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A pharmacological profile of the novel, peripherally-selective κ-opioid receptor agonist, EMD 61753

[1]A. Barber, G.D. Bartoszyk, *H.M. Bender, R. Gottschlich, H.E. Greiner, J. Harting, F. Mauler, K.-O. Minck, R.D. Murray, **M. Simon & C.A. Seyfried

Preclinical Pharmaceutical Research, E. Merck, 64271 Darmstadt, Germany; *Institute of Pharmacokinetics and Metabolism, E. Merck, 85567 Grafing, Germany and **Merck-Clevenot S.A., 5/9 Rue Anquetil, F-94731 Nogent-sur-Marne, France

1 The pharmacological properties of the novel diarylacetamide κ-opioid receptor agonist, EMD 61753, have been compared with those of ICI 197067 (a centrally-acting κ agonist) and ICI 204448 (a peripherally-selective κ agonist).

2 EMD 61753 binds with high affinity (IC_{50} 5.6 nM) and selectivity (κ:μ:δ:σ binding ratio 1:536:125:> 1,786) to κ-opioid receptors and is a full and potent (IC_{50} 54.5 nM) agonist in an *in vitro* assay for κ-opioid receptors (rabbit vas deferens preparation).

3 Systemically-applied [^{14}C]-EMD 61753 is found in high concentrations in the lungs, liver, adrenal glands and kidneys. Considerably less radioactivity is detected in the whole brain, and this radioactivity is concentrated in the region of the cerebral ventricles in the choroid plexuses. EMD 61753 penetrates only poorly into the CNS.

4 EMD 61753 was weakly effective in pharmacological tests of central activity. This compound reversed haloperidolol-induced DOPA accumulation in the nucleus accumbens of the rat only at a dose of 30 mg kg^{-1}, s.c., (doses of 0.1, 1.0 and 10 mg kg^{-1}, s.c., and 1.0, 10 and 100 mg kg^{-1}, p.o., were inactive). Hexobarbitone-induced sleeping in mice was prolonged by EMD 61753 at threshold doses of 10 mg kg^{-1}, s.c., and 100 mg kg^{-1}, p.o., whereas the motor performance of rats in the rotarod test was impaired by EMD 61753 with an ID_{50} value of 453 mg kg^{-1}, s.c.

5 EMD 61753 produced dose-dependent, naloxone-reversible antinociception in the mouse formalin test (1st phase ID_{50} 1.9 mg kg^{-1}, s.c., and 10.4 mg kg^{-1}, p.o.; 2nd phase ID_{50} 0.26 mg kg^{-1}, s.c., and 3.5 mg kg^{-1}, p.o.) and rodent abdominal constriction test (ID_{50} mouse 1.75 mg kg^{-1}, s.c., and 8.4 mg kg^{-1}, p.o.; ID_{50} rat 3.2 mg kg^{-1}, s.c., and 250 mg kg^{-1}, p.o.). EMD 61753 was inactive, or only weakly effective, in the rat pressure test under normalgesic conditions. After the induction of hyperalgesia with carrageenin, however, this compound elicited potent, dose-dependent (ID_{50} 0.08 mg kg^{-1}, s.c., and 6.9 mg kg^{-1}, p.o., after remedial application, and 0.2 mg kg^{-1}, s.c., and 3.1 mg kg^{-1}, p.o., after prophylactic application) and naloxone-reversible antinociception. The antinociceptive action of systemically-applied (50 mg kg^{-1}, p.o.) EMD 61753 in the hyperalgesic pressure test was completely inhibited by injection of the κ-opioid antagonist norbinaltorphimine (100 μg) into the inflamed tissue, a result which indicates that this opioid effect is mediated peripherally.

6 Cutaneous plasma protein extravasation produced by antidromic electrical stimulation of the rat saphenous nerve was dose-dependently inhibited by systemically-applied EMD 61753 (ID_{50} values 3.7 mg kg^{-1}, s.c., and 35.8 mg kg^{-1}, p.o.), and this effect was completely antagonized by intraplantar application of norbinaltorphimine (50 μg). Extravasation elicited by the intraplantar application of substance P (10 μg) was not influenced by the administration of EMD 61753.

7 EMD 61753 produced dose-dependent diuresis in non-hydrated rats at doses of and above 1.0 mg kg^{-1}, s.c., and 10 mg kg^{-1}, p.o., and in saline-loaded rats at doses of and above 10 mg kg^{-1}, s.c., and 30 mg kg^{-1}, p.o.

8 The prostaglandin-mediated fall in mean arterial blood pressure elicited in anaesthetized rats by i.v. application of arachidonic acid was not inhibited by prior treatment with EMD 61753 (10 mg kg^{-1}, p.o.). Thus, a blockade of prostaglandin synthesis via inhibition of cyclo-oxygenase activity does not contribute to the *in vivo* effects of EMD 61753 and its metabolites.

9 The present experiments therefore indicate that EMD 61753 is a potent, selective and orally-effective full κ-opioid receptor agonist which has a limited ability to penetrate the blood-brain barrier and elicit centrally-mediated sedation, putative aversion, diuresis, and antinociception. The inhibitory actions of systemically-applied EMD 61753 against hyperalgesic pressure nociception and neurogenic inflammation are mediated peripherally, probably by opioid receptors on the endings of sensory nerve fibres.

Keywords: EMD 61753; ICI 197067; ICI 204448; κ-opioid agonist; peripherally-selective; hyperalgesia; antinociception

Introduction

A successful strategy for improving the side-effect profile of peripherally-acting drugs has been to restrict the access of such compounds to the CNS. For example, a new generation of antihistamines are now available which have peripheral antiallergic effects without the drowsiness and impaired performance associated with centrally-acting antihistamines. They achieve this by virtue of their chemical structure, which prevents easy crossing of the blood-brain barrier (Nicholson, 1987).

Similar considerations have led to attempts to develop peripherally-selective κ-opioid receptor agonists. These compounds should be able to produce peripherally-mediated analgesia against pain of inflammatory origin (see Barber & Gottschlich, 1992, and Stein, 1993, for recent reviews) with-

[1] Author for correspondence.

out eliciting the unpleasant side effects of psychotomimesis/dysphoria and sedation found with centrally-acting κ agonists (Pfeiffer et al., 1986; Peters & Gaylor, 1989).

Various chemical approaches have been used in attempts to make a particular molecule less accessible to the brain without simultaneously reducing κ agonist activity. The introduction of zwitterionic groups led to the development of the highly polar hydrophilic κ-opioid receptor agonist, ICI 204448 (Shaw et al., 1989). Another strategy has been to introduce one or more moderately hydrophilic groups; GR 94839 (Rogers et al., 1992), BRL 52974 (Brooks et al., 1993), and EMD 60400 (Barber et al., 1994) are examples of such κ-opioid receptor agonists. However, while these hydrophilic compounds may be more or less peripherally selective in their actions (Shaw et al., 1989; Rogers et al., 1992; Barber et al., 1994) little has been revealed about some other important pharmacological properties, such as their bioavailability ofter oral application.

We now describe the pharmacological characteristics of the new κ-opioid receptor agonist, EMD 61753. This κ agonist is structurally novel since it contains the hydro*phobic* diphenylmethyl group in combination with a hydrophilic hydroxy group (Figure 1). This amphiphilic molecule therefore possesses the hydrophobic structural element found in the peripherally-selective antihistamines terfenadine and ebastine (Moragues & Roberts, 1990). We here compare the pharmacological properties of EMD 61753 with those of ICI 204448 and ICI 197067 (Costello et al., 1988), the latter being a κ-opioid receptor agonist which readily penetrates the blood-brain barrier (Shaw et al., 1989; Barber et al., 1994). Some of these results have been published previously in a preliminary form (Gottschlich et al., 1993; Barber et al., 1993).

Methods

Those experimental procedures which have been recently described (Barber, 1993; Barber et al., 1994) will be dealt with only briefly here.

In vitro *tests*

Binding Receptor binding was carried out in the presence of 1.6 nM [^{3}H]-U 69593 (κ), 0.5 nM [^{3}H]-PL 017 (μ), 1 nM [^{3}H]-[D-Pen^{2,5}]enkephalin (δ), or 4 nM [^{3}H]-SKF 10047 (σ) in 50 mM Tris (2-amino-2-hydroxymethyl-propan-1,3-diol) buffer (pH 7.7) using membranes prepared from guinea-pig cerebellum (κ), rat cerebrum (μ and δ) or guinea-pig whole brain (σ). Assays were run for 30 (μ and δ) or 40 (κ and σ) min at 25°C and non-specific binding was established by the use of 10 μM naloxone (κ), 0.3 μM morphine (μ), 1 μM [D-Ala^2, D-Leu^5]enkephalin (δ), or 10 μM haloperidol (σ).

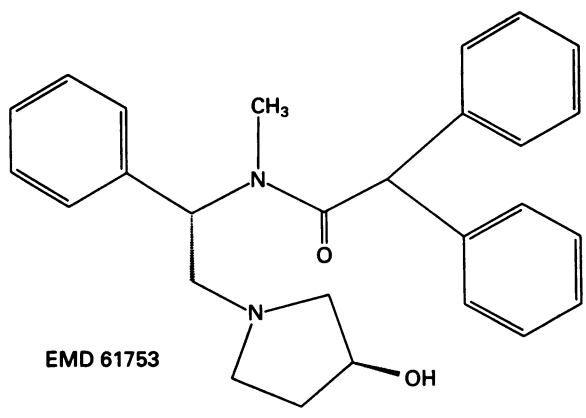

Figure 1 Structure of EMD 61753.

Rabbit vas deferens Male New Zealand rabbits (2.5–3 kg) were stunned and exsanguinated. The vasa deferentia were isolated and mounted in organ baths containing 50 ml of oxygenated (95% O_2/5% CO_2) physiological salt solution. The tissue was stimulated electrically with square-wave pulses (0.1 Hz, 2 ms, 20–40 V) via platinum field-stimulating electrodes. Contractions were recorded isometrically under a load of 0.5 g. After 60 min equilibrium a concentration-response curve to U 69593 was constructed and, after a 60 min washout period, a further curve was constructed for EMD 61753.

Central effects of EMD 61753

Studies with [^{14}C]-EMD 61753 In the macroautoradiography experiments, male Wistar rats (220–290 g) were injected i.v. with a radioactive dose of approximately 4 MBq kg^{-1} [^{14}C]-EMD 61753 (chemical dose 1 mg kg^{-1}). Five minutes later the rats were anaesthetized with ether, frozen and embedded in 3% carboxymethyl cellulose (Henkel, Düsseldorf, Germany). Sections of 20 μm thickness were then cut in a cryomicrotome (Type 450 MB, LKB, Sweden) and exposed at − 80°C for 25 to 64 days against X-ray film (Osray M3, Agfa Gevaert). The X-ray films were then developed in a modified X-ray developer (OP 22, Agfa) and fixed.

In the quantitive distribution studies, animals received a radioactive dose of approximately 2 MBq kg^{-1} [^{14}C]-EMD 61753 (chemical doses were 1 mg kg^{-1}, i.v., and 10 mg kg^{-1}, p.o.). At predetermined times after administration the animals were laparotomised under ether anaesthesia, exsanguinated by puncture of the abdominal aorta and the organs removed. Heparinised blood samples were centrifuged to separate plasma, while the organs were homogenized, dried, oxidized in a Tri-Carb 306 B oxidizer (Packard, Frankfurt) and counted in a TriCarb 460 CD or 4640 liquid scintillation spectrometer (Packard, Frankfurt).

Reversal of haloperidol-induced 3, 4-dihydroxyphenylalanine (DOPA) accumulation Male Wistar rats (175–250 g) received 0.3 mg kg^{-1} haloperidol, p.o., or vehicle (methocel; E. Merck, Darmstadt). One hour later the animals were treated s.c. or p.o. with the test compound or vehicle. Twenty-five minutes later the animals were injected i.p. with 100 mg kg^{-1} of the DOPA-decarboxylase inhibitor, NSD 1015. After a further 35 min the animals were decapitated and the N. accumbens were dissected and processed for determination of DOPA content using high performance liquid chromatography (h.p.l.c.) (Seyfried et al., 1986).

Hexobarbitone-induced sleeping time Male NMRI mice (20–30 g) were treated s.c. or p.o. with test compounds or vehicle. Thirty minutes later the mice were injected with hexobarbitone (Serva, Heidelberg, Germany; 100 mg kg^{-1}, i.p.) and the time necessary for the recovery of the righting reflex was measured.

Rotarod Male Wistar rats (190–270 g) were tested for their ability to balance on a rotating rod (diameter 9 cm; rate of rotation 7 revolutions per min). Selected rats were then injected s.c. or i.v. with test compounds. The rats were tested again 30 min after treatment; a rat falling off the bar more than twice within 2 min was considered to be showing motor impairment.

Antinociceptive tests

Pressure nociception Male Wistar rats (280–310 g) were fitted with a cuff at the base of the tail and treated with different doses of test compound by the s.c. or p.o. routes of administration. Thirty minutes later the proportion of animals in each treatment group which responded to painful pressure (4 bar, 5 s) delivered to the tail via a blunt pin built into the cuff was measured. This level of pressure produces a

behavioural response (vocalisation, turning and/or biting the cuff) in 100% of animals tested which have not received an analgesic.

Experiments were also carried out on rats in which hyperalgesia had been induced by the injection of a 1% solution of carrageenin (Marine Colloids Inc., Springfield, N.J., U.S.A.) into the tail tissue under the cuff. In this case a pressure of 2.5 bar was sufficient to produce a 100% response from untreated animals. All measurements were made 3 h after the application of carrageenin. Opiates were applied either 30 min or 3 h before testing (i.e., remedial and prophylactic application, respectively). The effect of naloxone on the remedial antinociceptive action of EMD 61753 was examined by applying naloxone 20 min before testing. Local effects of systemically-applied EMD 61753 in inflamed tissue were demonstrated by injecting the κ-opioid receptor antagonist norbinaltorphimine dissolved in 25 μl saline into the tail 5 min before testing.

Formalin test Male NMRI mice (27–33 g) were treated s.c. or p.o. with test compound or vehicle. Thirty minutes later 20 μl of a 3% solution of formalin (E. Merck, Darmstadt) was injected into the dorsal surface of the right hind paw. The amount of time spent licking the injected paw was measured 0–10 and 20–30 min after the injection of formalin (i.e., in the non-inflammatory first phase and the inflammatory second phase of the reaction, respectively; Hunskaar & Hole, 1987) and expressed as % inhibition compared to the respective vehicle group. In tests of naloxone antagonism naloxone was injected s.c. 10 min before the application of formalin.

Abdominal constriction (writhing) test Male NMRI mice (20–30 g) and Wistar rats (115–240 g) were injected s.c. or p.o. with test compound or vehicle. Thirty or 180 min later the animals were injected i.p. with a saturated aqueous solution of 2-phenyl-1,4-benzoquinone (1 ml 100 g^{-1}; E. Merck, Darmstadt). The number of abdominal constriction movements made in the subsequent 20 min were counted and expressed as % inhibition compared to the respective vehicle group. Naloxone antagonism of the action of EMD 61753 in the mouse abdominal constriction test was performed by applying naloxone at the same time as EMD 61753. Peripheral effects of EMD 61753 in the rat abdominal constriction test were looked for by applying this substance i.p. together with the irritant.

Other in vivo *tests*

Neurogenic inflammation Male Wistar rats (270–330 g) which had been pretreated s.c. or p.o. with test compound or vehicle were anaesthetized with pentobarbitone sodium (Sanofi, Paris; 60 mg kg^{-1}, i.p.) and their saphenous nerves were exposed and sectioned at the level of the thigh. Evans blue (E. Merck, Darmstadt; 50 mg kg^{-1}) was dissolved in a 0.9% solution of NaCl and applied i.v. through a catheter in a tail vein. One hour after application of the test substance or vehicle, and 15 min after the injection of Evans blue, the right saphenous nerve was stimulated antidromically with square-wave electrical pulses (5 mA, 1 ms, 2 Hz) for 10 min, after which the rat was killed with an overdose of anaesthetic. Dye was extracted by incubating hind paw skin in formamide (E. Merck, Darmstadt) and was measured spectrophotometrically at 620 nm. The dye level resulting from neurogenic extravasation was taken to be the mean difference between the stimulated and unstimulated paws in each dose group and was expressed as % inhibition in comparison to the respective vehicle group.

Plasma extravasation was also elicited by 10 μg substance P dissolved in 25 μl saline. This was injected into the paw 15 min after the application of Evans blue and was allowed to act for 10 min before the experiment was terminated. Local inhibitory actions of systemically-applied EMD 61753 were tested for by injecting norbinaltorphimine dissolved in 25 μl saline into the paw 10 min after the application of Evans blue and 5 min before the beginning of nerve stimulation. The control side was injected with 25 μl saline at the same time.

Diuresis in conscious rats Male Wistar rats (180–250 g) received water *ad libitum* and were deprived of food for 18 h before the start of the experiments. Groups of rats had their urinary bladders emptied by light pressure and were placed in metabolism cages (three rats in each cage) for 6 h. EMD 61753 or vehicle was given s.c. or p.o. just before the rats were placed in their cages. In experiments with volume-loaded rats, a 0.6% solution of NaCl (10 ml per 100 g, i.p.) was also injected at this time.

In vivo *inhibition of prostaglandin synthesis* These experiments were performed as described by Damas & Mousty (1978). Briefly, male Wistar rats (330–380 g) which had been pretreated orally with either vehicle, indomethacin or EMD 61753 were anaesthetized with pentobarbitone sodium (Sanofi, Paris; 50 mg kg^{-1}, i.p. and 40 mg kg^{-1}, s.c.). Blood pressure was recorded from a carotid artery by means of a Statham pressure transducer. Following a stabilisation period, arachidonic acid (Sigma, Deisenhofen, Germany) was applied i.v. at a dose of 5 mg kg^{-1}. In the vehicle-treated group this led to a fall in mean arterial blood pressure caused by the synthesis of prostaglandin from arachidonic acid by cyclo-oxygenase.

Substances

The following substances were synthesized at E. Merck, Darmstadt, unless otherwise indicated. [D-Ala^2, D-Leu^2]enkephalin acetate (Bachem, Basel, Switzerland), EMD 61753 (N-methyl-*N*-[(1S)-1-phenyl-2-((3S)-3-hydroxypyrrolidine-1-yl)-ethyl]-2,2-diphenylacetamide HCl; Figure 1), haloperidol HCl, ICI 197067 ((2S)-N-[2-(N-methyl-3,4-dichlorphenylacetamido)-3-methylbutyl]-pyrrolidine HCl), ICI 204448 (2-[3-(1-(3, 4-dichlorphenyl-N-methylacetamido)-2-pyrrolidonoethyl) - phenoxy] acetic acid HCl), indomethacin (Research Biochemicals International, Cologne, Germany), morphine HCl, naloxone HCl (Sigma, Deisenhofen, Germany), substance P tetratrifluracetate (Bachem, Basel, Switzerland) and U 69593 ((5-α, 7-α, 8-β)-(−)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)-dec-8-yl)-phenylacetamide; Research Biochemicals International, Cologne, Germany). For *in vivo* applications these compounds were dissolved in physiological saline or distilled water, or were suspended in gum arabic or methocel (for p.o. application). The ligands [^{3}H]-U 69593, [^{3}H]-PL 017 ([N-MePhe^3, D-Pro^4]morphiceptin), [^{3}H]-[D-Pen^{2,5}]enkephalin and [^{3}H]-SKF 10047 ((+)-(2S, 6R, 11S)-3-allyl-1, 2, 3, 4, 5, 6-hexahydro-6, 11-dimethyl-2, 6-methanobenzo(D)azocine-8-ol) were from NEN Research Products (Dreieich, Germany), while [^{14}C]-EMD 61753 was synthesized at the Institute of Pharmacokinetics and Metabolism of E. Merck (Grafing, Germany).

Statistics

The Dunnett test for multiple comparisons with control was used to determine statistical significance. Ninety five percent confidence intervals were calculated by the method of Litchfield & Wilcoxon (1949), except in the case of the pressure nociception and rotarod tests, where the staircase method of Dixon & Mood (1948) was applied.

Results

In vitro *tests*

Binding EMD 61753 binds to κ-opioid receptors with an IC_{50} value (mean ± s.d.) of 5.6 ± 1.4 nM ($n = 3$). The binding

Table 1 ^{14}C-concentrations (ng-eq g^{-1} and for plasma ng-eq ml^{-1}) in selected organs and tissues after single administration of [^{14}C]-EMD 61753 to male Wistar rats

	Time after application			
EMD 61753 (1 mg kg^{-1}, i.v.)	5 min	1 h	6 h	24 h
Plasma	595 ± 64	131 ± 10	22 ± 3	7 ± 1
Whole brain	242 ± 3	48 ± 4	49 ± 9	4 ± 0.4
Pituitary	2110 ± 189	480 ± 152	52 ± 4	10 ± 4
Lung	13700 ± 688	1940 ± 290	115 ± 14	13 ± 0.8
Liver	4030 ± 441	2470 ± 226	758 ± 44	401 ± 32
Kidney	3470 ± 287	757 ± 77	275 ± 4	175 ± 4
Adrenals	6630 ± 382	871 ± 103	123 ± 5	33 ± 6
Muscles	873 ± 52	107 ± 7	11 ± 0.2	2 ± 0.4
EMD 61753 (10 mg kg^{-1}, p.o.)	1 h	3 h	6 h	24 h
Plasma	353 ± 85	391 ± 17	369 ± 39	62 ± 9
Whole brain	168 ± 21	170 ± 20	190 ± 14	33 ± 8
Pituitary	888 ± 320	969 ± 211	798 ± 83	151 ± 23
Lung	4250 ± 1477	2540 ± 601	1540 ± 193	82 ± 4
Liver	9140 ± 1909	9820 ± 561	9840 ± 711	2340 ± 148
Kidney	1640 ± 403	1850 ± 198	1530 ± 144	431 ± 9
Adrenals	2010 ± 674	2350 ± 310	1620 ± 140	211 ± 28
Muscles	228 ± 47	236 ± 24	162 ± 23	18 ± 1.6

Values are mean ± s.e.mean, $n = 3$.

affinity of EMD 61753 to μ-opioid, δ-opioid, and σ-receptors was much weaker, with IC_{50} values of 3000 ± 900, 700 ± 120, and > 10,000 nM (all $n = 3$), respectively.

Rabbit vasa deferentia EMD 61753 was a full and potent κ-opioid receptor agonist, which produced concentration-dependent inhibition of electrically-induced contractions of rabbit isolated vasa deferentia. The IC_{50} value (with 95% confidence interval) of this effect was 54.5 (37.6–79) nM ($n = 4$), a value very simiar to that of the κ-opioid agonist, U 69593 (57.7 (47.6–70) nM; $n = 4$).

Central actions of EMD 61753

In vivo *distribution of [^{14}C]-EMD 61753* The distribution of radioactivity at various times following the application of [^{14}C]-EMD 61753 to male Wistar rats at a dose of 1 mg kg^{-1}, i.v., is shown in Table 1. The plasma concentration of 595 ng eq ml^{-1} found 5 min after i.v. administration (Table 1) indicates an initial distribution volume of 1.7 l kg^{-1}, which means that the substance is distributed rapidly to organs and tissues. The radioactivity in the plasma and brain at this timepoint represents unmetabolized substance, as indicated by radio-h.p.l.c. measurements (not shown). Initial concentrations were highest in the lungs, but relatively high concentrations were also measured in the adrenal glands, liver and kidneys (Table 1). Considerably less radioactivity, in comparison to the above organs, was found at all timepoints in the whole brain, although noticeably higher concentrations were detected in the pituitary (Table 1). Autoradiographs taken from rats 5 min after i.v. application (Figure 2) revealed that the radioactivity measured in the whole brain was highly concentrated in the region of the cerebral ventricles (3rd, 4th and lateral). These regions of high radioactivity were identified histologically as choroid plexus.

The distribution pattern of [^{14}C]-EMD 61753 following application at a dose of 10 mg kg^{-1}, p.o., was comparable to that after i.v. administration (Table 1). Comparisons of plasma concentrations of unmetabolised EMD 61753 over time after i.v. and p.o. application indicate a bioavailability of EMD 61753 of 13%; EMD 61753 was eliminated from the

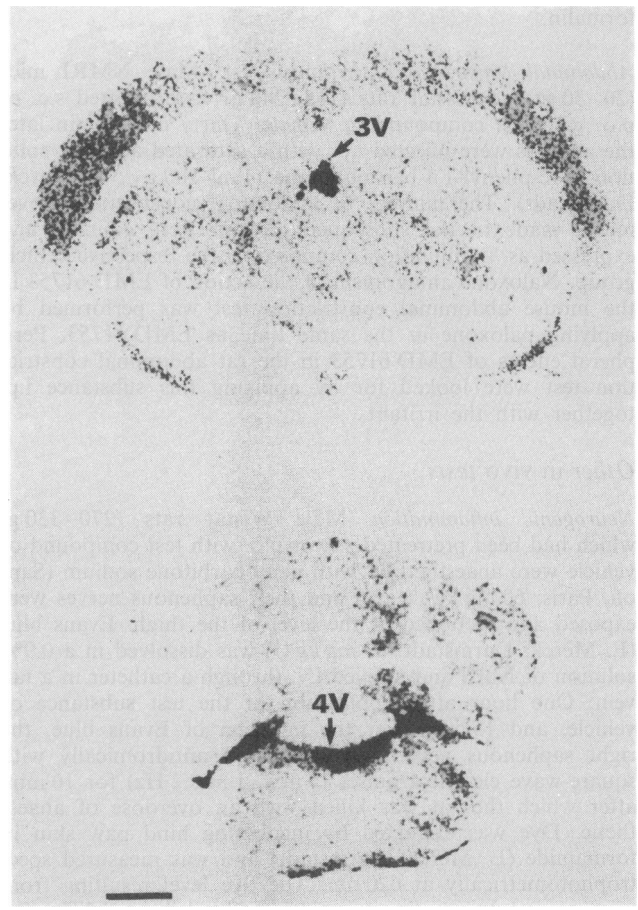

Figure 2 Autoradiographs of coronal sections of a rat brain showing the distribution of ^{14}C radioactivity 5 min after i.v. administration of 1 mg kg^{-1} [^{14}C]-EMD 61753. Radioactivity was concentrated in the ventricular system in the choroid plexuses (cf. Figure 1 of Risau & Wolburg, 1990, and Figure 56 of Paxinos & Watson, 1982). The calibration bar indicates 150 μm. 3V: 3rd ventricle; 4V: 4th ventricle.

plasma with a half life of 0.67 h (H.M. Bender, unpublished observations).

Haloperidol-induced DOPA accumulation In a first series of experiments the accumbal DOPA level (mean ± s.e.mean) in the vehicle-treated control group was 1565 ± 266 ($n = 4$), in the haloperidol-treated control group was 4012 ± 205 ($n = 6$), and in the animals treated with EMD 61753 at doses of 0.1, 1 and 10 mg kg^{-1}, s.c. was 3448 ± 414, 4322 ± 215, and 3607 ± 363 (all $n = 4$) ng g^{-1} fresh weight, respectively. These values for the EMD 61753-treated groups were not significantly different from haloperidol-treated controls. In the next set of experiments control and haloperidol-treated control animals had DOPA values of 2143 ± 71 ($n = 4$) and 6070 ± 496 ($n = 6$) ng g^{-1}, respectively, whereas rats treated with 30 mg kg^{-1} EMD 61753, s.c., had accumbal DOPA concentrations of 3969 ± 305 ng g^{-1} ($n = 4$). The latter represents a statistically significant ($P < 0.05$) reversal of the haloperidol effect by EMD 61753 (Table 2). The corresponding values after oral application were 2616 ± 139 ($n = 3$) and 6396 ± 277 ($n = 5$) ng g^{-1} for the control and haloperidol-treated control, respectively, and 6582 ± 527 ($n = 4$), 6239 ± 275 ($n = 4$) and 5117 ± 469 ($n = 3$) ng g^{-1} in animals given 1, 10 and 100 mg kg^{-1} EMD 61753, p.o., respectively. None of these levels in the EMD 61753-treated groups was significantly different from the haloperidol-treated control (Table 2).

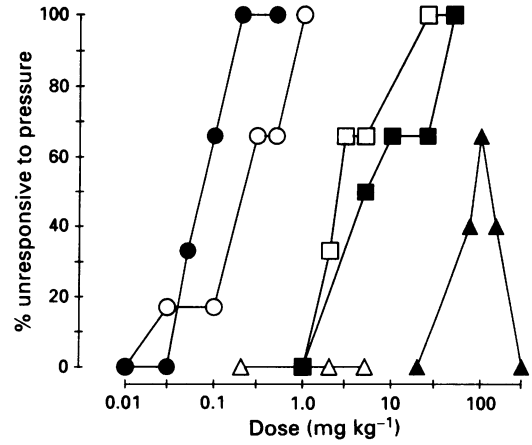

Figure 3 Effect of EMD 61753 on pressure nociception in the rat. Measurements were either made in rats without inflammation 30 min after application s.c. (△) or p.o. (▲) or animals with inflammation after s.c. (○, ●) or p.o. (□, ■) administration. Pressure testing in rats with inflammation was carried out either 3 h after the application of carrageenin and EMD 61753 (i.e. prophylactic application; □ and ○) or 3 h after the application of carrageenin and 30 min after the application of EMD 61753 (i.e. remedial application; ■ and ●). Each point represents the % of animals ($n = 3$–6) which did not respond to standard noxious pressure.

Table 2 Summary of effectiveness in tests of central activity

Test	*EMD 61753* (s.c.)	*EMD 61753* (p.o.)	*ICI 204448*[a] (s.c.)	*ICI 197067*[a] (s.c.)
DOPA accumulation[b]	30	>100	>30	0.3
Hexobarbitone sleeping[b]	10	100	>30	1.0
Rotarod[c]	453 (237–750)	–	>100	0.71 (0.45–1.12)

[a]From Barber *et al.* (1994).
[b]Threshold dose (mg kg^{-1}) for statistically significant effect.
[c]ID_{50} value (with 95% confidence intervals) in mg kg^{-1}.

Table 3 Summary of ID_{50} values (with 95% confidence intervals) in antinociceptive and related tests

Test	*EMD 61753* (s.c.)	*EMD 61753* (p.o.)	*ICI 204448* (s.c.)	*ICI 204448* (p.o.)	*ICI 197067* (s.c.)
Pressure nociception					
Normalgesic	5.0	c.100[a]	30[b]	100	0.08[b]
	inactive		inactive	inactive	(0.02–0.2)
Hyperalgesic	0.2[c]	3.1[c]	0.8[b,c]	30[c]	0.004[b,c]
	(0.08–0.46)	(1.8–5.2)	(0.3–2.4)	inactive	(0.001–0.01)
	0.08[d]	6.9[d]	0.4[d]	30[d]	0.004[d]
	(0.05–0.12)	(2.6–18.4)	(0.2–0.9)	inactive	(0.001–0.01)
Formalin					
1st phase	1.9	10.4	18.9[b]	>100	0.04[b]
	(0.4–9.5)	(2.1–52)	(7.3–49.1)		(0.02–0.1)
2nd phase	0.26	3.5	12.6[b]	77.7	0.02[b]
	(0.06–1.2)	(1.3–9.1)	(8.4–18.9)	(23.5–256.4)	(0.01–0.04)
Abdominal constriction					
Mouse	1.75	8.4	1.9[b]	22[c]	0.02[b]
	(1.4–2.3)	(6.2–11.3)	(0.6–5.9)		(0.01–0.03)
Rat	3.2	250	4.5[b]	200	0.04[b]
	(0.8–12.0)	(89.3–700)	(0.6–31.5)	inactive	(0.01–0.11)
Neurogenic inflammation	3.7	35.8	5.2	100	3.1
	(1.2–11.1)	(13.8–93.6)	(1.7–15.6)	inactive	(0.9–10.85)

All values expressed in mg kg^{-1}.
[a]Bell-shaped dose-response curve; [b]From Barber *et al.* (1994); [c]Prophylactic application, i.e., rats tested 180 min after application of opiates and carrageenin; [d]Remedial application, i.e., rats tested 180 min after application of carrageenin and 30 min after receiving opiates; [e]No 100% effect. Maximum effect 56% after 100 mg kg^{-1}, p.o.

Sleeping time Control sleeping times (mean ± s.e.mean) were 74.7 ± 4.8 ($n = 30$) and 70.8 ± 6.3 ($n = 15$) min in mice which had received vehicle s.c. and p.o., respectively. Sleeping times were 72.3 ± 4.8, 133.9 ± 14.3, 175.5 ± 13.9, and 211.3 ± 8.0 min (all $n = 10$) in mice injected with 3, 10, 30 or 100 mg kg^{-1} EMD 61753, s.c., respectively. The sleeping times after the last three of these doses represent statistically significant ($P < 0.01$) potentiation of narcosis (Table 2). Mice treated with EMD 61753 at doses of 10, 30 and 100 mg kg^{-1}, p.o., had sleeping times of 88.5 ± 9.4, 89.9 ± 11.7 and 169.2 ± 5.0 min (all $n = 10$), respectively, of which only the latter was significantly different ($P < 0.01$) from control (Table 2).

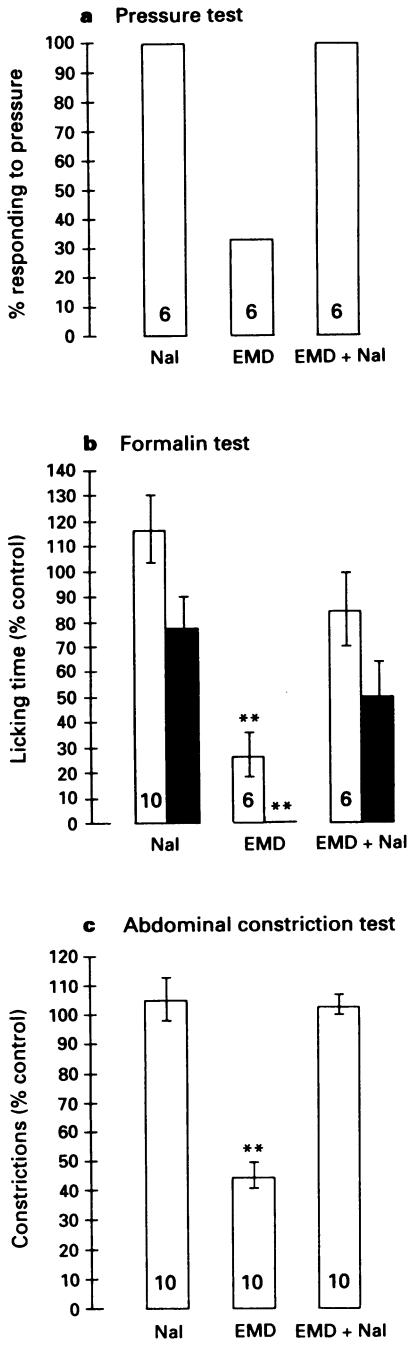

Figure 4 Effects of naloxone on the antinociceptive action of EMD 61753. (a) Pressure test: the proportion of animals responding to noxious pressure on the inflamed tail after the application of 0.5 mg kg^{-1} naloxone, s.c. (Nal), 10 mg kg^{-1} EMD 61753, p.o. (EMD), or EMD 61753 and naloxone (EMD + Nal) are shown. (b) Formalin test: licking times in the 1st (open columns) and 2nd (solid columns) phases of the response to formalin are expressed as % control (absolute licking times in the control group were 64.7 ± 6.0 and 103.7 ± 11.0 s (mean ± s.e.mean, $n = 10$), respectively). Responses after naloxone (Nal) applied at a dose of 1.0 mg kg^{-1}, s.c., EMD 61753 (EMD) at 30 mg kg^{-1}, p.o., and EMD 61753 and naloxone (EMD + Nal) are displayed. (c) Abdominal constriction test: constrictions are expressed as % of the control group, in which the absolute number of constrictions was 60.5 ± 2.8 (mean ± s.e.mean; $n = 15$). Responses after 1 mg kg^{-1} naloxone, s.c., (Nal), 2 mg kg^{-1} EMD 61753, s.c., (EMD), and EMD 61753 and naloxone (EMD + Nal) are shown. The number of animals used is shown in each column. (**$P < 0.01$).

Rotarod The ID_{50} value (with 95% confidence intervals) of EMD 61753 after administration by the s.c. route was 453 (237–750) mg kg^{-1} (Table 2). Motor performance was not affected by EMD 61753 at doses of 1 and 3 mg kg^{-1}, i.v., but the ability to balance on the rotarod was completely inhibited 2 and 10 (but not 20) min after the application of 10 mg kg^{-1} EMD 61753, i.v.

Antinociceptive actions of EMD 61753

Pressure test EMD 61753 was ineffective against pressure nociception in normalgesic rats after s.c. application at doses between 0.2 and 5.0 mg kg^{-1} (Figure 3; Table 3). Oral administration of EMD 61753 in this test revealed a bell-shaped dose-response relationship (Figure 3; Table 3), with a maximum effect (66% antinociception) after the application of a dose of 100 mg kg^{-1}.

EMD 61753 was considerably more potent following the induction of inflammatory hyperalgesia. Dose-dependent antinociception was found after prophylactic and remedial application via both oral and s.c. routes of administration (Figure 3; see Table 3 for ID_{50} values). In contrast, ICI 204448 was inactive in this test at a dose of 30 mg kg^{-1}, p.o. ($n = 3$; Table 3).

The antinociceptive action of EMD 61753 (remedial application of 10 mg kg^{-1}, p.o.) against pressure nociception under hyperalgesic conditions could be completely inhibited by systemic application of the opioid-receptor antagonist naloxone (0.5 mg kg^{-1}, s.c.; Figure 4a).

Formalin-induced paw licking EMD 61753 elicited dose-dependent antinociception against both early and late phase nociception after s.c. and p.o. administration in the mouse (Figure 5; see Table 3 for ID_{50} values). ICI 204448 was only weakly active after oral application (Figure 5; Table 3).

Systemic naloxone (1.0 mg kg^{-1}, s.c.) was able to antagonize the action of EMD 61753 (30 mg kg^{-1}, p.o.) in this test (Figure 4b).

Rodent abdominal constriction EMD 61753 produced an antinociceptive action in this test, although this effect showed some species-dependency. In rats the dose-response curve to EMD 61753 was not as steep as in the mouse, and EMD 61753 was only very weakly active after oral application (Figure 6; Table 3). Another finding in the rat was that EMD 61753 had an ID_{50} value (with 95% confidence interval) of 3.06 (1.1–8.6) mg kg^{-1} when applied i.p. simultaneously with phenylbenzoquinone. ICI 204448 was inactive in the rat abdominal constriction test at a dose of 200 mg kg^{-1}, p.o. ($n = 5$; Table 3), and was active but unable to produce 100% inhibition of abdominal constriction in the mouse following its oral administration (Figure 6).

The opioid nature of the antinociceptive action of EMD 61753 (2 mg kg^{-1}, s.c.) in the mouse abdominal constriction test was demonstrated by antagonism with naloxone (1 mg kg^{-1}, s.c.; Figure 4c).

Other in vivo *effects*

Neurogenic inflammation Neurogenic plasma extravasation was dose-dependently inhibited by s.c. or oral application of EMD 61753 (Figure 7; see Table 3 for ID_{50} values). ICI

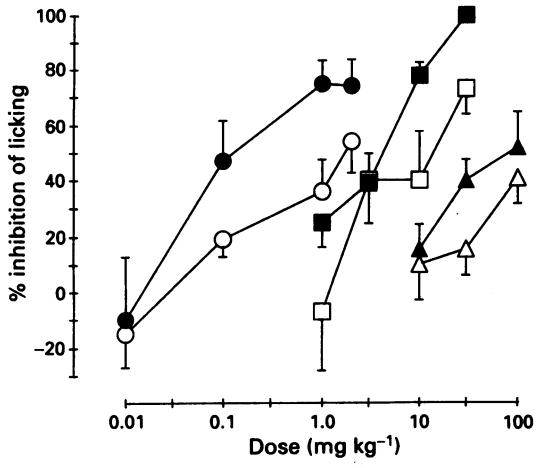

Figure 5 Effects of EMD 61753 and ICI 204448 on formalin-induced paw licking observed 0–10 (○, □, △) and 20–30 (●, ■, ▲) min after the intraplantar injection of formalin. EMD 61753 was applied s.c. (○, ●) and p.o. (□, ■), and ICI 204448 was applied p.o. (△, ▲), 30 min before formalin. Absolute licking times (mean ± s.e.mean; 1st phase/2nd phase) in the vehicle-treated groups were 73.1 ± 8.4/107 ± 11.7 (n = 17), 77.4 ± 8.3/153.7 ± 18.0 (n = 10), and 66.7 ± 6.0/105.3 ± 14.5 (n = 14) s for EMD 61753 s.c., EMD 61753 p.o., and ICI 204448 p.o., respectively. Each point in the figure represents the mean ± s.e.mean of measurements from 5 or 6 mice.

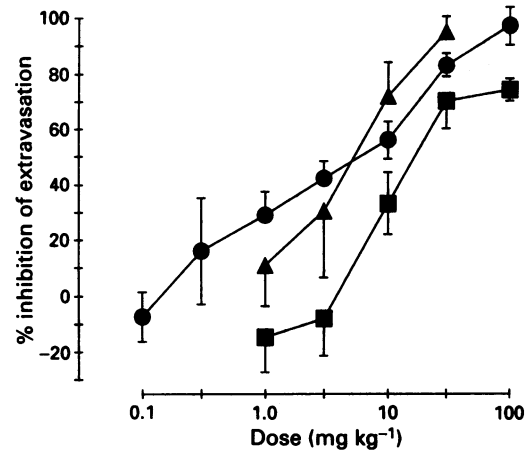

Figure 7 Effect of EMD 61753 and ICI 204448 on neurogenic plasma extravasation elicited by 10 min antidromic electrical stimulation of the rat saphenous nerve. EMD 61753 s.c. (●), EMD 61753 p.o. (■), and ICI 204448 s.c. (▲) were applied 60 min before the beginning of nerve stimulation. Mean dye levels (± s.e.mean) resulting from electrical stimulation (i.e. the difference between stimulated and control paws) were 7.1 ± 0.7 (n = 19), 5.4 ± 0.5 (n = 10), and 5.9 ± 0.9 (n = 6) μg dye per 100 mg skin in the EMD 61753 s.c., EMD 61753 p.o., and ICI 204448 s.c. vehicle-treated control groups, respectively. Each point in the figure represents the mean ± s.e.mean from 5–12 animals.

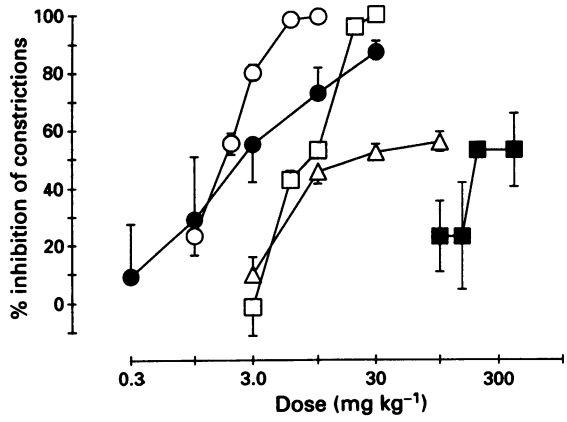

Figure 6 Effects of EMD 61753 and ICI 204448 on constriction movements measured 0–20 min after the i.p. injection of 2-phenyl-1,4-benzoquinone. EMD 61753 was applied s.c. (○, ●) 30 min, or p.o. (□, ■) 30 min (mouse) or 180 min (rat), before 2-phenyl-1,4-benzoquinone, whereas ICI 204448 (△) was allowed to act for 30 min p.o. before injection of the irritant. The numbers of constriction movements (mean ± s.e.mean) in the vehicle-treated groups in mice were 45.3 ± 3.2, 42.5 ± 3.7 and 50.9 ± 3.3 (all n = 15) and in rats were 42.9 ± 2.5 (n = 25) and 46.7 ± 3.1 (n = 20) for EMD 61753 s.c., EMD 61753 p.o., ICI 204448 p.o., EMD 61753 s.c. and EMD 61753 p.o., respectively. Each point in the figure represents the mean ± s.e.mean of measurements from 5 rats (●, ■) or 10 mice (○, □, △).

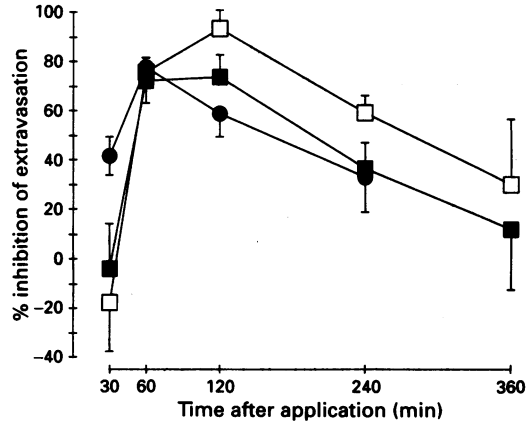

Figure 8 Time course of the inhibitory effect of EMD 61753 on neurogenic plasma extravasation. EMD 61753 was applied at doses of 4 mg kg^{-1}, s.c., (●), 30 mg kg^{-1}, p.o. (■), and 100 mg kg^{-1}, p.o., (□), and nerve stimulation was begun at various times thereafter. The mean (± s.e.mean) amounts of dye in the control groups (measured 60 min after application of vehicle) were 6.8 ± 0.5 (n = 24) and 5.8 ± 0.5 (n = 20) μg dye per 100 mg skin in the s.c. and p.o. groups, respectively. Each point represents mean ± s.e.mean from 5–11 animals.

204448 was also effective via the s.c. route of administration (Figure 7), but was inactive in this test at a dose of 100 mg kg^{-1}, p.o. (n = 5).

Levels of Evans blue (mean ± s.e.mean) after the injection of 10 μg substance P, i.pl., were 14.6 ± 1.2 μg dye per 100 mg skin (n = 5) in rats treated with vehicle p.o., and 11.2 ± 1.1 μg dye per 100 mg skin (n = 5) in animals treated with 100 mg kg^{-1} EMD 61753, p.o. This difference was not statistically significant ($P > 0.05$). Dye levels in paws injected with 25 μl saline were 5.6 ± 0.3 (n = 5) and 5.0 ± 0.62 (n = 5) μg per 100 mg skin in the vehicle- and EMD 61753-treated groups, respectively.

Eliciting neurogenic extravasation at various times after the application of EMD 61753 revealed that at single doses of 4.0 mg kg^{-1}, s.c., and 30 mg kg^{-1}, p.o. (i.e. approximate ID_{50} doses s.c. and p.o.), this compound still had a statistically significant ($P < 0.01$) inhibitory effect 2 but not 4 h after administration (Figure 8). After a dose of 100 mg kg^{-1}, p.o., the action of EMD 61753 was undetectable after 6 h (Figure 8).

Diuresis EMD 61753 elicited dose-dependent diuresis after its s.c. or oral administration in non-hydrated (Figure 9) and hydrated (data not shown) rats. A statistically significant

diuretic effect ($P<0.05$) of EMD 61753 was observed at doses of and greater than 1.0 mg kg^{-1}, s.c., and 10 mg kg^{-1}, p.o., in non-hydrated animals and at doses of 10 mg kg^{-1}, s.c., and 30 mg kg^{-1}, p.o., in hydrated rats. An antidiuretic action of EMD 61753 at low doses was not observed in either non-hydrated or hydrated animals.

Effects on prostaglandin biosynthesis The i.v. administration of 5 mg kg^{-1} arachidonic acid in anaesthetized rats produced a hypotensive response due to the *in vivo* formation of prostaglandins. Arterial blood pressure (mean ± s.e.mean) was reduced from 112.4 ± 2.5 to 48.8 ± 1.2 mmHg ($n = 12$) in rats pretreated with vehicle p.o. These values were changed from 91.2 ± 3.1 to 53.0 ± 1.5 mmHg ($n = 6$) in animals given 10 mg kg^{-1} EMD 61753, p.o., and from 103.5 ± 5.1 to 114.3 ± 6.6 mmHg ($n = 6$) in animals which had received 1 mg kg^{-1} indomethacin, p.o.

Peripheral effects of systemically-applied EMD 61753

The antinociceptive action of 50 mg kg^{-1} EMD 61753, p.o. in the hyperalgesic pressure test was completely antagonized by the application of 100 μg of the κ-opioid receptor antagonist norbinaltorphimine directly into the inflamed portion of the tail (Figure 10a). The action of the antagonist appeared to be a local inhibitory effect, since norbinaltorphimine at this dose did not produce a systemic antagonism of the action of EMD 61753 (Figure 10a).

The action of 50 mg kg^{-1} EMD 61753, p.o. against neurogenic inflammation was also inhibited by the local application of norbinaltorphimine (Figure 10b), in this case into the paw. On the other hand, the systemic application of the same dose of norbinaltorphimine had no effect on the systemic action of EMD 61753 (Figure 10b).

Discussion

EMD 61753 has been shown in the present *in vitro* experiments to be a selective, potent and full κ-opioid receptor agonist. The binding affinity of EMD 61753 for the κ-opioid receptor as indicated by the IC_{50} value (5.6 nM) is equivalent to that of other established κ agonists, such as ICI 197067 (6.3 nM, Costello *et al.*, 1988; 1.19 nM, Shaw *et al.*, 1989; 1.5 nM, Barber *et al.*, 1994), ICI 204448 (33.2 nM, Shaw *et al.*, 1989; 13 nM, Barber *et al.*, 1994), EMD 60400 (2.8 nM, Barber *et al.*, 1994), and U 69593 (9.5 nM, Lahti *et al.*, 1985). EMD 61753 also binds selectively to κ receptors with a κ:μ:δ binding ratio of 1:536:125. For ICI 197067 and EMD 60400 these ratios were 1:67:800 and 1:179:119, respectively (Barber *et al.*, 1994), and U 69593 has a κ:μ binding ratio of 1:484 (Lahti *et al.*, 1985). ICI 204448, on the other hand, is relatively unselective in its binding affinity (κ:μ:δ binding ratio 1:17.9:12.8, Barber *et al.*, 1994). The IC_{50} value of EMD 61753 in the rabbit vas deferens test of functional κ agonist potency (54.5 nM) is also comparable to that of EMD 60400 (41.8 nM), ICI 197067 (15.7 nM) and ICI 204448 (15 nM; Barber *et al.*, 1994).

The distribution of a radioactively labelled compound after i.v. injection occurs without distortion through absorption or first-pass metabolism. In the case of [^{14}C]-EMD 61753 it was shown that radioactivity in plasma and brain 5 min after i.v. administration represents exclusively the parent drug and not radioactive metabolites. EMD 61753 distributes rapidly from plasma, although its concentration in the whole brain was found to be low at all timepoints relative to that in other organs (Table 1). Moreover, these measurements from the whole brain include the high concentrations in the choroid plexus revealed on autoradiographs (Figure 2); the actual concentrations of EMD 61753 in nervous tissue will thus be less than those given in Table 1. These results therefore indicate that EMD 61753 penetrates poorly into the CNS and so can be termed peripherally-selective in its distribution after systemic application.

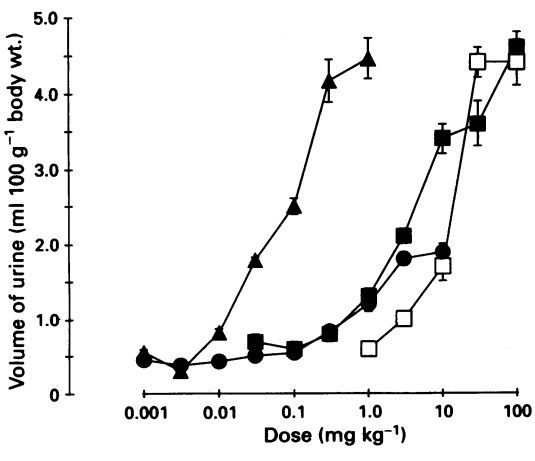

Figure 9 Effect of κ agonists on urinary volume in non-hydrated rats in the 6 h following s.c. application of ICI 197067 (▲), ICI 204448 (●), and EMD 61753 (■), and the p.o. application of EMD 61753 (□). The mean (± s.e.mean) amounts of urine in the vehicle-treated groups were 0.49 ± 0.03 ($n = 20$ cages), 0.44 ± 0.03 ($n = 30$ cages), 0.6 ± 0.1 ($n = 15$ cages), and 0.8 ± 0.1 ($n = 10$ cages) ml per 100 g body weight, respectively. Each point in the figure represents the mean ± s.e.mean of 5 or 10 cage measurements. The data with ICI 197067 and ICI 204448 are from Barber *et al.* (1994).

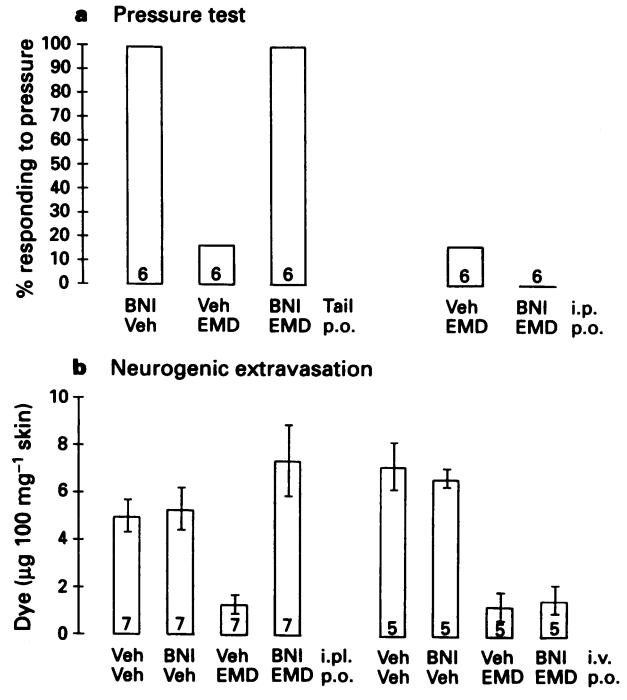

Figure 10 Demonstration of the peripheral action of systemically-applied EMD 61753. (a) Pressure test: the proportion of animals responding to noxious pressure on the inflamed tail after the application of 100 μg norbinaltorphimine (BNI) into the tail, 50 mg kg^{-1} EMD 61753, p.o. (EMD), and EMD 61753 and norbinaltorphimine are shown. Also displayed are the proportion of animals responding to pressure after administration of 10 mg kg^{-1} EMD 61753, p.o., and after EMD 61753 together with 100 μg norbinaltorphimine, i.p. (b) Neurogenic extravasation: the amount of dye extravasated after treatment with vehicle (Veh), with 50 μg norbinaltorphimine after intraplantar (i.pl.) application, with 50 mg kg^{-1} EMD 61753, p.o., and EMD 61753 and norbinaltorphimine are shown. Also indicated are the results obtained when this experiment was repeated with the same doses of the test compounds but the i.v. route of administration for norbinaltorphimine. The number of animals used is indicated in each column.

The pharmacological profile of EMD 61753 is consistent with that of a κ-opioid receptor agonist which has only weak central activity (Table 2). The major central side effects of κ agonists are sedation and aversive psychotomimesis/dysphoria (Pfeiffer *et al.*, 1986; Peters & Gaylor, 1989). Sedation was detected in the rotarod and narcosis potentiation models at much higher doses with EMD 61753 and the peripherally-selective κ-opioid receptor agonist ICI 204448 than with the centrally-active κ-agonist, ICI 197067 (Table 2). The same was true in our animal model of 'putative aversion', that is, the detection of the inhibitory effect of κ agonists on dopamine turnover in the nucleus accumbens as measured by DOPA accumulation (Table 2). κ agonists are known to inhibit the activity of mesolimbic dopaminergic neurones in this model (Manzanares *et al.*, 1991), and this characteristic is believed to be the physiological basis of their aversive action (Di Chiara & Imperato, 1988; Spanagel *et al.*, 1990; Shippenberg *et al.*, 1993).

Rank order of potency comparisons between various κ ligands in the diuresis, rodent abdominal constriction, formalin and normalgesic pressure tests indicate that activity in these models is probably due to penetration of the blood-brain barrier and activation of central κ-opioid receptors. Although the κ agonists tested all have a similar functional κ agonistic activity, the effectiveness of these substances after s.c. application (ICI 197067 > EMD 60400 > EMD 61753 and ICI 204448 in the normalgesic pressure, abdominal constriction, and diuresis tests and ICI 197067 > EMD 60400 > EMD 61753 > ICI 204448 in the formalin test; Barber *et al.*, 1994; Table 3; Figure 9) corresponds much better to their ability to produce central actions following systemic administration (Barber *et al.*, 1994; Table 2).

Our observation that the ability of a κ agonist to elicit diuresis corresponds to its effectiveness in stimulating central κ-opioid receptors confirms the findings of Brooks *et al.* (1993), who concluded that diuresis is mediated by κ receptors located beyond the blood-brain barrier. Furthermore, diuresis induced by the centrally-acting κ ligands ethylketazocine, spiradoline or CI 977 is found in the same dose-range as central discriminative stimulus properties in Rhesus monkeys (Dykstra *et al.*, 1987) and aversive and subjective effects in man (Peters *et al.*, 1987; Reece *et al.*, 1989), all of which indicates that water diuresis is a sensitive indicator of the activation of central κ-opioid receptors.

By this measure EMD 61753 compares favourably with other peripherally-selective κ-opioid receptor agonists. ICI 204448 elicits diuresis at doses of and above 0.3 mg kg^{-1},s.c., (Figure 9) while BRL 52974, which has an IC_{50} value of 42 nM in the rabbit vas deferens assay and an ID_{50} value > 10 mg kg^{-1}, s.c., in the rat rotarod test, elicits diuresis in the rat at doses above 0.03 mg kg^{-1}, i.v. (Brooks *et al.*, 1993). The threshold dose of EMD 61753 for the induction of diuresis (1.0 mg kg^{-1}, s.c., and 10 mg kg^{-1}, p.o., in non-hydrated rats) is relatively low in comparison to other measures of central activity (Table 2), possibly because EMD 61753 may have preferential access (see Table 1 for radioactivity levels in the pituitary) to hypothalamo-neurohypophysial κ-opioid receptors (Rossi & Brooks, 1993).

Peripheral antinociceptive effects of systemically- (Smith *et al.*, 1985; Follenfant *et al.*, 1988) and locally-applied (Bentley *et al.*, 1981) opioid receptor agonists have been claimed in the abdominal constriction test, although we, like Hayes & Hayward (1988) found no difference in ID_{50} values when our κ agonist was given by the i.p. or s.c. routes of administration. Moreover, since the action of systemically-applied EMD 61753 could not be inhibited by i.p. injection of norbinaltorphimine (100 μg; F. Mauler, unpublished observations) our findings are consistent with the contention of Rogers *et al.* (1992) that activity in this test is due to penetration into the CNS and activation of central κ receptors. This would further imply that there are species differences in the ability of EMD 61753 to activate central κ-opioid receptors since, in contrast to EMD 60400, ICI 197067, and ICI 204448 (Barber *et al.*, 1994), the dose-response curve of EMD 61753 in the abdominal constriction test is much shallower in the rat than in the mouse (Figure 6).

The ability of EMD 61753 to produce what is probably centrally-mediated antinociception in the normalgesic pressure test after p.o. but not s.c. application (Figure 3) was surprising. First-pass metabolism in the liver could generate active metabolites which are able to penetrate the blood-brain barrier more easily than the parent compound. The decreased potency of EMD 61753 in this test after very high oral doses (150 and 300 mg kg^{-1}; Figure 3) may reflect altered metabolism in the liver compared to that at lower doses. This must remain speculative, however, because activity of EMD 61753 after only oral but not s.c. administration was not observed in any other central test applied here.

In contrast to the weak central effects of EMD 61753, antinociception in the hyperalgesic pressure test was observed at relatively low doses (threshold doses in this test were approximately 0.03 mg kg^{-1}, s.c., and 1.0 mg kg^{-1}, p.o.; Figure 3). It seems likely that systemically-applied EMD 61753 is producing its antinociceptive effect in this test in the periphery. This is because the action of EMD 61753 was completely inhibited by local injection of the κ-opioid receptor antagonist norbinaltorphimine into the inflamed tissue at a dose (100 μg) which produced no systemic inhibition of κ-mediated antinociception (Figure 10a). Another indication that the actions of EMD 61753 in this model are peripherally mediated is that the rank order of potency of different κ ligands in this test under hyperalgesic conditions (EMD 60400 > ICI 197067 > EMD 61753 > ICI 204448; Table 3; Barber *et al.*, 1994) does not correlate with their ability to produce activation of central κ-opioid receptors (Table 2; Barber *et al.*, 1994). The difference in the activity of EMD 61753 in the pressure test under normalgesic and hyperalgesic conditions (Figure 3) confirms that the presence of an inflammatory response is important for the manifestation of peripheral antinociceptive effects (Stein, 1993).

The inhibitory action of EMD 61753 on neurogenic inflammation (Figure 7) is also likely to be peripheral in nature since the actions of opiates in this test are known to be largely independent of their CNS and systemic effects (Barber, 1993). As in the hyperalgesic pressure test, the effect of systemically-applied EMD 61753 in this model was antagonized by the local application of norbinaltorphimine (50 μg; Figure 10b). Moreover, EMD 61753, ICI 204448 and ICI 197067 were equipotent after s.c. application (Table 3; ICI 204448 and ICI 197067 are also equipotent after i.v. administration; Barber, 1993; Barber *et al.*, 1994), although they have very different tendencies to cross the blood-brain barrier. The observation that plasma extravasation elicited by the injection of substance P, the putative neurotransmitter mediating neurogenic inflammation (Lembeck & Holzer, 1979), was not influenced by treatment with EMD 61753 indicates that this compound has a presynaptic site of action.

The peripheral mechanism of action of EMD 61753 in the neurogenic inflammation test is likely to be presynaptic inhibition of the release of substance P and other inflammatory mediators from the nerve endings of sensory C fibres (Yonehara *et al.*, 1992). In the case of the hyperalgesic pressure model, on the other hand, the most important peripheral action of EMD 61753 is probably an inhibition of the sensory impulse flow from the inflamed area (Russell *et al.*, 1987; Haley *et al.*, 1990; Meßlinger *et al.*, 1993). Opioid receptors which could mediate these actions have been detected on the peripheral terminals of sensory neurones (see Stein, 1993). The lower potency of EMD 61753 in the neurogenic inflammation model in comparison to the hyperalgesic pressure test (Table 3) may be related to the different nature of the inflammatory stimuli used in these tests (electrical stimulation vs. carrageenin, respectively) or the different lengths of time between initiation of the inflammatory reaction and testing (0–10 min and 3 h, respectively).

The present experiments indicate that EMD 61753 is a compound which despite its lipophilic diphenylmethyl group (Figure 1) has a low tendency to penetrate into the CNS. EMD 61753 has, like terfenadine and astemizole, a high binding affinity to plasma proteins (97% in the rat; R. Kunert, unpublished observations) which will not facilitate crossing of the blood-brain barrier endothelial membrane (Seelig *et al.*, 1994). A further possibility is that free EMD 61753 may be trapped in the hydrophobic interior membrane of blood-brain barrier endothelial cells and so be unable to reach the extracellular fluid of the brain (Seelig *et al.*, 1994). One of the advantages of amphiphilic structures such as EMD 61753 is their generally improved oral activity in comparison to hydrophilic compounds (Kuschinsky & Lüllmann, 1981). This was confirmed in the present study; the hydrophilic molecule, ICI 204448, was in contrast to EMD 61753 only very poorly active after oral application (Table 3).

To summarise, EMD 61753 is a potent and selective, orally-active κ-opioid receptor agonist which has a low propensity to cross the blood-brain barrier and produce unwanted side effects (sedation and putative aversion) through activation of central κ-opioid receptors. The strong antinociceptive actions of EMD 61753 against pressure nociception under inflammatory conditions are mediated peripherally, possibly by opioid receptors on the endings of sensory nerve fibres. EMD 61753 therefore represents progress towards the development of a new class of analgesic compounds with which pain of inflammatory origin may be alleviated without evoking unacceptable central side effects.

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