Characterization of receptors for glucagon-like peptide-1(7–36)amide on rat lung membranes

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Specific binding of ^{125}I-labelled GLP-1(7 36)amide to rat lung membranes was dependent upon time and temperature and was proportional to membrane protein concentration. Binding was inhibited in a concentration-dependent manner by unlabelled GLP-1(7-36)amide consistent with the presence of a single class of binding sites with a dissociation constant (K_d) of 1 67 ± 0 29 nmol/l GLP-1(1-36)amide was 260 times less potent in inhibiting the binding of ^{125}I-labelled GLP-1(7 36)amide to lung membranes (K_d of 448 ± 93 nmol/l) Vasoactive intestinal polypeptide and peptide-histidine-isoleucine also displaced ^{125}I-labelled GLP-1(7 36)amide from the receptor concentration-dependently; the K_d was 4 31 ± 0 8 and 7 93 ± 4 79 nmol l, respectively Guanine nucleotides (GTP-γ-S, GDP-β-S) decreased the binding of ^{125}I-labelled GLP-1(7 36)amide to rat lung membranes as was found for GLP-1(7-36)amide receptors in RINm5F cells which were also shown to be coupled to the adenylate cyclase system

GLP-1(7–36)amide, Receptor, Lung, Guanine nucleotide, Adenylate cyclase

1. INTRODUCTION

Glucagon-like peptide-1(7–36)amide is a posttranslational processing product of proglucagon in the mammalian intestine [1–3]. Since it is released in response to oral glucose loads and has been shown to be a powerful stimulator of the glucose-induced insulin release [2,4–6], it was concluded to represent an important incretin candidate. Receptors for GLP-1(7–36)amide are present in different tissues. Previously, we identified and characterized high-affinity receptors for GLP-1(7–36)amide in a rat insulinoma-derived cell line (RINm5F) [7,8] which are coupled to the adenylate cyclase system [9]. Uttenthal and Blázquez demonstrated the presence of GLP-1(7–36)amide receptors on isolated rat gastric glands [10]. Binding of GLP-1(7–36)amide to gastric glands resulted in an increase of cAMP suggesting that like in RINm5F cells the receptor for GLP-1(7–36)amide is linked to the adenylate cyclase. Several studies showed that in brain the thalamus, hypothalamus, pituitary gland and medulla oblongata are rich in GLP-1(7–36)amide binding sites [11,12]. Recently, it has been shown that GLP-1(7–36)amide binds specifically to rat lung membranes and that this binding is pH dependent [12]. The present study was performed to further characterize GLP-1(7–36)amide receptors on rat lung membranes.

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2. MATERIALS AND METHODS

2.1 *Reagents*

GLP-1(7–36)amide, GLP-1(1–36)amide, glucagon-like peptide-2 (GLP-2), oxyntomodulin, glucagon, vasoactive intestinal peptide (VIP) and peptide-histidine-isoleucine (PHI) were purchased from Peninsula Laboratories (St. Helens, Merseyside, UK). Glucagon-related pancreatic peptide (GRPP) was kindly donated by Dr A. Moody (Novo Research Institute, Denmark). GTP-γ-S and GDP-β-S were from Sigma Chemicals (Deisenhofen, FRG).

2.2. *Iodination of GLP-1(7–36)amide*

Iodination of GLP-1(7–36)amide was carried out using a modified IODOGEN method [13] as described in detail previously [7]. The specific activity of the label was estimated to be approximately 74 TBq/mmol

2.3. *Preparation of rat lung membranes*

Membranes were prepared as described in detail previously [8] with minor modifications. Briefly, male albino Wistar rats (200–220 g body weight) were killed by decapitation, the lungs were removed and homogenized in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 30 mM NaCl, 1 mM dithiothreitol, 5 μM phenylmethylsulfonylfluoride (PMSF), pH 7.5, with an Ultra-Turrax followed by a further homogenization using a glass-glass homogenizer. The homogenate was layered over a 41% (w/v) sucrose solution and centrifuged for 60 min at 4°C at 95 000 × g. The band at the interface of the layers represented the membranes and was collected, diluted and centrifuged for 30 min at 4°C at 40 000 × g. The pellet was resuspended in a modified Krebs-Ringer buffer (KRB) (Tris-HCl 2.5 mM, NaCl 120 mM, MgSO₄ 1 2 mM, KCl 5 mM, CH₃COONa 15 mM; pH 7 4) containing 1% (w/v) human serum albumin, 0 1% (w/v) bacitracin and EDTA 1 mM, frozen in liquid nitrogen and stored at −80°C Protein concentration was determined as described by Bradford [14].

2.4 *Binding assay*
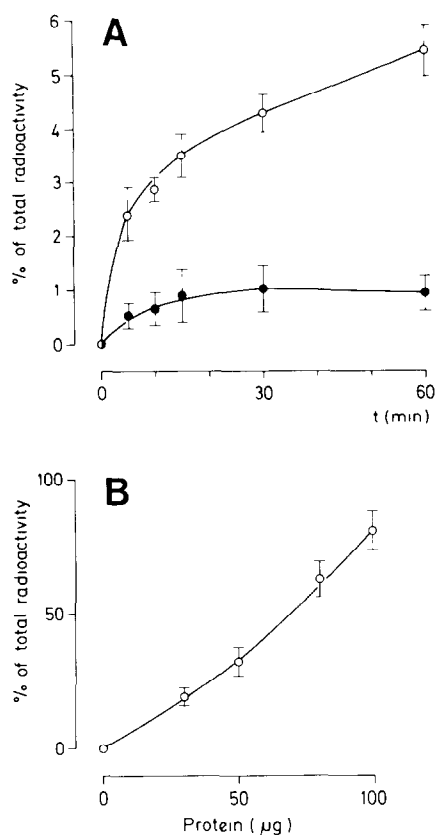
Membranes (1 mg/ml) were incubated with ^{125}I-labelled

Fig. 1. (A) Specific binding of ^{125}I-labelled GLP-1(7–36)amide to lung membranes (1 mg/ml) at 37°C (○) and 10°C (●) (means ± SE, n = 4). (B) Specific binding of ^{125}I-labelled GLP-1(7–36)amide to lung membranes as a function of protein concentration (means ± SE; n = 4).

Fig. 2. Inhibition of binding of ^{125}I-labelled GLP-1(7–36)amide to lung membranes by GLP-1(7–36)amide (○) (n = 4). (A) GLP-1(1–36)amide (●) (n = 4), glucagon (■) (n = 6) and GLP-2 (□) (n = 6). (B) VIP (●) (n = 5), PHI (□) (n = 6) and oxyntomodulin (■) (n = 6). Data points show means ± SE.

GLP-1(7–36)amide (approx. 40000 cpm) in modified KRB at 37°C for 30 min in the presence of the indicated substances. After centrifugation for 5 min at 4°C at 11500 × g the pellet was counted in a γ-counter (Hydragamma 16, Innotron, Oxford, UK). Nonspecific binding was defined as binding in the presence of 1 μmol/l GLP-1(7–36)amide and was subtracted in every case from the total-binding value.

3. RESULTS

Binding of ^{125}I-labelled GLP-1(7–36)amide to rat lung membranes (1 mg/ml protein) was temperature-dependent. Binding was rapid at 37°C and markedly reduced at 10°C (Fig. 1). Binding was dependent on membrane protein concentration over the protein concentration range of 10–100 μg/sample (Fig. 1).

Binding of ^{125}I-labelled GLP-1(7–36)amide was inhibited in a concentration-dependent manner by GLP-1(7–36)amide consistent with the presence of a single class of binding sites with a dissociation constant (K_d) of 1.67 ± 0.29 nmol/l (Fig. 2). Binding of ^{125}I-labelled GLP-1(7–36)amide was also inhibited in a concentration-dependent manner by GLP-1(7–36)amide but the binding affinity was ap-

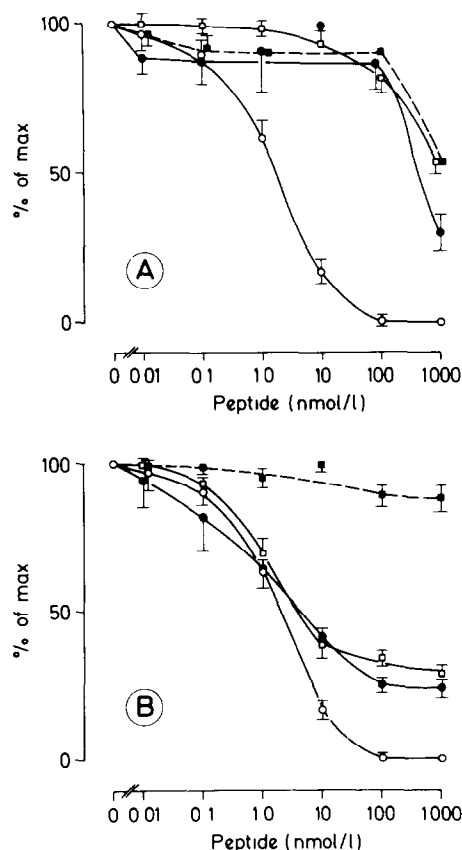proximately 260-fold less than that of the truncated peptide. The K_d was 448 ± 93 nmol/l (Fig. 2). VIP and PHI were also able to displace ^{125}I-labelled GLP-1(7–36)amide concentration-dependently with a K_d of 4.31 ± 0.8 and 7.93 ± 4.79 nmol/l, respectively (Fig. 2). Binding of tracer was weakly, but reproducibly, inhibited by glucagon and GLP-2. Thus, at a concentration of 1 μmol/l, glucagon and GLP-2 inhibited binding of tracer by 45% (Fig. 2). Binding of label was not significantly inhibited by oxyntomodulin and GRPP.

Table I

Effect of GDP-β-S and GTP-γ-S on binding of ^{125}I-labelled GLP-1(7–36)amide to rat lung membranes (means ± SE; n = 6)

	0.1 μmol/l	1.0 μmol/l	10 μmol/l	100 μmol/l
Control	100			
GDP-β-S	62.0 ± 1.73	24.17 ± 0.83	11.33 ± 0.88	9.0 ± 0.58
GTP-γ-S	–	64.83 ± 5.30	15.33 ± 1.12	11.50 ± 0.34

Binding is expressed as % of tracer max. specifically bound per 100 μg membrane protein

In further experiments lung membranes (1 mg/ml protein) were incubated for 30 min at 37°C with ^{125}I-labelled GLP-1(7−36)amide in the absence or presence of a range of concentrations of GTP-γ-S and GDP-β-S. As shown in Table I, binding of tracer was inhibited in a concentration-dependent manner by the nucleotides.

4. DISCUSSION

Our results demonstrate the presence of high affinity receptors for GLP-1(7−36)amide on isolated rat lung membranes. Consistent with the data of Kanse et al. [12], we found that GLP-1(1−36)amide competes with ^{125}I-labelled GLP-1(7−36)amide much less strongly than GLP-1(7−36)amide. In contrast to their results, we found that VIP and PHI were more potent in displacing ^{125}I-labelled GLP-1(7−36)amide than other proglucagon-derived peptides, except GLP-1(7−36)amide itself. The order of potency was GLP-1(7−36)amide > VIP > PHI > GLP-1(1−36)amide > glucagon and GLP-2.

Previously we identified and characterized receptors for GLP-1(7−36)amide on rat insulinoma-derived RINm5F cells [7,8]. Our results show that the binding affinity of GLP-1(7−36)amide on rat lung receptors is 10 times less compared to the receptors on RINm5F cells (1.67 ± 0.29 nmol/l vs 0.204 ± 0.08 nmol/l). While VIP and PHI did not bind to GLP-1(7−36)amide receptors on insulinoma cells both peptides were able to displace ^{125}I-labelled GLP-1(7−36)amide with a K_d which was 2.5 and 4.7 times, respectively, less than that of GLP-1(7−36)amide.

Previously, we demonstrated that GLP-1(7−36)amide receptors on RINm5F cells are coupled to the adenylate cyclase system [9]. We now show that binding of ^{125}I-labelled GLP-1(7−36)amide to lung membranes is decreased in the presence of guanine nucleotides suggesting that these receptors are also linked to adenylate cyclase system.

The presence of GLP-1(7−36)amide receptors on rat lung membranes indicates that the peptide may not only act, as has been suggested, as an incretin [2,4−6] but may have additional, so far undefined, functions. Studies are currently underway to obtain information on the possible functional relevance of these findings.

Interestingly, there are several reports of the presence of GLP-1(7−36)amide binding sites in the brainstem [11,15−19]. These findings along with ours infer that GLP-1(7−36)amide may have a role in peripheral and central regulation of pulmonary function.

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