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Cibacron blue-induced enhancement of agonist binding to cholecystokinin (CCK) receptors in solubilized pancreatic membranes

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The pancreatic receptor for cholecystokinin (CCK) typifies many G protein-coupled receptors in that its ability to bind agonist can be reduced by GTP or the solubilization of membranes. We found, however, that a dye, cibacron blue, caused up to a 6-fold increase in binding of the CCK receptor agonist, ^{125}I-CCK-8, to rat pancreatic membranes solubilized with digitonin. Binding optimally enhanced in this manner was comparable to binding of ^{125}I-CCK-8 to native membranes with respect to time-course, maximal amount bound, reversibility, and sensitivity to inhibition by various CCK receptor ligands. Increases in affinity of the CCK receptor for CCK-8 accounted fully for the enhancement of binding of ^{125}I-CCK-8. Cibacron blue did not enhance binding of ^{125}I-CCK-8 to native membranes, and also failed to enhance binding of the CCK receptor antagonist, [^{3}H]L-364,718, to solubilized or native membranes. The ability of cibacron blue to enhance binding of agonist but not that of antagonist suggests that this dye may mimic or perhaps stimulate the effects of G protein on CCK receptors. Such a phenomenon may provide new insights into the mechanisms by which receptors distinguish agonists from antagonists.

Introduction

Receptors that interact with guanine nucleotide-binding regulatory proteins (G proteins) mediate the actions of numerous hormones and neurotransmitters [1,2]. Central to the function of these receptors is the ability of biologically active receptor ligands (agonists) when bound to the receptor to promote receptor-G protein coupling. In the presence of GTP, agonist-receptor complexes interact with coupled G proteins to catalyze the dissociation of G protein, resulting in an α subunit-GTP complex that serves as an activator of the biological response [3] and a β-γ fragment that may also promote biological activity [4]. Biologically inactive receptor ligands (antagonists), on the other hand, bind to receptors but fail to promote receptor-G protein coupling.

The mechanisms by which G protein-coupled receptors distinguish agonist from antagonist are unknown. It appears, however, that G proteins themselves participate in these mechanisms, because G proteins generally increase the affinity of G protein-coupled receptors for agonist without a corresponding effect on the binding of antagonist [3,5]. This can be demonstrated directly by reconstituting purified receptors with purified G proteins [6–8]. Conversely, dissociation of receptor-G protein complexes by GTP or chemically modified analogues of GTP generally results in a decrease in receptor affinity for agonist but not for antagonist [9,10]. Indeed, this effect of guanine nucleotides is widely used as a criterion to infer that a given receptor is coupled to a G protein [11–14]. In addition, solubilization of receptor preparations with detergents frequently results in a loss of affinity for agonist but not for antagonist [6,15]. This effect of solubilization may also involve the disruption of receptor-G protein coupling, because maneuvers that stabilize receptor-G protein complexes during solubilization are capable of preserving the ability of solubilized preparations to bind agonist with high affinity [15,16].

The pancreatic receptor for cholecystokinin (CCK) appears to be a G protein-coupled receptor [17,18], and solubilized preparations of this receptor have a reduced affinity for agonist but not for antagonist [19,20]. In the course of using various affinity columns

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for the purification of this receptor, however, we found that binding of agonist to solubilized receptor preparations could be increased significantly by a dye, cibacron blue. Cibacron blue appeared to mimic the effects of G proteins on CCK receptors, in that it enhanced binding of the CCK receptor agonist, ^{125}I-CCK-8 [21], but not that of the CCK receptor-specific antagonist, [^{3}H]L-364,718 [22,23], to solubilized receptor preparations. Direct demonstration of involvement of G proteins, however, remains to be determined. The present report describes our initial characterization of this phenomenon.

Materials

Pancreata from male Sprague-Dawley rats (trimmed of loose fat, rinsed in saline, frozen at −70°C) were obtained from Zivic-Miller Laboratories, Zelienople, PA; digitonin from Gallard-Schlesinger, Carle Place, NY; cibacron blue F3G-A (C.I. 61211) from Fluka Chemical, Ronkonkoma, NJ; COOH-terminal octapeptide of cholecystokinin (CCK-8) from Research Plus, Bayonne, NJ; ^{125}I-Bolton-Hunter-CCK-8 (^{125}I-CCK-8) (2200 Ci/mmol) and [^{3}H](±)L-364,718 (70 Ci/mmol) from New England Nuclear, Boston, MA; Trp-Met-Asp-Phe-NH_2 (CCK-4), N^2,2′-*O*-dibutyryl-guanosine 3′:5′-cyclic monophosphate (Bu_2cGMP), guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF) and bovine γ globulin from Sigma, St. Louis, MO; 2-(*N*-morpholino)ethanesulfonic acid (Mes) from US Biochemical, Cleveland, OH; leupeptin sulfate from Boehringer-Mannheim, Indianapolis, IN; and poly(ethylene glycol) (average molecular weight 6000–7500) from JT Baker, Phillipsburg, NJ. All other chemicals used were of the highest purity commercially available. Membrane filters (cellulose nitrate, 0.2 μm pore diameter) were from Nalge, Rochester, NY; glass fiber filters (GF/B) were from Whatman LabSales, Hillsboro, OR. L-364,718 and L-365,260 were gifts from Drs. Paul Anderson and Ben Evans, Merck Sharp and Dohme Laboratories, West Point, PA.

Methods

Preparation of digitonin solutions

Digitonin was dissolved in water at 10% (w/v) by boiling for 1 min, followed by cooling in tepid water with continuous stirring and filtration through a 0.2 μm cellulose nitrate filter. The solution used for solubilizing membranes, containing 1% digitonin, was prepared by mixing one part 10% digitonin with nine parts Mes 10 mM (pH 6.5), $MgCl_2$ 5 mM, followed by boiling, cooling and filtering as described above.

Preparation of native and solubilized membranes from rat pancreas

Rat pancreas (1 g, −70°C) was dispersed as a coarse powder into 10 ml ice-cold Mes 10 mM (pH 6.5), $MgCl_2$ 5 mM, NaCl 150 mM, Na_2EGTA 1 mM, and PMSF 1 mM. Subsequent steps were performed at 0–4°C. After centrifugation at 250 × *g* for 2 min and aspiration of fatty particles, the suspension was homogenized with the 11 mm probe of a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) operated at power '4' for 30 s. (While efficient cell disruption was essential, excessive homogenization seemed to cause marked variability in the binding assay for ^{125}I-CCK-8.) After centrifugation at 42 000 × *g* for 10 min the pellet was resuspended in 10 ml Mes 10 mM (pH 6.5), $MgCl_2$ 5 mM, Na_2EGTA 1 mM, and PMSF 1 mM. The resulting suspension was divided into two portions for preparing native and solubilized membranes. After another centrifugation at 42 000 × *g* for 10 min, one pellet was resuspended in 10 ml Mes 10 mM (pH 6.5), $MgCl_2$ 5 mM, PMSF 1 mM, and leupeptin 10 μM; this suspension was termed 'native membranes'. The other pellet was resuspended similarly except with 1% digitonin [19], rotated end-over-end for 60 min, and centrifuged at 42 000 × *g* for 60 min; the resulting supernatant was termed 'solubilized membranes'. Native membranes were used within 12 h of preparation and solubilized membranes within 2 days; binding activity did not decay significantly during these periods.

Radioligand binding

Aliquots (20 μl) of native or solubilized membranes, each corresponding to 1 mg of frozen pancreas, were incubated with 7 pM ^{125}I-CCK-8 or 300 pM [^{3}H]L-364,718 in 1 ml of Mes 10 mM (pH 6.5), $MgCl_2$ 5 mM, $MnCl_2$ 10 mM [24] and digitonin 0.02%, at 25°C for 60 min unless noted otherwise. Preliminary experiments revealed that binding of ^{125}I-CCK-8 or [^{3}H]L-364,718 reached a steady-state at 30 min and remained constant for up to 90 min of incubation, and that saturable binding of ^{125}I-CCK-8 was maximal at pH 6.5 in an incubation solution containing 10 mM $MnCl_2$. Digitonin was necessary to prevent ^{125}I-CCK-8 from sticking to test tubes; however, concentrations greater than 0.02% significantly decreased binding of both ^{125}I-CCK-8 and [^{3}H]L-364,718 (data not shown). Binding was terminated as described by Szecowka et al. [19] by precipitating bound radioactivity with polyethylene glycol, added at a final concentration of 10%, and collecting precipitated radioactivity on glass fiber filters. Results are expressed as saturable binding, i.e., total binding (with radioligand alone) minus nonsaturable binding (with radioligand plus 1 μM nonradioactive ligand). Nonsaturable binding was always less than 30% of total binding.

Protein assay

Protein concentrations were determined by the method of Bradford [25] using a commercially available reagent (Bio-Rad Laboratories, Richmond, CA). Bovine γ globulin was used as a standard.

Results

Table I shows that when native pancreatic membranes were solubilized with digitonin, binding of ^{125}I-CCK-8 decreased by 73%. Further treatment of solubilized membranes with 100 μM GTPγS did not significantly alter binding of ^{125}I-CCK-8, but treatment of native membranes with 100 μM GTPγS decreased binding of ^{125}I-CCK-8 by 79%. Similar results were obtained using 1 μM instead of 100 μM GTPγS (results not shown). In contrast, solubilization of native membranes with digitonin increased binding of the CCK receptor antagonist [^{3}H]L-364,718 by 51%. Treatment of native or solubilized membranes with 100 μM GTPγS did not significantly alter binding of [^{3}H]L-364,718.

As illustrated in Fig. 1, binding of ^{125}I-CCK-8 to solubilized membranes (4 fmol/mg protein) was 25 percent of that measured using native membranes (16 fmol/mg protein). Cibacron blue caused up to a 6-fold enhancement of binding of ^{125}I-CCK-8 to solubilized membranes. Detectable enhancement occurred with 30 nM cibacron blue and maximal enhancement occurred with 1 μM cibacron blue. Concentrations of cibacron blue above 1 μM caused a progressive decrease in binding of ^{125}I-CCK-8 to solubilized membranes. Adding 1 μM GTPγS did not alter the effect of cibacron blue on binding of ^{125}I-CCK-8 to solubilized membranes. Cibacron blue did not enhance binding of ^{125}I-CCK-8 to native membranes alone or incubated with 1 μM GTPγS and at concentrations above 300 nM caused a progressive decrease in binding of ^{125}I-CCK-8.

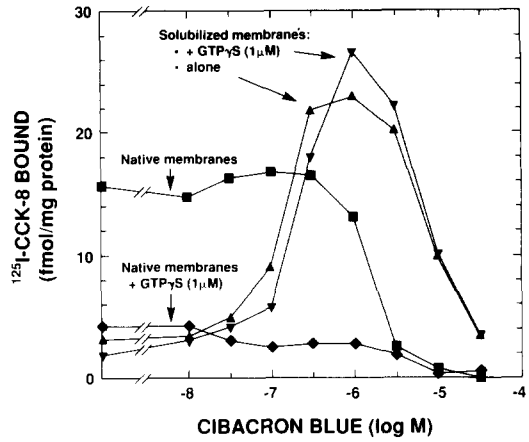

Fig. 1. Effects of varying concentrations of cibacron blue on binding of ^{125}I-CCK-8 to native or solubilized pancreatic membranes. Saturable binding of ^{125}I-CCK-8 was measured using native (45 μg protein/ml) or solubilized (24 μg/ml) membranes incubated at 25°C for 60 min with 7 pM ^{125}I-CCK-8 plus the indicated concentrations of cibacron blue or GTPγS, alone or plus 1 μM CCK-8. Results for saturable binding are from a single experiment and this experiment is representative of four others.

Fig. 2 (left panel) illustrates the time course for binding of ^{125}I-CCK-8 to native membranes or to solu-

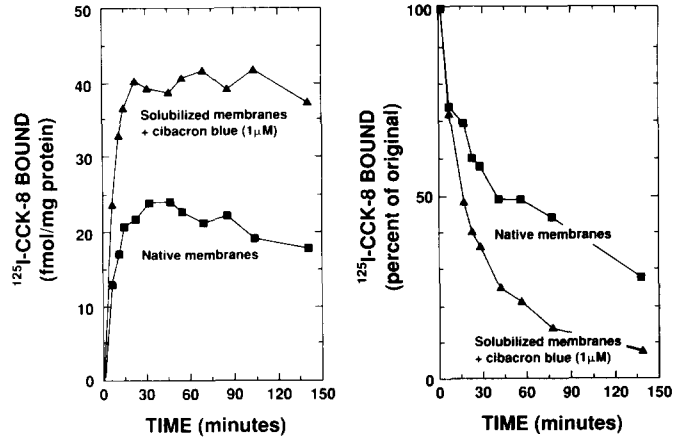

Fig. 2. (Left panel) Time course for binding of ^{125}I-CCK-8 to native pancreatic membranes or to solubilized membranes incubated with 1 μM cibacron blue. Membranes (native, 54 μg protein/ml; solubilized, 30 μg/ml) were incubated at 25°C with 7 pM ^{125}I-CCK-8, alone or plus 1 μM CCK-8, for up to 140 min. Saturable binding of ^{125}I-CCK-8 was determined at the times indicated. Results given are from a single experiment and this experiment is representative of two others. (Right panel) Time course for dissociation of bound ^{125}I-CCK-8 from native pancreatic membranes or from solubilized membranes incubated with 1 μM cibacron blue. Membranes (native, 54 μg protein/ml; solubilized, 30 μg/ml) were incubated at 25°C with 7 pM ^{125}I-CCK-8, alone or plus 1 μM CCK-8, for 60 min. At the end of 60 min of incubation, dissociation of bound ^{125}I-CCK-8 was initiated by adding 1 μM CCK-8 to incubations containing ^{125}I-CCK-8 alone. Saturable binding of ^{125}I-CCK-8 was determined at the times indicated. Results are expressed as the percent of saturably bound ^{125}I-CCK-8 at the beginning of dissociation. Results given are from a single experiment and this experiment is representative of two others.

TABLE I

Binding of ^{125}I-CCK-8 or [^{3}H]L-364,718 to native or solubilized membranes

Binding of ^{125}I-CCK-8 or [^{3}H]L-364,718 to native or solubilized membranes was measured with or without 100 μM GTPγS. Results are expressed as means $\pm$1 S.D. of triplicate determinations in a single experiment. This experiment is representative of two others. Protein concentrations in the assay were: native membranes, 45 μg/ml; solubilized membranes, 24 μg/ml.

Ligand	Ligand bound (fmol/mg protein)	
	native membranes	solubilized membranes
^{125}I-CCK-8 (7 pM)	38.7 $\pm$ 1.7	10.3 $\pm$ 1.3
plus GTPγS (100 μM)	8.0 $\pm$ 1.6	8.3 $\pm$ 2.3
[^{3}H]L-364,718 (300 pM)	1190 $\pm$10	1800 $\pm$50
plus GTPγS (100 μM)	1250 $\pm$20	2000 $\pm$30

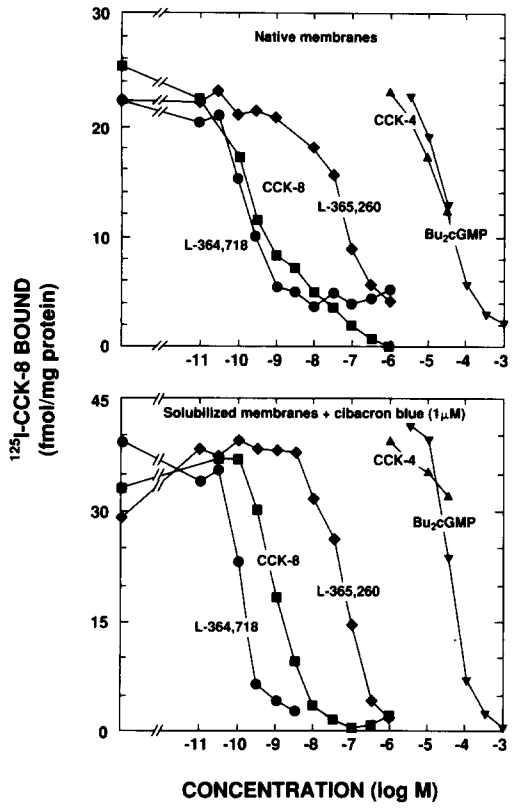

Fig. 3. Effects of CCK receptor agonists and antagonists on binding of ^{125}I-CCK-8 to native pancreatic membranes or to solubilized membranes incubated with 1 μM cibacron blue. (Top panel) Native membranes (57 μg protein/ml) were incubated at 25°C for 60 min with 7 pM ^{125}I-CCK-8 plus the agents indicated. Results given are for saturable binding. (Bottom panel) Same conditions as top panel except for the use of solubilized membranes (26 μg protein/ml) plus 1 μM cibacron blue. Results given are from a single experiment and this experiment is representative of two others.

bilized membranes incubated with 1 μM cibacron blue. In native membranes as well as solubilized membranes binding reached a steady-state by 30 min and did not change significantly during the subsequent 60 min. As illustrated in Fig. 2 (right panel), ^{125}I-CCK-8 bound to native membranes dissociated relatively slowly, with a $t_{1/2}$ of approx. 40 min. In contrast, ^{125}I-CCK-8 bound to solubilized membranes incubated with 1 μM cibacron blue dissociated more rapidly, with a $t_{1/2}$ of 12 min.

Fig. 3 illustrates the abilities of various CCK receptor agonists and antagonists [26,27] to inhibit binding of ^{125}I-CCK-8 to native membranes (top panel) or to solubilized membranes incubated with 1 μM cibacron blue (bottom panel). With each agent tested the pattern of inhibition of binding was essentially the same in solubilized membranes as it was in native membranes. Of the agents tested CCK-8 and L-364,718 were the most potent at inhibiting binding of ^{125}I-CCK-8. L-365,260 was approx. 100-fold less potent than L-364,718 or CCK-8, and CCK-4 and Bu_2cGMP were approx. 1000-fold less potent than L-365,260.

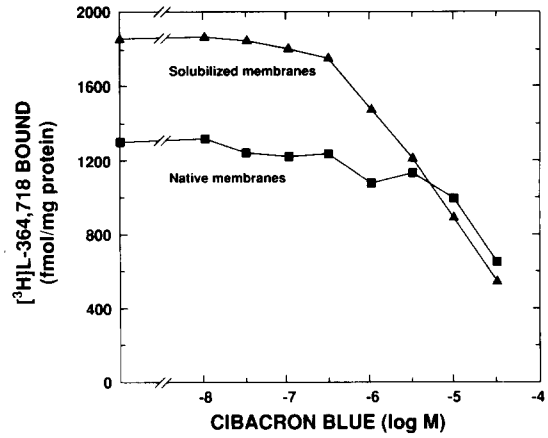

Fig. 4. Effects of cibacron blue on binding of [^{3}H]L-364,718 to native or solubilized pancreatic membranes. Saturable binding of [^{3}H]L-364,718 was measured using native (50 μg protein/ml) or solubilized (32 μg/ml) membranes incubated at 25°C for 60 min with 300 pM [^{3}H]L-364,718 with the indicated concentrations of cibacron blue, alone or plus 1 μM L-364,718. Results are from a single experiment and this experiment is representative of two others.

With native membranes cibacron blue not only inhibited binding of ^{125}I-CCK-8 (Fig. 1) but also inhibited binding of the CCK receptor antagonist [^{3}H]L-364,718 (Fig. 4). Whereas detectable inhibition of binding of ^{125}I-CCK-8 occurred with 1 μM cibacron blue (Fig. 1), detectable inhibition of binding of [^{3}H]L-364,718 occurred with 30 μM cibacron blue (Fig. 4). With solubilized membranes detectable inhibition of binding of [^{3}H]L-364,718 occurred with 1 μM cibacron

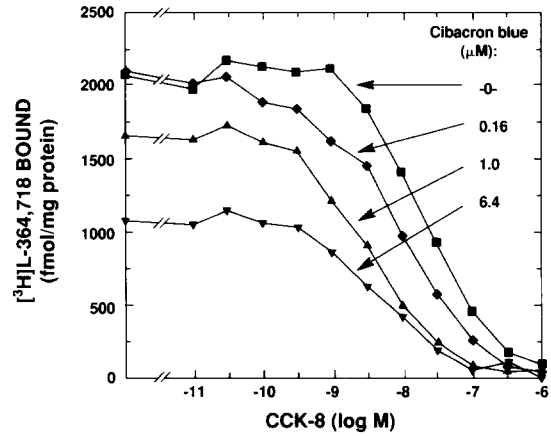

Fig. 5. Effects of cibacron blue on the ability of CCK-8 to inhibit binding of [^{3}H]L-364,718 to solubilized pancreatic membranes. Saturable binding of [^{3}H]L-364,718 was measured using solubilized membranes (24 μg protein/ml) incubated at 25°C for 60 min with 300 pM [^{3}H]L-364,718 plus the concentrations of cibacron blue and CCK-8 indicated. The four concentrations of cibacron blue were selected to represent different effects of cibacron blue on binding of ^{125}I-CCK-8 to solubilized membranes: 0 μM, no effect; 0.16 μM, half-maximal enhancement; 1.0 μM, maximal enhancement; 6.4 μM, half-maximal inhibition (see Fig. 1). Results are from a single experiment and this experiment is representative of two others.

blue (Fig. 4), approximately the same concentration of cibacron blue that produced detectable inhibition of binding of ^{125}I-CCK-8 to solubilized membranes (Fig. 1).

To examine the effect of cibacron blue on the affinity of the CCK receptor for CCK-8, we measured the ability of CCK-8 to inhibit binding of [^{3}H]L-364,718 to solubilized membranes incubated with different concentrations of cibacron blue. We chose concentrations of cibacron blue that caused half-maximal enhancement of binding of ^{125}I-CCK-8 (0.16 μM), maximal enhancement of binding of ^{125}I-CCK-8 (1.0 μM), or half-maximal inhibition of binding of ^{125}I-CCK-8 (6.4 μM). As the concentration of cibacron blue was increased to 1 μM there was a progressive 6-fold leftward shift in the dose-response curve for CCK-8 inhibition of binding of [^{3}H]L-364,718 (Fig. 5). This shift corresponded to a reduction in the IC_{50} for CCK-8 from 20 nM to 3 nM. Although increasing the concentration of cibacron blue from 1 μM to 6.4 μM reduced binding of [^{3}H]L-364,718 with no CCK-8 present, it did not produce a further leftward shift in the dose-response curve for CCK-8 inhibition of binding of [^{3}H]L-364,718.

Discussion

In the present study we found that the guanine nucleotide analogue, GTPγS, greatly reduced binding of the CCK receptor agonist, ^{125}I-CCK-8, in native membranes from rat pancreas. Treatment with GTPγS did not alter binding of the CCK receptor antagonist, [^{3}H]L-364,718. We also found that solubilization of rat pancreatic membranes with the nonionic detergent digitonin reduced binding of agonist but increased that of antagonist. These results confirm previous findings by others [17,19,23,24,28] and suggest that the affinity of CCK receptors for agonist is modulated by G proteins [17,18]. Although binding of antagonist in solubilized membranes was greater than that in native membranes, this increase (expressed as fmol/mg protein) most likely represents an enrichment of receptor protein relative to other proteins during solubilization, as noted previously with solubilized preparations of the CCK receptor [20] as well as those of other receptors [29–31].

It is generally believed that the effects of solubilization on the affinity of G protein-coupled receptors for agonist are irreversible unless the receptors are reconstituted with G proteins in liposomes [6]. We found, however, that binding of ^{125}I-CCK-8 to solubilized membranes could be increased up to 6-fold by a dye, cibacron blue. Four findings indicate that binding of ^{125}I-CCK-8 to solubilized membranes, incubated with an optimal concentration of cibacron blue (1 μM), is comparable to the binding of ^{125}I-CCK-8 to native membranes. First, the time-course for binding of ^{125}I-CCK-8 to solubilized membranes incubated with cibacron blue was essentially the same as that for binding of ^{125}I-CCK-8 to native membranes. Second, at the steady-state the amount of ^{125}I-CCK-8 bound to solubilized membranes incubated with cibacron blue was nearly equal to that bound to equivalent aliquots of native membranes alone. Third, while bound ^{125}I-CCK-8 dissociated slightly more rapidly from solubilized membranes incubated with cibacron blue than from native membranes, binding of ^{125}I-CCK-8 in solubilized membranes was clearly as reversible as that in native membranes. Fourth, dose-response curves for the abilities of various CCK receptor agonists and antagonists to inhibit binding of ^{125}I-CCK-8 to solubilized membranes incubated with cibacron blue were basically the same as those for the abilities of these same agents to inhibit binding of ^{125}I-CCK-8 to native membranes.

We assessed the effects of cibacron blue on the affinity of CCK receptors in solubilized membranes for CCK-8 by measuring the potency with which CCK-8 inhibited binding of [^{3}H]L-364,718 to these membranes. These measurements made use of the fact that binding of [^{3}H]L-364,718, unlike binding of ^{125}I-CCK-8, is largely preserved in solubilized membranes. Using this approach, we found that 1 μM cibacron blue caused a 6-fold increase in the affinity of CCK receptors in solubilized membranes for CCK-8. This was consistent with the 6-fold enhancement of binding of ^{125}I-CCK-8 observed when solubilized membranes were incubated with the same concentration of cibacron blue. With 0.16 μM cibacron blue we found that half-maximal enhancement in binding of ^{125}I-CCK-8 corresponded similarly with a half-maximal increase in affinity for CCK-8. Thus, cibacron blue enhances binding of ^{125}I-CCK-8 to solubilized membranes by increasing the affinity of CCK receptors in these membranes for CCK-8. Because cibacron blue failed to enhance binding of [^{3}H]L-364,718 to solubilized or native membranes, cibacron blue appears to be capable of increasing the affinity of CCK receptors for agonist but not for antagonist, similar to the effects of receptor-G protein coupling. This unanticipated effect of cibacron blue occurs only in solubilized membranes, however, as cibacron blue was unable to enhance binding of ^{125}I-CCK-8 to native membranes.

An additional effect of cibacron blue was its ability at concentrations above 1 μM to inhibit binding of both ^{125}I-CCK-8 and [^{3}H]L-364,718 to both solubilized and native membranes. In particular, binding of ^{125}I-CCK-8 to solubilized membranes incubated with 6.4 μM cibacron blue was only half of that obtained with 1.0 μM cibacron blue. Inhibition was not simply the reversal of enhancement, however, because the affinity

of receptors for CCK-8 in these solubilized membranes, determined from the ability of CCK-8 to inhibit binding of [^{3}H]L-364,718, did not change. These findings indicate that inhibition and enhancement have different mechanisms.

The mechanisms that account for the actions of cibacron blue on the CCK receptor are unknown. We can, however, suggest two general possibilities. The first involves interaction of cibacron blue with G protein in a way that restores receptor-G protein coupling. By postulating that G proteins mediate the actions of cibacron blue, such a mechanism explains the ability of cibacron blue to mimic, in solubilized membranes, those effects on binding of agonist to receptor that are usually attributed to G proteins in native membranes. In particular, one might surmise that cibacron blue blocks nucleotide binding sites in G proteins, given that GTPγS did not alter binding of ^{125}I-CCK-8 in solubilized membranes incubated with cibacron blue. This possibility is consistent with the well-documented use of cibacron blue as an affinity ligand in the purification of nucleotide binding proteins [32], and with the pivotal role that guanine nucleotides play in the regulation of receptor-G protein coupling [3].

An alternative mechanism, not involving G proteins, postulates that cibacron blue simulates the effects of receptor-G protein coupling on the receptor by means of some change in receptor conformation, induced either by an effect on the receptor itself or by a perturbation of the membrane environment of the receptor. Indeed, micelles of nonionic detergents spontaneously encapsulate cibacron blue [33]. Because cibacron blue carries a strong negative charge, the presence of this molecule near receptors in solubilized membranes might well alter the conformation of these receptors. There are several reports of compounds that appear to interact specifically with the agonist-preferring conformation of G protein-coupled receptors by allosteric mechanisms, albeit in experimental systems not entirely analogous to the one described here [34–39].

In the present report we have described the unanticipated ability of a commonly used dye to modulate the affinity of a peptide hormone receptor in solubilized membranes for agonist. Our results may be useful in binding assays of CCK receptors in solubilized membranes, and may provide the basis for a novel form of biospecific affinity chromatography, one in which retention of CCK receptors on a CCK column is controlled allosterically by the concentration of cibacron blue. Further studies of the mechanisms underlying the effects of cibacron blue will be important to clarify the extent to which these effects are generalizable, and may add to an understanding of how receptors distinguish agonists from antagonists [40].

References

1 Dohlman, H.G., Thorner, J., Caron, M.G. and Lefkowitz, R.J. (1991) Annu. Rev. Biochem. 60, 653–688.
2 Dohlman, H.G., Caron, M.G. and Lefkowitz, R.J. (1987) Biochemistry 26, 2657–2664.
3 Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–649.
4 Tang, W.J. and Gilman, A.G. (1991) Science 254, 1500–1503.
5 Ross, E.M. (1989) Neuron 3, 141–152.
6 Cerione, R.A. and Ross, E.M. (1991) Methods Enzymol. 195, 329–342.
7 Tota, M.R., Kahler, K.R. and Schimerlik, M.I. (1987) Biochemistry 26, 8175–8182.
8 Freissmuth, M., Selzer, E. and Schütz, W. (1991) Biochem. J. 275, 651–656.
9 De Lean, A., Stadel, J.M. and Lefkowitz, R.J. (1980) J. Biol. Chem. 255, 7108–7117.
10 Birnbaumer, L., Abramowitz, J. and Brown, A.M. (1990) Biochim. Biophys. Acta 1031, 163–224.
11 Siciliano, S.J., Rollins, T.E. and Springer, M.S. (1990) J. Biol. Chem. 265, 19568–19574.
12 Couvineau, A., Amiranoff, B. and Laburthe, M. (1986) J. Biol. Chem. 261, 14482–14489.
13 Coffer, A., Fabregat, I., Sinnett-Smith, J. and Rozengurt, E. (1990) FEBS Lett. 263, 80–84.
14 Sheikh, S.P., Hansen, A.P. and Williams, J.A. (1991) J. Biol. Chem. 266, 23959–23966.
15 Poyner, D. (1990) in Receptor-Effector Coupling: A Practical Approach (Hulme, E.C., ed.), IRL Press at Oxford University Press, New York.
16 Kilpatrick, B.F. and Caron, M.G. (1983) J. Biol. Chem. 258, 13528–13534.
17 Williams, J.A. and McChesney, D.J. (1987) Regul. Pept. 18, 109–117.
18 Lambert, M., Svoboda, M., Furnelle, J. and Christophe, J. (1985) Eur. J. Biochem. 147, 611–617.
19 Szecòwka, J., Goldfine, I.D. and Williams, J.A. (1985) Regul. Pept. 10, 71–83.
20 Duong, L.T., Hadac, E.M., Miller, L.J. and Vlasuk, G.P. (1989) J. Biol. Chem. 264, 17990–17996.
21 Miller, L.J., Rosenzweig, S.A. and Jamieson, J.D. (1981) J. Biol. Chem. 256, 12417–12423.
22 Chang, R.S.L. and Lotti, V.J. (1986) Proc. Natl. Acad. Sci. USA 83, 4923–4926.
23 Chang, R.S.L., Lotti, V.J., Chen, T.B. and Kunkel, K.A. (1986) Mol. Pharmacol. 30, 212–217.
24 Innis, R.B. and Snyder, S.H. (1980) Proc. Natl. Acad. Sci. USA 77, 6917–6921.
25 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
26 Gardner, J.D. and Jensen, R.T. (1981) In Physiology of the Gastrointestinal Tract (Johnson, L.R., ed.), Raven Press, New York.
27 Huang, S.C., Zhang, L., Chiang, H.-C.V., Wank, S.A., Maton, P.N., Gardner, J.D. and Jensen, R.T. (1989) Am. J. Physiol. 257, G169–G174.
28 Chang, R.S.L., Lotti, V.J. and Chen, T.B. (1987) Biochem. Pharmacol. 36, 1709–1714.
29 Benovic, J.L. (1990) In Receptor Biochemistry: A Practical Approach (Hulme, E.C., ed.), IRL Press at Oxford University Press, New York.
30 Guijarro, L., Couvineau, A., Calvo, J.R. and Laburthe, M. (1989) Regul. Pept. 25, 37–50.
31 Grigoriadis, D.E., Zaczek, R., Pearsall, D.M. and DeSouza, E.B. (1989) Endocrinology 125, 3068–3077.
32 Lowe, C.R. and Pearson, J.C. (1984) Methods Enzymol. 104, 97–113.

33 Robinson Jr., J.B., Strottmann, J.M., Wick, D.G. and Stellwagen, E. (1980) Proc. Natl. Acad. Sci. USA 77, 5847–5851.
34 Bruns, R.F. and Fergus, J.H. (1990) Mol. Pharmacol. 38, 939–949.
35 Bleakman, D. and Naftalin, R.J. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 366–372.
36 Gillard, M., Brunner, F., Waelbroeck, M., Svoboda, M. and Christophe, J. (1989) Eur. J. Pharmacol. 160, 117–124.
37 Huang, R.-R.C., DeHaven, R.N., Cheung, A.G., Diehl, R.E., Dixon, R.A.F. and Strader, C.D. (1990) Mol. Pharmacol. 37, 304–310.
38 Kirilovsky, J., Eimerl, S., Steiner-Murdoch, S. and Schramm, M. (1987) Eur. J. Biochem. 166, 221–228.
39 Bevan, J.A., Bevan, R.D. and Shreeve, S.M. (1989) FASEB J. 3, 1696–1704.
40 Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G. and Lefkowitz, R.J. (1992) J. Biol. Chem. 267, 1430–1433.