Stereoselective opiate antagonist induced hyperalgesia: evidence for a dopaminergic involvement

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It is believed that under normal physiological conditions endogenous opiates are released within the central nervous system which serve to modulate pain perception and transmission (Carmody et al 1979). Consequently it would follow that opiate antagonists should enhance nociceptive responses by blockade of endogenous opiates via opiate receptor occupation. Naloxone has been reported to augment nociceptive responses and produce hyperalgesia in both rats and mice, in a variety of experimental models employing thermal, mechanical, electrical and chemical noxia (Sawynok et al 1979).

It has been established that substances such as apomorphine, L-dopa and nomifensine evoke hyperalgesic responses which involve dopaminergic mechanisms (Tulunay et al 1975; Gonzalez et al 1980, 1981, 1982). We have investigated the possibility that there may be a dopaminergic component in the expression of steroselective opiate antagonist-induced hyperalgesia.

Materials and methods

Male albino mice, GBI variants of an ICI derived strain, 20–22 g, were allowed free access to standard rat and mouse breeding diet and tap water, both being withdrawn 2 h before experimentation. Nociceptive sensitivity (reaction latency in s) was determined using the hot plate method of Wolfe & MacDonald (1944) employing a surface stimulus temperature of 55 ± 0·5 °C. The nociceptive end point was taken to be the appearance of paw licking, flicking of the hind limbs or attempts to escape, and a cut-off time of 30 s was imposed to prevent tissue damage and the resultant change in skin sensitivity. Before treatment, nociceptive latencies of naive animals were determined, 'non-responders' within 10 s being excluded from the study. Drug pretreatments were administered intraperitoneally (i.p.) 30 min before subcutaneous (s.c.) administration of test agents and all dose volumes were restricted to 10 ml kg^{-1}.

Drugs used: Racemic naloxone hydrochloride (Endo), cis-flupenthixol (Lundbeck), dopamine hydrochoride (Sigma), Mr1452 (−)-(3-furylmethyl)-2¹-hydroxy-5,9-dimethyl-6,7-benzomorphan and its (+)-isomer Mr1453 (Boehringer Ingelheim) were dissolved in sterile 0·9% apyrogenic NaCl (saline). Sulpiride (Revizza) was dissolved in 1% tartaric acid solution and tetrabenazine (Roche) in 0·1M hydrochloric acid, both solutions were then back titrated to approximate neutrality. Control animals

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were treated with either saline or vehicle and all doses refer to weight of respective salts where appropriate. Statistics: all results are expressed as means ± standard errors. The statistical difference between groups was compared using the two tailed Student's t-test and significance was assumed where P values were less than 0·05.

Results

Effects of Mr1452, Mr1453 and naloxone in the hot plate test (55 °C). The opiate antagonist Mr1452, in doses up to 50 mg kg^{-1} produced a quantifiable linear dose-related decrease in nociceptive reaction latency compared with saline controls. However it is inactive (+)-isomer Mr1453 did not facilitate or enhance the nociceptive reactivity (Fig. 1). In addition, racemic naloxone, though inactive at 5 and 10 mg kg^{-1}, produced significant hyperalgesia at doses in the region of 20 mg kg^{-1}, the effect being less potent than Mr 1452. In subsequent experiments, mid-range doses of Mr1452 (20 mg kg^{-1}), Mr1453 (20 mg kg^{-1}) and naloxone (20 mg kg^{-1}) were selected for interaction studies with modifiers of dopaminergic function.

Effects of cis-flupenthixol and sulpiride on the hyperalgesic response to opiate antagonists. Pretreatment (30 min) with
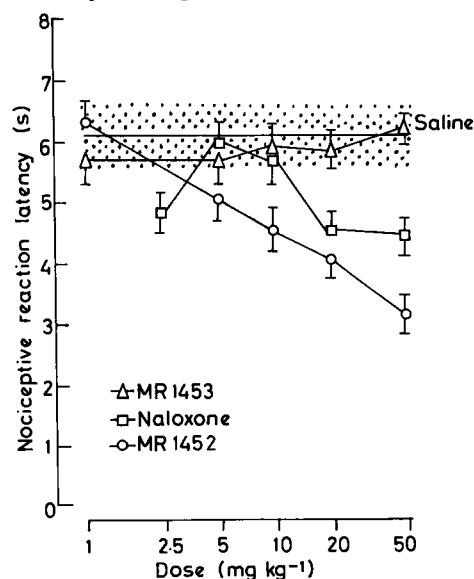

FIG. 1. Effects of naloxone, Mr1452 and Mr1453 on nociceptive reaction latencies on the hot plate (55 °C) 30 min after subcutaneous injection in the mouse.

Table 1. Effects of the dopamine antagonists *cis*-flupenthixol (0·25 mg kg⁻¹ i.p.) and sulpiride (25 mg kg⁻¹ i.p.) on nociceptive responses latencies evoked on the hot plate (55 °C) 30 min after subcutaneous injection of naloxone (20 mg kg⁻¹), Mr1452 (20 mg kg⁻¹) and Mr1453 (20 mg kg⁻¹) in the mouse.

Pretreatment (time −30 min)	Treatment (zero time)	Mean nociceptive reaction time ±s.e.m. (n = 8)	Significance P of difference from controls
Vehicle	Vehicle	5·50 ± 0·33	
cis-Flupenthixol	Vehicle	5·80 ± 0·48	NS
Vehicle	Mr1452	2·62 ± 0·26	<0·001
cis-Flupenthixol	Mr1452	4·50 ± 0·19	NS
Vehicle	Mr1453	5·14 ± 0·50	NS
cis-Flupenthixol	Mr1453	5·50 ± 0·53	NS
Vehicle	Naloxone	3·86 ± 0·53	<0·001
cis-Flupenthixol	Naloxone	5·50 ± 0·49	NS
Vehicle	Vehicle	5·71 ± 0·35	
Sulpiride	Vehicle	6·00 ± 0·37	NS
Vehicle	Mr1452	2·86 ± 0·26	<0·001
Sulpiride	Mr1452	2·85 ± 0·26	<0·001
Vehicle	Mr1453	5·14 ± 0·50	NS
Sulpiride	Mr1453	5·28 ± 0·18	NS
Vehicle	Naloxone	4·00 ± 0·26	<0·001
Sulpiride	Naloxone	4·00 ± 0·50	<0·001

Table 2. Effects of pretreatment with tetrabenazine (50 mg kg⁻¹ i.p.) on the hyperalgesia produced by Mr1452 (20 mg kg⁻¹ s.c.) on the hot plate (55 °C) and its subsequent modification by intracerebroventricular (i.c.v.) injection of dopamine (10 μg per animal) in the mouse.

Pretreatment (time −165 min)	Treatment*	Mean nociceptive reaction time ±s.e.m. (n = 8)	Significance P of difference from controls
Vehicle	Vehicle + Saline i.c.v.	5·87 ± 0·48	
Tetrabenazine	Vehicle + Saline i.c.v.	6·0 ± 0·50	NS
Vehicle	Vehicle + Dopamine i.c.v.	6·35 ± 0·4	NS
Vehicle	Mr1452 + Saline i.c.v.	2·85 ± 0·34	<0·001
Tetrabenazine	Mr1452 + Saline i.c.v.	4·62 ± 0·37	NS
Vehicle	Mr1452 + Dopamine i.c.v.	3·25 ± 0·31	<0·001
Tetrabenazine	Mr1452 + Dopamine i.c.v.	3·36 ± 0·28	<0·001

* Vehicle + Mr1452 administered at zero time; i.c.v. dopamine and saline at zero + 15 min.

the dopamine (D-1) receptor antagonist *cis*-flupenthixol (0·25 mg kg⁻¹ i.p.) abolished the hyperalgesic response produced by both Mr1452 and naloxone but had no effect on that of Mr1453 (Table 1). In contrast, pretreatment (30 min) with sulpiride (25 mg kg⁻¹ i.p.) a reputed D-2 receptor antagonist (Kebabian & Calne 1979) did not significantly modify the responses evoked either by Mr1452 or naloxone. In each case pretreatment with dopamine antagonists by themselves as positive controls did not alter levels of reactivity on the hot plate.

Effects of tetrabenazine and i.c.v. dopamine on opiate antagonist hyperalgesia. After pretreatment (165 min) with tetrabenazine (50 mg kg⁻¹ i.p.), a depletor of central catecholamines and 5-hydroxytryptamine (Quinn 1959), the hyperalgesia produced by Mr1452 was abolished and reaction times returned to levels which were not significantly different from controls ($P > 0·05$) (Table 2). However, intracerebroventricular injection (i.c.v.) of dopamine at a non-hyperalgesic dose (10 μg per animal) completely restored the hyperalgesia in Mr1452 treated animals, pretreated with tetrabenazine.

Discussion

In these studies a stereospecific attenuation of nociceptive responses by the opiate antagonist Mr1452 has been demonstrated, suggesting a possible interaction at a specific opiate receptor site. This finding confirms the observations made by Jacob & Ramabadran (1978) who used other (−)-isomers or opiate antagonists to reduce the latency of the jumping reaction of mice on the hot plate test. The difference in potency between Mr1452 and naloxone in producing hyperalgesia may be partially attributed to the fact that a racemic mixture of naloxone has been employed in the current study. However others have reported Mr1452 to possess a fifth of the antagonist potency of naloxone in vitro (Waterfield & Kosterlitz 1975) which may reflect differences in these experimental models.

The observation that *cis*-flupenthixol reversed the hyperalgesia produced by Mr1452 and naloxone would imply a D-1 receptor involvement, since *cis*-flupenthixol is reputed to be a specific ligand at this site (Hyttel 1980). Moreover, sulpiride at doses well in excess of threshold antagonist doses at D-2 receptors (Costall et al 1980) failed to modify opiate-antagonist induced hyperalgesia and this therefore may exclude any involvement of D-2 receptors in this particular phenomenon. The ability of tetrabenazine to abolish hyperalgesic effects may be attributable to its depleting action on central stores of catecholamines or 5-hydroxytryptamine. In addition, the reversal of the antagonism produced by tetrabenazine when dopamine was administered i.c.v. would further suggest the specific involvement of dopamine pathways in these effects. In conclusion, it is hypothesized that this central dopaminergic component in the expression of stereoselective opiate antagonist hyperalgesia is linked to the D-1 class of opiate receptors.

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A comparison of the effects of Pro-Leu-Gly NH₂ and L-leucine on tremorine-induced tremor and rigidity in rats

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The mechanism of action of the potential anti-Parkinson tripeptide Pro-Leu-GlyNH₂ (PLG) remains obscure despite numerous animal studies. Tremorine, and its active metabolite oxotremorine, provide an animal model for testing potential anti-Parkinson agents. Acute administration of PLG was reported to antagonize tremorine-induced tremor in rats (Plotnikoff et al 1972) and oxotremorine-induced tremor in mice (Plotnikoff & Kastin 1974), although others were unable to confirm these findings (Kruse 1977; Björkman et al 1980). A recent study (Dickinson et al 1981) has demonstrated a partial antagonism of tremorine tremor and rigidity in rats treated chronically with PLG, whereas acute doses of the peptide were ineffective. One explanation of these findings may be the accumulation of an active metabolite of PLG during chronic treatment with the peptide. PLG has a short half life in rats and the only significant metabolite is L-leucine (Witter et al 1980). This paper compares the effects of chronic administration of PLG and L-leucine on tremorine tremor and rigidity in rats. Tremorine was used rather than oxotremorine because preliminary studies had established that tremorine produces more sustained tremor and rigidity.

Methods
Female Sprague Dawley rats, 180–220 g, were used. Pro-Leu-GlyNH (PLG, Sigma, 2 mg kg⁻¹) and L-leucine (Sigma, 1 mg kg⁻¹) were dissolved in 0·9% (NaCl saline) solution and administered i.p. once daily for 5 days. Control rats were given saline. Every rat was given 1 mg kg⁻¹ of atropine methylnitrate 15 min before tremorine.

Normal limb tone and tremorine-induced rigidity were measured using a mechanical apparatus (Dickinson et al 1981) which measured the force required to partly flex one hind leg of a conscious, lightly restrained rat. Rats were placed singly in the apparatus and 10–12 measurements min⁻¹ of limb resistance to flexion were taken for 5–10 min. Tremorine dihydrochloride (20 mg kg⁻¹ u.p.) was administered without removing the rat and limb tone measurements made for 35 min.

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Tremor was recorded from one hind leg of rats held singly in a plastic, cylindrical restraining box with 2 slots through which the hind legs hung free. A small permanent magnet was taped to one leg. The leg was surrounded by a coil of 1400 turns of 0·5 mm polyurethane-coated copper wire. Limb movements induced a proportional voltage in the coil which was amplified by a Grass 7P1 preamplifier. The circuit included a filter which eliminated the large voltages induced by gross movements so that only tremors were recorded. The amplified, filtered d.c. signal was divided. One signal was integrated (Grass 7P10) to provide a chart record of tremor intensity measured at 1 min intervals. The other signal triggered a pulse generator. Each tremor generated a constant pulse. The pulses were accumulated by a calibrated (0–25 Hz) 7P10 integrator which re-set every min. A chart record displayed the average tremor frequency.
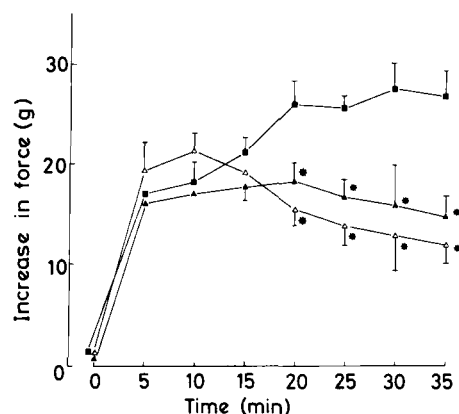

FIG. 1. Effects of PLG and L-leucine on tremorine-induced increase in hind limb muscle tone in rats. Tremorine dihydrochloride (20 mg kg⁻¹) was administered at time 0 to 8 saline-treated rats (■——■), 10 rats pretreated with PLG 2 mg kg⁻¹ once daily for 5 days (▲——▲), 10 rats pretreated with L-leucine 1 mg kg⁻¹ once daily for 5 days (△——△). Each point is the mean increase in the force required to displace 1 hind leg. Vertical bars show s.e.m. Significance of difference between saline and PLG/leucine groups at the corresponding time interval *P < 0·5 (Student's unpaired, 2-tailed t-test).