GLP-1 receptor agonists show neuroprotective effects in animal models of diabetes

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Abstract

Enzyme-resistant receptor agonists of the incretin hormone glucagon-like peptide-1 (GLP-1) have shown positive therapeutic effects in people with type 2 diabetes mellitus (T2DM). T2DM has detrimental effects on brain function and impairment of cognition and memory formation has been described. One of the underlying mechanisms is most likely insulin de-sensitization in the brain, as insulin improves cognitive impairments and enhances learning. Treatment with GLP-1 receptor agonists improves memory formation and impairment of synaptic plasticity observed in animal models of diabetes-obesity. Furthermore, it has been shown that diabetes impairs growth factor signalling in the brain and reduces energy utilization in the cortex. Inflammation and apoptotic signalling was also increased. Treatment with GLP-1 receptor agonists improved neuronal growth and repair and reduced inflammation and apoptosis as well as oxidative stress. In comparison with the diabetes drug metformin, GLP-1 receptor agonists were able to improve glycemic control and reverse brain impairments, whereas metformin only normalized blood glucose levels. Clinical studies in non-diabetic patients with neurodegenerative disorders showed neuroprotective effects following administration with GLP-1 receptor agonists, demonstrating that neuroprotective effects are independent of blood glucose levels.

Highlights
- enzyme-resistant receptor agonists of GLP-1 are effective in treating diabetes
- GLP-1 plays important additional roles as a growth factor
- GLP-1 receptor agonists have protective effects in the brain
- impaired insulin signaling is restored by GLP-1 drugs
- other, glucose independent neuroprotective effects are found

1. Introduction

Glucagon-like peptide 1 (GLP-1) is a gut hormone secreted from enteroendocrine L-cells and is best known for its ability to enhance glucose-induced beta cell insulin secretion. In addition, GLP-1 also possesses a range of extrapancreatic actions which helps control blood glucose concentrations. Based on these observations, several GLP-1 receptor agonists that are resistant to enzyme degradation and have enhanced biological half-life in the blood stream have been developed as treatments for type 2 diabetes mellitus (T2DM) (Madsbad et al., 2008; Christensen et al., 2011; Campbell and Drucker, 2013). GLP-1 receptor agonists are effective, well received with few side effects, and widely used throughout the world to treat people with T2DM.

2. Diabetes and cognitive impairment

Studies have found evidence of cross-sectional and prospective associations between T2DM and cognitive impairment, memory and executive function (Stewart and Liolitsa, 1999; Yaffe et al., 2004). As vascular health is impaired by T2DM (Brands et al., 2004), both vascular and non-vascular factors are likely to play a role in causing this effect. Furthermore, T2DM has been identified as a risk factor for developing dementia (Luchsinger et al., 2004; Li et al., 2017). Epidemiological studies have shown a correlation between T2DM and the development of dementia in later life (Leibson et al., 1997; Luchsinger et al., 2004; Ristow, 2004; Strachan, 2005; Biessels et al., 2006; Haan, 2006). In one study, T2DM had been identified as a risk factor that doubled the likelihood of developing dementia (Janson et al., 2004). In a longitudinal study monitoring the health status of people over time, there was an increased risk of developing dementia in people with significantly elevated blood glucose levels (Schrijvers et al., 2010; Ohara et al., 2011; Li et al., 2017).

2.1 Insulin desensitization in the brain

A key parameter in developing T2DM is the desensitization of insulin signaling. Insulin signaling in T2DM is not only affected in the periphery, but in the brain as well (Gispen and Biessels, 2000; Biessels et al., 2002; Baker et al., 2011). In cognitive tests and in labelled deoxyglucose ^{18}FDG -PET brain imaging scans, it was found that people with T2DM exhibited poor performance and much reduced glucose uptake in cortical areas during cognitive testing (Baker et al., 2011). Reduced uptake of ^{18}FDG demonstrated reduced energy turnover and neuronal function. Moreover, brain tissue analysis of people with diabetes showed clear signs of neuropathology (Beeri et al., 2008). In animal models of T2DM, insulin signaling in the

brain was found to be markedly impaired (Yang et al., 2013; Agrawal et al., 2014).

2.2 Insulin boosts brain function

When insulin was administered via a nasal spray, people showed improved performance in several tests of attention, cognition and memory formation (Biessels et al., 2004; Freiherr et al., 2013). In clinical trials, verbal and spatial memory was improved after administration of a single dose of insulin (Benedict et al., 2008; Krug et al., 2010). Another study investigated the effects of 8 weeks of intranasal administration of insulin on memory and attention in healthy subjects in a double blind, placebo controlled trial. Blood glucose and plasma insulin levels were measured and did not differ between placebo and insulin treatment groups. After treatment, the delayed recall of words improved significantly (Benedict et al., 2004). Improvements in memory was even greater using insulin aspart, a fast-acting and long-lasting insulin analogue (Benedict et al., 2007).

2.3 Insulin plays important roles in brain function and neuronal growth

Insulin not only controls glucose utilization in the periphery (Hallschmid et al., 2004; Benedict et al., 2011; Hallschmid et al., 2012; Ott et al., 2012), but has additional roles in the brain. Insulin receptors have been identified in a variety of brain areas; the highest densities can be found in the cortex, olfactory bulb, hippocampus, and hypothalamus (Havrankova et al., 1978a; Havrankova et al., 1978b). Neurons express insulin receptors and their stimulation activates growth factor second messenger cascades that are vital for cell growth, repair and synaptic functions (de la Monte and Wands, 2005; Holscher, 2014). The observed cognitive impairments observed in people with T2DM and in animal models of diabetes can be explained by the loss of growth factor signaling. This in turn reduces the ability to withstand stressors and to repair damage that accumulates over time (Neth and Craft, 2017). In brain tissue of dementia patients, insulin signaling has also been shown to be severely impaired (Moloney et al., 2010; de la Monte, 2011; Talbot et al., 2012).

3. Protective effects of GLP-1 signaling in the brain
3.1 GLP-1 receptor agonists can reverse insulin de-sensitization in the brain

The incretin hormone GLP-1 is a growth factor and has similar properties to insulin. The GLP-1 receptor is a classic 7 membrane spanning G-protein coupled receptor of the glucagon class (Perry and Greig, 2002; Baggio and Drucker, 2007; Doyle and Egan, 2007; Holscher, 2014). GLP-1 and several of its analogues can cross the blood-brain-barrier (BBB) and exert

neuroprotective effects (Kastin et al., 2002; Kastin and Akerstrom, 2003; McClean et al., 2011; Hunter and Holscher, 2012; Christensen et al., 2015; Athauda et al., 2017). GLP-1 receptors are expressed in the brains of rodents, primates and humans (Merchenthaler et al., 1999; Cork et al., 2015; Heppner et al., 2015; Farr et al., 2016). GLP-1 receptor agonists such as exendin-4 or liraglutide can reverse insulin de-sensitization in the brain (Bomfim et al., 2012; Long-Smith et al., 2013). The localization and distribution of the insulin receptor and increased levels of insulin receptor substrate (IRS)-1 phosphorylated at serine 616 (IRS-1 pS(616)), a key marker of insulin resistance, was normalized in the brains of mice by treatment with liraglutide (Long-Smith et al., 2013). Liraglutide also improved key biomarkers in the brains of diabetic rats. The levels of insulin found in the brain were reduced, and there was a decrease in the phosphorylation of protein kinase B (AKT) and glycogen synthase kinase-3beta (GSK-3beta), which indicated decreased insulin signaling in rats with T2DM. Liraglutide treatment not only ameliorated hyperglycemia and peripheral insulin resistance, but also reversed brain insulin de-sensitization in a time-dependent manner (Yang et al., 2013). In the STZ model, insulin signaling was re-sensitized following activation of GLP-1 receptors, as illustrated by reduction of phospho-IRS1^{Ser1101} levels and by pAkt^{Ser473} upregulation and reactivation (Shi et al., 2017). Through GLP-1 receptor activation, cAMP/PKA/CREB growth factor signaling cascade is activated thus increasing gene expression of the insulin receptor, insulin, IRS-1, Akt and other growth factor-related proteins (Perfetti et al., 2000; Doyle and Egan, 2007; Park et al., 2010; Holscher, 2014; Talbot, 2014).

3.2 GLP-1 receptor agonists normalize cognitive impairments in T2DM

Several studies examining learning and memory in animal models show clear cognitive impairments induced by diabetes-obesity. In the high fat diet mouse model, memory formation was impaired and treatment with exendin-4 reversed this (Gault et al., 2010). Exendin-4 also protected streptozotocin (STZ)-induced diabetic rats from learning impairments as demonstrated using an elevated plus maze task and passive avoidance task (Gumuslu et al., 2016). Treatment with native GLP-1 also protected memory formation in STZ-treated rats (Iwai et al., 2009). Liraglutide protected STZ treated rats from impairments of learning a water maze task and a passive avoidance task, and improved motor impairments observed in the forced swimming test, open field, elevated plus maze, and rotarod motor coordination tests (Palleria et al., 2017). Liraglutide also normalized object recognition memory impairments in mice were maintained on a high fat diet (Porter et al., 2010). Furthermore, the DPP-4 inhibitor Sitagliptin, which elevates GLP-1 concentrations by reducing GLP-1 degradation, protected

memory formation in a high fat diet mouse model (Gault et al., 2015). Importantly, this effect is not entirely due to the normalization of blood glucose levels. When comparing effects of the enzyme-resistant GLP-1 analogue (Val8)GLP-1(GluPAL) with the diabetes drug metformin, it was found that both drugs effectively controlled blood glucose levels in high fat fed mice. However, the memory impairment observed in diabetic mice was not reversed in the metformin drug group alone, but only in the (Val8)GLP-1(GluPAL) treated group, see fig. 1 (Lennox et al., 2014). This clearly indicates that the neuroprotective effect of GLP-1 signaling goes beyond the regulation of glucose levels. GLP-1 receptor agonists have also shown neuroprotective effects in non-diabetic patients of Alzheimer's or Parkinson's disease, underscoring the protective effects that are independent of blood glucose regulation (Gejl et al., 2016; Athauda et al., 2017). In contrast, metformin enhances the risk of developing Alzheimer's or Parkinson's disease in people with T2DM, demonstrating that control of blood glucose is not sufficient to protect the brain in the same way that GLP-1 receptor agonists do (Hsu et al., 2011; Kuan et al., 2017).

3.3 GLP-1 receptor agonists normalize synaptic plasticity in the brain

Neurons communicate via synaptic activity, and long-term potentiation of synaptic activity (LTP) is considered to be the cellular correlate of memory (Bliss and Collingridge, 1993; Hölscher, 1999). When stimulating pyramidal neurons in area CA3 of hippocampal formation, the synapses projecting to CA1 neurons are upregulated. In diabetic animals, LTP has been found to be impaired. When treating high fat fed mice with liraglutide, the diabetes-induced block of LTP in the hippocampus was found to be reversed (Gault et al., 2010). Liraglutide also protected LTP formation in mice were maintained on a high fat diet (Porter et al., 2010). Native GLP-1 was also able to rescue impairments in synaptic transmission in STZ-treated rats (Iwai et al., 2009). In the *ob/ob* mouse model of diabetes, liraglutide rescued LTP in the hippocampus (Porter et al., 2013). GLP-1 has direct modulatory effects on synaptic activity, independent of the growth factor related effects, as shown in acute drug treatment in electrophysiological recording experiments (Gault and Holscher, 2008; Wang et al., 2013; Korol et al., 2014).

Similar to the effects on memory formation, when comparing the effects of the enzyme-resistant GLP-1 analogue (Val8)GLP-1(GluPAL) with the diabetes drug metformin, it was found that both drugs effectively controlled blood glucose levels in high fat fed mice, but the block of LTP observed in diabetic mice was not reversed in the metformin drug group, only in

the (Val8)GLP-1(GluPAL) treated group (Lennox et al., 2014) (see fig. 2).

3.4 Other neuroprotective effects of GLP-1 receptor agonists

3.4.1 Growth factor expression

GLP-1 receptor activation not only normalizes insulin signaling, but the impaired signaling of several other key growth factors, such as insulin-like growth factor 1 (IGF-1) (Moloney et al., 2010; Torres-Aleman, 2010), brain-derived neurotrophic factor (BDNF) (Park et al., 2010; Gumuslu et al., 2016), glia-derived neurotrophic factor (GDNF) (Allen et al., 2013; Yuan et al., 2017), and others. Exendin-4 normalized BDNF expression in the STZ rat model of diabetes (Gumuslu et al., 2016). Treatment of high fat fed mice with (Val8)GLP-1(GluPAL) normalized the expression of vascular endothelial growth factor (VEGF) (Lennox et al., 2014). Sitagliptin also enhanced VEGF expression (Gault et al., 2015). Other studies found normalization in expression and function of other growth factors after treatment with GLP-1 receptor agonists (Holscher, 2014; Yuan et al., 2017). These growth factors have neuroprotective effects and protect synapses and keep them functional under conditions of cellular stress (Cheng and Mattson, 1994; Yamada et al., 2001; Allen et al., 2013; Holscher, 2014).

3.4.2 Neurogenesis

While neurons do not divide and regenerate in most parts of the brain, there are specific brain regions such as the hippocampus/dentate gyrus where neurogenesis is still observed even in the adult brain. Neurogenesis is impaired in diabetic animals (Lang et al., 2009; Guo et al., 2010; Park et al., 2010). Exendin-4 normalized neurogenesis in STZ treated rats (Solmaz et al., 2015), while liraglutide normalized neurogenesis in *ob/ob* mice (Porter et al., 2013). Liraglutide or lixisenatide can enhance neurogenesis in wild type mice (Hunter and Holscher, 2012). Treatment with Sitagliptin also rescued neurogenesis in T2DM mice (Gault et al., 2015). Continuous neurogenesis is considered to be an important factor in long-term memory formation (Winocur et al., 2006).

3.4.3 Second messenger signalling for cell growth, repair, energy utilization and autophagy

GLP-1 signaling can compensate for the loss of other growth factors and insulin signaling in the brain. The main second messenger signaling pathway is the cAMP-PKA-CREB expression pathway (Doyle and Egan, 2007). However, other pathways such as Akt/PKB, AMPk and ERK kinase activity are also enhanced by GLP-1 receptor activation (Sharma et al., 2013; Jalewa et

al., 2016; Palleria et al., 2017). Genes that are activated include those relevant to energy utilization, for example, glucose uptake, mitochondrial function and replacement of damaged mitochondria (Lennox et al., 2014; Jalewa et al., 2016; Palleria et al., 2017); cell signaling that is linked to blocking apoptosis, for example, Bcl2 and Bax/BAD signaling and caspase activation (Baggio and Drucker, 2007; Kimura et al., 2009; Lupi et al., 2010); genes that control DNA repair (Yang et al., 2017), as well as control of chronic inflammation response in the brain that is observed in diabetics and that enhances oxidative stress (Parthsarathy and Holscher, 2013; Gault et al., 2015; Qin et al., 2016). In addition, autophagy, an important protective process that helps to eliminate cell debris that can become toxic if left to accumulate, is also enhanced and controlled by GLP-1 signaling (Jalewa et al., 2016; Panagaki et al., 2017).

4. Is GLP-1 unique?

Neuroprotective hormones that are released to signal energy availability and have cytoprotective properties form a large family. They include glucagon (Lund et al., 2011), insulin (Dailey, 2007), IGF-1 (Levine et al., 2012), leptin (Harvey, 2013), ghrelin (Gomez et al., 2009), oxyntomodulin (Pocai, 2014), adinopectin (Katsiki et al., 2011), GLP-1 (Baggio and Drucker, 2007), GLP-2 (Lund et al., 2011), GIP (Finan et al., 2016) and others. One might speculate if GLP-1 has a unique role to play in physiology or if its success is just a random finding, simply dictated by the fact that it was one of the first incretin hormones to be identified. However, it appears that there are differences between these hormones. The main reason why GIP had not been chosen to act as a novel treatment for type II diabetes even though it had been discovered first is because it was found to desensitize in diabetic patients (Vilsboll et al., 2002; Mohammad et al., 2014). Insulin obviously desensitizes, as does IGF-1 (Cohen et al., 2009), ghrelin (Theodoropoulou et al., 2012), leptin (Clemmensen et al., 2013), adinopectin (Satoh et al., 2005) and others. It appears that GLP-1 does not desensitize. What could be the reason for this? Perhaps analysing the mechanisms that cause desensitization will cast some light on this issue. In an acute inflammation response, the role of pro-inflammatory cytokines that are released by immune cells is to close down growth factor signalling (Musolino et al., 2017). Inflammation is observed in obesity, diabetes, Alzheimer's and Parkinson's disease (Craft, 2005; Holmes et al., 2009; Tansey and Goldberg, 2010; Ferrari and Tarelli, 2011; Stafeev et al., 2017). Pro-inflammatory cytokines such as TNF-α and growth factors/ anti-inflammatory cytokines counteract each other (Rossert et al., 2000; Calixto et al., 2004; Cotman et al., 2007; Bomfim et al., 2012; Musolino et al., 2017). The purpose appears to be to preserve energy and

to protect cells that are exposed to free radicals released during the inflammation response. When the acute inflammation response is coming to an end, anti-inflammatory cytokines are released in order to re-activate cell growth and energy utilization (Herder et al., 2013; Musolino et al., 2017). GLP-1 is one of such anti-inflammatory cytokines (Dozier et al., 2009; Shiraki et al., 2012; Parthsarathy and Holscher, 2013). Therefore, the reason why GLP-1 analogues are so successful in re-sensitizing insulin, IGF-1, and other growth factor signalling pathways is because GLP-1 signalling does not desensitize. If all growth factor signalling desensitized in the affected tissue, it would not be possible to reverse that situation. Some signalling pathways have to remain open to be accessible and to signal the end of the inflammatory response. GLP-1 appears to be one of those privileged signalling pathways. There are many more, and perhaps there is a field of treasures right there, waiting to be discovered.

5. Conclusion

GLP-1 receptor agonists are effective treatments for T2DM and are widely used throughout the world. The evidence presented here documents that the beneficial effects exceed those of simply enhancing insulin release during hyperglycemic episodes and helping to normalize blood glucose levels. Additional beneficial effects are observed that are directly induced by GLP-1 receptor activation in the brain and that are visible even in non-diabetic people, and not visible in diabetic people that show good control of T2DM by non-GLP-1 diabetes drugs. Further research is required to investigate the underlying mechanisms of these additional neuroprotective processes.

Acknowledgements

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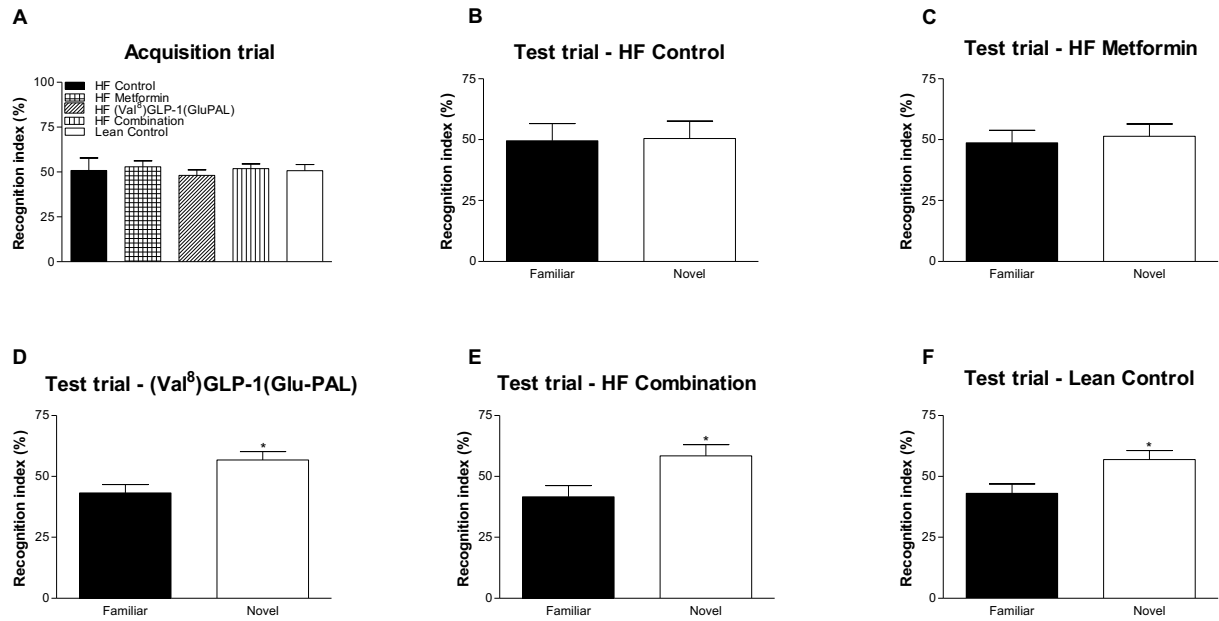
Figure 1: Effects of 20 days treatment with (Val[8])GLP-1(GluPAL), metformin or combined drug administration on recognition memory in high fat fed mice. Acquisition (A) and test (B-F) tasks in high fat fed mice. The recognition index (RI) was defined as the amount of time exploring the familiar (tA) or novel object (tB) over the total time spent exploring both objects x 100: (tA or tB/(tA+tB))*100. Values are means ± SE for ten mice. *P < 0.05 compared with saline-treated HF control mice. For technical details, see (Lennox et al., 2014). This Figure has been reproduced with permission (Lenox et al., 2014).
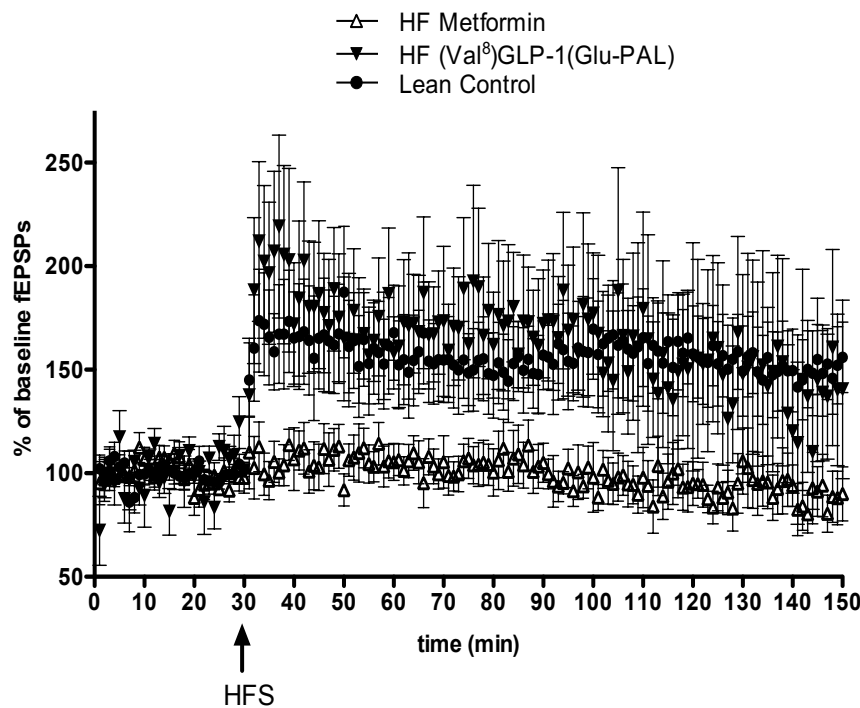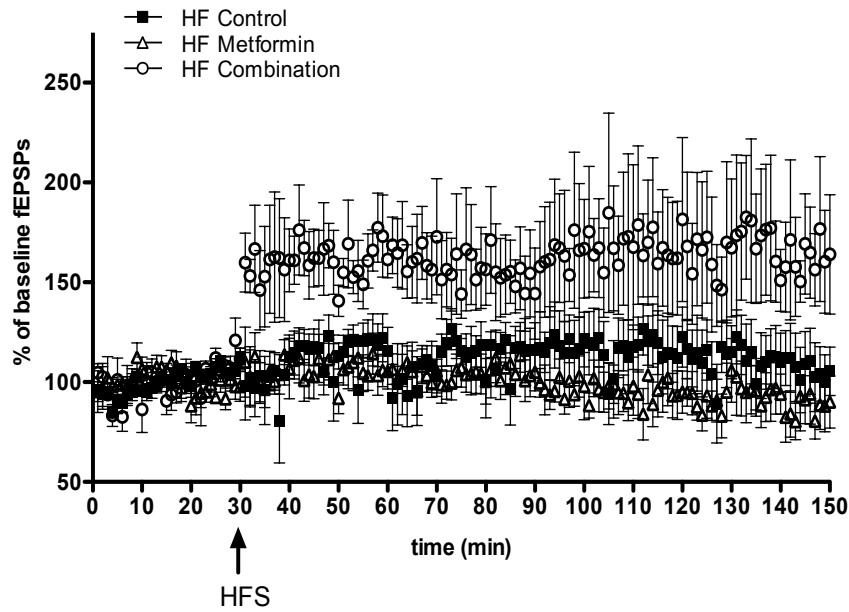
Figure 2: Effects of 20 days treatment with (Val[8])GLP-1(GluPAL), metformin or combined drug administration on measurements of LTP in the hippocampal CA1 region in high fat fed mice. Field excitatory postsynaptic potentials were recorded from stratum radiatum in the CA1 region of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral/commissural pathway. Values are means ± SEM for six mice. Treatment with the GLP-1 analogue ameliorated LTP as shown by a two-level two-way ANOVA indicating a significant difference between *HF* saline controls and GLP-1 analogue -treated mice ($P < 0.001$) and over time ($P <$

0.001). Similarly, a two-level two-way ANOVA showed a significant difference in LTP between *HF s*aline controls and the combination treatment metformin and GLP-1 analogue ($P < 0.001$) and over time ($P < 0.001$). However, no statistical difference was found between metformin treated HF mice and Saline treated HF mice. For technical details, see (Lennox et al., 2014). This Figure has been reproduced with permission (Lenox et al., 2014).