Adrenomedullin and glucagon-like peptide-1 have additive effects on food intake in mice

Bech, Esben M.; Voldum-clausen, Kristoffer; Pedersen, Søren L.; Fabricius, Katrine; Rudkjær, Lise C.B.; Hansen, Henrik H.; Jelsing, Jacob

Published in:
Biomedicine & Pharmacotherapy

DOI:
10.1016/j.biopha.2018.10.040

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Biomedicine & Pharmacotherapy 109 (2019) 167–173

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Adrenomedullin and glucagon-like peptide-1 have additive effects on food intake in mice

Esben M. Bech[a,b,1], Kristoffer Voldum-Clausen[a,1], Søren L. Pedersen[a], Katrine Fabricius[a], Lise C.B. Rudkjær[a], Henrik H. Hansen[a], Jacob Jelsing[a,*]

[a] Gubra ApS, Hørsholm, Denmark
[b] Department of Chemistry, University of Copenhagen, Frederiksberg, Denmark

ARTICLE INFO

Keywords:
Adrenomedullin
ADM
Food intake
Insulin secretion
Glucose tolerance
Obesity
Diabetes
Glucagon-like peptide 1
GLP-1

ABSTRACT

Adrenomedullin (ADM) is a vasoactive peptide expressed in several peripheral organs and known primarily for its beneficial vasoactive effects. However, ADM is also known to inhibit insulin secretion, and central administration of ADM has been shown to elicit anorexigenic effects. Here, we investigated if peripheral co-administration of ADM and glucagon-like peptide 1 (GLP-1) could subdue the hypoglycaemic effects of ADM while enhancing its anorectic properties.

The effects of mono- and combination therapy of ADM and GLP-1 on appetite regulation and glucose homeostasis were assessed acutely in male NMRI mice for 12 h, while effects on glucose homeostasis were assessed by oral glucose tolerance tests (OGTT).

While the monotherapy with GLP-1 and ADM resulted in modest anorexigenic effects, co-administration of the two peptides led to a marked additive reduction in food intake. Moreover, while OGTT-evoked blood glucose-excursions were significantly increased by ADM monotherapy, co-administration of ADM with a lower dose of GLP-1 normalized glucose excursions.

In conclusion, we demonstrate additive anorectic effects of ADM and GLP-1, and that GLP-1 co-administration prevents ADM-induced impairment of glucose tolerance, suggesting that ADM could be potential anti-obesity target when combined with GLP-1 agonist therapy.

1. Introduction

Adrenomedullin (ADM) is a 52-amino acid peptide hormone belonging to the calcitonin gene-related peptide (CGRP) superfamily and is primarily secreted by endothelial- and vascular smooth muscle cells [1]. Humans have two adrenomedullin receptors, ADM1 and ADM2, both being hetero-dimers of the calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein (RAMP) 2 and 3, respectively [2]. In addition, ADM might have cross-reactivity with other CGRP receptor family members, especially the CGRP receptor, a heterodimer of CLR and RAMP1 [2]. In humans, ADM have been found to increase cardiac output, induce hypotensive effects and elicit vasoprotection [1,3,4], and these vasoactive effects have spurred interest in developing ADM as a novel cardioprotective treatment concept [4,5]. Interestingly, ADM has also been proposed to exert effects on glucose homeostasis and food intake. However, whether ADM constitutes an attractive target within diabetes and obesity treatment is presently unclear [6–14].

Previous studies in animal models have shown that glucose homeostasis is negatively influenced by ADM through the inhibition of insulin secretion [7,14]. Thus, in rats, intravenous administration of ADM delays glucose-stimulated insulin responses resulting in elevated blood glucose levels [6,7]. Furthermore, a subset of diabetic patients has been found to have increased plasma concentrations of ADM, indicating further that ADM may play a causative role in the development of diabetes type 2 [6,14–16]. Preclinical and clinical studies have also revealed that ADM plasma concentrations are positively correlated to body weight gain and that ADM expression is increased in adipose tissues [12,13,17,18]. Conversely, weight loss may lead to reduced circulating ADM levels as demonstrated after Roux-en-Y gastric bypass (RYGB) surgery in morbidly obese subjects [17]. Finally, studies in chicks and rats have indicated a short-lasting food intake inhibitory effect of ADM following either central administration [10,11] or intravenous infusion [19]. Collectively, these data suggest that ADM may

* Corresponding author.
E-mail address: jacob@gubra.dk (J. Jelsing).
[1] Esben M. Bech and Kristoffer Voldum-Clausen contributed equally to this work.

https://doi.org/10.1016/j.biopha.2018.10.040
Received 13 June 2018; Received in revised form 9 October 2018; Accepted 9 October 2018

constitute a target within diabetes and obesity treatment.

The mechanistic basis for the anorectic and glucose regulatory of ADM is presently not clear. It has been suggested that the anorectic properties may be partly mediated through a direct activation of centrally located CGRP receptors, which leads to an inhibition of gastric emptying and increased sensation of satiety [10,20]. However, this hypothesis relies on antagonistic studies using $CGRP_{8-37}$, which also antagonize other members of the CGRP receptor family including the ADM receptors, albeit at a lower potency [2]. Conversely, activation of ADM receptors expressed in the islets of the pancreas is believed to be directly involved in the insulin regulatory properties [14,21].

The effects of ADM on glucose and body weight homeostasis are in a way similar to the metabolic effects of glucagon, which also raise blood glucose levels and reduce food intake [22]. Interestingly, GLP-1 and glucagon co-agonism has been shown to induce enhanced metabolic effects and is currently pursued by several pharmaceutical companies as a new therapeutic treatment for diabetes and obesity (for a review, see [23]). Similarly, synergy or additivity with GLP-1 has been demonstrated for several other satiety-regulatory peptides, including the amylin analogue salmon calcitonin [24], another member of the CGRP-superfamily albeit with different receptor preferences. A combined dose (*s.c.*) of salmon calcitonin and a GLP-1R agonist (exendin-4) have been reported to provide synergetic anorectic effects in non-human primates [25]. With this in mind, we were curious to examine if GLP-1 co-agonism could subdue the hypoglycaemic effects of ADM while enhancing its anorectic properties.

2. Materials & methods

2.1. Animals

Male NMRI mice (5 weeks of age, 25–30 g) were purchased from Taconic (Denmark). Following acclimatization (5 mice per cage), NMRI mice were single-housed for oral glucose tolerance tests (OGTT) or group-housed (four per cage) for automated real-time food intake monitoring (12/12 h light-dark cycle, lights off at 01:00 PM; temperature: 22 ± 2 °C; 50% relative humidity). Animals had ad libitum access to regular chow (Altromin 1324, Brogaarden, Denmark) and domestic-quality tap water.

All animal experiments were conducted in accordance to internationally accepted principles for the care and use of laboratory animals. The experiments were covered by a license (2013-15-2934-00784) issued by the Danish Animal Experiments Inspectorate.

2.2. Peptide synthesis

Human native ADM and GLP-1(7–37) − OH were synthesized using fully automated, standard Fmoc-based solid-phase peptide synthesis on a SyroII peptide synthesizer (Biotage AB, Sweden) with reagents from Iris Biotech GmbH (Germany) or Sigma Aldrich (Denmark). Crude peptides were purified using reversed-phase HPLC, quantified and characterized by LC–MS (Acquity UPLC equipped with a SQD2 mass spectrometer, Waters, Denmark). Subsequently, the disulphide bridge of ADM was formed using 1.2 eq. aldrithiol (Sigma, Denmark) after which ADM was again purified by C18 reversed-phase HPLC. The final purities of both GLP-1 and ADM were above 95% as determined by UPLC.

2.3. Compounds

For oral glucose tolerance studies, the vehicle consisted of 10% DMSO in water. In the food intake studies, the vehicle consisted of 10% DMSO in water with linagliptin (212 nmol/mL; 0.5 mg/kg, Toronto Research Chemicals, Canada) to extend the half-life of GLP-1 [26]. Compounds were administrated at 5–10 mL/kg.

2.4. Acute food intake

Real-time food intake of mice was monitored using a fully automated food intake monitoring system (HM-2; MBRose ApS, Denmark). Animals were randomized to treatment (n = 8 per group) based on baseline body weight. Animals were fasted for four hours prior to dosing. Animals were dosed *s.c.* 30 min prior to lights out, and food intake data were collected automatically for a total of 18 h post-dosing.

2.5. Oral glucose tolerance test (OGTT)

The single housed mice were randomized to treatment (n = 8 per group) according to body weight on day -1. On the day of the OGTT (day 0), animals were fasted for four hours prior to the test. Animals were dosed *s.c.* with vehicle or compound 30 min before receiving an oral glucose load (2 g/kg, at t = 0). Blood glucose was measured from tail vein blood samples at time points before glucose administration (t = -30 min) and after glucose administration (t = 15, 30, 60, 120, and 240 min). Blood samples were collected into 10 µl heparinized glass capillary tubes, immediately suspended in buffer (0.5 mL of glucose/lactate system solution) and analysed for glucose concentrations on the test day using a BIOSEN c-Line glucose meter according to manufacturer's instructions (EKF-diagnostics, Germany).

For analysis of insulin, blood samples were collected at t = 60 and 240 min to microvettes with anticoagulant. Subsequently, samples were mixed by inversion 5 times and plasma was isolated by centrifugation (3000 g, 10 min, 4 °C). Plasma insulin was measured in duplicate using a single-plex assay (Meso Scale Diagnostics, USA) according to the manufacturer's instructions.

2.6. Statistics

Data were analysed using GraphPad Prism (v7.0, Graphpad Software, USA). Results are presented as mean ± standard error of mean (S.E.M.). For statistical analysis, one-way ANOVA against the vehicle group with Bonferroni's post-hoc test (Bar-graphs; Food intake, OGTT-AUC, plasma insulin), or two-way ANOVA against the vehicle group with Bonferroni's post-hoc test (time-response data on body-weight and OGTT) was used. P-values < 0.05 were considered statistically significant.

3. Results

3.1. ADM reduces food intake in NMRI mice

ADM (3000 nmol/kg, *s.c.*) significantly reduced food intake at 1–3 h post-dosing but the effect had worn off at the 12-hour measuring point (Fig. 1A–C). The magnitude and duration of the food intake suppressive effect of ADM was comparable to an equivalent dose of GLP-1 (3000 nmol/kg, *s.c.*).

3.2. ADM impairs glucose tolerance in NMRI mice

ADM (3000 nmol/kg, *s.c.*) significantly increased glucose excursion at t = 60 and 120 min ($p < 0.001$) compared to vehicle controls (Fig. 1D). The effect was also reflected in the corresponding glucose-AUC values (0–120 min, $p < 0.01$) (Fig. 1E). Conversely, GLP-1 (300 nmol/kg, *s.c.*) reduced blood glucose levels at all timepoints measured, as well as glucose-AUC (0–120 min, $p < 0.001$) (Fig. 1D–E).

3.3. ADM potentiates the anorectic effect of GLP-1 in NMRI mice

A high dose of GLP-1 (3000 nmol/kg, *s.c.*) significantly reduced food intake throughout the study period, while a lower dose (1000 nmol/kg, *s.c.*) was effective only during the first hour post-dosing (Fig. 2). A low dose of ADM (1000 nmol/kg, *s.c.*) did not significantly influence food

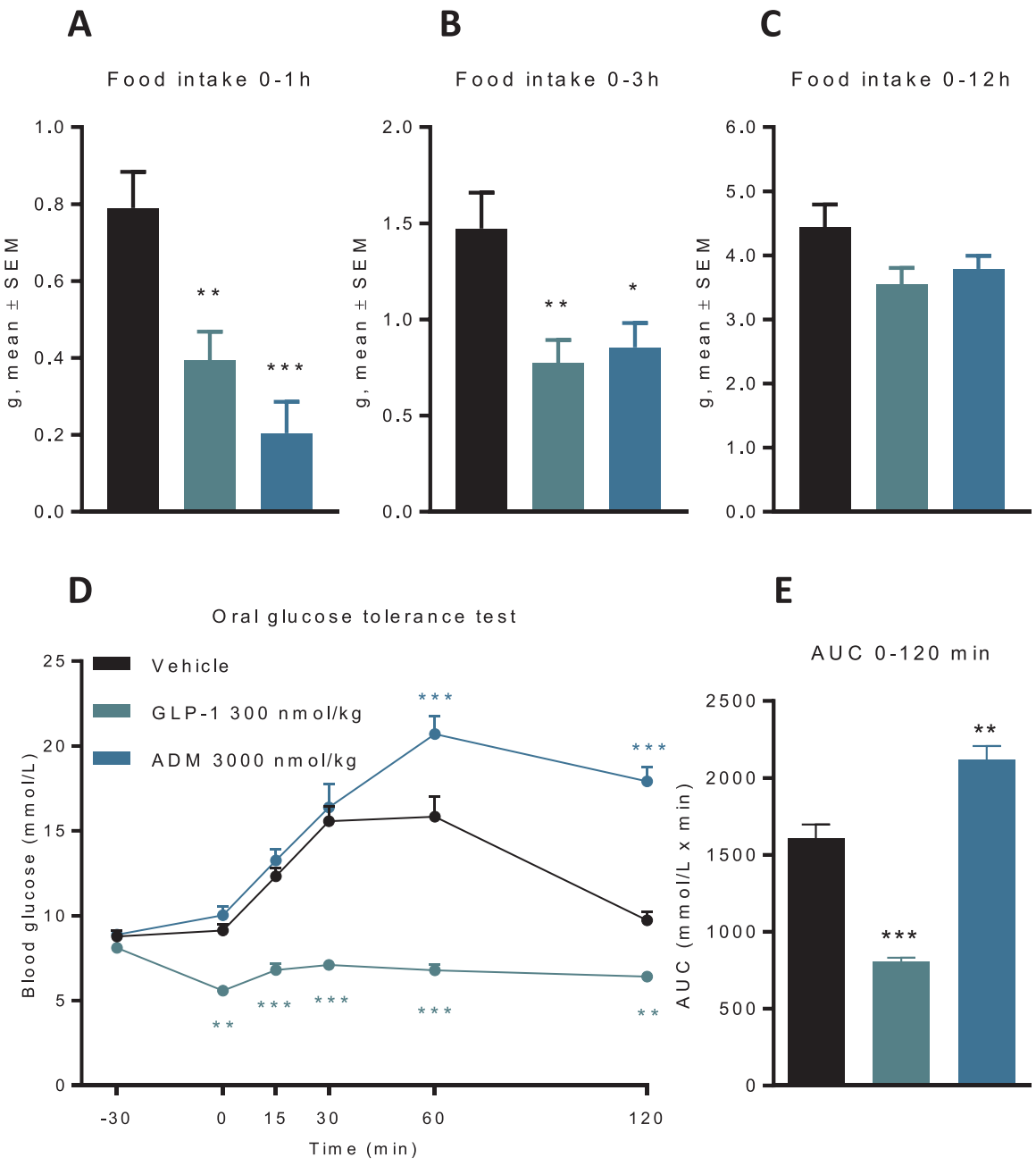

Fig. 1. ADM and GLP-1 reduces acute food intake but have opposing effects on oral glucose tolerance in lean NMRI mice. (A–C) Acute cumulative food intake of lean NMRI mice administered with vehicle, ADM or GLP-1 measured 1, 3, and 12 h post-dosing (10 mL/kg, 3000 nmol/kg). (D) Blood glucose excursion curves of lean NMRI mice during an OGTT. Vehicle, ADM (3000 nmol/kg), and GLP-1 (300 nmol/kg) were administered (5 mL/kg) 30 min prior to the glucose bolus. (E) Area-under-the-curve (AUC 0–120 min) calculated from the glucose excursion curves. Statistics: *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle derived from either one-way ANOVA with Bonferroni's post hoc test (A, B, C, and D) or two-way ANOVA with Bonferroni's post hoc test (E).

intake although animals tended to eat less than the vehicle animals; particularly in the first hour post-dosing. In contrast, combined treatment with the low doses of ADM and GLP-1 significantly suppressed food intake in NMRI mice, as compared to vehicle controls ($p < 0.05$, Fig. 2). The co-administration of ADM (1000 nmol/kg, *s.c.*) with a higher dose of GLP-1 (3000 nmol/kg, *s.c.*) augmented the anorectic actions (Fig. 2). The additive anorectic effects of ADM and GLP-1 were most pronounced 1–3 h post-dosing but remained throughout the entire monitoring period.

3.4. GLP-1 prevents impaired glucose tolerance in ADM-treated NMRI mice

The effects of ADM on glucose homeostasis were assessed in the context of monotherapy, as well as in combination with GLP-1 administration. When administered alone, ADM dose-dependently (300–1000 nmol/kg, *s.c.*) impaired glucose tolerance in NMRI mice (Fig. 3A). Administration of 300 nmol/kg (*s.c.*) resulted in elevated blood glucose levels at t = 30 and 60 min ($p < 0.001$; Fig. 3A), and glucose excursions were further augmented when administering a moderate dose of ADM (1000 nmol/kg, *s.c.*). The effect of a moderate dose of ADM (1000 nmol/kg, *s.c.*) on glucose excursions was comparable to that attained by a higher dose of ADM (3000 nmol/kg, *s.c.*) and resulted in a similar area-under-the-curve 120 min post dosing (Figs. 1E & 3 B). Additionally, ADM appeared to reduce or delay glucose-stimulated plasma insulin levels, with insulin levels being slightly reduced as compared to vehicle treated animals at t = 60 min of the OGTT

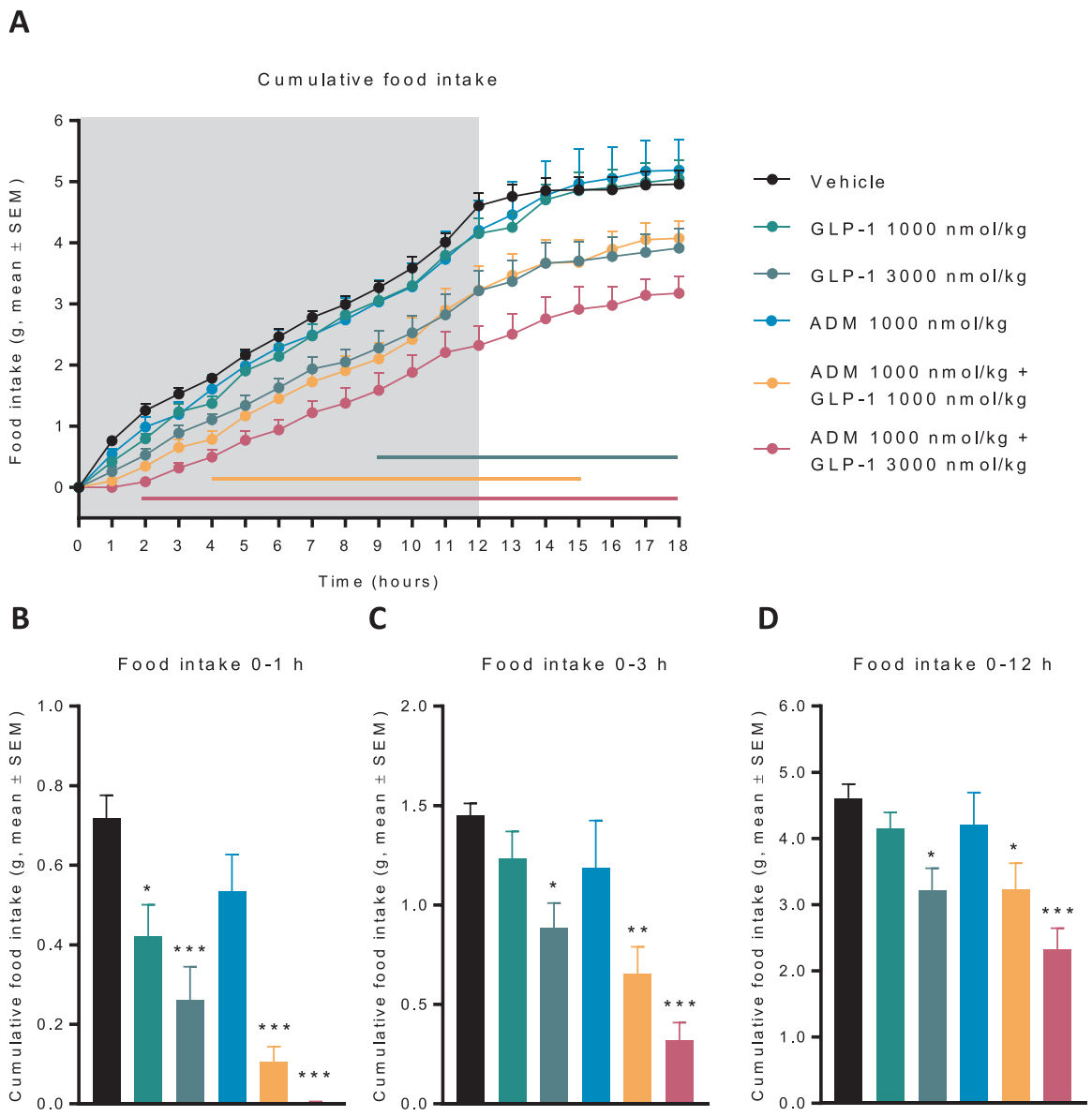

Fig. 2. ADM and GLP-1 shows additive inhibitory effects on food intake in NMRI mice. (A–D) Acute effects on food intake measured 1, 3, and 12 h after dosing (5 mL/kg) with vehicle, GLP-1 (1000–3000 nmol/kg), ADM (1000 nmol/kg), and combinations of these (ADM 1000 nmol/kg + GLP-1 1000–3000 nmol/kg). For figure A, shaded area represents the dark phase, and lines beneath graphs indicates $p < 0.05$ vs. vehicle derived from a two-way ANOVA with Bonferroni's post hoc test. For figure B–D, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle derived from one-way ANOVA with Bonferroni's post hoc test.

(Fig. 3C). However, the reduction was not found to be significant ($p = 0.09$).

As expected, GLP-1 (30 nmol/kg, *s.c.*) decreased blood glucose levels at t = 15, 30 and 60 min ($p < 0.001$) as well as the glucose-AUC (0–120 min, $p < 0.001$) compared to vehicle controls (Fig. 3A). Co-administration of GLP-1 (30 nmol/kg, *s.c.*) and ADM (1000 nmol/kg, *s.c.*) fully prevented the reduced glucose tolerance afforded by ADM administration alone (Fig. 3A). This effect was also reflected by normalized insulin responses in the OGTT upon combined ADM and GLP-1 administration (Fig. 3C). No treatment affected blood-glucose or insulin levels 240 min after the glucose bolus (Fig. 3A & D).

4. Discussion

Whereas the vasoactive properties of ADM have been well characterized, less is known about the metabolic effects of ADM. Here, we demonstrated that acute systemic ADM administration suppressed food intake and impaired glucose tolerance in mice. Furthermore, we demonstrated for the first time that GLP-1 and ADM acted additively to reduce food intake in an acute setting, and that GLP-1 prevented reduced oral glucose tolerance following acute ADM administration in lean mice.

An anorectic response to acute administration ADM has previously been reported following central administration and *i.v.* infusion in rats. In line with this, we found that subcutaneous administration of ADM had an inhibitory effect on the acute food intake of mice, being equi-efficacious to GLP-1. In addition, and in contrast to GLP-1, we demonstrated that *s.c.* ADM administration led to increased glucose excursions in an OGTT. Hence, ADM and GLP-1 had opposing effects on glucose homeostasis. A similar finding has been reported by Martínez and co-workers who demonstrated how *i.v.* administration of ADM to rats increased glucose excursions by delaying glucose-stimulated insulin release [7].

Based on these data, we hypothesized that co-administration of ADM with GLP-1 could attenuate ADM's insulin suppressing effects and potentially augment its anorectic properties. GLP-1 is a powerful

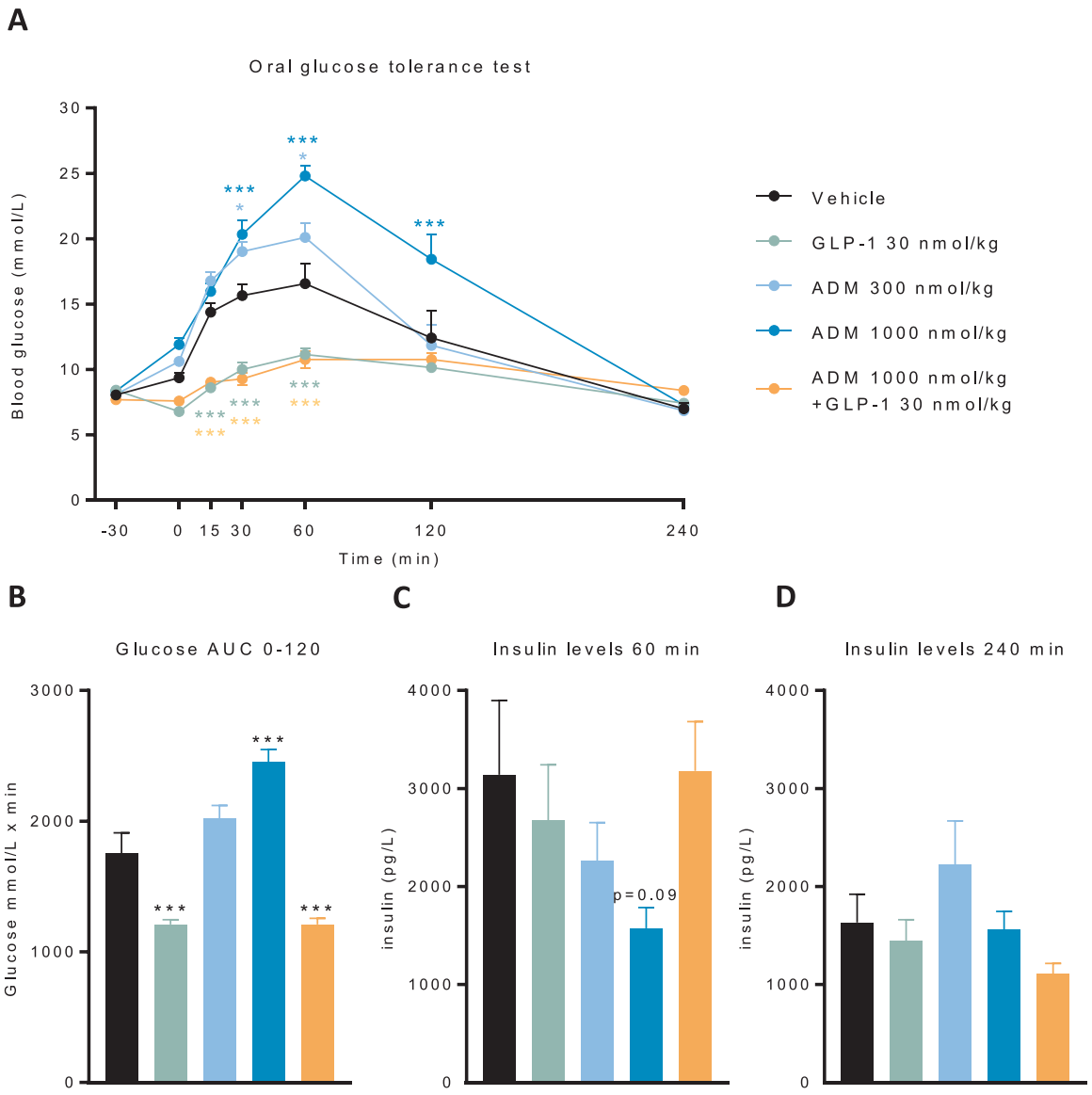

Fig. 3. GLP-1 prevents ADM-induced impaired glucose tolerance in NMRI mice (A) Glucose excursion curves in NMRI mice after dosing (5 mL/kg) with vehicle, ADM (300 or 1000 nmol/kg), native GLP-1 (30 nmol/kg) or a combination of ADM (1000 nmol/kg) and GLP-1 (30 nmol/kg). Animals were dosed 30 min prior to the glucose bolus. (B) Area-under-the-curve (AUC 0–120 min) calculated from the glucose excursion curves. (C & D) Plasma insulin levels during an oral glucose tolerance test at t = 60 min and t = 240 min. *p < 0.05, ***p < 0.001 vs. vehicle derived from two-way ANOVA with Bonferroni's post hoc test (A) or one-way ANOVA with Bonferroni's post hoc test (B–D).

incretin that reduces blood glucose levels through the amplification of insulin secretion in response to increased blood glucose levels, and is known to reduce food intake by central mechanisms of action [27–29]. In line with this hypothesis, we observed that ADM and GLP-1 acted additively to reduce food intake in an acute setting. This additive anorectic effect was most marked in the first few hours post dosing where it led to a near abolishment of cumulative food intake. The relative short-acting effects of ADM and GLP-1 are in agreement with the short circulatory half-lives of native GLP-1 ($t_{\frac{1}{2}}$ < 2 min, humans) [27] and ADM ($t_{\frac{1}{2}}$ = 22 min, humans) [30].

In respect to blood glucose homeostasis, we found that GLP-1 co-administration abolished the unfavourable effects of ADM on glucose homeostasis in lean mice. In an OGTT, ADM treated mice were found to have a dose-dependent increase in glucose excursions, coupled to a delayed glucose-stimulated insulin response, whereas low dose GLP-1 monotherapy did not affect insulin levels but blunted glucose excursions. Co-administration of ADM and a low dose of GLP-1 led to OGTT-evoked insulin responses identical to that observed for GLP-1 monotherapy (Fig. 3D–E). Thus, GLP-1 was able to fully prevent ADM's negative effects on glucose tolerance, even when administered in a much lower dose than ADM (30 nmol/kg vs 1000 nmol/kg).

The molecular mechanisms underlying ADM's anorectic effects are poorly understood. Taylor and co-workers found that anorectic effects observed following central administration of ADM in rats could be reduced by 50% through concomitant *i.c.v.* administration of the CGRP receptor family antagonist $CGRP_{8-37}$ [10]. Likewise, Martinez and colleagues reported that central administration of ADM (75–150 pmol/rat) inhibited gastric emptying in rats while an *i.v.* dose (150 pmol/rat) did not [20]. The ADM-mediated effects on gastric emptying rates could be fully restored by *i.c.v.* administration of $CGRP_{8-37}$ [20]. Together, this indicates that ADM's effect on acute food intake is mediated through central CGRP receptors. However, since $CGRP_{8-37}$ does not exclusively inhibit the CGRP receptor, but all receptors in the CGRP family, activation of central ADM1/2 receptors cannot be excluded [2]. Additionally, more research is needed to fully understand the insulin suppressive properties of ADM. Mulder and co-workers showed how

nanomolar concentrations of ADM promoted insulin secretion in isolated rat islets [31]. Contrarily, other studies have found that ADM inhibited insulin secretion in INS-1 cells and in isolated rat- and mouse islets [7,14,32], possibly through inhibition of exocytosis from pancreatic β-cells through activation of pertussis-toxin sensitive $G_{i/o}$ proteins [14]. The demonstration that ADM delayed OGTT-evoked insulin release and increased glucose excursions in rats [6,7] and in mice, supports the latter. Thus, ADM's effects on insulin secretion may balance between inhibitory and stimulatory depending on concentration, but at the pharmacological doses applied *in vivo* ADM appears to inhibit insulin secretion. Finally, it is presently not known if secondary effects of ADM contributed to the anorexia, hyperglycaemia, and insulin inhibition observed in this study. For example, intravenous administration of ADM is known to yield an acute hypotensive response in anesthetized mice at 12 nmol/kg lasting for 3–5 min [33]. To sustain hemodynamic stability, hypotension might lead to a rise in sympathetic tone, which in turn has been linked to hepatic glucose release and a reduction in insulin release [34]. In this study, ADM were investigated in non-anesthetized animals and administrated s.c., which will protract circulatory absorption. However, despite the deviations in conduction of the two studies, it cannot be ruled out that the observed effects at least partly can be ascribed to hypotension. Further studies are needed.

Peptides with GLP-1 additivity or synergy are of high interest for the development of better treatments against obesity and diabetes - particularly, chimeric peptides acting on two-three distinct receptors may elicit benefits beyond those achievable with monotherapies [35]. Our results with ADM GLP-1 co-administration lends rationale to a combined ADM and GLP-1 peptide, which could potentially elicit GLP-1's positive effects on blood glucose homeostasis while reducing food intake further than mono-therapy. Furthermore, ADM is known to elicit hypotensive and cardioprotective effects [1,3,36–38] and, thus, a ADM GLP-1 chimer might boost the positive cardiovascular effects already observed for marketed GLP-1 agonists [39]. However, further studies including chronic evaluation in diet- or genetically induced obese animal models are required to further access the potential of ADM/GLP-1 co-agonism for the treatment of obesity

5. Conclusion

In conclusion, we validated the acute effects of native ADM on food intake and glucose homeostasis in lean mice following peripheral administration. Additionally, co-administration of ADM and GLP-1 promoted additive inhibitory effects on food intake and prevented ADM-induced impairment of glucose tolerance. Consequentially, ADM if combined with GLP-1 could be interesting as a potential target for the treatment of obesity.

Disclosures

KF, HBH, SLP and JJ have shares in Gubra ApS.

References

[1] R. Schönauer, S. Els-Heindl, A.G. Beck-Sickinger, Adrenomedullin - new perspectives of a potent peptide hormone, J. Pept. Sci. 23 (2017) 472–485, https://doi.org/10.1002/psc.2953.

[2] D.L. Hay, M.L. Garelja, D.R. Poyner, C.S. Walker, Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR review 25, Br. J. Pharmacol. 175 (2018) 3–17, https://doi.org/10.1111/bph.14075.

[3] X. Xian, T. Sakurai, A. Kamiyoshi, Y. Ichikawa-Shindo, M. Tanaka, T. Koyama, H. Kawate, L. Yang, T. Liu, A. Imai, L. Zhai, K. Hirabayashi, K. Dai, K. Tanimura, T. Liu, N. Cui, K. Igarashi, A. Yamauchi, T. Shindo, Vasoprotective activities of the adrenomedullin-RAMP2 system in endothelial cells, Endocrinology 158 (2017) 1359–1372, https://doi.org/10.1210/en.2016-1531.

[4] J. Kato, K. Kitamura, Bench-to-bedside pharmacology of adrenomedullin, Eur. J. Pharmacol. 764 (2015) 140–148, https://doi.org/10.1016/j.ejphar.2015.06.061.

[5] R. Schönauer, S. Els-Heindl, J.P. Fischer, J. Köbberling, B. Riedl, A.G. Beck-Sickinger, Adrenomedullin 2.0: adjusting key levers for metabolic stability, J. Med. Chem. 59 (2016) 5695–5705, https://doi.org/10.1021/acs.jmedchem.6b00126.

[6] A. Martínez, T.H. Elsasser, S.J. Bhathena, R. Pío, T.A. Buchanan, C.J. Macri, F. Cuttitta, Is adrenomedullin a causal agent in some cases of type 2 diabetes? Peptides 20 (1999) 1471–1478, https://doi.org/10.1016/S0196-9781(99)00158-8.

[7] A. Martínez, C. Weaver, J. López, S.J. Bhathena, T.H. Elsasser, M.J. Miller, T.W. Moody, E.J. Unsworth, F. Cuttitta, Regulation of insulin secretion and blood glucose metabolism by adrenomedullin, Endocrinology 137 (1996) 2626–2632, https://doi.org/10.1210/endo.137.6.8641217.

[8] T.C. Murphy, W.K. Samson, The novel vasoactive hormone, adrenomedullin, inhibits water drinking in the rat, Endocrinology 136 (1995) 2459–2463, https://doi.org/10.1210/endo.136.6.7750467.

[9] W.K. Samson, T.C. Murphy, Adrenomedullin inhibits salt appetite, Endocrinology 138 (1997) 613–616, https://doi.org/10.1210/en.138.2.613.

[10] G.M. Taylor, K. Meeran, D. O'Shea, D.M. Smith, M.A. Ghatei, S.R. Bloom, Adrenomedullin inhibits feeding in the rat by a mechanism involving calcitonin gene-related peptide receptors, Endocrinology 137 (1996) 3260–3264, https://doi.org/10.1210/endo.137.8.8754748.

[11] G. Wang, B. Brumfield, M. DiCroce, L. Nelson, B.A. Newmyer, J. Flower, K. Hipskind, S. Sharma, E.R. Gilbert, M.A. Cline, Anorexigenic effects of central adrenomedullin are associated with hypothalamic changes in juvenile Gallus gallus, Gen. Comp. Endocrinol. 204 (2014) 223–228, https://doi.org/10.1016/j.ygcen.2014.05.032.

[12] Y. Li, C. Jiang, X. Wang, Y. Zhang, S. Shibahara, K. Takahashi, Adrenomedullin is a novel adipokine: adrenomedullin in adipocytes and adipose tissues, Peptides 28 (2007) 1129–1143, https://doi.org/10.1016/j.peptides.2007.03.005.

[13] S. Kawano, Y. Kawagoe, K. Kuwasako, S. Shimamoto, K. Igarashi, M. Tokashiki, K. Kitamura, J. Kato, Gender-related alterations in plasma adrenomedullin level and its correlation with body weight gain, Endocr. Connect. 4 (2015) 43–49, https://doi.org/10.1530/EC-14-0131.

[14] N. Sekine, K. Takano, N. Kimata-Hayashi, T. Kadowaki, T. Fujita, Adrenomedullin inhibits insulin exocytosis via pertussis toxin-sensitive G protein-coupled mechanism, Am. J. Physiol. Endocrinol. Metab. 291 (2006) E9–E14, https://doi.org/10.1152/ajpendo.00213.2005.

[15] M. Hayashi, T. Shimosawa, M. Isaka, S. Yamada, R. Fujita, T. Fujita, Plasma adrenomedullin in diabetes, Lancet (London, England) 350 (1997) 1449–1450.

[16] H. Kinoshita, K. Kato, M. Kuroki, S. Nakamura, K. Kitamura, S. Hisanaga, S. Fujimoto, T. Eto, Plasma adrenomedullin levels in patients with diabetes, Diabetes Care 23 (2000) 253–261.

[17] G. Vila, M. Riedl, C. Maier, J. Struck, N.G. Morgenthaler, A. Handisurya, G. Prager, B. Ludvik, M. Clodi, A. Luger, Plasma MR-proADM correlates to BMI and decreases in relation to leptin after gastric bypass surgery, Obesity 17 (2009) 1184–1188, https://doi.org/10.1038/oby.2009.22.

[18] I. Nomura, J. Kato, M. Tokashiki, K. Kitamura, Increased plasma levels of the mature and intermediate forms of adrenomedullin in obesity, Regul. Pept. 158 (2009) 127–131, https://doi.org/10.1016/j.regpep.2009.08.003.

[19] R.D. Reidelberger, L. Kelsey, D. Heimann, Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 282 (2002) R1395–R1404, https://doi.org/10.1152/ajpregu.00597.2001.

[20] V. Martínez, F. Cuttitta, Y. Taché, Central action of adrenomedullin to inhibit gastric emptying in rats, Endocrinology 138 (1997) 3749–3755, https://doi.org/10.1210/en.138.9.3749.

[21] E. Zudaire, F. Cuttitta, A. Martínez, Regulation of pancreatic physiology by adrenomedullin and its binding protein, Regul. Pept. 112 (2003) 121–130, https://doi.org/10.1016/S0167-0115(03)00030-2.

[22] J.W. Day, N. Ottaway, J.T. Patterson, V. Gelfanov, D. Smiley, J. Gidda, D. Bruemmer, D.J. Drucker, N. Chaudhary, J. Holland, J. Hembree, W. Abplanalp, E. Grant, J. Ruehl, H. Wilson, H. Kirchner, S.H. Lockie, S. Hofmann, S.C. Woods, R. Nogueiras, P.T. Pfluger, D. Perez-tilve, R. Dimarchi, M.H. Tschöp, A new glucagon and GLP-1 co-agonist eliminates obesity in rodents 5 (2009) 749–757, https://doi.org/10.1038/nchembio.209.

[23] M.A. Sánchez-Garrido, S.J. Brandt, C. Clemmensen, T.D. Müller, R.D. DiMarchi, M.H. Tschöp, GLP-1/glucagon receptor co-agonism for treatment of obesity, Diabetologia 60 (2017) 1851–1861, https://doi.org/10.1007/s00125-017-4354-8.

[24] D.L. Hay, S. Chen, T.A. Lutz, D.G. Parkes, J.D. Roth, Amylin : pharmacology, physiology, and clinical potential, Pharmalog. Rev. 67 (2015) 564–600.

[25] N.T. Bello, M.H. Kemm, E.M. Ofeldt, T.H. Moran, Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates, Am. J. Physiol. Integr. Comp. Physiol. 299 (2010) R945–R952, https://doi.org/10.1152/ajpregu.00275.2010.

[26] H.H. Hansen, G. Hansen, S. Paulsen, N. Vrang, M. Mark, J. Jelsing, T. Klein, The DPP-IV inhibitor linagliptin and GLP-1 induce synergistic effects on body weight loss and appetite suppression in the diet-induced obese rat, Eur. J. Pharmacol. 741 (2014) 254–263, https://doi.org/10.1016/j.ejphar.2014.08.010.

[27] J.J. Holst, The physiology of glucagon-like peptide 1, Physiol. Rev. (2007) 1409–1439, https://doi.org/10.1152/physrev.00034.2006.

[28] G. Hansen, J. Jelsing, N. Vrang, Effects of liraglutide and sibutramine on food intake, palatability, body weight and glucose tolerance in the gubra DIO-rats, Acta Pharmacol. Sin. 33 (2012) 194–200, https://doi.org/10.1038/aps.2011.168.

[29] E. Naslund, J. Bogefors, S. Skogar, P. Gryback, H. Jacobsson, J.J. Holst, P.M. Hellstrom, GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans, Am. J. Physiol. 277 (1999) R910–R916, https://doi.org/10.1152/ajpregu.1999.277.3.R910.

[30] K. Meeran, D.O. Shea, P.D. Upton, C.J. Small, M.A. Ghatei, P.H. Byfield, S.R. Bloom, Circulating adrenomedullin does not regulate systemic blood pressure but increases plasma prolactin after intravenous infusion in humans : a pharmacokinetic study, J. Clin. Endocrinol. Metab. 82 (1997) 95–100.

[31] H. Mulder, B. Ahrén, S. Karlsson, F. Sundler, Adrenomedullin: localization in the gastrointestinal tract and effects on insulin secretion, Regul. Pept. 62 (1996) 107–112, https://doi.org/10.1016/0167-0115(96)00005-5.
[32] G. Aggarwal, V. Ramachandran, N. Javeed, T. Arumugam, S. Dutta, G.G. Klee, E.W. Klee, T.C. Smyrk, W. Bamlet, J.J. Han, N.B. Rumie Vittar, M. De Andrade, D. Mukhopadhyay, G.M. Petersen, M.E. Fernandez-Zapico, C.D. Logsdon, S.T. Chari, Adrenomedullin is up-regulated in patients with pancreatic cancer and causes insulin resistance in β cells and mice, Gastroenterology 143 (2012) 1510–1517, https://doi.org/10.1053/j.gastro.2012.08.044 e1.
[33] J.B. Pawlak, S.E. Wetzel-Strong, M.K. Dunn, K.M. Caron, Cardiovascular effects of exogenous adrenomedullin and CGRP in Ramp and Calcrl deficient mice, Peptides 88 (2017) 1–7, https://doi.org/10.1016/j.peptides.2016.12.002.
[34] M. Schlaich, N. Straznicky, E. Lambert, G. Lambert, Metabolic syndrome: a sympathetic disease? Lancet Diabetes Endocrinol. 3 (2015) 148–158, https://doi.org/10.1016/S2213-8587(14)70033-6.
[35] M.H. Tschöp, B. Finan, C. Clemmensen, V. Gelfanov, D. Perez-Tilve, T.D. Müller, R.D. DiMarchi, Unimolecular polypharmacy for treatment of diabetes and obesity, Cell Metab. 24 (2016) 51–62, https://doi.org/10.1016/j.cmet.2016.06.021.
[36] A. García-Ponce, S. Chánez Paredes, K.F. Castro Ochoa, M. Schnoor, Regulation of endothelial and epithelial barrier functions by peptide hormones of the adrenomedullin family, Tissue Barriers. 4 (2016) e1228439, , https://doi.org/10.1080/21688370.2016.1228439.
[37] Y. Shimekake, K. Nagata, S. Ohta, Y. Kambayashi, H. Teraoka, K. Kitamura, T. Eto, K. Kangawa, H. Matsuo, Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca2+ mobilization, in bovine aortic endothelial cells, J. Biol. Chem. 270 (1995) 4412–4417.
[38] K. Kitamura, K. Kangawa, M. Kawamoto, Y. Ichiki, S. Nakamura, H. Matsuo, T. Eto, Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma, Biochem. Biophys. Res. Commun. 192 (1993) 553–560, https://doi.org/10.1006/bbrc.1993.1451.
[39] S. Madsbad, U. Kielgast, M. Asmar, C.F. Deacon, S.S. Torekov, J.J. Holst, An overview of once-weekly glucagon-like peptide-1 receptor agonists-available efficacy and safety data and perspectives for the future, Diabetes Obes. Metab. 13 (2011) 394–407, https://doi.org/10.1111/j.1463-1326.2011.01357.x.