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# α-Adrenoceptor and opioid receptor modulation of clonidine-induced antinociception

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1 The antinociceptive action of clonidine (Clon) and the interactions with $\alpha_1$, $\alpha_2$ adrenoceptor and opioid receptor antagonists was evaluated in mice by use of chemical algesiometric test (acetic acid writhing test).

2 Clon produced a dose-dependent antinociceptive action and the $ED_{50}$ for intracerebroventricular (i.c.v.) was lower than for intraperitoneal (i.p.) administration (1 ng $kg^{-1}$ vs 300 ng $kg^{-1}$). The parallelism of the dose-response curves indicates activation of a common receptor subtype.

3 Systemic administration of prazosin and terazosin displayed antinociceptive activity. Pretreatment with prazosin produced a dual action: i.c.v. Clon effect did not change, and i.p. Clon effect was enhanced. Yohimbine i.c.v. or i.p. did not induce antinonciception, but antagonized Clon-induced activity. These results suggest that $\alpha_1$- and $\alpha_2$-adrenoceptors, either located at the pre- and/or post-synaptic level, are involved in the control of spinal antinociception.

4 Naloxone (NX) and naltrexone (NTX) induced antinociceptive effects at low doses ($\mu$g $kg^{-1}$ range) and a lower antinociceptive effect at higher doses (mg $kg^{-1}$ range). Low doses of NX or NTX antagonized Clon antinociception, possibly in relation to a preferential $\mu$ opioid receptor antagonism. In contrast, high doses of NX or NTX increased the antinociceptive activity of Clon, which could be due to an enhanced inhibition of the release of substance P.

5 The results obtained in the present work suggest the involvement of $\alpha_1$-, $\alpha_2$-adrenoceptor and opioid receptors in the modulation of the antinociceptive activity of clonidine, which seems to be exerted either at spinal and/or supraspinal level.

**Keywords:** Antinociception; writhing test; clonidine; α-adrenoceptor antagonists; opioid receptor antagonists

## Introduction

Clonidine (Clon), an $\alpha_2$-adrenoceptor agonist clinically useful as an antihypertensive drug, has a number of other pharmacological properties due to effects in the CNS (sedation, mydriasis, xerostomia, anti-convulsant effect, increase in growth hormone secretion). In addition, it has been reported that Clon exhibits strong antinociceptive activity. This effect has been observed in animal models (Schmitt *et al.*, 1974; Ossipov *et al.*, 1990) and in clinical studies (Mendez *et al.*, 1990; Bernard *et al.*, 1991). In laboratory animals, Clon has been reported to be as potent as morphine (Fielding *et al.*, 1978), acting both at supraspinal and spinal levels (McCleary & Leander, 1981; Yaksh & Reddy, 1981; Ossipov *et al.*, 1990).

The antinociceptive effect of Clon has been studied mainly in relation to its $\alpha_2$-adrenoceptor agonist activity, but the interaction with other adrenoceptors has not been fully evaluated. In addition, the interactions of Clon with opioid receptors are complex and controversial, since supra-additive effects between Clon and morphine have been reported (Spaulding *et al.*, 1979; Gurtu *et al.*, 1994) and a reduction of the antinociceptive action of clonidine and morphine following prolonged administration of Clon or morphine has also been observed (Suematsu *et al.*, 1993). In addition, reports on the interaction of opioid systems and Clon are confusing and inconsistent. In some, naloxone, a known opioid antagonist, was not able to antagonize the antinociceptive activity of Clon (Yaksh & Reddy, 1981; Yaksh, 1985). However, naloxone at low doses significantly antagonized the Clon-induced analgesia in the rat tail-flick test (Kumar *et al.*, 1993). Furthermore, Clon suppressed the noxiously evoked activity of single wide dynamic range neurones of the spinal dorsal horn, an effect reversed by naloxone (Omote *et al.*, 1991). The hypoalgesia observed during prolonged naloxone administration was inhibited by Clon (Rochford & Dawes, 1993). In addition, naxolone did not change the increase produced by Clon in the threshold for vocalization (Paalzow & Paalzow, 1976).

The purpose of this work was to evaluate the antinociceptive action of Clon administered by the i.c.v. and i.p. routes in mice by use of a chemical algesiometric test (acetic acid writhing test), and to examine the interactions between Clon and $\alpha_1$- and $\alpha_2$-adrenoceptor and opioid receptor antagonists.

## Methods

### *Animals*

CF-1 mice, weighing 20-25 g, were used for the experiments. The animals were acclimatized to the laboratory environment for at least 2 h before being used, the ethical standards guidelines of the IASP (1983) were followed and the experimental protocols were approved by the local Animal Experimentation Ethics Committee. In particular, the duration of experiments was as short as possible, the number of animal involved was kept to a minimum and the animals were killed immediately after the recording period by the administration of an anaesthetic overdose (urethane 10 g $kg^{-1}$). Each animal was used only once and received only one dose of the drugs tested. All observations during the assay were performed by the authors in a randomized and 'blind' manner.

### *Antinociceptive assay*

Evaluation of antinociceptive activity was carried out as previously reported (Sierralta & Miranda, 1993). Thirty min after i.c.v. or i.p. injection of drugs, mice were injected i.p. with 10 ml $kg^{-1}$ of 0.6% acetic acid. The number of writhes was counted during a 5 min period, starting 5 min after the ad-

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ministration of acetic acid solution. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body. Control animals (saline) were run interspersed concurrently with the drug-related animals, which prevented all of the controls being run on a single group of mice at one time during the course of the investigation.

The antinociceptive activity of Clon was measured by a dose-response curve. Then, the dose that produced 50% of antinociception ($ED_{50}$) was selected and tested in conjunction with different opioid and/or agents acting on adrenoceptors. Antinociceptive activity was expressed as % inhibition of the usual number of writhes observed in i.c.v. ($24.7 \pm 2.3$, $n = 10$) or i.p. saline control animals ($27.2 \pm 1.7$, $n = 32$).

### *Drugs and routes of administration*

Drugs and saline control solutions were administered i.p. (10 ml $kg^{-1}$) or i.c.v. (5 $\mu$l per mice) 30 min before the algesiometric test. The i.c.v. administration was made under light ethyl ether anaesthesia as previously described (Sierralta & Miranda, 1993). Clon and yohimbine (Yoh) were injected i.c.v. or i.p. Naloxone (NX), naltrexone (NTX), prazosin (Pra) and terazosin (Ter) were administered i.p.

Clonidine hydrochloride, naloxone hydrochloride, naltrexone hydrochloride and yohimbine hydrochloride were all purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Prazosin hydrochloride was purchased from RBI, Natick, MA, U.S.A. Terazosin monohydrochloride was a gift from Laboratorios Abbott de Chile.

### *Analysis of results*

Results are presented as mean values $\pm$ s.e.mean. Student's two tailed $t$ test with Bonferroni correction was used to compare results between groups. The 0.05 level of probability was accepted as significant. All tests were performed with a computer programme (GB-Stat Professional Statistics & Graphics software).

## Results

### *Dose-dependent antinociception of clonidine*

Clon administered either i.c.v. or i.p. produced a dose-dependent antinociceptive effect in the mouse writhing test (Figure 1). The $ED_{50}$ for i.c.v. was lower than for i.p. administration (1 ng $kg^{-1}$ vs 300 ng $kg^{-1}$). Furthermore, the dose-response curves obtained with i.c.v. and i.p. administration did not deviate significantly from parallelism.

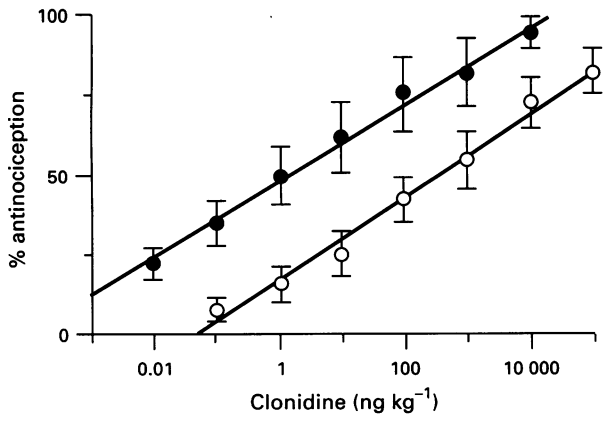


**Figure 1** Dose-response curves for the antinociceptive activity of clonidine in the writhing test in mice: (●) i.c.v. and (○) i.p. administration. Each point represents mean value $\pm$ s.e.mean of data obtained from 6 to 19 mice.

### *Effect of $\alpha_1$-adrenoceptor antagonists on Clon antinociceptive activity*

The i.p. administration of Pra or Ter induced a dose-dependent antinociception in the writhing test, with $ED_{50}$ values of 1.3 and 3.0 mg $kg^{-1}$, respectively. The dose-response curves were parallel, as can be seen in Figure 2.

The pretreatment of mice with 0.1 – 10 mg $kg^{-1}$, i.p. of Pra had no effect on the antinociceptive activity of i.c.v. administered Clon. By contrast, Pra 1 and 10 mg $kg^{-1}$ i.p. significantly increased the antinociception induced by i.p. injection of Clon (Table 1).

### *Effect of $\alpha_2$-adrenoceptor antagonists on clonidine antinociceptive activity*

The i.p. administration of Yoh (0.3 – 10 mg $kg^{-1}$) was not able to induce antinociception in the algesiometric test used in the present work. Similarly, i.c.v. administration of 0.1, 1 and 10 $\mu$g $kg^{-1}$ of Yoh did not produce any effect in the writhing test. The pretreatment of mice with Yoh administered either i.c.v. or i.p. antagonized the antinociceptive activity induced by i.c.v. Clon. The results are summarized in Table 2.

### *Effect of opioid receptor antagonists on clonidine antinociceptive activity*

The effect of the opioid antagonists NX and NTX on the antinociceptive activity of i.c.v. administered Clon was different depending on the dose. The i.p. administration of 1 $\mu$g $kg^{-1}$ of NX or NTX, which possess antinociceptive properties, significantly antagonized the effect of Clon, while the same opioid

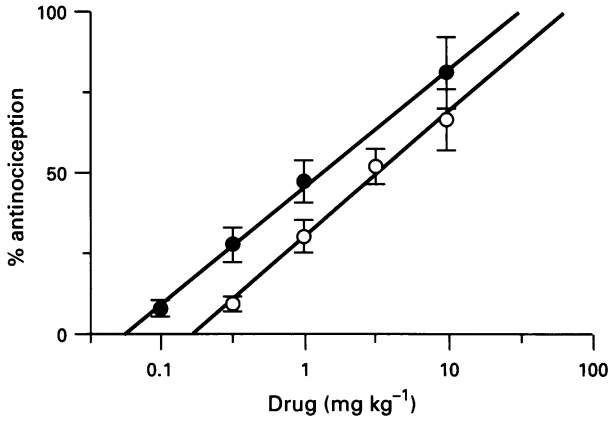


**Figure 2** Dose-response curves for the antinociceptive activity of prazosin and terazosin in the writhing test in mice: (●) prazosin and (○) terazosin. Each point represents mean value $\pm$ s.e.mean of data obtained from 6 to 11 mice.

**Table 1** Effect of prazosin (Pra) on the antinociceptive activity of clonidine (Clon) in the mouse writhing test

| *Treatment* (dose $kg^{-1}$) | *% antinociception* | n |
|---|---|---|
| Clon 1 ng, i.c.v | $50.9 \pm 8.5$ | 19 |
| Plus Pra 0.1 mg, i.p | $60.3 \pm 13.5$ | 9 |
| 1.0 mg, i.p. | $67.6 \pm 16.0$ | 6 |
| 10.0 mg, i.p. | $36.4 \pm 6.6$ | 11 |
| Clon 300 ng, i.p. | $52.3 \pm 6.8$ | 18 |
| Plus Pra 0.1 mg, i.p | $46.0 \pm 8.1$ | 6 |
| 1.0 mg, i.p. | $90.4 \pm 14.2$* | 11 |
| 10.0 mg, i.p. | $94.5 \pm 16.5$* | 10 |

Data are expressed as mean $\pm$ s.e.mean of the number of experiments ($n$). *$P < 0.05$ with respect to Clon i.p.

**Table 2** Effect of yohimbine (Yoh) on the antinociception induced by clonidine (Clon) in the writhing test in mice

| *Treatment* (dose $kg^{-1}$) | *% antinociception* | n |
|---|---|---|
| Clon 1 ng, i.c.v. | 50.9±8.5 | 19 |
| Plus Yoh 0.3 mg, i.p. | 16.3±1.9* | 8 |
| 1.0 mg, i.p. | 13.5±2.0* | 6 |
| 10.0 mg, i.p. | 23.0±2.8* | 8 |
| Plus Yoh 0.1 mg, i.c.v. | −19.2±10.1* | 6 |
| 10.0 mg, i.c.v. | 21.6±3.0* | 6 |

Data are expressed as mean±s.e.mean of the number of experiments (*n*). *$P<0.05$ with respect to Clon.

**Table 3** Effect of naloxone (NX) and naltrexone (NTX) on the antinociception induced by clonidine (Clon) in the writhing test in mice

| *Treatment* (dose $kg^{-1}$) | *% antinociception* | n |
|---|---|---|
| NX 1.0 μg, i.p. | 52.6±9.9 | 6 |
| 10.0 mg, i.p. | 25.0±4.4* | 8 |
| NTX 1.0 μg, i.p. | 32.5±3.6 | 6 |
| 10.0 mg, i.p. | 6.5±2.1* | 6 |
| Clon 1 ng, i.c.v | 50.9±8.5 | 19 |
| Plus NX 1.0 μg, i.p. | 27.4±1.1** | 12 |
| 10.0 mg, i.p. | 93.3±17.0** | 6 |
| Plus NTX 1.0 μg, i.p. | 17.8±1.8** | 12 |
| 10.0 mg, i.p. | 90.4±18.7** | 6 |

Data are expressed as mean±s.e.mean of the number of experiments (*n*). *$P<0.05$ with respect to the low dose. **$P<0.05$ with respect to Clon.

antagonists at 10 mg $kg^{-1}$ i.p., doses which are less active than 1 μg $kg^{-1}$, significantly increased the Clon-induced antinociception (Table 3).

## Discussion

The administration of Clon either i.p. or i.c.v. induced a dose-dependent antinociceptive effect in the mouse writhing test. These findings are in agreement with previous reports of similar Clon-induced antinociception observed in the formalin test in rats (Gurtu *et al.*, 1994), Haffner test in mice and tail-flick in rats (Ossipov *et al.*, 1988), tail-flick in mice (Fujimoto & Arts, 1990), formaline test in mice (Kanui *et al.*, 1993), vocalization threshold to paw pressure in normal and arthritic rats (Kayser *et al.*, 1992), carrageenan-induced inflammation/hyperalgesia in rats (Hylden *et al.*, 1991), writhing test in amphibians (Brenner *et al.*, 1994) and radiant heat-evoked hind paw withdrawal in rats (Naguib & Yaksh, 1994). However, the antinociceptive activity displayed by Clon administered i.c.v. differs from the findings of Fujimoto & Arts (1990), who reported that Clon administered by this route was unable to produce antinociception in the tail flick test in mice. The differences might be explained on the basis of the type of algesiometric test used and the experimental protocol. In addition, it has been suggested that Clon may not have analgesic properties, but merely impair the ability of the animal to respond to the nociceptive stimulation (Izenwasser & Kornetsky, 1990).

The parallelism of the dose-response curves for i.c.v. and i.p. Clon administration indicates a common receptor subtype activation, even if the antinociceptive effect might be exerted both at central (Ossipov *et al.*, 1985) and spinal level (Yaksh & Reddy, 1981).

The antinociceptive activity displayed by Pra in the writhing test of mice is in agreement with a previous report by Kanui *et al.* (1993), but not with the finding obtained in the rat hot-plate test (Carter, 1991). The pretreatment of mice with Ter, also produced an antinociceptive activity and the potency (calculated by transforming doses to molar concentration) compared with Pra was 2.14. These findings suggest that $\alpha_1$-adrenoceptors are involved in the antinociception, since both drugs are effective $\alpha_1$ antagonists and it has been postulated that $\alpha_1$-adrenoceptors are activated during the antinociceptive process (Howe *et al.*, 1983). However, the pretreatment of mice with Pra produced a dual effect on Clon-induced antinociception depending on the route of administration. Thus, antinociception induced by i.c.v. administration did not change, and following i.p. administration, antinociception activity increased. These findings are difficult to explain, but previous works have demonstrated that Pra had no effect (Solomon *et al.*, 1989), antagonized (Ono *et al.*, 1991; Danzebrink & Gebhart, 1990) or increased (Brenner *et al.*, 1994) the Clon induced antinociception.

The i.c.v. or i.p. administration of Yoh did not induce antinociception in the writhing test of mice, but antagonized Clon-induced activity, a result that is concordant with those obtained by McCleary & Leander, 1981; Howe *et al.*, 1983; Murata *et al.*, 1989; Ossipov *et al.*, 1988; Danzebrink & Gebhart, 1990; Kumar *et al.*, 1993; Naguib & Yaksh, 1994.

The results obtained with Pra and Yoh in the chemical acetic acid nociceptive test, independent of their pharmacological profile, suggest that $\alpha_1$- and $\alpha_2$-adrenoceptors, either located at pre- and/or post-synaptic level, are involved in the control of spinal antinociception.

The pretreatment of mice with the opioid antagonists, NX and NTX, induced a biphasic effect, characterized by a significant antinociceptive response at low doses (μg $kg^{-1}$ range) and a lower antinociception effect at higher doses (mg $kg^{-1}$ range). These results are in agreement with previous reports (Wheeler-Aceto & Cowan, 1993; Hamann & Martin, 1994; Noble *et al.*, 1994). In addition, hypoalgesia has been reported following NX treatment (Foo & Westbrook, 1993; Rochford & Dawes, 1993). The interaction of NX and NTX with i.c.v. administered Clon suggests the coexistence of opioid receptor subtypes at the noradrenergic terminals of CNS fibres, where Clon is modulating antinociception transmission. Thus, the antagonism of the antinociception of Clon induced by the lower doses of NX or NTX could be related to a preferential μ opioid receptor antagonism (Illes, 1989). In contrast, higher doses of NX or NTX increased the antinociceptive activity of Clon, which could be due to an enhanced inhibition of the release of substance P, since it has been reported that Clon and opioids inhibit the release of the peptide (Kuraishi *et al.*, 1985; Illes, 1989; Hama *et al.*, 1981). The interaction between opioids and $\alpha_2$-adrenoceptors has been suggested previously as a mechanism of inhibition of nociceptive input at the spinal level (Ossipov *et al.*, 1989). Nevertheless, it has been reported that analgesia produced by Clon is independent of the endogenous opioid system and is not antagonized by NX, but is antagonized by Yoh (Yaksh & Reddy, 1981; Howe *et al.*, 1983; Yaksh, 1985).

The action of NX is not related exclusively to opioid receptors, since it has been reported to antagonize the antinociceptive activity of tricyclic and atypical antidepressants (Biegon & Samuel, 1980; Reichenberg *et al.*, 1985). A biphasic effect of NX has been demonstrated in the antinociception induced by nitrous oxide, since pretreatment with high (mg $kg^{-1}$) doses of NX produced antagonism and lower doses (pg $kg^{-1}$) potentiation (Quock *et al.*, 1993). Furthermore, NX induced hypoalgesia which is not related to the GABA-benzodiazepine receptor complex (Rochford & Stewart, 1992).

The results obtained in the present work suggest the involvement of several neuronal systems in the modulation of the antinociceptive activity of Clon in the chemical algesiometric test used (acetic acid writhing test in mice). This modulation by $\alpha_1$-, $\alpha_2$-adrenoceptor antagonists and by opioid receptor antagonists seems to be exerted either at spinal and supraspinal levels, since it was observed both after i.c.v. and systemic administration of Clon.

## References


BERNARD, J.M., HOMMERIL, J.L., PASSUTI, N. & PINAUD, M. (1991). Postoperative analgesia by intravenous clonidine. *Anesthesiology*, **75**, 577–582.

BIEGON, A. & SAMUEL, D. (1980). Interaction of tricyclic antidepressants with opiate receptors. *Biochem. Pharmacol.*, **29**, 460–462.

BRENNER, G.M., KLOPP, A.J., DEASON, L.L. & STEVENS, C.W. (1994). Analgesic potency of alpha adrenergic agents after systemic administration in amphibians. *J. Pharmacol. Exp. Ther.*, **270**, 540–546.

CARTER, R. (1991). Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioural endpoint. *Pain*, **47**, 211–220.

COMMITTEE FOR RESEARCH AND ETHICAL ISSUES OF THE IASP. (1983). Ethical standards for investigations of experimental pain in animals. *Pain*, **16**, 109–110.

DANZEBRINK, R.M. & GEBHART, G.F. (1990). Antinociceptive effects of intrathecal adrenoceptor agonists in a rat model of visceral nociception. *J. Pharmacol. Exp. Ther.*, **253**, 698–705.

FIELDING, S., WILCKER, J., HYNES, M., SZEWCZAK, M., NOVICK, W.J. & LAL, H. (1978). A comparison of clonidine with morphine for antinociceptive and anti-withdrawal actions. *J. Pharamcol. Exp. Ther.*, **207**, 899–905.

FOO, H. & WESTBROOK, R.F. (1993). Naloxone-induced hypoalgesia: evidence from the formalin test. *Pharmacol. Biochem. Behav.*, **45**, 501–505.

FUJIMOTO, J.M. & ARTS, K.S. (1990). Clonidine, administered intracerebroventricularly in mice, produces an antianalgesic effect which may be mediated spinally by dynorphin A (1-17). *Neuropharmacology*, **29**, 351–358.

GURTU, S., SHUKLA, S. & MUKERJEE, D. (1994). Morphine, clonidine coadministration in subanalgesic doses: effective control of tonic pain. *Neuroreport*, **5**, 715–717.

HAMA, A.T., NAKATA, Y. & SEGAWA, T. (1991). Clonidine reduces substance P binding in rat spinal cord membrane preparation. *Jpn. J. Pharmacol.*, **55**, 157–160.

HAMANN, S.R. & MARTIN, W.R. (1994). Hyperalgesic and analgesic actions of morphine, U50-488, naltrexone, and (-)lobeline in the rat brainstem. *Pharmacol. Biochem. Behav.*, **47**, 197–201.

HOWE, J.R., WANG, J.Y. & YAKSH, T.L. (1983). Selective antagonism of the antinociceptive effect of intrathecally applied alpha adrenergic agonists by intrathecal prazosin and intrathecal yohimbine. *J. Pharmacol. Exp. Ther.*, **224**, 552–558.

HYLDEN, J.L., THOMAS, D.A., IADAROLA, M.J., NAHIN, R.L. & DUBNER, R. (1991). Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms. *Eur. J. Pharmacol.*, **194**, 135–143.

ILLES, P. (1989). Modulation of transmitter and hormone release by multiple neuronal opioid receptors. *Rev. Physiol. Biochem. Pharmacol.*, **112**, 139–233.

IZENWASSER, S. & KORNETSKY, C. (1990). Effects of clonidine and yohimbine, alone and in combination with morphine, on supraspinal analgesia. *Neuropharmacology*, **29**, 25–29.

KANUI, T.I., TJOLSEN, A., LUND, A., MJELLEM-JOLY, N. & HOLE, K. (1993). Antinociceptive effects of intrathecal administration of alpha adrenoceptor antagonists and clonidine in the formalin test in the mouse. *Neuropharmacology*, **32**, 367–371.

KAYSER, V., GUILBAUD, G. & BESSON, J.M. (1992). Potent antinociceptive effects of clonidine systemically administered in an experimental model of clinical pain, the arthritic rat. *Brain Res.*, **593**, 7–13.

KUMAR, A., RAGHUBIR, R. & DHAWAN, B.N. (1993). Analgesic effect of morphine, clonidine and serotonin microinjected into the PTN of rats. *Neuroreport*, **4**, 944–946.

KURAISHI, Y., HIROTA, N., SATO, Y., KANEKO, S., SATOH, M. & TAKAGI, H. (1985). Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn. *Brain Res.*, **359**, 177–182.

MCCLEARY, P.E. & LEANDER, J.D. (1981). Clonidine analgesia and suppression of operant responding: dissociation of mechanism. *Eur. J. Pharmacol.*, **69**, 63–69.

MENDEZ, R., EISENACH, J.C. & KASHTAN, K. (1990). Epidural clonidine analgesic after cesarean section. *Anesthesiology*, **73**, 848–852.

MURATA, K., NAKAGAWA, I., KUMETA, Y., KITAHATA, L.M. & COLLINS, J.G. (1989). Intrathecal clonidine suppresses noxiously evoked activity of spinal wide dynamic range neurons in cats. *Anesth. Analg.*, **69**, 185–191.

NAGUIB, M. & YAKSH, T.L. (1994). Antinociceptive effects of spinal cholinesterase inhibition and isobolographic analysis of the interaction with mu and alpha 2 receptor systems. *Anesthesiology*, **80**, 1338–1348.

NOBLE, F., FOURNIER-ZALUSKI, M.C. & ROQUES, B.P. (1994). Paradoxical analgesia induced by low doses of naloxone is not potentiated by complete inhibition of enkephalin degradation. *Neuropharmacology*, **33**, 135–140.

OMOTE, K., KITAHATA, L.M., COLLINS, J.G., NAKATANI, K. & NAKAGAWA, I. (1991). Interaction beteen opiate subtype and alpha-2 adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. *Anesthesiology*, **74**, 737–743.

ONO, H., MISHIMA, A., ONO, S., FUKUDA, H. & VASKO, M.R. (1991). Inhibitory effects of clonidine and tizanidine on release of substance P from slices of rat spinal cord and antagonism by alpha-adrenergic receptor antagonists. *Neuropharmacology*, **30**, 585–589.

OSSIPOV, M.H., CHATTERJEE, T.K. & GEBHART, G.F. (1985). Locus Coeruleus lesions in the rat enhance the antinociceptive potency of centrally administrered clonidine but not morphine. *Brain Res.*, **341**, 320–330.

OSSIPOV, M.H., LOZITO, R., MESSINEO, E., GREEN, J., HARRIS, S. & LLOYD, P. (1990). Spinal antinociceptive synergy between clonidine and morphine, U69593, and DPDPE: isobolographic analysis. *Life Sci.*, **46**, PL-71–PL-76.

OSSIPOV, M.H., SUAREZ, L.J. & SPALDING, T.C. (1988). A comparison of the antinociceptive and behavioral effects of intrathecally administered opiates, α-2-adrenergic agonists and local anesthetic agents in mice and rats. *Anesth. Analg.*, **67**, 616–624.

OSSIPOV, M.H., SUAREZ, L.J. & SPALDING, T.C. (1989). Antinociceptive interactions between alpha 2-adrenergic and opiate agonists at the spinal level in rodents. *Anesth. Analg.*, **68**, 194–200.

PAALZOW, G. & PAALZOW, L. (1976). Clonidine antinociceptive: effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **292**, 119–126.

QUOCK, R.M., CURTIS, B.A., REYNOLDS, B.J. & MUELLER, J.L. (1993). Dose-dependent antagonism and potentiation of nitrous oxide antinociception by naloxone in mice. *J. Pharmacol. Exp. Ther.*, **267**, 117–122.

REICHENBERG, K., GAILLARD-PLAZA, G. & MONTASTRUC, J.L. (1985). Influence of naloxone on the antinociceptive effects of some antidepressant drugs. *Arch. Int. Pharacodyn.*, **275**, 78–85.

ROCHFORD, J. & DAWES, P. (1993). Effects of naloxone on the habituation of novelty-induced hypoalgesia: the collateral inhibiion hypothesis revisited. *Bicohem. Behav.*, **46**, 117–123.

ROCHFORD, J. & STEWART, J. (1992). Naloxone-induced hypoalgesia: lack of involvement of the GABA-benzodiazepine receptor complex. *Pharmacol. Biochem. Behav.*, **43**, 321–328.

SCHMITT, H., LE DOUAREC, J.C. & PETILLOT, N. (1974). Antinociceptive effects of some α-sympathomimetic agents. *Neuropharmacology*, **13**, 289–294.

SIERRALTA, F. & MIRANDA, H.F. (1993). Adenosine modulates the antinociceptive action of benzodiazepines. *Gen. Pharmacol.*, **24**, 891–894.

SOLOMON, R.E., BRODY, M.J. & GEBHART, G.F. (1989). Pharmacological characterisation of alpha adrenoceptors involved in the antinociceptive and cardiovascular effects of intrathecally administered clonidine. *J. Pharmacol. Exp. Ther.*, **251**, 27–38.

SPAULDING, T.C., FIELDING, S., VENAFRO, J. & LAL, H. (1979). Antinociceptive activity of clonidine and its potentiation of morphine analgesia. *Eur. J. Pharmacol.*, **58**, 19–25.

SUEMATSU, M., OHNISHI, T., SHINNO, E., MAEDA, S. MATSUMOTO, K., SAKUDA, M. & SAITO, K. (1993). Effects of prolonged administration of clonidine on [$^{3}$H]PN 200-110 and [$^{125}$I]omega-conotoxin binding in mouse brain. *Neursci. Lett.*, **163**, 193–196.

WHEELER-ACETO, H. & COWAN, A. (1993). Naloxone causes apparent antinociception and pronociception simultaneously in the rat paw formalin test. *Eur. J. Pharmacol.*, **236**, 193–199.

YAKSH, T.L. (1985). Pharmacology of spinal noradrenergic systems which modulate spinal nociceptive processing. *Pharmacol. Biochem. Behav.*, **22**, 845–858.

YAKSH, T.L. & REDDY, S.V.R. (1981). Studies in the primate on the analgesic effects associated with intrathecal actions of opiates, alpha adrenergic agonists and baclofen. *Anesthesiology*, **54**, 451–467.


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