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The Non-Genomic Effects of the PPARβγ agonist GW0742 on STZ treated rat aorta

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Background: The ubiquitous nuclear receptor PPARβ/δ is increasingly being studied in regards to numerous diseases including diabetes following on the finding that PPARβ/δ agonist GW0742 controls Type 1 Diabetes in rats. Studies have shown that GW0742 has off target, non- PPARβ/δ effects in the cell although there are some key questions that remain to be addressed in respect to the significance of this control on vascular tone.

Methods: Using isometric organ baths, rat aorta rings were exposed to ROCK inhibitors and the changes in contraction and dilation measured.

Results: Our data shows that the PPARβ/δ agonist GW0742 (10^{-7}M) inhibits contractile responses to U46619 and phenylephrine, and that these responses are similar in normal and diabetic rat aorta. ROCK inhibitors Fasudil and Y27632 significantly reduced GW0742 mediated dilation of naïve rat aorta, but Fasudil had no effect on GW0742 dilation in STZ diabetic rat aorta. In contrast, STZ diabetic rat aorta pre-contracted with high [K^+] Krebs lacked a dilatory response to GW0742, which taken together indicates that the mechanism of action of GW0742 mediated dilation changes in the diabetic state compared to non-diabetic state.

Conclusion: This is the first direct evidence demonstrating the non- PPARβ/δ effect of GW0742 on contraction is irrespective to the diabetic state, and that GW0742 has the potential to induce vasodilation via multiple off-target mechanisms.

Keywords: PPAR, ROCK, Vasodilation, Artery, diabetes.

1. INTRODUCTION

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. PPARβ/δ is present in all animal cells, from close versions in *C. elegans* and drosophila to all mammals tested, yet the biology of how this ubiquitous protein controls gene expression is poorly understood. Signaling via PPARβ/δ has a number of effects on cell function, including lipid metabolism [1], glucose metabolism [2], insulin sensitivity [2], and inflammation [3]. There are complex mechanisms by which PPARβ/δ controls gene transcription through induction and transrepression of other nuclear receptors which are yet to be fully defined [4].

1.1. GW0742 is a potential therapeutic agent

The PPARβ/δ agonist GW501516 completed proof-of-concept clinical trials successfully for hypocholesteremia [1], with further potential as treatments for Type I diabetes [5, 6]. Direct activation of PPARβ/δ with agonist GW0742 (a close mimetic of GW501516) in rats treated with Streptozotocin (STZ; a model of Type I diabetes) improves insulin signaling [5] and glucose homeostasis [6]. Therefore GW0742 has the potential to be a novel therapeutic agent.

1.2. Non-Genomic Effects of PPARβ/δ

Surprisingly for a nuclear receptor, PPARβ/δ has non-genomic roles within the cell, showing direct binding and activity with proteins to alter the function of cells. The first example of this phenomenon was demonstrated in platelets, where the direct binding of activated PPARβ/δ receptor to PKCα inhibited aggregation [7]. Previous studies have shown that GW0742 inhibits contraction [8], but little is known about the concentration range at which this occurs, and whether the same phenomenon is seen in diabetes. The PPARβ/δ agonist GW0742 induces full vasodilation of aorta, pulmonary artery and mesenteric artery, and it has been proposed that this occurs due to the inhibition of GTP-RhoA formation [8]. Active GTP-RhoA activates Rho associated protein kinase (ROCK), the downstream effector protein; inhibition of either RhoA or ROCK will induce a lack of contraction. Direct pharmacological evidence of the effects of GW0742 on inhibiting ROCK mediated contraction has so far not been demonstrated possibly due to the complication of ROCK inhibition inducing a lack of contractile tone. ROCK is involved in contractile responses to phenylephrine and to a much lesser extent U46619 [9]. Here we used a protocol that allowed measurement of GW0742 on ROCK independent U46619 contraction. Our results demonstrate that GW0742 inhibits contraction of aorta from naïve and STZ Type 1 diabetic rat, and that GW0742 induces vasodilation mediated in part by the RhoA/ROCK pathway in naïve rat aortas and potassium channels in STZ diabetic rat aortas.

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2. MATERIALS AND METHODS

Male Wistar rats (350-450g) sourced from Charles River were housed in pairs and given a single injection of 65mg/kg Streptozotocin (STZ; dissolved in 20mM citrate buffer, pH 4.5) intraperitoneally (i.p. 10ml/kg). For 48 hours following STZ or control injection (20mM citrate buffer) an additional choice of 2% sucrose solution was provided to avoid the initial hypoglycemia that is seen following STZ. Rats were monitored for a minimum of 2 weeks post-injection, and diabetes was confirmed by testing a drop of tail vein blood (Accu-check blood glucose monitor); rats showing an elevated blood glucose of >16mmol/L were considered diabetic and used in this study.

The rats were killed by CO_2 asphyxiation and their thoracic aortas were rapidly removed and dissected in Krebs's buffer (pH 7.4, 118mM NaCl, 4.7mM KCl, 1.2mM $MgSO_4$, 1.2mM KH_2PO_4, 2.5mM $CaCl_2$, 25.0mM $NaHCO_3$ and 11.0mM glucose). Aortas were cut into 2mm wide rings after removing the surrounding connective tissue and fat. Each ring was suspended with silk thread in a 20ml organ bath filled with Krebs's buffer and maintained at 37°C. The upper end of the silk was connected to a force transducer and the lower one connected to an L-shaped mounting hook, and the aorta rings maintained an isometric force of 2g for a one hour equilibration time and then throughout the experiments. The bath solution was continuously bubbled with 95% O_2 and 5% CO_2.

Aorta rings from naïve and STZ diabetic rats were incubated with 10^{-9}, 10^{-8} or 10^{-7}M GW0742 or vehicle control (DMSO; final bath concentration 0.01%) for 30 minutes, followed by increasing concentrations of phenylephrine (10^{-8} to 10^{-5}M) or U46619 (10^{-9} to $3x10^{-7}$M). Some tissues were incubated with ROCK inhibitors Fasudil (10^{-6}M) or Y27632 (10^{-6}M) for 30 minutes followed by a response to increasing concentrations of U46619 (10^{-9} to $3x10^{-7}$M).

In order to measure the ROCK inhibitory effects of W0742, arteries were exposed to 10^{-6}M Fasudil or 10^{-6}M Y27632 for 30 minutes, pre-contracted with 10^{-7}M U46619 and exposed to increasing concentrations of GW0742 (10^{-6}M to $3x10^{-5}$M). In order to determine the extent to which potassium channels are involved in GW0742 mediated dilation, some aorta rings were pre-contracted with high potassium Krebs's buffer (pH 7.4, 124mM KCl, 1.2mM $MgSO_4$, 1.2mM KH_2PO_4, 2.5mM $CaCl_2$, 25.0mM $NaHCO_3$ and 11.0mM glucose) followed by increasing concentrations of GW0742 (10^{-6}M to $3x10^{-5}$M).

3. EXPERIMENTAL

Vasodilation is expressed as percentage of the U46619 mediated pre-contraction obtained prior to the addition of GW0742. Data are presented as mean ± SEM; n refers to the number of rats used. Statistical analysis was performed by two way analysis of variance using GraphPad Prism 5.0, and differences were considered to be significant when P was less than 0.05.

4. RESULTS

Effect of increasing concentrations of GW0742 on rat aorta contraction

Phenylephrine induced contraction of aorta from diabetic rats is significantly increased compared to aorta from naïve rat aortas (Figure 1A), in comparison to U46619 mediated contraction which shows no significant difference (Figure 1B). U46619 may only be tested up until a concentration of $3x10^{-7}$M since any further addition induces an irreversible contraction.

*Figure 1. Change in tension induced by A. Phenylephrine (PE), B. U46619 in naïve and STZ diabetic rat aorta; data is represented as mean ± SEM. Significant difference compared to vehicle controls was analysed by two-way ANOVA and denoted by ***= p<0.001, 'ns'= non-significance; n=4-6*

Aorta segments from naïve and STZ diabetic rats responded to increasing concentrations of GW0742 in a similar manner. Incubation in the presence of 10^{-9}M and 10^{-8}M GW0742 had no effect on U46619 or phenylephrine (Table 1) mediated contraction in aorta from naïve or STZ diabetic rats. Whereas 10^{-7}M GW0742 significantly decreased contraction to U46619 and phenylephrine (Table 1) in both naïve and STZ diabetic rat aorta.

*Table 1. Change in tension induced by U46619 in naïve control and STZ diabetic rat aorta following incubation with 10^{-9}M, 10^{-8}M or 10^{-7}M GW0742. E_{max} is represented as mean ± SEM. Significant difference compared to vehicle controls was analysed by two-way ANOVA and denoted by * = p<0.05, ***= p<0.001; n=4-6 for all groups*

Effects of ROCK inhibitors on aorta contraction

ROCK inhibitors Y27632 and Fasudil led to a loss of phenylephrine mediated contraction in naïve rat aorta segments from E_{max} 0.427 ± 0.05g Vehicle control to 0.053 ± 0.02g Y27632 treated and 0.174 ± 0.062g Fasudil treated rat aortas (Figure 2A). In contrast, ROCK inhibitors reduced U46619 mediated contraction in naïve rat aorta with E_{max} 0.551 ± 0.07g to E_{max} 0.313 ± 0.04 when incubated with Y27632 and E_{max} 0.45 ± 0.09g with Fasudil (Figure 2B). STZ diabetic rat aorta contraction in response to U46619 (E_{max} from 0.811 ± 0.07g in Vehicle control) was significantly reduced by Fasudil to E_{max} 0.449 ± 0.03g, and completely inhibited by Y27632 aortas to Fasudil treated aortas (E_{max} 0.121 ± 0.03g; Figure 2C).

*Figure 2. Contraction in response to A. Phenylephrine (PE, B. U46619 in naïve rat aorta, C. U46619 in STZ diabetic rat aorta following incubation with 10^{-5}M Y27632 and 10^{-5}M Fasudil; data is represented as mean ± SEM. Significant difference compared to vehicle controls was analysed by two-way ANOVA and denoted by **= p<0.01, ***= p<0.001, 'ns' non-significance; fff=p<0.001 by Bonferroni's post hoc test; n=4.*

Effects of ROCK inhibitors on GW0742 mediated vasodilatation

Aorta pre-contracted in the presence of EC_{80} U46619 and exposed to increasing concentrations of GW0742 results in a full relaxation response (E_{max} relaxation -79.4 ± 4.5%; EC_{50} $1.5x10^{-5}$M; Figure 3A), which is significantly reduced in STZ diabetic rat aorta (E_{max} relaxation -54.5 ± 8.6% EC_{50} 1.7 $x10^{-5}$M; Figure 3A). When pre-contracted by high [K^+] Krebs, the E_{max} relaxation in response to GW0742 in naïve control rat aorta was -39.3 ± 1.9%, significantly reduced in STZ diabetic rat aorta to E_{max} relaxation -20.7 ± 2.5% (Figure 3B). Fasudil significantly reduced the GW0742 mediated dilation in naïve rat aorta from E_{max} relaxation 79.4 ± 4.5% in vehicle controls to -54.2 ±3.7% Fasudil treated naïve rat aortas (Figure 3C), but not in STZ diabetic rat aorta (E_{max} relaxation -54.5 ± 8.6% vehicle control and -64.3 ± 8.4% Fasudil incubated STZ diabetic rat aorta; Figure 3D).

*Figure 3. Dilation of Naïve and STZ diabetic rat aorta to GW0742 following pre-contraction to A. U46619 and B. high potassium solution (124mM K^+). The effects of 10^{-5}M Fasudil on GW0742 mediated vasodilation in C. naïve rat aorta and D. STZ diabetic rat aorta. Rat aorta were incubated with 10^{-5}M Fasudil for 30 minutes, pre-contracted with EC_{80} U46619 and exposed to increasing concentrations of GW0742. Data is represented as mean ± SEM. Significant difference compared to vehicle controls was analysed by two-way ANOVA and denoted by *= p<0.05, ***= p<0.001, 'ns' non-significance; f =p<0.05, fff=p<0.001 by Bonferroni's post hoc test; n=4.*

5. CONCLUSION

While it has previously been noted that 10^{-5}M GW0742 significantly inhibits U46619 mediated vasocontraction [8], the concentration at which this inhibition occurs was not known, nor whether this would be altered in a diabetic state [8]. Here we show that in both naïve and STZ diabetic rats 10^{-7}M GW0742 significantly reduces U46619 and phenylephrine mediated contraction, a concentration selective for PPARβ/δ [10, 11]. While GW0742 direct actions on PPARβ/δ function can reduce the effects of diabetes, the off target effects of GW0742 were deemed to also be beneficial in controlling vascular tone. Our data indicates that these off target effects of GW0742 on vascular tone is similar in naïve and STZ diabetic aorta, although the mechanism of actions differs.

In previous studies, GW0742 was shown to inhibit the formation of GTP bound RhoA using aorta tissue extracts in an ELISA assay [8]. While this study is of interest in outlining an important relationship between GW0742 and RhoA-GTP formation, the consequence of this interaction on contractility remained to be defined in native aorta tissue bath experiments.

The RhoA/ROCK pathway mediates smooth muscle contraction; inhibition of this pathway decreases cardiomyopathy in Type 1 diabetic rat models [12]. Inhibition of ROCK/RhoA prevents contraction [9], making the investigation of a ROCK inhibitor's effects on contraction difficult to determine directly. Our approach was to use a contractile agent that induced a non-RhoA mediated contractile response, and measure the effects of GW0742 on tone. In order to achieve this, we incubated aorta with ROCK inhibitors Fasudil and Y27632, and contracted with U46619 or phenylephrine. The phenylephrine mediated contraction was largely abolished following ROCK inhibition in naïve aorta as shown previously [9, 13] thus precluding the use of this contractile agent in the GW0742 dilatory protocol. In contrast, U46619 contraction was significantly reduced following ROCK inhibition but still of sufficient tone for further experimentation. This result confirms previous data showing that U46619 contracts using partially different contractile machinery to phenylephrine [13].

Studies have been conducted to determine the mechanisms by which the activated PPARβ/δ induces vasodilation in arteries, possibly mediated by direct interaction with RhoA [8, 14], activation of PI3-Akt-eNOS pathways [15] or activation of K^+ channels [16]. In order to address the question as to whether PPARβ/δ directly interferes with the RhoA/ROCK pathway and K^+ channels, we took advantage of the dilatory properties of the agonist GW0742. The dilatory effect of GW0742 was significantly reduced in STZ diabetic rat aorta compared to naïve controls, which indicates that the mechanism by which PPARβ/δ induces a loss of tone is affected by the diabetic state.

In order to investigate the contribution of potassium channels to GW0742 mediated dilation, we induced depolarization of the smooth muscle cells with high [K^+] Krebs which inhibited GW0742 induced depolarization. While GW0742 mediated dilation was inhibited in naïve rat aorta pre-contracted with high [K^+] Krebs, it was abolished in STZ diabetic rat aorta, which indicates that the GW0742 mediated dilation of aorta involves K^+ channels more in the diabetic state than in the normal physiological state.

In our protocol, aortas were incubated with Y29632 or Fasudil for 30 minutes, a time frame that is too short to allow for new genes to be inducted. As expected from previous results, both Y29632 and Fasudil reduced U46619 EC_{80} contraction, although to a level that can still be used to maintain a contractile state to witness relaxation induced by GW0742. While the ROCK inhibitors reduced the U46619 mediated contraction, there was sufficient tone to measure GW0742 mediated dilation, although Y27632 abolished the U46619 mediated contraction in STZ diabetic rat aortas, making dilatory responses impossible to determine. The vasodilatory response elicited by 10^{-5}M GW0742 in naïve rat aorta was significantly inhibited by Fasudil incubation, providing direct pharmacological evidence that GW0742 stimulation of PPARβ/δ inhibits the RhoA/ROCK pathway in contracted arteries. Interestingly, Fasudil had no effect on GW0742 mediated dilation in STZ diabetic rat aorta. Taken together with the data of aorta pre-contracted with high [K^+] Krebs, this indicates that there is a shift in PPARβ/δ mediated dilation from ROCK pathway mechanism in naïve rats to more of a K^+ channel involvement in STZ diabetic rat aortas.

PPARβ/δ agonist GW0742 decreases contractile responses to U46619 in both naïve and STZ diabetic rat aorta. Here we show direct pharmacological evidence that GW0742 induces vasodilation mediated in part by the RhoA/ROCK pathway

in naïve rat aortas and potassium channels in STZ diabetic rat aortas..

6. STANDARD PROTOCOL ON ANIMAL PROTECTION

The care and use of all rats in this study was carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act of 1986 under PPL70/7732.

LIST OF ABBREVIATIONS

PPAR; Peroxisome proliferator activated receptors, STZ; Streptozotocin, ROCK; Rho associated protein kinase

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article. The conduct of the research and preparation of the article was funded by the University of Hertfordshire, UK.

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REFERENCES

[1] Olson, E.J.; Pearce, G.L.; Jones, N.P.; Sprecher, D.L., Lipid effects of peroxisome proliferator-activated receptor-delta agonist GW501516 in subjects with low high-density lipoprotein cholesterol: characteristics of metabolic syndrome. Arterioscler Thromb Vasc Biol, 2012, 32, (9), 2289-2294.

[2] Lee, C.H.; Olson, P.; Hevener, A.; Mehl, I.; Chong, L.W.; Olefsky, J.M.; Gonzalez, F.J.; Ham, J.; Kang, H.; Peters, J.M.; Evans, R.M., PPARdelta regulates glucose metabolism and insulin sensitivity. Proc Natl Acad Sci U S A, 2006, 103, (9), 3444-3449.

[3] Barish, G.D.; Atkins, A.R.; Downes, M.; Olson, P.; Chong, L.-W.; Nelson, M.; Zou, Y.; Hwang, H.; Kang, H.; Curtiss, L.; Evans, R.M.; Lee, C.-H., PPAR delta regulates multiple proinflammatory pathways to suppress atherosclerosis. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105, (11), 4271-4276.

[4] Perez-Diaz, N.; Mackenzie, L.S., Linking Induction and Transrepression of PPARβ/δ with Cellular Function. Annual Research and Review in Biology, 2015, 6, (4), 253-263.

[5] Quintela, A.M.; Jimenez, R.; Gomez-Guzman, M.; Zarzuelo, M.J.; Galindo, P.; Sanchez, M.; Vargas, F.; Cogolludo, A.; Tamargo, J.; Perez-Vizcaino, F.; Duarte, J., Activation of peroxisome proliferator-activated receptor-beta/-delta (PPARbeta/delta) prevents endothelial dysfunction in type 1 diabetic rats. Free Radic Biol Med, 2012, 53, (4), 730-741.

[6] Niu, H.S.; Ku, P.M.; Niu, C.S.; Cheng, J.T.; Lee, K.S., Development of PPAR-agonist GW0742 as antidiabetic drug: study in animals. Drug Des Devel Ther, 2015, 9, 5625-5632.

[7] Ali, F.Y.; Hall, M.G.; Desvergne, B.; Warner, T.D.; Mitchell, J.A., PPAR beta/delta agonists modulate platelet function via a mechanism involving PPAR receptors and specific association/repression of PKC alpha-Brief report. Arteriosclerosis Thrombosis and Vascular Biology, 2009, 29, (11), 1871-1873.

[8] Harrington, L.S.; Moreno, L.; Reed, A.; Wort, S.J.; Desvergne, B.; Garland, C.; Zhao, L.; Mitchell, J.A., The PPARbeta/delta agonist GW0742 relaxes pulmonary vessels and limits right heart hypertrophy in rats with hypoxia-induced pulmonary hypertension. PLoS One, 2010, 5, (3), e9526.

[9] Chan, C.K.; Mak, J.C.; Man, R.Y.; Vanhoutte, P.M., Rho kinase inhibitors prevent endothelium-dependent contractions in the rat aorta. J Pharmacol Exp Ther, 2009, 329, (2), 820-826.

[10] Sznaidman, M.L.; Haffner, C.D.; Maloney, P.R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M.L.; LeGrumelec, C.; Xu, H.E.; Montana, V.G.; Lambert, M.H.; Willson, T.M.; Oliver, W.R., Jr.; Sternbach, D.D., Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPARdelta)--synthesis and biological activity. Bioorg Med Chem Lett, 2003, 13, (9), 1517-1521.

[11] Mackenzie, L.S.; Lione, L., Harnessing the benefits of PPARbeta/delta agonists. Life Sci, 2013, 93, (25-26), 963-967.

[12] Guan, S.J.; Ma, Z.H.; Wu, Y.L.; Zhang, J.P.; Liang, F.; Weiss, J.W.; Guo, Q.Y.; Wang, J.Y.; Ji, E.S.; Chu, L., Long-term administration of fasudil improves cardiomyopathy in streptozotocin-induced diabetic rats. Food Chem Toxicol, 2012, 50, (6), 1874-1882.

[13] Budzyn, K.; Paull, M.; Marley, P.D.; Sobey, C.G., Segmental differences in the roles of rho-kinase and protein kinase C in mediating vasoconstriction. J Pharmacol Exp Ther, 2006, 317, (2), 791-796.

[14] Wang, Y.; Yu, L.; Cui, N.; Jin, X.; Zhu, D.; Jiang, C., Differential sensitivities of the vascular KATP channel to various PPAR activators. Biochem Pharmacol, 2013, 85, (10), 1495-1503.

[15] Jimenez, R.; Sanchez, M.; Jose Zarzuelo, M.; Romero, M.; Maria Quintela, A.; Lopez-Sepulveda, R.; Galindo, P.; Gomez-Guzman, M.; Manuel Haro, J.; Zarzuelo, A.; Perez-Vizcaino, F.; Duarte, J., Endothelium-dependent vasodilator effects of peroxisome proliferator-activated receptor beta agonists via the phosphatidyl-inositol-3 Kinase-Akt pathway. Journal of Pharmacology and Experimental Therapeutics, 2010, 332, (2), 554-561.

[16] Li, Y.; Connolly, M.; Nagaraj, C.; Tang, B.; Balint, Z.; Popper, H.; Smolle-Juettner, F.M.; Lindenmann, J.; Kwapiszewska, G.; Aaronson, P.I.; Wohlkoenig, C.; Leithner, K.; Olschewski, H.; Olschewski, A., Peroxisome proliferator-activated receptor-beta/delta, the acute signaling factor in prostacyclin-induced pulmonary vasodilation. American Journal of Respiratory Cell and Molecular Biology, 2012, 46, (3), 372-379..

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