**Short Communication**

# Effect of Peroxisome Proliferator-Activated Receptor $\alpha$ Ligand Fenofibrate on $K_v$ Channels in the Insulin-Secreting Cell Line HIT-T15

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**Abstract.** Ligands for peroxisome proliferator-activated receptors $\alpha$ (PPAR$\alpha$) are clinically used for the treatment of patients with hyperlipidemia. As we have previously shown, a synthetic ligand of PPAR$\alpha$, fenofibrate, has a stimulatory effect on insulin secretion in clonal hamster insulinoma $\beta$-cell line HIT-T15 cells. We have also demonstrated that fenofibrate directly inhibits ATP-sensitive potassium ($K_{ATP}$) channels, an effect independent of PPAR$\alpha$. In this study, fenofibrate was shown to be able to reduce voltage-dependent $K^+$ ($K_v$) channel currents in voltage-independent manner. Therefore, fenofibrate may modulate insulin secretion not only *via* inhibition of $K_{ATP}$ channels but also *via* reduction of the $K_v$ channel current.

**Key words:** PPAR — Fenofibrate — $K_v$ channel — Insulin secretion — Pancreatic $\beta$-cell

Insulin secretion is stimulated by the closure of the ATP-sensitive potassium ($K_{ATP}$) channels in the $\beta$-cell membrane (Rorsman and Trube 1985; Ashcroft and Rorsman 1989; Ashcroft and Gribble 1999). The $K_{ATP}$ channel plays a major role in regulating the membrane potential of $\beta$-cells as it is able to respond to the metabolic state of the cell. The reduction in membrane $K^+$ permeability by closure of the $K_{ATP}$ channels depolarizes the $\beta$-cell membrane. This activates the voltage-dependent

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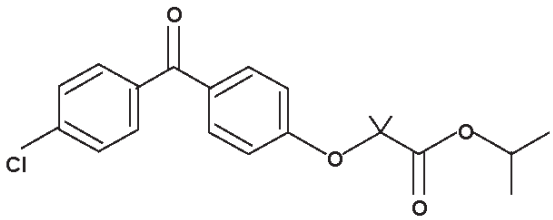


**Figure 1.** Chemical structure of fenofibrate.

$Ca^{2+}$ channels (VDCC) and resulting $Ca^{2+}$ influx stimulates insulin release (Rorsman 1997; Ashcroft and Gribble 1999). Voltage-dependent $K^+$ ($K_v$) channels modulate insulin secretion through repolarizing $\beta$-cell membrane potential during their activation (MacDonald and Wheeler 2003).

The intracellular nuclear receptors, peroxisome proliferator-activated receptors (PPAR), control gene transcription activity and affect protein synthesis (Braissant et al. 1996). PPAR subtypes, $\alpha$, $\gamma$ and $\delta$, show distinctive tissue distributions, and are associated with selective ligands (Braissant et al. 1996). Once activated by ligands, PPAR heterodimerize with the retinoic X receptor and alter the transcription of target genes after binding to response elements. PPAR$\alpha$ is known to regulate genes involved in fatty acid metabolism in liver. Fenofibrate, a synthetic ligand for PPAR$\alpha$ with a molecular weight of 360.8 g/mol (Fig. 1), reduces serum triglycerides concentration levels in patients with hyperlipidemia. This effect is mediated *via* the induction of fatty acid oxidation through activation of PPAR$\alpha$ (Lee et al. 2003).

We have previously demonstrated that fenofibrate directly inhibits $K_{ATP}$ channels in pancreatic $\beta$-cell membrane and stimulates insulin secretion. In the present study, we show that fenofibrate also reduces $K_v$ channel currents. Inhibition of $K_v$ channels by fenofibrate is expected to prolong the depolarization of $\beta$-cell membrane; consequently, it may enhance calcium influx *via* VDCC and thus modulate the insulin secretion.

Fenofibrate was kindly provided by Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Fetal bovine serum was purchased from GIBCO (Grand Island, NY, USA), F-12K medium in powder form was purchased from Flow Laboratories Inc. (Irvine, Scotland, UK). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

HIT-T15 cells were purchased from Flow Laboratories Inc. (Irvine). The cells were cultured in F-12K medium containing 7 mmol/l glucose and supplemented with 10% fetal bovine serum, and incubated in a 95% $O_2$-5% $CO_2$ incubator at 37°C.

Conventional whole-cell patch-clamp recordings were performed using Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA). The standard extracellular solution contained (in mmol/l): 160 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10

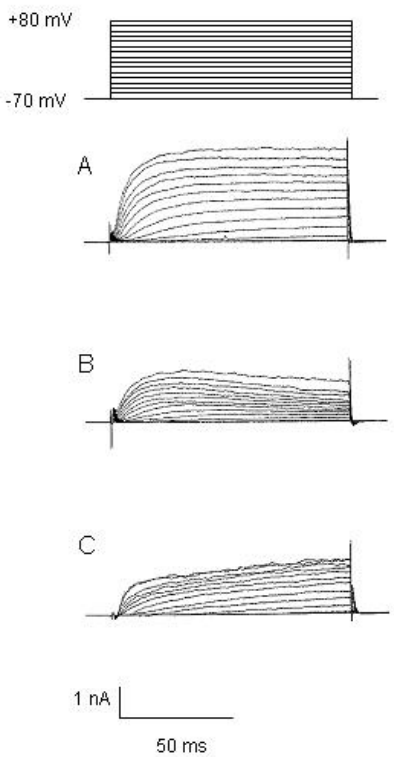


**Figure 2.** Block of $K_v$ currents in HIT-T15 fenofibrate. Outward $K_v$ currents were elicited by a voltage protocol schematically drawn on top. **A.** control solution; **B.** 100 $\mu$mol/l fenofibrate; **C.** fenofibrate-sensitive $K_v$ currents derived by digital subtraction of currents in the presence of the drug from those in the absence.

HEPES, 0.5 glucose (pH 7 adjusted with NaOH). The pipette solution contained (in mmol/l): 150 KCl, 10 HEPES, 1 EGTA (to inhibit $Ca^{2+}$-sensitive $K_v$ channels), 1 $MgCl_2$, 0.1 $CaCl_2$, 5 MgATP (to block the $K_{ATP}$ channel current). The pH of the pipette solution was adjusted to 7.2 by KOH. The resistance of patch pipettes when filled with the pipette solution ranged from 2–4 M$\Omega$. Following G$\Omega$ seal formation, a negative pressure was applied to the pipette to rupture the membrane and establish the whole-cell mode. In order to study outward $K_v$ currents, the cells were held at $-70$ mV and the currents were evoked by 100 ms voltage depolarizations to voltage values between $-60$ and $+80$ mV with a 10 mV increment. All experiments were performed at room temperature (22–25°C). All data represent mean $\pm$ S.E. The statistical analysis of the data was performed by using the Students $t$-test.

The effect of fenofibrate on outward $K_v$ channels recorded from HIT-T15 cells is presented in Fig. 2. Fig 2A shows the control current. As shown in Fig. 2B, application of 100 $\mu$mol/l fenofibrate to the intracellular solution dramatically reduced the amplitude of the outward $K^+$ current. In addition, fenofibrate also induced a mild time-dependent inactivation of current (Fig. 2B). Fig. 2C shows fenofibrate-sensitive $K_v$ components, derived by digital subtraction of current in the presence

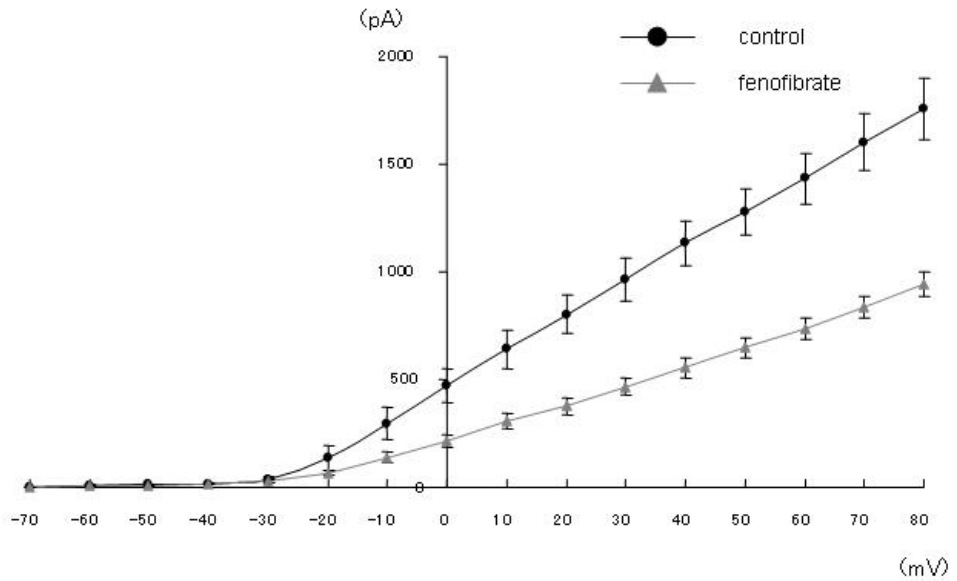


**Figure 3.** The current-voltage relationship of the mean maximum sustained current, plotted for both control solution (closed circle) and in the presence of 100 $\mu$mol/l fenofibrate (triangle). $n = 6$ in each group.

of fenofibrate from current in its absence. It is clear from the analyzed data that the effect of fenofibrate on the amplitude of the $K_v$ current is voltage-independent; the mean extent of block amounted to $60.4 \pm 7.3\,\%$ ($n = 6$, $p < 0.01$; Fig. 3). This inhibition of $K_v$ channel currents by fenofibrate was reversible upon washing after exposure of 10 min. We have also observed that the stimulatory effect on insulin secretion by fenofibrate did not persist when the compound was applied for more than 2 h, which suggests that fenofibrate does not affect stimulus-secretion coupling *via* modulation of gene transcription activity in HIT-T15 cells. The physiological role of $K_v$ channel in pancreatic $\beta$-cells is to restore the cell membrane to hyperpolarized state after the activation of VDCC (Misler et al. 1992; MacDonald and Wheeler 2003; Proks and Lippiat 2006). By restoring cell membrane to hyperpolarized state, $K_v$ channel serve as a determinant of the frequency and duration of $Ca^{2+}$-dependent action potentials in $\beta$-cells (Bokvist et al. 1990; Satin et al. 1994). Enhanced level of $K_v$ channel activity have been reported to impair insulin secretion through shortening of action potential duration (Phillipson et al. 1994). Conversely, inhibition of $K_v$ channels by their antagonist, tetraethylammonium, induced prolonged action potentials and enhancement of insulin secretion in $\beta$-cells (Atwater et al. 1979; Henquin et al. 1979; MacDonald et al. 2002).

There are 11 mammalian $K_v$ channel families currently known (MacDonald and Wheeler 2003). Recent work identified $K_v2.1$ as a major contributor to $K_v$

currents in rodent pancreatic $\beta$-cells and insulinoma cells, including HIT-T15 cells, which were used in our experiments. By using dominant-negative approach, MacDonald and co-workers have reported that $K_v2.1$ contributes 60–70 % of the $K_v$ current in HIT-T15 cells and regulates glucose-induced insulin secretion (MacDonald et al. 2001).

We have previously demonstrated the existence of a PPAR-independent pathway of fenofibrate action, which directly inhibits $K_{ATP}$ channels in the $\beta$-cell membrane (Shimomura et al. 2004). In the present study, we have shown that fenofibrate also exerts an effect on $K_v$ channels. It has been shown that hanatoxin, the most specific $K_v2.1$ channel inhibitor known, profoundly affects oscillatory intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) responses in human and mouse islets. At high glucose concentration, addition of hanotoxin induces slow $[Ca^{2+}]_i$ oscillations in human and mouse islets (Tamarina et al. 2005). The mathematical model used in this study predicts 5–20 % reduction of $K_v$ conductance which is sufficient to induce slow $[Ca^{2+}]_i$ oscillations and membrane potential spikes with increased spike frequency and amplitude in the active period. Fenofibrate at 100 $\mu$mol/l (the clinically relevant concentration; Caldwell 1989) inhibited $K_v$ channel up to 60 %; thus, applications of clinically relevant concentrations of this drug should be enough to induce the change in $[Ca^{2+}]_i$ oscillations and spike activity. Therefore, in addition to $K_{ATP}$ channel inhibition, fenofibrate is likely to cause a reduction in $K_v$ channel currents which prolongs the depolarization of the cell membrane and promotes insulin secretion.

Compounds which not only stimulate insulin secretion *via* closure of $K_{ATP}$ channels but can also augument insulin secretion by inhibition of $K_v$ channels, would have an additional beneficial effect of lowering glucose level in diabetic patients. To date, nateglinide, which is considered to be a $K_{ATP}$ channel inhibitor, was found to inhibit $K_v$ channels (Hu and Wang 2001). Our study suggests that, like nateglinide, fenofibrate could be useful for the treatment of type 2 diabetic patients due to its ability to block both $K_{ATP}$ and $K_v$ channels. However, since $K_v2.1$ channels are expressed in a number of extrapancreatic tissues, the possibility of side-effects needs to be investigated. Thus, further studies are required to determine the suitability of fenofibrate as a therapeutic agent in the treatment of type 2 diabetes.

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