J. Pharm. Pharmacol. 1991, 43: 640–643
Received January 15, 1991

Investigation of the Different Types of Opioid Receptor Involved in Electroconvulsive Shock-induced Antinociception and Catalepsy in the Rat

HELEN C. JACKSON AND D. J. NUTT

Reckitt and Colman Psychopharmacology Unit, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK

Abstract—The effects of novel opioid antagonists on the behavioural syndrome induced by electroconvulsive shock (ECS) in rats have been examined and compared with those of the established agent naloxone. A single ECS produced catalepsy and significantly increased tail immersion response times during the 15 min following the seizure. These responses were inhibited by a low dose of naloxone (1 mg kg^{-1}, i.p.) and also by RX8008M (16-methylcyprenorphine; 1 mg kg^{-1}, i.p.) which blocks μ- and δ- but not κ-opioid receptor function. In comparison, the antinociception and catalepsy induced by ECS was not attenuated by the selective δ-receptor antagonist naltrindole (1 mg kg^{-1}, i.p.). These results suggest that ECS-induced antinociception and catalepsy may be mediated by endogenous opioids acting at μ-opioid receptors and are consistent with biochemical studies showing the release of β-endorphin in both animals and man following this procedure.

Electroconvulsive shock (ECS) produces both antinociception and catalepsy in rats. These responses appear to be mediated by endogenous opioids since they are reversed by opioid antagonists (see review by Jackson & Nutt 1990). Most studies have employed the opioid antagonists naloxone and naltrexone which unfortunately cannot discriminate between the different types of opioid receptor in-vivo. More selective compounds have recently become available including RX8008M (16-methylcyprenorphine) and naltrindole. RX8008M is of interest as it has been characterized in isolated tissue preparations as a pure competitive opioid antagonist with high affinity for μ- and δ-receptors and low affinity for κ-receptors (Smith 1987). It can also clearly distinguish between μ- and κ-receptor function in whole animal work (Birch & Hayes 1987; Birch et al 1988; Hayes & Birch 1988; Jackson & Kitchen 1989a, b). Naltrindole is a stable non-peptide opioid antagonist which has been shown to act selectively at δ-receptors in a number of different paradigms (Portoghese et al 1988, 1990; Rogers et al 1990).

In the current study we have compared the effects of naloxone, RX8008M and naltrindole on ECS-induced antinociception and catalepsy in rats in order to investigate the relative roles played by different opioid receptors in these responses.

Materials and Methods

Animals

Male Wistar rats (Bantin & Kingman), 110–150 g, were housed in groups of 8 on a 14:10 h light-dark cycle (lights on at 0500 h) at a temperature of 23 ± 2°C and with free access to standard rat diet and drinking water at all times. Animals were used on only one occasion.

Correspondence: H. C. Jackson, Reckitt and Colman Psychopharmacology Unit, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK.

Experimental procedures

On the day of the test, rats were transferred to a quiet, air-conditioned laboratory (23 ± 2°C) and acclimatized to these conditions for at least 2 h before use. All experiments were carried out between 1300 and 1700 h. In studies of the effects of opioid antagonists on ECS-induced antinociception and catalepsy 3 treatment groups were employed: sham-vehicle; ECS-vehicle and ECS-antagonist. ECS was applied to conscious rats by ear-clip electrodes (80 mA, unipolar, 0·5 s duration) using a constant current generator (Applegarth Electronics, Oxford, UK). Electrodes were clipped onto the sham controls but the current was not switched on. Only animals exhibiting full tonic hindlimb seizures to ECS were used in the study and to account for this and for any ECS-induced fatalities ECS-treatment groups initially contained a larger number of animals (14) than the sham-treatment groups (7 or 8). Experiments examining the effects of naloxone and RX8008M on ECS-induced catalepsy and antinociception, were performed in parallel, using the same sham- and ECS-vehicle treated groups. The effects of naltrindole on these parameters were examined on a separate occasion using separate control groups.

Antinociception was assessed, immediately before injection and 5, 10, 15, 30 and 60 min after ECS, using the tail immersion test. Rats were hand-held over a hot-water bath with the terminal 2–3 cm portion of their tails in water at a temperature of 55°C. This noxious stimulus produced a distinct tail flick response and response times were determined to the nearest 0·1 s using a stop-watch.

Catalepsy was measured 15 min after ECS. Animals were held with their forepaws resting over a horizontal bar (5 cm from the ground) for 3 s and then gently released. Catalepsy was defined as present if animals remained on the bar for over 60 s.

Drugs

RX8008M and naltrindole were synthesized at Reckitt &

Colman, Hull. Naloxone was purchased from Research Biochemicals Incorporated. All drugs were dissolved in pH 3 0·9% NaCl (saline) and injected intraperitoneally (i.p.) using a dose volume of 5 mL kg^{-1}. The experimenter was not aware of the drug treatment of the animals given ECS. Animals were given naloxone (1 mg kg^{-1}) or RX8008M (1 mg kg^{-1}) 5 min before ECS and naltrindole (1 mg kg^{-1}) 30 min before ECS. Pretreatment times and doses of antagonist were based on those shown to be effective in other studies in our laboratory and by other workers (Birch & Hayes 1987; Birch et al 1988; Hayes & Birch 1988; Jackson & Kitchen 1989b, c; Jackson et al 1989; Gacel et al 1990).

Statistical analysis

In antinociceptive studies, treatment group means were statistically compared using analysis of variance followed by Dunnett's test. Statistical comparisons between the number of animals exhibiting catalepsy in each treatment group were made using the Fisher exact test.

Results

Nociceptive thresholds of control animals showed a small increase in the first 15 min of the experiment (Figs 1, 2, 3). A single ECS induced a further 2-fold increase in tail immersion response times over the sham-treated control levels. This antinociceptive response began to develop 5 min post-ECS and peaked after 10 min before decreasing to baseline values 30 to 60 min after the seizure (Figs 1, 2, 3).

The antinociception induced by a single ECS was significantly attenuated by pretreatment with the opioid antagonist naloxone (1 mg kg^{-1}; Fig. 1). This effect was not apparent at 5 min but began to emerge at the 10 min reading and by 15 min the tail immersion response times of animals given naloxone and ECS were not significantly different from those of the sham and vehicle-treated controls. The μ/δ-opioid antagonist RX8008M (1 mg kg^{-1}) significantly reduced ECS-induced antinociception in a similar manner (Fig. 2). Thus, the inhibition showed a delay in development but by 15 min had reduced the elevated response times of ECS-treated animals back to control levels. In contrast, the δ-opioid antagonist naltrindole (1 mg kg^{-1}) did not modify the increase in tail immersion response time induced by ECS at either the 5, 10 or 15 min reading (Fig. 3).

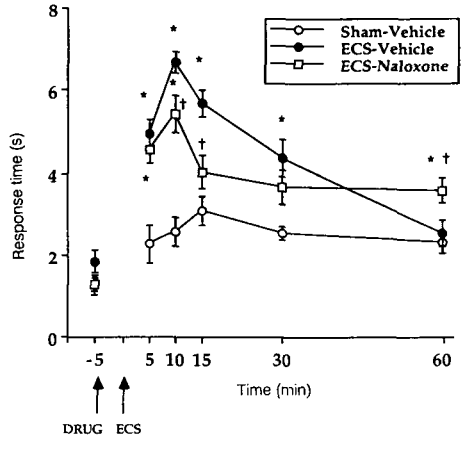

FIG. 1. Antagonism of ECS-induced antinociception by naloxone (1 mg kg^{-1}, i.p.). Values represent means ± s.e.m. for groups of 8 (sham-vehicle) or 13 (ECS-treated) rats. * $P<0.05$ compared with sham-vehicle, † $P<0.05$ compared with ECS-vehicle.

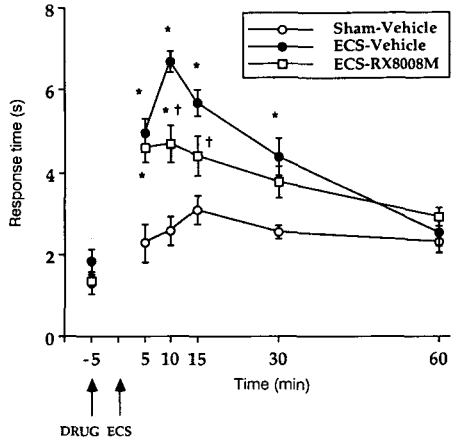

FIG. 2. Antagonism of ECS-induced antinociception by RX8008M (1 mg kg^{-1}, i.p.). Values represent means ± s.e.m. for groups of 8 (sham-vehicle); 13 (ECS-vehicle) or 14 (ECS-RX8008M) rats. * $P<0.05$ compared with sham-vehicle, † $P<0.05$ compared with ECS-vehicle.

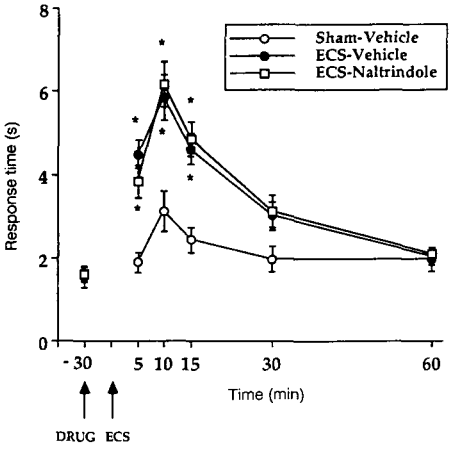

FIG. 3. Lack of effect of naltrindole (1 mg kg^{-1}, i.p.) on ECS-induced antinociception in the rat. Values represent means ± s.e.m. for groups of 7 (sham-vehicle); 13 (ECS-vehicle) or 14 (ECS-naltrindole) animals. * $P<0.05$ compared with sham-vehicle.

Table 1. Effect of opioid antagonists on ECS-induced catalepsy in rats.

Treatment	Number cataleptic/number tested
Sham-vehicle (i.p.)	0/8
ECS-vehicle (i.p.)	9/13*
ECS-naloxone (1 mg kg^{-1}, i.p.)	4/13§
ECS-RX8008M (1 mg kg^{-1}, i.p.)	5/14
Sham-vehicle (i.p.)	1/7
ECS-vehicle (i.p.)	9/13*
ECS-naltrindole (1 mg kg^{-1} i.p.)	11/14*

*$P<0.05$ compared with sham-vehicle, §$P<0.05$ compared with ECS-vehicle.

The catalepsy produced by a single ECS was also significantly reversed by naloxone (1 mg kg^{-1}) as shown in Table 1. ECS-induced catalepsy was also attenuated by RX8008M (1 mg kg^{-1}) and although this response was not significant ECS did not produce significant catalepsy in RX8008M-treated animals suggesting some antagonism had occurred (Table 1). In contrast, naltrindole (1 mg kg^{-1}) had no effects on the catalepsy induced by ECS (Table 1).

Naloxone (1 mg kg^{-1}), RX8008M (1 mg kg^{-1}) and naltrindole (1 mg kg^{-1}) do not have any effects in the tail immersion test (data not shown) and do not produce any overt behaviours in animals not given ECS treatment.

Discussion

In this study we confirmed that a single ECS produced antinociception and catalepsy in rats. The antinociception was of short duration and was reversed by a low dose of the opioid antagonist naloxone. Sham-treated animals which were injected with vehicle also showed a small increase in nociceptive thresholds. This was of similar magnitude to the small stress-induced increase seen on injection alone—a response which is not altered by administration of naloxone (own observations and see Jackson & Kitchen (1989c) and Jackson et al (1989)). Our finding that naloxone attenuates ECS-induced antinociception is consistent with results from others who have reported naloxone-sensitive increases in tail flick response times (using a radiant heat source) following ECS (Urca et al 1981; Furui et al 1986). On the other hand, Holaday & Belenky (1980) observed that the increases in tail flick responses induced by ECS were resistant to naloxone. However, their results are difficult to interpret since the high doses they used would not necessarily act selectively at opioid receptor systems (Sawynok et al 1979) and also produced antinociception when given alone.

A novel finding in the present study was that the antinociceptive effects of ECS in rats were also attenuated by the stable non-peptide opioid antagonist RX8008M. This compound differs from naloxone in that it discriminates between μ- and κ-opioid receptors. Such selectivity has been demonstrated in a range of isolated tissue preparations in-vitro (Smith 1987) and also in the whole animal. For instance, the dose of RX8008M used in this study (1 mg kg^{-1}) produced large (over twentyfold) shifts in the antinociceptive dose-response curves for the μ-agonists morphine and fentanyl in mice without effecting the antinociception induced by κ-agonists, even in doses 30 times higher (Birch et al 1988; Hayes & Birch 1988). Moreover, a low dose of RX8008M blocked the antinociception induced by the μ-agonist D-Ala^2-$MePhe^4$-Gly-ol^5-enkephalin in neonatal rats (Jackson & Kitchen 1989b) whereas RX8008M was inactive against κ-responses in tenfold higher doses (Jackson & Kitchen 1989a). Additionally, RX8008M (1 mg kg^{-1}) blocked the anti-diuretic effects of μ-agonists in water-loaded rats, but did not block the diuretic effects of κ-agonists in doses up to 16 times higher (Birch & Hayes 1987). Therefore, our observation that ECS-induced antinociception is blocked by RX8008M suggests that it is mediated by μ- rather than κ-opioid receptors. Final proof of this supposition would be obtained by the use of the recently developed selective κ-antagonist norbinaltorphimine (Portoghese et al 1987; Takemori et al 1988). Unfortunately, this compound has poor systemic bioavailability and is very expensive, therefore it generally has to be given by the intracerebroventricular (i.c.v.) route. The good bioavailability of RX8008M after peripheral administration and its low affinity for κ-receptors makes it a useful alternative approach for studying the κ-receptor component of endogenous opioid activity.

However, in addition to blocking μ-receptors, RX8008M also antagonises δ-receptor function in isolated tissues (Smith 1987) and has been shown to reduce the antinociceptive effects of the δ-agonist D-Pen^2, D-Pen^5-enkephalin following i.c.v. administration (Fanselow et al 1989). Thus, the effects of this compound on ECS-induced antinociception could be due to δ-receptor blockade. To examine this possibility we used the opioid antagonist naltrindole which acts selectively at δ-opioid receptors in receptor binding studies and in isolated tissues (Portoghese et al 1988, 1990; Rogers et al 1990). Moreover, naltrindole blocks the antinociceptive effects of selective δ-, but not μ- and κ-opioid agonists, in mice (Portoghese et al 1988; Gacel et al 1990). In addition, the dose used in the current study has no effect on the antinociception induced by the μ-opioid agonist sufentanil in rats (unpublished observations) although it blocks δ-mediated swim-stress-induced antinociception in this species (Jackson et al 1989). Thus, the absence of an effect of naltrindole on ECS-induced antinociception in rats, precludes a major role for δ-opioid receptors in the ECS-induced response. The doses of RX8008M and naltrindole used in this study had no effects on tail immersion response times in animals not exposed to ECS (own observations), although, at high doses of naltrindole, agonist-like properties do emerge (Jackson et al 1989).

The only previous study examining the contribution of μ- and δ-receptors to ECS-antinociception was by Belenky et al (1983). They reported that the peptide δ-antagonist ICI 154, 129 given i.c.v. reduced post-ictal increases in hot plate escape times while 24 h pretreatment with the non-competitive μ-antagonist β-funaltrexamine did not. These findings are the reverse of those reported here. The much longer duration of antinociception measured using the hot plate test (over an hour) suggests it may be quite different from that detected by the tail immersion test. Furthermore, ICI 154, 129 elevated hot plate escape times itself and these partial agonist properties could have influenced the results.

The lack of ability of either naloxone or RX8008M to antagonize ECS-induced antinociception in the immediate post-ictal period is interesting. Similar delayed effects of opioid antagonists have been noticed by other workers (Furui et al 1986) who have suggested that the initial antinociceptive response following ECS may be associated with activation of a spinal noradrenergic system. Moreover, the brief time-lag before onset of naloxone-sensitive antinociception may merely reflect the time required for endogenous opioids released by ECS (see below) to reach their site of action.

The catalepsy induced by a single ECS in rats was reversed by a low dose of naloxone confirming its mediatation by opioid receptors as previously reported by others (using higher doses of opioid antagonist; Holaday & Belenky (1980), Urca et al (1981) and Frenk & Stein (1984). It was

also antagonized, although to a lesser extent, by RX8008M. In contrast ECS-induced catalepsy was not modified by naltrindole. Hence ECS-induced catalepsy resembles ECS-induced antinociception in that it appears to be mediated by μ-, but not δ- (or κ-) opioid receptors. The time course of ECS-induced catalepsy, however, is much longer than the post-ictal increase in response times showing that these effects are under independent control (unpublished observations; Furui et al 1986).

In conclusion, the results of this preliminary study show blockade of ECS-induced antinociception and catalepsy by naloxone and RX8008M, but not naltrindole. These findings suggest that these responses may be mediated via the release of an endogenous opioid which acts at μ-opioid receptors. A possible candidate for such a ligand is β-endorphin (Lord et al 1977; Wuster et al 1979; Shook et al 1988). There is some evidence from animal studies that levels of this peptide are reduced in the pituitary and the hypothalamus following ECS, presumably reflecting release (Dias et al 1981; Lason et al 1987). Moreover, elevated β-endorphin levels in plasma have been reported following this procedure (Lason et al 1987; Thiagarajan et al 1989). Finally, since β-endorphin is also increased by electroconvulsive therapy in man (Weizman et al 1987) it is conceivable that this peptide may play a role in some of the therapeutic effects of ECT in the clinic.

Acknowledgements

We would like to thank Miss Tamzin Ripley and Dr David Ball for their assistance with some of the experiments.

References

Belenky, G. L., Gelinas-Sorell, D., Kenner, J. R., Holaday, J. W. (1983) Evidence for δ-receptor involvement in the post-ictal antinociceptive responses to electroconvulsive shock in rats. Life Sci. 33 (Suppl. I): 583–585

Birch, P. J., Hayes, A. G. (1987) Effect of the antagonist 16-methyl cyprenorphine (RX8008M) on opioid-induced changes in urine output in the water-loaded rat. Br. J. Pharmacol. 91: 472P

Birch, P. J., Hayes, A. G., Sheehan, M. J., Tyers, M. B. (1988) In-vivo studies with the opioid antagonist, 16-methylcyprenorphine. J. Pharm. Pharmacol. 40: 213–214

Dias, R. D., Perry, M. L. S., Carrasco, M. A., Izquierdo, I. (1981) Effect of electroconvulsive shock on β-endorphin immunoreactivity of rat brain, pituitary gland, and plasma. Behav. Neural Biol. 32: 265–268

Fanselow, M. S., Calcagnetti, D. J., Helmstetter, F. J. (1989) Delta opioid antagonist, 16-Me cyprenorphine, selectively attenuates conditional fear- and DPDPE-induced analgesia on the formalin test. Pharmacol. Biochem. Behav. 32: 469–474

Frenk, H., Stein, B. E. (1984) Endogenous opioids mediate ECS-induced catalepsy at supraspinal levels. Brain Res. 303: 109–112

Furui, T., Harty, G. J., Yaksh, T. L. (1986) Studies on the effects of opioid, noradrenergic and serotonergic antagonists on the antinociceptive effects of electroconvulsive shock. Ibid. 367: 162–168

Gacel, G. A., Fellion, E., Baamonde, A., Dauge, V., Roques, B. P. (1990) Synthesis, biochemical and pharmacological properties of BUBUC, a highly selective and systemically active agonist for in vivo studies of δ-opioid receptors. Peptides 11: 983–988

Hayes, A. G., Birch, P. J. (1988) Reversal by β-funaltrexamine and 16-methyl cyprenorphine of the antinociceptive effects of opioid agonists in the mouse and guinea-pig. Neuropharmacology 27: 813–816

Holaday, J. W., Belenky, G. L. (1980) Opiate-like effects of electroconvulsive shock in rats: a differential effect of naloxone on nociceptive measures. Life Sci. 27: 1929–1938

Jackson, H. C., Kitchen, I. (1989a) Behavioural effects of selective μ-, κ-, and δ-opioid agonists in neonatal rats. Psychopharmacology 97: 404–409

Jackson, H. C., Kitchen, I. (1989b) Antinociceptive effects of the μ-selective agonist [D-Ala^2-MePhe^4-Gly-ol^5] enkephalin in 10-day old neonatal rats. Adv. Biosci. 75: 487–490

Jackson, H. C., Kitchen, I. (1989c) Swim-stress-induced antinociception in young rats. Br. J. Pharmacol. 96: 617–622

Jackson, H. C., Nutt, D. J. (1990) Does electroconvulsive shock therapy work through opioid mechanisms? Human Psychopharmacology 5: 3–23

Jackson, H. C., Ripley, T. L., Nutt, D. J. (1989) Exploring δ-receptor function using the selective opioid antagonist naltrindole. Neuropharmacology 28: 1427–1430

Lason, W., Przewlocka, B., Przewlocki, R. (1987) Single and repeated electroconvulsive shock differentially affects the prodynorphin and pro-opiomelanocortin system in the rat. Brain Res. 403: 301–307

Lord, J. A. H., Waterfield, A. A., Hughes, J., Kosterlitz, H. W. (1977) Endogenous opioid peptides: multiple agonists and receptors. Nature 267: 495–499

Portoghese, P. S., Lipkowski, A. W., Takemori, A. E. (1987) Binaltorphimine and nor-binaltorphimine, potent and selective κ-opioid receptor antagonists. Life Sci. 40: 1287–1292

Portoghese, P. S., Sultana, M., Takemori, A. E. (1988) Naltrindole, a highly selective and potent non-peptide δ opioid receptor antagonist. Eur. J. Pharmacol. 146: 185–186

Portoghese, P. S., Sultana, M., Takemori, A. E. (1990) Design of peptidomimetic δ opioid receptor antagonists using the message-address concept. J. Med. Chem. 33: 1714–1720

Rogers, H., Hayes, A. G., Birch, P. J., Traynor, J. R., Lawrence, A. J. (1990) The selectivity of the opioid antagonist, naltrindole, for δ-opioid receptors. J. Pharm. Pharmacol. 42: 358–359

Sawynok, J., Pinsky, C., LaBella, F. S. (1979) Minireview on the specificity of naloxone as an opiate antagonist. Life Sci. 25: 1621–1632

Shook, J. E., Kazmierski, W., Wire, W. S., Lemcke, P. K., Hruby, V. J., Burks, T. F. (1988) Opioid receptor selectivity of β-endorphin in vitro and in vivo: mu, delta and epsilon receptors. J. Pharmacol. Exp. Ther. 246: 1018–1025

Smith, C. F. C. (1987) 16-Me cyprenorphine (RX 8008M): a potent opioid antagonist with some δ selectivity. Life Sci. 40: 267–274

Takemori, A. E., Ho, B. Y., Naeseth, J. S., Portoghese, P. S. (1988) Nor-binaltorphimine, a highly selective kappa-opioid antagonist in analgesic and receptor binding assays. J. Pharmacol. Exp. Ther. 246: 255–258

Thiagarajan, A. B., Gleiter, C. H., Mefford, I. N., Eskay, R. L., Nutt, D. J. (1989) Effect of single and repeated electroconvulsive shock on the hypothalamic-pituitary-adrenal axis and plasma catecholamines in rats. Psychopharmacology 97: 548–552

Urca, G., Yitzhaky, J., Frenk, H. (1981) Different opioid systems may participate in post-electroconvulsive shock (ECS) analgesia and catalepsy. Brain Res. 219: 385–396

Weizman, A., Gil-Ad, I., Grupper, D., Tyano, S., Laron, Z. (1987) The effect of acute and repeated electroconvulsive treatment on plasma β-endorphin, growth hormone, prolactin and cortisol secretion in depressed patients. Psychopharmacology 93: 122–126

Wuster, M., Schulz, R., Herz, A. (1979) Specificity of opioids towards the μ-, δ- and ε-opiate receptors. Neurosci. Lett. 15: 193–198