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ENHANCEMENT OF A NOCICEPTIVE REACTION BY OPIOID ANTAGONISTS IN MICE

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1 The opioid antagonists, naloxone, GPA 2163, levallorphan and Mr-2266 reduced the latency of the jumping reaction of mice in the hot plate test. The (+)-isomers of levallorphan and Mr-2266 which are devoid of antagonistic activity did not increase this latency.

2 In the same nociceptive reaction test, the enhancing effect of naloxone progressed in a dose-range similar to that required for the antagonism by naloxone of the depressive action of morphine.

3 The facilitatory effect of naloxone was not blocked by the previous administration of morphine or etorphine but it was prevented by pretreatment with a high dose of buprenorphine.

4 The antagonism by naloxone of morphine and of buprenorphine did not follow the same pattern.

5 The factors which are or may be involved in the efficacy of naloxone in enhancing nociceptive reactions are discussed.

6 The enhancing effect of naloxone may be due to an antagonism of endogenous ligands for the opiate receptor. If so, these ligands would be involved in reaction to but not in perception of nociceptive stimuli which need not be harmful ones.

Introduction

Endogenous ligands for the opiate receptor were first discovered by Terenius & Wahlström (1974) and by Hughes (1975). These ligands were further characterized by several workers (Cox, Opheim, Teschemacher & Goldstein, 1975; Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975; Terenius & Wahlström, 1975; Pasternak, Goodman & Snyder, 1975; Bradbury, Smyth, Snell, Birdsall & Hulme, 1976; Graf, Ronai, Bajusz, Cseh & Szekely, 1976; Guillemin, Ling & Burgus, 1976; Li & Chung, 1976). The administration of specific opioid antagonists to non-dependent animals may help to elucidate the functions of endogenous ligands for the opiate receptor. It has been shown that naloxone enhances nociceptive reaction (licking and jumping) of mice and rats in the hot plate test, which suggests that these reactions may be normally limited by the release of endogenous ligands (Jacob, Tremblay & Colombel, 1974). This observation has been confirmed by several workers (Frederickson, Nickander, Smithwick, Schuman & Norris, 1976; Pomeranz & Chiu, 1976; Grevert & Goldstein, 1977b) and extended to two other opioid antagonists, naltrexone and diprenorphine (Tremblay, Colombel & Jacob, 1976).

Naloxone also enhances various spinal reflexes in spinal cats (Goldfarb & Hu, 1976; Bell & Martin, 1977). In the tail-flick test, the effect of naloxone varies according to the investigator; Tulunay, Sparber Takemori (1975), Pert & Walter (1976) and Yaksh, Yeung & Rudy (1976) found naloxone ineffective, whereas King, Hughey, Massareno, Codd & Byrne (1977) and Berntson & Walker (1977) reported a decrease in reaction time induced by naloxone. However, naloxone does not affect other nociceptive reactions such as escape from foot-shock in trained rats (Goldstein, Pryor, Otis & Larsen, 1976), escape from tail-pinch (Berntson & Walker, 1977), writhing in mice (Elliott, Spieghler & Navarro, 1976) or pain rating in humans (Sobky, Dostrovsky & Wall, 1976; Grevert & Goldstein, 1977a).

Endogenous ligands may be involved in analgesia produced by stress (Akil, Madden, Patrick & Barchas, 1976; Madden, Akil, Patrick & Barchas, 1977), percutaneous electrical stimulation (Woolf, Barrett, Mitchell & Myers, 1977) or acupuncture (Pomeranz, Cheng & Law, 1977; Mayer, Price & Rafii, 1977) but not by hypnosis (Goldstein & Hilgard, 1975). The results obtained in stimulation-produced analgesia vary from author to author (Pert & Walter, 1976; Yaksh *et al.*, 1976; Akil, Mayer & Liebeskind, 1976; Oliveras, Hosobuchi, Redjemi, Guilbaud & Besson, 1977).

In this paper, we demonstrate that the enhancement of the nociceptive reaction by opioid antagonists results from an action on stereospecific receptors and analyze some possible factors which are or may be involved in the efficacy of these drugs in the used and related tests. Possible roles of the endogenous ligands are discussed.

Methods

Male Swiss mice (18 to 28 g, obtained from the CNRS and IFFA CREDO) were maintained in a constant environment with free access to commercial food (Extralabo) and water. At least one week was allowed for acclimatization to the new environment before the mice were used in the experiments.

The technique used in this study has been described previously (Jacob *et al.*, 1974). The apparatus consisted of a hot plate on which was placed a restraining glass cylinder (height 17 cm; diameter 13 cm). Unless otherwise stated, the temperature of the hot plate was 64.5 ± 0.5°C. The latency of the jumping reaction was determined by an observer who was unaware of the drug treatment. Animals which did not respond after 2 min were removed from the hot plate; in some experiments this 'cut-off' time was prolonged up to 5 min. The hot plate tests were carried out between 14 h 00 min and 18 h 00 min.

Groups of 8 to 10 mice were used in each experiment. Control groups received at appropriate times 0.9% w/v NaCl solution (saline) or the respective solvent. In general, naloxone, levallorphan, dextrallorphan, Mr-2266, Mr-2267 and GPA 2163, were injected 10 min before hot plate testing. Morphine was administered 0.5 or 1.5 h, etorphine 1.5 h and buprenorphine 2 h before naloxone. Buprenorphine was also administered 5 min after naloxone, the hot plate test being performed 35 min after naloxone. When the effect of a drug on the action of naloxone was studied, the following groups were used: (a) saline plus saline, (b) modifying drug plus saline, (c) saline or respective solvent plus naloxone, and (d) modifying drug plus naloxone. Mice were exposed individually to the hot plate and one mouse from each group was tested alternately.

For the intracerebroventricular (i.c.v.) administration of naloxone, the technique of Haley & McCormick (1957) was followed.

Drugs

Morphine hydrochloride (Francopia, France), levallorphan tartrate and dextrallorphan hydrobromide (Hoffmann La Roche, Switzerland), GPA 2163 ((−)-β-2-propargyl-5-phenyl-9-methyl-2′-hydroxy-6,7-benzomorphan) (Ciba-Geigy, France) were dissolved in saline. Buprenorphine hydrochloride and etorphine (Reckitt & Colman), Mr-2266 and Mr-2267 ((−)-5,9-α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan and its (+)-isomers, respectively) (Boehringer, West Germany) were dissolved in a few drops of 0.1 N HCl and made up with distilled water. All solutions were freshly prepared and administered in a volume of 0.5 ml/20 g (s.c.) or 0.02 ml/20 g (i.c.v.).

Statistical analysis

The statistical significance was calculated by the rank-correlation method of Wilcoxon (Wabeke & Van Eeden, 1955). The results are expressed as percentage of corresponding controls with standard error of the mean. The absolute values for the latencies of the control mice ranged from 62 to 99 s at 55°C and 13 to 51 s at 64.5°C.

Results

Reproducibility of the facilitatory effect

The facilitatory effect of naloxone on the jumping response was highly reproducible. A significant reduction in the latency to jumping was obtained in all 16 groups of mice which received 10 mg/kg naloxone (range: 54 to 75% of saline-treated mice). There was a significant reduction in the latency to jumping in 56 out of 59 groups of mice which received 1 mg/kg naloxone (range: 32 to 69% of saline-treated mice) and in 8 out of 10 groups which received 0.1 mg/kg naloxone (range: 29 to 51% of saline-treated mice). Naloxone (1 mg/kg) produced a significant enhancement even when the control latencies were as short as 16 s.

Stereospecificity of the facilitatory effect

Three opiate antagonists (levallorphan, Mr-2266 and GPA 2163) and the inactive (+)-isomers of two of them, dextrallorphan (Leimgruber, Mohacsi, Baruth & Randall, 1974) and Mr-2267 (Merz, Langbein, Stockhaus. Walther & Wick, 1974) were studied to assess the specificity of the facilitatory effect. All three antagonists shortened the reaction time (Figure 1), although they were less active than naloxone in this respect. Levallorphan, Mr-2266 and GPA 2163 are also less potent than naloxone as antagonists of opiate agonists (Merz *et al.*, 1974; Clarke, Hill, Saelens & Yokoyama, 1974). The facilitatory effect of levallorphan and Mr-2266, like that of naloxone, increased as the dose was increased (Figure 1). For each drug, an optimal dose was reached whereupon further increases in dose produced a smaller response. Dextrallorphan and Mr-2267 did not facilitate the

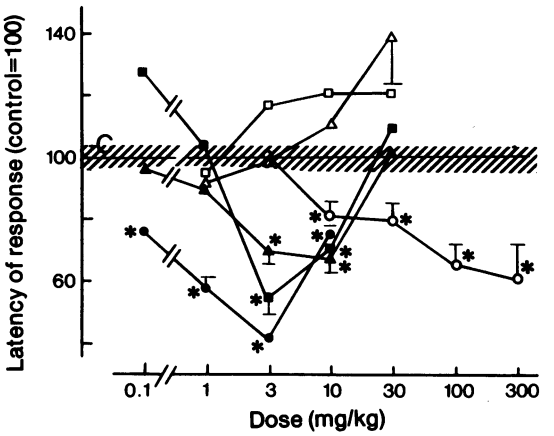

Figure 1 Stereospecificity of the facilitatory effect of the opioid antagonists on the jumping response of mice in the hot plate test at 55°C. Ordinate scale: latency of the jumping reactions in percentage of corresponding control mice (C). Abscissa scale: doses of the antagonists in mg/kg (log scale). The drugs were injected s.c. 10 min before test. Groups of 10–20 mice were used; cut-off time, 2 min; $^*P < 0.05$; in fact, P was <0.01 or even 0.001 for some points but no distinction has been made for sake of clarity. Vertical bars represent the standard error of the means. Hatched area, mean ± standard error of the means for the corresponding control mice (C). (●) Naloxone; (▲) levallorphan; (△) dextrallorphan; (○) GPA 2163; (■) Mr-2266; (□) Mr-2267. Mr-2267 at a dose of 30 mg/kg produced convulsions, excessive salivation and hyperirritability. The maximum dose of GPA 2163 that could be administered was 300 mg/kg.

nociceptive reaction indicating the stereospecificity of the facilitatory effect.

Naloxone was also injected into the lateral cerebral ventricles in mice to see if its effect was mediated by a central action. It was found effective; jumping times measured 30 min after the injection were 58 ± 13% for 0.3 mg/kg ($P < 0.05$) and 63 ± 11% ($P < 0.02$) for 1 mg/kg of the corresponding times obtained in controls injected with saline. Lower doses were not effective. Thus, naloxone is not more active by the intraventricular than by the subcutaneous route. The low potency of naloxone when injected intraventricularly may be due to its rapid egress from the brain (Berkowitz, 1976). The procedure of intracerebroventricular injection itself induced a depressive state which may have obscured the effect of naloxone.

Effects of morphine, etorphine and buprenorphine in naloxone facilitation

Morphine (10 mg/kg) given alone markedly increased the latency to jumping (Figure 2a). When naloxone was administered after morphine the increase in latency produced by morphine was reduced. The antagonism of morphine by naloxone and the enhancing effect exerted by naloxone on the jumping reaction in the absence of morphine increased in a similar dose-range (Figure 2a).

When the dose of morphine was increased, there was no evidence that the decrease in the latency of the jumping reaction produced by naloxone was blocked by the highest tolerated doses of morphine (Figure 2b). The facilitatory effect of naloxone 1 mg/kg was still present, almost unchanged, after 10, 30 and 100 mg/kg of morphine. The interaction between morphine and a lower dose of naloxone (0.1 mg/kg) was studied but even then it was not possible to ascertain whether the effect of naloxone had been reduced by morphine since 100 mg/kg morphine plus 0.1 mg/kg naloxone was much less effective in increasing the latency of the jumping reaction (272%) than morphine alone (451%). Antagonism of morphine by naloxone is certainly involved in this difference to which the facilitatory effect of naloxone may however also contribute. Similar results were obtained with etorphine (Figure 2c). It was not possible to ascertain whether the facilitatory effect of 1 mg/kg naloxone was inhibited by 1 mg/kg etorphine (Figure 2c). Higher doses of etorphine could not be used because a profound depressive state was induced. The influence of 1 mg/kg etorphine on the facilitatory effect of a smaller dose of naloxone (0.1 mg/kg) could not be assessed because the latencies after etorphine alone and after etorphine plus naloxone were beyond the cut-off time (4 min).

Buprenorphine possesses mixed agonist-antagonist properties and binds strongly to the opiate receptors (Hambrook & Rance, 1976); it was therefore of interest to determine whether it would block the enhancing effect of naloxone. Buprenorphine induced moderate increases in the latency of the jumping reaction; this antinociceptive effect was dose-related up to 1 mg/kg. In doses up to 1 mg/kg the effect of buprenorphine was reduced by 1 mg/kg of naloxone administered 2 h after buprenorphine (Figure 3a). When a higher dose of buprenorphine (10 mg/kg) was used, its antinociceptive effect was less than that observed for smaller doses and this residual effect was not antagonized by naloxone. Under these circumstances naloxone had no facilitatory effect.

When buprenorphine was given before naloxone, the reductions of the antinociceptive effect by naloxone of the lower doses (0.02, 0.04, 0.1 and 1 mg/kg) of buprenorphine did not follow the pattern observed for morphine, since they were not significantly greater than the facilitatory effect of naloxone even when the dose of naloxone was increased from 1 to 10 mg/kg s.c. (Figure 3b).

However, when buprenorphine was given after

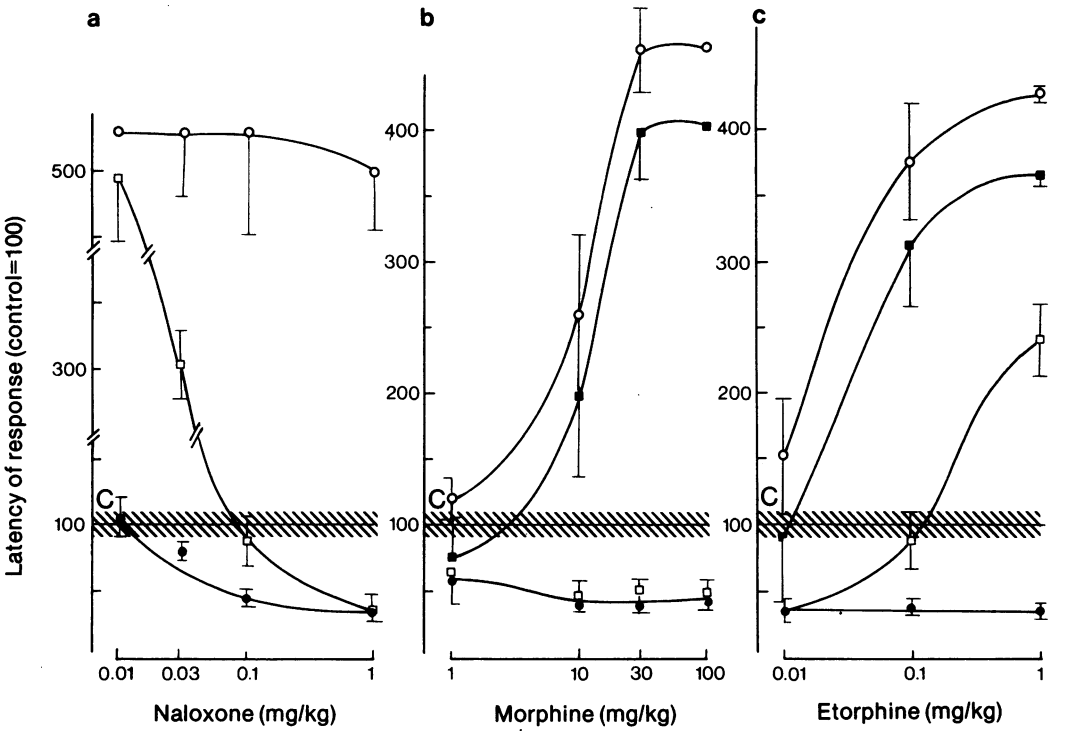

Figure 2 Effects of morphine and etorphine on naloxone facilitation. The temperature of the hot plate was 64.5°C ± 0.5°C. Ordinate scale, vertical bars, C and hatched area as in Figure 1. (a) Increase in the enhancing and antagonist actions with increasing dose of naloxone. Abscissae, dose of naloxone s.c. (log scale). (○) Morphine alone; (□) morphine plus naloxone; (●) naloxone alone. The dose of morphine was constant (10 mg/kg s.c.); the time interval between the administration of morphine and naloxone was 30 min. 14/20 mice which received morphine alone had not jumped by 2 min (cut-off time). (b) Lack of antagonism of the facilitatory effect of a constant dose of naloxone (1 mg/kg s.c.) by graded doses of morphine. Abscissae, graded doses of morphine in mg/kg s.c. (log scale). (○) Morphine alone; (■) curve calculated by the algebraic summation of the antinociceptive effect of the opiate and the facilitatory effect of naloxone; (□) morphine plus naloxone; (●) naloxone alone. Pretreatment interval between morphine and naloxone was 1.5 h. 2/10 (morphine 10 mg/kg), 7/10 (morphine 30 mg/kg) and 10/10 (morphine 100 mg/kg) mice had not jumped by 2 min. (c) Interaction between a constant dose of naloxone (1 mg/kg) and graded doses of etorphine. Etorphine was less readily antagonized by naloxone than morphine, but the maximum dose used was still antagonized (see text). Abscissae, dose of etorphine in mg/kg s.c. (log scale). (○) Etorphine alone; (■) as in (b); (□) etorphine plus naloxone; (●) naloxone alone. Pretreatment interval between etorphine and naloxone was 1.5 h. 9/10 (etorphine 0.1 mg/kg) and 10/10 (etorphine 1 mg/kg) mice had not jumped by 2 min.

naloxone, the antagonism was similar to that observed with morphine. Thus, the antagonism of buprenorphine by naloxone was greater than the facilitatory effect of naloxone; the latencies of the jumping reaction were for buprenorphine alone (0.1 mg/kg) 200% of the saline controls and for naloxone (1 mg/kg) plus buprenorphine (0.1 mg/kg) 40% of the saline controls; therefore, the antagonism was 160%, i.e. greater than the enhancing effect of naloxone in the absence of buprenorphine (50%).

These differences, depending on the time of administration, are probably linked to the slow dissociation of the buprenorphine-receptor complex. Similar results were also observed by Cowan, Lewis & Macfarlane (1977). However, these authors did not observe any effect when naloxone was given alone.

Discussion

The results obtained in this paper support the view (Jacob *et al.*, 1974) that the enhancing effect of opiate antagonists on the jumping reaction in the hot plate test is due to an interaction with specific opiate recep-

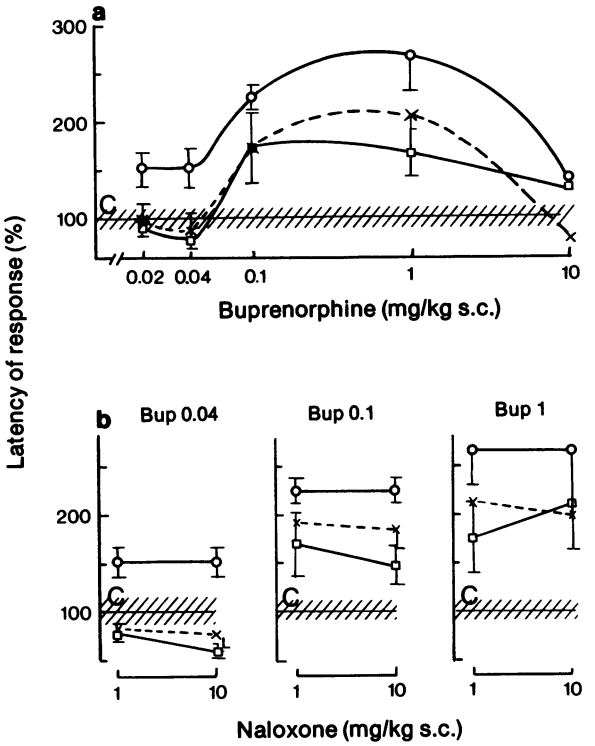

Figure 3 Interactions between buprenorphine and a subsequent dose of naloxone on the jumping response in mice at 64.5 ± 0.5°C. Ordinates, vertical bars, C and hatched area as in Figure 1. The time interval between the administration of buprenorphine and naloxone was 2 h. The enhancing effect of naloxone was determined in corresponding groups of mice but it has not been illustrated for the sake of clarity. (a) Influence of a constant dose of naloxone (1 mg/kg s.c.) on the effect of graded doses of buprenorphine. Abscissae, graded doses of buprenorphine s.c. (log. scale). (○) Buprenorphine; (□) buprenorphine plus naloxone; (×) curve calculated by the algebraic summation of the antinociceptive effect of the opiate and the facilitatory effect of naloxone. The antinociceptive effects of buprenorphine alone (0.02, 0.04, 0.1 and 1 mg/kg) and the association of these doses with naloxone (1 mg/kg) were significant: $P < 0.05$. (b) Influence of increasing doses of naloxone on the effects of various doses of buprenorphine (Bup). Abscissae, doses of naloxone in mg/kg s.c. (log scale). Symbols as in (a).

tors. We have shown, firstly, that levallorphan, Mr-2266 and GPA 2163 decreased the latency of the jumping reaction and that the potency of each drug to produce this effect was similar to its opiate antagonist potency (Merz *et al.*, 1974; Leimgruber *et al.*, 1974; Clarke *et al.*, 1974). Secondly, dextrallorphan and Mr-2267, the inactive (+)-isomers of levallorphan and Mr-2266, did not decrease the latency. Thirdly, the decrease in the latency of the jumping reaction and the antagonism of opiate agonists by naloxone was evident over the same dose-range. Finally, the facilitatory effect of naloxone was blocked by pretreatment with buprenorphine, a ligand of opiate receptors with a slow dissociation rate (Hambrook & Rance, 1976). However, to obtain this effect it was necessary to use a high dose of buprenorphine which by itself had almost no effect on the latency of the jumping reaction. On the other hand, lower doses of buprenorphine, which were antinociceptive, did not reduce the facilitatory effect of naloxone. This finding suggests that an almost complete occupancy of the antagonist conformation of the opiate receptors is needed for the blocking effect of buprenorphine.

The high reproducibility of the enhancing effect of naloxone in our experiments seems to be dependent on the doses used and may be related to the complex nature of the jumping response (Jacob, 1966) and the nature, intensity and duration of the superficial thermal stimulus. It is also important to use naive animals. The doses of the antagonists, administered covered the whole dose-response curve which was U shaped for naloxone, levallorphan and Mr-2266; such a relationship was also obtained by Pert & Walter (1976) for the antagonism by naloxone of the analgesia induced by central stimulation in rats. Effects of antagonists might have been overlooked by authors who used too few, too low or too high doses. The falling-off of the enhancing effect with high doses may be due to a toxic effect or of an opioid agonist effect of the molecule itself or of a dealkylated metabolite. There is evidence that even low doses of naloxone might have 'agonist' actions (Jacob & Michaud, 1976). The complexity of the response is not relevant as effects of naloxone have been also observed with simpler responses, such as monosynaptic and polysynaptic spinal reflexes in spinal animals (Goldfarb & Hu, 1976; Bell & Martin, 1977), the tail flick test in rats (Berntson & Walker, 1977; King *et al.*, 1977) and the licking response in mice (Jacob *et al.*, 1974). The licking response, however, is less influenced than the jumping response by either naloxone (Jacob *et al.*, 1974; Grevert & Goldstein, 1977b) or morphine (Jacob & Blozovski, 1961). In contrast to nociceptive responses in animals, pain perception of humans is not influenced by naloxone (Sobky *et al.*, 1976), a difference observed some time ago for morphine (Hardy, Wolf & Goodell, 1940; Beecher, 1957). Superficial thermal nociception as used also by Berntson & Walker (1977) and by Bell & Martin (1977) appears to be more easily influenced than deep nociception since naloxone has no effect on responses to pressure on the skin of the tail (Berntson & Walker, 1977), to chemical stimulation of the peritoneum (Elliott *et*

al., 1977) and to ischaemic pain (Grevert & Goldstein, 1977a). However, deep nociception was used in a study where naloxone was effective on the analgesia produced by central stimulation in the cat (Oliveras *et al.*, 1977). High intensity stimulation is not necessary and may even interfere since we have shown previously (Jacob *et al.*, 1974) that the influence of naloxone on the latency of the paw lick response was observed only when the temperature of the hot plate was low. Similarly, effects of naloxone on spinal reflexes are relatively greater with electrical stimulation of low frequency (Goldfarb & Hu, 1976). Naive animals were used because even a few re-exposures reduce the latency of the jumping reaction considerably (Jacob & Blozovski, 1961). The ineffectiveness of naloxone on the escape threshold of trained rats in the experiments of Goldstein *et al.* (1976) may have been due to the fact that the training has already reduced the reaction time to its minimum.

The simplest interpretation of the enhancing effect of naloxone and related drugs is an antagonism of endogenous ligands released by the nociceptive stimulation. If this view is correct, the endogenous ligands would modulate the response to, but not the perception of, the nociceptive stimuli. Another possibility is that perception involves receptors with low affinity for naloxone such as those described recently by several authors (Lord, Waterfield, Hughes & Kosterlitz, 1976; Simantov & Snyder, 1976; Audigier, Malfroy-Camine & Schwartz, 1977; Terenius, 1977; Lord, Waterfield, Hughes & Kosterlitz, 1977). Further, this regulatory endorphin system would be triggered already by stimuli of low intensity which may not necessarily be painful ones. This concept is at variance with the view of Barker & Smith (1977) who poposed that endorphins are released only in dramatic conditions. The possible involvement of endogenous ligands in acupuncture (Pomeranz *et al.*, 1977; Mayer *et al.*, 1977) and electrodermal analgesia (Woolf *et al.*, 1977) points in the same direction. After training, this system may become inoperative and be replaced by pathways not controlled by endogenous ligands.

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References

AKIL, H., MADDEN, J., PATRICK, R.L. & BARCHAS, J.D. (1976). Stress-induced increase in endogenous opiate peptides: In *Opiates and Endogenous Opiate Peptides.* ed. Kosterlitz, H.W. pp. 63–70. Amsterdam: North-Holland Biomedical Press.

AKIL, H., MAYER, D.J., & LIEBESKIND, J.C. (1976). Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science*, N.Y., **191**, 961–962.

AUDIGIER, Y., MALFROY-CAMINE, B. & SCHWARTZ, J.C. (1977). Binding of ^{3}H-Leu-enkephalin in rat striatum: Partial inhibition by morphine or naloxone. *Eur. J. Pharmac.* **41**, 247–248.

BARKER, J.L. & SMITH, T.G. Jr. (1977). Peptides as neurohormones. In *Approaches to the Cell Biology of Neurons, Neurosci. Symposia*, Vol. 2. ed. Cowan, W.M. & Ferrendelli, J.A. pp. 340–373, Bethesda: National Institute of Mental Health.

BEECHER, H.K. (1957). The measurement of pain. *Pharmac. Rev.*, **9**, 59–209.

BELL, J.A. & MARTIN, W.R. (1977). The effects of narcotic antagonists, naloxone, naltrexone and nalorphine on spinal cord C-fibre reflexes evoked by electrical stimulation or radiant heat. *Eur. J. Pharmac.*, **42**, 147–154.

BERKOWITZ, B.A. (1976). The relationship of pharmacokinetics to pharmacological activity: morphine, methadone and naloxone. *Clin. Pharmacokinet.*, **1**, 219–230.

BERNTSON, G.G. & WALKER, J.M. (1977). Effect of opiate receptor blockade on pain sensitivity in the rat. *Brain Res. Bull.*, **2**, 157–159.

BRADBURY, A.F., SMYTH, D.G., SNELL, C.R., BIRDSALL, N.Y. & HULME, E.C. (1976). C fragment of lipotropin has a high affinity for brain opiate receptors. *Nature, Lond.*, **260**, 793–795.

CLARKE, F.H., HILL, R.T., SAELENS, J.K. & YOKOYAMA, N. (1974). Antagonists in the 5-Phenyl-benzomorphan series. In *Narcotic Antagonists Adv. Biochem. Psychopharmac.* Vol. 8, ed. Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. & Villarreal, J.E. pp. 81–89. New York: Raven Press.

COWAN, A., LEWIS, J.W. & MACFARLANE, I.R. (1977). Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br. J. Pharmac.*, **60**, 537–545.

COX, B.M., OPHEIM, K.E., TESCHEMACHER, H. & GOLDSTEIN, A. (1975). A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. *Life Sci.* **16**, 1777–1782.

ELLIOTT, H.W., SPIEGHLER, V. & NAVARRO, G. (1976). Effect of naloxone on antinociceptive activity of phenoxybenzamine. *Life Sci.*, **19**, 1637–1644.

FREDERICKSON, R.C.A., NICKANDER, R., SMITHWICK, E.L., SCHUMAN, R. & NORRIS, F.H. (1976). Pharmacological activity of Met-enkephalin and analogues *in vitro* and *in vivo*. Depression of single neuronal activity in specific brain regions. In *Opiates and Endogenous Opiate Peptides* ed. Kosterlitz, H.W. pp. 239–246. Amsterdam: North-Holland Biomedical Press.

GOLDFARB, J. & HU, J.W. (1976). Enhancement of reflexes by naloxone in spinal cats. *Neuropharmac.*, **15**, 785–792.

GOLDSTEIN, A. & HILGARD, E.R. (1975). Failure of the opiate antagonist, naloxone to modify hypnotic analgesia. *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2041–2043.

GOLDSTEIN, A., PRYOR, G.T., OTIS, L.S. & LARSEN, F. (1976). On the role of endogenous opioid peptides: Failure of naloxone to influence shock escape threshold in the rat. *Life Sci.* **18**, 599–604.

GRAF, L., RONAI, A.Z., BAJUSZ, S., CSEH, G., & SZEKELY, J.I. (1976). Opioid agonist activity of β-lipotropin fragments: A possible biological source of morphine-like substances in the pituitary. *FEBS Lett.*, **64**, 181–184.

GREVERT, P. & GOLDSTEIN, A. (1977a). Effects of naloxone on experimentally-induced ischaemic pain and on mood in human subjects. *Proc. natn. Acad. Sci. U.S.A.*, **74**, 1291–1294.

GREVERT, P. & GOLDSTEIN, A. (1977b). Some effects of naloxone on behaviour in the mouse. *Psychopharmac.* **53**, 111–113.

GUILLEMIN, R., LING, N. & BURGUS, R. (1976). Endorphins, peptides, d'origine hypothalamique et neurophysaire à activité morphinomimétique. Isolement et structure moléculaire de l'α-endorphine. *C.R. Acad. Sci. (Paris)*, **282**, 783–785.

HALEY, T.J. & McCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in conscious mouse. *Br. J. Pharmac. Chemother.*, **12**, 12–15.

HAMBROOK, J.M. & RANCE, M.J. (1976). The interaction of buprenorphine with the opiate receptor: Lipophilicity as a determining factor in drug-receptor kinetics. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W. pp. 295–301. Amsterdam: North-Holland Biomedical Press.

HARDY, J.D., WOLFF, H.G., & GOODELL, H. (1940). Studies on pain. A new method for measuring pain threshold: observations on spatial summation of pain. *J. clin. Invest.* **19**, 649–657.

HUGHES, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88**, 295–308.

HUGHES J., SMITH, T.W., KOSTERLITZ, H.W., FOTHERGILL, L.A., MORGAN, B.A., & MORRIS, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.*, **258**, 577–579.

JACOB, J. (1966). Evaluation of narcotic analgesics. In *Methods in Drug Evaluation*. ed. Mantegazza, P. & Piccinni, F. pp. 278–296. Amsterdam: North-Holland Biomedical Press.

JACOB, J. & BLOZOVSKI, M. (1961). Actions de divers analgésiques sur le comportement de souris exposées à un stimulus thermoanalgésique (II): Apprentissage nociceptive d'urgence. Actions différentielles de substances analgésiques et psychoréactives sur les réactions de lèchement et le bond. *Arch. Int. Pharmacodyn.*, **133**, 296–309.

JACOB, J.J. & MICHAUD, G.M. (1976). Production par la naloxone d'effets inverses de ceux de la morphine chez le Chien éveillé. *Archs int. Pharmacodyn.*, **222**, 332–340.

JACOB, J.J., TREMBLAY, E.C. & COLOMBEL, M.C. (1974). Facilitation de réactions nociceptives par la naloxone chez la Souris et chez le Rat. *Psychopharmacologia (Berl.)*, **37**, 217–223.

KING, C.D., HUGHEY, L.J., MASSARENO, M., CODD, E.E. & BYRNE, W.L. (1977). Endorphin and naloxone: Insomnia and seizures in cats and hyperalgesia in rats. *Fedn Proc.*, **36**, 965.

LEIMGRUBER, W. MOHACSI, E., BARUTH, H., & RANDAL, L.O. (1974). Levallorphan and related compounds. In *Narcotic Antagonists, Adv. Biochem. Psychopharmac.*, Vol 8. ed. Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. & Villarreal, J.E. pp. 45–50. New York: Raven Press.

LI, C.H. & CHUNG, D. (1976). Primary structure of human β-lipotropin, *Nature, Lond.*, **260**, 622–624.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1976). Multiple opiate receptors. In *Opiates and Endogenous Opiate Peptides*. ed. Kosterlitz, H.W. pp. 275–280., Amsterdam: North-Holland Biochemical Press.

LORD, J.A.H., WATERFIELD, A.A., HUGHES & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature, Lond.*, **267**, 495–499.

MADDEN, J., AKIL, H., PATRICK, R.L. & BARCHAS, J.D. (1977). Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. *Nature, Lond.*, **265**, 358–360.

MAYER, D.J., PRICE, D.D. & RAFII, A. (1977). Antagonism of acupuncture analgesia in man by the narcotic antagonist, naloxone. *Brain Res.*, **121**, 368–372.

MERZ, H., LANGBEIN, A., STOCKHAUS, K., WALTHER, G., & WICK, H. (1974). Structure-activity relationships. In *Narcotic Antagonists, Adv. Biochem. Psychopharmac.* Vol. 8. ed. Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. & Villarreal, J.E. pp. 91–107. New York: Raven Press.

OLIVERAS, J.L., HOSOBUCHI, Y., REDJEMI, F., GUILBAUD, G. & BESSON, J.M. (1977). Opiate antagonist, naloxone strongly reduces analgesia induced by stimulation of a raphe nucleus (centralis inferior). *Brain Res.* **120**, 221–229.

PASTERNAK, G.W., GOODMAN, R. & SNYDER, S.H. (1975). An endogenous morphine-like factor in mammalian brain. *Life Sci.*, **16**, 1765–1769.

PERT, A. & WALTER, M. (1976). Comparison between naloxone reversal of morphine and electrical stimulation induced analgesia in the rat. *Life Sci.*, **19**, 1023–1032.

POMERANZ, B., CHENG, R. & LAW, P. (1977). Acupuncture reduces electrophysiological and behavioural responses to noxious stimuli: Pituitary is implicated. *Exp. Neurol.*, **54**, 172–178.

POMERANZ, B. & CHIU, D. (1976). Naloxone blockade of

acupuncture analgesia: Endorphin implicated. *Life Sci.*, **19,** 1757–1762.

SIMANTOV, R. & SNYDER, S.H. (1976). Brain pituitary opiate mechanisms: Pituitary opiate receptor binding, radioimmunoassays for methionine enkephalin and leucine enkephalin and ^{3}H-enkephalin interaction with the opiate receptor, in *Opiates and Endogenous Opioid Peptides.* ed. Kosterlitz, H.W. pp. 41–48, Amsterdam: North-Holland Biomedical Press.

SOBKY, A.E., DOSTROVSKY, J.O. & WALL, P.D. (1976). Lack of effect of naloxone on pain perception in humans. *Nature, Lond.*, **263,** 783–784.

TERENIUS, L. (1977). Opioid peptides and opiates differ in receptor selectivity. *Psychoneuroendocrinology*, **2,** 53–58.

TERENIUS, L. & WAHLSTRÖM, A. (1974). Inhibitor(s) of narcotic receptor binding in brain extracts and cerebrospinal fluid. *Acta. Pharmac. Copenh*, **35,** suppl. I, 55.

TERENIUS, L. & WAHLSTRÖM, A. (1975). Morphine-like ligand for opiate receptors in human CSF. In *The Opiate Narcotics: Neurochemical Mechanisms in Analgesia and Dependence.* ed. Goldstein, A. pp. 7–12. New York: Pergamon Press.

TREMBLAY, E., COLOMBEL, M.C. & JACOB, J.J. (1976). Précipitation et prévention de l'abstinence chez le Rat et chez la Souris en état de dépendance aiguë. *Psychopharmac.*, **49,** 41–48.

TULUNAY, F.C., SPARBER, S.B. & TAKEMORI, A.E. (1975). The effect of dopaminergic stimulation and blockade on the nociceptive and antinociceptive responses in mice. *Eur. J. Pharmac.*, **33,** 65–70.

WABEKE, D. & VAN EEDEN, C. (1955). *Handleiding voor de Toests van Wilcoxon.* Amsterdam: Mathematische Centrum, statische Afdeling.

WOOLF, C.J., BARRETT, G.D., MITCHELL, D. & MYERS, R.A. (1977). Naloxone-reversible peripheral electroanalgesia in intact and spinal rats. *Eur. J. Pharmac.*, **45,** 311–314.

YAKSH, T.L., YEUNG, J.C. & RUDY, T.A. (1976). An inability to antagonise with naloxone the elevated thresholds resulting from electrical stimulation of the mesencephalic central gray. *Life Sci.*, **18,** 1193–1198.

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