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RESEARCH PAPER

Characterization of spinal α-adrenergic modulation of nociceptive transmission and hyperalgesia throughout postnatal development in rats

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Background and purpose: The selective α_2-adrenergic agonist dexmedetomidine is used clinically for analgesia and sedation, but effects in early life are not well characterized. Investigation of age-related effects of dexmedetomidine is important for evaluating responses to exogenously administered analgesics and provides insight into postnatal function of noradrenergic pathways.
Experimental Approach: We examined effects of epidural dexmedetomidine in anaesthetized rat pups (3, 10 and 21 postnatal days) using a quantitative model of nociception and C-fibre induced hyperalgesia. Electromyographic recordings of withdrawal responses to hindpaw mechanical stimuli measured effects of dexmedetomidine upon the baseline reflex and the response to mustard oil application on the hindpaw (primary hyperalgesia) or hindlimb (secondary hyperalgesia). In addition, we compared epidural with systemic administration, examined effects of spinal transection and evaluated heart rate changes following dexmedetomidine.
Key Results: Epidural dexmedetomidine dose-dependently prevented mustard oil-induced hyperalgesia at all ages but dose requirements were lower in the youngest pups. Higher doses also suppressed the baseline nociceptive reflex when given epidurally, but had no effect when given systemically. Analgesic efficacy was the same for primary and secondary hyperalgesia, and was not diminished by spinal cord transection.
Conclusions and Implications: Our laboratory studies predict that spinally mediated α_2-agonist analgesia would be effective throughout postnatal development, dose requirements would be lower in early life and selective anti-hyperalgesic effects could be achieved with epidural administration at doses lower than associated with antinociceptive or cardiovascular effects. Clinical trials of α_2 agonists in neonates and infants should consider developmentally regulated changes.
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Keywords: dexmedetomidine; α_2-adrenergic agonist; postnatal development; primary hyperalgesia; epidural

Abbreviations: AUC, area under curve; DRG, dorsal root ganglion; ERK, extracellular signal-regulated kinase; MPE, maximum possible effect; P, postnatal age; RMS, root mean square; TRP, transient receptor potential

Introduction

Dexmedetomidine is a potent highly selective α_2-adrenergic agonist (Buerkle and Yaksh, 1998) that is increasingly used in clinical practice for sedation and analgesia (Paris and Tonner, 2005). In paediatric patients, preliminary reports of systemic dexmedetomidine use include short-term procedural sedation or premedication (Koroglu *et al*., 2006; Mason *et al*., 2006), reduction of postoperative pain and agitation (Guler *et al*., 2005) and more prolonged infusion for sedation or facilitation of opioid withdrawal in intensive care patients (Finkel *et al*., 2005; Hammer *et al*., 2005; Chrysostomou *et al*., 2006). The pharmacodynamic profile of dexmedetomidine has been investigated in adult volunteers (Cortinez *et al*., 2004; Hsu *et al*., 2004), but effects in infants and children are not well characterized (Paris and Tonner, 2005).

Investigation of the postnatal pharmacodynamic properties of α_2 agonists is important for understanding mechanisms and evaluating responses to exogenously administered analgesics, but also provides an insight into the function of noradrenergic pathways. The developmental regulation of these pathways affects the ability of the immature nervous system to control responses to acute noxious stimuli through endogenous mechanisms. Inadequate modulation of nociceptive input in early life may lead to persistent

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changes in somatosensory processing (Fitzgerald and Walker, 2003).

The antinociceptive properties of α_2-adrenergic agonists are well described in adult animal models (Bol *et al*., 1999; Asano *et al*., 2000), but age-related changes during early development have only recently been investigated in rat pups. The efficacy of systemic dexmedetomidine in the formalin test has been reported to be independent of age from postnatal day (P)7 to adult (Sanders *et al*., 2005), but at earlier ages (P3–5), lower doses suppressed the response to formalin (Otsuguro *et al*., 2005). The dose of epidural dexmedetomidine required to reverse behavioural inflammatory hyperalgesia was also lower at P3 than P10 and P21 (Walker *et al*., 2005). Age-dependent sedative effects have been reported following both epidural and systemic administration, again with increased sensitivity in younger rat pups (Sanders *et al*., 2005; Walker *et al*., 2005), but the effect of postnatal age on cardiovascular side effects has not been investigated.

Recently, we developed a model of mustard oil-induced hyperalgesia in anaesthetized rat pups, which allows quantitative evaluation of the reflex response (Walker *et al*., 2007). This has several advantages for testing antinociceptive and antihyperalgesic efficacy. Electromyogram (EMG) recordings provide better quantification of the reflex response than behavioural observations, and allow evaluation of responses to both threshold and suprathreshold stimuli, which may be more relevant to the clinical experience of pain. Dose-dependent effects can be measured upon nociceptive baseline responses and upon hyperalgesic changes in the reflex response produced by mustard oil application. We have used this model to further examine the pharmacodynamic effects of epidural dexmedetomidine at three different postnatal ages. To establish the site of action, we have compared epidural with systemic administration, examined the effects of spinal transection and also compared the dose–response for epidural dexmedetomidine against primary and secondary hyperalgesia. Furthermore, we evaluated changes in heart rate following epidural dexmedetomidine at all ages. Significant age-dependent changes in the pharmacodynamic profile of epidural dexmedetomidine were found, with increased sensitivity to spinally mediated antihyperalgesic, antinociceptive and cardiovascular effects in early development.

Methods

All experiments were performed under personal and project licences in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986. Male and female Sprague–Dawley rat pups aged 3,10 and 21 days (P3, P10 and P21) were obtained from the UCL Biological Services Unit.

Surgical preparation

The model for primary and secondary hyperalgesia using EMG recordings in lightly anaesthetized rat pups has been described previously (Walker *et al*., 2007). Briefly, animals were anaesthetized with halothane (2–4% initially) in oxygen and subsequently ventilated (Harvard Apparatus Ltd) via a tracheostomy. Heart rate was continuously monitored with an electrocardiograph (Vektronics ERM-8010, UK) and recorded at 5-min intervals. Body temperature was monitored with a rectal probe in P10 and P21 pups and a surface electrode in P3 pups. Normothermia was maintained with a thermostatically controlled heat source. Pups were placed in a small animal spinal frame and one hindlimb was secured in slight extension and plantar flexion on a fixed platform using a double-sided self-adhesive pad with the plantar surface of the paw exposed for cutaneous stimulation.

The effect of spinal transection was investigated in P21 pups. A single level laminectomy was performed in anaesthetized pups and the upper thoracic cord was visualized and divided. After 4-6 h recovery, reflex movements of the hindlimbs had returned and the animals were then prepared as above.

Epidural injection technique

Epidural injections were performed with a sterile 30G needle attached to a glass 50 or 100 μl Hamilton syringe. A midline lumbar incision was performed and a lower lumbar transverse process identified after blunt dissection of the right paraspinal muscles. The epidural needle was placed on the transverse process and then advanced in a cephalad direction toward the midline until a loss of resistance was felt on entry into the epidural space. Using this technique, a volume of $1\,\mu l\,g^{-1}$ produced spread of solution over lumbar and low thoracic segments. Epidural solutions contained 1% Evans blue. Data were only included from animals in which epidural placement could be confirmed by midline extradural spread of solution at the end of the experiment, and dural puncture was excluded by lack of cerebrospinal fluid and spinal cord staining.

Dose and age group

In preliminary experiments, doses of $10\,\mu g\,kg^{-1}$ and above of epidural dexmedetomidine (Abbott Australasia Pty Ltd, Kurnell, Australia) markedly reduced or abolished the EMG response and produced significant bradycardia in P10 and P21 pups. Similar effects were seen at lower doses in P3 pups ($1\,\mu g\,kg^{-1}$ and above). Therefore, 0.5, 1, 2 or $5\,\mu g\,kg^{-1}$ epidural dexmedetomidine was administered to P10 and P21 pups, and 0.1, 0.2 and $0.5\,\mu g\,kg^{-1}$ epidural dexmedetomidine in P3 pups. Solutions of saline and different concentrations of dexmedetomidine were prepared and then coded by an independent colleague. As the same volume ($1\,\mu l\,g^{-1}$) of different concentrations of drug was administered, doses could be adjusted for body weight without compromising blinding. The sample size was six to eight for each treatment group. Epidural injections were performed 30 min before reflex recordings, based on a previous study of the time course of behavioural responses to epidural dexmedetomidine in rat pups (Walker *et al*., 2005).

To compare effects of epidural and systemic administration, the maximum tolerated epidural dose ($5\,\mu g\,kg^{-1}$ in P10 and P21 pups; $0.5\,\mu g\,kg^{-1}$ in P3 pups) was administered by subcutaneous injection within the same experimental protocol.

Electromyographic recording

Bipolar EMG electrodes (Ainsworks, London, UK) comprising stainless steel 30G needles with a central copper wire core were placed through a small skin incision into the belly of the biceps femoris muscle. Raw signals were recorded and stored using an analog-to-digital signal converter for online display and later analysis (PowerLab 4S, AD Instruments, Castle Hill, Australia). Von Frey hairs were applied to the plantar surface of the hindpaw for 1 s and the EMG response was recorded. Hairs were applied in descending order from a maximum of von Frey hair 17 (50 g bending force) in P3 and P10 animals and hair number 18 (75 g) in P21 animals until no response was recorded.

Experimental protocol

Following surgical preparation, positioning of the animal and injection of epidural solution, the halothane concentration was reduced to an age-appropriate concentration (1.1% in P3, 1% in P10 and 0.9% in P21 animals), which produced similar recording conditions across the age groups, that is, animals tolerated mechanical ventilation, gross or bilateral hindlimb movements were prevented and specific quantifiable EMG responses to mechanical stimuli could be obtained. The halothane concentration was allowed to equilibrate for 30 min before EMG recordings and remained at the same level throughout the recording period.

The flexion reflex EMG response to plantar mechanical stimulation was recorded before and 10 min following topical application of 100% mustard oil on the plantar surface of the hindpaw as indicated in earlier studies (Walker *et al*., 2007). The volume of mustard oil was adjusted at each age (3.5 μl at P3; 7 μl at P10; and 12 μl at P21), to cover a similar surface area (Jiang and Gebhart, 1998). In P21 pups, the degree of mustard oil-induced secondary hyperalgesia is similar to the degree of primary hyperalgesia, but is less in P10 pups and absent in P3 pups (Walker *et al*., 2007). Therefore, in P21 pups, the effect of epidural dexmedetomidine on secondary hyperalgesia was determined by quantifying the hindpaw reflex response before and 10 min after distant application of mustard oil on the lateral hindlimb and the dose–response compared with primary hyperalgesia experiments. At the end of the experiments, animals were terminally anaesthetized with intraperitoneal pentobarbitone (100 mg kg^{-1}).

Data analysis and statistics

The duration of the EMG response was established from the raw data, and the integral of the root mean square (RMS) of the signal was calculated (Chart, Powerlab AD Instruments). As von Frey hairs are numbered on a linear scale with a consistent log difference between hairs, the stimulus was measured in terms of von Frey hair number as described previously (Howard *et al*., 2001). The integral of the RMS (response) was plotted against the von Frey hair number (mechanical stimulus strength) and the area under the resulting stimulus–response curve (AUC) calculated. Data were also expressed as the percentage change following mustard oil, that is, % change = ((post AUC−pre AUC/pre AUC) × 100). This analysis allowed each animal to act as its own control and facilitated construction of a dose–effect relationship. The maximum possible effect (MPE) was designated from 0 to 100%, where 0% suppression of hyperalgesia equates with no difference from the control saline group and 100% MPE is complete prevention of hyperalgesia and no change in reflex response following mustard oil. This was calculated as (1−(% change in reflex/mean % change in control saline group)) × 100. The effect of epidural dexmedetomidine on heart rate was analysed 20 min after injection, as this allowed time for halothane equilibration and absorption of dexmedetomidine, but was before testing. Data are expressed as mean ± s.e.m. Statistical comparisons between treatment groups were analysed using paired, two-tailed Student's *t*-test (pre vs post mustard oil values) or one-way analysis of variance (ANOVA) with *post hoc* comparisons (GraphPad Prism 4, San Diego, USA). $P<0.05$ was considered statistically significant.

Results

Epidural dexmedetomidine prevents mustard oil-induced hyperalgesia at all postnatal ages

Figure 1 shows how EMG responses were used to measure the baseline reflex, mustard oil-induced hyperalgesia and the effect of epidural dexmedetomidine upon those measures. In the left panel (Figure 1a–c), the baseline EMG response to mechanical hindpaw stimulation (Figure 1a) was markedly altered 10 min after mustard oil application (Figure 1b). The mustard oil-induced decrease in mechanical threshold and increased suprathreshold response represents hyperalgesia, which was reflected in the leftward shift and increased AUC of the graph in Figure 1c. This hyperalgesic response was not prevented by epidural dexmedetomidine 0.5 μg kg^{-1}. In the right panel (Figure 1d-f), the same measures are shown in the presence of 2 μg kg^{-1} epidural dexmedetomidine. This dose of dexmedetomidine prevented primary hyperalgesia, as there was no change in the EMG response (Figure 1e) or AUC following mustard oil (Figure 1f).

The effect of age and dexmedetomidine dose on mustard oil-induced primary hyperalgesia was investigated. Figure 2 shows that primary hyperalgesia, measured as an increase in the reflex response following hindpaw application of mustard oil, occurred at all postnatal ages. This primary hyperalgesia was prevented in P3 pups by 0.1 μg kg^{-1} epidural dexmedetomidine (Figure 2a), but doses of 1 μg kg^{-1} were required in P10 and P21 pups (Figure 2b and c). When data were analysed as the percentage change in reflex response, suppression of hyperalgesia was seen at the same doses. Following epidural dexmedetomidine, 0.1 μg kg^{-1} in P3 and 1 μg kg^{-1} in P10 and P21 pups, there was no significant increase in the baseline reflex ($P<0.05$ one-way ANOVA with Dunnett's comparison to zero). The increased sensitivity to epidural dexmedetomidine in early development is also reflected by the left shift of the log-dose–response relationship for P3 pups (Figure 3).

The antihyperalgesic effect of epidural dexmedetomidine was independent of the effects upon baseline nociception. Comparison of the baseline reflex responses (that is,

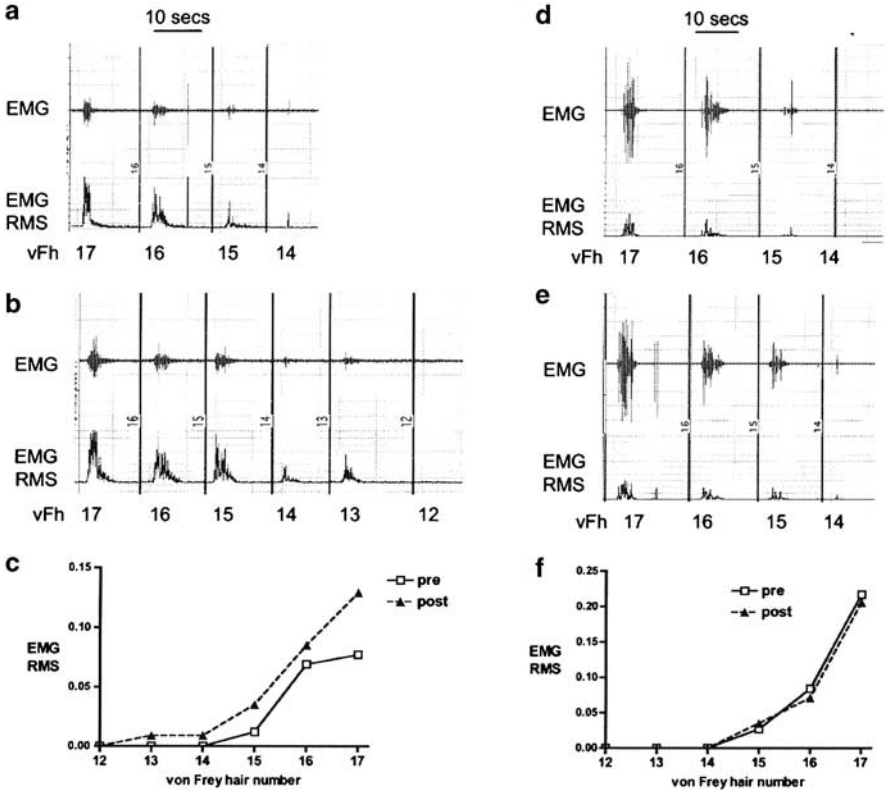

Figure 1 The effects of epidural dexmedetomidine on mustard oil-induced hyperalgesia. The left panel shows development of hyperalgesia in a P10 rat pup despite epidural dexmedetomidine 0.5 $\mu g\,kg^{-1}$. (**a**) Baseline EMG recordings (upper trace, raw EMG; lower trace, EMG; root mean square, RMS) in response to von Frey hair stimuli of descending strength (17–14); (**b**) 10 min after mustard oil, the threshold is reduced and the response to suprathreshold stimuli is increased; (**c**) hyperalgesia is shown as a leftward shift in the stimulus–response relationship. The right panel shows data from a P10 rat pup following epidural dexmedetomidine 2 $\mu g\,kg^{-1}$. (**d**) Baseline EMG recordings in response to von Frey hair stimuli (17–14); (**e**) there is no change in the reflex response following mustard oil (that is, hyperalgesia is prevented); (**f**) there is no shift in the stimulus–response curve. EMG, electromyogram; P, postnatal age.

pre-application of mustard oil) following different doses of dexmedetomidine found significant reductions from the saline group only with the highest doses used at each age (0.5 $\mu g\,kg^{-1}$ at P3 and 5 $\mu g\,kg^{-1}$ at P10 and P21; $P<0.05$ one-way ANOVA with Tukey's *post hoc* comparison).

Epidural dexmedetomidine was equally effective at preventing primary and secondary hyperalgesia in P21 pups. Doses of 1 and 2 $\mu g\,kg^{-1}$ epidural dexmedetomidine prevented increases in the reflex response induced by distant hindlimb application of mustard oil (Figure 4a).

Effects of epidural dexmedetomidine are spinally mediated

Disruption of supraspinal descending pathways did not influence primary hyperalgesia or the efficacy of epidural dexmedetomidine. Mustard oil significantly increased the reflex response in rats following spinal transection in P21 pups, but this was prevented by 1 $\mu g\,kg^{-1}$ epidural dexmedetomidine (Figure 4b).

Despite producing both antinociceptive and antihyperalgesic effects when given by the epidural route, the maximum dose of dexmedetomidine (5 $\mu g\,kg^{-1}$ in P21 and P10 pups; 0.5 $\mu g\,kg^{-1}$ in P3 pups) had no effect when administered subcutaneously. The degree of primary hyperalgesia following these doses of systemic dexmedetomidine did not differ from the epidural saline groups (data not shown).

Epidural dexmedetomidine produces dose- and age-dependent effects on heart rate

Heart rate across treatment groups within each age group did not differ significantly at baseline (P21: 339 ± 8.4; P10: 270 ± 4.5 and P3: 238 ± 10 beats min^{-1}) or within the first 10 min. At 20 and 30 min after injection, heart rate was significantly reduced by 5 $\mu g\,kg^{-1}$ epidural dexmedetomidine at P21 when compared with epidural saline and lower doses of dexmedetomidine. The same was true with 2 and 5 $\mu g\,kg^{-1}$ at P10 and 0.5 $\mu g\,kg^{-1}$ at P3 ($P<0.05$ one way ANOVA with Tukey's comparison). Figure 5 shows dose-dependent changes in heart rate 20 min following injection of epidural dexmedetomidine at different ages. Physiologically significant changes in heart rate (that is, 20% reduction from baseline) were produced by lower doses of epidural dexmedetomidine in younger pups.

Discussion and conclusions

Epidural dexmedetomidine produces dose-dependent antihyperalgesic and antinociceptive effects at all postnatal ages in the rat pup. Mustard oil-induced hyperalgesia is selectively prevented by doses of epidural dexmedetomidine that have no effect on the baseline nociceptive reflex response. Effects of epidural dexmedetomidine are spinally mediated,

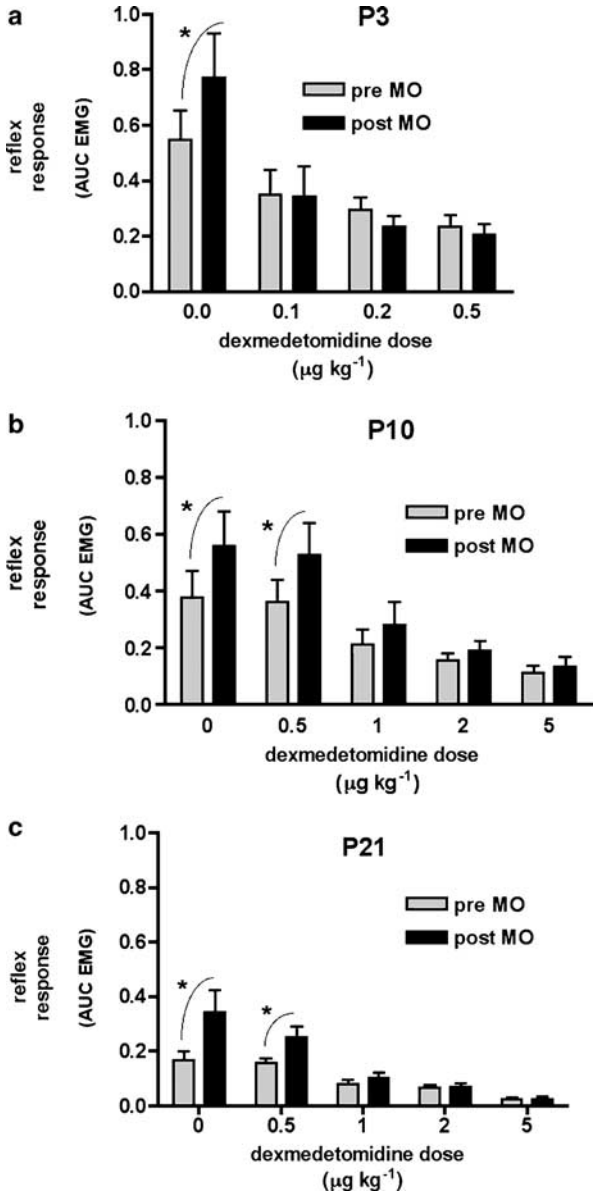

Figure 2 Effect of mustard oil and epidural dexmedetomidine on the quantified reflex response in P3 (**a**), P10 (**b**) and P21 (**c**) rat pups. Data are presented for the reflex response (area under curve of the EMG stimulus–response relationship) before (pre MO) and 10 min after hindpaw application of mustard oil (post MO). At all ages, mustard oil produces a significant increase in the reflex response in the control epidural saline group. Epidural dexmedetomidine 0.1–0.5 μg kg^{-1} in P3 pups, and 1–5 μg kg^{-1} in P10 and P21 pups prevented significant increases in the reflex response. *$P<0.05$, two-tailed paired Student's *t*-test. Bars = mean ± s.e.m.; $n=6$–8, all groups. EMG, electromyogram; P, postnatal age.

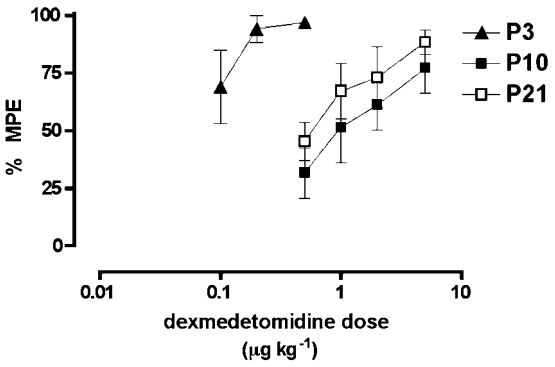

Figure 3 Postnatal age and antihyperalgesic effects of epidural dexmedetomidine. The dose–response relationship for prevention of mustard oil-induced primary hyperalgesia is shown for P3, P10 and P21 rat pups. For each dose, the percentage change in reflex response is represented as a proportion of the change seen in the control group, and ranges from zero (same degree of hyperalgesia as epidural saline group) to 100% maximum possible effect (prevention of hyperalgesia and no change in reflex response). Data points = mean ± s.e.m.; $n=6$–8, all groups. P, postnatal age.

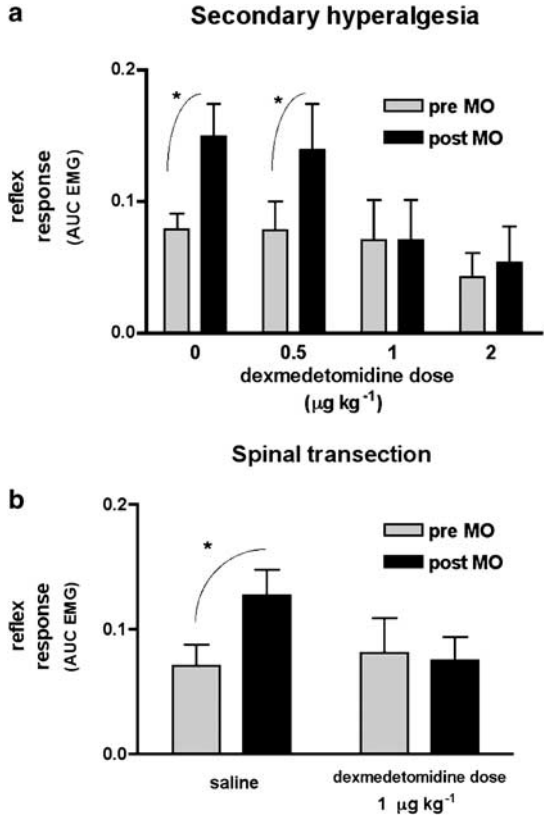

Figure 4 (**a**) Effect of epidural dexmedetomidine on mustard oil-induced secondary hyperalgesia in P21 pups. Following epidural saline or 0.5 μg kg^{-1} dexmedetomidine, the reflex response is significantly increased. Secondary hyperalgesia is prevented by 1 and 2 μg kg^{-1} epidural dexmedetomidine. *$P<0.05$ two-way paired Student's *t*-test. Bars = mean ± s.e.m.; $n=6$–7, all groups. (**b**) Effect of thoracic spinal transection on primary hyperalgesia and response to epidural dexmedetomidine in P21 pups. In rats with prior spinal transection, mustard oil significantly increases the reflex response, but this is prevented by epidural dexmedetomidine (spinal transection + epi dex 1 μg kg^{-1}). *$P<0.05$ two-way paired Student's *t*-test. Bars = mean ± s.e.m.; $n=4$. P, postnatal age.

as antinociceptive epidural doses have no effect when given systemically, and are not dependent on descending inhibitory pathways as efficacy is maintained in animals with complete transection of the spinal cord. Finally, there is increased sensitivity to both antihyperalgesic and cardiovascular effects in early development.

The current results extend our previous study of the effects of epidural dexmedetomidine on behavioural reflex thresh-

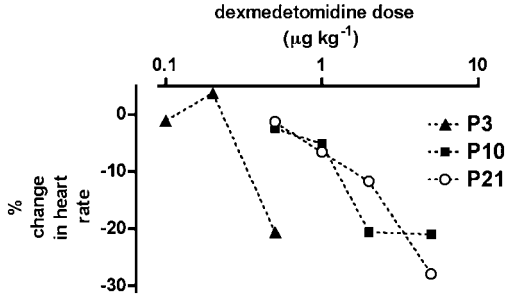

Figure 5 Effect of epidural dexmedetomidine on heart rate. Within each age group, the percentage change in heart rate for each dose of epidural dexmedetomidine is compared to the epidural saline group 20 min following injection. Heart rate was decreased to a similar degree (20% reduction) by epidural dexmedetomidine 0.5 μg kg^{-1} in P3 pups, 2 μg kg^{-1} and higher in P10 pups and 5 μg kg^{-1} in P21 pups. P, postnatal age.

olds following hindpaw inflammation (Walker *et al*., 2005). Here, we have quantified the antihyperalgesic effects using electrophysiological measures of flexor muscle activity that allow quantification of the reflex response to graded mechanical stimuli at threshold and suprathreshold intensities. The AUC provides a measure of overall responsiveness of the reflex (Walker *et al*., 2007). Halothane provides a stable plane of anaesthesia during electrophysiological recordings and does not affect the EMG pattern of the withdrawal reflex (Schouenborg and Kalliomaki, 1990) or prevent C-fibre-induced sensitization (Dickenson and Sullivan, 1987; Schouenborg and Dickenson, 1988). In accordance with age-related changes in anaesthetic potency (Orliaguet *et al*., 2001), slightly higher concentrations of halothane were required in younger pups to obtain the same recording conditions, but are not sufficient to explain the 10-fold difference in dexmedetomidine dose requirement.

Mustard oil stimulates C-fibre nociceptors via the TRPA1 receptor (Bandell *et al*., 2004; Jordt *et al*., 2004), resulting in primary hyperalgesia (owing to sensitization of peripheral nociceptors within an area of injury) and a surrounding zone of secondary hyperalgesia (mediated by central changes in nociceptive processing) (Reeh *et al*., 1986; Treede *et al*., 2004). High-intensity stimulation of peripheral C fibres acutely increases levels of endogenous noradrenaline in the spinal cord (Yaksh and Tyce, 1981; Men *et al*., 1996), and α_2 agonists can suppress C-fibre nociceptive pathways (Mansikka and Pertovaara, 1995; Mansikka *et al*., 2004). In neonatal (P3–6) ventral root preparations, dexmedetomidine suppresses high-intensity excitatory postsynaptic potentials produced by activation of C-fibre primary afferents (Kendig *et al*., 1991; Faber *et al*., 1998; Otsuguro *et al*., 2005), suggesting that these inhibitory mechanisms are functional in early life. In the current *in vivo* study, we have demonstrated inhibition of mustard oil-induced hyperalgesia by dexmedetomidine at all postnatal ages, with an increased sensitivity in early development. Previous studies using inflammatory hyperalgesia (Walker *et al*., 2005) and the formalin response (Otsuguro *et al*., 2005; Sanders *et al*., 2005) also show reduced dexmedetomidine dose requirements in the first postnatal week. Spinally administered dexmedetomidine has high binding affinity and high intrinsic efficacy at α_2-adrenergic sites within the cord (Takano and Yaksh, 1991; Asano *et al*., 2000) and competitive interaction studies support a predominant action at α_{2A} and minimal effect at α_1 and imidazole sites (Takano and Yaksh, 1992). Antinociceptive effects of dexmedetomidine are lost in D79N mice with a point mutation in the α_{2A} adrenoceptor, but maintained in α_{2B} and α_{2C} receptor knockouts (Hunter *et al*., 1997; Stone *et al*., 1997; Malmberg *et al*., 2001). Therefore, changes in α_{2A} receptor distribution and function may contribute to the increased efficacy of dexmedetomidine in early life. Messenger RNA for the α_{2A}-adrenergic receptor is present in the dorsal horn prenatally (Huang *et al*., 2002) and levels vary throughout development (high at P5–14, moderate at P21)(Winzer-Serhan *et al*., 1997). α_{2A} mRNA is present in adult dorsal root ganglia (DRG) (Shi *et al*., 2000), but age-related changes have not been investigated.

The current data also cast light on the site and mechanism of action of α_2-adrenergic analgesia. α_2-Adrenergic agonists reduce excitatory glutamatergic transmission in the spinal cord by both presynaptic (Feng *et al*., 2002; Pan *et al*., 2002) and postsynaptic mechanisms (North and Yoshimura, 1984; Li and Zhuo, 2001), and by activating descending inhibitory noradrenergic tracts from the brainstem (Jones, 1991; Nuseir *et al*., 1999). The relative importance of these different mechanisms is unclear. In the first postnatal week, primary afferent responses to mustard oil can be observed in DRG (Fitzgerald, 1987) but postsynaptic responses to C-fibre stimuli in the dorsal horn are immature (Jennings and Fitzgerald, 1998; Baccei *et al*., 2003). As dexmedetomidine was effective at all ages, its major effect may be mediated by presynaptic suppression of activity in C-fibre afferents, either in the dorsal horn or possibly by diffusion of epidural solution to the DRG. We have recently shown differential effects of postnatal age and extracellular signal-regulated kinase (ERK) activation in primary and secondary hyperalgesia (Walker *et al*., 2007). Intrathecal administration of an ERK inhibitor had no effect on primary hyperalgesia, but secondary hyperalgesia was suppressed in P21 pups, as this enzyme is required for dorsal horn neuronal sensitization. In the current study, epidural dexmedetomidine was equally effective against primary and secondary hyperalgesia at P21. Again, effects may be pre- or postsynaptic, but prevention of induction of secondary hyperalgesia rather than specific postsynaptic mechanisms (as seen with ERK inhibition) may be the predominant action. As the duration of mustard oil-induced hyperalgesia is relatively brief in the current model (reflex responses are back to baseline by 20 min; Walker *et al*., 2007), it is not possible to test differential effects on the induction or maintenance phase of secondary hyperalgesia.

In adult animals, mustard oil-induced hyperalgesia is prevented by doses of dexmedetomidine that have no effect on the nociceptive withdrawal threshold of the contralateral paw or the hindpaw of control animals (Mansikka and Pertovaara, 1995; Mansikka *et al*., 1996). The decreased dose requirement for reversal of injury effects may relate to activity-dependent changes in neurotransmitter release, increased receptor expression or affinity or an increase in efficiency of coupling between α_2 receptors and G-proteins (Bantel *et al*., 2005). In the current study, we also observed

antihyperalgesic effects at lower doses than required for antinociceptive effects (that is, suppression of the baseline reflex response) throughout postnatal development, demonstrating that activity-dependent mechanisms are functional from an early age. In adult rats, the potency of systemic medetomidine has been shown to vary with time after inflammation (4–44 h), suggesting that different mechanisms may be recruited sequentially (Molina and Herrero, 2006), but as the changes following mustard oil reported in the present study occur within minutes, this is unlikely here.

Epidural analgesia is frequently used for perioperative analgesia in infants and children (Ansermino *et al*., 2003), but a selective spinal action of α_2 agonists has not been confirmed in clinical paediatric trials (Ivani *et al*., 2002; Hansen *et al*., 2004). In adult animals, epidural dexmedetomidine is rapidly absorbed into the cerebrospinal fluid (Eisenach *et al*., 1994) and the same antinociceptive effect is achieved with approximately 20% of the systemic dose (Asano *et al*., 2000). Binding sites for [^{3}H]dexmedetomidine have been identified in both adult and neonatal (P1and P2) rat spinal cord (Savola and Savola, 1996) and at all postnatal ages in the current study selective spinally mediated effects were demonstrated, as the maximum epidural dose had no effect when given systemically. The contribution of supraspinal descending inhibitory pathways to α_2-adrenergic analgesia continues to be debated (Molina and Herrero, 2006), but the current data support a spinal site of action. Following lumbar epidural administration, dexmedetomidine circulating in cerebrospinal fluid could activate supraspinal mechanisms, but this is not a significant effect in the current model as complete spinal transection did not alter the efficacy of epidural dexmedetomidine. In addition, descending inhibitory pathways are not fully functional in the first three postnatal weeks (Fitzgerald and Koltzenburg, 1986; van Praag and Frenk, 1991) and yet the sensitivity to exogenous α_2 agonists is increased in early life.

α_2-Adrenergic agonists produce dose-related hypotension and bradycardia, predominantly by central depression of sympathetic drive (Paris and Tonner, 2005). As α_2-adrenergic receptors are highly expressed in the brainstem during the early postnatal period in the rat (Happe *et al*., 2004), and binding of [^{3}H]*p*-aminoclonidine is higher in human neonates than infants (Mansouri *et al*., 2001), susceptibility to centrally mediated cardiovascular side effects of α_2 agonists may be increased in early life. Similar to a study in adult rats (Asano *et al*., 2000), we found dose-dependent reductions in heart rate in the first 30 min following epidural dexmedetomidine at all ages. Heart rate is higher in older pups (Schuen *et al*., 1997), but a similar degree of bradycardia was produced by lower doses of dexmedetomidine in younger pups. Following systemic administration of dexmedetomidine, the therapeutic window is narrow and dose requirements for analgesia and side effects overlap (Hunter *et al*., 1997; Sanders *et al*., 2005). The decreased dose requirements with epidural compared to systemic administration allowed separation between antihyperalgesic effects and cardiovascular side effects at all postnatal ages.

In adults, the clinical use of dexmedetomidine is increasing, although further investigation is required before routine 'off-label' use (Paris and Tonner, 2005) and the safety of spinal administration has not been established. In children, controlled trials are required to evaluate age-related changes in the pharmacokinetic profile and clinical utility of α_2-adrenergic agonists (Serlin, 2004). This study shows that, in rat pups, epidural dexmedetomidine has dose-dependent, spinally mediated, antihyperalgesic effects at all postnatal ages and higher doses are required to suppress the nociceptive reflex. The sensitivity to antihyperalgesic, antinociceptive and cardiovascular effects was increased in the youngest pups. These developmentally regulated pharmacodynamic changes have implications for dosing of α_2 agonists in neonates and infants and should be considered in the design of clinical paediatric trials.

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Conflict of interest

The authors state no conflict of interest.

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