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FURTHER STUDIES ON OPIATE RECEPTORS THAT MEDIATE ANTI-NOCICEPTION: TOOTH PULP STIMULATION IN THE DOG

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1 The antinociceptive activities of morphine, codeine and dextropropoxyphene (μ-agonists), buprenorphine and Mr 2034 [(−)5,9-dimethyl-2-(tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan](k-agonists) have been determined against nociceptive responses to electrical stimulation of the tooth pulp in the conscious dog.

2 Dose-dependent increases in nociceptive thresholds were obtained for all of the analgesic drugs tested at doses within their antinociceptive range as determined in nociceptive pressure and chemical tests in rodents.

Introduction

Opioid analgesic drugs may be classified in terms of their interactions with μ- or k-opiate receptors according to the profile of their antinociceptive actions in the chronic spinal dog (Martin, Eades, Thompson, Huppler & Gilbert, 1976) or in rodent tests (Tyers, 1980). In rodents, analgesic drugs, such as morphine, codeine and dextropropoxyphene, which act on μ-opiate receptors, are effective against heat, pressure and chemical pain. But drugs such as nalorphine, pentazocine and certain N-tetrahydrofurfuryl substituted benzomorphans, which have agonist actions predominantly on k-receptors, are effective against pressure and chemical pain but are essentially inactive against heat pain. Preliminary data obtained in the conscious dog (Skingle & Tyers, 1979) suggest that both μ- and k-agonists are effective against nociceptive responses to electrical stimulation of the tooth pulp. This latter study has now been extended to determine the anti-nociceptive activities of several μ- and k-agonists.

Methods

Mature, male Beagle dogs weighing from 10 to 13.5 kg were used. The surgical implantation of tooth pulp electrodes, training procedures and methods for determination of nociceptive thresholds have been described previously (Skingle & Tyers, 1979). Dosing was carried out blind such that the operator was unaware of the treatment each dog had received. Drugs were given either subcutaneously (s.c.) or sublingually (s.l.). Sublingual doses were applied in a dose-volume of 0.5 ml under the tongue by use of a 1 ml hypodermic syringe without a needle attached. Dogs that received sublingual doses were premedicated with atropine, 0.05 mg/kg intramuscularly, to prevent excessive salivation.

Various doses of the analgesic drugs were compared with placebo (normal saline) using a randomized, cross-over designed test. Drugs were tested at 2 or more dose-levels in 6 dogs for each dose. Dogs were tested on only two occasions each week with at least 3 days between doses.

Nociceptive thresholds to electrical stimulation of the tooth pulp were determined for each dog before, and then at 15 and 30 min after drug administration and then at 30 min intervals thereafter up to 3 h. Antinociceptive activity was expressed for each dog as the percentage increase over the pre-drug control threshold. The mean changes (%) in threshold for each group of dogs receiving the same treatment were plotted as ordinate against time after dosing. Each point was compared statistically by means of Student's *t* test to determine any significant difference between drug and placebo-treated groups. Any changes that occurred in the behaviour of the dogs following drug treatment were also noted.

Drugs

The following drugs were used: morphine hydrochloride, codeine phosphate (McFarlane Smith); dextropropoxyphene (Dista); (±)-ethylketazocine methanesulphonate, pentazocine base (Sterling-Winthrop); buprenorphine hydrochloride (Reckitt & Colman); (−)-α-(1R, 5R, 9R, 2^{11}R)-5, 9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6, 7-benzomorphan

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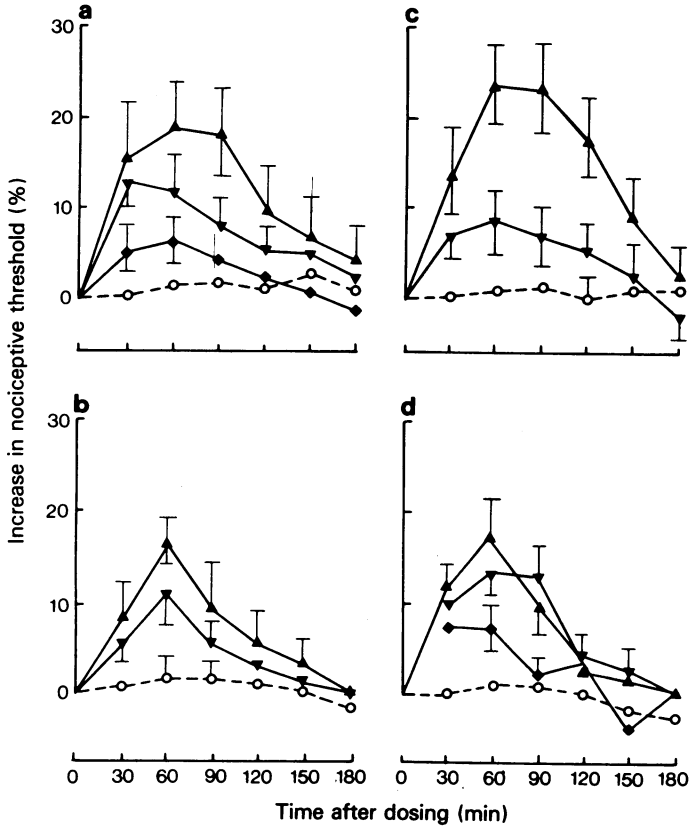

Figure 1 Antinociceptive effects of codeine 1.0 (◆), 2.0 (▼) and 4.0 (▲) mg/kg subcutaneously (a) and 2.0 (▼) and 4.0 (▲) mg/kg sublingually (b), and morphine 0.1 (▼) and 0.2 (▲) mg/kg subcutaneously (c) and 0.4 (◆), 0.8 (▼) and 1.6 (▲) mg/kg sublingually (d) against electrical stimulation of the tooth pulp in the conscious dog. Each point is the mean change (%) in nociceptive thresholds determined in 6 dogs by means of a randomised, cross-over designed experiment; vertical lines show s.e. mean. All doses produced effects that were significantly different from placebo (○).

base (Mr 2034, Dr H. Merz, Boehringer-Ingelheim); nalorphine HBr (Burroughs-Wellcome). Doses given in the text refer to the free parent compound of each salt.

Results

Dose-dependent increases in nociceptive thresholds to electrical stimulation of the tooth pulp in the dog were obtained with subcutaneous doses of morphine, 0.05 to 0.2 mg/kg, codeine, 2 to 8 mg/kg (Figure 1), pentazocine, 0.1 to 0.5 mg/kg (Figure 2), ethylketazocine, 0.006 to 0.1 mg/kg, dextropropoxyphene, 2 to 8 mg/kg (Figure 3), nalorphine, 0.05 to 0.2 mg/kg and buprenorphine, 0.02 to 0.04 mg/kg (Figure 4) and with sublingual doses of morphine, 0.4 to 1.6 mg/kg, codeine, 2 to 4 mg/kg (Figure 1), pentazocine, 1 to 2 mg/kg (Figure 2), Mr 2034, 0.0025 to 0.025 mg/kg and buprenorphine, 0.02 to 0.04 mg/kg (Figure 4).

Nociceptive thresholds were at least 30% above pre-injection values. Occasionally, with the higher doses of some drugs, the thresholds exceeded 40% but these responses were always accompanied by sedation and degrees of motor incoordination. For example, sublingual doses of buprenorphine, 0.04 mg/kg and Mr 2034, 0.025 mg/kg (Figure 4) which caused 50 to 60% increases in threshold values also caused marked sedation, ataxia and respiratory depression. However, ethylketazocine, 0.05 and 0.1 mg/kg subcutaneously, also caused moderate to marked sedation but this was not accompanied by any great increase in nociceptive

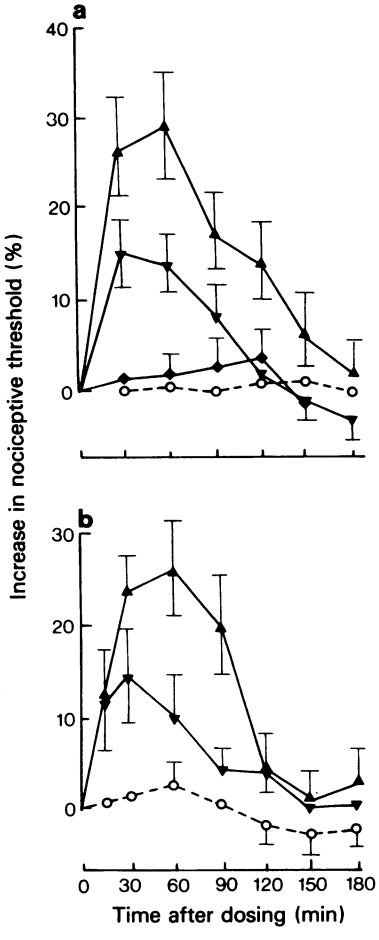

Figure 2 Antinociceptive effects of pentazocine 0.1 (◆, NS), 0.25 (▼) and 0.5 (▲) mg/kg subcutaneously (a) and 1.0 (▼) and 2.0 (▲) mg/kg sublingually (b) against electrical stimulation of the tooth pulp in the conscious dog. Each point is the mean change (%) in nociceptive thresholds determined in 6 dogs by means of a randomised, cross-over designed experiment; Vertical lines show s.e. mean. All doses, with the exception of pentazocine 0.1 mg/kg s.c., produced effects that were significantly different ($P < 0.05$) from placebo (○).

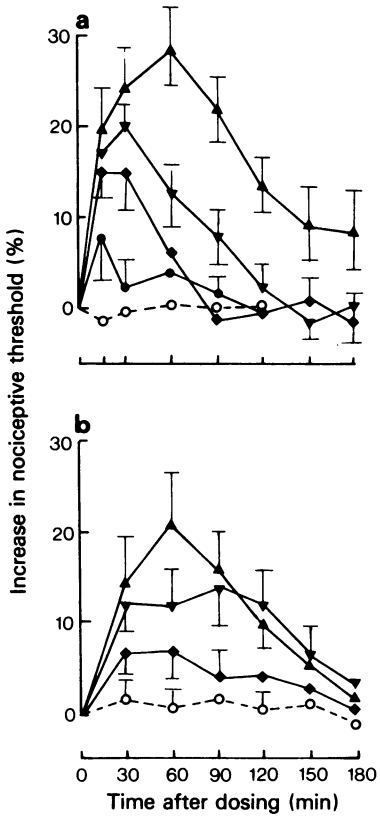

Figure 3 Antinociceptive effects of subcutaneous doses of ethylketazocine 0.006 (●), 0.013 (◆), 0.05 (▼) and 0.1 (▲) mg/kg (a) and dextropropoxyphene, 2.0 (◆ NS), 4.0 (▼) and 8.0 (▲) mg/kg (b) against electrical stimulation of the tooth pulp in the conscious dog. Each point is the mean change (%) in nociceptive thresholds determined in 6 dogs by means of a randomised, cross-over designed experiment; Vertical lines show s.e. mean. All doses, with the exception of D-propoxyphene, 2.0 mg/kg s.c., produced effects that were significantly different ($P < 0.05$) from placebo (○). Ethylketazocine, 0.05 and 0.1 mg/kg s.c. also caused marked sedation.

threshold. Lower doses of these and other drugs tested, increased the nociceptive thresholds without causing any observable changes in behaviour. Drug effects were very rapid in onset by either route of injection: the higher doses of each drug produced significant increases in nociceptive activity 30 to 60 min after injection. The duration of action for most drugs was 2 to 2.5 h but the antinociceptive action of buprenorphine, given by either route, lasted for longer than 3 h. With the exception of buprenorphine, the drugs were more potent subcutaneously than sublingually. Buprenorphine was about 4 times more potent sublingually which may be attributable to its high lipophilicity, a property favourable to more rapid absorption through mucous membranes.

Discussion

The results obtained show that nociceptive responses to electrical stimulation of the tooth pulp in

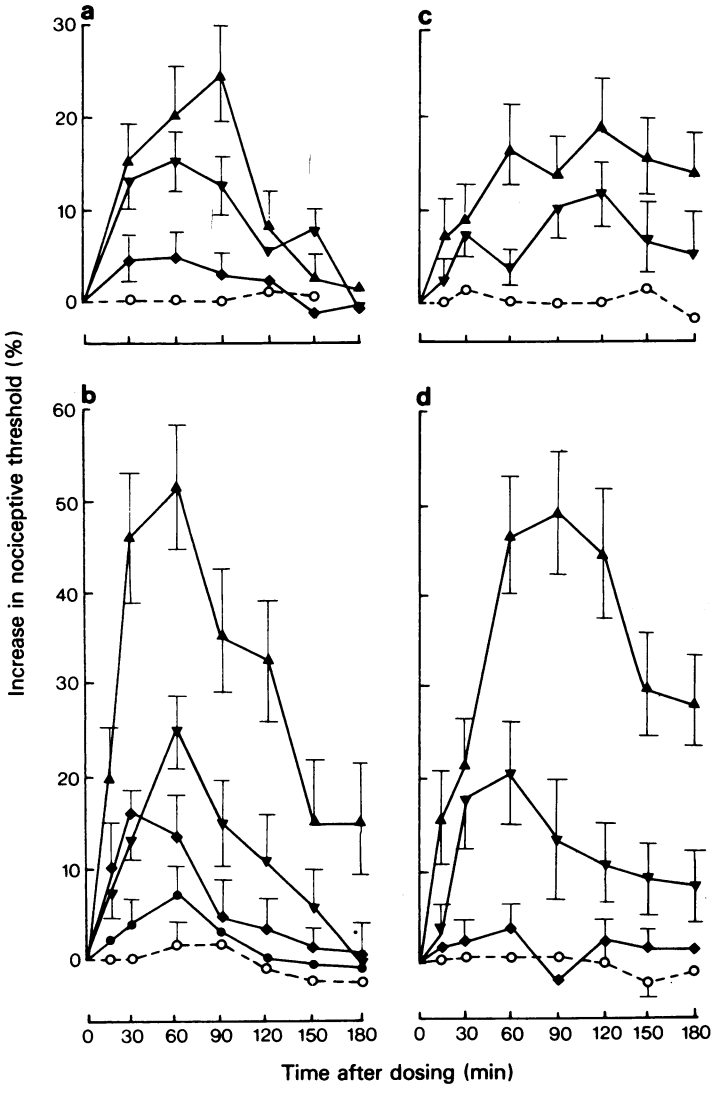

Figure 4 Effects of nalorphine, 0.05 (◆), 0.1 (▼) and 0.2 (▲) mg/kg subcutaneously (a); Mr 2034, 0.0025 (●), 0.005 (◆), 0.01 (▼) and 0.025 (▲) mg/kg sublingually (b); and buprenorphine 0.02 (▼) and 0.04 (▲) mg/kg subcutaneously (c) and 0.01 (◆ NS) and 0.02 (▼) and 0.04 (▲) sublingually (d) against electrical stimulation of the tooth pulp in the conscious dog. Each point is the mean change (%) in nociceptive thresholds determined in 6 dogs using a randomised, cross-over designed experiment; vertical lines show s.e. mean. All doses, with the exception of buprenorphine 0.01 mg/kg s.l., produced effects that were significantly different from placebo (○). Buprenorphine, 0.04 mg/kg s.l. and Mr 2034, 0.025 mg/kg s.l. also produced marked sedation (see text).

the conscious dog are inhibited by both μ- and k-opiate receptor agonists. The potencies of these drugs in the dog are very similar to their antinociceptive potencies against noxious pressure and chemical stimulation in rodents (Tyers, 1980). For the μ-agonists there is also a correlation between the potencies in these tests and against heat noxia. The k-agonists are essentially inactive against noxious heat stimulation.

Tooth pulp afferent fibres are probably exlusively

pain fibres (Anderson, Hannam & Matthews, 1970), they are mostly unmyelinated and, like the peripheral unmyelinated fibres (Hokfelt, Kellerth & Nilsson & Pernow, 1975) they contain considerable amounts of substance P (Olgart, Hokfelt, Nilsson & Pernow, 1977). It has been suggested that substance P is the neurotransmitter at primary afferent terminals associated with nociception both in the dorsal horn (Henry, 1976; Randic & Miletic, 1977), where the peripheral afferent nerves synapse, and in the trigeminal nucleus (Andersen, Lund & Puil, 1978) where afferent nerves from the tooth pulp enter the brain stem. However, some recent work (Hayes & Tyers, 1980) shows that substance P may not be involved in the transmission of heat-induced nociception. These latter experiments showed in the rat that depletion of substance P from primary afferent fibres, using capsaicin, increased only nociceptive chemical and pressure thresholds while nociceptive heat thresholds were unaffected or even slightly reduced. Therefore, the antinociceptive action of k-agonists may be exclusively one of inhibition, either directly or indirectly, of nociceptive input from primary afferents for which substance P is the likely neurotransmitter. The μ-agonists have a similar action via μ-receptors but have an additional action, also via μ-receptors, which inhibits heat-induced nociception.

In conclusion, nociception from electrical stimulation of the tooth pulp in the dog is analogous to nociception resulting from noxious chemical or pressure stimulation of the skin in that substance P is likely to be the neurotransmitter at the primary afferent terminals and nociceptive responses may be inhibited by both μ- and k-agonists.

References

ANDERSEN, R.K., LUND, J.P. & PUIL, E. (1978). Enkephalin and substance P effects related to trigeminal pain. *Can. J. Physiol. Pharmac.*, **56**, 216–222.

ANDERSON, D.J., HANNAM, A.C. & MATTHEWS, B. (1970). Sensory mechanisms in mammalian teeth and their supporting structures. *Physiol. Rev.*, **50**, 171–195.

HAYES, A.G. & TYERS, M.B. (1980). Effects of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. *Brain Res.*, (in press).

HENRY, J.L. (1976). Effects of substance P on functionally identified units in cat spinal cord. *Brain Res.*, **114**, 439–451.

HOKFELT, T., KELLERTH, J.O., NILSSON, G., & PERNOW, B. (1975). Experimental immunohistochemical studies on the localisation and distribution of substance P in cat primary sensory neurones. *Brain Res.*, **100**, 235–252.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E., & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

OLGART, L., HOKFELT, T., NILSSON, G. & PERNOW, B., (1977). Localisation of substance P-like immunoreactivity in nerves in the tooth pulp. *Pain*, **4**, 153–159.

RANDIC, M. & MILETIC, V. (1977). Effect of substance P in cat dorsal horn neurones activated by noxious stimuli. *Brain Res.*, **128**, 164–169.

SKINGLE, M. & TYERS, M.B. (1979). Evaluation of antinociceptive activity using electrical stimulation of the tooth pulp in the conscious dog. *J. Pharmac. Methods*, **2**, 71–80.

TYERS, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmac.*, (in press).

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