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Indomethacin/Ibuprofen-like Anti-inflammatory Agents Selectively Potentiate the γ-Aminobutyric Acid-Antagonistic Effects of Several Norfloxacin-like Quinolone Antibacterial Agents on [^{35}S]*t*-Butylbicyclophosphorothionate Binding

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SUMMARY

Four piperazinoquinolone antibacterial drugs (norfloxacin, ciprofloxacin, enoxacin, and pipemidic acid), known to be γ-aminobutyric acid (GABA) antagonists, fully reversed the inhibitory effect of GABA on [^{35}S]*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to rat brain membranes *in vitro*. Twelve indomethacin/ibuprofen-like arylalkanoic acid (AAA) anti-inflammatory drugs alone had no effect on [^{35}S]TBPS binding, or on its inhibition by GABA, but potentiated the GABA-antagonistic effects of the four quinolones. Felbinac (4-biphenylacetic acid) was most potent in this respect (EC_{50} = 110 nM, together with 5 μM norfloxacin), followed by flurbiprofen > anirolac > metiazinic acid > tolmetin = ketoprofen = fenbufen = indomethacin > fenoprofen > ibuprofen = (+)-naproxen = sulindac. Other anti-inflammatory analgesic drugs, including aspirin, diclofenac, diflunisal, meclofenamic acid, mefenamic acid, nambumetone, phenacetin, piroxicam, and phenylbutazone, failed to potentiate the GABA-antagonistic effect of norfloxacin. Felbinac (1 μM) increased the GABA-antagonistic potencies of norfloxacin and enoxacin about 26-fold, while increasing those of ciprofloxacin and pipemidic acid 7-fold and 2.3-fold, respectively. Using subsaturating concentrations of the four quinolones, concentration-response curves for felbinac yielded EC_{50} values ranging from 110 nM with 5 μM norfloxacin to 1.3 μM with 100 μM pipemidic acid. Three other piperazinoquinolone antibacterial agents (amifloxacin, difloxacin, and fleroxacin) and four nonpiperazinoquinolone antibacterial agents (oxolinic acid, cinoxacin, nalidixic acid, and piromidic acid) were much weaker GABA antagonists and were not significantly potentiated by felbinac. All other known $GABA_A$ receptor blockers tested, including R 5135, pitrazepin, bicuculline, SR 95531, strychnine, D-tubocurarine, thebaine, securinine, theophylline, and caffeine, were not potentiated by felbinac. Our results suggest that norfloxacin and related piperazinoquinolones, acting at $GABA_A$ receptors, may induce a high affinity binding site for indomethacin/ibuprofen-like anti-inflammatory agents (the AAA site) that, when occupied, reciprocally increases the affinities of the quinolones for $GABA_A$ receptors. The AAA binding site may be a new site in the $GABA_A$ receptor complex.

The binding of [^{35}S]TBPS to picrotoxin sites in $GABA_A$ receptor complexes can be fully, but noncompetitively, inhibited by GABA and all known $GABA_A$ receptor agonists, with a rank order of potencies similar to those found using electrophysiological and radioligand binding systems involving $GABA_A$ receptors (muscimol > GABA > 4,5,6,7-tetrahydroisoxazolopyridin-3-ol) (1). The inhibitory effect of GABA on [^{35}S]TBPS binding can be reversed by all known $GABA_A$ receptor blockers, also with a rank order of potencies similar to those found using electrophysiological and other radioligand binding systems (R 5135 ≫ bicuculline > securinine > caffeine) (2). Thus, in this system $GABA_A$ receptor blockers have the unique ability to selectively increase specific [^{35}S]TBPS binding that has been suppressed by GABA.

In a preliminary survey we found that the piperazinoquinolone antibacterial drugs norfloxacin and pipemidic acid, fully reversed the inhibitory effect of 5 μM GABA on [^{35}S]TBPS binding, with EC_{50} values near 30 μM and 1 mM, respectively (3). Norfloxacin was later shown to be a GABA antagonist in electrophysiological systems (4, 5).

In the early 1980s it was noted in Japan that some patients being treated with combinations of certain quinolone antibacterial agents and fenbufen, an AAA NSAID, had serious convulsions, leading the Japanese Ministry of Health and Welfare to issue a warning against the joint use of these two classes of drugs (4, 5). Electrophysiological studies revealed that several NSAIDs (fenbufen, indomethacin, ketoprofen, and naproxen, all AAAs), while having no effects of their own, potentiated the GABA-antagonistic effects of several piperazinoquinolone antibacterial agents, including norfloxacin, enoxacin, and ciprofloxacin (4, 5). Further, fenbufen potentiated the inhibitory effects of several piperazinoquinolones on [^{3}H]GABA binding

ABBREVIATIONS: TBPS, *t*-butylbicyclophosphorothionate; GABA, γ-aminobutyric acid; NSAID, nonsteroidal anti-inflammatory drug; AAA, arylalkanoic acid.

to rat brain membranes while having no effect alone (6). Similarly, Hori *et al.* (7) reported that the inhibitory effects of four quinolones (norfloxacin, enoxacin, ofloxacin, and ciprofloxacin) on the binding of [^{3}H]GABA to mouse brain membranes *in vitro* were enhanced remarkably by felbinac and to a lesser extent by flurbiprofen, indomethacin, and fenbufen but not aspirin. Further, Akahane *et al.* (8) reported that enoxacin, norfloxacin, lomefloxacin, ciprofloxacin, and pipemidic acid caused clonic convulsions and death in mice pretreated with felbinac (400 mg/kg), whereas five other quinolones, including ofloxacin and nalidixic acid, were inactive in this respect. Akahane *et al.* (8) also reported that felbinac (100 μM) also potentiated by up to several hundredfold the inhibitory effects of the convulsant quinolones on [^{3}H]muscimol binding to rat brain membranes *in vitro*, whereas the inhibitory effects of the nonconvulsant quinolones (e.g., nalidixic acid) were unaffected by felbinac.

We report here that 12 AAAs (all of those so far tested) alone have no effect on [^{35}S]TBPS binding or on its inhibition by GABA but potently potentiate the GABA-antagonistic effects of some but not all piperazinoquinolone antibacterial agents, including norfloxacin, ciprofloxacin, enoxacin, and pipemidic acid. The AAAs active in this system may define a new binding site in $GABA_A$ receptor complexes.

Materials and Methods

EDTA/water-dialyzed rat brain membrane preparations. Essentially as described earlier (1–3), Sprague-Dawley rats of either sex (30–90 days of age) were euthanized with CO_2 and decapitated, brains were removed, and whole forebrain and cerebellum were separated (the pons-medulla was discarded) and stored frozen at −20° for up to 8 weeks. On the day of membrane preparation, whole forebrain tissue was thawed, weighed, and homogenized in 50 volumes (w/v) of ice-cold 2 mM EDTA, pH 7.5, and a crude P_2 membrane fraction was prepared by conventional differential centrifugation. The P_2 pellet was resuspended in 50 volumes of ice-cold 2 mM EDTA and was dialyzed against 1 mM EDTA and two successive portions of water (ion exchanged twice) at 4–8° for 90 min each. The volume outside the dialysis bag (Spectrapor 2 membrane tubing, molecular weight cut-off of 12,000–14,000) was at least 20 times the volume of the P_2 suspension inside the bag. After dialysis, the membranes were pelleted once more by centrifugation (about 25,000 × *g* for 30 min) at about 4°. The supernatants were discarded and the pellets were stored frozen at −20° in the plastic centrifuge tubes. Stored in this way, [^{35}S]TBPS binding sites are stable for several weeks.

Chemicals. [^{35}S]TBPS was purchased from DuPont-New England Nuclear (Boston, MA). Other chemicals were purchased from Aldrich, Sigma Chemical Co., and Fisher. Some drugs were obtained from their manufacturers (pharmaceutical companies) as indicated in the text and tables.

[^{35}S]TBPS binding. Essentially as described earlier (1, 2, 9, 10), frozen P_2 pellets were dispersed in pure ice-cold water with a glass-Teflon homogenizer, and aliquots containing the equivalent of about 20 mg of original wet brain tissue were pipetted into glass assay tubes containing 5 mM Tris·HCl, pH 7.5, 200 mM KBr, and 2.0 nM [^{35}S]TBPS (final concentrations) plus test substances, in a final volume of 2.0 ml. Nonspecific [^{35}S]TBPS binding was defined as binding in the presence of 100 μM picrotoxin and was 10–15% of control binding. The samples were incubated at 25° for 100 min before being filtered through glass fiber filters (no. 25; Schleicher and Schuell) that had been previously dipped in 0.1% polyethyleneimine. The filters were washed twice with 5-ml portions of 200 mM NaCl containing 5 mM Tris·HCl, pH 7.5, and were then transferred to disposable polypropylene scintillation vials, to which 5.0 ml of liquid scintillation cocktail [Liquiscint (National Diagnostics, Somerville, NJ) containing 8% water] were added. The tightly capped vials were placed in an oven at 80° for 10 min to dissolve the material collected on the filters. After cooling, the samples were placed immediately in a scintillation counter for counting.

The concentration-response curves obtained were analyzed as described previously, to obtain EC_{50} values (1, 2, 9, 10). Briefly, seven or eight concentrations of the test compound were selected so that about half were below and half were above the EC_{50} value. Routinely we increase the concentration of test compound to a level where it starts to decrease [^{35}S]TBPS binding, yielding slightly "bell-shaped" concentration-response curves (see figures). The ΔB_{opt} value was calculated either from the average of the two or three highest values that were not significantly different from each other or from double-reciprocal plots ($1/c$ versus $1/\Delta B$) and was used in a Hill transformation of the data, i.e., log ($[\Delta B_{opt}/\Delta B) - 1]$ versus log (concentration), from which the EC_{50} value and a pseudo-Hill coefficient were obtained by using standard linear regression analysis. ΔB is total [^{35}S]TBPS binding in the presence of 1 μM GABA and a given concentration of antagonist minus binding in the presence of 1 μM GABA alone. ΔB_{opt} is [^{35}S]TBPS binding in the presence of 1 μM GABA and a concentration of GABA antagonist (or potentiator) giving maximum (optimum) binding minus [^{35}S]TBPS binding in the presence of 1 μM GABA alone and is expressed as a percentage of control ΔB in the tables and figures (2). Controls, nonspecific binding, and controls containing 10 nM R 5135 were included at the start, in the middle, and at the end of every experiment to verify that equilibrium had been reached. The inclusion of R 5135 (10 nM) is a measure of membrane contamination by endogenous GABA; the greater the concentration of endogenous GABA in the membrane preparation, the greater the stimulation of [^{35}S]TBPS binding by R 5135. Routinely, experiments consisting of 156 tubes, including the various controls and up to eight concentration-response curves for the drugs in question (with eight concentrations in duplicate), were performed. An experiment of this size takes roughly 80 min to filter, in addition to the 100-min incubation, so the last tubes to be filtered had been incubated almost 180 min. Controls performed at the beginning and the end of the experiment showed that binding equilibrium was maintained throughout the 80-min filtration period. The concentration-response curves for each drug were performed at least three times, rotating from the beginning to the end to somewhere in the middle of the experiment, so that one of the concentration-response curves was incubated 20–80 min longer than another. On average, the standard deviations for ΔB_{opt} and EC_{50} values were 10 and 18% of their mean values, respectively, indicating that binding equilibrium for all of the components of the system (GABA, TBPS, quinolones, and NSAIDs) had been reached.

Results

Preliminary experiments showed that the four quinolones and 12 NSAIDs, tested alone at various concentrations and in several combinations with 1 μM GABA (e.g., 5–500 μM norfloxacin plus 1 μM GABA, with or without 1 μM felbinac), had no effect on nonspecific [^{35}S]TBPS binding in the presence of 100 μM picrotoxin.

Of 11 quinolone antibacterial drugs, four (norfloxacin, ciprofloxacin, enoxacin, and pipemidic acid) fully reversed the inhibitory effect of 1 μM GABA on [^{35}S]TBPS binding to rat brain membranes *in vitro* (Table 1). Earlier we reported that norfloxacin and pipemidic acid fully reversed the inhibitory effect of 5 μM GABA on [^{35}S]TBPS binding (3). In the presence of 1 μM felbinac (4-biphenylacetic acid), the most potent of the 12 AAAs tested, the potencies of norfloxacin and enoxacin were increased about 26-fold, whereas those for ciprofloxacin and pipemidic acid were increased 7-fold and 2.3-fold, respectively (Table 1; Fig. 1).

TABLE 1

Potentiation by 1 μM felbinac of the GABA-antagonistic effects of four piperazinoquinolone antibacterial drugs on [^{35}S]TBPS binding, using 1 μM GABA

Felbinac (4-biphenylacetic acid) has no effect on [^{35}S]TBPS binding either alone or together with inhibitory concentrations of GABA. In most cases the ΔB_{opt} values in all Tables 1–3 are the means of the two or three largest ΔB values that were not significantly different from each other. The EC_{50} values and pseudo-Hill coefficients (α) were derived from Hill plots of the concentration-response curves by using standard linear regression analysis. Values are means ± standard deviations.

Antibacterial agent	Alone				+1 μM Felbinac				
	n^a	EC_{50}	α	ΔB_{opt}	n	EC_{50}	α	ΔB_{opt}	Enhancing factor
		μM		% of Δ control		μM		% of Δ control	
Norfloxacin	5	15 ± 2.6	1.2 ± 0.14	160 ± 12	3	0.57 ± 0.10	1.3 ± 0.15	120 ± 5.8	26
Ciprofloxacin	3	24 ± 3.8	1.2 ± 0.058	120 ± 5.8	3	3.3 ± 0.15	1.3 ± 0.15	120 ± 5.8	7
Enoxacin	3	46 ± 6.5	1.1 ± 0.10	130 ± 5.8	3	1.7 ± 0.30	1.4 ± 0.17	120 ± 10	27
Pipemidic acid	3	180 ± 29	1.3 ± 0.15	98 ± 6.6	3	78 ± 24	1.1 ± 0.16	120 ± 10	2.3

[a] n, Number of experiments.

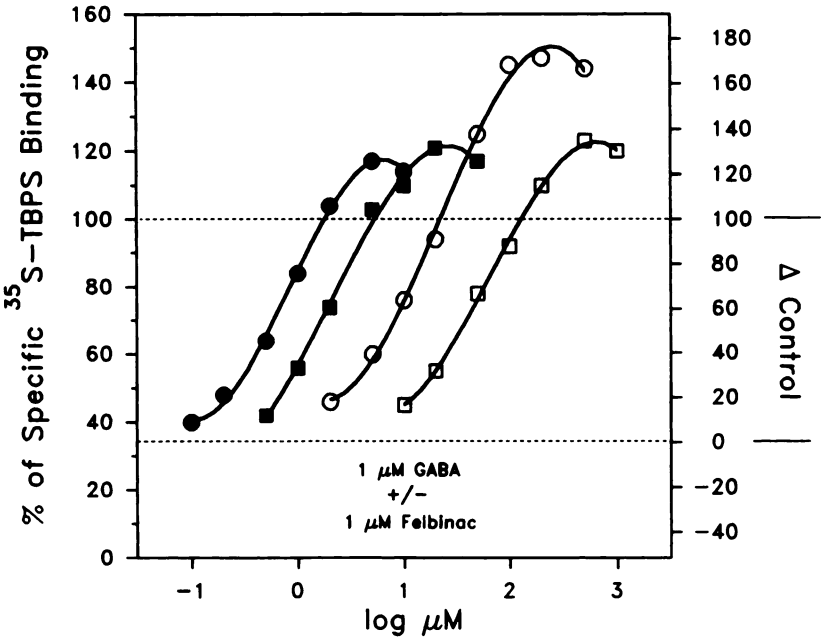

Fig. 1. Representative concentration-response curves for norfloxacin (●, ○) and enoxacin (■, □), in the absence (○, □) and presence (●, ■) of 1 μM felbinac, showing reversal of the inhibitory effect of 1 μM GABA on the binding of [^{35}S]TBPS to rat brain membranes *in vitro*.

In the presence of 5 μM norfloxacin, a concentration about one third the EC_{50} value for norfloxacin alone (Table 1), 12 AAA NSAIDs increased [^{35}S]TBPS binding suppressed by 1 μM GABA, with EC_{50} values ranging from 110 nM for felbinac to 18 μM for sulindac (Table 2; Fig. 2). In every case maximum specific [^{35}S]TBPS binding (ΔB_{opt}) was greater than control [^{35}S]TBPS binding (Table 2), an effect obtained with several established $GABA_A$ receptor blockers, including R 5135, pitrazepin, bicuculline, and securinine (2).

As shown in Tables 1–3, the concentration-response curves tended to yield pseudo-Hill coefficients greater than unity. In particular, the Hill coefficients for the concentration-response curves for one third of the NSAIDs in Table 2 ranged from 1.2 to 1.5, significantly different from the Hill coefficient for sulindac (1.0 ± 0.092). These high Hill coefficients suggest, perhaps, positive cooperative interactions between several binding sites for the AAA NSAIDs in the $GABA_A$ receptor complex.

Concentration-response curves for felbinac in the presence of subsaturating concentrations of the four quinolones yielded EC_{50} values ranging from 110 nM with 5 μM norfloxacin to 1.3 μM with pipemidic acid (Table 3), suggesting that each quinolone induces a different affinity for felbinac in the AAA binding site.

Discussion

Of 11 antibacterial quinolones, four piperazinoquinolones proved to be potent GABA antagonists in our [^{35}S]TBPS binding system, with a rank order of potencies of norfloxacin > ciprofloxacin > enoxacin > pipemidic acid when tested alone (Table 1). In the presence of 1 μM felbinac the rank order was slightly changed (norfloxacin > enoxacin > ciprofloxacin > pipemidic acid), because felbinac increases the potencies of norfloxacin and enoxacin 26–27-fold while increasing the potencies of ciprofloxacin and pipemidic acid only 7-fold and 2.3 fold, respectively. This rank order of potentices (norfloxacin > enoxacin > ciprofloxacin) is the same as that obtained by Yakushiji *et al.* (5) in their electrophysiological system in the presence of a fixed concentration of 4-biphenylacetic acid (felbinac). Similarly, the rank order of potencies reported by Yakushiji *et al.* (5) for the NSAIDs in the presence of a fixed concentration of enoxacin (felbinac > indomethacin = ketoprofen > naproxen) is very similar, but not identical, to our rank order of potencies obtained using a fixed concentration of norfloxacin (Table 2). Our present results are also in agreement with those of Hori *et al.* (7), who found that the inhibitory effects of norfloxacin and three other quinolones on the binding of [^{3}H]GABA to mouse brain membranes *in vitro* were remark-

TABLE 2

Enhancement by 12 AAA NSAIDS of the GABA-antagonistic effect of norfloxacin on [^{35}S]TBPS binding, using 5 μM norfloxacin and 1 μM GABA

Values are means ± standard deviations.

NSAID (source)	n^a	EC_{50}	α	$\Delta B_{opt}{}^b$
		μM		% of Δ control
Felbinac (4-biphenylacetic acid) (Sigma)	3	0.11 ± 0.01	1.2 ± 0.15	130 ± 10
Flurbiprofen (Sigma)	3	0.15 ± 0.035	1.2 ± 0.15	150 ± 12
Anirolac (Syntex)	3	0.83 ± 0.32	1.2 ± 0.058[c]	130 ± 17
Metiazinic acid (Rhône-Poulenc)	3	2.1 ± 0.69	0.97 ± 0.20	150 ± 12
Tolmetin (Sigma)	3	2.9 ± 0.87	1.3 ± 0.23	130 ± 12
Ketoprofen (Sigma)	3	3.3 ± 0.65	1.2 ± 0.10	160 ± 17
Fenbufen (Sigma)	3	3.6 ± 0.36	1.3 ± 0.15	140 ± 17
Indomethacin (Sigma)	3	3.6 ± 0.85	1.5 ± 0.12[d]	160 ± 15
Fenoprofen (Sigma)	3	5.2 ± 0.72	1.3 ± 0.15[e]	130 ± 15
Ibuprofen (Sigma)	3	13 ± 2.1	1.4 ± 0.10[f]	140 ± 15
(+)-Naproxen (Sigma)	3	15 ± 2.6	1.2 ± 0.32	160 ± 10
Sulindac (Merck)	3	18 ± 1.2	1.0 ± 0.092	140 ± 26

[a] *n*, Number of experiments.
[b] Δ Control is control [^{35}S]TBPS binding minus binding in the presence of 5 μM norfloxacin plus 1 μM GABA.
[c-f] The α values were significantly greater than that for sulindac (1.0 ± 0.092), with *p* values (Student's *t* test) as follows: [c] = 0.041, [d] = 0.0047, [e] = 0.050, and [f] = 0.011. All other α values were not significantly different from that for sulindac.

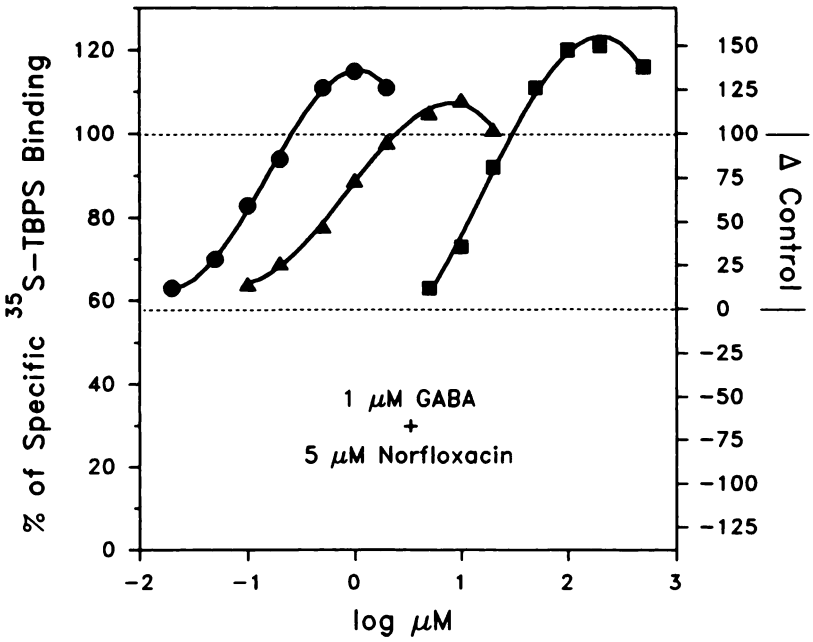

Fig. 2. Representative concentration-response curves for felbinac (●), anirolac (▲), and ibuprofen (■) in the presence of 1 μM GABA and 5 μM norfloxacin, showing reversal of the inhibitory effect of GABA, as in Fig. 1.

TABLE 3

Concentration-response curves for felbinac in the presence of subsaturating concentrations of piperazinoquinolones

The concentrations of quinolones were near one third of their EC_{50} values as GABA antagonists when tested alone (see Table 1). Values are means ± standard deviations.

Quinolone (source)	Concentration	n^a	EC_{50}	α	ΔB_{opt}
	μM		μM		% of Δ control
Norfloxacin (Merck)	5	3	0.11 ± 0.01	1.2 ± 0.15	130 ± 10
Enoxacin (Sigma)	20	3	0.21 ± 0.022	1.1 ± 0.20	140 ± 38
Ciprofloxacin (Sigma)	10	3	0.61 ± 0.065	1.2 ± 0.15	140 ± 21
Pipemidic acid (Dainippon)	100	3	1.3 ± 0.26	1.1 ± 0.12	160 ± 26

[a] *n*, Number of experiments.

ably enhanced by felbinac. Three other AAA NSAIDs (flurbiprofen, indomethacin, and fenbufen but not aspirin) also potentiated to some extent the inhibitory effects of the quinolones on [^{3}H]GABA binding. Hori *et al.* (7) found fleroxacin to be a weak inhibitor of [^{3}H]GABA binding that was not significantly potentiated by the AAAs, which is also in agreement with our results. Our results are also in agreement with those of Akahane *et al.* (8), who reported that enoxacin, norfloxacin, ciprofloxacin, and pipemidic acid caused clonic convulsions and death in mice that had been pretreated with felbinac (100 mg/kg). Further, felbinac (100 μM) increased up to several hundredfold the inhibitory effects of the convulsant quinolones on [^{3}H]muscimol binding to rat brain membranes *in vitro*, whereas the nonconvulsant quinolone nalidixic acid, at a concentration of 100 μM, did not inhibit [^{3}H]muscimol binding, either alone or together with felbinac.

Felbinac, by binding to an allosteric site, may induce a

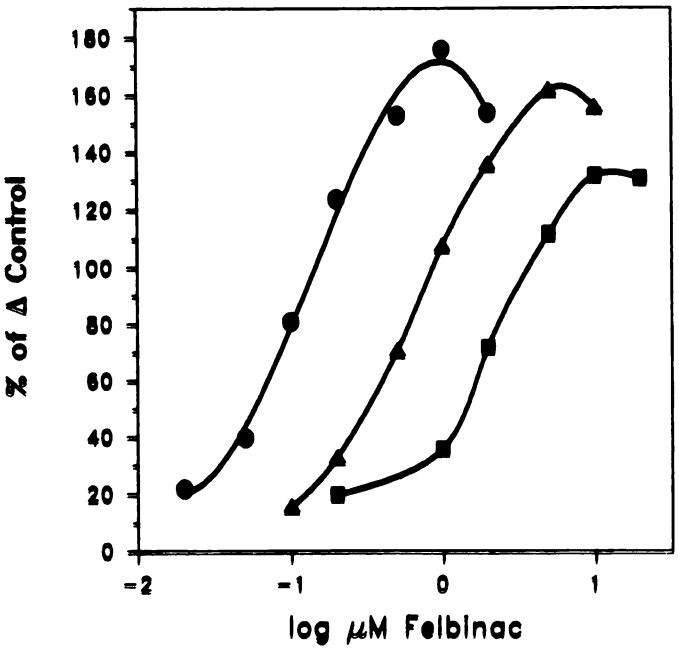

Fig. 3. Representative concentration-response curves for felbinac in the presence of 20 μM enoxacin (●), 10 μM ciprofloxacin (▲), and 100 μM pipemidic acid (■), showing reversal of the inhibitory effect of 1 μM GABA, as in Figs. 1 and 2.

conformational change in the $GABA_A$ receptor such that its affinities for norfloxacin and enoxacin are increased more than are those for ciprofloxacin and pipemidic acid (Table 1). Several other piperasinoquinolones (amifloxacin, difloxacin, and fleroxacin) exhibited little or no GABA-antagonistic activity in this system, either alone or in combination with felbinac, at up to 100 μM (data not shown). In some experiments but not others, oxolinic acid and cinoxacin exhibited slight GABA-antagonistic activity that could be slightly increased by felbinac (data not shown). A number of known GABA antagonists (R 5135, pitrazepin, bicuculline, SR 95531, strychnine, D-tubocurarine, thebaine, securinine, theophylline, and caffeine), when tested at concentrations that reversed the inhibitory effect of 1 μM GABA on [^{35}S]TBPS binding by about 20–30%, were not potentiated by concentrations of felbinac ranging from 1 to 100 μM. Therefore, although the quinolone structure is required for potentiation by AAAs (e.g., felbinac), it is not a sufficient condition for potentiation; thus far only four of 11 quinolone antibacterial agents tested have been found to be GABA antagonists that could be substantially potentiated by felbinac in our system, and all four are piperasino derivatives. Most mono-*N*-arylpiperasines are GABA antagonists in our system (9), and a few of these [norfloxacin, amoxapine, 1-(*o*-chlorophenyl)piperasine, and *N*-piperasine(*p*-acetophenone)] were subsequently shown to be GABA antagonists in electrophysiological systems (4, 5, 10). Conversely, two new GABA antagonists (enoxacin and ciprofloxacin) were recently identified in electrophysiological systems (5, 11), and we now show that they are also active in our *in vitro* TBPS systems. Thus, every GABA antagonist so far identified electrophysiologically has also been active in our TBPS system, but many compounds that we have identified as GABA antagonists in our system remain to be tested in electrophysiological systems, and some of these (e.g., clozapine) may act selectively on subsets of $GABA_A$ receptor complexes (9, 12).

Every AAA we have tested so far can potentiate the GABA-antagonistic effect of 5 μM norfloxacin. Of these, 12 AAA NSAIDs are listed in Table 2. The biphenyl group seems to increase potency; felbinac (4-biphenylacetic acid) and the related biphenyl derivative flurbiprofen are the two most potent AAAs in potentiating norfloxacin (Table 2). In contrast, a naphthyl group seems to reduce affinity for the AAA binding site [(+)-naproxen]. 5-Phenyl-2-pyrrolepropionic acid (Aldrich), which does not appear to have a therapeutic indication, is about equipotent with ibuprofen and (+)-naproxen in potentiating norfloxacin (EC_{50} ~14 μM) (data not shown). Interestingly, the central stimulant quinolone derivative amfonelic acid (Sterling-Winthrop) (13)[1] is not a GABA antagonist in our system but does weakly potentiate norfloxacin (data not shown).

When concentrations of the four quinolones corresponding to one third or more of their EC_{50} values as GABA antagonists alone were tested, the concentration-response curves for felbinac yielded quite different EC_{50} values, ranging from 110 nM with norfloxacin (5 μM) to 1.3 μM with pipemidic acid (100 μM) (Table 3; Fig. 3). The rank order of the EC_{50} values of felbinac (norfloxacin > enoxacin > ciprofloxacin > pipemidic acid) is the same as the rank order of EC_{50} values for the same quinolones in the presence of 1 μM felbinac. Taken together, our results suggest a reciprocal interaction between two allosterically linked sites, one for AAAs and the other the $GABA_A$ receptor, such that occupation of the $GABA_A$ receptor by one of the active quinolones induces a higher affinity for the AAA site and, conversely, occupation of the AAA site (for example, by felbinac) induces a higher affinity of the $GABA_A$ receptor for the active quinolones but not other GABA antagonists. The AAA binding site appears, therefore, to be a new site in the $GABA_A$ receptor complex.

Acknowledgments

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