Vol. 179, No. 1, 1991
August 30, 1991

GLP-$1_{(7-36)}$amide-STIMULATED INSULIN SECRETION IN RAT ISLETS IS SODIUM-DEPENDENT

Tord Fridolf[1] and Bo Ahrén[1,2]

Departments of [1]Pharmacology and [2]Surgery, Lund University, Sölvegatan 10 S-223 62 Lund, Sweden

Received June 13, 1991

Summary. We examined the Na^+-dependency of the effects of GLP-$1_{(7-36)}$amide in normal, overnight cultured rat islets. It was found that GLP-1(7-36)amide stimulated insulin secretion, $^{45}Ca^{2+}$-efflux, and $^{86}Rb^+$-efflux from prelabelled islets. All these effects were abolished by omitting Na^+ from the medium and replacing it with N-methyl-glucamine. This suggests that GLP-$1_{(7-36)}$amide stimulates insulin secretion by depolarizing the β-cells by increasing their permeability to Na^+.

It is now established that GLP-$1_{(7-36)}$amide potently stimulates insulin secretion in vivo, as demonstrated both in man (1) and in mice (2). The insulinotropic action of the peptide has also been demonstrated in vitro in the perfused rat and pig pancreas (3,4) and in tumoral RINm5F cells (3). A suggested mechanism whereby GLP-$1_{(7-36)}$amide exerts this action is by increasing the production of cAMP (5). However, GLP-$1_{(7-36)}$amide seems also to increase the cellular uptake of Ca^{2+} as evident by a Ca^{2+}-dependent $^{45}Ca^{2+}$-efflux from preloaded islets (6). Since at the same time, the peptide does not affect the K^+-permeability, the opening of the membraneous Ca^{2+}-channels might be caused by an increased Na^+-permeability. In fact, such an action has recently been shown to be of importance for the insulinotropic action of muscarinic agonism (7). Therefore, we examined the Na^+-dependency of GLP-$1_{(7-36)}$amide-stimulated insulin secretion and $^{45}Ca^{2+}$- and $^{86}Rb^+$-efflux in normal, overnight, cultured rat islets. Na^+ was omitted from the medium and replaced by N-methyl-glucamine, which is known to be a large impermeant cation (8).

Abbreviation: GLP, glucagon-like peptide.

0006-291X/91 $1.50

MATERIALS AND METHODS

Animals. The experiments were performed with pancreatic islets isolated from male Sprague-Dawley rats (Anticimex, Stockholm, Sweden), weighing 200-300 g. The animals were fed on a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum.

Preparation of islets. Islets were isolated by the collagenase digestion technique (Collagenase P, Boehringer, Mannheim Gmbh, Germany) (6). After overnight culture in RPMI 1640 (Biocrom KG, Berlin, Germany), the islets were washed three times in Krebs-Ringer bicarbonate (KRB) buffer. The KRB buffer consisted of (in mM): 114 NaCl, 4.4 KCl, 1.28 $CaCl_2$, 1.5 $KHPO_4$, 0.8 $MgSO_4$, 24 $NaHCO_3$ and supplemented with 10 mM Hepes (Boehringer, Mannheim, Gmbh, Germany), 0.2 % human serum albumin (Sigma Chemical Co., St.Louis, Mo, USA) and 3.3 mM glucose. Ten islets were preincubated for 45 min in chambers (Multidish, Nunclon^R, Nunc, Roskilde, Denmark) in 1 ml of KRB buffer supplemented with 3.3 mM glucose at 37° C. After preincubation, the islets were transferred into new chambers (Microwell^R Module F-8, Immunoquality, Medium binding capacity, Nunc , Roskilde, Denmark) in which 1 islet per chamber was incubated for 60 min in 100 µl of KRB supplemented, according to the protocols, with different substances. The solutions were gassed with 9% O_2 and 5% CO_2. After incubation, 50 µl of the medium from the chamber were taken for determination of immunoreactive insulin.

Perifusion of islets. After overnight culture, 160 islets were incubated for 120 min in the KRB medium supplemented with 16.7 mM glucose and 50 µl of $^{45}CaCl_2$ (50-100 µCi) which was added from a stock solution with the specific activity of 26-37 mCi/mg $^{45}CaCl_2$ or for 90 min in 3.3 mM glucose and 40-170 µCi $^{86}RbCl$ which was added from a stock solution with the specific activity of 1.6-6.4 mCi/mg $^{86}RbCl$. The islets were rinsed three times and divided into four groups. They were then transferred to a column and sandwiched between layers of gel (Bio-gel P-4, 200-400 mesh, Bio-rad Lab., Richmond, Ca, USA) and perifused with the KRB solution supplemented with 8.3 mM glucose with or without replacement of Na^+ by 114 mM N-methyl-glucamine (Sigma Chemical Co, St. Louis, Mo, USA). The perifusion rate was 0.1 ml/min. 100 nM of synthetic porcine $GLP\text{-}1_{(7\text{-}36)}$amide (Peninsula Lab., Belmonte, Ca, USA) was introduced according to the protocol. The medium was continously gassed with 95% O_2 and 5% CO_2. Samples were taken every second min. To 0.1 ml of the sample was added 5 ml of scintillation fluid (Ready Safe^{TM}, Beckman, Fullerton, USA) before counting in a scintillation counter (Packard Instrument Inc., Downers Groove, Ill., USA). The remainder of the sample was used for determination of immunoreactive insulin.

Determination of insulin. Insulin was determined radioimmunochemically by the use of guinea pig antiporcine insulin antiserum (MILAB, Malmö, Sweden), ^{125}I-labelled porcine insulin and as standard porcine insulin (Novo Res, Bagsvaerd, Denmark). The separation of bound and free radioactivity was performed with the dextran-coated charcoal technique (9).

Evaluation of results. The results are reported as means ± SEM. The data from the perifusions experiment is presented as the fractional outflow of $^{45}Ca^{2+}$ or $^{86}Rb^+$ from total contents in the islets, calculated for each time point. $P<0.05$ was considered significant with Students t-test.

RESULTS AND DISCUSSION

Fig.1 shows the effect of GLP-$1_{(7-36)}$amide (100 nM) on insulin release from normal, overnight cultured rat islets incubated in a solution with 114 mM Na^+ or without Na^+, which then was replaced by N-methyl-glucamine. In the presence of extracellular Na^+, GLP-$1_{(7-36)}$amide significantly stimulated insulin secretion at 6.7 and 8.3 mM glucose. In contrast, when Na^+ was omitted from the incubation medium, GLP-$1_{(7-36)}$amide was no longer able to induce insulin secretion. Fig.2 shows, similarly, that GLP-$1_{(7-36)}$amide significantly stimulated $^{45}Ca^{2+}$-efflux and insulin secretion from perifused, prelabelled, rat islets at 8.3 mM glucose in the presence of Na^+. In contrast, when Na^+ was omitted from the medium, GLP-$1_{(7-36)}$amide did not stimulate $^{45}Ca^{2+}$-efflux or insulin secretion. Fig.3 A shows that GLP-$1_{(7-36)}$amide, in the presence of Na^+, significantly stimulated ^{86}Rb-efflux from prelabelled islets. However in the absence of Na^+, the peptide was devoid of action (Fig.3 B). Thus, our results show that omission of extracellular Na^+ abolishes the stimulatory effects of the peptide on insulin release, $^{45}Ca^{2+}$-efflux, and

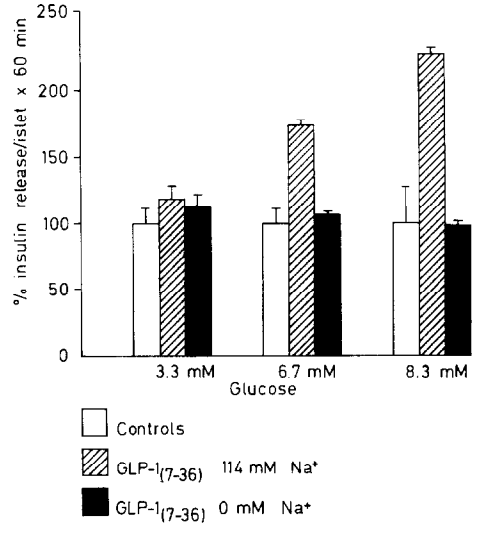

Fig.1. Effects of GLP-$1_{(7-36)}$amide (100 nM) on insulin secretion from normal, overnight cultured rat islets incubated for 60 min in KRB medium supplemented with either 3.3, 6.7, or 8.3 mM glucose. In experiments demonstrated by the black bars, Na^+ was omitted from the medium and replaced by N-methyl-glucamine. Each column represents means ± SEM for 8-24 observations.

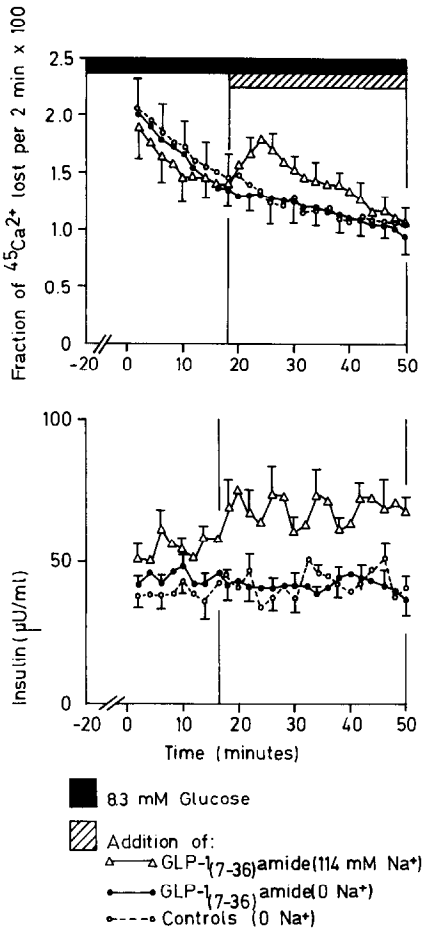

Fig.2. Effects of GLP-$1_{(7-36)}$amide (100 nM) on $^{45}Ca^{2+}$-efflux (upper panel) and insulin secretion (lower panel) from normal, overnight cultured rat islets prelabelled with $^{45}Ca^{2+}$ and perifused in a KRB medium with or without Na^+. In the latter, Na^+ was replaced by N-methyl-glucamine. Means ± SEM are shown for 4 experiments.

$^{86}Rb^+$-efflux in normal rat islets. This suggests that GLP-$1_{(7-36)}$amide depolarizes the β-cell membranes by increasing their permeability to Na^+. This process in turn opens voltage-sensitive Ca^{2+}-channels, which is a mechanism whereby insulin secretion is stimulated (10). This action of GLP-$1_{(7-36)}$amide is similar to that of acetylcholine which recently was shown to stimulate insulin secretion and $^{45}Ca^{2+}$-efflux from prelabelled islets in a Na^+-dependent manner (7). However, differences between the islet effects of GLP-$1_{(7-36)}$amide and cholinergic agonists exist. Thus, phospho-inositide hydrolysis is stimulated by the latter (7) but not by GLP-$1_{(7-36)}$amide (6). Conversely,

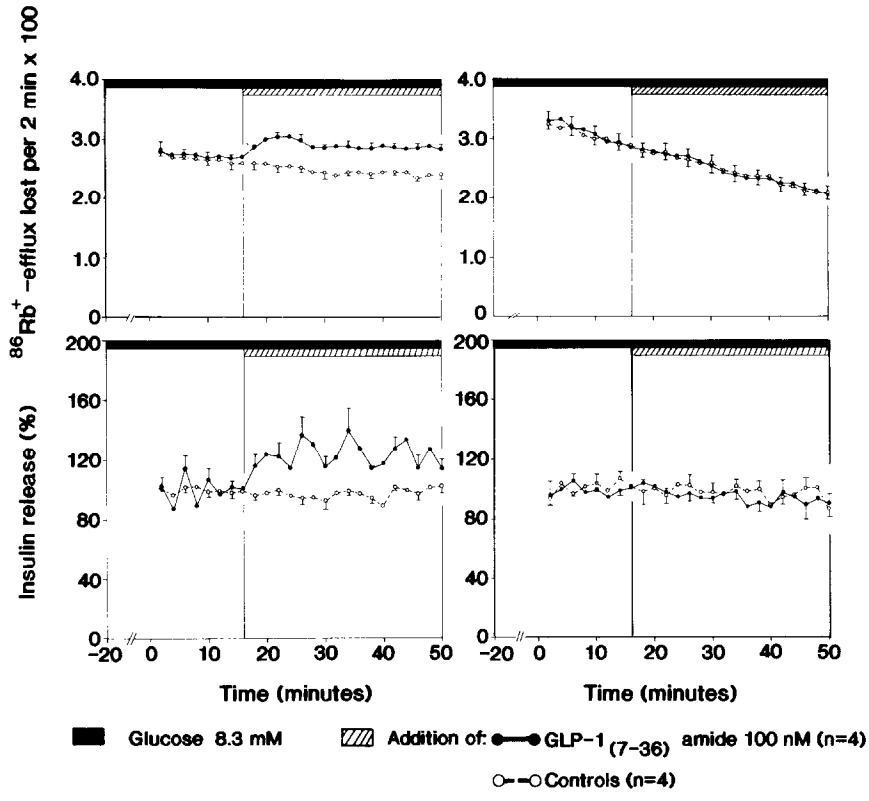

Fig.3. Effects of GLP-$1_{(7-36)}$amide (100 nM) of $^{86}Rb^+$-efflux (upper panel) and insulin release (lower panel) from normal, overnight cultured prelabelled rat islets in the presence of extracellular Na^+ (Fig.3A) or in the absence of extracellular Na^+ (Fig. 3B) in a KRB medium containing 8.3 mM glucose. Means ± SEM are shown for 4 experiments.

the peptide, but not cholinergic agonists, stimulates the production of cyclic AMP (5,11,12). Hence, GLP-$1_{(7-36)}$amide does not stimulate insulin secretion simply by activating the same mechanisms as does acetylcholine. Instead, our results suggest that the peptide, in addition to its stimulatory action on cyclic AMP production, stimulates insulin secretion also by Na^+-dependent stimulation of the uptake of extracellular Ca^{2+}.

ACKNOWLEDGMENTS

The technical assistance of Lena Kvist and Lilian Bengtsson is gratefully acknowledged. This study was supported by the Swedish Medical Research Council (grants No 14X-6834, 14P-8453 and 12X-712), Nordisk Insulinfond, Crafoordska Stiftelsen, Albert Påhlssons Stiftelse, Svenska Diabetesförbundet, Magnus Bergvalls Stiftelse, Svenska Hoechst Diabetesfond, and the Faculty of Medicine, Lund.

REFERENCES

1. Kreymann G., Williams G., Ghatei M.A., and Bloom S.A. (1989) Lancet 2:1300-1304.

2. Fridolf T., Böttcher G., Sundler F., and Ahrén B. (1990) Pancreas 6: 208-215.

3. Göke R., Fehmann H.C., Richter G., Trautmann M., and Göke B. (1989) Pancreas 4: 668-673.

4. Holst J.J., Örskov C., Nielsen O.V., and Schwartz T.W. (1987) FEBS Lett. 211: 169-174.

5. Drucker D.J., Philippe J., Mojsov S., Chick W.L., and Habener J.F. (1987) Proc. Natl. Acad. Sci. USA 84: 3434-3438.

6. Fridolf T., and Ahrén B. (1991) Diabet. Res. in press.

7. Henquin J.C., Garcia M.-C., Bozem M., Hermans M.P., and Nenquin M. (1988) Endocrinology 122: 2134-2142.

8. Reuss L., and Finn A.L. (1975) J. Membrane Biol. 25: 115-139.

9. Herbert V., Lau K.S., Gottlieb C.W., and Bleicher S.J. (1965) J. Clin. Endocrinol. Metab. 25: 1875-1884.

10. Prentki M. and Matschinsky F.M. (1987) Physiol. Rev. 67: 1185-1248.

11. Göke R., Trautmann M.E., Haus E., Richter G., Fehmann H.-C., Arnold R., and Göke B. (1989) Am. J. Physiol. 257: G397-G401.

12. Ahrén B., Karlsson S., and Lindskog S. (1990) Progress in Brain Research 84: 209-218.