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Antagonism of swim-stress-induced antinociception by the δ-opioid receptor antagonist naltrindole in adult and young rats

[1]Ian Kitchen & Sally R. Pinker

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

1 The availability of the non-peptide δ-opioid receptor antagonist naltrindole has provided the possibility for *in vivo* studies on the function of δ-opioid receptors. We have studied the effects of naltrindole on swim-stress-induced antinociception in adult and neonatal rats.

2 Adult, 25 and 20 day old rats were stressed by warm water (20°C) swimming for 3 min periods and antinociception was assessed by the tail immersion test (50°C).

3 Naltrindole (0.5 and 1 mg kg^{-1}) antagonized swim-stress-induced antinociception in adult and 25 day old rats but in 20 day old rats naltrindole (1 mg kg^{-1}) was without effect.

4 Antinociception induced by the highly μ-opioid receptor selective agonist alfentanil was completely antagonized by naloxone (1 mg kg^{-1}) but virtually unaffected by naltrindole (1 mg kg^{-1}).

5 Neither naloxone nor naltrindole (1 mg kg^{-1}) antagonized swim-stress-induced rises in plasma corticosterone in adult rats at the time of peak antinociception.

6 In conclusion, naltrindole shows *in vivo* antagonism of opioid-mediated responses. Swim-stress-induced antinociception is mediated through the δ-opioid receptor in 25 day old and adult rats and through the μ-opioid site in 20 day old animals.

Introduction

There is good evidence that opioid systems in the central nervous system are involved in the control of stress responses and there is a clearly definable opioid component in the phenomenon of stress-induced antinociception (Bodnar, 1984). The opioid component of stress-induced antinociception, for most stressors, appears to involve μ-opioid receptors but there are indications in both mice (Hart *et al.*, 1985) and rats (Jackson & Kitchen, 1989) that δ-opioid receptors may also contribute to this effect. However, studies on opioid receptor mechanisms have been hampered because of the lack of selective receptor antagonists. The evidence for a δ-involvement comes from studies with the δ-receptor antagonist ICI, 174864 (Cotton *et al.*, 1984) a peptide with low affinity, questionable stability and which poorly penetrates into the central nervous system after parenteral administration.

Recently, a highly selective, non-peptide, δ-opioid receptor antagonist, naltrindole has become available (Portoghese *et al.*, 1988) which in *in vitro* systems has relatively high affinity. Accordingly, to provide further evidence on the δ-receptor contribution to stress-induced antinociception we have studied the effects of naltrindole on swim-stress-induced antinociception using a protocol which induces only opioid-mediated responses. Further we have compared the effect of naltrindole on stress-induced antinociception in adult and in 20 and 25 day old neonatal rats, since previous experiments have indicated that responses at day 20 and 25 can differentiate a μ and a μ/δ contribution to stress-induced antinociception (Jackson & Kitchen, 1989).

Methods

Animals and experimental conditions

Male Wistar albino rats (University of Surrey strain) were used for studies on adult and 25 and 20 day old rats. Twenty day old pups (of either sex) remained with the mother except during drug administration, swimming and nociceptive testing to minimize stress due to maternal deprivation. All rats were housed in groups of no more than 10 and maintained at 21 ± 1°C in a constant 12 h light dark cycle (lights on at 07 h 00 min) and experimental procedures were carried out in a quiet, windowless, air conditioned laboratory. All procedures were carried out between 12 h 00 min and 17 h 00 min to minimize diurnal variation.

Swim stress procedures, nociceptive testing and corticosterone measurements

Animals were divided into treatment groups so that nociceptive testing took place for saline and drug-treated animals on at least two separate days, to minimize interday variation. Drugs (in 0.9% saline) were administered i.p. in dose volumes no greater than 0.2 ml; 10 min after dosing, animals were stressed by placing them individually in 20 ± 1°C water for a period of 3 min. For adult rats, the water was contained in a plastic tank (29 × 22 × 28 cm deep) and for neonates 2 litre glass beakers were employed as previously described (Jackson & Kitchen, 1989). At the end of the swimming period rats were removed from the water, dried and returned to the home cage before nociceptive testing. Nociceptive responses were assessed in adults and neonates by the tail immersion test (50°C) as previously described (Janssen *et al.*, 1963; Kitchen *et al.*, 1984). Responses were measured immediately before drug administration and at 1, 5, 10, 15, 30, 45 and 60 min following swimming stress. Control, unstressed rats, were tested at equivalent time points. For studies with alfentanil, tail immersion latencies were determined 2, 5, 10 and 15 min after administration. Opioid antagonists were administered 10 min before alfentanil.

In a parallel study adult rats were subjected to swim-stress procedures and trunk blood collected by decapitation 5 min after the end of the swim period (i.e. at the point of peak antinociception). Plasma corticosterone was measured by a fluorimetric assay as previously described (Kitchen & Rowan, 1984).

Drugs and statistical procedures

Drugs used were alfentanil (Janssen Pharmaceuticals), naloxone (Du Pont) and naltrindole (Reckitt and Colman). Nociceptive treatment groups were compared by the Least Significant Difference Test accounting for repeated measures. Comparison of corticosterone measurements in drug-treated groups was made by analysis of variance.

[1] Author for correspondence.

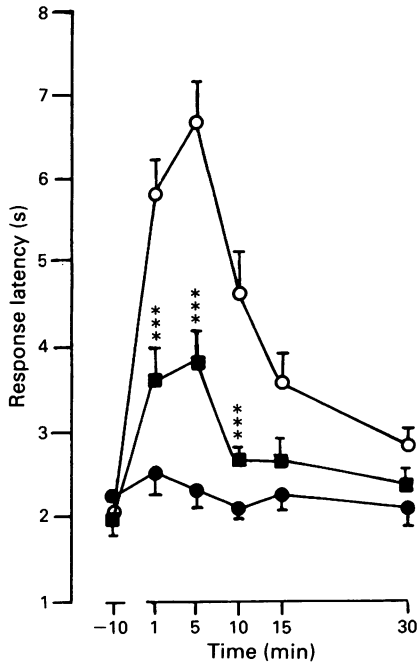

Figure 1 Effect of naloxone on antinociception induced by a 3 min swim in adult rats. Values represent means and vertical bars s.e.mean for groups of at least 10 animals. (●) Saline-injected, unstressed; (○) saline-injected, swim-stressed; (■) naloxone 1 mg kg^{-1}, swim-stressed. Significant differences from stressed controls, *** $P < 0.001$. Saline-injected, unstressed, significantly different at time points up to 15 min. Asterisks omitted for clarity.

Table 1 Effect of naloxone and naltrindole on plasma corticosterone levels in swim-stressed adult rats

Treatment	*Plasma corticosterone* (μg 100 ml^{-1})
Naive	6.8 ± 0.9
Saline-injected	15.7 ± 1.7
Naltrindole (1 mg kg^{-1})	17.3 ± 2.0
Naloxone (1 mg kg^{-1})	18.9 ± 0.8
Swim-stressed	12.4 ± 2.0
Saline-injected, swim-stressed	19.9 ± 2.1
Naltrindole (1 mg kg^{-1}), swim-stressed	24.3 ± 3.6
Naloxone (1 mg kg^{-1}), swim-stressed	19.2 ± 2.4

Values represent mean ± s.e.mean of 4–7 determinations of corticosterone 5 min after a 3 min period of warm water swimming. Saline, naloxone or naltrindole was administered 10 min prior to swim stress. For saline or drug-treated groups alone measurements were made 18 min after injection (i.e. at an equivalent time point to those animals undergoing swimming stress). No significant difference between saline and drug-treated groups. $P < 0.001$ for naive vs stressed groups (ANOVA).

Results

Naltrindole did not produce any overt behavioural changes in adult or neonatal animals. Animals given alfentanil at 60 mg kg^{-1} exhibited marked behavioural depression as shown by reduced locomotion and, in some animals, catatonia, an effect reversed by pretreatment with naloxone (1 mg kg^{-1}) but not naltrindole (1 mg kg^{-1}).

Three min swim-stress induced significant antinociception in adult, 25 and 20 day old rats (Figures 1 and 2) with peak effects 5 min after stress. Naloxone (1 mg kg^{-1}) significantly attenuated swim-stress-induced antinociception in adult rats (Figure 1). Naltrindole at 0.5 and 1 mg kg^{-1} significantly attenuated stress-induced antinociception in adult rats (Figure 2) and at 1 mg kg^{-1} the antagonism at the time of peak antinociception (5 min) was equivalent to that observed with naloxone. Naltrindole, 0.1 mg kg^{-1}, was without effect on the stress-induced antinociception (data not shown). Naltrindole (1 mg kg^{-1}) also attenuated stress-induced antinociception in 25 day old neonates but was without effect in 20 day old animals (Figure 2). In all studies (Figures 1 and 2) no significant changes were observed at 45 and 60 min time points; these are accordingly omitted from the figures.

In adult rats, swimming stress produced significant increases in plasma corticosterone in comparison with unstressed controls at the time of peak antinociception. Naloxone or naltrindole (1 mg kg^{-1}) did not alter the stress-induced elevation of corticosterone (Table 1).

The μ-receptor agonist alfentanil (10–60 mg kg^{-1}) produced dose-related antinociceptive responses in adult rats (data not shown) and naloxone (1 mg kg^{-1}) markedly reduced the response to 60 mg kg^{-1} alfentanil (Figure 3). In contrast, nal-

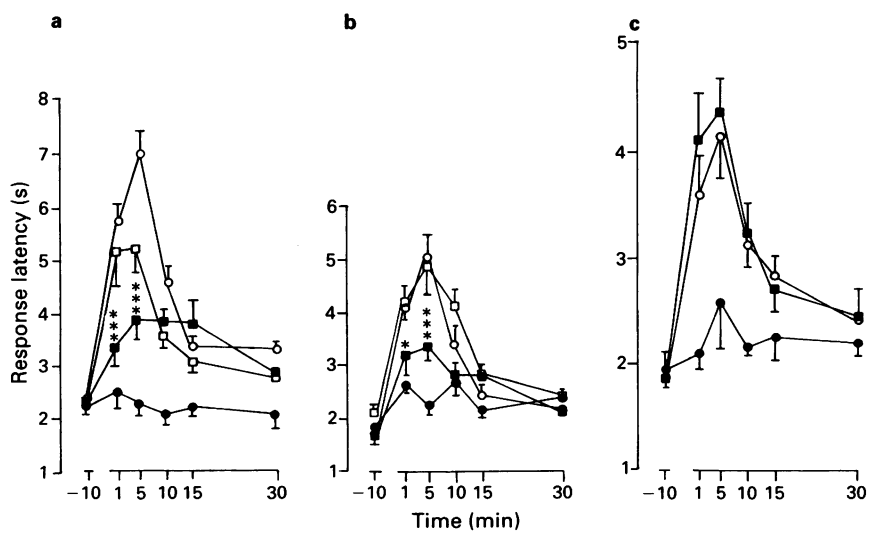

Figure 2 Effect of naltrindole on antinociception induced by a 3 min swim in (a) adult (b) 25 day and (c) 20 day old rats. Values represent means and vertical bars s.e.mean for groups of 4–10 animals. (●) Saline injected, unstressed; (○) saline injected, swim-stressed; (□) naltrindole 0.5 mg kg^{-1}, swim-stressed; (■) naltrindole 1 mg kg^{-1}, swim-stressed. Separate saline swim-stressed controls were run for each dose of naltrindole. Control stressed groups did not differ significantly and mean values are given for clarity. Significant differences determined from appropriate paired stressed controls: * $P < 0.05$; *** $P < 0.001$. Saline injected, unstressed, significantly different at time points up to 10 min. Asterisks omitted for clarity.

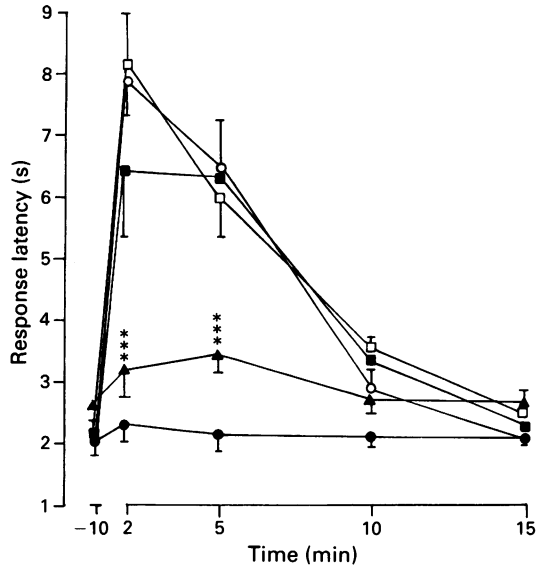

Figure 3 Effect of naltrindole and naloxone on alfentanil antinociception in adult rats. Values represent means and vertical bars s.e.mean for groups of at least 4 animals. (●) Naltrindole, 1 mg kg^{-1}; (○) alfentanil, 60 μg kg^{-1}; (□) alfentanil, 60 μg kg^{-1} and naltrindole 0.5 mg kg^{-1}; (■) alfentanil 60 μg kg^{-1} and naltrindole, 1 mg kg^{-1}; (▲) alfentanil, 60 μg kg^{-1} and naloxone, 1 mg kg^{-1}. Significant difference from alfentanil alone *** $P < 0.001$. Responses to naltrindole 0.5 mg kg^{-1} alone are omitted for clarity and did not differ from the naltrindole, 1 mg kg^{-1} response.

trindole at 0.5 mg kg^{-1} had no effect on alfentanil antinociception and only slightly attenuated the antinociception at 1 mg kg^{-1} (Figure 3). Further, naltrindole alone had no significant effects on nociceptive latencies.

Discussion

The role of δ-opioid receptors in antinociception has been very much an open question due to a lack of selective agonists and antagonists, though evidence with the newer selective δ-agonists clearly points to δ-receptor involvement in spinal and supraspinal antinociception (Rodriguez *et al.*, 1986; Heyman *et al.*, 1987). Since most selective agonists and antagonists at δ-receptors are peptides, this has posed problems for *in vivo* studies. Naltrindole, as a non-peptide δ-receptor antagonist offers the opportunity to re-examine δ-receptor control *in vivo*.

Studies with quaternary opioid antagonists have demonstrated swim-stress-induced antinociception to be centrally-mediated (Jackson & Kitchen, 1989). The ability of naltrindole to block this antinociception supports the assertion that this δ-receptor antagonist readily penetrates the brain after parenteral administration.

Naloxone is an opioid antagonist with only limited selectivity for the multiple opioid receptor subtypes. Most *in vitro* isolated tissue or binding studies indicate a μ/δ selectivity of only 10 fold (Lord *et al.*, 1977; Shaw *et al.*, 1982; James & Goldstein, 1984). As a δ-antagonist, naltrindole in *in vitro* isolated tissue studies shows a >100 fold selectivity for the δ-receptor in comparison with μ and κ receptors (Portoghese *et al.*, 1988) and this selectivity profile has been confirmed in receptor binding studies with brain homogenates (Traynor, personal communication). Alfentanil is a μ-opioid agonist with a μ/δ selectivity ratio of over 600 in binding studies (Yeadon & Kitchen, 1988) and exhibits a profile in nociceptive tests consistent with μ-receptor activation (Niemegeers & Janssen, 1981; Williams *et al.*, 1982). Antinociception induced by alfentanil was blocked by naloxone and only slightly antagonized by naltrindole in doses up to 1 mg kg^{-1} suggesting that naltrindole at these doses, *in vivo*, has negligible activity at μ-receptors. The ability of naltrindole to produce equivalent antagonism of stress-induced antinociception in adult rats to that observed with naloxone suggests that the δ-opioid receptor predominantly mediates this effect. Further, the ability to antagonize stress-induced antinociception in 25 day old rats but not in 20 day old animals confirms our previous suggestion (Jackson & Kitchen, 1989) that δ-opioid receptors are not involved in this response in preweanling animals. The ability of naloxone to block stress-induced antinociception at day 20 (Jackson & Kitchen, 1989) is presumably due to antagonism at μ-receptors at this age.

Though δ-receptors show a delayed development in the rat in comparison with μ-receptors, at postnatal day 20 over 75% of δ-binding sites are present in brain tissue (McDowell & Kitchen, 1986). The failure of δ-receptor antagonism by naltrindole to alter stress-induced antinociception at this early age might suggest that δ-receptors are not coupled to their second messengers or that a specific subpopulation of δ-receptors involved in the stress-induced antinociception response does not develop until the fourth postnatal week. Further there is evidence that stressors causing antinociception release endogenous opioids (see Akil *et al.*, 1986). Opioid peptides such as β-endorphin could mediate stress-induced antinociception in both the neonate and the adult since this peptide exhibits equivalent activity at μ- and δ-receptors (Paterson *et al.*, 1983).

Stress induces increases in plasma corticosterone and indeed stress-induced antinociception can be antagonized by suppression of the hypothalamus pituitary adrenal system with dexamethasone (Lewis *et al.*, 1980, Jackson & Kitchen, 1989). The elevation in corticosterone produced by swimming however appears to be independent of μ- or δ-opioid receptor activation since neither naloxone nor naltrindole attenuated stress-induced rises in corticosterone levels. The dissociation of corticosterone and antinociceptive effects is in agreement with observations in mice where changes in corticosterone induced by swimming are not antagonized by ICI, 174864 or naloxone at doses which block antinociception (Hart *et al.*, 1985).

In conclusion, we have demonstrated that naltrindole shows δ-receptor antagonist properties *in vivo* and that in 25 day old and adult rats, swim-stress-induced antinociception is mediated through the δ-opioid receptor. However, swim-stress-induced antinociception at day 20 appears to involve μ-receptor activation. The reasons and mechanisms underlying this neonatal transition warrant further investigation.

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