Perinatal lead exposure impairs opioid but not non-opioid stress-induced antinociception in developing rats

¹Helen C. Jackson & Ian Kitchen

Department of Biochemistry, Molecular Toxicology and Pharmacology Group, University of Surrey, Guildford, Surrey, GU2 5XH

- 1 The development of opioid systems has been shown to be sensitive to perinatal exposure to lead. We have studied the effects of such exposure on opioid and non-opioid mediated stress-induced antinociception in developing rats.
- 2 Lead was administered in the maternal drinking water from conception to postnatal day 14 at 300 and 1000 p.p.m. Twenty and 25 day old rats were subjected to swimming stress and antinociception measured using the tail immersion test.
- 3 A 3 min swim-stress induced an opioid-mediated antinociceptive response in 20 day old rats which was attenuated by 300 p.p.m. lead and by 1000 p.p.m. lead treatment in a dose-related manner. A 10 min swim-stress induced a non-opioid mediated antinociceptive response in 25 day old rats which was not antagonised by 300 or 1000 p.p.m. lead.
- 4 Naloxone antagonised the residual antinociception observed in 20 day old animals treated with 300 p.p.m. lead and had no effect on antinociception in control or lead-treated 25 day old rats.
- 5 Using a lead exposure model considered to represent subclinical lead toxicity in man, it was shown that perinatal lead exposure disrupts opioid but not non-opioid mediated stress antinociception.

Introduction

There are clear indications that the developing child is particularly susceptible to neurotoxic effects of lead and indeed that environmental exposure to this metal might be responsible for certain behavioural abnormalities (Royal Commission Report, 1983). Experimental animal studies also indicate susceptibility to lead toxicity during the perinatal period and some neurotransmitter systems have been identified as being particularly sensitive to exposure to this metal. In this respect we have observed marked toxic effects of low level perinatal lead exposure on the development of opioid peptides (Bailey & Kitchen, 1985), opioid receptors (McDowell & Kitchen, 1988) and opioid-induced antinociception (Kitchen *et al.*, 1984; Kitchen & McDowell, 1985) in the rat, using a model of lead exposure which gives blood levels representative of subclinical lead toxicity.

We have extended our investigations on opioid-induced antinociception to examine the effect of perinatal lead exposure on the phenomenon of stress-induced antinociception early in development. Recently we demonstrated that short swims produce opioid-mediated stress-induced antinociception in 20 day old rats, whereas longer swims at later ages (25 days) induce antinociception via non-opioid mechanisms (Jackson & Kitchen, 1989). Accordingly, the effects of lead exposure on opioid and non-opioid stress-induced antinociception have been studied at day 20 and day 25 respectively.

Methods

Animals and lead dosing

Wistar albino rats (University of Surrey strain) of either sex were used in all experiments and maintained at a constant temperature ($21 \pm 1^\circ\text{C}$) and

¹ Author for correspondence at: Reckitt & Colman Psychopharmacology Unit, The Medical School, University Walk, Bristol, BS8 1TD.

Table 1 Effect of lead on nociceptive responses in unstressed 20 and 25 day old rats using the tail immersion test

Dose of lead (p.p.m.)	Response latency (s)	
	20 days	25 days
0	2.0 ± 0.1	2.7 ± 0.2
300	1.6 ± 0.1	2.0 ± 0.1*
1000	1.5 ± 0.1*	2.0 ± 0.1*

Each value is the mean of at least 16 observations. The data represent pretest values for animals subsequently used as unstressed control groups or swim stressed groups. Significant differences from 0 p.p.m. groups are denoted by * $P < 0.05$.

light/dark cycle (lights on at 07h 00min). Litters were culled to 8 pups per dam at birth. Lead was administered as originally described by Carmichael *et al.* (1981) with the modifications of McDowell & Kitchen (1988). In brief, lead was administered in the maternal drinking water (as the acetate dissolved in distilled water acidified with glacial acetic acid) from conception (-21 days) to postnatal day 14. Two doses of lead were used in this study (300 p.p.m. and 1000 p.p.m.) and mothers of control litters were

treated with a corresponding solution of acetic acid (28 mM). Maternal weight and fluid intake were monitored throughout and litter size and neonate weight gain measured in the postnatal period. Neonates were weaned at day 21.

Stress-induced antinociception

Stress-induced antinociception was examined in 20 and 25 day old pups treated with 0 p.p.m., 300 p.p.m. or 1000 p.p.m. lead. Warm water swimming (20°C) for 3 min at day 20 or 10 min at day 25 was used as the stressor. All animals used in this study were handled regularly and injected on the day of test to control for any stressful effects of these procedures and to enable meaningful comparison between all experimental groups. Procedures were carried out in a quiet, windowless, air conditioned laboratory and animals equilibrated to these conditions for 2 h before use. Rat pups remained in the home cage except during drug administration, swimming and antinociceptive testing. Each series of determinations represents measurements made on at least four separate litters in each lead treatment group, on at least two separate days. Each animal was tested only once and all experiments were carried out between 13 h 00 min and 17 h 00 min.

Each litter was divided into four groups. One group received 0.9% saline i.p. and remained in the home cage as an unstressed control group. The other 3 groups were injected i.p. with either saline or with 1 or 10 mg kg⁻¹ doses of the opioid antagonist naloxone (using a blind procedure and a dose volume of 0.1 ml 20 g⁻¹). Ten minutes later animals were placed individually in a swimming area 14 cm in diameter and 12 or 20 cm deep for 20 and 25 day old rat pups, respectively. At the end of the swimming period pups were gently dried. Antinociception was assessed using the tail immersion test. Rats were hand-held over a hot water bath with the terminal 1–2 cm portion of their tails in water at a temperature of 50°C. This noxious stimulus produced a distinct tail flick response and tail immersion latencies (i.e. the time taken for rats to remove their tails from the water) were measured to the nearest 0.1 s using a stop-watch. Nociceptive responses were determined 10 min before swimming stress and 1, 5, 10, 15, 30, 45 and 60 min after swimming. Treatment groups were statistically compared by use of analysis of variance and Dunnett's test.

Figure 1 Effect of perinatal lead exposure on antinociception induced by a 3 min swim in 20 day old rats. Values represent means for groups of at least 8 animals (vertical lines show s.e.mean). (●) 0 p.p.m. Pb; (○) 300 p.p.m. Pb; (■) 1000 p.p.m. Pb. Significant differences from the 0 p.p.m. Pb control group are denoted by * $P < 0.05$.

Results

Baseline nociceptive latencies in unstressed lead-treated rats were lower than in the corresponding unstressed control (0 p.p.m.) group, an effect

Figure 2 Effect of naloxone on antinociception induced by a 3 min swim in 20 day old rats exposed to (a) 300 p.p.m. and (b) 1000 p.p.m. lead. Values represent means for groups of at least 8 animals (vertical lines show s.e.mean). (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (■) naloxone (1 mg kg^{-1}), swim-stressed; (□) naloxone (10 mg kg^{-1}), swim-stressed. Significant differences from unstressed controls are indicated by $*P < 0.05$ and from the swim-stressed controls by $\dagger P < 0.05$.

Figure 3 Effect of perinatal lead exposure on antinociception induced by a 10 min swim in 25 day old rats. Values represent means for groups of at least 8 animals (vertical lines show s.e.mean). (●) 0 p.p.m. Pb; (○) 300 p.p.m. Pb; (■) 1000 p.p.m. Pb. Significant differences from the 0 p.p.m. Pb control group are shown by $*P < 0.05$.

observed in both 20 and 25 day old animals (Table 1). A 3 min swim stress in 20 day old rats produced a 3–4 fold increase in nociceptive latency 5 min after the stress in the control (0 p.p.m.) group. This stress-induced antinociception persisted for 15 min (Figure 1). A 3 min swim produced significantly less antinociception in 20 day old rats treated with either 300 p.p.m. lead or 1000 p.p.m. lead than in the 0 p.p.m. control group. The nociceptive latencies of 300 p.p.m.-treated rats subjected to a 3 min swim stress were, however, still greater than those of the unstressed 300 p.p.m.-treated group, as shown in Figure 2a. The antinociception induced by swimming in these 20 day old animals was partially attenuated and completely antagonised by 1 and 10 mg kg^{-1} naloxone respectively, an effect also observed in the control 0 p.p.m. group (data not shown). The much smaller increases in tail immersion latencies induced by a 3 min swim in 1000 p.p.m.-treated pups were only significantly elevated over those of unstressed controls during the first 5 min post-stress (Figure 2b). These responses were not significantly reversed by naloxone, although nociceptive latencies of stressed animals treated with the highest dose of naloxone were similar to those of controls at 1 and 5 min after swimming, suggesting that naloxone was having

some effect on the residual stress-induced antinociception observed in these animals. The doses of naloxone used in the present study had no effects on baseline tail immersion latencies *per se* (data not shown).

In 25 day old rats, a 10 min swim produced a 2–3 fold increase in nociceptive latency 5 min after stress and this antinociception was observed in control and both lead-treated groups. However, nociceptive latencies in lead-treated animals returned to baseline values more rapidly and were significantly lower than those of the 0 p.p.m. group 45 and 60 min after stress (Figure 3). Swim stress-induced antinociception in 25 day old control groups was unaffected by 1 and 10 mg kg⁻¹ naloxone (data not shown) and this lack of effect of the opioid antagonist was also observed in 300 (Figure 4a) and 1000 p.p.m. lead-treated animals (Figure 4b). Figure 4a and b also show the tail immersion latencies of unstressed 300 and 1000 p.p.m.-treated pups. These animals showed small increases in response latencies during the first 15 min of the test. This effect was consistently seen throughout the study (see also Figure 2a and b) and is likely to reflect the stress induced by an injection of saline.

There were no significant effects of lead on maternal or neonate weight gain or on maternal fluid intake.

Discussion

Several studies have shown changes in nociception threshold after inorganic lead treatment and the majority point to a reduced sensitivity to pain (Baraldi *et al.*, 1985; Bondy *et al.*, 1985; Vickers & Paterson, 1986). In the present study there were indications of the opposite effect and previous experiments in our laboratory have suggested that lead-induced changes in response to noxious stimuli are age- and test-dependent (Kitchen *et al.*, 1984; Kitchen & McDowell, 1985). Antinociception induced by a short period of swimming stress is reversed by naloxone and is clearly opioid in nature (Terman *et al.*, 1986; Jackson & Kitchen, 1989). Stress-induced antinociception has been demonstrated to involve the release of endogenous opioid peptides (for review see Millan, 1981) and lead may produce antagonism of stress-induced antinociception by disruption of synthesis, storage or release of these peptides. In support of this, perinatal lead exposure has been shown to disrupt markedly opioid peptides in the postnatal period (Bailey & Kitchen, 1985) and disruption of dopamine/opioid interneurons by lead has also been proposed (Govoni *et al.*, 1980; Memo *et al.*, 1981). Alternatively, attenuation of this effect by lead might indicate

Figure 4 Lack of effect of naloxone on antinociception induced by a 10 min swim in 25 day old rats exposed to (a) 300 p.p.m. and (b) 1000 p.p.m. lead. Values represent means for groups of at least 8 animals (vertical lines show s.e.mean). (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (■) naloxone (1 mg kg⁻¹), swim-stressed; (□) naloxone (10 mg kg⁻¹), swim-stressed. There were no significant differences between swim-stressed controls and naloxone-treated groups. All 3 groups showed significant ($P < 0.05$) antinociception during the first 30 min post-stress.

an opioid receptor blocking activity of this metal. Indeed, reduced antinociceptive effects of morphine and ketocyclazocine have been observed after similar perinatal lead exposure (Kitchen *et al.*, 1984; Kitchen & McDowell, 1985). However, we have been unable to demonstrate antagonism by lead (up to 10^{-6} M) of μ -opioid responses in opioid-sensitive isolated tissues (Kitchen & Kelly, unpublished) and the effects of lead on binding do not indicate major disruption of μ -receptor sites (McDowell & Kitchen, 1988). Therefore lead may also be acting at the post receptor level.

The stress-induced antinociception produced by a 10 min swim in 25 day old rats is insensitive to antagonism by naloxone and hence is mediated via non-opioid mechanisms (Jackson & Kitchen, 1989). The lack of effect of lead on this type of stress-induced antinociception clearly points to toxic effects of lead on opioid function and not to generalised toxicity upon perception of noxious stimuli. Indeed,

there were no indications of overt toxicity using the lead dosing protocol and previous studies have demonstrated that blood lead levels at weaning are below $15 \mu\text{g } 100 \text{ ml}^{-1}$ at the highest lead dose (McDowell & Kitchen, 1988).

There is also evidence from biochemical studies that the development of opioid systems are more sensitive to the effects of lead than other neurochemical systems (Bailey & Kitchen, 1986). Whether this developmental disruption of opioid systems produces long-lasting toxic effects into adulthood is unclear, but the question of opioid functioning in man should be addressed where there is evidence of behavioural abnormalities associated with lead exposure.

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