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Inhibition by fluoroquinolones of K^+ currents in rat dissociated hippocampal neurons

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Abstract

The effects of four fluoroquinolones (sparfloxacin, fleroxacin, ofloxacin and levofloxacin) on K^+ currents were investigated in pyramidal neurons acutely isolated from rat hippocampus, to evaluate their relative potencies for inhibiting these channels. Using patch-clamp electrophysiological techniques, we found that all four compounds inhibited the delayed rectifier K^+ current (I_K), but with different potencies. Sparfloxacin was the most potent compound, displaying an IC_{50} value of 6.44×10^{-4} M, followed by fleroxacin, ofloxacin and levofloxacin, their IC_{50} values being 7.09×10^{-3}, 8.42×10^{-3} and 1.10×10^{-2} M, respectively. In contrast, the fast transient K^+ current (I_A) was blocked only by sparfloxacin ($IC_{50} = 2.86 \times 10^{-3}$ M) and fleroxacin ($IC_{50} = 4.38 \times 10^{-3}$ M), but not by ofloxacin and levofloxacin even at concentrations up to 1 mM. The K^+ current inhibition was reversible after washout of the compounds. Further study is needed to clarify the possible involvement of this novel action in the adverse effects of fluoroquinolones in the central nervous system (CNS).

Keywords: Fluoroquinolone; Hippocampus; K^+ current, transient; K^+ current, delayed rectifier

1. Introduction

The fluoroquinolones, such as ofloxacin, ciprofloxacin, levofloxacin and sparfloxacin, are very effective in the treatment of various bacterial infections. However, they have been shown to produce adverse effects on the central nervous system (CNS), such as headache, dizziness and paroxysmal convulsions, although the incidence is quite low (1–4%) (Christ, 1990). Among these adverse CNS effects, convulsive seizures have been observed more frequently in high-risk patients such as patients with a history of epilepsy (Stahlmann and Lode, 1999) or patients who are cotreated with nonsteroidal anti-inflammatory drugs such as fenbufen (Anastasio et al., 1988; Halliwell et al., 1993).

The exact mechanism by which fluoroquinolones cause epileptogenic activity is still unclear. Several lines of evidence suggest that fluoroquinolones are weak inhibitors of [^{3}H]γ-aminobutyric acid (GABA) and [^{3}H]muscimol binding to brain membranes (Segev et al., 1988; Tsuji et al., 1988; Akahane et al., 1989), and the inhibitory potency of fluoroquinolones is dramatically potentiated in combination with biphenylacetic acid, an active metabolite of fenbufen (Akahane et al., 1989, 1994a,b). Electrophysiological experiments using dissociated rat hippocampal neurons have demonstrated that fluoroquinolones suppress GABA-gated Cl^- currents (Akaike et al., 1991; Halliwell et al., 1995), but not ionotropic glutamate receptors (Shriasaki et al., 1991; Halliwell et al., 1995), in the presence of biphenylacetic acid. Nevertheless, it remains questionable whether GABA receptor antagonism alone can explain the convulsant activity of these antimicrobial agents, especially in the absence of biphenylacetic acid.

Several published studies showed that sparfloxacin was able to cause electrocardiographic changes in patients, manifested by a prolongation of the QT interval (Lipsky and Baker, 1999; Domagala, 1994; Adamantidis et al., 1998). More recently, fluoroquinolones were found to prolong the cardiac action potential and to block the human ether-a-go-go-related gene (HERG) K^+ channel, which plays an important role in the repolarization of ventricular action potentials, thus inducing QT prolongation (Bischoff et al., 2000; Kang

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et al., 2001). It seems reasonable to assume that fluoroquinolones also affect K^+ currents in the brain, which may contribute to their neurotoxicity by eliciting abnormal neuronal discharge, as was found with the convulsant 4-aminopyridine and tetraethylammonium (Segle et al., 1984). The present study was designed to investigate the effects of four commonly used fluoroquinolones, sparfloxacin, fleroxacin, ofloxacin and levofloxacin, on K^+ currents in pyramidal neurons acutely isolated from rat hippocampus and to determine their relative potencies for inhibiting these channels.

2. Materials and methods

2.1. Recording of K^+ current in pyramidal neurons

Dissociated neurons were prepared as described previously (Sodickson and Bean, 1998) with modifications. Briefly, hippocampi from 5- to 9-day-old Sprague–Dawley rats were dissected in oxygenated ice-cold dissociation solution containing 82 mM Na_2SO_4, 30 mM K_2SO_4, 5 mM $MgCl_2$, 10 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) and 10 mM glucose (pH 7.3). Transverse slices (500 μm) were cut and mini-slices of the CA1 region were prepared. The mini-slices were incubated in dissociation solution containing 3 mg/ml protease XXIII at 32 °C for 8 min. Then the solution was replaced with dissociation solution containing 1 mg/ml trypsin inhibitor type II-S and 1 mg/ml bovine serum albumin. The mini-slices were allowed to cool to room temperature under an oxygen atmosphere. Before recording, the mini-slices were triturated using a series of fire-polished Pasteur pipettes with decreasing tip diameters. Dissociated neurons were placed in a recording dish and perfused with external solution containing 135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose and 0.001 mM tetrodotoxin (pH 7.3 with NaOH). The neurons with large pyramidal-shaped cell bodies and thick apical dendritic stumps were chosen for study.

Recording was made in the whole-cell voltage-clamp configuration using an Axopatch 200 A amplifier (Axon Instruments, USA) at 21–23 °C. Electrodes (tip resistance 2–4 MΩ) pulled from borosilicate glass pipettes (Hilgenberg, Germany) were filled with pipette solution containing 125 mM potassium gluconate, 20 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES and 10 mM EGTA. Voltage command protocols were provided and currents were recorded via a DigiData-1200A interface controlled by pClamp 6.2 software (Axon Instruments). The holding potential was −50 mV. Currents were filtered at 2–10 kHz and sampled at frequencies of 10–40 kHz. Series resistance was compensated by 85–90%. Linear leak and residual capacitance currents were subtracted on-line using a P/6 protocol. The liquid junction potential was less than 3 mV and was not compensated.

2.2. Compounds

Sparfloxacin (yellow, purity >99%), fleroxacin, ofloxacin and levofloxacin (white, purity >99%) were provided by the Henan Institute for Drug Control, and all other chemicals were purchased from Sigma. The compounds were dissolved in external solution and the pH and osmolarity were readjusted. Compound-containing solutions were directly applied to the recorded neuron using RSC-100 Rapid Solution Changer with an 18-tube head (Bio-Logic France).

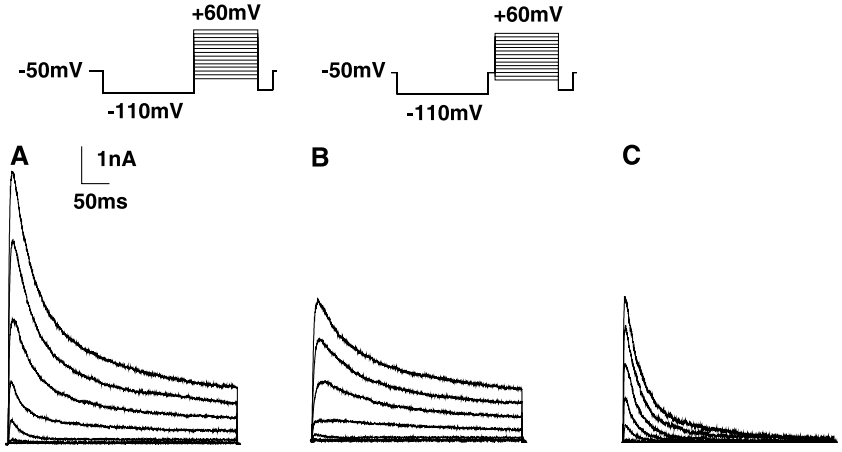

Fig. 1. Outward current families elicited in hippocampal pyramidal cells acutely isolated from CA1 region of rats at postnatal day (P) 6. Note that all recordings shown were performed in pyramidal cells isolated from the same animal. Voltage protocols used are illustrated in insets. Depolarizing voltage commands were applied in steps of +10 mV. Holding potential was −50 mV. (A) Depolarizing voltage commands (200 ms) were preceded by a hyperpolarizing prepulse (150 ms) to −110 mV (inset). (B) Similar protocol as in (A), but with a delay of 50 ms at a holding potential between prepulse and depolarizing command pulses. (C) Subtraction of current traces in (B) from those in (A) isolates a fast transient A current. Calibrations in (A) apply to all recordings in this figure.

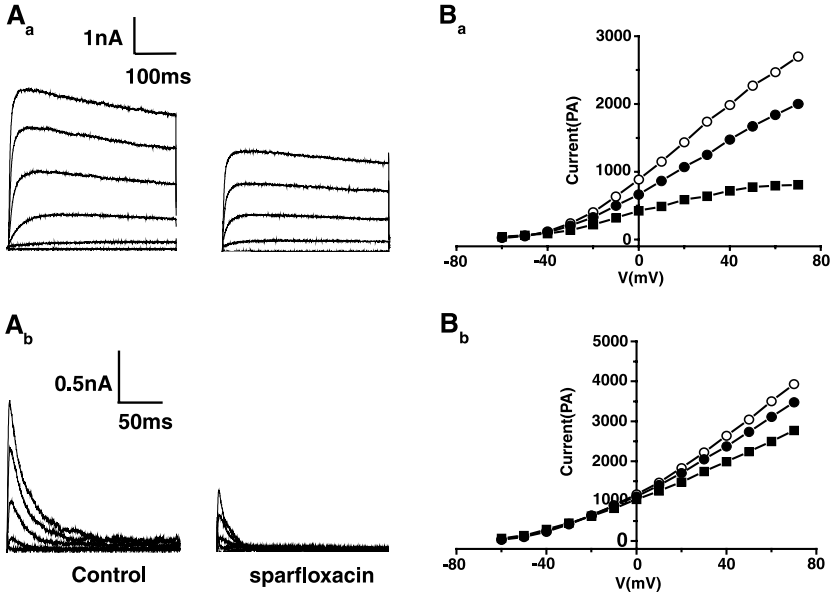

Fig. 2. The inhibitory effects of sparfloxacin on I_K and I_A. (Aa) Inhibitory effect of 1 mM sparfloxacin on I_K in a CA1 pyramidal cell, elicited with the voltage protocol shown in the inset in Fig. 1B. (Ab) Inhibitory effect of 1 mM sparfloxacin on I_A in a CA1 pyramidal cell, determined by the subtraction procedure described in Fig. 1C. (Ba, Bb) Current–voltage relationships for the I_K and I_A peak current in the presence of control solutions (○), 1 mM sparfloxacin (■) and washout (●), respectively.

2.3. Data analysis

The IC_{50} values for compounds were obtained using a computer software "Microcal Origin 6.0". The data are presented as means ± S.E.M.

3. Results

The outward current pattern of all CA1 pyramidal cells under investigation could be separated into a fast transient A current (I_A) and a delayed rectifier-like current (I_K). In our experiments, depolarizing command pulses of 200-ms duration followed directly on a 150-ms hyperpolarizing prepulse to − 110 mV (h.p. − 50 mV; "prepulse protocol") or a 50-ms interval at holding potential was interposed between the prepulse and the command pulses ("delayed prepulse protocol"). Families of outward currents elicited with voltage steps between − 60 and + 70 mV are shown in Fig. 1.

I_K was blocked by all of the compounds tested, whereas I_A was blocked by sparfloxacin and fleroxacin. The effect of these compounds on current–voltage relationships of I_K and I_A was typified by sparfloxacin, as shown in Fig. 2. The effects occurred immediately after exposure to the cells and reached a steady state. The compounds could be washed-out rapidly and almost completely. At the highest applied concentrations of fluoroquinolones, current amplitudes recovered to about 85–90% of the initial current amplitude.

The concentration–response relationships were calculated by a non-linear least-squares fit of equation: $f = 1/(1+(\chi/IC_{50})^{n_H})$ to the data (Fig. 3A and B). Hill coefficient (n_H) and the half-maximum inhibiting concentration (IC_{50}) were calculated (Table 1). Each compound inhibited I_K or I_A in a concentration-dependent manner and the order of the

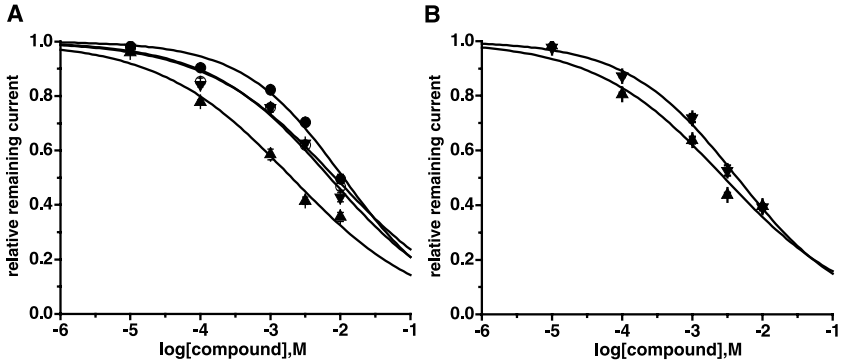

Fig. 3. Concentration–response relationships for sparfloxacin (▲), fleroxacin (▼), ofloxacin (○) and levofloxacin (●) on the I_K (A) and sparfloxacin (▲) and fleroxacin (●) on I_A (B).

Table 1
IC_{50}(M) of different fluoroquinolones calculated from electrophysiological experiments in pyramidal neurons acutely isolated from rat hippocampus

Compound	$I_K(\times 10^{-3})$ ($IC_{50} \pm$ S.E.M)	$n_H \pm$ S.E.M	$I_A(\times 10^{-3})$ ($IC_{50} \pm$ S.E.M)	$n_H \pm$ S.E.M.
Sparfloxacin	0.64 ± 0.03	0.46 ± 0.05	2.86 ± 0.70	0.47 ± 0.07
Fleroxacin	7.09 ± 1.74	0.50 ± 0.08	4.38 ± 0.43	0.55 ± 0.04
Ofloxacin	8.42 ± 1.59	0.47 ± 0.05	–	–
Levofloxacin	11.02 ± 2.08	0.60 ± 0.08	–	–

inhibitory effects of compounds on I_K was sparfloxacin> fleroxacin>ofloxacin>levofloxacin. The inhibitory effects of compounds on I_A was in the order of sparfloxacin>fleroxacin, whereas ofloxacin and levofloxacin were without effect at concentrations up to 1 mM.

4. Discussion

The present study is the first to examine the possible effects of fluoroquinolone antimicrobial agents on K^+ currents in pyramidal neurons acutely isolated from rat hippocampus. We found that all four drugs tested inhibited K^+ currents in a concentration-dependent fashion, albeit with widely differing potencies. IC_{50} values against I_K ranged from 6.44×10^{-4} M for sparfloxacin to 1.10×10^{-3} M for levofloxacin. Conversely, I_A was blocked only by sparfloxacin and fleroxacin, whereas ofloxacin and levofloxacin were without effect even at concentrations up to 1 mM. This indicated the existence of specific structural features in these molecules that contribute separately to their antimicrobial activity and to the inhibition of K^+ currents in pyramidal neurons.

K^+ currents are especially important for the regulation of neuronal excitability, because they repolarize neurons in response to depolarizing events and help to stabilize the membrane potential below the firing threshold. Interference with K^+ currents leads to abnormal neuronal discharge and this is believed to contribute to the convulsant activity of 4-aminopyridine (Segle et al., 1984).

It is noteworthy that, in this study, the four tested fluoroquinolones produced I_A and I_K inhibition in rat hippocampal pyramidal neurons at concentrations higher than 10^{-5} M. These concentrations were much higher than those observed in human serum and cerebrospinal fluid (CSF) in a clinical setting (Davey et al., 1994), thus preventing direct application of the present findings to humans. However, they fell within the range of concentrations of fluoroquinolones in the biophase of rats at the onset of convulsive seizures. In an in vivo study, sparfloxacin was administered intravenously to rats until maximal seizures were elicited. At this time, the concentration of the drug in plasma and CSF was 2.14×10^{-4} and 1.31×10^{-4} M, respectively (Delon et al., 1999), which is in the order of its IC_{50} (6.44×10^{-4} M) for I_K inhibition observed in this study.

The sensitivity of K^+ channels in human hippocampal pyramidal neurons to the inhibitory effect of fluoroquinolones is unknown, but if high it may still explain some of the CNS adverse effects of these drugs. In fact, fluoroquinolone-induced convulsive seizures occur more frequently in patients with some risk factors, such as a history of epilepsy and concomitant use of nonsteroidal anti-inflammatory drugs. It can be inferred that, in these situations, K^+ channels may be sensitive to the inhibitory effect of fluoroquinolones, thus predisposing patients to the epileptogenic action of fluoroquinolones even at therapeutic doses.

In conclusion, our study demonstrated that fluoroquinolones inhibited I_A and I_K in rat hippocampal pyramidal neurons. We hypothesized that this inhibition may be associated with the altered excitability of the CNS during clinical treatment with some fluoroquinolones. Further studies are needed to clarify the possible involvement of this novel action in the adverse effects of fluoroquinolones in the CNS.

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