ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1142–1146
0066-4804/91/061142-05$02.00/0

Vol. 35, No. 6

Hippocampal Activity in the Presence of Quinolones and Fenbufen In Vitro

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Received 17 August 1990/Accepted 4 April 1991

Rare side effects on the central nervous system including dizziness, restlessness, and even very rare convulsions as reported during the course of antibiotic treatment with quinolones were the topic of a well-controlled in vitro approach. The excitability of brain matter was tested by electrically evoking field potentials in the CA_1 region of the rat hippocampus in vitro. Direct effects of nalidixic acid, enoxacin, pefloxacin, norfloxacin, ofloxacin, and ciprofloxacin were found to occur as a dose-dependent increase in amplitude of this field potential, which is in line with the view that the quinolones increase excitability. The highest increase was found with enoxacin and nalidixic acid, and the lowest increase was found with ciprofloxacin. In order to keep the potential risk of the antibiotic therapy as low as possible, ciprofloxacin might be the drug of choice of the quinolones. In contrast to the quinolones, which only increased the amplitudes of electrically evoked potentials, fenbufen induced spontaneous firing in the pyramidal cell layer without stimulation in addition to its dose-dependent effects on the amplitudes of the evoked potentials. Threshold doses of the quinolones tested (0.25 μM) increased the amplitudes of evoked potentials in the presence of an otherwise ineffective concentration of fenbufen (1 μM) to different degrees, ranging from 39.2% for ciprofloxacin to 72.6% for enoxacin.

From the substantial amount of data accumulated in the literature, it is clear that quinolones are generally well tolerated. However, clinical experience with new quinolones has indicated a low incidence of effects on the central nervous system (CNS). Dizziness, headache, and restlessness have most commonly been reported. Seizures and hallucinations have been reported more frequently either in patients with underlying predisposition (e.g., cerebrovascular disease) to CNS effects or in patients receiving nonsteroidal antiinflammatory drugs (NSAID) concomitantly with quinolones. The convulsant actions of quinolones have been attributed to the inhibition of gamma-aminobutyric acid (GABA) binding to its receptor. The combination of quinolones and NSAID had an additive effect in reducing the binding of GABA to its receptor (9, 11, 15, 17).

However, the concentrations needed for an interaction of quinolones and GABA are rather high and vary among the different quinolones tested by a factor of ~100. These differences, recorded in the specific receptor binding and competition in in vitro experiments, do not mirror clinical experience with the various quinolones, as the total incidences of CNS effects are on the average 0.4 to 1.6% (1, 5, 7, 10, 16). Thus, it seems questionable that a specific interaction of quinolones with the GABA receptor can explain the CNS effects observed during quinolone or quinolone-NSAID therapy.

In contrast to the GABA-ergic mechanism postulated by Tsuji et al. (17), Segev et al. (15), and Hori et al. (11), Dette and Knothe (5) could not demonstrate any competition at all between quinolones and the GABA receptors by using synaptosomes isolated from rat brain. Data generated by Halliwell et al. (9), who adopted the patch clamp technique, suggest that GABA receptor binding studies may underestimate the complexity of NSAID-quinolone interactions with receptor(s). The in vivo studies performed by Christ et al. and Nozaki et al. indicate that dopamine receptors or opioid and glutaminergic receptors may also be involved in CNS effects of quinolones (3, 14). These data indicate, first, that GABA-ergic mechanisms are a necessary but not sufficient prerequisite for effects and, second, that specific ligand receptor studies do not reflect the complex mechanism leading to quinolone-mediated CNS excitation.

The aim of the present investigation was to test the ability of different quinolones alone and in combination with fenbufen to influence electrically evoked field potentials in the hippocampal slice preparation. The method of using brain tissue slices as physiologically intact units dates back to 1966 (18). In this ex vivo model, the complex physiology, i.e., the interconnections between neurons as well as between neurons and glial cells is maintained. In the meantime, this technique has been widely used in order to study the physiology of the hippocampus in vitro (for review, see reference 13). The modulatory contribution, especially, of various transmitters to hippocampal excitation and inhibition phenomena could be studied on a cellular level but within the pertinent network (6).

(Part of this study has been presented in abstract form at the 3rd International Symposium on New Quinolones, Vancouver, Canada, 1990.)

MATERIALS AND METHODS

Hippocampal slice preparations were obtained from 15 adult male CD rats (Charles River Wiga). The animals were exsanguinated under slight ether anesthesia, the brain was removed in total, and the hippocampal formation was isolated under microstereoscopic sight. The middle part of the hippocampus was fixed to the table of a Vibratom (Rhema Labortechnik, Hofheim, Federal Republic of Germany) with cyanoacrylat glue, submerged into chilled artificial cerebrospinal fluid (ACF) (124 mM NaCl, 5 mM KCl, 2 nM $CaCl_2$,

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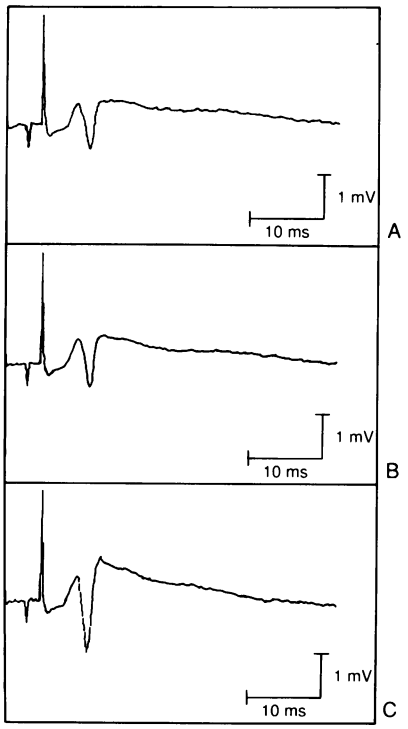

FIG. 1. Increase of pyramidal cell population spike amplitude in the hippocampal slice preparation by coadministration of fenbufen and pefloxacin. (A) Electrically evoked potential of the CA_1 region in drug-free ACF. (B) Potential recorded under superfusion with ACF containing 1 μmol of fenbufen per liter. (C) Potential recorded under superfusion with ACF containing 1 μmol of fenbufen per liter and 2 μmol of pefloxacin per liter. All signals are averages of four individual responses. All recordings were made on one single brain slice with intervals of 30 min. The first two sharp peaks are electrical stimulus artifacts.

2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose), and chopped into slices of 400-μm thickness. All slices were preincubated for at least 1 h in carbogen-saturated ACF in a prechamber before use. During the experiment, the slices were held and treated in a special superfusion chamber according to the method of Haas et al. (8). The preparation was superfused with 180 to 230 ml of ACF per hour.

Electrical stimulation (3.5 to 10 V, 300-μs pulse width) of the Schaffer collaterals within the CA_2 area and recording from the pyramidal cell layer of CA_1 were performed according to conventional electrophysiological methods using the LabTEAM computer system with the NeuroTOOL software package. Representative signals (averaged from four potentials evoked with a stimulus interval of 20 s) are given in Fig. 1. Regular measurements were performed only every 10 min in order to avoid potentiation mechanisms. After three stable predrug values were obtained, the perfusion was switched to a drug containing ACF and continued for at least 30 min. The main amplitudes of the three predrug signals were averaged and set to 100%. All averaged postdrug changes refer to this percentage value. In the first series of experiments, four doses of fenbufen (1, 5, 10, and 20 μM) were tested in order to characterize the actions of this drug and its dose dependence. In the second series, dose-response curves (0.25, 0.5, 1.0, and 2.0 μM) were evaluated for nalidixic acid, norfloxacin, ciprofloxacin, enoxacin, ofloxacin, and pefloxacin in order to define the minimally effective concentration of each quinolone, which in turn was needed to define the experimental conditions for the subsequent interaction experiments with fenbufen. Thus, in order to evaluate the risk potential of each of the antibacterial drugs in combination with fenbufen quantitatively, in the third series, the lowest dose of fenbufen which did not show any effects on the evoked potentials by itself was tested in the presence of the minimally effective concentration of each of the quinolones.

All results are given as mean ± standard error of the mean of n = six slices for each concentration.

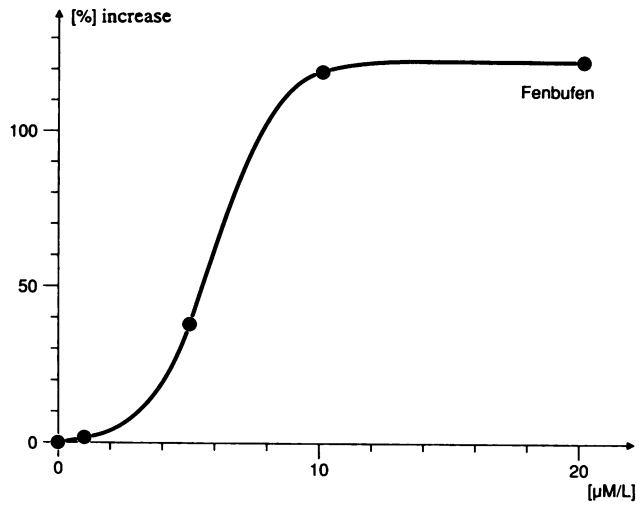

FIG. 2. Dose-dependent increase of pyramidal cell population spike amplitude expressed as percentage of predrug value under fenbufen.

RESULTS

In the first series of experiments testing the effects of fenbufen on the amplitude of the electrically evoked field potential, a dose-dependent increase of the amplitude of the field potential between 1 and 10 μM was observed (Fig. 2; Table 1). With 10 μM fenbufen, nearly the maximum enhancement is reached; an elevation of the fenbufen concentration to 20 μM did not increase the amplitude significantly. Superfusion with the higher dosages of fenbufen at first resulted in the appearance of spontaneous multiunit spiking activity, whereas during continuous superfusion, synchronous firing, i.e., small population spikes, were observed (Fig. 3).

In the second series of experiments, the low concentration (0.25 μM) of the quinolones tested resulted in 5 to 10% increases of the amplitude of the field potentials evoked in

TABLE 1. Dose-dependent increase of pyramidal cell population spike amplitude in the presence of different concentrations of fenbufen

Fenbufen concn (μmol/liter)	Mean increase ± SEM[a]
1	1.7 ± 3.0
5	38.7 ± 3.2
10	119.2 ± 4.5
20	122.9 ± 4.9

[a] Expressed as percentage of predrug value; n = 6.

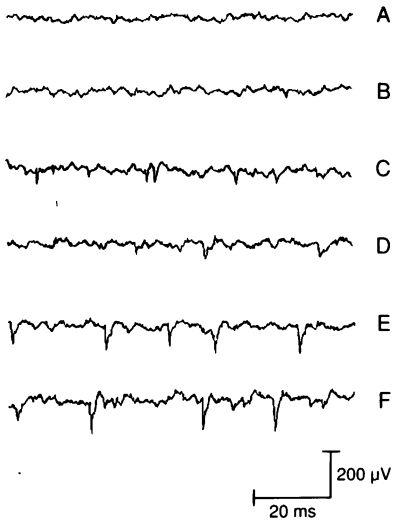

FIG. 3. Appearance of spontaneous multiunit spiking activity and small population spikes formed by synchronous multiunit firing. (A) Single signal trace of recording in the hippocampal CA_1 region superfused with drug-free ACF. (B through F) Single signal traces 10, 20, 30, 40, and 50 min after beginning of superfusion with ACF containing 20 μmol of fenbufen per liter.

the CA_1 area of the rat hippocampus in vitro. Table 2 summarizes the effects of the diverse quinolones. Opposite to the results with the higher concentrations of fenbufen, no tendency to an increased spontaneous spiking activity was observed with the higher concentrations of the quinolones.

For the interaction study, the borderline doses of 0.25 μM of the quinolones and the subthreshold, ineffective concentration of 1 μM of fenbufen were regarded as suitable. A typical example of the results of this sort of interaction is given in Fig. 1 for the combination of pefloxacin with fenbufen. Whereas the low concentration of pefloxacin hardly produces a visible increase of the amplitude by itself, the addition of fenbufen leads to a dramatic increase of the population spike amplitude. The effectiveness of the different quinolones tested to produce this enhancement is quite different, however.

In the presence of fenbufen, ciprofloxacin induces a 39% increase of the amplitude (Table 3; Fig. 4), whereas enoxacin (72%) or nalidixic acid (68%) was approximately twice as effective. The values for norfloxacin and ofloxacin (52% each) are in between. Thus, the quinolones tested reliably caused quantitatively different responsiveness within the hippocampus in vitro. An otherwise ineffective concentration of fenbufen (if applied alone) increases the effects of all quinolones tested. Interestingly, these interaction experiments on the concomitant application of fenbufen and quinolones resulted in the same rank order of potencies of the quinolones compared with the data generated in the second experimental series for the effects of quinolones on their own. Thus, quantitative, but no qualitative, differences could be demonstrated among the quinolones tested. Fenbufen obviously had a trigger and an amplificatory function.

DISCUSSION

Despite the fact that the incidence of CNS adverse drug reactions (ADR) of quinolones is reasonably low (on average, 0.4 to 1.6%) (1, 5, 7, 12), they may cause problems in individuals with an underlying predisposition to CNS effects. In addition, the simultaneous application of quinolones with NSAID increases the frequency and severity of CNS ADR. For example, in the course of numerous clinical studies on norfloxacin, the relative number of patients complaining of headache and dizziness ranged from 0.5 to 35.7% and from 1% to 37.5%, respectively (for summary, see reference 5). These differences may reflect different methods of asking patients about ADR either in general or in particular and of recording the data. Reviews on clinical efficacy and safety of various quinolones based on the analysis of comparable numbers of patients having been treated with one specific quinolone indicated that there may be differences among the quinolones with respect to the incidence of CNS ADR (7, 12). The well-controlled condition of the hippocampal in vitro slice preparation, which may represent an ex vivo model for the evaluation of CNS side effects in humans, allows a direct quantitative comparison of various quinolones with respect to their direct functional effect on the brain and their interaction with fenbufen. As concomitant recordings of intracellular activity (action potential) and

TABLE 2. Dose-dependent increase of pyramidal cell population spike amplitude in the presence of quinolones

Quinolone	Concn (μmol/liter)	Mean increase ± SEM[a]
Ciprofloxacin	0.25	5.2 ± 2.1
	0.50	29.0 ± 3.1
	1.00	62.2 ± 3.8
	2.00	97.6 ± 3.5
Enoxacin	0.25	9.9 ± 2.9
	0.50	72.7 ± 2.5
	1.00	128.7 ± 5.2
	2.00	132.9 ± 3.9
Nalidixic acid	0.25	5.2 ± 2.2
	0.50	61.9 ± 3.5
	1.00	122.9 ± 3.9
	2.00	131.5 ± 4.8
Norfloxacin	0.25	5.6 ± 3.3
	0.50	33.3 ± 2.5
	1.00	76.5 ± 5.3
	2.00	92.1 ± 4.1
Ofloxacin	0.25	10.3 ± 2.9
	0.50	39.9 ± 3.3
	1.00	78.5 ± 4.4
	2.00	130.1 ± 5.6
Pefloxacin	0.25	6.5 ± 3.3
	0.50	37.7 ± 3.3
	1.00	75.3 ± 4.5
	2.00	102.7 ± 5.0

[a] Expressed as percentage of predrug value; $n = 6$.

TABLE 3. Dose-dependent increase of pyramidal cell population spike amplitude during concomitant administration of fenbufen (1 μmol/liter) and different quinolones

Quinolone	Concn (μmol/liter)	Mean increase ± SEM[a]
Ciprofloxacin	0.25	39.2 ± 3.1
Enoxacin	0.25	72.6 ± 4.9
Nalidixic acid	0.25	68.2 ± 3.9
Norfloxacin	0.25	52.6 ± 4.4
Ofloxacin	0.25	52.7 ± 3.6
Pefloxacin	0.25	60.4 ± 4.5

[a] Expressed as percentage of predrug value; $n = 6$.

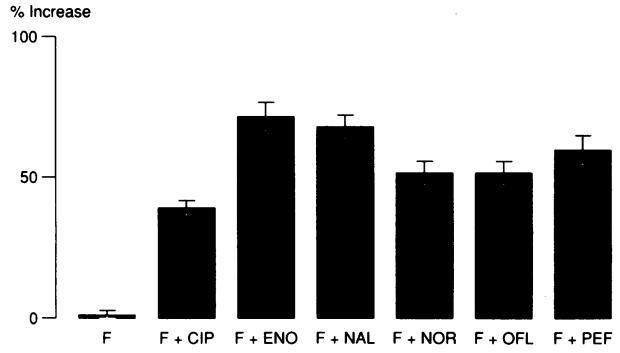

FIG. 4. Bar chart representing the enhancement of pyramidal cell population spike amplitude expressed as percentage of predrug value under fenbufen (1 μM) alone and in the simultaneous presence of fenbufen (1 μM) and different quinolones (0.25 μM). F, fenbufen; CIP, ciprofloxacin; ENO, enoxacin; NAL, nalidixic acid; NOR, norfloxacin; OFL, ofloxacin; PEF, pefloxacin.

extracellular activity (population spike) within the CA_1 area of the hippocampus by Heinemann et al. (10) have shown that the population spikes reflect the activity of multiple neuronal units in their transition from normal to epileptic responses, any increase in the amplitude of the population spike reflects higher excitability of the system.

In general, one can state from the results described above that the responsiveness of the neuronal network is increased in the presence of quinolones to different degrees depending on the compound tested, ciprofloxacin being the weakest amplifier of evoked activity and nalidixic acid and enoxacin being the strongest amplifiers. Quinolones, even at the highest concentrations tested, were unable to induce spontaneous activity. But quinolones are able to enhance evoked activity in a dose-dependent manner. Thus, quinolones, once they have passed the blood-brain barrier, interfere with information processing within the brain at low concentrations (>0.25 μM).

The quinolone concentrations used in our experiments are within the same range as the concentrations determined in human cerebrospinal fluid (CSF) (for summary, see reference 4). Following administration of either an oral dose of 500 mg or an intravenous dose of 200 mg of ciprofloxacin to patients with noninflamed meninges, mean maximal CSF concentrations were 0.14 and 0.27 mg/liter, corresponding to 0.42 and 0.81 μM, respectively. In patients with inflamed meninges, mean maximal CSF concentrations were 0.35 mg/liter (corresponding to 1.05 μM) and 0.56 mg/liter (corresponding to 1.69 μM) upon an oral dose of 500 mg or an intravenous dose of 200 mg of ciprofloxacin. Following repeated oral doses of 200 mg of ofloxacin twice a day to patients with inflamed meninges, mean maximal CSF concentrations on days 2 and 5 were 3.2 and 9.4 mg/liter, corresponding to 8.85 and 26.01 μM, respectively.

In contrast to the quinolones tested, fenbufen increased not only the responsiveness of the hippocampal pyramidal cells in response to electrical stimuli in a dose-dependent manner but also induced spontaneous action potentials measured extracellularly as multiunit activity or even synchronous discharges of small cell populations. This increase in excitability is reminiscent of the action of theophylline, which induced spontaneous activity under the same conditions. There is a great probability that this feature is related to the well-known epileptogenic action of this drug in humans (2). Thus, there is a major difference in the effects of fenbufen and quinolones on hippocampal activity.

It is quite remarkable that the rank order of potencies obtained in the experimental series with the quinolones alone is equal to the one obtained from the interaction study in which a subthreshold dosage of fenbufen was used to potentiate the action of the different quinolones. It might thus be suspected that the probability of inducing side effects in humans, particularly if the interaction with fenbufen is concerned, depends on the type of quinolone. With respect to the incidence of side effects of quinolones alone, Janknegt (12) gives a rank order of potencies which corroborates our findings: ciprofloxacin (n = 2,575), 0.4%; norfloxacin (n = 3,215), 0.8%; ofloxacin (n = 3,340), 1.0%; and enoxacin (n = 2,407), 1.2%. The fact that there is also only a factor of 3 between ciprofloxacin and enoxacin—very similar to our results—gives us the hope that we are tracing not just a coincidence.

The interaction of quinolones with fenbufen on a neuronal level may be explained by an amplification of the spontaneous excitations triggered by fenbufen or theophylline (likewise inducing spontaneous potentials in the hippocampus) leading possibly to epileptiform discharges after reaching a certain threshold. With respect to the factors which might be involved in this process, in general either a direct increase of the excitability or a disinhibition feature might be suspected. The interaction of quinolones with the GABA receptor in the CNS as described by others (9, 11, 15, 17) may well account for the effects monitored in the course of our experiments, as the pyramidal cells of the CA_1 region of the hippocampus are under considerable control of this transmitter. Other factors modulating this site, such as, for example, shifts of ionic strength (increase of calcium or removal of magnesium) resulting in the appearance of spontaneous potentials, as well possibly might explain the action of fenbufen.

Thus, though the exact mechanism of action of quinolones in this model has still to be elucidated by further experiments, the occurrence of epileptogenic seizures in the presence of fenbufen can well be explained by a primary induction of spontaneous hippocampal activity by fenbufen and its amplification by the quinolones. This means that the crucial primary event must be seen in the spontaneous activity as induced by fenbufen. For the same reason, namely, spontaneous neuronal activity, quinolones should be used with caution in epileptic patients, because under certain threshold conditions, small focal activity in the brain might be amplified into grand mal seizures.

The present findings support the hypothesis that studies with the hippocampal slice preparation might have a predictive value for the CNS side effects of quinolones in humans. If the information provided by the results of this study is of clinical relevance, it could lead to the proposal to prefer one of the two quinolones having the lowest potency of inducing excitability changes within the CNS (norfloxacin or ciprofloxacin), especially in cases in which the enhanced penetration of quinolones into the brain via a damaged blood-brain barrier cannot be excluded.

ACKNOWLEDGMENTS

Leoni Schombert is gratefully acknowledged for most excellent technical assistance in performing the experiments and taking care of the animals. We thank Carlos Hofmann for help in programming our laboratory computer system.

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