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Effects of the δ-opioid receptor antagonist naltrindole on antinociceptive responses to selective δ-agonists in post-weanling rats

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1 Antagonism, by the selective δ-opioid receptor antagonist naltrindole, of the antinociceptive effects of [D-Pen^2, D-Pen^5] enkephalin (DPDPE), [D-Ser^2, Leu^5, Thr^6] enkephalin (DSLET) and D-Ala^2 deltorphin I (DELT I) has been studied in 25 day old rats.

2 Antinociception was measured by the 50°C tail immersion test following i.p. administration of agonists and/or antagonists.

3 Dose-related antinociception was observed with DPDPE, DSLET and DELT I and ED_{75} doses were computed (0.66 mg kg^{-1}, 0.65 mg kg^{-1}, 0.032 mg kg^{-1} respectively) and used for antagonism studies.

4 Naltrindole (0.01 mg kg^{-1}) significantly attenuated the antinociceptive effects of DPDPE and DSLET with 0.1 mg kg^{-1} producing complete reversal of the effects of the ED_{75} dose. In contrast, naltrindole at 0.01 and 0.1 mg kg^{-1} did not alter antinociceptive responses to DELT I. Naltrindole at 1 mg kg^{-1} significantly attenuated DELT I antinociception.

5 Naloxone (1 mg kg^{-1}) produced equivalent degrees of antagonism of the antinociceptive effects of DPDPE, DSLET and DELT I. ICI 174,864 (1 mg kg^{-1}) also antagonized antinociception with a differential degree of attenuation (DSLET > DPDPE > DELT I).

6 Naltrindole (1 mg kg^{-1}) had no effect on the antinociception induced by the selective μ-agonist alfentanil (60 μg kg^{-1}). Naltrindole, naloxone or ICI 174,864 had no effect on nociceptive latencies.

7 The differential antagonism by naltrindole of the effects of three selective δ-agonists suggests δ-receptor heterogeneity. Further, the lower sensitivity of response to DELT I suggests that this agent may exert its antinociceptive effects at a different δ receptor subtype from DPDPE or DSLET.

Keywords: Antinociception; δ-opioid receptors; ontogeny; naltrindole; δ-agonists

Introduction

Results from *in vitro* and *in vivo* experimental studies strongly suggest the existence of three primary opioid receptor types (μ, δ and κ) (see Paterson *et al.*, 1983). Elucidation of the role of the δ-opioid receptor has in the past been hampered because of a lack of selective, stable, non-peptide agonists and antagonists. Several high affinity, δ-selective peptide agonists have now been produced (Grace *et al.*, 1980; Mosberg *et al.*, 1983; Erspamer *et al.*, 1989) and recently a selective non-peptide antagonist, naltrindole has become available (Portoghese *et al.*, 1988). These pharmacological tools have allowed a re-examination of the possible roles of the δ-opioid receptor and, in particular, studies in mice with naltrindole and its benzofuran analogue have suggested the existence of δ-receptor subtypes (Sofuoglu *et al.*, 1991). We further addressed this possibility by studying the ability of naltrindole to antagonize the antinociceptive responses to three highly selective δ-agonists, [D-Pen^2, D-Pen^5] enkephalin (DPDPE), [D-Ser^2, Leu^5, Thr^6] enkephalin (DSLET) and D-Ala^2 deltorphin I (DELT I) in postweanling rats.

Methods

Animals and experimental conditions

Male Wistar albino rats (University of Surrey strain) 25 days old and weighing 60–90 g, were used in all studies. All rats were housed in groups of 8–10 and maintained at 22 ± 1°C in a constant 12 h light-dark cycle (lights on at 07 h 00 min). Experimental procedures were carried out in a quiet, windowless, air-conditioned laboratory between 14 h 00 min and 18 h 00 min to minimize diurnal variation. Animals were allowed to acclimatize for 2 h before experimentation.

Nociceptive testing

Animals were divided into treatment groups so that nociceptive tests took place for saline- and drug-treated animals on at least three separate days. Drugs (in 0.9% w/v saline) were administered i.p. in a dose volume no greater than 0.1 ml. Nociceptive responses were measured by the 50°C warm water tail immersion test (Janssen *et al.*, 1963) adapted for young rats (Kitchen *et al.*, 1984). Nociceptive responses were defined as withdrawal of the tail from the surface of the water and a maximum 10 s cut-off was used. Response latencies were measured 15 min before administration of the δ-agonists DPDPE, DSLET, DELT I or the μ-agonist alfentanil and nociceptive responses measured 2, 5, 10 and 15 min after treatment.

When studied, antagonists (naloxone, naltrindole or ICI 174,864) were injected 10 min before agonist administration. The ED_{75} of each of the δ-agonists was chosen for antagonism studies and calculated as the 75% of maximum effect from full dose-response curves at peak antinociception (5 min) with saline control values defined as 0%.

Drugs and statistical procedures

Drugs used were [D-Pen^2, D-Pen^5], enkephalin (DPDPE), [D-Ser^2, Leu^5, Thr^6] enkephalin (DSLET), ICI 174,864 (Allyl$_2$-

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Tyr-Aib-Aib-Phe-Leu-OH; Cambridge Research Biochemicals), D-Ala^2 deltorphin I (DELT I) (Bachem), alfentanil HCl (Janssen Pharmaceuticals), naloxone (Dupont) and naltrindole HCl (NTI) (Research Biochemicals Incorporated).

Antagonism of the antinociceptive responses to the δ-agonists was analysed by analysis of variance followed by *post-hoc* analysis with Dunnett's test.

Results

Administration of increasing doses of DPDPE, DSLET and DELT I induced a dose-related antinociception with ED_{75}s of 0.66 mg kg^{-1}, 0.65 mg kg^{-1}, 0.032 mg kg^{-1} respectively. Responses to all three agonists were qualitatively similar with peak antinociception being observed 5 min after administration (Figure 1a,b,c).

Figure 2a,b,c shows the effects of NTI (0.01–1 mg kg^{-1}) on the antinociceptive responses to the ED_{75} dose of DSLET, DPDPE and DELT I respectively. NTI (0.01 mg kg^{-1}) significantly attenuated the peak response to DPDPE and DSLET, while higher doses (0.1 and 1 mg kg^{-1}) completely antagonized the antinociceptive response to DSLET. NTI (0.1 mg kg^{-1}) markedly reduced the response to DPDPE and the response following 1 mg kg^{-1} NTI was not significantly different from saline control values. In contrast, NTI (0.01 and 0.1 mg kg^{-1}) did not alter antinociception induced by DELT I, and only the highest dose of NTI (1 mg kg^{-1}) significantly antagonized antinociception induced by this agonist.

Naloxone (1 mg kg^{-1}) significantly antagonized response to DPDPE, DSLET and DELT I producing a similar degree of attenuation of the antinociceptive effects of all three agonists (Figure 3). ICI 174,864 (1 mg kg^{-1}) also significantly antagonized the antinociceptive effects of these agonists with a differential degree of attenuation (DSLET > DPDPE > DELT I). Further, ICI 174,864 (2 mg kg^{-1}) completely blocked antinociceptive responses to all the δ-agonists (data not shown) but at this dose in some animals this antagonist caused behavioural toxicity, including hindlimb stretching, flaccidity and barrel rolling. NTI and naloxone showed no overt behavioural toxicity at the highest doses used.

NTI (1 mg kg^{-1}) and ICI 174,864 (2 mg kg^{-1}) had no effect on the antinociceptive response induced by a submaximal dose (60 μg kg^{-1}) of the μ-agonist alfentanil (Figure 4).

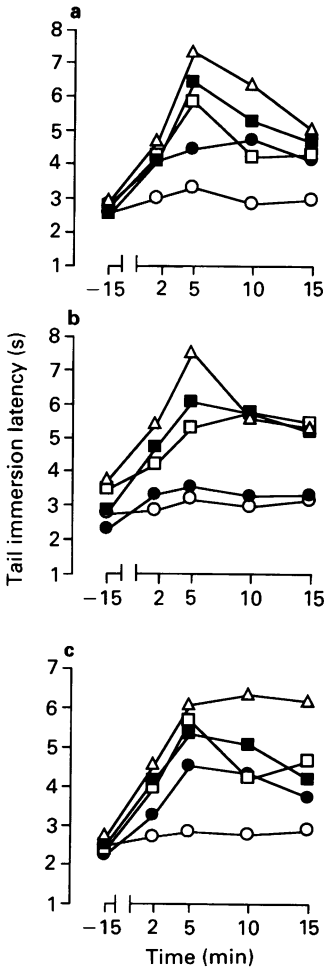

Figure 1 Time course of the antinociceptive effects of (a) [D-Ser^2, Leu^5, Thr^6] enkephalin (DSLET), (b) [D-Pen^2, D-Pen^5] enkephalin (DPDPE) and D-Ala^2 deltorphin (DELT I) in 25 day old rats using the tail immersion test. Values are means of at least six observations. Error bars are omitted for clarity of presentation; s.e.mean varied from 3–14% of the mean value. (○) Represents saline treated controls; (●) 0.25 (DSLET), 0.25 (DPDPE), 0.02 (DELT I) mg kg^{-1}; (□) 0.5 (DSLET), 0.5 (DPDPE), 0.05 (DELT I) mg kg^{-1}; (■) 0.75 (DSLET), 2 (DPDPE), 0.1 (DELT I) mg kg^{-1}; (△) 1 (DSLET), 4 (DPDPE), 0.15 (DELT I) mg kg^{-1}.

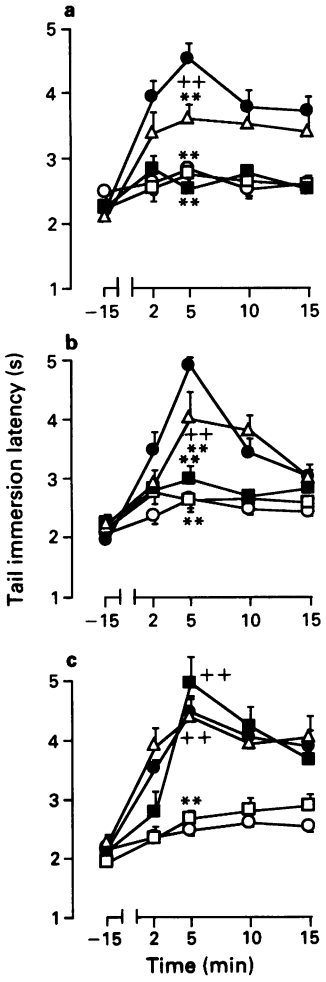

Figure 2 Effect of increasing doses of naltrindole (NTI) on the antinociceptive response to ED_{75} of (a) DSLET (b) DPDPE and (c) DELT I in 25 day old rats using the tail immersion test. Values are means ± s.e.mean of at least six observations. (○) Saline; (●) 0.65 mg kg^{-1} DSLET, 0.66 mg kg^{-1}, DPDPE, 0.032 mg kg^{-1}, DELT I; (□) agonists + 1 mg kg^{-1} NTI; (■) agonists + 0.1 mg kg^{-1} NTI; (△) agonists + 0.01 mg kg^{-1} NTI.
Significant differences vs saline control at 5 min $P<0.01$ DSLET alone, DSLET + 0.01 mg kg^{-1} NTI. Significant differences at 5 min $^{**}P<0.01$ vs agonist alone, $^{++}P<0.01$ vs saline control. Responses to antagonist alone were not significantly different from saline controls (data not shown). For abbreviations see legend to Figure 1.

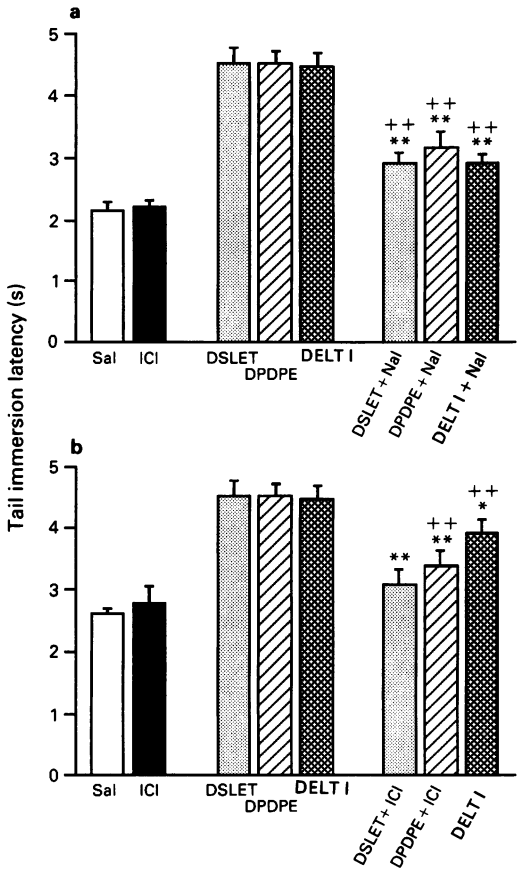

Figure 3 Effect of (a) naloxone and (b) ICI 174,864 on the peak antinociceptive response (5 min) to an ED_{75} of DSLET, DPDPE and DELT I in 25 day old rats in the tail immersion test. Values are the mean (± s.e.mean, vertical bars) of at least six observations. Sal = saline; Nal = naloxone 1 mg kg^{-1}; ICI = ICI 174,864, 1 mg kg^{-1}; DSLET 0.65 mg kg^{-1}; DPDPE 0.66 mg kg^{-1}; DELT I 0.032 mg kg^{-1}. For other abbreviations see legend to Figure 1. $^{**}P<0.01$; $^{*}P<0.05$ vs agonist alone; $^{++}P<0.01$ vs saline control.

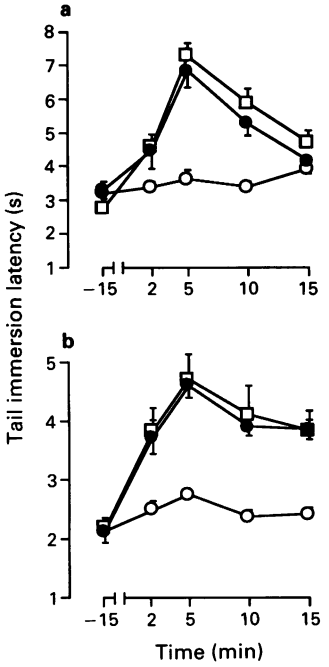

Figure 4 Effect of (a) naltrindole (NTI) and (b) ICI 174,864 on the antinociceptive response to alfentanil in 25 day old rats in the tail immersion test. Values are means (± s.e.mean, vertical bars) of at least six observations: (○) 1 mg kg^{-1} NTI (a), 2 mg kg^{-1} ICI 174,864 (b); (●) 60 μg kg^{-1} alfentanil; (□) 60 μg kg^{-1} alfentanil + 1 mg kg^{-1} NTI (a), 2 mg kg^{-1} ICI 174,864 (b). Alfentanil vs alfentanil + antagonist: not significant.

None of the antagonists (NTI, ICI 174,864 and naloxone) used had any effects on nociceptive latencies in the tail immersion test when administered alone.

Discussion

Determination of the importance of the δ-opioid receptor in mediating antinociception has been difficult because of the lack of suitable brain-penetrating selective agonists and antagonists. Studies with the selective ligand DPDPE implicate δ-sites in both spinal (Rodriguez *et al.*, 1986) and supraspinal (Heyman *et al.*, 1987) antinociception. Most studies have employed intrathecal or intracerebroventricular routes for administration of these agonists (Galligan *et al.*, 1984; Porreca *et al.*, 1987; Suh & Tseng, 1990) and administration by parental routes in the rat has been generally unsuccessful in inducing antinociception (Tavani *et al.*, 1989).

Our success in obtaining dose-related antinociceptive responses to DPDPE, DSLET and DELT I after i.p. injection most probably reflects the age of the animals used since in the rat, the blood brain barrier is not fully mature until postnatal day 30 (Keep *et al.*, 1986). We have also previously demonstrated δ-receptor operated stress-induced antinociception in 25 day old rats (Kitchen & Pinker, 1990).

NTI shows 100 fold selectivity for δ-receptors over other opioid receptors in isolated tissue preparations (Portoghese *et al.*, 1988) and in radioligand binding studies (Rogers *et al.*, 1991). The confirmation of the δ-receptor selectivity of NTI at doses used in this study was provided by lack of antagonism of the highly selective μ-agonist, alfentanil, and agrees with our previous assessment of *in vivo* selectivity of this antagonist (Kitchen & Pinker, 1990) and that of others (Calcagnetti & Holtzmann, 1991).

The observation that responses to DPDPE, DSLET and DELT I were antagonized to an equivalent degree by naloxone confirms opioid receptor mediation. The differential sensitivity to antagonism by NTI points to the possibility that these δ-agonists do not all mediate their effects via a common site. Others have found differential antagonism of DPDPE and DSLET by NTI and by its benzofuran analogue after intrathecal injection in mice (Sofuoglu *et al.*, 1991) and have suggested that these differences might be explained by the existence of δ-receptor subtypes. The differences between our work and that of Sofuoglu *et al.* (1991) could reflect the species used although route of administration may be important because Sofuoglu *et al.* (1991) did not see differential antagonism of DPDPE and DSLET after intracerebroventricular injection.

Our results also show there is not equal antagonism of the δ-agonists by ICI 174,864 and although the differences are not as marked as for NTI, it supports the possibility of δ-receptor heterogeneity. Further, the results in rats now show that DELT I provides the best discrimination of the effects of NTI with a 10 fold dose difference in sensitivity to either DPDPE or DSLET. The most marked difference is seen between DSLET and DELT I since NTI antagonism of DPDPE responses was not quite as marked as the antagonism of DSLET at 0.1 mg kg^{-1} (Figure 2b). This might suggest that DPDPE has affinity for both postulated sites. Further support for this conclusion is provided by differential functional antagonism of DPDPE and D-Ala^2 deltorphin II responses by tetraethylammonia and glibenclamide, respectively (Wild *et al.*, 1991). Other studies also suggest δ-receptor heterogeneity. For example, the two irreversible δ-antagonists, naltrindole 5′ isothiocyanate (Portoghese *et al.*, 1990) and D-Ala^2 Leu^5 Cys^6 enkephalin (Jiang

et al., 1990) differentially antagonize antinociceptive effects of D-Ala^2 deltorphin II, DSLET and DPDPE (Jiang et al., 1991).

Thus the results from our studies and that of others clearly demonstrate differential antagonism of the antinociceptive responses to selective δ-agonists by highly sensitive antagonists suggesting the presence of δ-opioid receptor subtypes in both rats and mice. The possibility that the differential antagonist effects of NTI might, in part, reflect pharmacokinetic differences in the distribution or metabolism of the three δ-agonists cannot be excluded. The differences in sensitivity to NTI are unlikely to be explained by μ-activity of DELT I since this peptide shows over 3000 fold selectivity for δ-sites in isolated tissue studies (Erspamer et al., 1989). Metabolism of DELT I to a non-selective μ/δ compound is also unlikely since its antinociceptive activity is fully reversed by ICI 174,864 and NTI at doses that have no effect on antinociception mediated by a highly selective μ-agonist (Figure 4).

In conclusion, the differential antagonism of the antinociceptive effects of three selective δ-agonists by NTI is in agreement with the proposal of the existence of δ-opioid receptor subtypes and suggests that DELT I exerts its antinociceptive effect at a different receptor subtype from DPDPE or DSLET in the rat.

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