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Inhibition of the preferential binding of actin to the N-terminal hydratase domain of the 78-kDa gastrin-binding protein by non-steroidal anti-inflammatory drugs and gastrin receptor antagonists

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Abstract

The 78 kDa gastrin-binding protein (GBP) is a likely target for the antiproliferative effects of gastrin receptor antagonists and non-steroidal anti-inflammatory drugs (NSAIDs) on colorectal carcinoma cells (Baldwin GS, Murphy VJ, Yang Z, and Hashimoto T. J Pharmacol Exp Ther 1998;286:1110–14). This study tested the hypotheses that the GBP bound actin, and that the interaction could be disrupted by gastrin receptor antagonists and NSAIDs. Binding of actin to the GBP was assessed by competition with ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ in a covalent cross-linking assay, and by comparison of ^{125}I-actin binding to the N- and C-terminal GBP domains, which had been expressed independently in *E. coli* as glutathione-S-transferase (GST) fusion proteins. The ability of gastrin receptor antagonists and NSAIDs to interfere with the actin-GBP interaction was measured by release of ^{125}I-actin from preformed complexes with the N- and C-terminal domain-GST fusion proteins. Actin purified from skeletal muscle or from gastric mucosal cytosol competed with ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ for binding to the GBP with IC_{50} values of 2.6 ± 0.7 μM, and 2.1 ± 0.7 μM, respectively. The amount of ^{125}I-actin from either source bound to the N-terminal GBP domain was 8.2 times greater than the amount bound to the C-terminal domain. Binding of actin to both domains was inhibited by the gastrin receptor antagonists proglumide and benzotript, and by NSAIDs. We conclude that the GBP may associate with the cytoskeleton via an interaction between its N-terminal domain and actin, and that the association may be disrupted either by gastrin receptor antagonists or by NSAIDs. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Actin; Benzotript; Gastrin; Gastrin binding protein; Mitochondrial trifunctional protein; NSAIDs

1. Introduction.

A 78-kDa GBP has been purified from porcine gastric mucosal membranes [1]. The amino acid sequence of the GBP, translated from the nucleotide sequence of the corresponding cDNA [2], was closely related to the sequence of the α-subunit of a rat MTP which catalyses the second and third steps in long chain fatty acid oxidation [3,4]. Recent cloning experiments have indicated that the GBP and the α-subunit of the MTP may be products of alternately spliced mRNAs which encode the same mature protein, but differ in the presence of signal peptides specific for the endoplasmic reticulum and for mitochondria, respectively [5]. Sequence comparisons with monofunctional relatives suggested that the N-terminal half of the GBP catalyzes enoyl CoA hydratase activity, and that the C-terminal half catalyzes L-3-hydroxyacyl CoA dehydrogenase activity [6]. Both the N- and C-terminal halves of the GBP bound gastrin and gastrin receptor antagonists when expressed independently in *Escherichia coli* as glutathione S-transferase fusion proteins [7].

The GBP is a likely target for the anti-proliferative effects of gastrin/CCK receptor antagonists on colorectal carcinoma cells [8]. Thus the gastrin receptor antagonists proglumide and benzotript inhibited cross-linking of iodinated gastrin to the GBP, and the IC_{50} values obtained by computer fitting correlated well with the IC_{50} values for inhibition of HCT 116 cell proliferation [9]. Gastrin and benzotript have also been shown to inhibit all three enzyme activities associated with the MTP [10]. Evidence has also been presented that the GBP binds NSAIDs [11], and that the consequent inhibition of long chain fatty acid oxidation contributes to the anti-proliferative effects of NSAIDs on colorectal carcinoma cells [12].

The D-bifunctional protein is an 80 kDa peroxisomal

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Abbreviations: CCK, cholecystokinin; GBP, gastrin-binding protein; GST, glutathione *S*-transferase; MTP, mitochondrial trifunctional protein; NSAID, non-steroidal anti-inflammatory drug.

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enzyme which was first characterized by its ability to oxidize estradiol [13,14]. More recently sequence comparisons have revealed that the D-bifunctional protein is related to a family of yeast and fungal multifunctional fatty acid oxidation enzymes, exemplified by the product of the Fox-2 gene of *Saccharomyces cerevisiae*, which is a 96 kDa protein, containing duplicated N-terminal D-3-hydroxyacyl-CoA dehydrogenase domains, and a C-terminal enoyl-CoA hydratase domain [15]. None of the domains show any similarity to the hydratase and dehydrogenase domains of the MTP/GBP family [6]. The D-bifunctional protein contains a single N-terminal D-3-hydroxyacyl-CoA dehydrogenase domain, a central enoyl-CoA hydratase domain, and a C-terminal domain related to sterol carrier proteins [16]. The individual domains, when expressed in *E. coli*, catalyzed the reactions predicted from the sequence comparisons [16], and human sterol carrier protein-2 binds long chain fatty acyl CoAs with nM affinities [17]. The observation that mutations in the D-bifunctional protein lead to a fatal form of Zellweger syndrome suggests that the D-bifunctional protein plays an essential role in the oxidation of long chain fatty acids in the peroxisome [18,19].

Some forms of the porcine D-bifunctional protein interact with actin [20]. For example, porcine endometrial cells contain a 32-kDa protein, which is generated by proteolytic release of the N-terminal domain of the 80 kDa primary translation product [20]. Approximately 20% of the 32-kDa protein is found as a 1:1 complex with β-actin, and in half of the complexes the protein is covalently bound to actin by an ε-(γ-glutamyl)-lysine linkage [20].

In order to determine whether or not similar non-covalent complexes existed between actin and members of the functionally similar, but structurally unrelated, MTP/GBP family, the ability of actin to inhibit the binding of iodinated gastrin to the GBP was investigated. The location of the actin binding site within the GBP sequence was then defined by measurement of the binding of iodinated actins to glutathione S-transferase fusion proteins containing the N- and C-terminal halves of the GBP. The ability of the gastrin/CCK receptor antagonists proglumide and benzotript, and of NSAIDs, to interfere with the binding of iodinated actin to the two fusion proteins was also studied. The observed interaction between actin and the enoyl-CoA hydratase domain of the GBP is consistent with an interaction between the GBP and the cytoskeleton. The data presented herein further suggests that the interaction may be disrupted by gastrin/CCK receptor antagonists or by NSAIDs.

2. Materials and methods

2.1. Materials

[Nle^{15}]-gastrin$_{2,17}$ and gastrin$_{17}$ were from Research Plus, Bayonne, NJ. The gastrin/CCK receptor antagonists proglumide (D,L-4-benzamido-N,N-dipropyl-glutaramic acid) and benzotript (*N-p*-chlorobenzoyl-L-tryptophan) were from Sigma and Calbiochem respectively. Porcine skeletal muscle actin and NSAIDs were from Sigma. The porcine 78-kDa GBP was purified from detergent extracts of gastric mucosal membranes by chromatography on concanavalin-A-Sepharose and DEAE-Sepharose as described previously [1].

2.2. Purification of actin

Actin was purified from gastric mucosal cytosol by precipitation with $MgCl_2$ in the presence of glycerol [21], followed by polymerization in the absence of glycerol, and chromatography on DE-52 cellulose [22]. All steps were at 4°. Cytosol was prepared from gastric mucosal extracts [1] by centrifugation at 100,000 *g* for 60 min. Actin was precipitated by addition of glycerol to 4 M, $MgCl_2$ to 10 mM, and phenylmethylsulphonyl fluoride to 0.5 mM. The pellet after centrifugation at 100,000 *g* for 30 min was resuspended in 10 mM Tris/HCl, pH 7.4, containing 1 mM ATP, 1 mM EDTA, 0.5 mM 2-mercaptoethanol, and 1 mM phenylmethylsulphonyl fluoride, and was dialysed against the same buffer overnight. After centrifugation at 100,000 *g* for 30 min actin was polymerized by addition of KCl to 100 mM, $MgCl_2$ to 1.1 mM and ATP to 1 mM. The actin pellet was collected by centrifugation at 100,000 *g* for 30 min, and was resuspended in 10 mM imidazole, pH 8, 100 mM KCl, 0.1 mM $CaCl_2$ 1 mM ATP, 0.2 mM 2-mercaptoethanol and 0.005% NaN_3 (buffer A), and rotated for 2 hr with 5 mL DE-52 cellulose, which had been pre-equilibrated with the same buffer. The resin was collected by centrifugation, and washed with 20 mL buffer A. Actin was eluted with 10 mM imidazole, pH 6.4, 300 mM KCl, 0.1 mM $CaCl_2$, 1 mM ATP, 0.2 mM 2-mercaptoethanol and 0.005% NaN_3. The purity of actin preparations, assessed by SDS-polyacrylamide gel electrophoresis [23], was >90%. The concentration of actin was determined by the Bradford assay [24], with porcine skeletal muscle actin as standard.

2.3. Iodinations

[Nle^{15}]-gastrin$_{2,17}$ was iodinated by the iodogen method, and purified by chromatography on a C18 Seppak (Waters) as described previously [25]. Actin was also iodinated by the iodogen method, but the reaction was stopped by the addition of sodium metabisulphite to 1 mg/mL, and the labelled actin was separated from unincorporated iodide by passage through a 10 mL column of Sephadex G25 Fine (Pharmacia).

2.4. Cross-linking assay

Binding of actin to the GBP was measured by inhibition of cross-linking of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ (10 fmol, approx. 30,000 cpm) with disuccinimidylsuberate as described previously [1,26]. Reaction products were separated by SDS-polyacrylamide gel electrophoresis and the radioactivity associated with the GBP was detected and quantified with a

FujiBas phosphorimager (Berthold). Estimates of IC_{50} values, and of the levels of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ bound in the absence of competitor, were obtained with the program SIGMASTAT (Jandel Scientific) by nonlinear regression to the equation $y = a/(1 + x/b)$, where y is the amount of iodinated gastrin bound expressed as a percentage of the value a observed in the absence of actin, x is the concentration of actin, and b is the IC_{50} value.

2.5. Preparation of GST fusion proteins

The N- and C-terminal halves of the GBP were expressed independently in *E. coli* as glutathione-*S*-transferase fusion proteins, solubilised with 1.5% Sarkosyl [27], and purified on glutathione-agarose beads, as described previously [7].

2.6. Binding of actin to fusion proteins

GST-fusion proteins bound to glutathione-agarose beads (200 μL) were washed twice with 1 mL 50 mM Na^+ Hepes, pH 7.6, 0.1% Triton X-100, 1 mM dithiothreitol and 0.02% NaN_3 (buffer B), and incubated for 2 hr at 4°C with 250,000 cpm iodinated actin. All subsequent steps were at 4°. The beads were then washed twice with 1 mL buffer B, and resuspended in 225 μL buffer B. Twenty-five microliter aliquots were transferred to tubes containing an equal volume of buffer with or without the desired antagonist, or of loading buffer [23] containing 50 mM dithiothreitol, and incubated for 2 hr. The beads were then removed by centrifugation (13000 *g*, 1 min), and 10-μL aliquots of the supernatant were counted in a γ-counter (LKB-Wallac). The amount of actin released was expressed as a percentage of the amount released in the presence of loading buffer containing dithiothreitol, after correction for the amount released in the presence of buffer alone.

In some cases the extent of residual binding of the GST-fusion proteins to the beads at the end of the experiment was assessed as follows. The beads were resuspended in loading buffer, boiled for 5 min, and centrifuged at 13000 *g* for 1 min. Aliquots of the supernatant were then electrophoresed on a 10% SDS-polyacrylamide gel [23], and proteins were detected by staining with Coomassie blue.

3. Results

A high affinity interaction between actin and the D-3-hydroxyacyl-CoA dehydrogenase domain of porcine peroxisomal 17β-hydroxysteroid dehydrogenase type IV has been described [20]. In order to determine whether or not a similar complex was formed between actin and the L-3-hydroxyacyl-CoA dehydrogenase domain of members of the functionally related, but structurally different, MTP family [6], the interaction between actin and the 78 kDa GBP was examined. We initially investigated the ability of actin to compete in a covalent cross-linking assay which measures the binding of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ to the 78-kDa GBP [1,26].

3.1. Binding of actin to the GBP

Actin from a variety of sources dose dependently inhibited covalent cross-linking of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ to the 78-kDa porcine GBP (Fig. 1). IC_{50} values ($\pm$ SEM, $n = 3$) of 110 $\pm$ 30 μg/mL (2.6 $\pm$ 0.7 μM) and 90 $\pm$ 30 μg/mL (2.1 $\pm$ 0.7 μM) were observed for α-actin from porcine skeletal muscle (Sigma) and for a mixture of β- and γ-actins prepared from porcine gastric mucosal cytosol, respectively. No competition was observed in the presence of similar concentrations of ovalbumin, which was chosen as a control because its molecular weight is the same as actin (43,000). These results are consistent with a model in which actin and gastrin compete for the same binding site on the GBP.

The region of the GBP responsible for binding was investigated by measuring the binding of iodinated actins to the N- and C-terminal halves of the GBP expressed as fusion proteins with glutathione S-transferase [7]. Since actin had previously been shown to interact with the D-3-hydroxyacyl-CoA dehydrogenase domain of porcine peroxisomal 17β-hydroxysteroid dehydrogenase type IV [20], we expected that actin would interact with the C-terminal L-3-hydroxyacyl-CoA dehydrogenase domain of the GBP. However binding to the fusion protein containing the N-terminal enoyl CoA hydratase domain of the GBP (Fig. 2B, lane N) was 8.2 times greater than to the fusion protein containing the C-terminal L-3-hydroxyacyl-CoA dehydrogenase domain (Fig. 2B, lane C). The interaction appeared to involve only the GBP sequences in the fusion proteins, since no binding was observed to glutathione *S*-transferase (Fig. 2B, lane G), or to glutathione-agarose beads (Fig. 2B, lane B).

The affinity of interaction between α-actin and GST-fusion proteins containing the N- and C-terminal halves of the GBP was measured by competition for ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ binding in the covalent cross-linking assay (Fig. 1C). The IC_{50} value ($\pm$ SEM, $n = 4$) of 260 $\pm$ 50 μg/mL for binding of α-actin to the N-terminal half of the GBP was only 2.3 fold higher than the IC_{50} value for binding to the entire molecule. In contrast the IC_{50} value for binding of α-actin to the C-terminal half of the GBP could not be accurately determined because inhibition did not exceed 20% at the maximum accessible concentration of actin, but is estimated to be greater than 1 mg/mL, or tenfold higher than the IC_{50} value for binding to the entire molecule. The lower affinity of binding of α-actin to the C-terminal half of the GBP provides a satisfying explanation for the reduced binding of iodinated actin to the C-terminal half compared to the N-terminal half of the GBP (Fig. 2).

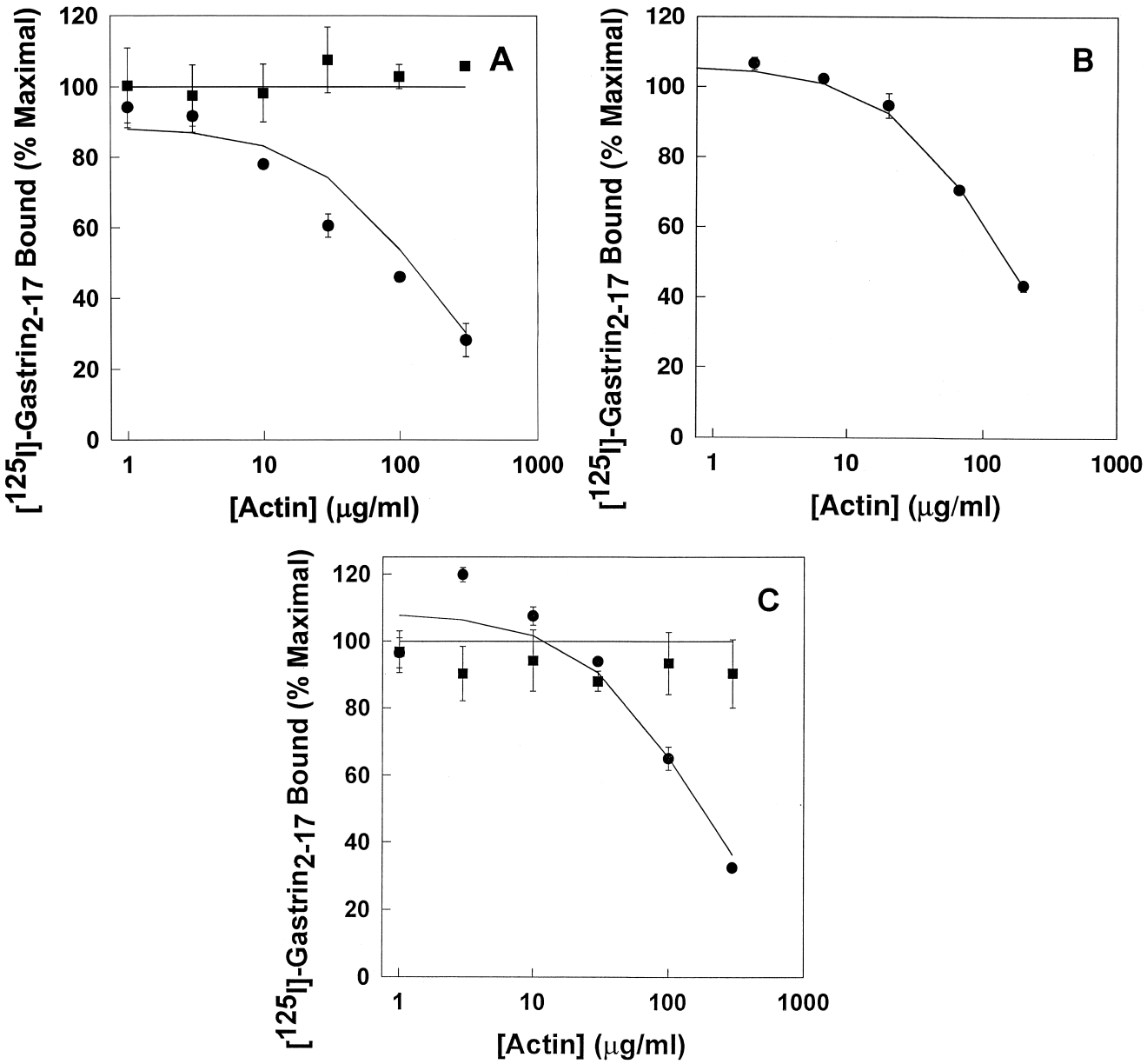

Fig. 1. Binding of actins to the GBP. ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ was cross-linked to the GBP (A, B) or to GST fusion proteins containing either the N-terminal (C, circles) or C-terminal (C, squares) halves of the GBP with disuccinimidylsuberate in the presence of increasing concentrations of actin as previously described [1,26]. Actin was from porcine skeletal muscle (A, circles; C, circles; C, squares) or porcine gastric mucosal cytosol (B, circles). Ovalbumin (A, squares) was chosen as a negative control because its molecular weight is the same as actin (43,000). Reaction products were separated by SDS-polyacrylamide gel electrophoresis and the radioactivity associated with the GBP was detected and quantified with a phosphorimager. Estimates of IC_{50} values, and of the levels of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ bound in the absence of competitor (A, 116 μg/mL, 87.7%; B, 138 μg/mL, 105.8%; C, 151 μg/mL, 108.5%), were obtained with the program SIGMASTAT (Jandel Scientific, San Rafael, CA) by nonlinear regression as described in the Materials and Methods section. These IC_{50} values were combined with the values obtained in at least two other experiments to obtain the mean values presented in the text.

3.2. Competition with gastrin receptor antagonists

We have demonstrated previously that the non-selective gastrin/CCK receptor antagonists proglumide and benzotript compete with gastrin for binding to both the intact GBP [8] and to GST fusion proteins containing the N- and C-terminal halves of the GBP [6]. No competition was observed in either assay with the CCK-A receptor-selective antagonist L364,718, or with the gastrin/CCK-B receptor-selective antagonist L365,260, at concentrations as high as 20 μM. In order to confirm that actins bound to the gastrin-binding site of the GBP we next investigated the effect of gastrin/CCK receptor antagonists on the binding of iodinated actin to GST-fusion proteins containing either the N- or C-terminal halves of the GBP. Although the gastrin/CCK receptor antagonists proglumide and benzotript inhibited the binding of iodinated actins to both the N- and C-terminal fusion proteins (Fig. 3), no inhibition was observed with the

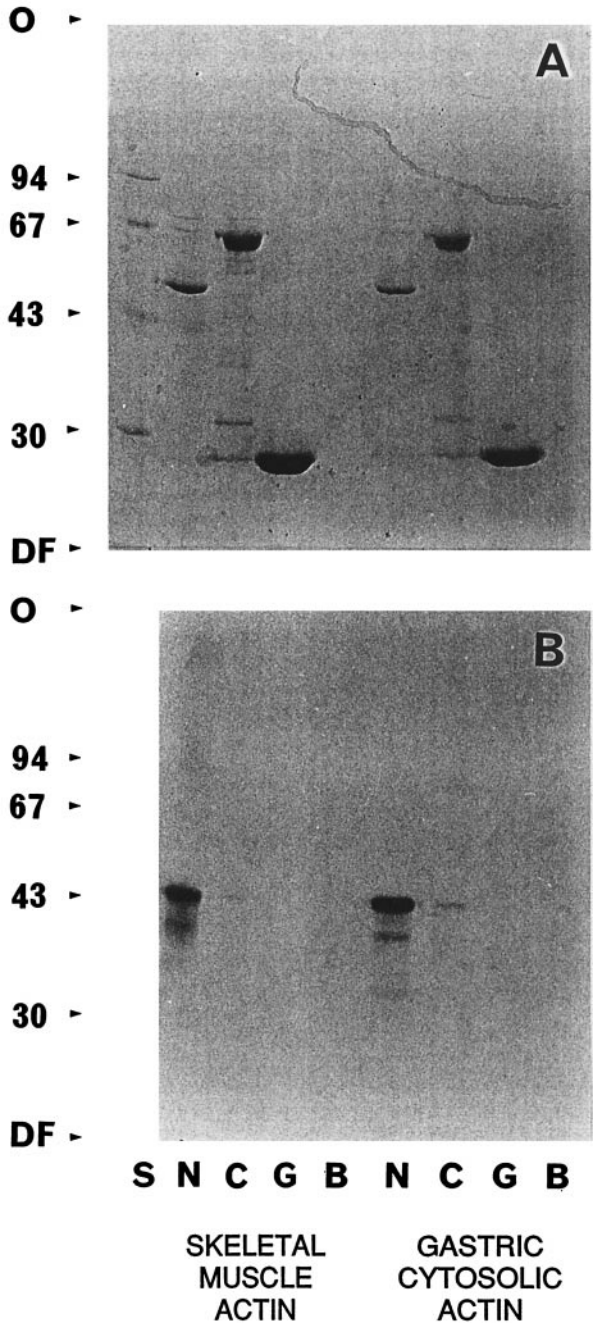

Fig. 2. Actins bind preferentially to the N-terminal half of the GBP. Iodinated actins from skeletal muscle and from gastric mucosal cytosol were incubated either with the N- (N) or C-terminal (C) halves of the GBP expressed as glutathione-*S*-transferase fusion proteins and bound to glutathione-agarose beads, or with GST (G) bound to glutathione-agarose beads, or with glutathione-agarose beads alone (B) as described in the Materials and methods section. After washing with buffer the bound actin was released by boiling the beads in the presence of loading buffer containing dithiothreitol. The supernatant after centrifugation was then electrophoresed on a 10% SDS-polyacrylamide gel and stained with Coomassie Blue (A), and the iodinated actins were detected by autoradiography (B). After correction for the different loading of fusion proteins (A), the amount of iodinated actin bound to the N-terminal enoyl CoA hydratase domain was 8.2-fold greater than to the C-terminal 3-hydroxyacyl CoA dehydrogenase domain of the GBP. The sizes of molecular weight standards (S) are given in kDa. O and DF indicate the origin and dye front, respectively.

CCK-A receptor-selective antagonist L364,718, or with the gastrin/CCK-B receptor-selective antagonist L365,260 (Fig. 3). Despite the fact that the amount of actins bound to the N-terminal fusion protein was 8.2-fold greater than to the C-terminal fusion protein, the percentage of actins displaced from either the N- or C-terminal halves by gastrin/CCK receptor antagonists was similar (Fig. 3). These results are consistent with a model in which actin, gastrin, and some gastrin/CCK receptor antagonists, compete for the same binding site on the GBP.

3.3. Competition with NSAIDs

We have also demonstrated previously that several NSAIDs compete with gastrin for binding to the GBP, with IC_{50} values which ranged from 40 μM for sulindac sulphide to 9.3 mM for aspirin [11]. In order to test the hypothesis that NSAIDs would interfere with the interaction between actins and the GBP, we next investigated the effect of several NSAIDs, at concentrations similar to (aspirin) or greater than (sulindac sulphide, indomethacin and ibuprofen) the previously determined IC_{50} values, on the binding of iodinated actin to GST-fusion proteins containing either the N- or C-terminal halves of the GBP. The NSAIDs sulindac sulphide, indomethacin and ibuprofen all inhibited the binding of iodinated actins to both the N- and C-terminal fusion proteins (Fig. 4). Little if any inhibition was observed with aspirin. Although the amount of actins bound to the N-terminal fusion protein was 8.2-fold greater than to the C-terminal fusion protein, the percentage of bound actins displaced from either the N- or C-terminal halves by NSAIDs was similar (Fig. 4). These results suggest that NSAIDs may interfere with the interaction between the GBP and cytoskeletal actins *in vivo*.

4. Discussion

The data presented in this paper indicates that the 78-kDa GBP binds actins from a variety of sources. Thus both α-actin from skeletal muscle and β- and γ-actins from gastric mucosal cytosol were able to inhibit the binding of iodinated gastrin to the GBP in a covalent cross-linking assay. Studies with GST-fusion proteins containing either the N- or C-terminal halves of the GBP indicate that actin binds primarily to the N-terminal enoyl CoA hydratase domain of the GBP. The observation that the interaction between actin and the GBP can be blocked by the gastrin receptor antagonist benzotript, which is a competitive inhibitor of enoyl CoA hydratase activity [10], further suggests that actin binds in the vicinity of the enoyl CoA hydratase active site. In contrast actin binds to the 3-hydroxyacyl CoA dehydrogenase domain of the D-bifunctional protein type IV [20].

The sequence within the enoyl CoA hydratase domain responsible for binding actin has not been located yet. Se-

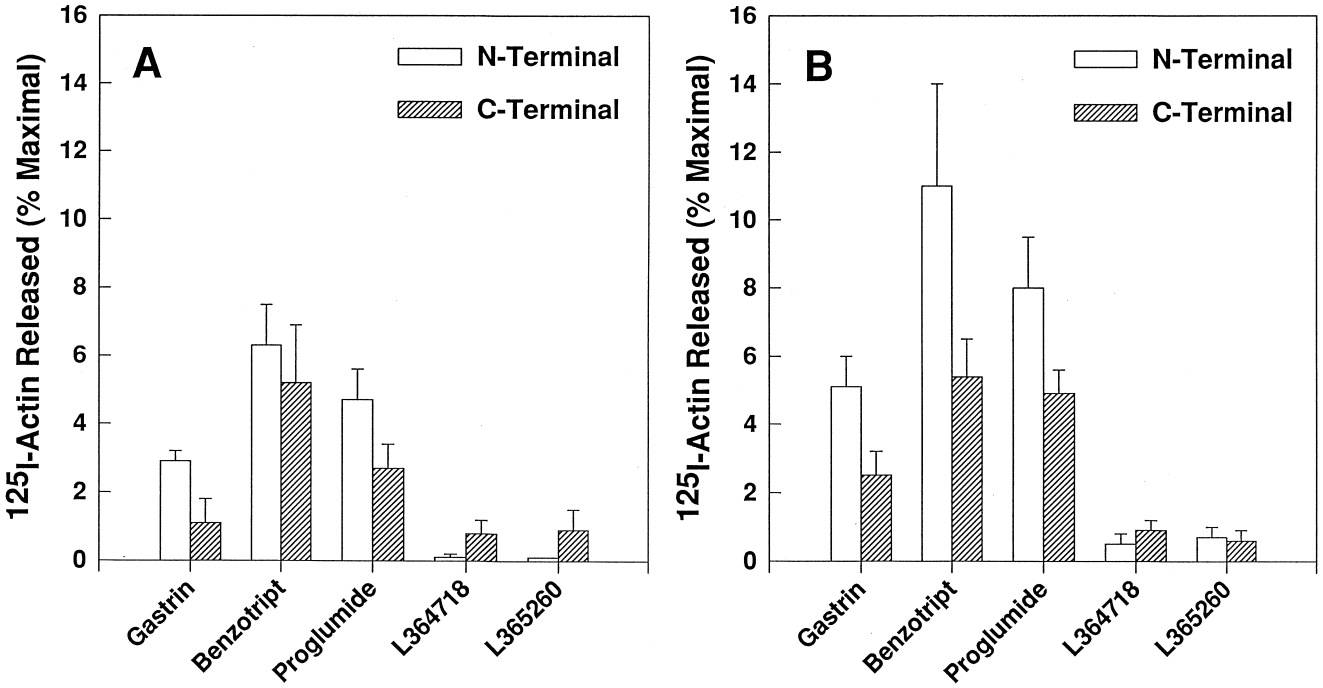

Fig. 3. Gastrin and gastrin/cholecystokinin receptor antagonists compete with actin for binding to the GBP. The release of iodinated porcine skeletal muscle actin (A), or porcine gastric cytosol actin (B), from GST fusion proteins containing the N- (open bars) and C-terminal (hatched bars) halves of the GBP was determined in duplicate as described in section 2. The amount of actin released in the presence of gastrin (10 μM) or the gastrin receptor antagonists benzotript (2.5 mM), proglumide (10 mM), L364,718 (10 μM) and L365,260 (10 μM) was expressed as a percentage of the total amount bound (defined as the amount released after boiling in loading buffer containing dithiothreitol, after correction for the amount released in the presence of buffer alone). Values are the mean ± SEM of at least 4 separate experiments.

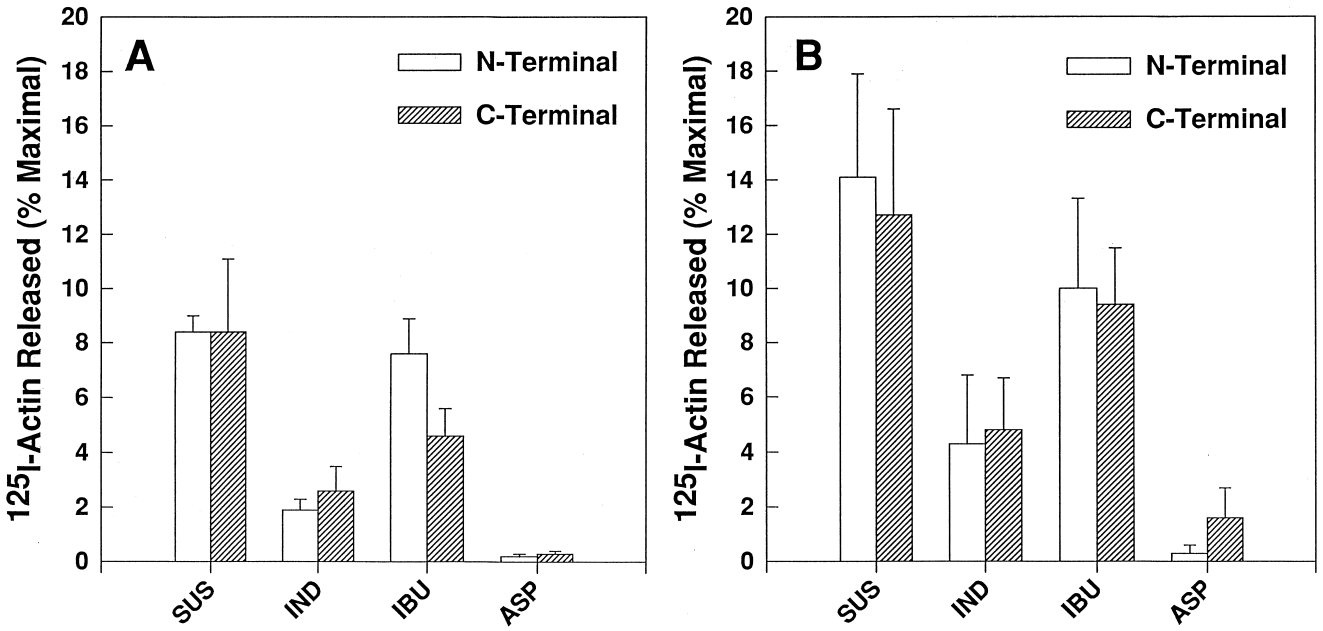

Fig. 4. NSAIDs compete with actin for binding to the GBP. The release of iodinated porcine skeletal muscle actin (A), or porcine gastric cytosol actin (B), from GST fusion proteins containing the N- (open bars) and C-terminal (hatched bars) halves of the GBP was determined in duplicate as described in section 2. The amount of actin released in the presence of sulindac sulphide (SUS, 0.75 mM), indomethacin (IND, 0.75 mM), ibuprofen (IBU, 7.5 mM) and aspirin (ASP, 7.5 mM) was expressed as a percentage of the amount released in the presence of loading buffer containing dithiothreitol, after correction for the amount released in the presence of buffer alone. Values are the mean ± SEM of at least 4 separate experiments.

quence comparisons have not revealed the presence in the GBP of an actin-binding domain similar to the consensus sequence E[AS]GXX[IVM][IV][KH] reported in the 3-hydroxyacyl CoA dehydrogenase domain of the D-bifunctional protein by Adamski and coworkers [28]. Furthermore there is no obvious similarity between the GBP and the actin-binding domains of members of the calponin, gelsolin, cofilin or profilin families [29]. The lack of similarity is not surprising as, although the actin-binding domains of gelsolin and cofilin share the same overall tertiary structure, no similarity is apparent between their protein sequences [29]. Measurement of the actin binding capacity of N- and C-terminally truncated variants of the GBP enoyl CoA hydratase domain would be one experimental approach to the solution of this problem in the future.

The subcellular location of the observed interaction between actin and the GBP is yet to be established. The initial isolation of the GBP from detergent extracts of gastric microsomal membranes [1,26], and evidence that the GBP was glycosylated [30], suggested that the GBP was extramitochondrial. Recent cloning experiments have indicated that the GBP and the α-subunit of the MTP may be products of alternately spliced mRNAs which encode the same mature protein, but differ in the presence of signal peptides specific for the endoplasmic reticulum and for mitochondria, respectively [5]. Hence current evidence is consistent with the hypothesis that the GBP in the endoplasmic reticulum may interact with cytoskeletal actin. On the other hand the recent development of resinless ultrathin section electron microscopy has revealed that cytoskeletal filaments serve as frameworks for organelles such as mitochondria [31]. Genetic studies in yeast have also indicated that mutations in the actin gene ACT1 cause mitochondrial aggregation, enlargement, and loss of mitochondrial motility [32], and that actin influences the mitochondrial/cytoplasmic distribution of some proteins found in both subcellular locations [33]. It is therefore possible that the observed interaction between the GBP/MTP and actin may influence the mitochondrial delivery of the protein.

The physiological relevance of the observed *in vitro* interaction between the GBP and actin remains to be established. We have demonstrated previously that several NSAIDs bind to the GBP [11], with a resultant inhibition of long chain fatty acid oxidation in colorectal carcinoma cell lines [12]. The good correlation observed between the IC_{50} values for inhibition of gastrin binding to the GBP by NSAIDs, and the IC_{50} values for inhibition of proliferation in colorectal carcinoma cell lines [11], suggested that the GBP might be a possible target for some of the anti-proliferative effects of NSAIDs. While the fact that deletion mutations in the GBP/MTP gene are not lethal clearly establishes that the GBP is not essential for cell proliferation, it is equally well established that under conditions of metabolic stress children with such mutations may present with nonketotic hypoglycaemia and hepatic encephalopathy progressing to coma and death [34]. Presumably under fasting conditions oxidation of long chain fatty acyl CoAs by the GBP/MTP provides a critical alternative energy supply. Inhibition of the GBP/MTP by NSAIDs might then decrease the supply of energy and consequently reduce cell proliferation. The observations that actin binds to the GBP, and that NSAIDs inhibit the interaction, raises the intriguing possibility that disruption of the interaction of the GBP with the cytoskeleton may also contribute to the inhibitory effects of NSAIDs on cell proliferation. Further experiments to investigate this mechanism *in vivo* are in progress.

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