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Effects of Chronic Opiate Administration on Spontaneous Activity of Fetal Rats

MARGARET L. KIRBY

Department of Anatomy, Medical College of Georgia, Augusta, GA 30912

AND

STEPHEN G. HOLTZMAN

Department of Pharmacology, Division of Basic Health Sciences, Emory University, Atlanta, GA

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KIRBY, M. L. AND S. G. HOLTZMAN. *Effects of chronic opiate administration on spontaneous activity of fetal rats.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 263–269, 1982.—No studies have been reported previously on fetal behavior after chronic morphine administration during the period of opiate receptor ontogenesis. In the present study, pregnant rats were injected with 20 mg/kg/day divided into either two or four daily doses. The injections were made from day 12 through the end of gestation. On days 18–20, the effect of morphine and naloxone on fetal activity in the pretreated animals was assessed by visual observation and quantification. Animals exposed twice daily to morphine showed a morphine-induced depression in spontaneous activity similar to animals pretreated with saline while animals injected four times daily developed tolerance to the depressant effect of the morphine. Animals injected with either morphine or levorphanol were hyperactive after challenge with naloxone. Animals injected with saline or dextrorphan or pair-fed were not hyperactive after exposure to naloxone. Even though behavioral changes were seen *in utero* after injection of morphine, 30-day neonates which had been treated identically prenatally did not show changes in the analgetic response to morphine.

Spontaneous activity Morphine Naloxone Tolerance Withdrawal Rat fetuses

SEVERAL behavioral and morphological aberrations have been identified in offspring of rats exposed chronically to morphine during gestation. Postnatal behavioral changes include alteration in spontaneous locomotor activity and seizure susceptibility [4,17] and decreased analgesic effect of morphine [8,18]. Morphological alterations include changes in total body weight and brain weight [20, 24, 25] decreases in cytoplasmic volume of selected brain nuclei, and decrease in cortical thickness [17]. In addition to these postnatal changes in behavior and morphology, Tsang and Ng [25] have shown alterations in the postnatal pattern of regional opiate receptor development in brains of animals exposed prenatally to morphine.

Recently, this laboratory has demonstrated that 18-day rat fetuses exposed to morphine four times daily from day 12 to 18 of gestation have a 20% reduction in spinal cord volume as compared to controls. Spinal cords of fetuses from animals pair-fed with morphine-injected animals suffer a 10% reduction in volume [13]. The rats in that study were treated from day 12 of gestation in order to coincide with the period of opiate receptor development in the fetuses. Opiate receptors appear on the 14th day of gestation in fetal rat brain [2,3] and on the 16th day in fetal rat spinal cord [14]. These receptors mediate a dose-dependent depression of fetal spontaneous activity by morphine which is reversed by naloxone [10]. The receptors in the spinal cord have been shown to be sensitive to morphine as soon as spontaneous activity begins, late on the 15th day of gestation [15]. The present study was undertaken to determine whether aberrations can be identified in 18–20 day fetal spontaneous activity after chronic exposure to morphine during the period of opiate receptor development.

METHOD

Pregnant Wistar rats were obtained from Harlan-Sprague-Dawley (Indianapolis, IN) on the 6th day of gestation. The rats were weighed and divided into six groups with the mean weight of each group approximately the same. All of the rats were placed in wire-bottomed metabolism cages so that food intake could be measured. The cages were located in one room with 12-hour light/dark cycle and constant temperature and humidity. The animals were weighed daily and all groups were fed powdered lab chow ad lib until the 12th day of gestation.

Injections

Beginning on the 12th day of gestation all the rats were injected every 6 hours as follows: group 1 received 1 ml of physiological saline. Groups 2 and 3 received 20 mg/kg/24-hour period of morphine (morphine sulfate, calculated as free base, Mallinckrodt) dissolved in physiological saline. In group 2, 5 mg/kg of the morphine was injected every 6 hours while in group 3, 10 mg/kg of the morphine was injected at 12-hour intervals with saline being injected at intervening 6-hour intervals. In this manner both groups received an

identical amount of morphine and four injections in every 24-hour period; however, group 2 received 5 mg/kg of morphine in every injection, while group 3 received 10 mg/kg of morphine in every other injection. Group 4 received 0.8 mg/kg of dextrorphan every 6 hours whereas group 5 received 0.8 mg/kg of levorphanol every 6 hours. This dose of levorphanol is equianalgesic with 5 mg/kg of morphine [19]. The levorphanol and dextrorphan were generously supplied by Hoffman-LaRoche.

Feeding Schedule

Groups 1–5 were fed ad lib. Each day the food intake for animals injected with saline, 5 mg/kg of morphine four times daily and 10 mg/kg of morphine twice daily, was measured. The mean amount of food intake for animals injected with 5 mg/kg of morphine four times daily was determined and that amount was made available to each animal in group 6 for the subsequent day. In this way group 6 was pair-fed with animals receiving 5 mg/kg of morphine four times daily.

Behavioral Studies

Spontaneous activity of one fetus in each dam was observed during an acute experiment (lasting one hour) on day 18, 19 or 20 of gestation. The observation period was scheduled such that the dam had not received morphine for a minimum of 12 hours or a maximum of 18 hours.

The method for observation of fetuses *in utero* and quantification of the data has been reported previously [10,11] and will be summarized here. The dams to be studied received midthoracic spinal cord transections under ether anesthesia. Ether has been shown previously not to affect fetal motility if appropriate times are used for recovery [11]. The dams were immobilized and the hindquarters and abdomen submerged in a bath of physiological saline (approximately 300 mOs at 37°C). The abdomen was opened and the uterine horns allowed to float outside the abdominal cavity in the warm saline. Twenty minutes was allowed for acclimatization of the fetuses and recovery from the ether.

Spontaneous activity of one fetus in each dam was observed through the uterine wall and recorded by an observer pressing an event marker wired into a Grass Model 3 polygraph. Activity was monitored constantly for 60 minutes divided into the following periods. During the first 20 minutes a control record of fetal activity was obtained. After the first 20 minutes, morphine was injected subcutaneously into the dam and the fetus observed for a second 20-minute period. After 40 minutes, naloxone (generously supplied by Endo Laboratories) was injected subcutaneously into the dam and the fetus observed for a final 20-minute period. Both drugs were injected in 1 ml of physiological saline. At the termination of the observation period, several fetuses from each dam were weighed and the fetal age determined using the formula of Knox and Lister-Rosenoer [16].

The amount of time each fetus spent in spontaneous movement was calculated as a percentage for each 1-minute interval of the record. These points were plotted on 1/10-inch grid graph paper and connected to obtain a time-activity curve. The area under a comparable 15-minute interval of the curve during the control period and after administration of each drug was determined using a planimeter. Area 1 represents the area measured during the control period, area 2 is the area after morphine administration and area 3 is the area measured after naloxone injection. Area 2 was compared across all drug treatments by analysis of covariance using area 1 as the covariate. Area 3 was compared also using area 1 as covariate.

TABLE 1

MATERNAL WEIGHT GAIN BETWEEN DAYS 12–18 OF GESTATION

Group	Treatment	n	Mean* Weight Gain ± S.E.M. (g)
1	Saline (Control)	16	46 ± 2
2	Morphine, 5 mg/kg four times daily	28	24 ± 3
3	Morphine, 10 mg/kg twice daily	24	26 ± 2
4	Dextrorphan	4	43 ± 6
5	Levorphanol	5	17 ± 6
6	Pair-fed with group 2	6	20 ± 3

*Weight gain is significantly different ($p<0.01$) by analysis of variance. Groups 1 and 4 are different from groups 2, 3, 5 and 6 using a Tukey multiple range test.

Postnatal Analgesia Testing

Some animals from groups 1, 2 and 3 were transferred to solid-bottom cages on the 21st day of gestation when all injections were terminated. These dams were allowed to deliver and suckle their offspring. Litters were adjusted to 8–9 pups 24-hours after parturition. At 29 and 30 days postnatally, analgesia was measured with the hotplate method of Eddy and Leimbach [6] as described previously [18]. A licking of the fore- or hindpaws was used as the endpoint for the determination of response latencies. In order to establish a control response latency, each subject received two test trials 30 minutes apart. The first trial acquainted the animals with the test procedure; the response latency in the second trial served as the control. The temperature of the hot-plate surface was maintained at 52.0±0.1°C. Animals failing to react to the thermal stimulus within 60 seconds were removed from the hot-plate surface and assigned a 60-second response latency.

Offspring were injected with 2.5 mg/kg or 5.0 mg/kg of morphine immediately after determining the control response latency. Analgesia was assessed by measuring response latencies at 20-minute intervals for the duration of the analgesic effect.

A time-effect curve was constructed for each animal and areas under the curves were calculated and compared using a Student's *t*-test.

RESULTS

As had been reported previously morphine-injected animals consumed 25–30% less lab chow than saline-injected controls [13]. This is true for animals injected with either 10 mg/kg of morphine twice daily or 5 mg/kg of morphine four times daily. Body weight gain in morphine and levorphanol-injected animals and pair-fed animals was significantly less than the weight gain of saline- or dextrorphan-injected animals (Table 1). The pregnant rats in all groups appeared healthy and did not evince great sensitivity in the injection site. The fetuses appeared healthy and no malformations were noted in fetuses in any of the litters.

TABLE 2

SPONTANEOUS ACTIVITY MEASURED IN RESPONSE TO VARYING DOSES OF MORPHINE AND NALOXONE IN 82 FETUSES FOLLOWING CHRONIC EXPOSURE TO DRUGS

Group	Treatment	n (Fetuses observed)	Area 1	Morphine mg/kg	Area 2	% Activity $\frac{\text{Area}^2}{\text{Area}^1} \times 100$	mg/kg	Naloxone Area 3	% Activity $\frac{\text{Area}^3}{\text{Area}^1} \times 100$
1	Saline (Control)	6	2202	1.25	1933	96	0.1 –0.5	3283	149
		5	2638	5.0	1610	61	0.25–1.0	2694	102
		5	2944	10.0	1318	45	0.5 –1.0	2168	70
		9	2667	20.0	936	35	0.5 –2.5	2019	76
		3	2685	40.0	1203	45	0.5 –5.0	2803	104
2	5 mg/kg Morphine 4 times daily	6	3224	10	3906	121	0.5 –1.5	4969	154
		2	626	15	481	77	0.25–	2213	354
		8	666	20	358	54	0.5 –1.0	1460	219
		5	719	40	458	64	0.1 –1.0	1689	235
		3	1194	80	787	66	0.1 –0.5	3196	268
3	10 mg/kg Morphine 2 times daily	2	1123	5	836	74	0.1 –0.5	2537	226
		6	4215	10	1716	41	0.25–1.0	6038	143
		4	937	20	500	53	0.25–0.5	2865	306
		5	2300	40	1395	61	0.1 –1.0	2945	128
4	0.8 mg/kg Dextrorphan 4 times daily	3	4336	10	1994	46	0.5 –1.0	3912	90
5	0.8 mg/kg Levorphanol 4 times daily	3	2718	10	877	32	0.5 –1.0	3999	147
6	Pair Fed with morphine-injected animals	7	995	20	534	54	1.0	1364	137

Fetal Spontaneous Activity

The effects of acute morphine administration on fetal spontaneous activity have been reported previously [10]. After injection of saline into the dam every six hours from day 12 to days 18–20 of gestation the fetal response to morphine administered acutely during the behavioral study is identical to that of fetuses in dams which have not been handled or injected prior to the observation period. Maximum depression of activity is seen after 20 mg/kg representing 36% of control activity. The ED_{50} for the depression (68% of control) is slightly greater than 1.25 mg/kg (Table 2).

Maternal injection of 5 mg/kg of morphine four times daily during the same period produces tolerance to the depressant effect of morphine on fetal activity. Thus the dose-response curve from 10 mg/kg to 20 mg/kg undergoes a shift to the right (Fig. 1). Injection of 10 mg/kg during the observation period causes a significant increase ($p<0.01$) in fetal activity to 120% of control activity. Maximum depression of activity occurs after 20 mg/kg as in uninjected animals. Maximum depression in animals treated with 5 mg/kg of morphine four times daily is 54% of control acitvity and is not significantly different from maximum depression in saline pretreated animals.

Fetuses exposed twice daily to 10 mg/kg of morphine do not show tolerance to challenge doses of morphine up to 10 mg/kg. However, maximum depression of activity is seen

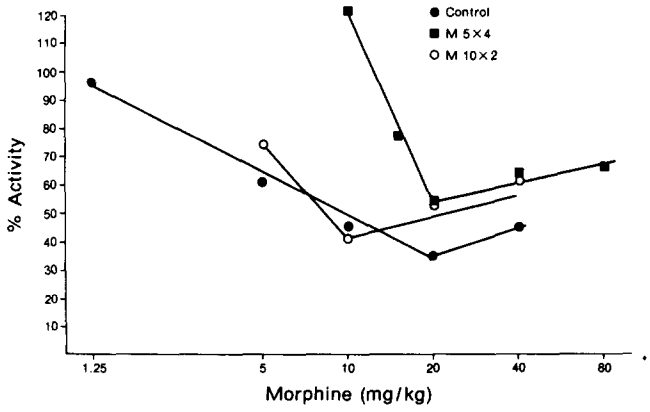

FIG. 1. Spontaneous activity of 18–20 day rat fetuses following injection of morphine during the observation period. Activity after morphine injection is expressed as % of control (Area 2/Area 1×100). The dams were pretreated with saline four times daily (Control); 5 mg/kg of morphine four times daily (M5×4); or 10 mg/kg of morphine two times daily (M10×2) as explained in the text.

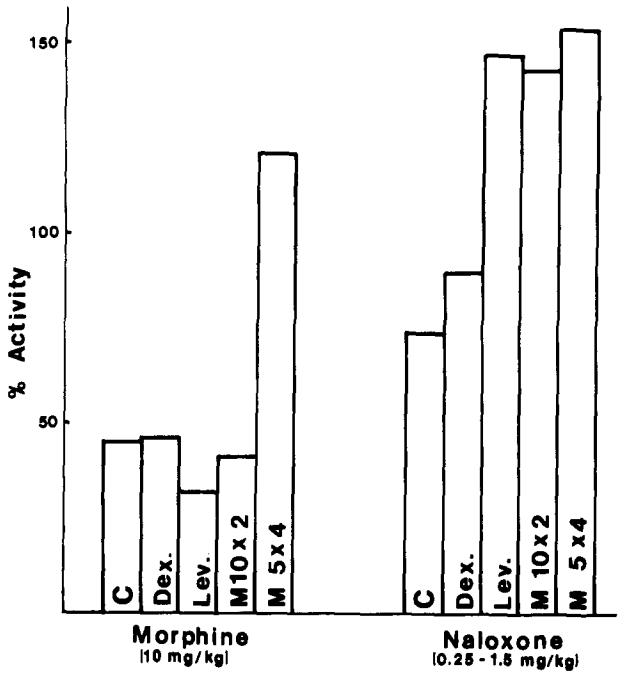

FIG. 2. Spontaneous activity of 18–20 day rat fetuses following morphine (10 mg/kg) or naloxone (0.25–1.5 mg/kg) injection during the observation period. Activity after the injection is expressed as % of control. The dams were pretreated with saline four times daily (C); 5 mg/kg of morphine four times daily (M5×4); 10 mg/kg of morphine two times daily (M10×2); 0.8 mg/kg of dextrorphan four times daily (Dex.); or 0.8 mg/kg of levorphanol four times daily (Lev.) as explained in the text.

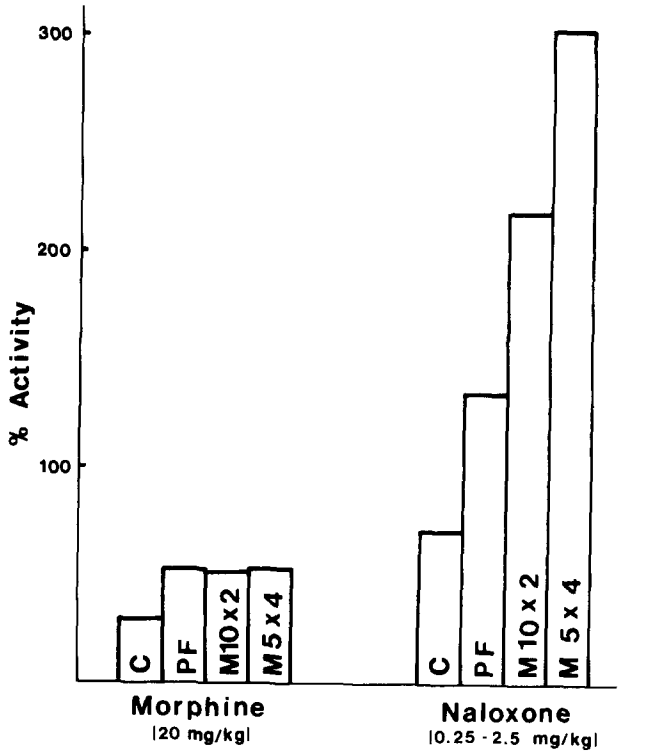

FIG. 3. Spontaneous activity of 18–20 day rat fetuses following morphine (20 mg/kg) or naloxone (0.25–2.5 mg/kg) injection during the observation period. Activity after the injections is expressed as % of control. The dams were pretreated with saline four times daily (C); 5 mg/kg of morphine four times daily (M5×4); 10 mg/kg of morphine two times daily (M10×2); or pair-fed with morphine treated animals (PF) as explained in the text.

after a 10 mg/kg challenge. Maximum depression is 41% of control activity. At 20 and 40 mg/kg, fetal activity is identical to that in animals treated with 5 mg/kg of morphine four times daily. Following a challenge of 10 mg/kg of morphine, activity in fetuses pretreated with either dextrorphan or levorphanol is similar to that in animals pretreated with saline or 10 mg/kg morphine twice daily (Fig. 2). Activity in animals pair-fed with morphine pretreated animals is similar to that of saline-pretreated animals after 20 mg/kg morphine challenge (Fig. 3).

Response to Naloxone

Animals which have been exposed to morphine prior to the observation period show an immediate increase in spontaneous activity followed by an extended period of hyperactivity after naloxone injection (Fig. 4). In animals injected with 10 mg/kg of morphine twice daily, varying doses of naloxone cause elevations in fetal activity to 130 to 170% of control activity. In animals treated with 5 mg/kg four times daily, 0.1 mg/kg of naloxone has no effect while doses of 0.25 mg/kg or greater cause elevations in activity between 180 and 274% of control activity. Although activity is increased to 166% of control in saline-injected animals after 0.1 mg/kg of naloxone, no elevation of activity above control levels occurred after higher doses of naloxone (Fig. 4).

Naloxone causes hyperactivity in animals pretreated with levorphanol (147%) but not with dextrorphan (90%) (Fig. 2). In pair-fed animals, naloxone reverses the effect of morphine without causing hyperactivity characteristic of withdrawal (Fig. 3). Figure 4 shows a reconstruction of % activity from Table 2 after injection of varying doses of naloxone in

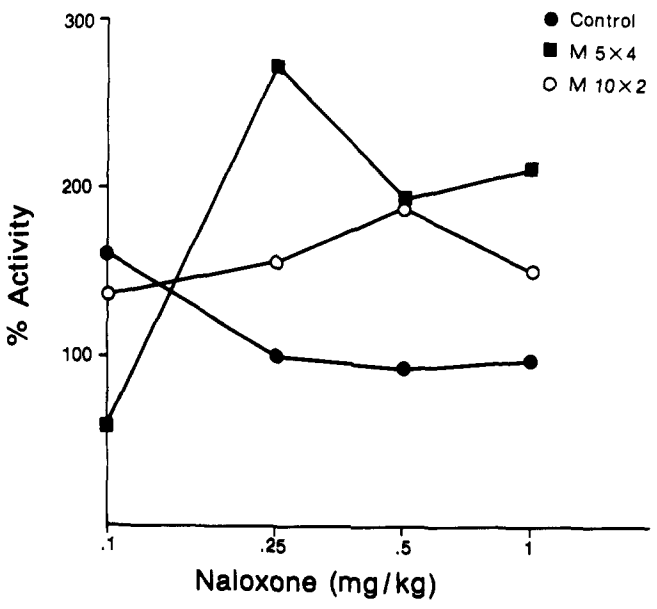

FIG. 4. Spontaneous activity of 18–20 day rat fetuses following injection of naloxone during the observation period. Activity after naloxone injection is expressed as % of control (Area 3/Area 1×100). The dams were pretreated with saline four times daily (Control); 5 mg/kg of morphine four times daily (M5×4); or 10 mg/kg of morphine two times daily (M10×2) as explained in the text.

TABLE 3

SPONTANEOUS ACTIVITY OF FETUSES RESPONDING TO NALOXONE FOLLOWING CHRONIC PRETREATMENT WITH VARIOUS OPIATES

Group	Treatment	n	Area 1	Naloxone	Area 2	% Activity $\frac{\text{Area}^2}{\text{Area}^1} \times 100$
1	Saline (Control)	2	5124	0.5	5912	115
3	10 mg/kg Morphine 2 times daily	2	609	0.25	6094	1001
4	0.8 mg/kg Dextrorphan 4 times daily	2	910	0.5	1194	131
5	0.8 mg/kg Levorphanol 4 times daily	1	17	0.5	77	453

Area 2 in Groups 3 and 5 are significantly different from Groups 1 and 4 ($p<0.01$).

TABLE 4

SENSITIVITY OF RATS EXPOSED TO SALINE OR MORPHINE *IN UTERO* TO THE ANALGESIC EFFECT OF MORPHINE AT 30 DAYS POSTNATALLY (MEAN ± SEM)

Group (n=8)	Treatment	Baseline (sec)	Morphine Dose 2.5 mg/kg (Area of Analgesia)	5.0 mg/kg (Area of Analgesia)
1	Saline (Control)	8.7 ± 0.3	872 ± 59	2069 ± 169
2	5 mg/kg Morphine 4 times daily	9.2 ± 0.5	803 ± 68	2231 ± 118
3	10 mg/kg Morphine 2 times daily	8.9 ± 0.6	879 ± 66	2341 ± 190

animals which were pretreated chronically with saline, morphine four times daily and morphine twice daily. All of these animals have been exposed to morphine during the observation period which might alter the fetal response to naloxone. Therefore, in a few observations, morphine was not injected prior to naloxone during the observation (Table 3). Naloxone was injected immediately after a control record of activity was obtained. In animals chronically treated with morphine, but not exposed to morphine during the observation period, the hyperactivity following naloxone injection is more striking than in animals which have been injected with morphine during the observation period.

Postnatal Analgesia

No differences in the control response latency between the morphine and saline offspring are observed. Morphine produces similar dose-related increases in response latency in the hot-plate test in both morphine and saline offspring at 30 days of age (Table 4).

DISCUSSION

Morphine abstinence syndrome has been reported previously in human [21] and rat fetuses [10]. However, this is the first report of the development of fetal tolerance *in utero* after chronic maternal injection of an opiate. We have also shown the development of dependence on morphine without the concomitant development of tolerance (measured using fetal spontaneous activity) by altering the injection schedule without changing the total daily dose of the drug. The prolonged period of hyperactivity following injection of naloxone indicates that the fetuses are dependent. As this manifestation of withdrawal is not seen in animals injected chronically with saline or dextrorphan, but only in animals injected with morphine or levorphanol, it does appear to be a reliable measure of opiate abstinence.

The depressant effect of morphine on fetal rat spontaneous activity cannot be predicted from morphine effects on adult spontaneous activity. Fetal spontaneous activity is depressed by all the doses of morphine used thus far [10]. This depression is seen throughout the period of fetal spontaneous activity, and is not altered by transection of the fetus' spinal cords [15]. In adult animals, small doses of morphine (1–4 mg/kg) produce an initial stimulation of locomotor activity. Doses larger than 4 mg/kg have a biphasic effect with an initial depression of activity which is followed by elevated activity. As the dose of morphine increases, the depression of activity lasts for a longer period of time [5]. Babbini and Davis [1] showed that chronic administration of morphine

results in tolerance development to the depressant but not the stimulant actions of the drug on rat locomotor activity. As tolerance develops to the depressant actions of morphine, the stimulant actions are enhanced [23].

In the preparation presented here, fetal activity is depressed at all doses in animals acutely injected with morphine. However, it must be kept in mind that the effect of morphine on fetal spontaneous activity is measured for only 20 minutes after the injections, since the preparation is inherently in a tenuous position. Thus, it would be possible not to observe a biphasic response to morphine. Since 10 mg/kg of morphine in tolerant fetuses caused an increase in fetal activity (120% of control), it appears that tolerance to the depressant effect may have caused an unmasking of the stimulating effect of morphine in the fetus. It is equally possible that chronic exposure to morphine has caused some basic change in the fetal response to morphine, the neural circuitry or biochemical homeostasis of the fetal nervous system.

The development of tolerance in animals injected every 6 hours but not in animals injected every 12 hours is difficult to explain. Morphine has been shown to decline rapidly in fetuses by about 6 hours after injection [12] even though traces remain for up to 12 hours after maternal administration. It is quite possible that fetuses injected every 12 hours undergo a period of withdrawal (hyperactivity) between injections while animals exposed to morphine every 6 hours do not manifest withdrawal signs between injections. Thus the fetuses exposed every 6 hours develop tolerance in order to overcome the depressant effects of the drug and maintain a certain circadian level of spontaneous activity. It is paradoxical that an equipotent dose of levorphanol injected every 6 hours does not produce tolerance since the duration of action of morphine and levorphanol in adult animals is similar [19]. Perhaps the disposition and fate of these drugs in fetuses and/or gestating females is different.

As tolerance and dependence have been demonstrated *in utero* using this drug schedule, it is surprising that the animals do not have an abnormal response to morphine-induced analgesia postnatally. Studies by Steele and Johannesson [20] and O'Callaghan and Holtzman [18] have shown changes in the analgesic effect of morphine in offspring of morphine-treated mothers. All of these studies have used the hot plate method to measure analgesia. The period of drug injection in these studies is different and this may explain the difference in the postnatal analgetic response. Johannesson and Becker [8] and Steele and Johannesson [20] administered 20 mg/kg subcutaneously to pregnant rats on days 17 to 20 of gestation and found postnatal tolerance to morphine analgesia accompanied by lower brain and body weights, with lower total amounts of brain DNA, RNA and protein which were paralleled by decreased incorporation of radioactive precursors into brain macromolecules. O'Callaghan and Holtzman [18] injected pregnant rats with 20 mg/kg/day administered twice daily on days 5–12 of gestation. These investigators were also able to demonstrate a change in the analgesic effect of morphine postnatally.

In conclusion, this is the first study illustrating fetal tolerance to morphine and characterizing the fetal withdrawal response.

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