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In Vitro Modulation of Hippocampal Pyramidal Cell Response by Quinolones: Effects of HA 966 and γ-Hydroxybutyric Acid

W. DIMPFEL,[1]* A. DALHOFF,[2] AND E. VON KEUTZ[3]

Pro Science Private Research Institute GmbH, D-35440 Linden,[1] and Institut für Chemotherapie[2] and Institut für Toxikologie,[3] Bayer AG, D-42113 Wuppertal, Germany

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The influence of quinolones on electrically evoked pyramidal cell activity in the rat hippocampus in vitro was studied by using the slice technique. We hoped to learn more about the possible mechanisms for the development of side effects of different quinolones and to find a possible treatment. As reported earlier (W. Dimpfel, M. Spüler, A. Dalhoff, W. Hofmann, and G. Schlüter, Antimicrob. Agents Chemother. 35:1142–1146, 1991), the amplitude of the population spike increased in the presence of ciprofloxacin, lomefloxacin, or ofloxacin about twofold in comparison with reference values. This increase could be prevented in a concentration-dependent manner by the concomitant presence of 3-amino-1-hydroxy-2-pyrrolidone (HA 966), a compound acting at the so-called glycine site of the *N*-methyl-D-aspartate (NMDA) receptor, but not in the presence of aminophosphonovaleric acid (APV), which acts at a different recognition site of the NMDA receptor. Another tool, 6,7-dinitroquinoxaline-2,3-dione, an antagonist of the so-called AMPA receptor (named after the binding of L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] to this site), could not antagonize the effect induced by the quinolones. Activation of the glycine site of the NMDA receptor induced by the presence of D-serine in the superfusion medium also resulted in a concentration-dependent increase in the population spike amplitude. This response remained unchanged in the presence of ciprofloxacin, whereas lomefloxacin and ofloxacin led to further increases in the amplitude, especially in the presence of higher concentrations of D-serine. These results also point to an involvement of the glycine site of the central NMDA receptor in the development of side effects by different quinolones. A complete attenuation of the quinolone-induced effects was obtained in the presence of 2.5 μM γ-hydroxybutyric acid (GHB), a physiological neuromodulator which is marketed in some countries of Europe as a sedative. It is therefore concluded that the excitatory adverse effects of quinolones might be treated by the administration of GHB.

The method of using brain tissue slices as physiologically intact units dates back to 1966 (17). In this in vitro model the complex physiology, i.e., the interconnections between neurons as well as those between nerve and glial cells, is maintained. Since then, this technique has been widely used for the study of the physiology of the hippocampus in vitro. In particular, the modulatory contribution of various transmitters in terms of hippocampal excitation and inhibition phenomena could be studied on a cellular level, but within the pertained network of neurons (5). This in vitro model therefore seems to be especially useful not only for studying the mechanism of action of drugs on the central nervous system but also for gaining information on its possible treatment. Quinolones are among the most frequently used antibacterial agents, and their use results in a reasonably low incidence of adverse drug reactions. Despite the existence of the blood-brain barrier, however, some side effects (0.4 to 1.6%) originating from the central nervous system have been described (11).

In order to study the origins of these side effects in more detail, the blood-brain barrier can be bypassed by exposing brain tissue directly to the action of quinolones in an in vitro approach. Recently, the rat hippocampus slice in vitro has been introduced successfully into quinolone research by showing that clinically relevant concentrations of diverse quinolones (0.25 to 2 μM) increase the amplitude of the population spike of pyramidal cells upon electrical stimulation of the Schaffer collaterals in correlation with their clinical incidence (3, 4).

The present study dealt with two neurotransmitter systems which have been recognized as especially important for the regulation of hippocampal excitability, namely, the glutamatergic system and the γ-hydroxybutyric acid (GHB) system.

Because electrical stimulation of the Schaffer collaterals releases glutamate, acting in an excitatory manner on the hippocampal pyramidal cells, drugs might directly or indirectly interact with synaptic efficacy by interacting postsynaptically with glutamate receptors or presynaptically by modulating glutamate release, respectively. Whereas glutamate increases the neuronal excitability of pyramidal cells by interacting with *N*-methyl-D-aspartate (NMDA) or L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainic acid receptors, the action of GHB is characterized by the attenuation of pyramidal cell activity by a receptor of its own. For each of the glutamate receptor types, specific antagonists are available, thus allowing investigators to try to antagonize the effects of quinolones. From the degree of interference of these specific tools with the effects of quinolones indirect conclusions first with respect to the mode of interaction of quinolones with physiological neuromodulation will be drawn. Second, different degrees of interference with this neuronal communication structure by different quinolones might allow conclusions as to their relative potency with respect to the incidence of adverse drug effects to be made, provided that the penetration through the blood-brain barrier does not differ to a major degree between quinolones.

* Corresponding author. Mailing address: Pro Science Private Research Institute GmbH, Kurt-Schumacher-Str. 9, 035440 Linden, Germany. Phone: 49/64 03/95 13-0. Fax: 49/64 03/95 13 95.

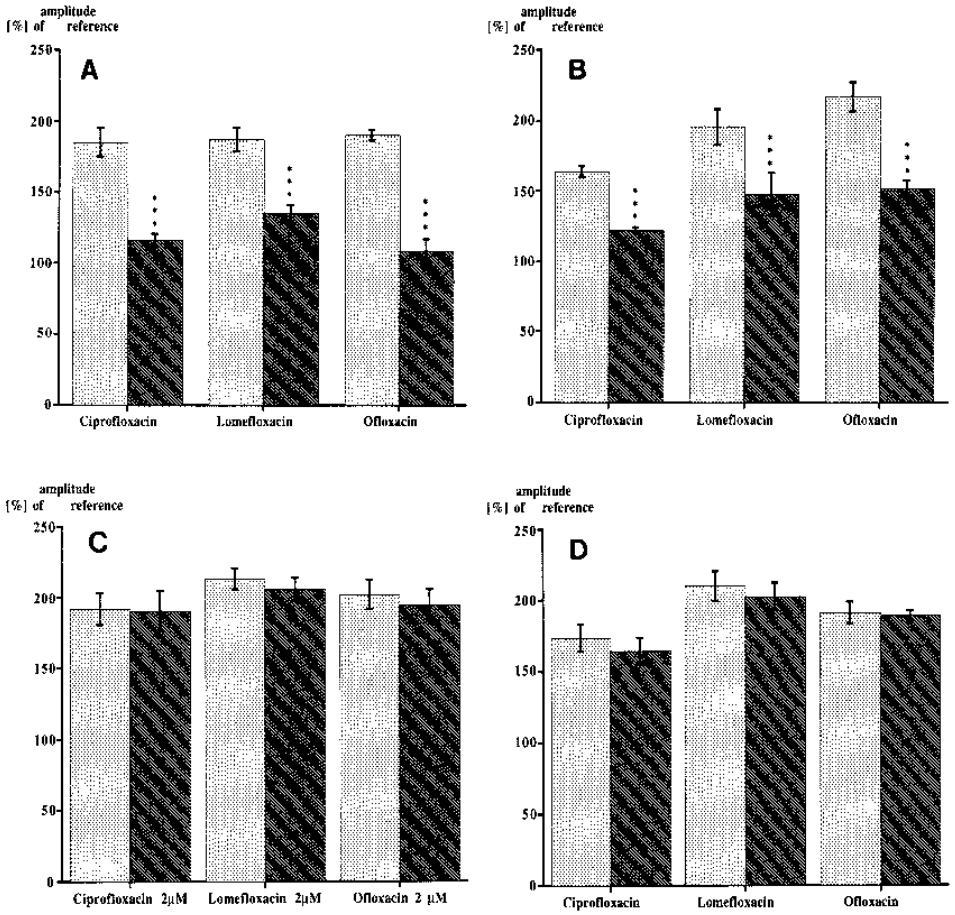

FIG. 1. Increase in pyramidal cell population spike amplitude in the hippocampal slice preparation by coadministration of quinolones and different antagonists of glutamate receptors. Left bar, mean amplitude in the presence of either quinolone at 2 μM; right bar, amplitude in the presence of GHB, HA 966, APV, or DNQX at the indicated concentrations. The concentrations of the antagonists themselves have no influence on the reference signal. Values are given as means ± SEMs for six independent slices. For statistical significance, the three asterisks correspond to $P < 0.01$ according to the Wilcoxon-Mann-Whitney U test. (A) ▨, drug; ▩, drug plus GHB at 2.5 μM. (B) ▨, drug; ▩, drug plus HA 966 at 50 μM. (C) ▨, drug; ▩, drug plus APV at 1 μM. (D) ▨, drug; ▩, drug plus DNQX at 0.1 μM.

MATERIALS AND METHODS

Hippocampal slice preparations were obtained from 23 day–night-reversed adult male rats (Charles River Wiga). The animals were exsanguinated under ether anesthesia, each brain was removed in total, and the hippocampal formation of each brain was isolated under a stereoscopic microscope. The midsection of the hippocampus was fixed to the table of a vibratom with cyanoacrylate glue, submerged in chilled phosphate-buffered saline (artificial cerebrospinal fluid [ACF], consisting of NaCl, 124 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgSO_4$, 2 mM; NaH_2PO_4, 2.25 mM; $NaHCO_3$, 26 mM; and glucose, 10 mM), and chopped into slices of 400 μm in thickness. All slices were incubated in a prechamber for at least 1 h before use in carbogen-saturated ACF.

During the experiment the slices were held and treated in a special superfusion chamber (7). The preparation was superfused with 180 to 230 ml of ACF per h.

Electrical stimulation (200 μA, 200-μs pulse width) of the Schaffer collaterals within the CA_2 area (submaximal stimulus) and recording from the pyramidal cell layer of CA_1 was performed by conventional electrophysiological methods by using the LabTEAM Computer System with the NeuroTOOL software package. Signals were averaged from four potentials evoked with a stimulus interval of 20 s. Measurements were performed at 10-min intervals to avoid potentiation mechanisms. Having obtained three stable predrug values, the perfusion was changed to drug containing ACF and was continued for a minimum of 30 min. The average amplitude of the three predrug signals was in the range of 1,065 to 1,439 μV (mean ± standard error of the mean, 1,263 ± 21.7 μV) and was set to 100%. All averaged postdrug changes refer to this percentage.

In the first series of experiments the three antagonists of glutamate receptors were checked in the presence of 2 μM (each) the three quinolones (ciprofloxacin, lomefloxacin, and ofloxacin): (i) aminophosphonovaleric acid (APV) for antagonizing a particular site of NMDA receptor-modulated action, (ii) 6,7-dinitroquinoxaline-2,3-dione (DNQX) for antagonizing the AMPA receptor-induced changes, and (iii) 3-amino-1-hydroxy-2-pyrrolidone (HA 966) for antagonizing the so-called glycine site of the NMDA receptor. In addition, GHB was used to suppress the quinolone-induced increase in amplitude of the electrically evoked population spike of the pyramidal cells.

In a second series of experiments D-serine was used to stimulate the glycine site of the NMDA receptor (12) in the absence or presence of the three quinolones mentioned above. Increasing cumulative doses of from 100 to 800 μM were used in order to uncover potential differences in the three quinolones with respect to the potentiation of the response.

Quinolones were obtained from Bayer AG, Wuppertal, Germany. D-Serine was from Sigma Chemie GmbH, Deisenhofen, Germany, and HA 966, APV, and DNQX were from RBI Chemikalien GmbH, Cologne, Germany. GHB was from Köhler Chemie, Alsbach-Hähnlein, Germany.

All results are given as the mean ± SEM for six slices for each concentration. For statistical purposes the Wilcoxon, Mann, and Whitney U test was used.

RESULTS

The presence of any of the quinolones at 2 μM was reproducibly able to increase the amplitude of the population spike amplitude up to about 200% of the original predrug value. This confirms the results published earlier (4). The additional presence of glutamate receptor antagonists at concentrations which do not interfere with the reference signal (tested in earlier experiments) led to different results. Whereas neither APV nor DNQX was able to attenuate the amplitude of the population spike, HA 966 prevented the quinolone-induced increase in a concentration-dependent manner (Fig. 1 and 2). GHB at a

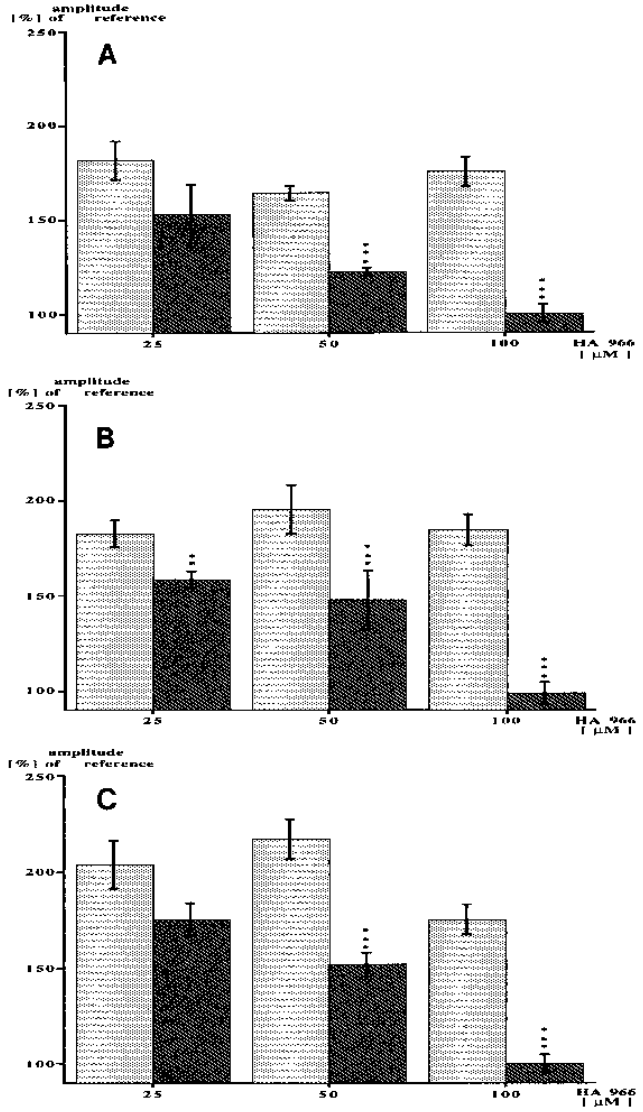

FIG. 2. Concentration dependence of antagonism by HA 966 for each of the three quinolones. Values are given as means ± SEM for six independent slices. Drug-induced values might vary from one experimental series to the other (depending on electrode positioning within the slice), but with respect to antagonism, the slices were used as their own controls. For statistical significance, three asterisks correspond to $P < 0.01$ and two asterisks correspond to $P < 0.02$ according to the Wilcoxon, Mann, and Whitney U test. (A) ▨, ciprofloxacin at 2 μM; ◪, ciprofloxacin at 2 μM plus HA 966. (B) ▨, lomefloxacin at 2 μM; ◪, lomefloxacin at 2 μM plus HA 966. (C) ▨, ofloxacin at 2 μM; ◪, ofloxacin at 2 μM plus HA 966.

concentration of 2.5 μM was similarly effective, as was the presence of 100 μM HA 966 (Fig. 1). The statistical significance of the inhibition produced by both compounds was high.

The presence of D-serine in the superfusion medium potentiated the stimulatory effect of the electrical stimulation in a concentration-dependent manner. The additional presence of 2 μM ciprofloxacin in the medium did not change the resulting amplitudes. In contrast, however, lomefloxacin and, even more, ofloxacin at the same concentration increased the D-serine response to a considerable degree in a statistically significant manner (Fig. 3). Especially at the highest dose of D-serine, the responses induced by ciprofloxacin, lomefloxacin, and ofloxacin could be differentiated from each other quite well.

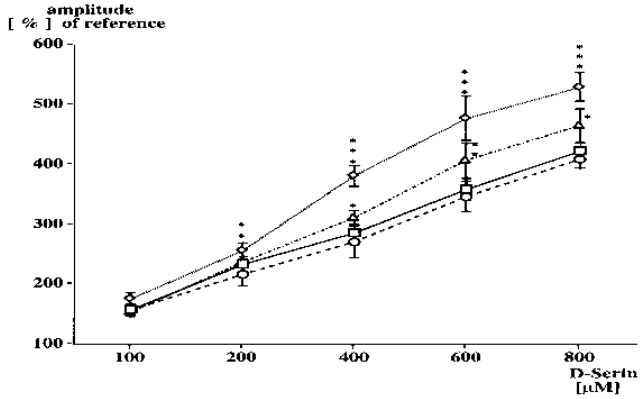

FIG. 3. Activation of the glycine site of the NMDA receptor by D-serine (D-Serin). The concentration dependence and its modulation by various quinolones, ciprofloxacin, lomefloxacin, and ofloxacin, is indicated. Values are given as means ± SEMs for six slices. D-serine was tested in a cumulative manner by increasing the concentration stepwise from 100 to 800 μM within one experiment. In statistical significance, three asterisks correspond to $P < 0.01$, two asterisks correspond to $P < 0.02$, and one asterisk corresponds to $P < 0.05$ according to the Wilcoxon, Mann, and Whitney U test. □, control; ◇, ofloxacin at 2 μM; ○, ciprofloxacin at 2 μM; △, lomefloxacin at 2 μM.

DISCUSSION

Modulation of rat hippocampal activity by the presence of quinolones provides a plausible interpretation for the occurrence of central nervous system side effects in humans (3). Various quinolones have different potentials for inducing adverse effects which might be reflected in the rank order of the in vitro testing (4). The new series of experiments gives further evidence that various quinolones have different potentials for modulating the pyramidal cell activity in response to electrical stimulation of the Schaffer collaterals.

The question that now arises is what the underlying biochemical mechanisms could be. Because hippocampal pyramidal cells are excited by the glutamatergic input from Schaffer collaterals, this transmitter was the first candidate to be tested. Glutamate receptors have been implicated in a wide range of nervous system disorders, from memory to epilepsy. Fortunately, quite specific tools that can be used to characterize the interaction between glutamate receptors and drugs on a molecular base are available. Among these, only HA 966, a synthetic compound acting at the so-called glycine site of the NMDA receptor, effectively blocked the actions of the quinolones. A direct interaction with the AMPA receptor can be excluded. This result is in line with reports of earlier studies, in which no binding between quinolones and the NMDA receptor could be found (6). The concentration-dependent antagonism of HA 966 against the quinolone-induced increase in pyramidal cell excitability, however, might be a valuable step in elucidating the mechanism of action of quinolones on brain matter.

Another test with respect to the interference of quinolones with the glycine site of the NMDA receptor is activation of this site by the addition of D-serine to the superfusion medium. In the presence of D-serine the amplitude of the electrically evoked population spike increases with increasing concentrations of D-serine. Whereas ciprofloxacin did not change this pattern, lomefloxacin and, even more, ofloxacin potentiated this response. This allows us to possibly discriminate among various quinolones in this rather simple in vitro test under defined experimental conditions. In vivo study of drug action must also consider penetration into the central nervous system,

but it might be valuable during the development of new quinolones to check their possible direct interaction with central nervous system matter. In cases in which direct interaction of quinolones with brain matter cannot be excluded (i.e., trauma of the skull), the choice of the particular quinolone to be used for treatment might be influenced by the in vitro capacity of the quinolone to interact with the brain. This is especially important because the quinolone concentrations used in our experiments were within the same range as the concentrations determined in human cerebrospinal fluid (0.25 μM) (i.e., according to the literature [2, 13], levels in cerebrospinal fluid amount to about 2.5% of the concentration in serum, which has been determined to be in the range of 2.5 mg/liter, corresponding to 62.5 μg/liter).

Considering the mechanism of action which might be involved in these drug effects, the interaction of quinolones with rat hippocampal pyramidal cells is also attenuated by the presence of 2.5 μM GHB, a physiological transmitter or neuromodulator (1, 16). Because GHB at this concentration does not interfere with the amplitude of the population spike in the absence of quinolones (data not shown), this result points to a high degree of specificity. In this light, reports in the literature of an involvement of γ-aminobutyric acid (GABA) in the mechanism of action of quinolones on the central nervous system (8–10, 14, 15) can be explained by a more indirect action. GHB can be converted from GABA via reduction of succinic semialdehyde to a certain degree. This might explain why the concentrations used in the studies dealing with quinolones and the GABA receptor greatly exceed clinically relevant amounts.

Because the central effects of quinolones are also attenuated by very low dosages of GHB, it might be worthwhile to try a concomitant application during clinical use. GHB passes the blood-brain barrier, is marketed in some countries of Europe as a sedative, and might be used to prevent quinolone-induced excitatory adverse side effects on the brain, like hallucinations, insomnia, or even seizures.

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