British Journal of Pharmacology (1998) 123, 1479–1481
http://www.stockton-press.co.uk/bjp

SPECIAL REPORT

Enhancement of opioid inhibition of GABAergic synaptic transmission by cyclo-oxygenase inhibitors in rat periaqueductal grey neurones

C.W. Vaughan

Department of Pharmacology, The University of Sydney, Sydney, NSW 2006, Australia

Cyclo-oxygenase (COX) inhibitors potentiate opioid inhibition of GABAergic synaptic transmission in rat periaqueductal grey (PAG) (Vaughan *et al*., 1997). In the present study, the relative contribution of cyclo-oxygenase-1 (COX-1) and COX-2 inhibition to this phenomenon was examined by use of whole-cell patch clamp recordings in brain slices. The μ-receptor partial agonist morphine (10 μM) had little effect on GABAergic synaptic transmission. Morphine reduced the frequency of spontaneous miniature inhibitory postsynaptic currents (m.i.p.s.cs) by 13%. The nonselective COX inhibitor, indomethacin, produced a dose-dependent potentiation of the morpine inhibition of m.i.p.s.c. frequency (maximum inhibition 42%, $IC_{50} = 6$ nM). More selective COX-2 inhibitors produced a similar potentiation of the morphine inhibition of m.i.p.s.c. frequency; however, at greater concentrations ($IC_{50} = 57$ nM piroxicam, 1.7 μM DFU). Maintaining slices in the protein synthesis inhibitor cycloheximide (1 μM), to prevent COX-2 induction, had no effect on the potentiation action of DFU (10 μM). These results demonstrate that the potentiation of opioid inhibition of GABAergic synaptic transmission in PAG is largely a result of inhibition of COX-1 activity. These findings suggest that COX-1, rather than COX-2 inhibition, mediates the synergistic analgesic actions of opioids and non-steroidal anti-inflammatory drugs (NSAIDs) in the midbrain PAG.

Keywords: Opioid; cyclo-oxygenase; synaptic transmission; GABA; analgesia; periaqueductal grey; central nervous system

Introduction The midbrain periaqueductal grey (PAG) is rich in opioid receptors and endogenous opioids and is a major site of analgesic actions of opioids in the central nervous system (Mansour *et al*., 1995). Microinjections of cyclo-oxygenase (COX) inhibitors into the PAG produces analgesia (Tortorici & Vanegus, 1995) and these non-steroidal anti-inflammatory drugs (NSAIDs) potentiate the analgesic actions of opioid agonists (Meade *et al*., 1993; Riendeau *et al*., 1997). It has been proposed that opioids produce analgesia within the PAG by inhibiting GABAergic inhibitory influences on neurones which form part of a descending antinociceptive pathway. It has recently been demonstrated that μ-opioid inhibition of GABAergic synaptic transmission within the PAG is mediated by modulation of a presynaptic, dendrotoxin-sensitive potassium conductance coupled via a phospholipase A2/arachidonic acid/12-lipoxygenase pathway (Vaughan *et al*., 1997). Opioid inhibition of GABAergic synaptic transmission is potentiated by COX inhibitors, presumably because more arachidonic acid is available for enzymic conversion to 12-lipoxygenase products.

Two isoforms of COX have been identified, COX-1 which is constitutively expressed and COX-2 an immediate early gene which is induced by inflammatory agents (Meade *et al*., 1993). Both COX-1 and COX-2 enzymes are expressed in a number of brain regions including the PAG (Breder *et al*., 1992; 1995). In the present study, I have examined the relative potencies of selective COX-1 and COX-2 inhibitors on the potentiation of opioid inhibition of GABAergic synaptic transmission in the PAG.

Methods Sprague-Dawley rats, 11–30 days old, were anaesthetized (halothane) and brain slices containing PAG were prepared in ice-cold artificial cerebrospinal fluid (ACSF). Slices were maintained in ACSF (34°C) containing a COX inhibitor, and in some experiments cycloheximide (1 μM), for at least 1 h before transfer to the recording chamber. Whole-cell patch clamp recordings (holding potential -70 mV) were made as described previously (Vaughan *et al*., 1997). Spontaneous miniature inhibitory postsynaptic currents (m.i.p.s.cs) were obtained in the presence of tetrodotoxin (TTX; 0.3 μM and CNQX; 3 μM), filtered at 2 kHz and recorded on video tape (via a SONY PCM501). Miniature p.s.cs were sampled at 5 kHz (Fetchex) for later off-line analysis (Axograph, Axon) as described previously (Vaughan *et al*., 1997).

Stock solutions of all drugs were diluted to working concentrations in ACSF immediately before use and applied by superfusion. Morphine hydrochloride was obtained from Glaxo (U.K.); naloxone hydrochloride, CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH_2) and CNQX (6-cyano-7-nitro-quinoxaline-2,3-dione) from Research Biochemicals Inc. (Natick, MA, U.S.A.); cycloheximide, indomethacin and piroxican from Sigma (St Louis, MO, U.S.A.); tetrodotoxin (TTX) from Alamone (Jerusalem, Israel); DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone) from Merck Frosst (Canada). All data are expressed as means $\pm$ s.e.mean.

Results To determine the effect of morphine on GABAergic synaptic transmission I examined its effect on spontaneous TTX-insensitive miniature i.p.s.cs. The μ-receptor partial agonist, morphine (10 μM), had little effect on either m.i.p.s.c. frequency (13 ± 7% inhibition) or amplitude (-6 ± 3% inhibition, $n = 6$), as shown previously (Vaughan *et al*., 1997). The nonspecific COX inhibitor, indomethacin, potentiated the morphine-induced inhibition of m.i.p.s.c. frequency in a dose-dependent manner, with an IC_{50} of 6 ± 2 nM (Figure 1a, b, e). In the presence of indomethacin (100 nM–1 μM), morphine (10 μM) produced a maximal inhibition of m.i.p.s.c. frequency of 42 ± 4%, without any significant effect on m.i.p.s.c.

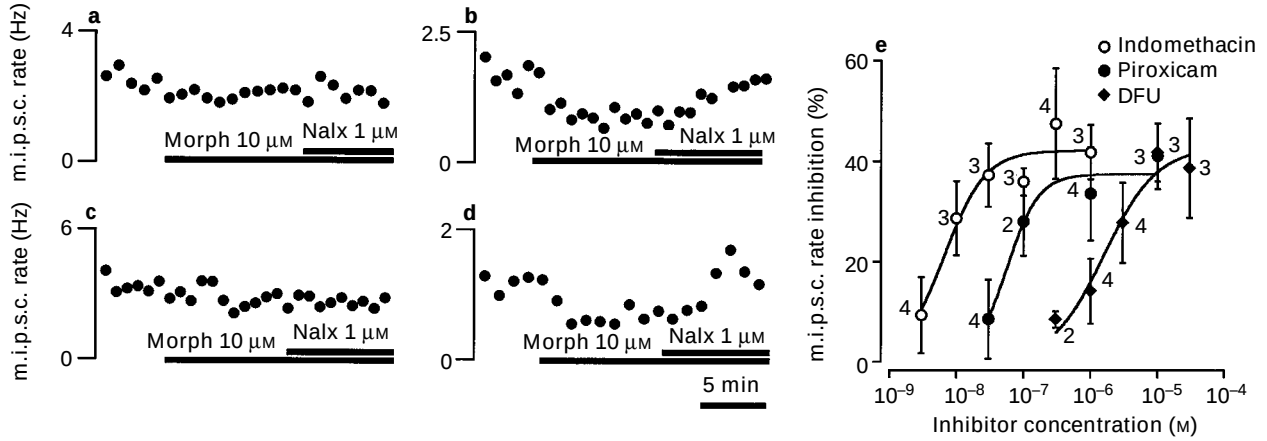

Figure 1 Potentiation of opioid inhibition of GABAergic synaptic transmission by COX-1 and COX-2 blockade. Time course of m.i.p.s.c. rate during superfusion of morphine (10 μM), then morphine plus naloxone (1 μM) in the presence of (a) 3 nM and (b) 30 nM indomethacin, and (c) 300 nM and (d) 10 μM DFU. (e) Concentration-response relationship for percentage m.i.p.s.c. rate inhibition by morphine (10 μM) in the presence of indomethacin, piroxicam and DFU. Each point shows the mean response of 2–4 different neurones; vertical lines indicate s.e.mean. A logistic function was fitted to the curves to determine the IC_{50}.

amplitude (-3 ± 5% inhibition, $n=10$). The morphine-induced inhibition was reversed by the addition of naloxone (1 μM, $n=6$), or CTAP (1 μM, $n=3$). A reduction in m.i.p.s.c. frequency without any effect on m.i.p.s.c. amplitude reflects a reduction in the probability of neurotransmitter release from the presynaptic GABAergic terminals.

To examine the relative contributions of COX-1 and COX-2 inhibition to the potentiation of opioid presynaptic inhibition, I examined the effects of more selective COX-2 inhibitors, piroxicam and DFU (Meade *et al*., 1993; Riendeau *et al*., 1997). Piroxican and DFU potentiated the morphine-induced inhibition of m.i.p.s.c. frequency in a dose-dependent manner (Figure 1c,d,e). Morphine (10 μM) produced a maximal inhibition of m.i.p.s.c. frequency of 37 ± 6% in the presence of piroxicam (1 and 10 μM, $n=7$) and of 40 ± 5% in the presence of DFU (10 and 30 μM, $n=6$). However, the potentiation of morphine-induced inhibition occurred at higher concentrations of these COX inhibitors. The IC_{50} for the potentiation of the morphine inhibition of m.i.p.s.c. frequency was 57 ± 18 nM for piroxicam and 1.7 ± 0.6 μM for DFU (Figure 1e). I then examined whether the effect of the COX-2 selective inhibitor DFU was affected by the protein synthesis inhibitor cycloheximide. The morphine-induced inhibition of m.i.p.s.c. frequency in the presence of DFU (10 μM) was 42 ± 6% in control slices ($n=3$) and was 34 ± 5% in slices maintained in cycloheximide from the time of preparation of tissue (1 μM, $n=5$).

Discussion The present study has demonstrated that inhibition of COX-1, rather than COX-2, enzymic activity potentiates the inhibitory action of opioids on GABAergic synaptic transmission. The lower IC_{50} for DFU potentiation of opioid inhibition of neurotransmission observed in the present study ($IC_{50}=1.7$ μM) might be due a number of factors. While indomethacin, piroxicam and DFU differ greatly in their potency at inhibiting COX-1 (IC_{50}s of 18–20 nM, 163–3,460 nM and 12–450 μM, respectively), they are nearly equipotent at inhibiting COX-2 (IC_{50} 26–51 nM) (Riendeau *et al*., 1997). The relative potency of COX-1 and COX-2 inhibitors varies between different preparations (Meade *et al*., 1993; Riendeau *et al*., 1997), and has not been evaluated in the terminals of GABAergic neurones in the PAG. COX-2 inhibition might have made a contribution to the observed effect because low levels of COX-2 enzyme are constitutively expressed in the brain (Breder *et al*., 1995). Expression of COX-2 is greatly increased by inflammatory mediators, possibly as a result of the procedures used to prepare brain slices. However, the protein synthesis inhibitor cycloheximide had no effect on the potentiation by DFU, suggesting that induction of COX-2 is unlikely to have influenced the potency of DFU.

NSAIDs inhibit cyclo-oxygenase activity, a property that accounts for their shared therapeutic and side effects. The present findings provide a mechanism for the synergistic central actions of opioids and cyclo-oxygenase inhibitors (Maves *et al*., 1994). In particular, COX-1 inhibition is likely to produce the central analgesic effects of NSAIDs. This contrasts with previous observations that inhibition of constitutively expressed COX-1 is associated with the toxicity of NSAIDs and inhibition of induced COX-2 is associated with the anti-inflammatory effects of NSAIDs in peripheral tissues (Meade *et al*., 1993).

This work was supported by the NH&MRC of Australia. DFU was supplied by Merck Frosst Canada. The author thanks Prof. M. Christie for his invaluable discussions.

References

BREDER, C.D., DEWITT, D. & KRAIG, R.P. (1995). Characterization of inducible cyclooxygenase in rat brain. *J. Comp. Neurol.,* **355,** 296–315.

BREDER, C.D., SMITH, W.L., RAZ, A., MASFERRER, J., SEIBERT, K., NEEDLEMAN, P. & SAPER, C.B. (1992). Distribution and characterization of cyclooxygenase immunoreactivity in the ovine brain. *J. Comp. Neurol.,* **322,** 409–438.

MANSOUR, A., FOX, C.A., AKIL, H. & WATSON, S.J. (1995). Opioid-receptor mRNA expression in the rat CNS– anatomical and functional implications. *Trends Neurosci.,* **18,** 22–29.

MAVES, T.J., PECHMAN, P.S., MELLER, S.T. & GEBHART, G.F. (1994). Ketorolac potentiates morphine antinociception during visceral nociception in the rat. *Anesthesiology,* **80,** 1094–1101.

MEADE, E.A., SMITH, W.L. & DEWITT, D.L. (1993). Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.,* **268,** 6610–6614.

RIENDEAU, D., PERCIVAL, M.D., BOYCE, S., BRIDEAU, C., CHARLESON, S., CROMLISH, W., ETHIER, D., EVANS, J., FALGUEYRET, J.P., FORD-HUTCHINSON, A.W., GORDON, R., GREIG, G., GRESSER, M., GUAY, J., KARGMAN, S., LEGER, S., MANCINI, J.A., O'NEILL, G., OUELLET, M., RODGER, I.W., THERIEN, M., WANG, Z., WEBB, J.K., WONG, E., XU, L., YOUNG, R.N., ZAMBONI, R., PRASIT, P. & CHAN, C.C. (1997). Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *Br. J. Pharmacol.,* **121,** 105–117.

TORTORICI, V. & VANEGUS, H. (1995). Anti-nociception induced by systemic or PAG-microinjected lysine-acetylsalicylate in rats. Effects on tail-flick related activity of medullary off- and on-cells. *Eur. J. Neurosci.,* **7,** 1857–1865.

VAUGHAN, C.W., INGRAM, S.L., CONNOR, M.A. & CHRISTIE, M.J. (1997). How opioids inhibit GABAergic neurotransmission. *Nature,* **390,** 611–614.

(Received January 7, 1998
Accepted February 9, 1998)