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Selective antagonism of the $GABA_A$ receptor by ciprofloxacin and biphenylacetic acid

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1 Previous studies have shown that ciprofloxacin and biphenylacetic acid (BPAA) synergistically inhibit γ-aminobutyric acid $(GABA)_A$ receptors. In the present study, we have investigated the actions of these two drugs on other neuronal ligand-gated ion channels.

2 Agonist-evoked depolarizations were recorded from rat vagus and optic nerves *in vitro* by use of an extracellular recording technique.

3 GABA (50 μM)-evoked responses, in the vagus nerve *in vitro*, were inhibited by bicuculline (0.3–10 μM) and picrotoxin (0.3–10 μM), with IC_{50} values and 95% confidence intervals (CI) of 1.2 μM (1.1–1.4) and 3.6 μM (3.0–4.3), respectively, and were potentiated by sodium pentobarbitone (30 μM) and diazepam (1 μM) to (mean $\pm$ s.e.mean) 168 $\pm$ 18% and 117 $\pm$ 4% of control, respectively. 5-Hydroxytryptamine (5-HT; 0.5 μM)-evoked responses were inhibited by MDL 72222 (1 μM) to 10 $\pm$ 4% of control; DMPP (10 μM)-evoked responses were inhibited by hexamethonium (100 μM) to 12 $\pm$ 5% of control, and $\alpha\beta$MeATP (30 μM)-evoked responses were inhibited by PPADS (10 μM) to 21 $\pm$ 5% of control. Together, these data are consistent with activation of $GABA_A$, 5-HT_3, nicotinic ACh and $P2_X$ receptors, respectively.

4 Ciprofloxacin (10–3000 μM) inhibited $GABA_A$-mediated responses in the vagus nerve with an IC_{50} (and 95% CI) of 202 μM (148–275). BPAA (1–1000 μM) had little or no effect on the $GABA_A$-mediated response but concentration-dependently potentiated the effects of ciprofloxacin by up to 33,000 times.

5 Responses mediated by 5-HT_3, nicotinic ACh and $P2_X$ receptors in the vagus nerve and strychnine-sensitive glycine receptors in the optic nerve were little or unaffected by ciprofloxacin (100 μM), BPAA (100 μM) or the combination of these drugs (both at 100 μM).

6 GABA (1 mM)-evoked responses in the optic nerve were inhibited by bicuculline with an IC_{50} of 3.6 μM (2.8–4.5), a value not significantly different from that determined in the vagus nerve. Ciprofloxacin also inhibited the GABA-evoked response with an IC_{50} of 334 μM (256–437) and BPAA (100 μM) potentiated these antagonist effects. However, the magnitude of the synergy was 48 times less than that seen in the vagus nerve.

7 These data indicate that ciprofloxacin and BPAA are selective antagonists of $GABA_A$ receptors, an action that may contribute to their excitatory effects *in vivo*. Additionally, our data suggest that the molecular properties of $GABA_A$ receptors in different regions of the CNS influence the extent to which these drugs synergistically inhibit the $GABA_A$ receptor.

Keywords: Quinolones; NSAIDs; $GABA_A$ receptors; ligand-gated channels; 5-HT_3; $P2_X$ receptors; glycine receptor; nicotinic cholinoceptors

Introduction

Fluoroquinolones, such as ciprofloxacin and norfloxacin, represent an important class of antimicrobial agents used in the treatment of a wide range of infectious diseases (Lietman 1995). However, these drugs are also associated with a low incidence of adverse effects related to gastrointestinal and central nervous system (CNS) function (Hooper & Wolfson, 1989). The adverse CNS effects include dizziness, headaches and insomnia (Domagala, 1994). In addition, convulsions are occasionally observed (Simpson & Brodie, 1985; Anastasio *et al*., 1988), an adverse effect that may markedly increase in patients concomitantly prescribed certain non-steroidal anti-inflammatory drugs (NSAIDs), particularly fenbufen (Christ, 1990; Janknegt, 1990; Stahlman, 1990; Lietman, 1995). This is a serious and unwanted clinical effect that has resulted in the Committee on Safety of Medicines (CSM) advising clinicians not to prescribe quinolones together with NSAIDs (CSM, 1991).

A number of studies have investigated possible mechanisms underlying the adverse CNS actions of quinolones (for review, see Halliwell *et al*., 1993). Electrophysiological studies in this and other laboratories have clearly demonstrated that several quinolones, including ciprofloxacin and norfloxacin, are weak antagonists of γ-aminobutyric acid $(GABA)_A$-mediated currents in CNS neurones (Akaike *et al*., 1991; Halliwell *et al*., 1991; Shirasaki *et al*., 1991). However, significantly, the NSAID, biphenyl-acetic acid (BPAA; the active metabolite of fenbufen), whilst having little or no effect itself, potentiates the antagonist effects of ciprofloxacin (and certain other fluoroquinolones) at the $GABA_A$ receptor by as much as 10,000 times (Akaike *et al*., 1991; Halliwell *et al*., 1991; 1995; Shirasaki *et al*., 1991). Consistent with these findings, radioligand binding studies in human (Motumoro *et al*., 1991) and animal synaptic plasma membranes have demonstrated a synergistic inhibition of [^{3}H]-GABA and [^{3}H]-muscimol binding and modulation of [^{35}S]-t-butylbicyclophosphorothionate ([^{35}S]-TBPS) binding by some quinolones and BPAA at neuronal $GABA_A$ receptors (Hori *et al*., 1987; Tsuji *et al*., 1988; Yamamoto *et al*., 1988; Akahane *et al*., 1989; Squires & Saederup, 1993; Domagala, 1994). It is well established that $GABA_A$ antagonists have proconvulsant effects (Matsumoto, 1989; Upton, 1994; Sieghart, 1995) and, therefore, the actions of quinolones in combination with BPAA at the $GABA_A$ receptor may contribute to their excitotoxic effects. In keeping

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with this hypothesis, in animal studies there is a close correlation between the epileptogenic activity of quinolones and BPAA and their potency at inhibiting [^{3}H]-muscimol binding to rodent brain synaptic plasma membranes (Akahane *et al*., 1989).

However, although these drugs do not affect ionotropic glutamate receptor-mediated currents in hippocampal neurones (Akaike *et al*., 1991; Halliwell *et al*., 1995), the effects of quinolones and BPAA at other neuronal ligand-gated ion channels are not known. In the present study, therefore, we have utilized an extracellular recording technique to investigate the effects of ciprofloxacin and BPAA alone, and in combination, on neuronal $GABA_A$, 5-hydroxytryptamine$_3$ (5-HT_3), nicotinic acetylcholine (nicotinic ACh) and $P2_X$ receptors in the rat isolated vagus nerve, and $GABA_A$ and glycine receptors in the rat isolated optic nerve. Parts of this study have been published in abstract form (Green *et al*., 1995).

Methods

Male Wistar rats (100–350 g, bred in-house) were killed by a rising concentration of CO_2. The cervical vagus nerves (15–20 mm) and/or the optic nerves (circa 8–12 mm long) were quickly dissected free. The connective tissue sheath which surrounds the vagus nerve bundle was removed. Each nerve was mounted onto a microscope slide straddling across a thin ($\approx$2 mm wide) seam of silicone grease. A further seam of silicone grease was layered over the first, essentially bissecting the nerve into two equal halves on the slide. The preparation was then placed on to a 'Perspex' holder housed inside a 'Faraday' cage.

Electrophysiological recordings

Silver-silver chloride recording electrodes (RC1, Clarke Electromedical), were placed onto the slide on either side of the grease-gap and secured by brackets onto the Perspex frame. Agonist-evoked changes in d.c. potential across the recording electrodes were relayed through miniature coaxial cable to a Neurolog AC-DC amplifier (NL106) and the signal filtered (d.c.—50 Hz) by a Neurolog filter unit (NL125). The recordings were displayed on a flat-bed pen chart recorder (Kipp & Zonen, BD111 or Ross Instruments, MDL 202). Agonists, which were dissolved in the recording solution, were applied to one side of the nerve via the perfusion system for approximately 2 min, once every 12 min to evoke a change in membrane polarization (the agonist-evoked response). Control experiments demonstrated that this protocol allowed the recording of stable and reproducible responses.

Drugs and solutions

The two sides of the nerves were separately superfused, at 2 ml min^{-1}, with a physiological recording solution gassed with O_2 and composed of the following (in mM): NaCl 118.0, KH_2PO_4 1.18, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, glucose 11.0 and HEPES 10.0. This solution was brought to pH 7.2 by the addition of NaOH. All drugs were applied to the nerve through the superfusion system.

Stock solutions of GABA (1 M), glycine (1 M), 1,1-dimethyl-4-phenylpiperazinium (DMPP, 1 mM), hexamethonium (1 M) and strychnine (1 mM), which were obtained from Sigma, were initially dissolved in twice-distilled deionized water and then further diluted to the required concentrations in the recording solution. α,β-Methylene adenosine 5′ triphosphate ($\alpha\beta$MeATP, 10 mM), 5-hydroxytryptamine (5-HT, 10 mM), picrotoxin (1 mM) and sodium pentobarbitone (10 mM) (all obtained from Sigma) were dissolved in the recording solution. Pyridoxal phosphate-6-azophenyl-2′4′-disulphonic acid (PPADS) and MDL 72222 (3-tropanyl-3-5-dichlorobenzoate) were obtained from Research Biochemicals and were also dissolved in the recording solution to give stock solutions of 1 mM. Ciprofloxacin (Bayer) was dissolved as a stock solution (at 10 mM) in twice-distilled deionized water and then diluted into the recording solution to the required concentrations. BPAA (Sigma) was made up as a stock solution of 100 or 300 mM in absolute ethanol and then diluted in the recording solution. The final concentration of ethanol did not exceed 0.1%, a concentration which had no effect on the response to GABA in control experiments. Diazepam (Roche) was first dissolved in 0.1 ml ethanol and then diluted in recording solution to form a 1 mM stock solution. Bicuculline (Sigma) was first dissolved in 0.1 ml conc. HCl to which twice-distilled deionised water was added to form a stock solution of 1 mM. Bicuculline was then diluted in the recording solution at the required concentrations. All salts, which were of analytical grade, were purchased from British Drug Houses.

Experimental protocol

Concentration-response curves to each of the agonists were obtained from which the EC_{50} value ($\pm$95%CI) was interpolated. A concentration of each agonist approximating its EC_{50} was then used in further selectivity studies. Three control agonist-evoked responses were obtained and then three further agonist-evoked responses were determined in the presence of each antagonist/modulator concentration. Pilot experiments demonstrated that all compounds produced their maximal effect after 20 min in contact with the preparations. All drug effects were therefore tested after 20 min contact time.

Data analysis

Agonist-evoked responses were measured at their peak amplitude and are expressed as the arithmetic mean ($\pm$s.e.mean of n experiments) of the response before the addition of any drugs. The data were pooled and log[agonist]-response plots and log[antagonist]-response plots fitted with a sigmoidal function by use of an automated least squares curve fitting routine (Graphpad Prism v2.0) to determine EC_{50} and IC_{50} ($\pm$95% CI) values. Control concentration-response curves to agonists were normalized by expressing all responses relative to the maximal response evoked for each agonist. Statistical comparisons of the data were carried out by use of one way ANOVA followed by Newman-Keuls Multiple Comparison *post hoc* test running on Graphpad Prism.

Results

Pharmacological characteristics of agonist-evoked responses in the rat vagus nerve in vitro

GABA (3–3000 μM), 5-HT (0.01–10 μM), DMPP (3–100 μM) and $\alpha\beta$MeATP (1–300 μM) each evoked concentration-dependent depolarizations of the isolated vagus nerve with EC_{50} values (and 95% Cl) of 69 μM (55–87, n=21), 0.8 μM (0.5–1.0, n=9), 13 μM (8–22, n=7) and 26 μM (19–34, n=4), respectively (see Figure 1).

Responses to GABA (50 μM) were inhibited by bicuculline (0.3–10 μM) and picrotoxin (0.3–10 μM) with IC_{50} values (and 95% Cl) of 1.2 μM (1.1–1.4, n=3–12) and 3.6 μM (3.0–4.3, n=3–5), respectively. GABA-evoked responses were potentiated by the barbiturate, sodium pentobarbitone (30 μM), and the benzodiazepine, diazepam (1 μM), to (mean$\pm$s.e. mean) 168$\pm$18% (n=6) and 117$\pm$4% (n=7) of control, respectively (not shown). These data are therefore consistent with GABA activating the $GABA_A$ receptor.

5-HT (0.5 μM)-evoked responses were inhibited by MDL 72222 (1 μM) to 10$\pm$4% (n=5) of control, consistent with activation of the 5-HT_3 receptor. The DMPP (10 μM) responses were inhibited by hexamethonium (100 μM) to 12$\pm$5% (n=4) of control, consistent with activation of a ni-

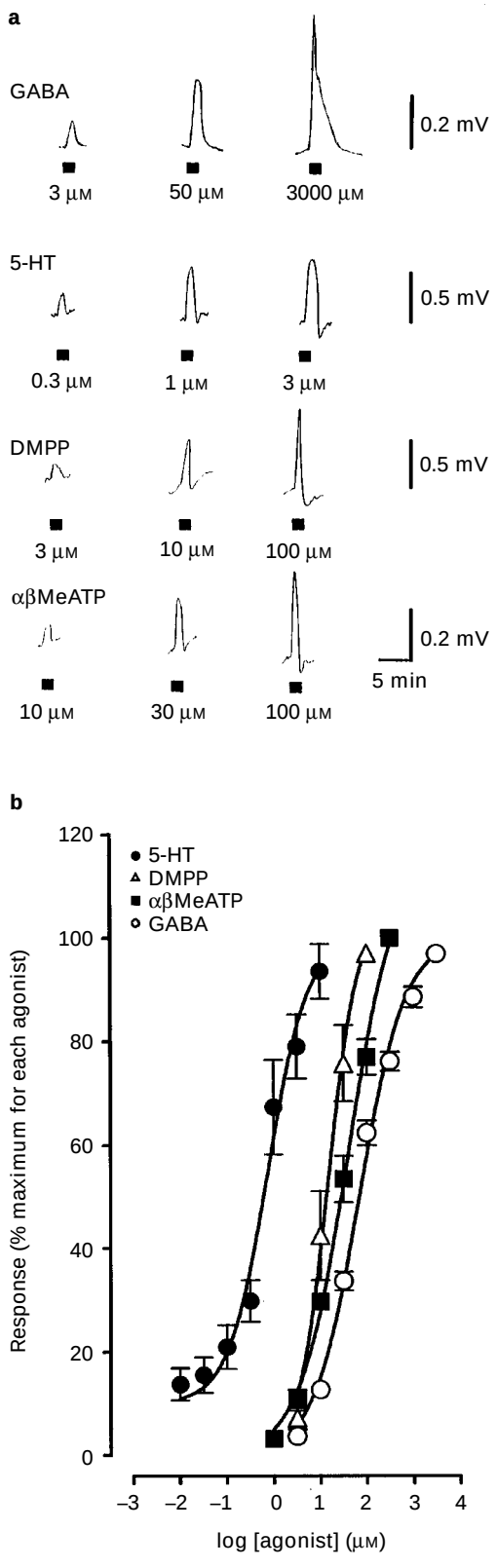

Figure 1 GABA, 5-HT, DMPP and $\alpha\beta$MeATP each evoked concentration-dependent depolarizations of the rat isolated vagus nerve. (a) Actual chart recorder traces of responses evoked by the four agonists used in this study at 3 different concentrations. The time and duration of agonist application are indicated by the black bars beneath each response. The responses for each of the agonists are from different vagus nerves. (b) A log agonist concentration (on the abscissa scale) versus response (on the ordinate scale) plot. The response evoked by each agonist has been normalized to a concentration that evoked a maximal response for that agonist. Each data point represents the mean (s.e.mean indicated by vertical lines) of 4–21 experiments.

cotinic ACh receptor. The $\alpha\beta$MeATP (30 μM) response was inhibited by PPADS (10 μM) to 21±5% (n=4) of control, consistent with activation of a $P2_X$ receptor (Figure 3).

Together, these data are in agreement with those of others recording from vagus nerves by use of extracellular techniques (Ireland & Tyers, 1987; Marsh, 1989; Trezise *et al*., 1994).

The effects of ciprofloxacin and BPAA on $GABA_A$, $5\text{-}HT_3$, nicotinic ACh and $P2_X$ receptors in the vagus nerve

Ciprofloxacin (10–3000 μM) weakly, but concentration-dependently inhibited $GABA_A$-mediated responses with an IC_{50} (and 95% Cl) of 202 μM (148–275). BPAA (1–1000 μM) had little or no effect on the GABA-evoked response but potentiated the antagonist effects of ciprofloxacin in a concentration-dependent fashion (see Figure 2). For example, in the presence of BPAA (300 μM), the IC_{50} for ciprofloxacin was reduced by more than 33,000 times to 6 nM (see Table 1). The effects of all drugs were reversible on wash.

In contrast, neither ciprofloxacin (100 μM), BPAA (100 μM) nor the combination of these drugs (both at 100 μM) greatly

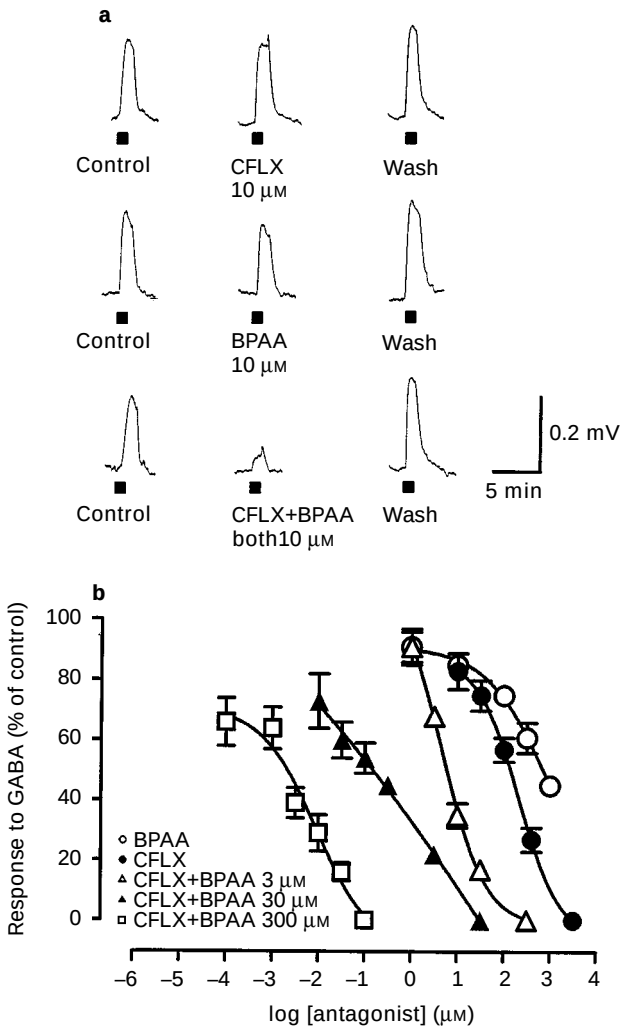

Figure 2 The antagonist effects of ciprofloxacin and BPAA on the GABA-evoked response were synergistic. (a) Actual chart recorder traces of GABA (50 μM)-evoked responses in the absence (control), presence and following the removal (wash) of ciprofloxacin (CFLX), BPAA and the combination of these drugs at the concentrations shown. (b) Depicts the GABA-evoked response, expressed as % of control (on the ordinate scale), in the presence of the log concentration (on the abscissa scale) of ciprofloxacin alone and together with BPAA. Also shown is the effect of BPAA alone. Each data point is the mean of 4–12 experiments; vertical lines show s.e.mean. The lines through the data points are a logistic curve fit.

affected 5-HT_3-, nicotinic ACh- or $P2_X$-mediated responses (see Figure 3).

Pharmacological characteristics of agonist-evoked responses in the rat optic nerve in vitro

Glycine (30 μM – 10 mM) and GABA (30 μM – 10 mM) evoked concentration-dependent depolarizations of the isolated optic nerve with EC_{50}values (and Cl) of 1.7 mM (1.4 – 2.2, $n=16$), and 1.1 mM (1.0 – 1.2, $n=6$), respectively (see Figure 4). Bicuculline (0.3 – 30 μM) inhibited GABA (1 mM)-evoked responses with an IC_{50} of 3.6 μM (2.8 – 4.5, $n=4-7$). An overall one way analysis of variance followed by *post hoc* analysis indicated that the antagonist effects of bicuculline were not different between the vagus and optic nerves ($F=0.86$, $P\geqslant 0.05$). Bicuculline (10 μM) had little or no effect on the response to glycine (1 mM). In contrast, strychnine (3 μM) antagonized glycine responses to $16\pm 7\%$ ($n=4$), but had little or no effect on the response to GABA (1 mM). These control data are consistent with those obtained previously by Simmonds (1983).

Table 1 The inhibitory effects of ciprofloxacin against the GABA-evoked response in the rat vagus nerve are concentration-dependently potentiated by BPAA

Drug treatment	*IC_{50}* (μM)	*(95% Cl)*	*Shift in potency*
CFLX alone	202	(148 – 275)	–
+ BPAA (μM)			
1	7.0	(4.6 – 11)	29
3	5.0	(4.9 – 5.1)	41
30	0.5	(0.4 – 0.7)	404
100	0.05	(0.02 – 0.12)	4,040
300	0.006	(0.003 – 0.009)	33,666

The IC_{50} value of ciprofloxacin (CFLX) alone against the GABA (50 μM)- evoked response and its IC_{50} when BPAA was co-applied, at the concentrations shown, are presented. The shift in the potency is the IC_{50} value of ciprofloxacin alone, divided by its IC_{50} in the presence of BPAA.

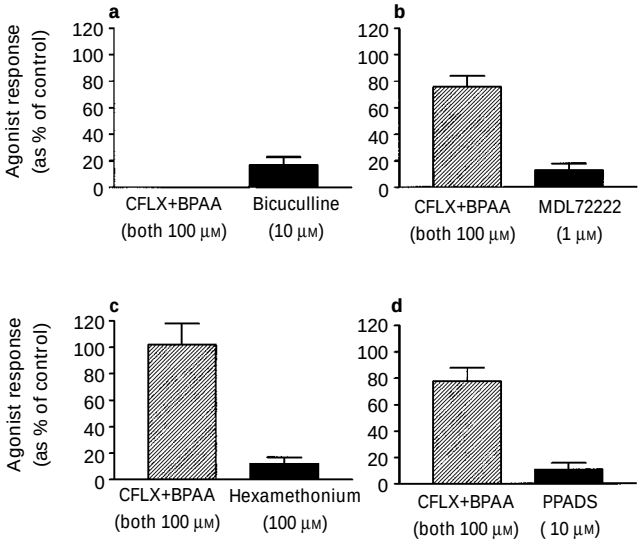

Figure 3 Ciprofloxacin and BPAA inhibited GABA-evoked responses but had little or no effect on 5-HT-, DMPP- or $\alpha\beta$MeATP-evoked responses. The 4 histograms represent the responses evoked by (a) GABA (50 μM), (b) 5-HT (0.5 μM), (c) DMPP (10 μM) and (d) $\alpha\beta$MeATP (30 μM), as a % of control. The effects of ciprofloxacin (CFLX) plus BPAA (both 100 μM) and a control antagonist are given on the x-axis. The vertical lines represent the s.e.mean of 6 – 12 experiments.

The effects of ciprofloxacin and BPAA on $GABA_A$ and glycine receptors in the optic nerve

Ciprofloxacin inhibited GABA-evoked responses with an IC_{50} of 334 μM (256 – 437, $n=4-6$). The concentration-inhibition relationship for ciprofloxacin against the GABA-evoked response was not significantly different between the two nerves ($P\geqslant 0.05$). BPAA (1 – 300 μM) had little or no effect on the

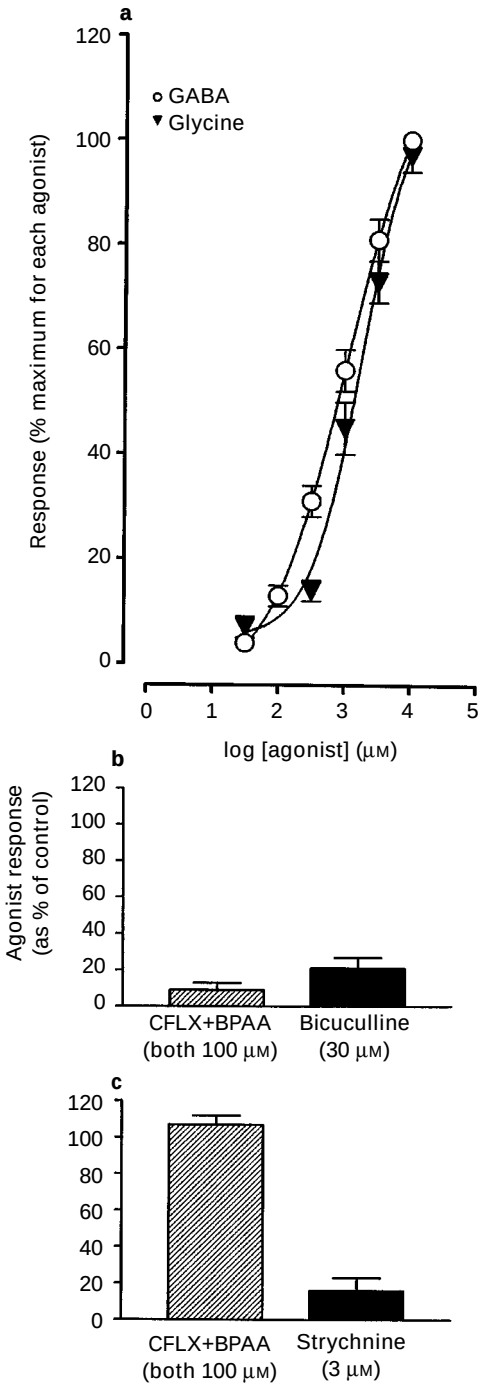

Figure 4 GABA and glycine evoked concentration-dependent depolarizations of the rat isolated optic nerve. (a) A log agonist concentration (on the abscissa scale) versus response (on the ordinate scale) plot. The response evoked by each agonist has been normalized to a concentration that evoked a maximal response for that agonist. Each data point represents the mean (s.e.mean indicated by vertical lines) of 6 – 16 experiments. (b and c) The 2 histograms show GABA (1 mM, b)- and glycine (1 mM, c)-evoked responses as a % of control. The effects of ciprofloxacin (CFLX) plus BPAA (both 100 μM) and a control antagonist are given on the x-axis. The vertical lines represent the s.e.mean of 4 – 8 experiments.

GABA-evoked response. However, BPAA (100 μM) potentiated the antagonist effects of ciprofloxacin at the $GABA_A$ receptor by 84 times, a value 48 times less than that seen in the vagus nerve with this combination of drugs (see Figure 5). Strychnine-sensitive glycine responses were little or unaffected by ciprofloxacin (100 μM), BPAA (100 μM) or the combination of these drugs (both at 100 μM): the responses were $113 \pm 8\%$ ($n=5$), $89 \pm 5\%$ ($n=4$) and $107 \pm 5\%$ ($n=8$) of control, respectively (see Figure 4).

Discussion

The present study has shown that ciprofloxacin is a weak antagonist of $GABA_A$ receptor-mediated responses in the rat isolated vagus nerve. Our experiments also show that BPAA, whilst having little or no effect itself at the $GABA_A$ receptor, potentiates the antagonist effects of ciprofloxacin by up to 33,000 times. These data are consistent with previous electrophysiological studies of the effects of these drugs on GABA-evoked whole cell currents recorded from rat and frog single dorsal root ganglion neurones (Halliwell *et al*., 1991; Yakushijii *et al*., 1992) and rat hippocampal neurones (Akaike *et al*., 1991; Halliwell *et al*., 1995). In addition, our findings are in keeping with radioligand binding experiments demonstrating that these drugs synergistically inhibit the binding of [^{3}H]-GABA and [^{3}H]-muscimol to rodent brain synaptic plasma membranes (Hori *et al*., 1987; Tsuji *et al*., 1988; Yamamoto *et al*., 1988; Akahane *et al*., 1989). Squires and Saederup (1993) have also shown that fluoroquinolones, including ciprofloxacin, reverse the inhibitory effect of GABA on [^{35}S]-TBPS binding and that this action is most markedly enhanced by BPAA, when compared with other NSAIDs. Taken together, these data are remarkably consistent and support the use of the isolated vagus nerve as a simple model with which to investigate the pharmacological properties of neuronal $GABA_A$ receptors.

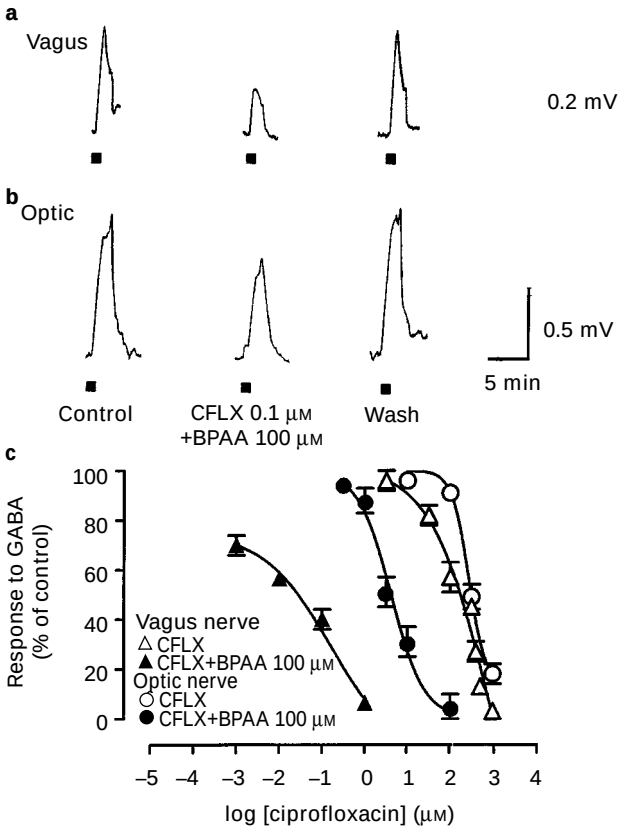

Figure 5 A comparison of the effects of ciprofloxacin and BPAA on GABA-evoked responses in the vagus and optic nerves. (a and b) Actual chart recorder traces of GABA-evoked responses recorded from the vagus nerve (a) and optic nerve (b) in the absence (control), presence and following the removal (wash) of ciprofloxacin (CFLX) plus BPAA at the concentrations shown. The GABA concentration was 50 μM for the vagus nerve and 1 mM for the optic nerve. (c) The GABA-evoked response as a % of control in the presence of the log concentration of ciprofloxacin alone and ciprofloxacin plus BPAA (100 μM) in the vagus and optic nerve. Each data point represents the mean of 4–12 experiments; vertical lines show s.e.mean.

The present study also demonstrates, for the first time, that ciprofloxacin and BPAA have little or no effect at 5-HT_3, nicotinic ACh or $P2_X$ receptors in the vagus nerve or strychnine-sensitive glycine receptors in the optic nerve. These data, together with previous radioligand binding and electrophysiological experiments showing that such drugs have no effect on ionotropic glutamate receptors (Dodd *et al*., 1989; Nozaki *et al*., 1990; Shirasaki *et al*., 1991; Halliwell *et al*., 1995), indicate that ciprofloxacin together with BPAA are highly selective antagonists of neuronal $GABA_A$ receptors. In addition, ciprofloxacin alone does not influence the binding of specific ligands to muscarinic cholinoceptors, β-adrenoceptors, naloxone-sensitive opiate or imipramine sites in rat CNS membranes (Segev *et al*., 1988). These compounds may, therefore, prove useful and selective tools for the investigation of $GABA_A$ receptors, although their effects have not yet been determined on voltage-gated ion channels or several other second messenger-coupled receptors.

At the present time, the mechanism of the synergy between ciprofloxacin and BPAA is unclear. Two hypotheses currently under investigation are: (a) that there is an intermolecular interaction between the quinolone and BPAA resulting in a molecule more active at the receptor and (b) that a novel binding site exists for the NSAID on the $GABA_A$ receptor complex. Evidence for both hypotheses has been presented. Thus, Akahane and colleagues have shown that a hybrid molecule, which flexibly links norfloxacin with BPAA, is a potent antagonist of [^{3}H]-muscimol binding and $^{36}Cl^-$ uptake in rat synaptic membranes, and also of GABA-activated whole cell currents recorded from rat hippocampal neurones. In contrast, a hybrid molecule linking norfloxacin with BPAA via a less flexible bridge had little effect (Akahane *et al*., 1994; Imanishi *et al*., 1996; Ito *et al*., 1996). These data were interpreted to indicate an intermolecular interaction between the quinolone and BPAA at the $GABA_A$ binding site. However, these data do not rule out a separate binding site for BPAA which cannot be accessed when it is rigidly and closely linked to norfloxacin. Indeed, norfloxacin (and ciprofloxacin) bind to their own (possibly the GABA binding) site and, therefore, this interaction would hinder the association of BPAA to other sites on or around the receptor. It is clearly of interest to determine if quinolones and NSAIDs interact with distinct binding sites on the $GABA_A$ receptor complex.

With reference to the second hypothesis, Squires and Saederup (1993) have, on the basis of [^{35}S]-TBPS binding studies, suggested that norfloxacin and related piperazinoquinolones, acting at $GABA_A$ receptors, induce a high affinity binding site for BPAA-like NSAIDs that when occupied, reciprocally increases the affinities of the quinolones for $GABA_A$ receptors. This site is postulated to be a new site on the $GABA_A$ receptor complex. Our present data are not inconsistent with a specific binding site for BPAA because the magnitude of the synergy was approximately 50 times less in the optic nerve than in the vagus nerve. The molecular (subunit?) properties of the $GABA_A$ receptor complex may therefore influence the interaction of quinolone/NSAIDs at $GABA_A$ receptors. Interestingly, Motomura and colleagues (1991) have shown that enoxacin and fenbufen synergistically inhibit [^{3}H]-muscimol binding to mouse and human hippocampal and cortical membranes, but do not synergistically inhibit muscimol binding to cerebellum membranes. These data, along with our own results, are the first evidence for regional differences in the magnitude of the

synergy across the CNS. Speculatively, these molecules may thus prove useful probes with which to identify different $GABA_A$ receptor isoforms.

Fluoroquinolones alone and, more especially, used in combination with certain NSAIDs (e.g. fenbufen) are associated with a number of unwanted neurological effects in both animals and man (most seriously convulsions, Hori *et al*., 1987; Akahane *et al*., 1989; Christ, 1990; Giardina, 1991; Leitman, 1995). This interaction is probably not the result of a pharmacokinetic interaction between these drugs *in vivo* (Fillastre *et al*., 1993; Tsutomi *et al*., 1994), but may be related to their selective antagonism of neuronal $GABA_A$ receptors. Consistent with this possibility, in ddY mice that develop clonic convulsions following administration of ciprofloxacin (40 mg kg^{-1}) and BPAA (50 mg kg^{-1}), brain concentrations were 1 μM and 25 μM, respectively (Tsutomi *et al*., 1994). These concentrations were also shown to reduce [^{3}H]-muscimol binding to synaptic plasma membranes by circa 30%, suggesting that a reduction of $GABA_A$ receptor function of 30% may result in convulsions. Moreover, when brain concentrations of ciprofloxacin reached 2.5 μM, achieved with 100 mg kg^{-1} ciprofloxacin, convulsions and subsequent death occurred in all mice tested; this concentration of ciprofloxacin (in the presence of BPAA [50 mg kg^{-1}]) inhibited [^{3}H]-muscimol binding by 45% (Tsutomi *et al*., 1994). Interestingly, the hybrid molecule of norfloxacin and BPAA (discussed above) has been shown to induce clonic seizures and subsequent death in mice when injected intracisternally at doses as low as 0.1 nmol (Akahane *et al*., 1994). These agents may therefore prove useful in the study of seizure mechanisms.

In conclusion, the present study shows that ciprofloxacin and BPAA synergistically and selectively inhibit $GABA_A$ receptors. The mechanism underlying this novel synergy is presently not understood, but the magnitude may be influenced by the properties of the $GABA_A$ receptor complex.

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