

Effects of prenatal morphine treatment of rats on mortality, bodyweight and analgesic response in the offspring

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A method is presented for the maternal ingestion of morphine in rats in order to investigate morphine's prenatal effects. Effects on the offspring of the treatment throughout pregnancy were compared with effects of treatment during specific periods of the pregnancy. Morphine treatment (25 mg/kg per day) throughout pregnancy resulted in 0% survival of the offspring. Morphine treatment up to gestational day 21 resulted in $65.4 \pm 7.7\%$ survival of the offspring, and up to gestational day 17 in $31.8 \pm 13\%$ survival. Reduction in the neonatal birth weight was maximal in animals treated with 25 mg/kg of morphine per day from gestational day 4 throughout pregnancy. The analgesic effect of morphine in the offspring was tested with the hot plate method. Male offspring treated with morphine from gestational day 1 to day 17 showed an elevated thermal response after morphine injections on day 80–90 post partum. These findings indicate a long-lasting, post-partum alteration in that part of the opiate system involved in the perception of noxious stimuli.

Key words: morphine; prenatal treatment; analgesia; rat

Introduction

It is well known that rats treated prenatally with morphine exhibit psychological and behavioural abnormalities as compared to untreated animals. Furthermore, an increased locomotor activity at an early age has been attributed to prenatal opiate exposure in rats and humans [1,5]. In addition, prenatal morphine treatment is known to induce a growth retardation of the offspring [1,2]. Long-lasting alterations in the thermal response after opiates have been reported [2,3]. Little is known of the underlying neurochemical mechanisms. The hazards of opiate treatment and its withdrawal in utero, as well as at delivery, have been elucidated in terms of increased numbers of stillborn pups, and perinatal death of prenatally exposed rodents [1,4].

The most common method used to administer morphine when studying prenatal exposure is via maternal i.p. injections. One possible

difficulty with this technique could be the need to avoid caloric imbalances between control and experimental animals. In addition, blood morphine levels vary between day and night. We have developed a method utilizing the administration of a morphine-admixed liquid diet [6]. This method allows morphine ingestion under equivalent caloric conditions for experimental and control animals. The animals become physiologically dependent and the concentrations indicate an even plasma level.

The aims of the present study were to investigate the effects of maternal morphine ingestion during specific periods of pregnancy on three parameters in the offspring: (1) mortality, (2) body weight, and (3) thermal response (analgesia).

Materials and methods

Female Sprague–Dawley rats weighing 250 g (A-lab Stockholm) were caged individually

(41 × 25 × 15 cm³) in a constant room temperature, lights on between 0700 and 1700 h. The fluid diet was administered in graded Richter tubes. The consumption and weight of the animals were determined daily between 1600 and 1700 h.

The ingredients of the diet were mixed to a stabilized solution [6] and 90 ml of liquid diet were administered to each rat every 24 h.

Analgesia was measured with the hot-plate method, [7] surface temperature maintained at 52 ± 0.1 °C. Licking of the fore or hind paw or attempts to jump were used as end points when determining response latency. In order to establish a basal response latency, each subject received one test trial 30 min before injection. The thermal response was determined immediately after the injection of 7.5 mg/kg of morphine and 20, 30, 40, 55, and 75 min after the injection. Animals not reacting within 60 s were removed from the surface and assigned a 60-s response latency. Control animals were injected with saline.

Statistical analyses of survival in offspring were performed according to the Kruskal Wallis one-way analysis of variance followed by the Mann-Whitney U test. Statistical analyses of weight and thermal response of offspring after morphine were done according to Student's *t*-test. S.E.M. values are given.

Protocols

Investigation of offspring mortality after maternal morphine treatment

Experiment I. Twenty naive female animals were divided into four groups of five animals per group and were mated. After a 4-day mating period ingestion was initiated and drug doses were gradually increased to reach 50, 100 and 150 mg/kg per day respectively (Fig. 1A). Drug treatment was maintained throughout the pregnancy and ended 2 days after delivery. Group four received no morphine and served as control.

Experiment II. Twenty-two female rats were divided randomly into three groups of five animals per treatment group, and seven

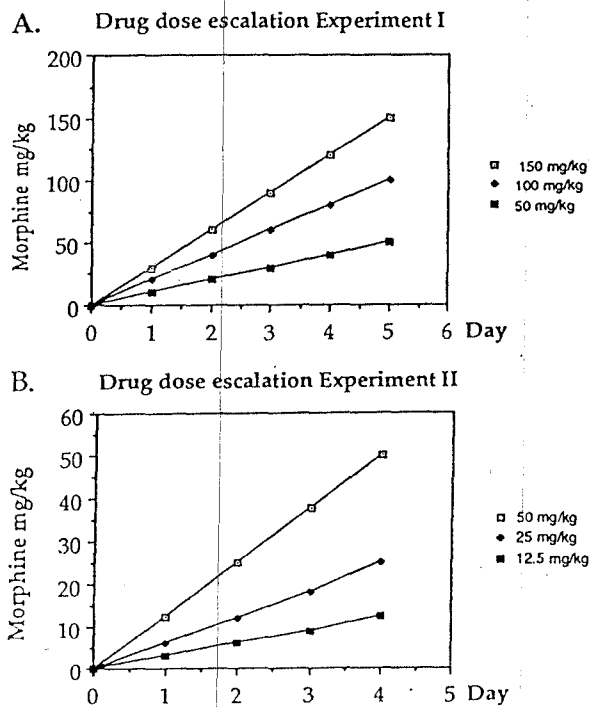


Fig. 1.

animals in the control group. Drug treatment was initiated after a 4-day mating period. The drug doses were escalated according to Fig. 1B. Drug treatment was maintained throughout pregnancy and ended 2 days after delivery. The control group received a liquid diet without morphine.

Mortality was calculated as the number of surviving pups day 16 post partum divided by the total number of delivered pups in one litter. The average survival rates were calculated within all treatment groups.

Offspring survival rate and alterations in sensitivity to the analgesic action of morphine after treatment of pregnant rats during selective periods of pregnancy

This experiment consisted of seven groups of female rats (Fig. 2). The animals were challenged at different gestational profiles of morphine exposure, with a maximum dose of 25 mg/

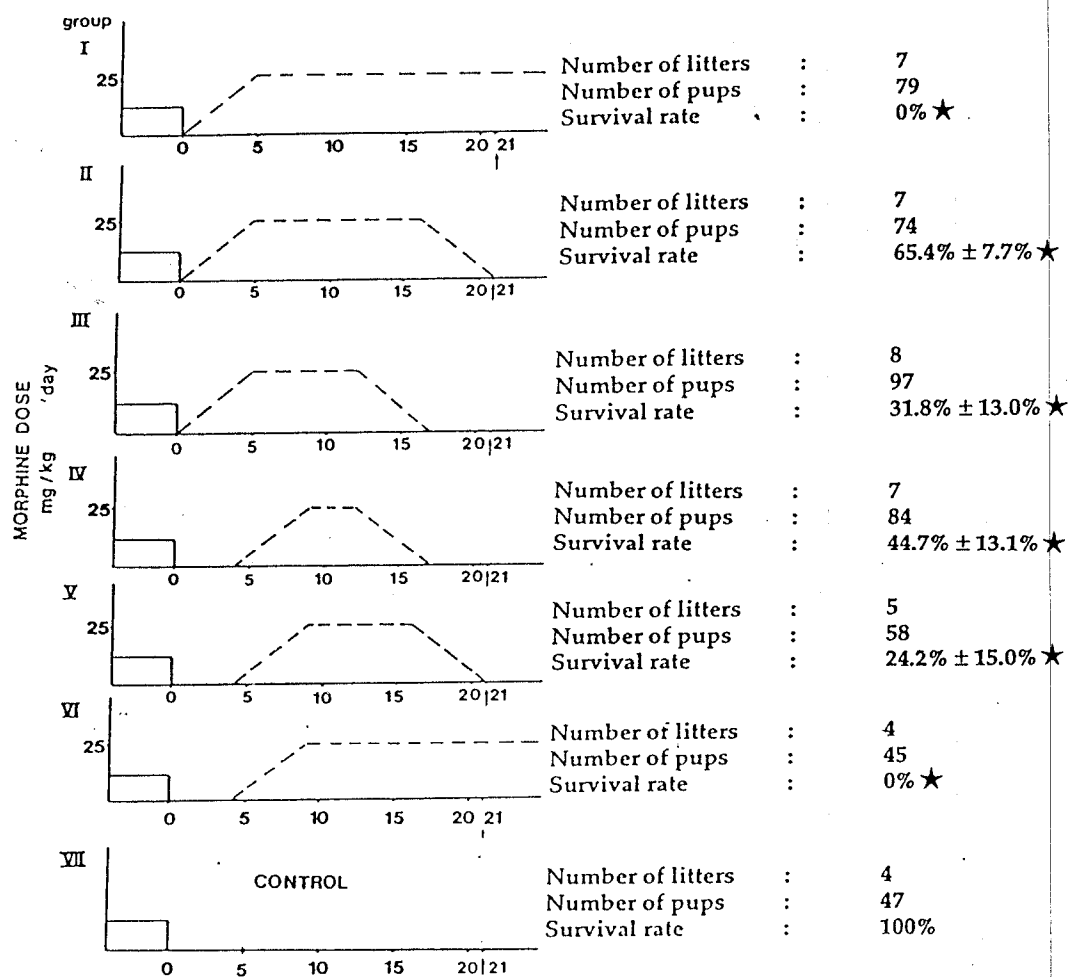


Fig. 2. Treatment schedules at different periods of gestation. The resulting survival rates are calculated as mean survival rate in one group based on survival in each litter. S.E.M. values are given. U-values in all experimental groups compared with controls were 0. Arrow = delivery. * $P < 0.01$.

kg per day. In group seven, four non-treated female rats were mated and allowed to litter normally. These control animals were kept under the same isocaloric conditions as the animals in the experimental groups. The day of estrus was established by examination of the vaginal secretions and was denoted as day 1 of pregnancy.

Male littermates were tested for their thermal response after injection of morphine at the age of 80–90 days. Twelve animals from four different litters were randomly selected from

each treatment group. Each animal was administered 7.5 mg/kg morphine.

Three control experiments were performed. In each experiment 12 male rats, 80–90 days old were injected with 5, 7.5, and 10 mg/kg of morphine, respectively.

Results

Offspring survival after maternal morphine treatment

In experiment I the survival was 0% in all

Table I. Survival rates (%). Survival rates were calculated as numbers of surviving offspring divided in total number of offspring per group. The mean survival rate in a group is based on survival in each litter. The numbers in parentheses designates the number of pregnant rats in each group. S.E.M. values are given. Statistical analyses according to Kruskal-Wallis one-way analysis of variance followed by Mann Whitney U-test. U-values in all experimental groups compared with controls were 0. * $P < 0.01$, ** $P < 0.001$.

Experiment I	Control	50 mg/kg	100 mg/kg	150 mg/kg
	100 (4)	0* (4)	0* (4)	0* (4)
Experiment II	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
	100 (12)	62 ± 11** (8)	5 ± 7** (7)	0** (7)

three experimental groups, while no mortality was recorded in the control group. In experiment II, the end doses were lowered. Animals prenatally treated with 12.5 mg/kg showed a survival rate of 62 ± 11%. In the group treated with 25 mg/kg the survival rate was 5 ± 7%, and treatment with 50 mg/kg, resulted in 0% survival rate (Table I).

Offspring survival rate and alterations in sensitivity to the analgesic action of morphine after treatment of pregnant rats during selective periods of pregnancy

The maternal weight gain did not differ statistically among the treated females compared with controls during pregnancy. The offspring in groups I and VI showed a 0% survival rate (Fig. 2). Offspring of animals in groups II, III, IV and V, showed a survival rate of 65.4 ± 7.7%, 31.8 ± 13.0%, 44.7 ± 13.1% and 24.2 ± 15.0%, respectively. All groups differed significantly from the control group in respect to survival. No sex differences were noted among the offspring. The birth weight of all members of treated litters was significantly lower compared with controls (Fig. 3). The major part of the mortality among offspring to treated animals was noted during the first 5 days post partum (Fig. 4). The weight gain of all members of treated litters was significantly reduced up to day 19 compared with controls. Thereafter, no significant differences in weight gain were recorded (Fig. 5).

In male control animals the thermal response after 5, 7.5 and 10 mg/kg of morphine reached peak values in 20 min in a dose

dependent manner (Fig. 6). In groups II, III and IV, a hyposensitivity to the thermal stimulus was recorded, compared with controls. The number of surviving pups from group V was too small for analgesia testing. The thermal response was elevated in all tested male groups. Prenatal treatment according to group III gave the most prominent alterations toward morphine hypersensitivity in male rats.

Discussion

Prenatal exposure to opiates is considered to affect a variety of developmental processes. Several investigators have noted the retarded growth rate in the offspring of rats following pre- and perinatal opiate administration [1,2,8,9,10]. Other more complex parameters

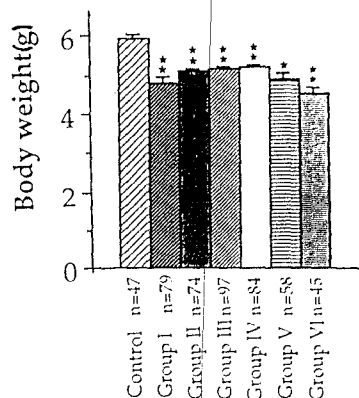


Fig. 3. Neonatal mean birth weights in all treated groups compared with controls. Pups were weighed immediately after delivery. Statistical evaluation according to Student's *t*-test. * $P < 0.01$; ** $P < 0.001$.

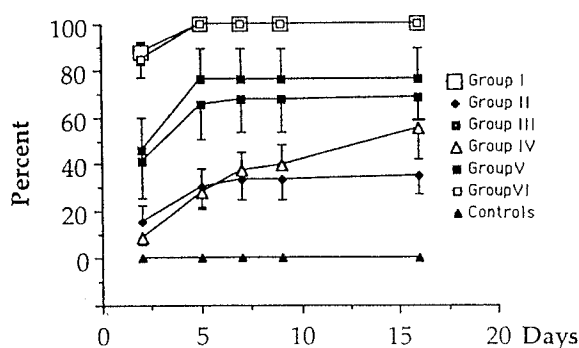


Fig. 4. Time scale of mortality for the different groups. S.E.M. values are given.

affected in rats are altered sexual behaviour [11] and differences in postnatal motor activity, with hyperactivity [1,12]. Postnatal motor alterations have also been reported among infants born to opiate-addicted mothers [5]. A possible teratogenic effect of morphine has been suggested to be responsible for the decreased postnatal weight and increased peri- and postnatal death rate in rats [14]. Johannesson et al. [2] were unable to demonstrate any teratogenic effect after prenatally administered morphine. No gross anomalies were found in the present study either.

It has been pointed out that abstinence in utero could possibly result in an increased perinatal mortality [15]. The peri- and postnatal mortality has been noted to increase by several investigators [1,16] after prenatal morphine treatment. However, Johannesson et al. [2] found a significant increase in maternal mortality without any significant effects on the number of implanted fetuses, neither on peri- nor postnatal mortality. In the present study we found a marked increase in peri- and postnatal mortality in prenatally treated litters, but no difference in the amount of pups per litter between experimentals and controls. The most prominent offspring mortality in experiment II was noted when animals in groups I and VI were subjected to abrupt morphine withdrawal. This could support the role of abstinence as a lethal factor in the offspring.

The reduced birth weight of litters in all morphine-treated groups could possibly be attributed to the general growth-retarding effect of morphine [4,17] and/or an endocrine disturbance at the hypothalamic level [8]. Furthermore, body growth has been shown to be stimulated by opiate receptor blockade [18]. The weight gain of the surviving pups was lower than that of the controls. The treated offspring show a catch-up in weight gain and, after day 19 postnatally, no significant differences in body weight were recorded as to controls. This could partly be due to an over-mortality among the animals of lowest weight.

An increased sensitivity to the analgesic effect of morphine in 80–90-day-old prenatally treated male offspring was noted in the present study. This is in line with earlier studies in which enhanced sensitivity to the analgesic effect of opiates has been reported following prenatal or perinatal treatment [19–21], but is not in line with studies in which prenatally treated animals were tolerant to that effect [2,3,22]. The discrepancy could be attributed to differences in the time of drug administration, as well as the time of analgesia testing. In our study animals were tested on days 80–90, but on days 21–22 as by Johannesson and Becker [2]. A biphasic pattern in the number of naloxone binding sites in prenatally treated rats has been found, with an initial phase of decreased binding sites, followed after day 60 by an increase in naloxone binding sites in the spinal cord, as compared with controls [23]. Prenatal treatment with methadone (Delta and μ -receptors agonist) has been shown to induce a decrease in opioid receptor and in α_2 adrenoceptor density [24] in the cerebral cortex of the rat. Alterations within the striatum after prenatal treatment with μ -receptor agonists have been shown in terms of decreased receptor density in the striatal dopamine system [25]. Thus, the striatum cannot be ruled out as a possible target. However, these changes are probably coupled mainly to motor alterations. Since the thalamus receives input from the spinal cord and the para-aqueductal gray as part of the pain signal system, and since these areas are

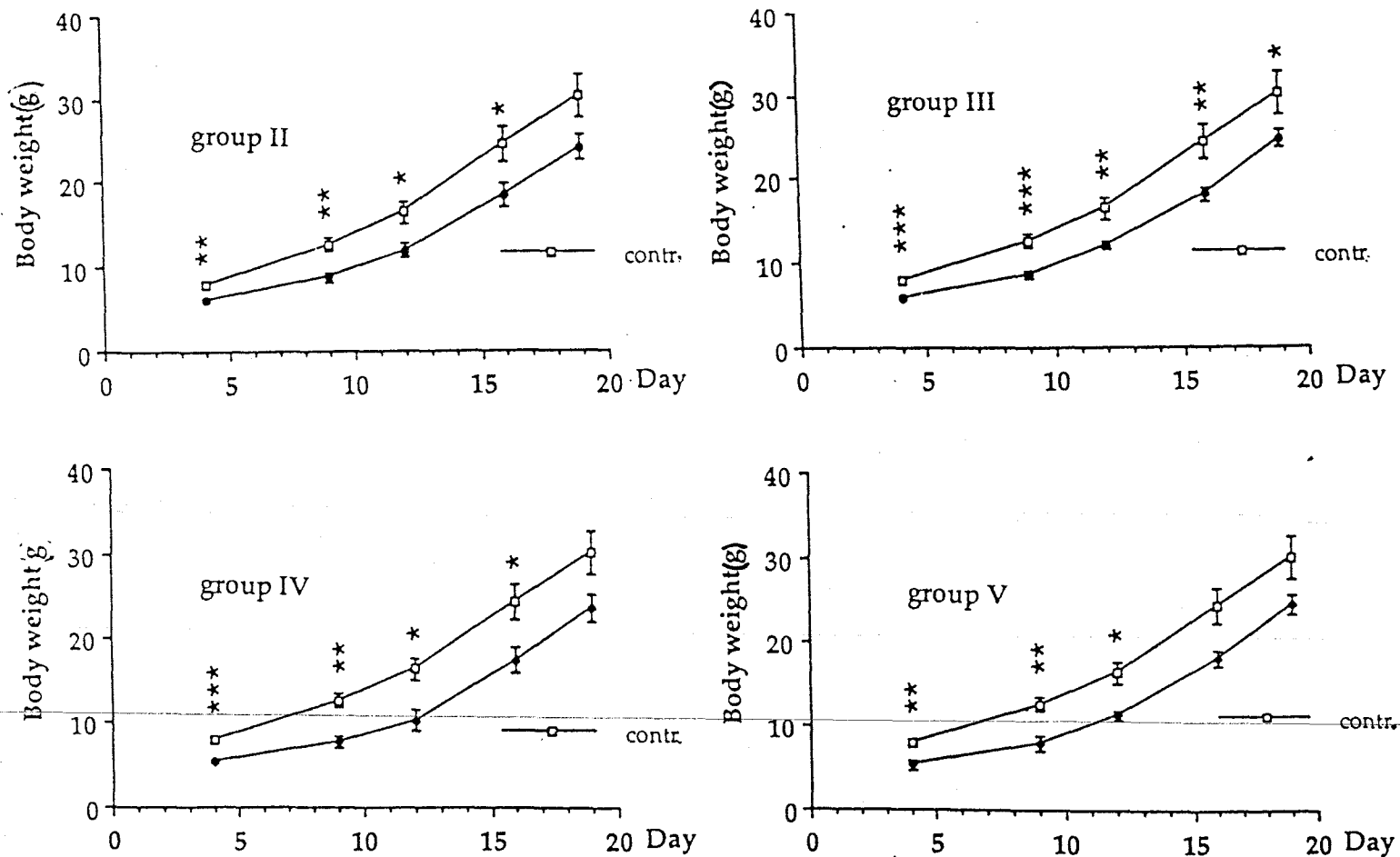


Fig. 5. Weight gain of surviving pups in experimental groups II, III, IV, V (see Fig. 2) vs. weight gain in the control group. The control group consisted of 41 pups from 4 different litters. The male/female ratio was approximately 1 ($\pm 7\%$) in all groups. S.E.M. values are given. Statistical evaluation according to Student's *t*-test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

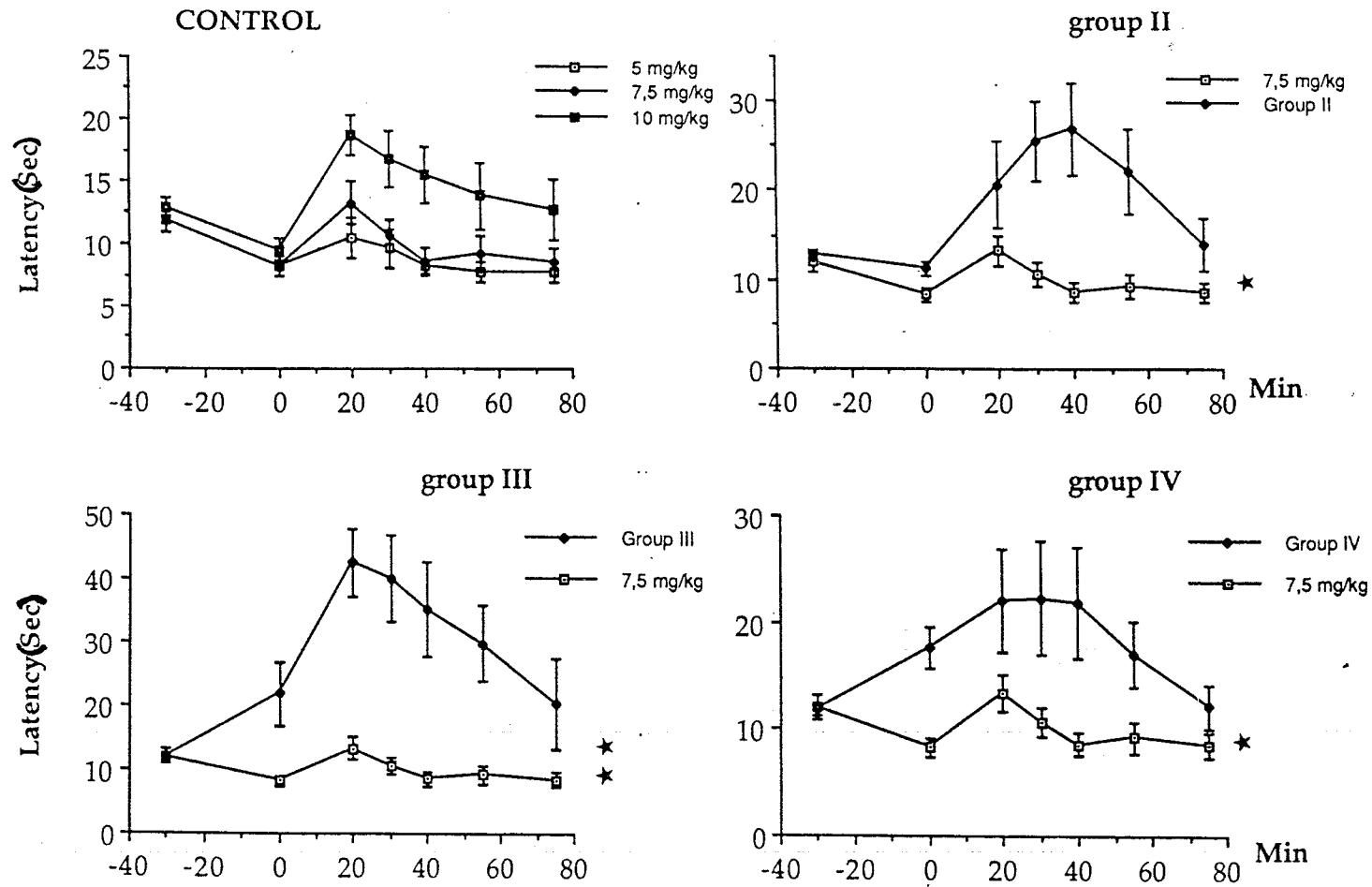


Fig. 6. Thermal response to morphine. Three control experiments were performed each using twelve male rats injected with 5, 7.5, or 10 mg/kg of morphine, respectively. Hot plate latency of 12 male rats each from treatment groups II, III, and IV, respectively, compared with 12 controls. Areas under the curve were calculated and compared with control areas. (Student's *t*-test). **P* < 0.01; ***P* < 0.001.

known to exhibit μ -receptors [26], the thalamus could possibly also be affected.

Endogenous opiate receptors have also been shown to be involved in the regulation of neuro-ontogeny since the blockade of opioid receptors with receptor antagonists results in an increased neuronal maturation in the rat brain [27], as well as an altered neurobehavioural development [28]. Since the most prominent effect on morphine-analgesia was registered in offspring treated prenatally from day 1 to day 17, morphine may exert its effects in ontogenically older parts of the CNS involved in pain sensation, such as the substantia gelatinosa of the spinal cord, the peri-aqueductal gray or the thalamus [28–30]. In our study the peak mortality did not coincide with this pattern, indicating partly different mechanisms.

An enhanced sensitivity to the morphine analgesia was noted in all animals prenatally treated with morphine. This could indicate a common mechanism or point of action on specific parts of the pain system depending on the stage of ontogenic development at the time of drug administration. The common point of action could be the opioid receptors (preferentially the μ -receptor), e.g. alterations in the receptor density or in the transducing systems. In search for mechanisms explaining the effects of prenatal morphine treatment, one possible way could be to study the opiate system at the cellular and receptor level.

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