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Behavioural and biochemical effects in C57BL/6J mice after a prolonged treatment with the δ-opiate antagonist ICI 154129

A. VOLTERRA, N. BRUNELLO, R. CAGIANO†, V. CUOMO†, G. RACAGNI*, *Institute of Pharmacology and Pharmacognosy, University of Milan and †Institute of Pharmacology, University of Bari, Italy*

A long term treatment with the δ-selective opiate antagonist *NN*-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH (ICI 154129) produces an increase in the number of δ-opiate binding sites, whereas the same treatment with the non selective opiate antagonist naloxone results in an enhancement of both μ- and δ-binding sites. This biochemical effect in naloxone-pretreated mice is paralleled by a more pronounced increase in locomotor activity induced by a challenge dose of morphine. In contrast, no difference in the effect of morphine was seen in ICI 154129-pretreated mice with respect to control. These data suggest that the locomotor response to morphine in C57 mice is not mediated through δ-opiate receptors.

The C57BL/6J (C57) inbred strain of mice seems to be an interesting experimental tool for investigating the effects of opiate agonists and antagonists. In particular, C57 mice exhibit a peculiar pattern of responses following morphine administration, such as a rapid development of tolerance to the pharmacological action of the opiate (Berti et al 1978; Frigeni et al 1978), an increase in locomotor activity and an improvement in active avoidance behaviour after a single injection of the drug (Cuomo et al 1981a, b).

Furthermore, our recent findings have shown that the non-selective opiate antagonist naloxone, given on a chronic basis, was able to enhance the morphine-induced increase in locomotor activity; these results were paralleled by biochemical data showing that a repeated administration of naloxone significantly increased the density of [^{3}H]dihydromorphine ([^{3}H]-DHM) and D-Ala^2-D-Leu^5-[^{3}H]enkephalin ([^{3}H]-DADLE) binding sites in C57 brain areas, thus suggesting a possible correlation between the behavioural response to morphine and the higher number of opiate receptors in the striatum (Brunello et al 1984).

A new synthetic peptide, *NN*-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH (ICI 154129), showed a pattern of actions interpreted as a δ-selective antagonism both in in-vitro and in in-vivo preliminary tests. In fact, ICI 154129 is 30 times more effective in antagonizing Leu-enkephalin than normorphine effects on the field-stimulated mouse vas deferens. Furthermore it does not display any antagonism against morphine-induced analgesia, but it is active in slowing the head-turn induced by etorphine (Shaw et al 1982).

It was therefore of interest to investigate the effects of prolonged treatment with ICI 154129 on the above reported biochemical and behavioural parameters.

* Correspondence to: Via A. del Sarto, 21, 20129 Milan, Italy.

Material and methods

Male mice strain C57 BL/6J (18–20 g) were injected either with saline 0·9% NaCl, morphine (10 mg kg^{-1} i.p.), ICI 154129 (30 mg kg^{-1} s.c.) or naloxone (1 mg kg^{-1} i.p.). Treatment schedules are in the legends of Table 1 and Fig. 1.

Binding studies were performed on brain areas of mice decapitated according to Wood et al (1981). Briefly, tissues were homogenized in 50 volumes of Tris-HCl 50 mM pH 7·7, centrifuged at 50 000*g* for 20 min; the pellet was then resuspended in 50 volumes of the same buffer and incubated at 37 °C for 40 min.

After centrifugation at 50 000*g* for 20 min the final pellet was resuspended in Tris-HCl buffer yielding a protein concentration of 0·5 mg ml^{-1}. 0·8 ml of the membrane suspension was incubated at 25 °C for 1 h in the presence of the ^{3}H-ligand with or without 10^{-5} M displacer.

When the δ-selective agonist [^{3}H]DADLE (1–15 nM) was used, 100 μg of bacitracin were added to the assay and 10^{-5} M D-Ala^2-Met-enkephalinamide was used as a displacer.

Levorphanol, 10^{-5} M, was added as displacer in the preparation of saturation curves of the binding of the

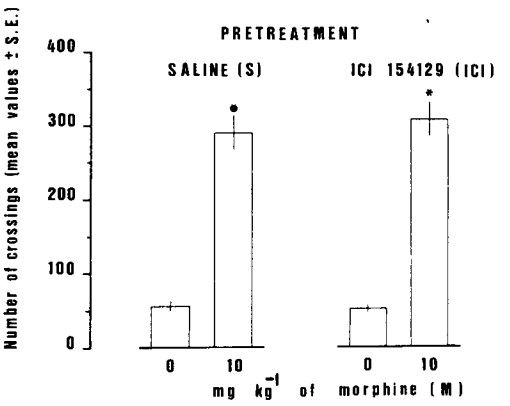

FIG. 1. Influence of repeated subcutaneous injection of ICI 154129 on morphine-induced increase of locomotor activity during a 30 min session. Mice received eight single doses of ICI 154129 (30 mg kg^{-1}); the injections were given at intervals of 12 h. The challenge dose of morphine (10 mg kg^{-1} i.p.) was administered 12 h after the last injection of ICI 154129 and 1 h before the activity session. Each group included 8 mice. Individual comparisons were obtained by the test of the least significant difference: S + O vs S + M: $P < 0.001$; ICI + O vs ICI + M: $P < 0.001$.

Table 1. Density of μ and δ binding sites in brainstem and striatal membranes from saline, naloxone and ICI 154129 treated C57 mice.

	Striatum		Brainstem	
	[^{3}H]DHM (fmol mg^{-1} prot.)	[^{3}H]DADLE	[^{3}H]DHM (fmol mg^{-1} prot.)	[^{3}H]DADLE
Saline	229 ± 16	204 ± 19	158 ± 13	149 ± 16
Naloxone	282 ± 18*	249 ± 12*	206 ± 15*	203 ± 15*
ICI 154129	233 ± 17	258 ± 11*	168 ± 9	235 ± 13*

*$P < 0.05$ vs saline-treated mice with Dunnet's test.

Data are expressed as fmol ^{3}H-ligand bound mg^{-1} protein and are the mean of the B_{max} ± s.e.m. of at least 3 saturation curves.

K_D values, 1·2 nM and 1·8 nM for [^{3}H]DHM in striatum and brainstem, 1·5 nM and 7·2 nM for [^{3}H]DADLE in striatum and brainstem respectively, were not affected by either of the treatments.

Animals were treated either with saline, naloxone (1 mg kg^{-1} i.p.) or ICI 154129 (30 mg kg^{-1} s.c.) for 3 days, twice daily and killed 6 h after the last injection.

μ-selective ligand [^{3}H]DHM (0·5–10 nM). K_D and B_{max} values were obtained plotting the experimental data according to Scatchard (1949).

Locomotor activity was measured with a series of toggle-floor boxes as described by Oliverio & Castellano (1974). Each toggle-floor box (divided into 19 × 9·5 cm compartments connected by an opening of 5 × 3 cm) was encased in an individual sound attenuating chamber with a dim light as the source of illumination. For each animal, the number of spontaneous crossings from one compartment to the other during a 30-min session was automatically recorded by a microswitch connected to the tilting floor of the box.

Results

Opiate receptor densities in naloxone and ICI 154129-pretreated mice. According to our previous results, a repeated administration of the non-selective opiate antagonist naloxone produced a marked increase in the number of [^{3}H]DHM and [^{3}H]DADLE binding sites in striatum and brainstem of C57 mice (Table 1). Conversely, prolonged treatment with the δ-antagonist ICI 154129 significantly increased the number of [^{3}H]DADLE binding sites both in striatum and brainstem, without affecting the density of [^{3}H]DHM binding sites.

Locomotor activity. Results are reported in Fig. 1. A two-factor analysis of variance (ANOVA) for spontaneous crossings showed the following differences: between saline- and ICI 154129-pretreated groups (F: 0·23; df: 1/28; n.s.), between treatments (F: 216·4; df: 1/28; $P < 0.001$) and between groups × treatments (F: 0·40; df: 1/28; n.s.).

Since ANOVA did not show any significant difference between saline- and ICI 154129-pretreated groups, no further analyses were made to obtain individual between groups comparisons.

Individual comparisons between treatments showed that morphine (10 mg kg^{-1}) significantly increased the spontaneous locomotor activity of C57 mice.

Discussion

An increase in the number of opiate binding sites has been reported after both naloxone and naltrexone prolonged administration in the rat (Lahti & Collins 1978; Schulz et al 1979; Zukin et al 1982; Bardo et al 1983). The present results are in line with these data and further confirm that naloxone, given on a chronic basis, induces an enhancement in the number of both μ and δ sites in the striatum as well as in the brainstem of C57 mice (Brunello et al 1984). Our previous behavioural studies are in agreement with these biochemical data, since the more pronounced increase in locomotor activity induced by a challenge dose of morphine in naloxone-pretreated mice is paralleled by an increase in the number of opiate binding sites.

On the other hand, in order to establish whether the supersensitivity of both μ and δ binding sites elicited by a repeated treatment with naloxone is involved in the development of the behavioural supersensitivity to morphine, we have investigated the effects of prolonged treatment with a selective δ-antagonist, ICI 154129, on the plasticity of opiate receptors as well as on the morphine-induced hyperactivity in C57 mice.

The results of these experiments show that, unlike naloxone, the prolonged exposure to ICI 154129 produced a significant increase in the number of [^{3}H]DADLE binding sites in the striatum and in the brainstem, but did not affect the density of [^{3}H]DHM binding sites. This is further evidence for the existence of a δ binding component somewhat distinct from the μ component.

Moreoever, pretreatment with the selective δ-receptor antagonist did not influence the stimulant effect of morphine on locomotion of C57 mice.

Thus, the present data suggest that the enhanced behavioural response to morphine elicited by pretreatment with the non-selective opiate antagonist naloxone is not mediated through the modulation of δ receptor function.

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The development of a relative hyperthermia during continuous clonidine infusion in the rat

C. MACCARRONE*, S. J. LEWIS, B. JARROTT, *University of Melbourne, Department of Medicine, Clinical Pharmacology & Therapeutics Unit, Austin Hospital, Heidelberg, Vic., 3084 Australia*

In rats the continuous infusion of clonidine (10 μg kg^{-1} h^{-1}) produces a relative hyperthermia of approximately 0·6 °C during the light phases but not the dark phases of a 10 day infusion period. It appears that the relative hyperthermia results from a clonidine infusion-induced attenuation of the fall in body temperature which normally occurs at the onset of the light phases.

In rats, the acute systemic injection of clonidine produces a dose-dependent hypothermia (Ozawa et al 1977; Bugajski et al 1980). This response most probably arises from the direct stimulation of α_2-adrenoceptors (Zacny 1982) within the thermoregulatory control centres of the hypothalamus and brainstem (Tsoucaris-Kupfer & Schmitt 1972a, b). In addition, it has been suggested that this fall in body temperature results from a decrease in metabolic heat production as well as increases in evaporative heat loss and cutaneous vasodilatation (Lin et al 1981). At present, little is known concerning the thermoregulatory effects of longer term clonidine administration. Lewis et al (1981) examined the effects of twice daily intraperitoneal injections of clonidine (50 μg kg^{-1}) for 7 days and reported that a hypothermia of 3–4 h duration followed each injection. However, this drug has a relatively short elimination half-life (1 h) in the rat (Conway & Jarrott 1980) and so this injection regimen cannot be regarded as a suitable form of chronic administration. However, with the availability of implantable osmotic minipumps which can infuse drugs continuously at a constant rate for up to 14 days, this problem can be circumvented.

We now report that rats receiving a subcutaneous infusion of clonidine (10 μg kg^{-1} h^{-1} via osmotic minipumps), at a rate which is essentially devoid of any initial changes in body temperature, produced a relative hyperthermia by day 2 of a 10 day infusion period. Moreover, the elevation in body temperature is restricted to the light phases (08.00–20.00 h) rather than the dark phases (20.00–08.00 h) of the infusion period suggesting that these effects may result from an interaction with circadian control mechanisms.

* Correspondence.

Methods

Female Wistar-Kyoto rats weighing between 205–225 g were used. Animals with similar body temperatures (within a range of 0·3 °C) were deliberately selected for the experiments. Immediately before and during the experiments the animals were kept in individual cages with food and water freely available. The room was maintained at a temperature of 21 ± 1 °C and had a 12 h light (08.00–20.00 h)—dark cycle. In order to obtain constant tissue and fluid concentrations of clonidine, this drug was continuously infused at a rate of 10 μg (base) kg^{-1} h^{-1} in 8 rats via subcutaneously implanted osmotic minipumps (Model 2002; ALZA Corporation, California, USA). This infusion rate was selected on the basis that it does not initially lower the body temperature in the rats. Higher infusion rates of 50–100 μg kg^{-1} h^{-1} do produce hypothermia (Maccarrone, unpublished observation). The minipumps were implanted at 20.00 h (beginning of day 1) under light halothane anaesthesia whilst sham operated rats (n = 8) served as controls. The body temperatures of these unrestrained rats were measured once every hour during days 1, 2, 6 and 10 of the infusion by means of a thermistor probe inserted 6 cm into the rectum and recorded with the aid of a tele-thermometer (Model 423, Yellow Springs Instrument Co., USA). Preliminary studies have demonstrated that the infusion of 0·9% NaCl (saline) from these minipumps does not affect a variety of physiological parameters including body temperature (Jarrott & Lewis, in preparation).

The values represented here are the mean ± s.e.m. Statistical analysis of differences between means were performed by one way or two way analysis of variance (ANOVA). The analysis is by one way ANOVA unless stated otherwise.

Results

The body temperatures of the control rats displayed a circadian rhythm with the values being consistently higher during the dark phases compared with the light phases on each day of the experiment. For example, on day 6, the body temperature was on average 0·9 °C higher during the dark phase than the following light phase (Table 1). These changes were clearly light/dark