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Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor

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Abstract

Cyclooxygenase (COX), which is present in two isoforms (COX1 and 2), synthesizes prostaglandins from arachidonic acid; it plays a crucial role in inflammation in both central and peripheral tissues. Here, we describe its role in synaptic plasticity and spatial learning *in vivo* via an effect on brain-derived neurotrophic factor (BDNF) and prostaglandin E2 (PGE2; both measured by Elisa). We found that broad-spectrum COX inhibition (BSCI) inhibits the induction of long-term potentiation (LTP; the major contemporary model of synaptic plasticity), and causes substantial and sustained deficits in spatial learning in the watermaze. Increases in BDNF and PGE2 following spatial learning and LTP were also blocked. Importantly, 4 days of prior exercise in a running wheel increased endogenous BDNF levels sufficiently to reverse the BSCI of LTP and spatial learning, and restored a parallel increase in LTP and learning-related BDNF and PGE2. In control experiments, we found that BSCI had no effect on baseline synaptic transmission or on the nonhippocampal visible-platform task; there was no evidence of gastric ulceration from BSCI. COX2 is inhibited by glucorticoids; there was no difference in blood corticosterone levels as measured by radioimmunoassay in any condition. Thus, COX plays a previously undescribed, permissive role in synaptic plasticity and spatial learning via a BDNF-associated mechanism.

Introduction

Cyclooxygenase (COX), which synthesizes prostaglandins (PGs) from arachidonic acid (AA; Smith *et al*., 2000), has been investigated largely in relation to central and peripheral tissue inflammation. COX is widely distributed in CNS neurons (especially hippocampus, amygdala, periventricular regions and brainstem; Yamagata *et al*., 1993; Breder *et al*., 1995; Kaufmann *et al*., 1996). The COX1 isoform is expressed in most tissues and increases 2–4-fold under appropriate stimulation (Samad *et al*., 2001). COX2 shares 60% identity with COX1 (O'Banion *et al*., 1991) and is undetectable in most non-neuronal tissues under basal conditions; cytokines can increase its expression 10–80-fold (Masferrer *et al*., 1994). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit both isoforms, preventing PG production (DeWitt, 1999); glucocorticoids also inhibit COX2.

Few studies have investigated the role of COX in synaptic plasticity. COX2 and PGE2 levels increase after NMDA-receptor activation through high-frequency stimulation (Yamagata *et al*., 1993; Lazarewicz & Salinska, 1995). MK-801 blocks NMDA-receptor-dependent long-term potentiation (LTP; a model of synaptic plasticity and of the putative biological processes underlying memory) and COX2 mRNA increases, suggesting NMDA-receptor activation regulates COX2. The slow, time-dependent, reversible COX inhibitor indomethacin (Dannhardt & Kiefer, 2001) does not, however, block the induction of LTP (Williams & Bliss, 1989; Yamagata *et al*., 1993); it is possible that the particular pharmacological profile of indomethacin accounts for this lack of an inhibitory effect on LTP. To date, there has been no systematic evaluation of the effects of the many other COX-inhibiting drugs on synaptic plasticity. Apart from these studies, there appear to be few data available on the effects of COX inhibition or activation on synaptic plasticity or learning; the NMDA-receptor-related regulation of COX activity is suggestive of a role for COX in synaptic plasticity (see also Miettinen *et al*., 1997; who have shown that spreading depression, another form of neuroplasticity, also induces COX-2 activation in cortical neurons). Ischemia activates COX (Miettinen *et al*., 1997); ischemic damage is attenuated in COX2-knockout mice (Iadecola *et al*., 2001). During Alzheimer's disease (AD), hippocampal COX2 expression increases in proportion to the disease's severity (Ho *et al*., 2001); chronic NSAID treatment appears to reduce AD risk (Halliday *et al*., 2000), perhaps by inhibiting COX2 cell-death-associated overexpression (Kadoyama *et al*., 2001). Kainic acid-induced seizures stimulate COX2 mRNA as well as brain-derived neurotrophic factor (BDNF) mRNA production in hippocampal neurons (Lazarewicz & Salinska, 1995; Hashimoto *et al*., 1998), suggesting BDNF synthesis occurs in response to COX2-induced neuronal injury. COX and BDNF may therefore interact directly or indirectly during the intracellular cascades associated with synaptic plasticity. BDNF plays a vital role in neuronal survival, growth (Lindsay *et al*., 1994) and synaptic plasticity (Thoenen, 1995; Schinder & Poo, 2000). Hippocampal BDNF expression increases during spatial learning (Gooney *et al*., 2002; Kesslak *et al.,* 1998; Mizuno *et al*., 2000); BDNF-knockout mice show deficits in spatial learning (Linnarsson *et al*., 1997) and synaptic plasticity (Korte *et al*., 1995). Sustained physical

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activity (such as daily running in a running wheel) increases endogenous BDNF levels in dentate gyrus (DG) and hippocampus (Neeper *et al*., 1995, 1996; Oliff *et al*., 1998; Russo-Neustadt *et al*., 1999) and decreases experimental stroke-induced neuronal loss (Radak *et al*., 2001). Exogenous BDNF protects the neonatal brain from ischemic injury *in vivo* (Han & Holtzman, 2000).

We investigate here the consequences of broad-spectrum COX inhibition (BSCI) on synaptic plasticity in the hippocampal formation and on a task which engages the hippocampal formation: spatial learning in the water maze, using the rapid and competitive COX 1 and 2 inhibitor ibuprofen (Dannhardt & Kiefer, 2001). We predict that BSCI does not affect synaptic transmission but does impair synaptic plasticity and processes which hypothetically (Martin *et al*., 2000) depend on synaptic plasticity, namely memory. We suggest that these deficits will appear as a consequence of the inhibition of the molecular events that occur downstream of NMDA and TrkB receptor activation, given the centrality of these receptors in synaptic plasticity, memory and a variety of pathophysiological processes (e.g. Bliss & Collingridge, 1993; Bi & Poo, 2001). Furthermore, given the tentative evidence for a reciprocal or regulatory interaction between COX and BDNF, manipulations of endogenous BDNF via exercise should rescue deficits in synaptic plasticity and LTP induced by BSCI (these data have been previously published in abstract form: O'Mara *et al*., 2001b, 2002).

Materials and methods

Adult male Wistar rats (BioResources Unit, University of Dublin; weight 200–300 g), pair-housed in a temperature-controlled laminar airflow cupboard, on a 12-h light : 12-h dark cycle, were used. All local, national and EU guidelines on the care and welfare of animals were obeyed. All electrophysiological experiments were conducted *in vivo* under urethane anaesthesia (1.5 g/kg i.p.; Commins & O'Mara, 2000). Stimulating electrodes (8.1 mm posterior to bregma and 4.4 mm lateral to the midline) were aimed at the perforant path, and recording electrodes (3.9 mm posterior and 2.5 mm lateral) at DG (Paxinos & Watson, 1997). Signals were filtered (0.1 Hz−1 kHz) and amplified (×100; DAM-50 differential amplifier, WPI, UK). Recordings were digitized online using a PC connected to a CED−1401 interface (CED, UK). Electrodes were slowly lowered to a depth of 2.5 mm; test stimuli were administered during electrode movement at 0.05 Hz. The final depths were adjusted until maximal field excitatory postsynaptic potentials (fEPSPs) were obtained; electrodes were allowed to settle for 10 min before recordings were conducted at half-maximum fEPSPs (using individually determined input–output curves). Field EPSPs are expressed as percent baseline slope (mean ± SEM). In all cases, rats were injected i.p. with either 30 mg/kg of ibuprofen, a potent inhibitor of COX 1 and 2 [Dannhardt & Kiefer, 2001; Weggen *et al*., 2001; ibuprofen (IBU) sodium salt: alpha-methyl-4-(isobutyl)phenylacetic acid; Sigma, USA; dissolved according to manufacturer's instructions], or saline (0.9% NaCl) 1 h prior to behavioural or electrophysiological procedures. The dose of ibuprofen is 50% of that which is effective in reducing the neuropathology associated with β-amyloid expression in a transgenic mouse model of AD (60 mg/kg; Lim *et al*., 2000). Preliminary testing in our laboratory with differing doses indicated that there is a clear dose–response effect of the dose used (doses of IBU of up to 110 mg/kg; data not shown). The dose used was not associated with any morbidity and was chosen to allow easy comparison between the electrophysiological and behavioural experiments. We have also exhaustively examined the fEPSPs recorded after IBU treatment and have not observed any change in the incidence of afterdischarges or other anomalous events relative to control (untreated) conditions (such events occurred on $\ll 0.1\%$ of recordings); the figure insets from our recordings showing a smooth decay of fEPSPs are typical of our recordings. Other data from our lab (O. Gobbo and S. O'Mara, unpublished observations) show that the putative COX2-selective antagonist celecoxib (Celebrex™; Searle-Monsanto) does not affect spatial learning in the watermaze ($n = 7$; 6 mg/kg, i.p.). There are no COX1-selective inhibitors available at this time.

LTP

LTP was induced by biologically submaximal high-frequency stimulation (HFS) consisting of 10 trains of 20 stimuli at 200 Hz, intertrain interval of 2 s (Commins *et al*., 1998), after which baseline stimulation was resumed and fEPSPs recorded for 30 min. The stimulation intensity was not changed during HFS. In our experience using this stimulation protocol at differing synapses (for example, the anterior thalamic nucleus–prefrontal projection, Gemmell & O'Mara, 2002; the ipsi- to contralateral prefrontal projection, Gemmell & O'Mara, 2000; the CA1-subicular projection, O'Mara *et al*., 2001a, b) and here at the perforant path (PP)–DG synapse, we typically do not induce post-tetanic potentiation and have never observed transient seizure activity, in contrast to this possibility after stronger forms of stimulation.

Exercise protocol

Rats ran for 4 consecutive nights in a running wheel prior to experimental manipulation. Distance and time spent running were recorded and rats had to run at least 100 meters each night to be included in the experiment.

Watermaze training and the visible-platform task

All watermaze experiments were conducted using an automated computerized digital tracking system (EthoVision, Noldus, Netherlands), using standardized protocols (Commins *et al*., 1999; Shaw *et al*., 2001). The water maze was a black circular pool (2 m diameter, 35 cm deep; water, 20 ± 1 °C) filled to 31 cm. Rats could escape the water by climbing on to a hidden platform (29 × 9 cm). The hidden platform was placed in the north-western quadrant of the pool and submerged 2 cm below the water surface so it was invisible at water level; the location of the platform was fixed during the experiment. Distal cues were standard room objects (e.g. doors, shelving and curtains). Rats received five trials per day for 5 days, in which they had to search for the hidden platform. The rat was allowed 60 s to find the platform; otherwise, the rat was led to the platform by the researcher. The rat remained on the platform for 15 s and the intertrial interval was 5 s. All rats entered the maze at a fixed start position. We have found (O. Gobbo and S. O'Mara, unpublished observations) that animals with histologically verified hippocampal area CA3 and CA1 lesions induced by the excitotoxin kainic acid (≈ 50% neuronal loss relative to controls) do not learn the watermaze under these training conditions, but do acquire the visible-platform task (see below), suggesting that these animals do not suffer from visuomotor deficits. The visible-platform task was run in an identical fashion to the standard watermaze, except that the water level was lowered to 2.5 cm below the level of the platform and a beacon was attached to the platform to aid its visibility.

Measurement of BDNF, PGE2 and blood corticosterone

After each experiment, the animals were stunned, immediately decapitated quickly and the brain removed. Thus, the brains were removed ≈45 min after HFS in the *in vivo* anaesthetized neurophysiological experiments and, after 5 days of training, within 1 h of the last training session on the final training day. The hippocampus was dissected out bilaterally according to standard methods (Gooney *et al*., 2002) and the

DG was isolated, chopped and stored (−70 °C) pending subsequent biochemical analysis. Samples were divided in advance as necessary; concentrations of BDNF and PGE2 were determined using Elisa in duplicate or triplicate in homogenized DG. All like conditions were pooled for biochemical analysis.

BDNF enzyme-linked immunosorbent assay protocol

The BDNF Emax™ ImmunoAssay System; Promega UK Ltd was used. Slices of DG were preincubated in 250 μL Krebs solution containing 2 mM $CaCl_2$ for 3 min, and the supernatant was removed and discarded. This step was repeated in a volume of 100 μL and at the end of the 3 min incubation samples were centrifuged at 1000 ***g*** for 3 min and the supernatant was retained. This step was repeated in the presence of 40 mM KCl to depolarize the slices and supernatant was retained. Both samples of supernatant were stored at −70 °C for later analysis of BDNF by two-site immunoassay. BDNF concentration was determined in the supernatant. Dentate gyri were homogenized in ice-cold Krebs solution 25 times, centrifuged and the supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at −80 °C. Ninety-six-well plates were coated with 100 μL anti-BDNF monoclonal antibody diluted (1 : 1000) in 0.025 M carbonate–bicarbonate buffer. Plates were covered, incubated overnight at 4 °C and subjected to interceding washes to remove excess antibody. Plates were blocked for nonspecific binding for 1 h at room temperature and washed (composition of wash buffer, in mM: Tris–HCl, 20; NaCl, 150 containing 0.05% Tween (v/v); pH 7.6). Samples of dentate gyrus (50 μL), supernatant (50 μL) or BDNF standards (50 μL; ranging from 0.0078 to 1 ng/mL) were added to the wells, which were then covered, incubated for 2 h at room temperature with shaking and then incubated overnight at 4 °C, and washed. Aliquots (100 μL) of antihuman BDNF pAb (diluted 1 : 500) were added to the wells, plates were incubated for 2 h at 37 °C and washed. Aliquots (100 μL) of anti-immunoglobulin Y horseradish peroxidase (1 : 2000 dilution) were added to wells and incubated for 1 h at 37 °C. During this incubation, the enzyme substrate was prepared. Plates were washed and 100 μL of this substrate was added to the wells and incubated for ≈15 min until a blue colour formed in the wells. The reaction was stopped by the addition of 100 μL of 1 M phosphoric acid to the wells. Plates were read at 450 nm in a 96-well plate reader, and BDNF concentrations were estimated for the standard curve (expressed as ng/mg protein).

PGE2 high-sensitivity enzyme immunoassay kit protocol

The Assay Designs Correlate-EIA PGE2 kit, 93001; Metachem, Nottingham, UK was used. PGE2 expression was used as the principal assay of COX activity, given the known difficulties of measuring COX directly in brain. Slices of dentate gyrus were preincubated in 250 μL Krebs solution containing 2 mM $CaCl_2$ for 3 min and the supernatant was removed and discarded. This step was repeated in a volume of 100 μL and at the end of the 3-min incubation samples were centrifuged at 1000 ***g*** for 3 min and the supernatant was retained. This step was repeated in the presence of 40 mM KCl to depolarize the slices and supernatant was retained. Both samples of supernatant were stored at −80 °C for later analysis of PGE2 by immunoassay. PGE2 concentration was determined in the supernatant. To prepare the samples, dentate gyri were homogenized in ice-cold Krebs 25 times. Samples were centrifuged and supernatant retained. Protein was assessed and samples were diluted to give equal protein concentrations and stored at −80 °C. Samples of supernatant (100 μL) or PGE2 standards (100 μL; ranging from 7.81 pg/mL to 1 ng/mL) were added to the 96-well plates. The plates were precoated with goat antibody specific to mouse IgG. Fifty microlitres of PGE2 HS-EIA conjugate was then added to each well, followed by 50 μL of PGE2 HS-EIA antibody; the wells were covered, incubated overnight at 4 °C and washed. Two hundred microlitres of p-Npp Substrate solution was then added to every well and the plates were incubated for 1 h at 37 °C. The reaction was stopped by the addition of 50 μL trisodium phosphate to the wells. Plates were read at 405 nm in a 96-well plate reader and PGE2 concentrations were estimated for the standard curve and expressed as pg/mg protein.

Corticosterone levels

These were measured by radioimmunoassay in trunk blood, which was collected on decapitation and rapidly frozen at −20 °C and stored for subsequent analysis (DPC, USA; Shaw *et al.*, 2001). With this procedure serum samples are assayed directly, without preparation. Samples were thawed to room temperature and 50 μL of either serum or calibrator (ranging from 0 to 2000 ng/mL corticosterone) was added to the rat corticosterone AB-coated test tubes. These tubes were precoated with antibodies to rat corticosterone. ^{125}I rat corticosterone (100 mL) was added to every tube and incubated for 2 h at room temperature. The tubes were then decanted and counted for 1 min in a gamma counter. Concentrations were estimated from the calibrator standard curve and expressed as ng/mL of corticosterone.

Statistics

All data were analysed using one-way or two-way ANOVAs or *t*-tests, as appropriate; a value of $P < 0.05$ was considered to be significant. *Post hoc* comparisons were made using Tukey's method.

Results

Broad-spectrum COX inhibition (BSCI) did not affect baseline synaptic transmission

We first examined the effect of BSCI, using i.p.-administered IBU (see Materials and methods), on baseline synaptic transmission in the DG of anaesthetized rats. We found that there were no significant differences (Fig. 1a and b) in mean fEPSPs (99.6 ± 2.8, 95.7 ± 4.6 and 103.3 ± 6% at 10, 30 and 60 min) recorded in saline-treated ($n = 7$) and IBU-treated animals ($n = 9$; 101.7 ± 4.9, 99.01 ± 7.1 and 104.6 ± 6.7% at 10, 30 and 60 min). BSCI therefore did not affect baseline synaptic transmission. In other experiments (data not shown) we found there were no differences in baseline synaptic transmission in animals treated with IBU after anaesthesia. We also found that BSCI did not result in significant differences in the expression of BDNF or PGE2 (our index of COX activity; see Materials and methods) concentrations in DG, as measured by Elisa ($P > 0.05$ in all cases; Fig. 1c). Thus, BSCI did not affect baseline synaptic transmission or associated BDNF or PGE2 protein expression.

Broad-spectrum COX inhibition inhibited the induction of long-term potentiation

We induced LTP in anaesthetized saline-treated animals ($n = 18$) using high-frequency stimulation (HFS); mean fEPSPs post-HFS were 136.3 ± 8.5, 145.3 ± 8.7 and 152.12 ± 8.8% of pre-HFS responses at 5, 15 and 30 min, respectively ($P < 0.001$; Fig. 2a and b), indicating that LTP was successfully induced. In contrast, LTP was substantially blocked in IBU-treated animals ($n = 20$): mean fEPSPs at 5, 15 and 30 min post-HFS were 110.7 ± 6.4, 114.5 ± 7.5 and 116 ± 9.2%, respectively, of pre-HFS responses (Fig. 2a; a significant increase, $P < 0.001$, compared to the pre-HFS period). Mean fEPSP responses were significantly higher ($P < 0.001$) in saline-treated controls than in IBU-treated animals. Furthermore, concentrations of BDNF ($P < 0.001$) and PGE2 ($P < 0.05$) in DG were significantly higher in saline-treated controls than in IBU-treated animals (Fig. 2c). In other

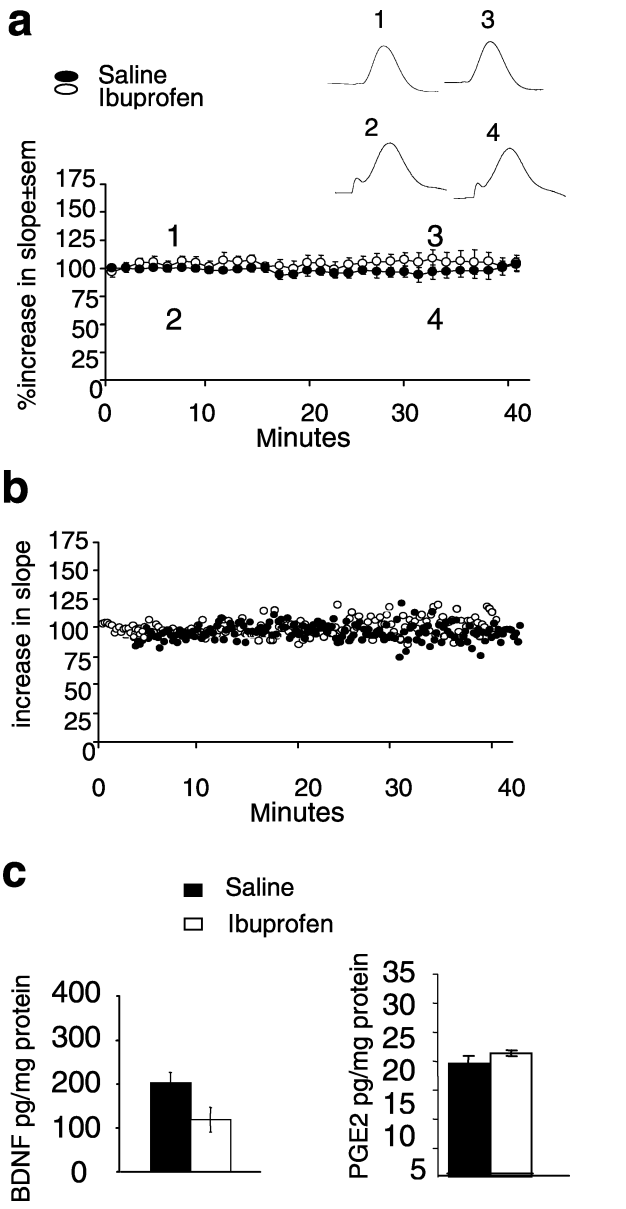

FIG. 1. BSCI did not affect baseline synaptic transmission or BDNF and PGE2 concentrations in DG. (a) fEPSPs recorded for 40 min *in vivo* in saline-treated and IBU-treated animals; insets are representative nonaveraged single-experiment recorded fEPSPs waveforms; (b) a representative nonaveraged single experiment of recorded fEPSPs; (c) protein concentrations of BDNF and PGE2 in DG (as measured by Elisa; see Materials and methods).

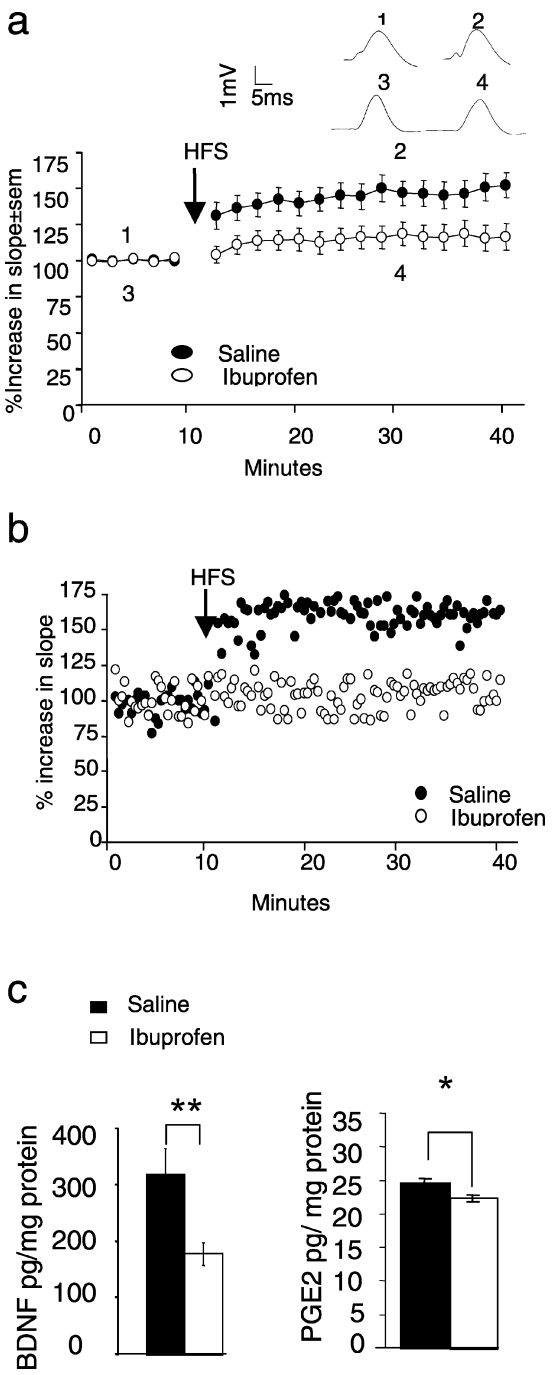

FIG. 2. BSCI blocked LTP and associated increases of BDNF and PGE2 concentrations in DG. (a) LTP induced by HFS in saline-treated and blocked by HFS in IBU-treated animals; insets are representative nonaveraged single-experiment recorded fEPSPs waveforms; (b) representative nonaveraged single experiment of recorded fEPSPs; (c) protein concentrations of BDNF and PGE2. $^{*}P < 0.05$, $^{**}P < 0.001$.

experiments (data not shown) we have found that this inhibition of LTP varies in a dose-dependent fashion, with higher doses of IBU (up to 110 mg/kg) causing an even greater inhibition of LTP (see Materials and methods). Thus, BSCI blocked the induction of LTP and there was less BDNF and PGE2 after the ibuprofen treatment than with LTP treatment alone.

Broad-spectrum COX inhibition caused deficits in spatial learning in the water maze

Models of the LTP–memory relationship (Martin *et al*., 2000) predict that treatments disrupting LTP should also disrupt learning. We found that IBU-treated animals ($n = 6$) were significantly slower in learning the watermaze (ANOVAs, $P < 0.01$; days 2–5) than were the saline-treated animals ($n = 6$; Fig. 3a and b). Furthermore, we found BDNF ($P < 0.001$) and PGE2 ($P < 0.01$) concentrations were significantly higher in the DG of the saline-treated group than the IBU-treated group (Fig. 3c), indicating that both BDNF and PGE2 were increased after learning, and paralleling the effect of BSCI on the induction of LTP. In other experiments (data not shown) we have found that the deficit in learning is enhanced by daily doses of IBU.

In order to verify that the drug-induced behavioural deficits observed did not result from either motor problems or deficits in

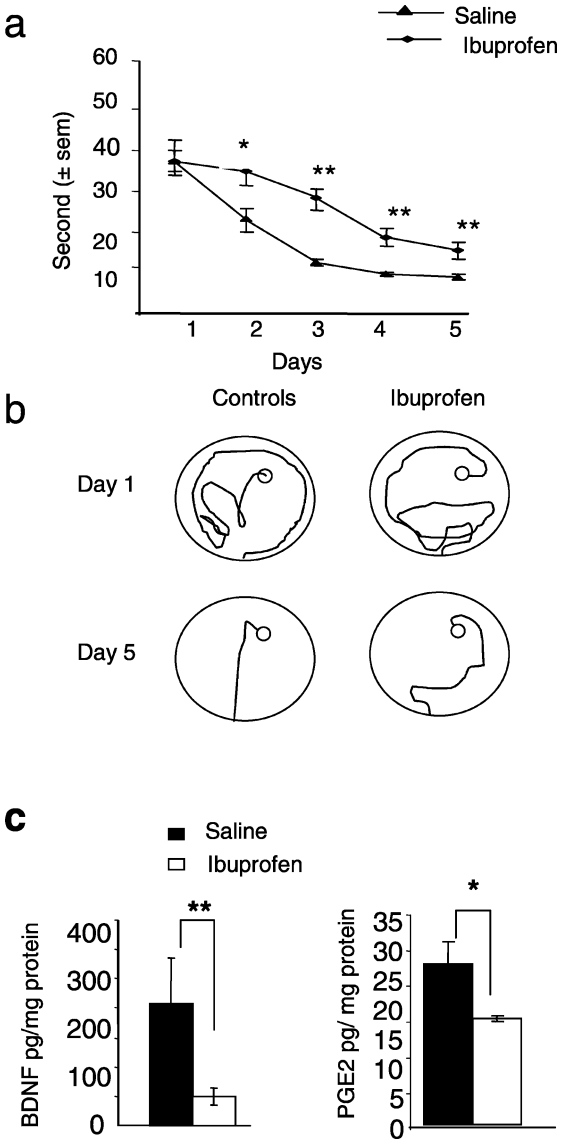

FIG. 3. BSCI caused deficits in spatial learning in the watermaze, as well as in associated BDNF and PGE2 increases in DG. (a) Escape latencies of saline-treated and IBU-treated animals; (b) representative swim-paths recorded at time-points indicated; (c) protein concentrations of BDNF and PGE2. $^{*}P < 0.05$, $^{**}P < 0.001$.

visual discrimination, we examined, in a separate series of control experiments, the performance of IBU- and saline-treated groups (each $n = 5$) in the visible-platform task version of the watermaze. This is a nonhippocampal-dependent task requiring an intact caudate–putamen (Packard & McGaugh, 1992), and is used commonly as a control for visuomotor deficits in the watermaze (Stewart & Morris, 1992; Hölscher & O'Mara, 1997). We found there were no escape latency or velocity differences between the saline-treated and IBU-treated groups on the visible-platform task (data not shown; ANOVAs, $P > 0.05$, days 1–5), indicating that deficits in learning were unlikely to be a consequence of either motor problems or deficits in visual discrimination.

Increasing endogenous BDNF prevented a broad-spectrum COX-inhibition-induced deficit in long-term potentiation

We first examined whether exercise in a running wheel increased endogenous hippocampal BDNF levels. We found a significant increase in BDNF in animals ($n = 5$) who ran 100 m/day for 4 days in exercise wheels, compared to the sedentary group ($n = 5$) who remained in their home cage ($P < 0.05$; Fig. 5a); this exercise regime therefore increased levels of endogenous BDNF. There was no exercise-induced increase in PGE2 ($P > 0.05$; Fig. 5b). We also found that hippocampal BDNF was significantly increased in groups of animals who ran for 100 m/day for days but who were treated with IBU 1 h prior to being killed ($n = 5$), indicating that IBU had no direct effect on already synthesized BDNF protein (at least for 1 h *in vivo*).

In separate experiments, we found that the deficit induced in LTP by BSCI was reversed by periods of exercise that were sufficiently sustained to increase endogenous BDNF (Fig. 4a and b: in exercise–IBU animals ($n = 15$, mean fEPSPs at 5, 15 and 30 min post-HFS were 119.6 ± 8.9, 128.2 ± 6.6 and $132.4 \pm 8.4\%$, respectively, of pre-HFS responses ($P < 0.001$), indicative of a slowly developing potentiation which takes >15 min to peak. LTP was also induced in exercise–saline animals ($n = 10$): mean fEPSPs at 5, 15 and 30 min post-HFS were 133.7 ± 9.3, 149.8 ± 13.2 and $143.0 \pm 10.8\%$, respectively, of pre-HFS responses ($P < 0.001$). There were no significant differences in BDNF or PGE2 concentrations ($P > 0.05$) in the DG between the two groups (Fig. 4c), indicating that the increase in both BDNF and PGE2 expected after the induction of LTP via HFS had occurred. Mean fEPSPs at 20–30 min post-HFS of the IBU-only treated group ($116.5 \pm 0.5\%$; Fig. 2a) were significantly lower (ANOVA, $P < 0.01$) than the IBU–exercise ($132.46 \pm 0.48\%$), saline–exercise ($145.75 \pm 1.1\%$) and saline-only treated ($148 \pm 1.4\%$) groups (Fig. 3a).

Increasing endogenous BDNF prevented a broad-spectrum COX-inhibition-induced learning deficit

After 4 days exercise prior to training in the watermaze, the exercise–saline and exercise–IBU groups acquired the task at the same rate (Fig. 5b and c; $P > 0.05$) and both groups were significantly faster than the IBU-only treated group (days 1–5: $P < 0.001$, $P < 0.05$, $P < 0.001$, $P < 0.001$, $P < 0.01$), suggesting that there were no performance differences between the former two groups. Furthermore, BDNF and PGE2 levels were the same in the exercise–saline and exercise–IBU groups ($P > 0.05$; Fig. 5d).

We found that there were no significant differences between saline- and ibuprofen-treated groups in gastrointestinal lesions (assayed by visual inspection after gastrointestinal dissection) in any condition tested, suggesting that the doses of IBU used did not cause gastric distress; in fact, there were no ulcers present in any animal examined. Corticosterone levels were measured by radioimmunoassay in the trunk blood of all animals in all conditions after killing at the completion of each experiment; corticosterone levels did not differ between animals in any condition, suggesting that there were no unintended effects of glucorticoids on COX2 regulation (data not shown).

Discussion

We examined the effects of broad-spectrum cyclooxygenase inhibition (BSCI) on the induction of LTP in the dentate gyrus *in vivo*, as well as on a task which engages the hippocampal formation, namely spatial learning in the water maze, using the rapid and competitive COX 1 and 2 inhibitor ibuprofen. We found that BSCI blocked the induction of LTP and caused deficits in spatial learning; we also found that the ibuprofen treatment resulted in smaller amounts of BDNF and PGE2 than those that occur after spatial learning and LTP alone. Furthermore, a period of prior exercise reversed the BSCI-induced block of LTP and of spatial learning, most probably via an increase in pre-existing endogenous BDNF levels. We therefore have provided the first

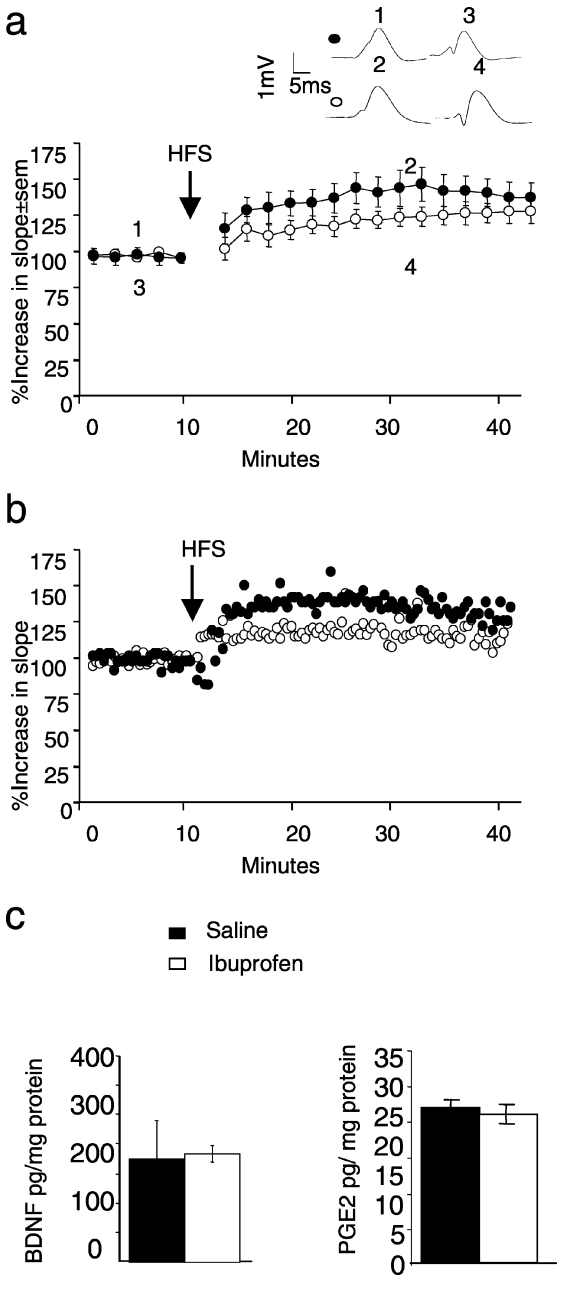

FIG. 4. Prior exercise rescued the LTP and associated BDNF and PGE2 increases in DG caused by BSCI. (a) LTP induced by HFS in exercise–saline and exercise–IBU animals; insets are representative nonaveraged single-experiment recorded fEPSPs waveforms; (b) representative nonaveraged single experiment of recorded fEPSPs; (c) protein concentrations of BDNF and PGE2.

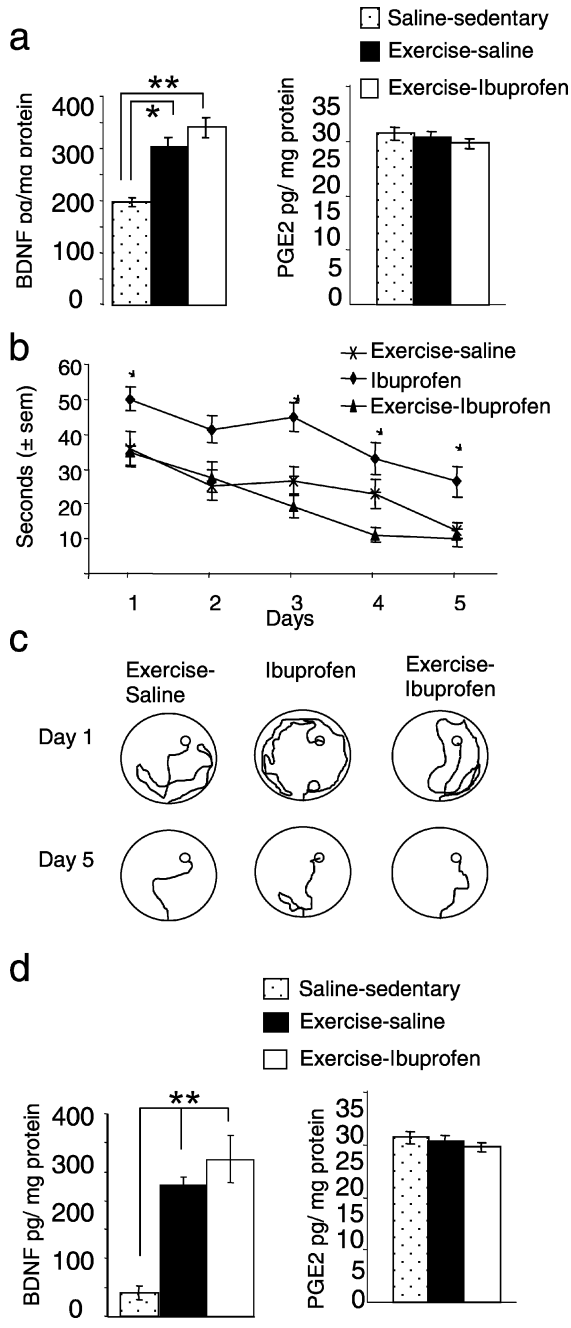

FIG. 5. Prior exercise rescues deficits in spatial learning, BDNF and PGE2 concentration in DG caused by BSCI. (a) Protein concentrations of BDNF and PGE2 in animals who have spent 4 days exercising in a running wheel; (b) escape latencies of saline-treated and IBU-treated animals; (c) representative swim paths recorded at time points indicated; and (d) protein concentrations of BDNF and PGE2 in exercise–saline, exercise–IBU and IBU-only animals. $^{*}P < 0.05$; $^{**}P < 0.01$.

evidence that COX is involved in BDNF expression, LTP and spatial learning; we have provided the first evidence that exercise, which increases levels of endogenous BDNF, also reverses ibuprofen-induced deficit in LTP and spatial learning. We have shown for the first time that PGE2 plays an important regulatory role in synaptic plasticity and learning. We have also confirmed here our previous data (Gooney *et al*., 2002) showing that both spatial learning and LTP are associated with an increase in BDNF. In control experiments, we found that BSCI did not affect baseline synaptic transmission, blood corticosterone levels or performance of the nonhippocampal-dependent visible-platform task, suggesting these effects are not nonspecific pharmacological effects. These data suggest that broad-spectrum cyclooxygenase inhibition reversibly inhibits LTP and spatial learning via a BDNF-associated mechanism. Furthermore, these data also suggest that there is yet another major and unexplored molecular mechanism regulating synaptic plasticity and the biological bases of memory.

Several caveats must be entered regarding the interpretation of the data presented here: it is generally hypothesized that the cellular mechanisms underlying synaptic plasticity and those underlying memory are very likely to be similar (Martin *et al*., 2000), but they will not necessarily be exactly the same (Bliss & Collingridge, 1993;

Martin *et al*., 2000; Gooney *et al*., 2002). The parameters we have measured before and after BSCI and its interaction with LTP induction and spatial learning generally parallel each other and thus these processes probably rely on the regulation or modulation of a similar set of cellular mechanisms. However, it is clear that the time courses of the biological changes in the behaving animal in the watermaze and in the anaesthetized animals in which neurophysiological changes are induced are very different. LTP induction in the present experiments is an acute event induced and measured in the septal third of the septo-temporal axis of the hippocampus and has a time course of <1 h, whereas learning in the watermaze takes place over a period of days and involves repetitive activation of the entire septo-temporal axis of the hippocampus. Thus, direct comparisons between the two (whether at the biochemical or some other level) can only be tentatively made and are not directly comparable in the absence of data which make use of other technologies (such as neurogenomic, structural, imaging or chronic neurophysiological recordings) and other, as yet unknown, relationships between synaptic plasticity and learning.

We conducted an extensive series of control behavioural, electrophysiological and neuropharmacological experiments. COX inhibition is often associated with gastric distress and/or gastric ulceration (Fitzgerald & Patrono, 2001). We found no instances of ulceration in the large or small intestines of any animal examined in any condition, which suggests our treatment was without effect in this regard. Our observations also suggest there was no disturbance of food and liquid intake, and the presence of faeces in all cages suggests that there was no effect of the drug treatment on gastric motility. The drug treatment was without effect on baseline electrophysiological activity, suggesting that the observed effects were specific only to activity-dependent processes occurring subsequent to high-frequency synaptic transmission. We observed no effects of the drug treatment on performance in the visible-platform task, a nonhippocampal task that depends on an intact caudate-putamen. The lack of effect on this task, which is essentially a visuomotor stimulus–response chaining task, suggests that the animals did not have visual or motor deficits induced by COX inhibition. Finally, several studies have indicated that COX2 and BDNF are both regulated by glucorticoids. We measured the presence of corticosterone in trunk blood after the completion of each experiment by each animal. There were no differences in corticosterone levels between animals treated in any condition, suggesting that our treatment did not result in any effect on corticosterone and therefore there was unlikely to be any collateral effect of corticosterone on either BDNF or COX.

What intracellular mechanisms might give rise to the interactions described above? Activation of *TrkB* is among the first step in a cascade of reactions triggered by BDNF following training in a spatial task and following induction of LTP. The identity of the effector molecules transducing the signal following phosphorylation of *TrkB* remains to be established; one possible effector is extracellular regulated kinase (ERK), which is activated downstream of *TrkB* phosphorylation in cultured hippocampal neurons (Marsh *et al*., 1993). We have previously found (Gooney *et al*., 2002) that ERK phosphorylation is increased following activation of *TrkB* by BDNF induced by spatial learning or tetanic stimulation. Hippocampal LTP is blocked after application of PD98059, an ERK inhibitor (English & Sweatt, 1997 McGahon *et al*., 1999). Blum *et al*. (1999) found hippocampal ERK activation in rats trained on the watermaze task, but when rats were given a MEK blocker, an upstream mechanism of ERK, they were unable to form long-term memories of the task. Similarly, Atkins *et al*. (1998) found that MEK inhibition resulted in deficits in fear-conditioning learning and the inability to induce LTP in rats. Gooney *et al*. (2002) found that ERK activation is stimulated by BDNF in circumstances where glutamate receptor activation occurs; activation of ERK can be also achieved through the receptor tyrosine kinase-induced activation of the *ras/raf* cascade (Graves & Krebs, 1999). The findings of Gooney *et al*. (2002) are consistent with the view that the increase in ERK activation that accompanies spatial learning and LTP may be activated because of BDNF-stimulated *TrkB* phosphorylation.

Kaufmann *et al*., 1996) reported that prostaglandins diffuse to distal sites of the same neuron or to contiguous cells where they serve as a substrate for the production of other PGs. It appears that no study has yet examined the effects of direct application of PGE2 on the activity of central cortical or hippocampal neurons; Baba *et al*. (2001) found that peripheral application of bath-applied exogenous PGE2 directly activates rat spinal dorsal horn neurons, probably via an EP2-like receptor (see also Bevan & Storey, 2002). It is possible that this increase in excitability plays a role in the physiological conditions necessary for the induction of synaptic plasticity by, for example, decreasing the threshold for the induction of synaptic plasticity through prior increases in levels of intracellular calcium. Salvemini *et al*. (1993) found that COX2 is modulated by nitric oxide (NO), a gaseous molecule that plays a role in synaptic plasticity and cellular death. In view of the many regulatory signals involved in COX2 activity and its localization in spines, Kauffman *et al*. (1996) suggest that COX2 may generate a diffusible signal as a function of the activity at specific synapses. C-fos is rapidly induced by hippocampal NMDA activation and blocked by COX inhibition (Lerea & McNamara, 1993). Because c-fos activity must involve events in the nucleus, it suggests a COX-dependent signal linking receptor activation and gene expression.

COX has mostly been investigated in relation to pathophysiological processes such as inflammation. How do we reconcile the apparently pathophysiological consequences of COX (and especially COX2) expression with the permissive role in synaptic plasticity and learning that we describe here? Basal levels of neuronal COX expression may be altered by either activity-dependent neuronal stimulation or inhibition; we suggest there are proportionate, positive and activity-dependent relationships between neuronal COX levels, synaptic plasticity and pathophysiological processes. The present data suggest that spatial learning and induction of LTP rely on modulation of similar cellular mechanisms and that both are associated with increased release of BDNF. BDNF stimulates phosphorylation of postsynaptic *TrkB* receptors and ERK and in turn enhances presynaptic glutamate release. At moderate levels of stimulation and thus moderate levels of COX expression, COX participates in synaptic plasticity and learning in a permissive and regulatory fashion exerted, at these normal physiological levels of stimulation, via a direct or indirect effect on BDNF synthesis. COX over-expression may occur when neurons are over-stimulated (e.g. by kainic acid-induced convulsions or increases in circulating cytokines), and progressive cellular death may result in the hippocampus (Manev *et al*., 2000; Ho *et al*., 2001; Kadoyama *et al*., 2001; Kim *et al*., 2001), perhaps because the sustained and dysregulated overproduction of PGs is neuropathological (see Nogawa *et al*., 1997).

In conclusion, we suggest that COX plays an important role in synaptic plasticity and learning via a BDNF-associated mechanism, and that there is an important and consequent role played by PGE2 in these processes. Furthermore, sustained physical activity such as running in a running wheel increases endogenous BDNF levels sufficiently to reverse the effects of broad-spectrum COX inhibition. We conclude that broad-spectrum cyclooxygenase inhibition reversibly blocks long-term potentiation and spatial learning via a brain-derived neurotrophic factor-associated mechanism.

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Abbreviations

AA, arachidonic acid; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; BSCI, broad-spectrum COX inhibition; COX, cyclooxygenase; DG, dentate gyrus; ERK, extracellular regulated kinase; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; IBU, ibuprofen; LTP, long-term potentiation; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PP, perforant path.

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