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Increase in insulin release from rat pancreatic islets by quinolone antibiotics

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1 The present study was undertaken to elucidate the mechanism(s) of hypoglycaemia caused by quinolone antibiotics. We investigated the effects of various quinolone antibiotics on insulin release in rat pancreatic islets.

2 At a non-stimulatory concentration of 3 mM glucose, lomefloxacin (LFLX) or sparfloxacin at 1 mM and pipemidic acid (0.1-1 mM) induced slight insulin release but tosufloxacin or enoxacin up to 100 μM did not.

3 At the stimulatory concentration of 10 mM glucose, all quinolones augmented insulin release in a dose-dependent manner. LFLX (100 μM) shifted the dose-response curve of glucose-induced insulin release to the left without altering the maximal response.

4 At 10 mM glucose, LFLX (100 μM) increased insulin release augmented by forskolin (5 μM) or 12-O-tetradecanoyl phorbol-13-acetate (100 nM) but not by raising the K^+ concentration from 6 to 25 mM.

5 Verapamil (50 μM) or diazoxide (50-400 μM) antagonized the insulinotropic effect of LFLX.

6 These data suggest that quinolone antibiotics may cause hypoglycaemia by increasing insulin release via blockade of ATP-sensitive K^+ channels.

Keywords: Quinolone antibiotics; insulin release; hypoglycaemia; ATP-sensitive K^+ channel

Introduction

The older quinolone antibiotics, such as nalidixic acid, have been used for the treatment of urinary tract infections for many years. They are of relatively minor significance because of the narrow spectrum of antibacterial activity and the rapid development of bacterial resistance. These drawbacks were overcome by the introduction of fluorinated quinolones such as enoxacin (ENX) and lomefloxacin (LFLX), which are used for the treatment of a wide variety of infectious diseases. Recently these drugs were reported to induce hypoglycaemia in geriatric patients (Toyoda *et al.*, 1991; Ministry of Health and Welfare of Japan, 1992). In one of those reports, the serum level of immunoreactive insulin (IRI) was found to be higher than the normal range. In addition, quinine with some structural resemblance to quinolones was reported to increase insulin release by decreasing the K permeability (Henquin, 1982). In the present study, we investigated the possible effects of quinolone antibiotics (ENX, LFLX, pipemidic acid (PPA), sparfloxacin (SPFX), and tosufloxacin (TFLX)) on insulin release and adenosine 3': 5'-cyclic monophosphate (cyclic AMP) production from rat pancreatic islets in order to elucidate the involvement of ATP-sensitive K^+ channels (K^+-ATP channels) in their actions.

Methods

Islets were isolated from pancreata of fed male Wistar rats (250-350 g) by collagenase digestion, followed by preincubation for 30 min in Krebs-Ringer bicarbonate buffer gassed with O_2/CO_2 (19:1) supplemented with 3 mM glucose and bovine serum albumin (BSA) 5 mg ml^{-1}.

For the measurement of insulin release, groups of 3 islets were incubated at 37°C for 1 h in 0.5 ml of solutions gassed with O_2/CO_2 (19:1) containing (mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 20, HEPES 10 and 5 mg ml^{-1} BSA under the indicated conditions. When K^+ was raised to 25 mM, Na^+ was reduced by an equivalent amount in order to keep the osmolarity constant. The pH of the solution was adjusted to 7.4 with NaOH. All media for incubation of islets and enzyme immunoassay contained 0.2% dimethyl sulphoxide. Insulin content in the medium was determined by enzyme immunoassay using an EIA kit Mitsui II (Mitsui pharmaceutical industry, Tokyo).

For the measurement of cyclic AMP, groups of 5 islets were incubated at 37°C for 1 h in 0.2 ml of the same solutions as those for insulin release experiments. Then HCl and EDTA were quickly added to the reaction mixture to make final concentrations of 0.1 N and 5 mM, respectively, as described by Katada & Ui (1979). The acidified mixture was then put in a boiling water bath for 3 min to extract cyclic AMP. After centrifugation, cyclic AMP in the supernatant was succinylated and determined with a commercially available kit (Yamasa Syoyu Co., Ltd., Tokyo). Thus cyclic AMP accumulation was expressed as the total amount of cyclic AMP which was released in the medium and accumulated in the islets.

ENX [1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid sesquihydrate], pipemidic acid (PPA) {8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl) pyrido [2,3- d] pyrimidine-6-carboxylic acid trihydrate}, sparfloxacin (SPFX) [5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid] were from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). LFLX [(±)-1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride], and tosufloxacin (TFLX) [(±)-7-(3-amino-1-pyrrolidinyl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid *p*-toluenesulphonate hydrate] were from Shionogi & Co., Ltd. (Osaka) and Toyama Chemical Industry Co., Ltd. (Tokyo),

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respectively. They were dissolved in 0.1 N NaOH to obtain stock solutions of 25 mM. Verapamil hydrochloride was from Eisai Co., Ltd. (Tokyo). Forskolin (Calbiochem, La Jolla, CA) was dissolved in 95% ethanol, 12-O-tetradecanoyl phorbol-13-acetate (TPA) (Sigma) in dimethyl sulphoxide, and diazoxide (Sigma) in 0.1 N NaOH to obtain stock solutions of 20 mM, 100 μM and 40 mM, respectively. Data are given as means ± s.e. mean and statistical significance was assessed by ANOVA with Duncan's multiple range test or Williams-Wilcoxon's test (for data that were not normally distributed).

Results

Effects of various quinolones on insulin release at 3 and 10 mM glucose

In the presence of 3 mM glucose, basal insulin release from 3 islets for 1 h was below the minimum detectable limit (0.04 ng h^{-1} per islet) of the immunoassay used. When ENX, LFLX, or TFLX at concentrations of 10 and 100 μM was added, insulin release remained undetectable. However, LFLX or SPFX at 1 mM and PPA at concentrations of 100 μM or more induced insulin release (LFLX: 0.80 ± 0.13, SPFX: 0.24 ± 0.03, PPA (100 μM): 0.14 ± 0.02, PPA (1 mM): 0.29 ± 0.04 ng h^{-1} per islet, $n = 15-20$). ENX or TFLX at a concentration of 1 mM could not be dissolved and were not tested. The amount of insulin release induced by 10 mM glucose was 4.9 ± 0.5 ng h^{-1} per islet. Addition of the quinolones augmented the release in a dose-dependent manner (Figure 1). The augmenting effects of LFLX and PPA were significant ($P \leq 0.05$) at 10 μM or more and at 100 μM or more, respectively. Those of both quinolones were maximal at a concentration of 100 μM and decreased at 1 mM ($0.01 < P \leq 0.05$ between 100 μM and 1 mM LFLX; not significant for PPA). TFLX produced a similar pattern but the dose-response curve was shifted to the left by one order. The maximal insulin release was 159% of the control for LFLX, 135% for PPA, and 172% for TFLX. ENX at 10 and 100 μM or SPFX at concentrations between 10 μM and 1 mM continued to augment glucose-induced insulin release significantly ($P \leq 0.05$); ENX at 100 μM induced insulin release to 154% of the control and SPFX at 1 mM to 185%. Thus all the quinolones tested in the present study had a stimulatory effect on insulin release. In the following experiments, 100 μM LFLX was used to study the mechanism underlying the insulinotropic effects.

Effect of LFLX on insulin release induced by various concentrations of glucose

Addition of 100 μM LFLX shifted the dose-response curve of glucose-induced insulin release to the left without affecting the basal release in the absence or presence of 3 mM glucose and the maximal release at 30 mM glucose (Figure 2). The increments in percentage change of insulin release augmented by LFLX were 505%, 96% 26%, and 12% (not significant) of that induced by glucose alone at concentrations of 8, 10, 20, and 30 mM, respectively. The K_M value was reduced from 10.0 mM to 8.2 mM by quinolone. Thus the augmenting effect of LFLX was more marked near the threshold concentration of glucose than at higher concentrations.

Effect of LFLX on insulin release induced by high K^+, forskolin or TPA in the presence of 10 mM glucose

Three experimental conditions were tested to investigate the mechanisms by which the quinolone could exert its effect on insulin release: the concentration of K^+ was increased to 25 mM to depolarize the plasma membrane and fully open Ca^{2+} channels (Arkhammar *et al.*, 1987); 5 μM forskolin (Malaisse *et al.*, 1984) and 100 nM TPA (Hughes & Ashcroft, 1988) were used to activate adenylate cyclase and protein kinase C, respectively. In these conditions the selected concentrations were almost maximally effective. Figure 3 shows that LFLX failed to affect glucose-induced insulin release augmented by high K^+ but increased the release augmented by

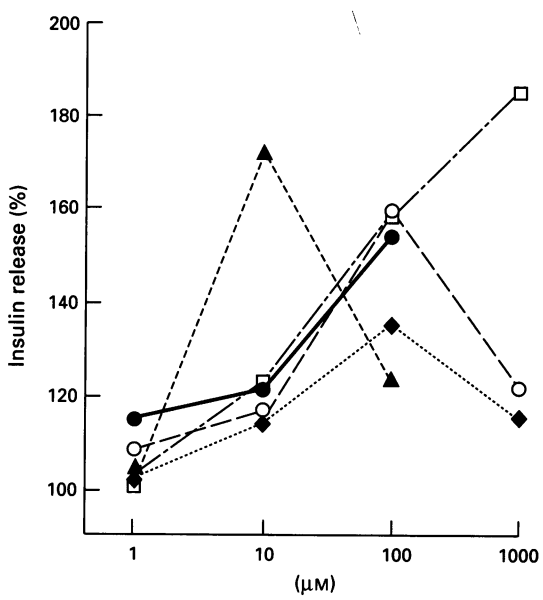

Figure 1 Augmentation of insulin release by various quinolones from rat pancreatic islets in the presence of 10 mM glucose. Groups of 3 islets were incubated for 1 h at 37°C in a medium containing 10 mM glucose and indicated concentrations of ENX (●), LFLX (○), PPA (◆), SPFX (□) and TFLX (▲). Insulin release is expressed as a % of the value found in paired groups of islets incubated in the presence of 10 mM glucose alone. Values are means for 20 observations from 4 independent experiments; s.e.mean is not shown for clarity of presentation. For abbreviations in this and subsequent figures, see text.

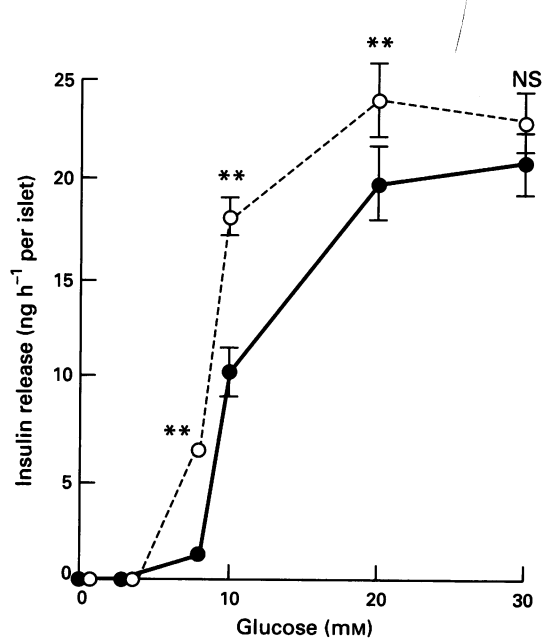

Figure 2 LFLX augmentation of glucose-induced insulin release from rat pancreatic islets. Groups of 3 islets were incubated for 1 h at 37°C in the medium containing the indicated concentrations of glucose with (○) or without 100 μM LFLX (●). Values are means ± s.e.mean for 20 observations from 4 independent experiments. Significance levels: $^{**}P \leq 0.01$ vs the value with glucose alone; NS, not significant.

addition of forskolin or TPA. Thus LFLX seemed to exert its effect on insulin release in a similar way to high K^+ but not activation of adenylate cyclase or protein kinase C.

Effect of LFLX on cyclic AMP accumulation

In the presence of 10 mM glucose, cyclic AMP accumulation was 43 ± 4 fmol h^{-1} per islet ($n=20$), which was not influenced by addition of LFLX (48 ± 3 fmol h^{-1} per islet, $n=20$). Forskolin augmented glucose-induced cyclic AMP accumulation by about 9 times (382 ± 15 fmol h^{-1} per islet, $n=20$). This effect was not augmented but rather reduced by further addition of LFLX (233 ± 16 fmol h^{-1} per islet, $n=20$, $P \leqslant 0.01$).

Effect of verapamil on LFLX-augmented insulin release

The results obtained in the above experiments suggested that the insulinotropic action of LFLX was mediated via a calcium signal. Therefore verapamil, a Ca^{2+} channel blocker, was used to clarify the action of LFLX. As shown in Figure 4, verapamil (50 μM) abolished insulin release caused by glucose alone or by the combination of glucose and LFLX, or glucose and high K^+. Since it was possible that verapamil affected only glucose-induced insulin release and thereby influenced the release augmented by LFLX indirectly, the effect of verapamil was also tested in the presence of a non-stimulatory concentration of glucose. In the presence of 3 mM glucose, 1 mM LFLX-induced insulin release (1.3 ± 0.07 ng h^{-1} per islet, $n=20$) was markedly inhibited by 50 μM verapamil (0.28 ± 0.03 ng h^{-1} per islet, $n=20$, $P \leqslant 0.01$).

Effect of diazoxide on LFLX-induced insulin release

At 3 mM glucose, 400 μM diazoxide, which is known to open K^+-ATP channels (Trube *et al.*, 1986), did not influence basal insulin release. Over the range of 50–400 μM, diazoxide inhibited insulin release caused by 1 mM LFLX in a dose-dependent manner ($IC_{50}=70$ μM) (Figure 5). At 10 mM glucose, insulin release was stimulated and was inhibited in a dose-dependent manner by diazoxide ($IC_{50}=15$ μM) (Figure 6) as reported previously (Henquin *et al.*, 1982). This dose-response curve for diazoxide-inhibition of glucose-induced release was shifted to the right by 100 μM LFLX ($IC_{50}=160$ μM).

Discussion

The present data provide the first evidence that quinolone antibiotics (PPA, ENX, LFLX, SPFX, and TFLX) at concentrations of 10–1000 μM increase insulin release from rat pancreatic islets. The maximal serum levels of these antibiotics found in healthy subjects are up to about 30 μM (Shimizu *et al.*, 1975; Soejima, 1990). In one case where the serum concentration of ENX was measured when hypoglycaemia occurred, it was reported to be 48 μM and the serum concentration of IRI 1.02 nM (Toyoda *et al.*, 1991). After

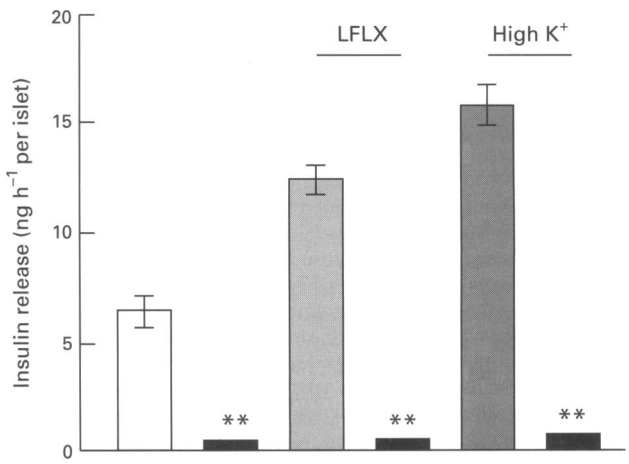

Figure 4 Verapamil inhibition of insulin release augmented by LFLX or high K^+ in the presence of 10 mM glucose. Groups of 3 islets were incubated for 1 h at 37°C in the medium containing 10 mM glucose with or without the indicated test substance (100 μM LFLX, 25 mM K^+ or 50 μM verapamil (■)). Values are means ± s.e.mean for 20 observations from 4 independent experiments. Significance levels: $^{**}P \leqslant 0.01$ vs each control without verapamil.

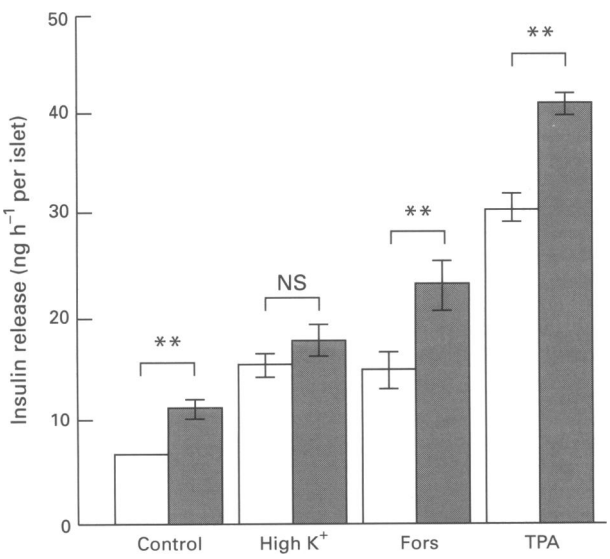

Figure 3 Effects of LFLX on insulin release augmented by high K^+, forskolin and TPA in the presence of 10 mM glucose. Groups of 3 islets were incubated for 1 h at 37°C in the medium containing 25 mM K^+ (high K^+), 5 μM forskolin (Fors), or 100 nM TPA with (■) or without (□) 100 μM LFLX in the presence of 10 mM glucose. Values are means ± s.e.mean for 20 observations from 4 independent experiments. Significance levels: $^{**}P \leqslant 0.01$ vs each control without LFLX; NS, not significant.

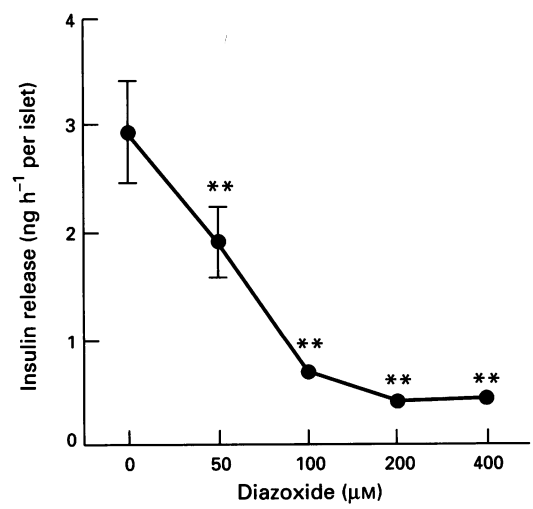

Figure 5 Diazoxide inhibition of insulin release induced by 1 mM LFLX in the presence of 3 mM glucose. Groups of 3 islets were incubated for 1 h at 37°C in a medium containing 3 mM glucose and 1 mM LFLX in the presence of the indicated concentrations of diazoxide. Values are means ± s.e.mean for 20 observations from 4 independent experiments. Significance levels: $^{**}P \leqslant 0.01$ vs the control without diazoxide.

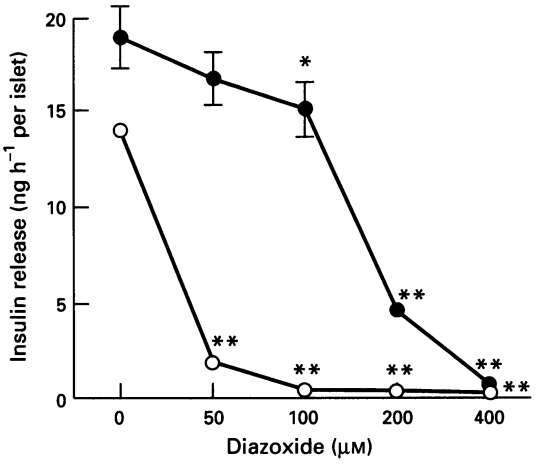

Figure 6 Diazoxide inhibition of insulin release augmented by LFLX in the presence of 10 mM glucose. Groups of 3 islets were incubated for 1 h at 37°C in a medium containing 10 mM glucose alone (○) or with glucose and 100 μM LFLX (●) in the presence of the indicated concentrations of diazoxide. Values are means ± s.e. mean for 20 observations from 4 independent experiments. Significance levels: $0.01 < *P \leqslant 0.05$; $**P \leqslant 0.01$ vs each control without diazoxide.

haemodiafiltration treatment, hypoglycaemia disappeared and the serum concentrations of ENX and IRI decreased to 35 μM and 660 pM, respectively. Thus it is possible that a serum level of the quinolone in patients whose renal function is impaired may reach a level 10 times as high as the maximal serum level (about 4 μM) in healthy subjects. It is known that *in vivo* there are substances other than glucose which can stimulate insulin release, e.g. other sugars like mannose and fructose, amino acids, fatty acids, and some gastrointestinal hormones (Henquin, 1994). Taken together, the present finding that ENX at 10 μM or more induced insulin release in the presence of a stimulatory concentration of glucose may explain the ENX-induced hyperinsulinaemia in man. Although 1 mM LFLX induced insulin release in the presence of 3 mM glucose, this would not happen *in vivo* because such a concentration is far above the maximal serum level of LFLX in healthy subjects. However, it is possible that PPA may cause hypoglycaemia in man because PPA at a concentration of 100 μM induced insulin release and the maximal serum level of PPA is reported to be 15–30 μM.

The mechanism of stimulation of insulin release by quinolones was explored. Since LFLX shifted the dose-response curve of glucose-induced insulin release to the left without affecting the maximal release, the quinolone was considered to act through mechanisms also involved in glucose-induced insulin release. The sugar is known to activate three major second messenger systems (Malaisse-Lagae & Malaisse, 1971; Fex & Lernmark, 1972; Charles *et al.*, 1973; Tanigawa *et al.*, 1982; Rorsman *et al.*, 1984; Prentki & Matschinsky, 1987). Thus we used three experimental conditions to determine by which system LFLX influenced insulin release and found that Ca^{2+} influx was important for the action of the quinolone based on the following lines of evidence: (1) the effects of LFLX and high K^+ on glucose-induced insulin release were not additive in contrast to those of LFLX and forskolin or those of LFLX and TPA; (2) verapamil, a Ca^{2+} channel blocker, prevented the insulinotropic action of the quinolone; (3) LFLX did not increase cyclic AMP accumulation on augmenting insulin release induced by glucose or by the combination of glucose and forskolin. Unexpectedly it inhibited cyclic AMP accumulation in the presence of glucose and forskolin. This may be due to activation of cyclic AMP phosphodiesterase by an increase in the cytosolic Ca^{2+} concentration (Sugden & Ashcroft, 1981; Lipson & Oldham, 1983), which will be caused by LFLX.

It is known that many insulin secretagogues, e.g. glucose and hypoglycaemic sulphonylureas, close K^+-ATP channels (Ashcroft *et al.*, 1984; Trube *et al.*, 1986) and decrease K^+ efflux (Sehlin & Taljedal, 1975; Henquin, 1978; Henquin & Meissner, 1982). The subsequent depolarization of the plasma membrane results in opening of voltage-dependent Ca^{2+} channels and an increase in cytosolic Ca^{2+} concentration (Arkhammar *et al.*, 1987). Conversely, diazoxide inhibits insulin release by opening K^+-ATP channels. Since LFLX could overcome the effect of diazoxide as shown in Figure 6, we suggest that the quinolone increases Ca^{2+} influx by closing K^+-ATP channels. There are many other substances which also act at the same level (Henquin, 1990). Quinolone antibiotics may be added to this list.

Quinine is considered to increase insulin release by blocking K^+-ATP channels (Henquin, 1990). Because both this drug and quinolone antibiotics have quinoline in their structure, quinoline may be important in the insulinotropic actions. Although PPA augmented glucose-induced insulin release, the maximal insulin release that it caused was considerably less than any other quinolone tested in this study. Since only PPA does not have fluorine attached to the 6-carbon of the quinolone ring, fluorine may augment the insulinotropic action of quinolones. The most potent quinolone in the insulinotropic action was TFLX, which differs from ENX by the presence of the 2,4-difluorophenyl group substituted for the ethyl group attached to the 1-nitrogen and the aminopyrrolidinyl moiety substituted for the piperazinyl moiety attached to the 7-carbon of the quinolone ring.

In conclusion, quinolone antibiotics may cause hypoglycaemia by increasing insulin release via blockade of K^+-ATP channels.

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