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Perinatal lead exposure alters the development of δ- but not μ-opioid receptors in rat brain

Julia McDowell & [1]Ian Kitchen

Department of Biochemistry, Division of Pharmacology & Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH

1 Low level lead exposure has been shown to impair the development of opioid peptide levels in the brain, and to impair antinociceptive responses to opioid drugs. We have now studied the effects of lead exposure on the development of opioid receptors using ligand binding studies.

2 The ontogenesis of μ- and δ-opioid binding sites was studied using rat whole brain membranes and [^{3}H]-[D-Ala^{2}MePhe^4-Gly-ol]enkephalin and [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin as binding ligands. Rats were exposed to lead during development by addition of lead acetate (at 100–1000 p.p.m.) to the maternal drinking water from conception to postnatal day 14.

3 Perinatal lead exposure had no significant effects on the binding affinity (K_D) or binding capacity (B_{max}) for the μ-opioid receptor measured at postnatal days 10, 21 and 30. Lead exposure (at 1000 p.p.m.) increased the K_D for the δ-opioid receptor at postnatal days 15, 21, 35 and 50 but had no effect on the binding capacity. No indications of overt toxicity were observed and blood lead levels were in the ranges considered to represent subclinical lead toxicity in man.

4 The lack of effect of lead on μ-receptor binding contrasts with previously described impairment of antinociceptive effects of μ-agonists suggesting that the toxicity is not manifested at the μ-binding site. However, the δ-opioid receptor appears to be more sensitive to lead exposure and the persistent changes in δ-site affinity after cessation of lead exposure suggest irreversible damage in the production of the receptor protein.

Introduction

Certain behavioural abnormalities have been associated with environmental exposure to lead in children (Royal Commission Report, 1983) and the possible mechanisms underlying this behavioural toxicity has been the subject of several experimental animal studies over the last 20 years (for review see Winder & Kitchen, 1984). The dopaminergic system has been identified as being sensitive to low level lead exposure and, recently, in our laboratory we have shown disruption of endogenous opioid systems using an experimental model of lead exposure which gives blood lead levels in neonatal rats corresponding to levels representative of sub-clinical lead toxicity (Royal Commission Report, 1983). This disruption is indicated by delayed ontogeny of striatal proenkephalin products which can be reduced by 90–100% in lead-treated animals at postnatal day 10 (Bailey & Kitchen, 1985). These toxic effects appear to be selective, since monoamines and γ-aminobutyric acid are unaffected by the same lead treatment (Bailey & Kitchen, 1986). Other indications of toxic effects on opioid systems include impaired responses to the antinociceptive actions of the opioid drugs morphine (Kitchen *et al.*, 1984) and ketocyclazocine (Kitchen & McDowell, 1985).

In the light of impaired peptide function and antinociceptive effects of opioids, we have entertained the possibility that the ontogeny of opioid receptor sites in the brain may be altered by perinatal exposure to lead. We describe here the ontogeny of μ- and δ-opioid receptors, using ligands highly selective for these sites, in lead-treated rats.

Methods

Animals and lead dosing

Lead (as the acetate in distilled water) was administered to female Wistar albino rats (University of Surrey strain) in the drinking water as described by

[1] Author for correspondence.

Table 1 Effect of perinatal lead exposure on the ontogenesis of μ-opioid receptor binding capacity

Age (days)	Lead dose (p.p.m.)	[^{3}H]-DAGOL Binding capacity (B_{max}) pmol/brain	fmol mg^{-1} wet wt.	fmol mg^{-1} protein
10	0	3.5 ± 0.46	3.1 ± 0.37	56.2 ± 7.1
	100	3.2 ± 0.26	3.2 ± 0.29	61.5 ± 6.8
	300	3.8 ± 0.42	3.7 ± 0.40	69.4 ± 5.3
	1000	3.6 ± 0.29	3.7 ± 0.28	68.6 ± 5.9
21	0	14.2 ± 1.50	9.4 ± 1.03	134 ± 15.9
	100	15.6 ± 0.60	10.2 ± 0.30	141 ± 4.1
	300	12.6 ± 1.94	8.1 ± 1.19	118 ± 17.8
	1000	12.9 ± 0.97	8.4 ± 0.70	129 ± 9.8
30	0	14.8 ± 1.48	8.8 ± 0.79	120 ± 9.8
	100	19.0 ± 0.85	11.5 ± 0.42	150 ± 10.7
	300	16.6 ± 1.30	10.1 ± 0.90	127 ± 12.9
	1000	15.4 ± 0.77	9.6 ± 0.44	125 ± 7.7

Lead was administered in the maternal drinking water from conception to postnatal day 14. μ-Receptor binding was determined using [^{3}H]-[D-Ala^2-MePhe^4-Gly-ol^5]enkephalin ([^{3}H]-DAGOL) as the binding ligand. Specific binding was >80% in all determinations. Each value represents mean ± s.e. mean of at least 5 Scatchard analyses. One-way analysis of variance for lead groups at each age – no significant differences.

Carmichael *et al.* (1982), with modifications of the dose and duration of administration. Control groups were treated with acetic acid and lead groups were given lead acetate made up in acetic acid. For this study 3 doses of lead were employed: 100, 300 and 1000 p.p.m. and were administered from conception (−21 days) to postnatal day 14. Maternal weight gain and fluid intake were monitored throughout, and litter size and neonate weight gain measured in the postnatal period. Neonates were killed at 10, 21 and 30 days for studies of μ-receptor development and at 15, 21, 35 and 50 days for studies of δ-receptor development. Trunk blood was collected for determination of blood lead levels as previously described (Bailey & Kitchen, 1985) and brain tissue rapidly removed for use in receptor binding assays.

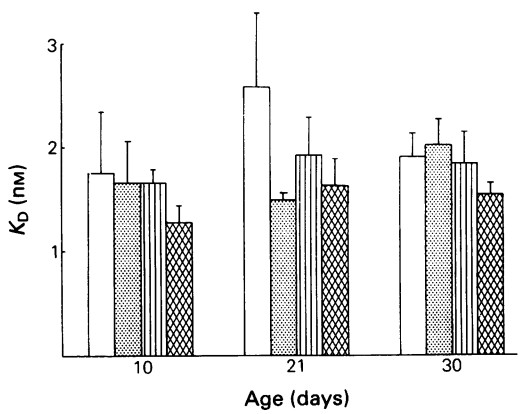

Figure 1 Effects of perinatal lead exposure on the ontogenesis of μ-opioid receptor affinity. Lead (Pb) was administered in the maternal drinking water from conception to postnatal day 14. Open columns, 0 p.p.m. Pb; stippled columns, 100 p.p.m. Pb; hatched columns, 300 p.p.m. Pb; cross-hatched columns, 1000 p.p.m. Pb. μ-Binding was determined using [^{3}H]-[D-Ala^2-MePhe^4-Gly-ol^5]enkephalin as the binding ligand and each value represents mean of dissociation constants determined from at least 5 Scatchard analyses; vertical lines indicate s.e. mean. One-way analysis of variance for lead groups – no significant difference.

Receptor binding assays

Whole brain homogenates were prepared as described by Gillan and Kosterlitz (1982). For neonates up to 21 days, pooled brain tissue was used for each binding assay and for animals >21 days a single brain was employed. For μ-receptor binding [^{3}H]-[D-Ala^{2}MePhe^{4}Gly-ol]enkephalin (DAGOL, 57–60 Ci mmol^{-1}, Amersham) was used as a binding ligand, and for δ-receptor binding [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin (DPDPE, 25–27 Ci mmol^{-1}, Amersham) was used. Both ligands were purified by h.p.l.c. (Bailey & Kitchen, 1985) before use.

Twelve concentrations of DAGOL (40 pM–11 nM) and ten concentrations of DPDPE (0.2–25 nM) were employed in each assay. Binding incubations were carried out in Tris HCl, pH 7.4 at 25°C for 40 min to produce saturation curves; 1 μM diprenorphine and 10 μM naloxone were used to determine non-specific binding of DAGOL and DPDPE, respectively. Scat-

Table 2 Effect of perinatal lead exposure on the ontogenesis of δ-opioid receptor binding capacity

Age (days)	*Lead dose* (p.p.m.)	[^{3}H]-*DPDPE* *Binding capacity* (B_{max}) pmol/brain	fmol mg^{-1} wet wt.	fmol mg^{-1} protein	*Specific binding ratio*
15	0	3.7 ± 0.34	2.7 ± 0.26	47.5 ± 3.6	0.33
	300	3.6 ± 0.41	2.7 ± 0.25	55.3 ± 5.8	0.30
	1000	3.6 ± 0.31	2.8 ± 0.23	49.8 ± 4.5	0.29
21	0	7.4 ± 0.32	4.8 ± 0.23	79.0 ± 3.9	0.48
	300	6.3 ± 0.62	4.2 ± 0.46	73.4 ± 12.7	0.42
	1000	7.4 ± 0.36	4.9 ± 0.17	75.8 ± 3.2	0.45
35	0	10.7 ± 0.77	6.2 ± 0.49	93.1 ± 8.9	0.53
	300	10.5 ± 0.39	6.4 ± 0.13	94.2 ± 5.1	0.53
	1000	14.0 ± 0.51	8.1 ± 0.40	127.2 ± 14.2	0.51
50	0	12.1 ± 1.25	6.4 ± 0.62	102.5 ± 11.0	0.53
	300	10.7 ± 0.71	5.9 ± 0.35	92.7 ± 3.9	0.49
	1000	12.2 ± 1.60	6.6 ± 0.79	97.7 ± 9.9	0.47

Lead was administered in the maternal drinking water from conception to postnatal day 14. δ-Binding was determined using [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin ([^{3}H]-DPDPE) as the binding ligand. Specific binding ratio represents the mean of specific binding divided by total binding. All values represent mean ± s.e. mean of at least 5 Scatchard analyses. One-way analysis of variance for lead groups at each age – no significant differences.

chard analysis was used to determine the equilibrium dissociation constant (K_D) and maximal binding capacity (B_{max}). Values for B_{max} were determined per mg wet weight tissue and per mg protein after determination of protein content by the method of Lowry *et al.* (1951).

Results

Effect of lead on receptor ontogeny

μ-Opioid receptor affinity remained constant during postnatal development but the number of binding sites increased 2–3 fold in the third postnatal week. Perinatal lead exposure had no significant effects on either K_D or B_{max} for [^{3}H]-DAGOL binding at any of the ages studied (Table 1, Figure 1). δ-Opioid receptor affinity remained constant during postnatal development but the number of binding sites increased 2–3 fold between day 15 and day 35 (Table 2, Figure 2). The number of δ-sites measured throughout the postnatal period and up to 50 days was not significantly altered by perinatal lead exposure and in animals treated with 300 p.p.m. lead DPDPE affinity was also not altered. However, in the 1000 p.p.m. lead-treated groups the K_D for DPDPE was increased by up to 50% at all ages studied (Figure 2).

Blood lead levels and effects on body weight

Blood lead levels are shown in Table 3. Peak blood leads were observed at postnatal day 15 immediately after cessation of lead administration to the mother. Thereafter blood lead levels fell and reached control levels by day 30. There were no significant correlations between blood lead levels and DPDPE K_D in

Table 3 Blood lead levels (μg 100 ml^{-1}) in postnatal rats determined by atomic absorption spectrophotometry

Dose of lead (p.p.m.)	*Age* (days) *10*	*15*	*21*	*30*	*35*	*50*
0	1.5 ± 0.9	1.0 ± 0.7	0.39 ± 0.29	0.14 ± 0.1	ND	0.8 ± 0.8
100	16.6 ± 3.0	—	0.90 ± 0.4	1.4 ± 0.5	—	—
300	21.9 ± 3.4	23.9 ± 3.0	7.4 ± 1.3	1.6 ± 0.7	1.2 ± 1.2	0.4 ± 0.4
1000	27.6 ± 2.4	28.2 ± 3.0	15.0 ± 2.1	6.3 ± 2.2	6.5 ± 3.6	ND

Each value represents the mean ± s.e. mean of 5–24 determinations. Lead was administered in the maternal drinking water from conception (−21 days) to postnatal day 14. ND = non detectable.

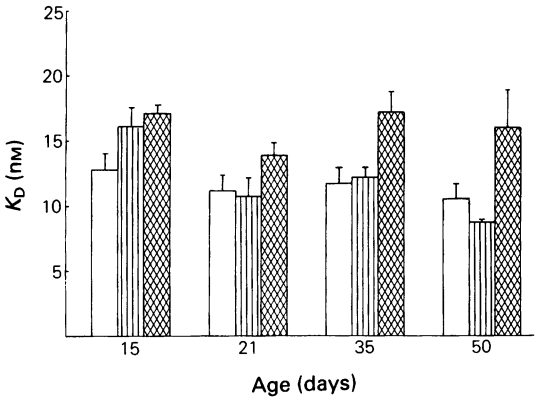

Figure 2 Effect of perinatal lead exposure on the ontogenesis of δ-opioid receptor affinity. Lead (Pb) was administered in the maternal drinking water from conception to postnatal day 14. Open columns, 0 p.p.m. Pb; hatched columns, 300 p.p.m. Pb; cross-hatched columns, 1000 p.p.m. Pb. [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin was used as the binding ligand and each value represents mean of dissociation constants determined from at least 5 Scatchard analyses; vertical lines indicate s.e. mean. One-way analysis of variance for lead groups * $P < 0.05$.

those groups where lead caused changes in site affinity.

There were no significant effects of lead on maternal or neonate weight gain or maternal fluid intake (data not shown).

Discussion

The profile of postnatal development of both μ- and δ-sites in the control groups (0 p.p.m.) was essentially similar to that obtained by ourselves (McDowell & Kitchen, 1986) and others (Spain *et al.*, 1985) previously. Thus, with respect to opioid receptor binding, the acetic acid component is without toxic effect. Despite suggestions that this component may in some instances adversely affect behaviour (Barrett & Livesey, 1982), it is clearly preferable to chronic administration of sodium nitrate which has been shown to increase the number of opioid binding sites (Baraldi *et al.*, 1984). DPDPE non-specific binding was high in both control and lead-treated groups, a problem in the use of this binding ligand for developmental studies (McDowell & Kitchen, 1986).

The failure of perinatal lead exposure to alter binding characteristics at the μ-opioid site is surprising, since opioid-mediated responses such as antinociception are impaired at ages equivalent to those used in this study (Kitchen *et al.*, 1984; Kitchen & McDowell, 1985). This perhaps indicates that the toxic effects on antinociceptive response to opioids are manifested at a site other than the μ-opioid receptor; an obvious possible candidate is the second messenger systems coupled to the μ-site. It is noteworthy that this protocol of perinatal exposure severely suppresses the development of peptide products of proenkephalin (Bailey & Kitchen, 1985) and at ages where these opioid peptides are virtually undetectable after lead exposure (day 10), it does not result in disruption of μ-opioid binding. There are indications, however, from this study that the ontogeny of δ-opioid sites is susceptible to lead exposure, as affinity for DPDPE is decreased. This effect persists into adult animals and abnormal K_D values were recorded 5 weeks after cessation of lead treatment. Since the time for turnover of opioid receptors is likely to be in the order of days, it is probable that the toxicity is manifested at the level of the genetic message for the receptor protein. This effect appears irreversible and is of particular concern, since blood levels of lead correspond to the lower limit for subclinical lead poisoning in man (Royal Commission Report, 1983). It is conceivable that the toxic effects on δ-receptors result from the loss of proenkephalin peptide in early postnatal development, since these ligands are considered to show selectivity at δ-sites and may function as physiological mediators at these receptors (Paterson *et al.*, 1983).

One other study of the effects of lead on opioid receptor development has been presented in the literature (Baraldi *et al.*, 1985) and in this study [^{3}H]-naloxone was used as a binding ligand. A significant increase in the number of sites was observed without a change in affinity. The disparity of these data may reflect the use of the nitrate salt of lead given by gastric intubation since the nitrate salt itself causes changes in B_{max} (Baraldi *et al.*, 1985), and intubation may produce compounding problems of stress-related effects which have been shown to be different in lead-exposed animals (Barrett & Livesey, 1985).

In conclusion, perinatal lead exposure does not alter the ontogeny of μ-opioid receptors but doses of 1000 p.p.m. in the maternal drinking water decrease the affinity of δ-opioid receptors. This change in δ-sites persists after cessation of lead exposure, possibly suggesting irreversible damage in the production of receptor protein.

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