obtained, a handheld computer (Psion Organizer LZ II, Psion PLC, London, UK) was programmed to acquire data for 60 min. Off-line, the stored data were fed into a personal computer running a software package developed for human FHR analysis (System 8002, Oxford Sonicaid, Abingdon, UK; paper speed 3 cm/min) was placed on the maternal abdominal wall over the fetal heart.

Table 1. Distribution of fetal heart rate and/or movement recordings according to gestational age made in catheterized pig fetuses in each of three groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Fetus No. (Total n = 21)</th>
<th>Gestational Age at FHR Recording, days</th>
<th>Gestational Age at FM Recording, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 3)</td>
<td>1</td>
<td>102, 103, 105</td>
<td>102, 103, 107</td>
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<tr>
<td></td>
<td>2</td>
<td>104, 109</td>
<td>104, 106</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>109, 111, 113</td>
<td></td>
</tr>
<tr>
<td>Morphine (n = 9)</td>
<td>4</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>103, 105</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>106, 108, 110</td>
<td></td>
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<td></td>
<td>8</td>
<td>101</td>
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<td>9</td>
<td>104</td>
<td></td>
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<tr>
<td></td>
<td>10</td>
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<td>107, 109</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>104, 106</td>
<td></td>
</tr>
<tr>
<td>Naloxone (n = 9)</td>
<td>13</td>
<td>94, 96, 98</td>
<td>92, 94, 96</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>102, 104</td>
<td></td>
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<tr>
<td></td>
<td>15</td>
<td>102</td>
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<tr>
<td></td>
<td>21</td>
<td>105, 107, 109, 111</td>
<td>105, 107, 109, 111</td>
</tr>
</tbody>
</table>

n, No. of animals; FHR, fetal heart rate; FM, fetal movement.
isolated movements of the head (HM), isolated movements of
a forelimb (LM), and rotation of the fetus along its longitu-
dinal axis (Rot), using a specially designed software package
(Poly 5, Inspector Research Systems, Amsterdam, The Neth-
erlands). A single investigator analyzed the tapes and was
not informed about the kind of treatment. The recordings
were split into four periods of 15 min each (1 period before
and 3 periods after drug administration). The incidence of
each movement pattern was expressed as a percentage of
observation time (15 min). The sum of the four percent
incidences per 15 min was also calculated [total fetal activity
(TFA)].

Statistical analysis. Because the data were not normally
distributed they were expressed as median and range or
interquartile range (IQR), and nonparametric tests were
used. The Wilcoxon test was used to compare paired mea-
surements, the Kruskal-Wallis test for comparison of the
three treatment groups, and the Friedman test for repeated
measurements within each group with the Neuman-Keuls
method for multiple comparison. The relationship between
two variables was studied with the Spearman test. Signifi-
cant differences were assumed at the level of \( P < 0.05 \)
(2-tailed). To achieve optimal visual presentation in Figs. 1
and 3, data were presented as means ± SE, but statistical
significance was tested nonparametrically.

RESULTS

The median values for gestation length, litter size,
and the proportion of stillborn piglets were 113 days
(range 111–115 days), 11 piglets (range 9–14 piglets),
and 12% (range 8–14%), respectively, and these values
did not differ significantly between the three treatment
groups. The percentage of stillborn piglets was rela-
tively high (8% on average in this herd) because, in
addition to the noninstrumented fetuses, the instru-
mented fetuses also were allowed to be born vaginally
and several of them were born dead. Median birth
weight of the operated fetuses was 1.1 kg (range 0.9–
1.2 kg) and was within the normal range of all litters
born.

Effects of morphine and naloxone on FHR. A total of
29 1-h FHR recordings was obtained from 15 fetuses
and used for analysis (Table 1). The quality of these
recordings varied widely among the fetuses. Whenever
an ECG signal was recorded \((n = 10)\), signal loss (SL; 
percentage of recording time) was <0.5%, whereas the
median SL was 45% (range 4–74%) for the Doppler
ultrasound recordings \((n = 20)\). Maternal movements
and FMs were the major reasons for SL.

During the control period \((-15–0 \text{ min})\), there were
no significant differences between the three treatment
groups for any of the FHR parameters (Kruskal-Wallis
test). Saline injection had no significant effect on FHR
and its variation (Friedman test; Fig. 1). After mor-
phine administration, FHR decreased dramatically
\((P < 0.001)\) within 55 s (median; IQR 30–80 s) and
basal FHR remained reduced until 30 min after injec-
tion (Fig. 1), whereas both LTV \((P < 0.01)\) and STV
\((P < 0.05)\) were increased for ~15 min compared with
pretreatment values (Fig. 1). Naloxone administration
resulted in a significant increase in FHR \((P < 0.001)\)
within 48 s (median; IQR 20–90 s), whereas no signif-
ificant changes were observed in STV and LTV. The
effect of naloxone on basal FHR remained present until
45 min after the injection. There was no significant
relationship between the gestational age at recording
and the fetal responses to either drug (i.e., change in
FHR 0–15 min postinjection compared with control
level; Spearman test). Examples of the rapid changes
in the FHR pattern after administration of morphine
and naloxone are presented in Fig. 2, A and B, respec-

Fig. 1. Changes in basal fetal heart rate (FHR), long-term variation
(LTV), and short-term variation (STV) of FHR recorded from 15 min
before until 45 min after intravenous bolus administration (time = 0
min) of saline \((n = 5 \text{ animals})\), morphine \((n = 8 \text{ animals})\), or naloxone
\((n = 16)\) (both drugs 1 mg per fetus). Values are means ± SE; \(n\), no.
of animals; bpm, Beats/min. Significance was tested with the Fried-
man test. *\(P < 0.05\). **\(P < 0.01\). ***\(P < 0.001\).
Basal FHR was negatively correlated with LTV (Spearman rank correlation coefficient \( r_S = -0.36, n = 30 \) animals, \( P < 0.05 \)) and STV (\( r_S = -0.30 \), not significant) when all control periods were regarded. During the first 30 min after drug administration, however, only the correlation between basal FHR and LTV in the naloxone group was statistically significant (\( r_S = -0.40, n = 17, P < 0.05 \)).

**Effects of morphine and naloxone on FM.** A total of thirty-two 1-h recordings of FM was obtained from 16 fetuses and used for analysis (Table 1). The quality of these recordings was good, with SL (% of recording time not suitable for analysis) <15%. No significant differences were found among the three treatment groups during the control periods (Kruskal-Wallis test; Fig. 3). Saline injections caused no significant changes in any movement pattern (Friedman test). Morphine administration had no effect on GM, HM, and Rot, but it resulted in a dramatic increase in LMs within 115 s (median; IQR 99–123 s) after injection (Fig. 3). LMs were significantly increased until the end of observation (Fig. 3) and occurred in clusters of 45-s duration (median; IQR 24–65 s) alternating with epochs without LM (median duration 110 s; IQR 51–240 s, Fig. 4). The increase in LM resulted in a significant increase in TFA (Fig. 3). Administration of naloxone increased the incidence of GM (\( P < 0.05 \)), which occurred within 115 s (median; IQR 99–123 s) after injection (Fig. 3). Naloxone had no significant effect on either HM or LM. Fetal rotations were rare, and their incidence was not affected by any treatment (data not shown). There was no significant relationship between the gestational age at recording and the fetal responses to either drug (i.e., change in FM 0–15 min postinjection compared with control level; Spearman test).
The results demonstrate that intravenous administration of morphine and naloxone had differential effects on basal FHR, FHR variation, and FM during late gestation in the pig.

Fetal age at treatment ranged from 92 to 114 days. Although the development of fetal pigs during late gestation is rapid, we could not find any statistically significant age effect in our results. We therefore considered the differences in fetal age in our study to be of minor importance. Mean birth weight of the catheterized fetuses was 1.1 kg (range 0.9–1.2 kg). Therefore, it could only be concluded at birth that the dose of 1 mg per fetus had been actually higher in some fetuses but lower in others than the envisaged dose of 1 mg/kg body wt.

Intravenous administration of 1 mg of morphine to the pig fetus induced a rapid decrease in FHR, accompanied by increased LTV and STV. Naloxone administration (1 mg iv) caused an immediate increase in FHR without significant changes in LTV and STV. Available data on the effects of opioids and their antagonists on FHR in other species are inconsistent. Intramuscular administration of the opioid agonist pethidine (50 and 75 mg) (21) or fentanyl (31) to women during labor diminished FHR variation for up to 30 min. Naloxone injection and therefore further changes in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation but only in the naloxone group.

A strong negative relationship between basal FHR and LTV or STV has been found in the human fetus (23, 24). A negative correlation between basal FHR and LTV has also been described in the pig fetus (7) and confirmed in the present study in the pretreatment control recordings. Interestingly, this negative relationship was still present after drug administration but only in the naloxone group. The increase in basal FHR seen after naloxone administration was not accompanied by a significant decrease in FHR variation. Naloxone blocks or terminates fetal arousal in the sheep (18) but when administered to near-term pregnant women (0.4 mg iv) an increase in fetal body movements was seen (2). We found that intravenous administration of 1 mg of morphine to the pig fetus induced an increase in the incidence of LM, whereas the administration of naloxone (1 mg iv) caused an increase in the incidence of GM. Interestingly, except for the increase in FHR, naloxone does not exert effects different from those of morphine in the pig. Such an apparent paradoxical action of naloxone in the pig has already been described for other fetal functions. Bolus administration of both morphine (1 mg) and naloxone (1 mg) resulted in impaired luteinizing hormone secretion in fetal and neonatal pigs (4, 29). Whether these paradoxical effects of naloxone are due to the absence of the δ-receptor in the perinatal period in the pig is not known (20, 28). However, it is important to recognize...
that although morphine and naloxone induced an increase in FM, the stimulated movement patterns were different in nature. This suggests that morphine and naloxone act at different receptors in the nervous system controlling FM.

An interesting phenomenon was observed after morphine administration. LMs not only increased in frequency but also they occurred in clusters (Fig. 4). Apart from direct observation of these LMs during ultrasound scanning, this clustering was also subjectively felt by the hand of the observer during Doppler FHR monitoring. This type of movement occurred in a stereotyped manner (i.e., repetitively and with no obvious function) and is abnormal because it was never seen in the saline-treated fetuses or in other pig fetuses (8). To the best of our knowledge, this is the first time that a stereotyped behavior has been observed in a mammalian fetus.

The role of endogenous opioids in stereotyped behavior of adult sows has been extensively studied (32). When a sow is tethered she develops stereotyped behavior within 1 mo, which is probably regulated via both μ- and κ-receptors in the brain cortex (41). Naloxone administration to a sow showing stereotyped behavior reduces the total time spent on stereotyping, mainly by reducing the mean duration of single bouts of stereotypies (41). In the present experiment, morphine initiated stereotyped behavior in the fetus by increasing the duration and incidence of LM bouts.

In conclusion, our study demonstrates that an opioid tonus is present in the pig fetus during the last 3 wk of gestation and that opioids are apparently involved in controlling FHR and fetal kinetics. A more prolonged treatment with naloxone or more specific antagonists should disclose whether the previously observed decreases in FHR and FM occurring during late gestation (7, 8) can be influenced.

We thank the technical staff of the Department of Physiology (Institute for Animal Science and Animal Behaviour, PAL, Neustadt, Germany) for skillful and dedicated assistance. We also thank Pie (Institute for Animal Science and Animal Behaviour, FAL, Neustadt, D-Neuenrade, Germany) for skillful and dedicated assistance. We also thank Pie (Institute for Animal Science and Animal Behaviour, FAL, Neustadt, D-Neuenrade, Germany) for skillful and dedicated assistance.

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