Differential effects of suramin on the coupling of receptors to individual species of pertussis-toxin-sensitive guanine-nucleotide-binding proteins

Sandra J. BUTLER,* Elaine C. H. KELLY,† Fergus R. MCKENZIE,† Simon B. GUILD,*†
Michael J. O. WAKELAM† and Graeme MILLIGAN*††

Molecular Pharmacology Group, Departments of *Pharmacology and †Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

The anti-helminthic drug suramin inhibited the basal high-affinity GTPase activity of both C6 BU1 glioma and NG 108-15 neuroblastoma × glioma hybrid-cell membranes with an IC_{50} (concentration causing half-maximal inhibition) value close to $30 \mu\text{g/ml}$. This effect was shown to occur via a non-competitive mechanism in which the binding affinity of the G-proteins for GTP was not altered, but the maximal velocity of the subsequent hydrolysis was reduced. In NG 108-15 membranes, both opioid peptides and foetal-calf serum stimulated high-affinity GTPase activity in a pertussis-toxin-sensitive manner. These effects have previously been shown to be mediated by different G-proteins [McKenzie, Kelly, Unson, Spiegel & Milligan (1988) *Biochem. J.* **249**, 653–659]. Suramin completely prevented the opioid-peptide-stimulated increase in GTP hydrolysis, but did not prevent the opioid peptide from binding to its receptor. Suramin, however, did not block the foetal-calf-serum-stimulated GTPase response. This selective action of suramin provides further evidence for distinct roles for two separate pertussis-toxin-sensitive G-proteins in signal transduction in NG 108-15 membranes and provides the first evidence for a selective effect of a drug on the functions of different G-proteins.

INTRODUCTION

Hormones, neurotransmitters and growth factors which act to modulate levels of intracellular second messengers do so via the intermediacy of members of a family of guanine-nucleotide-binding proteins (G-proteins) [1,2]. These include G_s and G_i , which respectively mediate stimulation and inhibition of adenylate cyclase [3], transducin, which couples the photon receptor rhodopsin to a cyclic GMP phosphodiesterase [4], and G_o [5,6], which may couple receptors to the modulation of Ca^{2+} channels. Each of these transducer proteins consists of three non-identical subunits, α , β and γ , and it is the α -subunit which is unique to each individual member of the family. Although the α -subunits of these proteins are distinct, they are highly homologous [7,8]. Attempts to distinguish between them thus far have been based on the ability of certain exotoxins produced by either *Vibrio cholerae* or *Bordetella pertussis* to catalyse selectively specific ADP-ribosylation of these proteins [9,10] or, more recently, on the use of specific polyclonal and anti-peptide antisera [11–14].

It would be useful if selective drugs were available which could discriminate interactions between receptors and particular G-proteins. However, to date no such drugs have been described, and the marked sequence homology between the α -subunits of different G-proteins limits the potentially selective sites of drug interaction.

The anti-helminthic drug suramin is a polyanionic compound which has been reported to interact non-specifically with a wide range of plasma proteins [15]. It

has also been described as a relatively selective competitive antagonist at a range of growth-factor receptors [16,17]. Here we demonstrate that suramin can non-competitively inhibit the GTPase activity of certain G-proteins such as G_i and hence disrupt the function of δ -opioid receptors which couple to this G-protein in the neuroblastoma × glioma hybrid cell line NG 108-15. However, suramin does not inhibit the receptor-stimulated GTPase activity of a second pertussis-toxin-sensitive G-protein which has been shown to interact with a growth-factor receptor in this cell line [18].

MATERIALS AND METHODS

Materials

Foetal-calf serum was from Gibco and was heat-inactivated by treatment at 56°C for 1 h before use. $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ was prepared as described elsewhere [19] or purchased from Amersham International. $^{32}\text{P}[\text{NAD}^+]$ was obtained from New England Nuclear, and suramin was from Bayer. All other materials were obtained from commercial sources and were of the highest grade available.

Methods

Rat glioma C6 BU1 cells, which were kindly given by Dr. G. Wilkin, Department of Biochemistry, Imperial College of Science and Technology, London S.W.7, U.K., were grown routinely in 80 cm^2 tissue-culture flasks in Dulbecco's Modified Eagle's Medium con-

Abbreviations used: IC_{50} , concentration causing half-maximal inhibition; DADLE, $[\text{D-Ala}^2, \text{Leu}^5]\text{enkephalin}$; DALAMID, $[\text{D-Ala}^2, \text{Met}^5]\text{-enkephalinamide}$; EC_{50} , concentration causing half-maximal effect; GABA, γ -aminobutyric acid.

† To whom correspondence and reprint requests should be addressed.

Fig. 1. Basal GTPase activity in C6 BU1 membranes: the effect of suramin

Basal high-affinity GTPase activity was measured in membranes of rat glioma C6 BU1 cells, as described in the Materials and methods section, in the presence of increasing concentrations of suramin. The data represent means $\pm$ S.D. for quadruplicate assays in a single experiment. The experiment was repeated four times with similar results.

taining 5% (v/v) foetal-calf serum and 100 units of penicillin and streptomycin/ml as previously described [20]. Neuroblastoma $\times$ glioma hybrid NG 108-15 cells, which were kindly given by Dr. W. Klee, National Institute of Mental Health, Bethesda, MD, U.S.A., were grown as in [13], except that the concentration of foetal-calf serum was 10% (v/v). Cells were harvested at confluency, and cell pastes and membranes were prepared [21] and stored at -70°C until use.

GTPase activities of the membranes were measured as previously described [18]. In all cases, receptor-stimulated GTPase activities represent the increase in hydrolysis of GTP measured in the presence of a ligand over that in its absence, under identical conditions. Less than 15% of the available nucleotide was hydrolysed during the period of the assays.

Opioid-receptor binding in NG108-15 membranes was performed in 10 mM-Tris/HCl/0.1 mM-EDTA, pH 7.5, for 15 min at 25°C , using 10 nM ^3H -labelled [D-Ala², -Leu⁵]enkephalin (^3H]DADLE). Non-specific binding was assessed by parallel incubations performed in the presence of 10 μM -[D-Ala², Met⁵]enkephalinamide (DALAMID). Protein was measured as described by Lowry *et al.* [22], with bovine serum albumin as standard.

RESULTS

The basal rate of hydrolysis of GTP by high-affinity GTPases in membranes prepared from cells of the rat glioma cell line C6 BU1 was characterized by an apparent K_m of $0.24 \pm 0.03 \mu\text{M}$ and a $V_{\max}$ of $36.6 \pm 1.9 \text{ pmol/min}$

Table 1. Basal high-affinity GTPase activity in NG108-15 and C6BU1 cell membranes: the effect of suramin

Kinetic parameters of the basal GTPase activity of membranes of NG108-15 and C6 BU1 cells were assessed as described in the Materials and methods section and in Fig. 1 in the absence and presence of suramin (30 $\mu\text{g/ml}$).

Basal high-affinity GTPase activity	Suramin ($\mu\text{g/ml}$) ...	Parameter value	
		0	30
NG108-15			
V_{max} (pmol/min/mg of protein)		14.7 ± 0.5	4.6 ± 0.3
K_{m} (μM)		0.26 ± 0.02	0.18 ± 0.03
C6 BUI			
V_{max} (pmol/min/mg of protein)		36.6 ± 1.9	15.6 ± 0.8
K_{m} (μM)		0.24 ± 0.03	0.25 ± 0.03

per mg of membrane protein (means $\pm$ S.D.). This activity was inhibited by suramin (Fig. 1). In the presence of suramin at 30 $\mu\text{g/ml}$ the estimated $V_{\max}$ for the reaction was reduced to $15.6 \pm 0.8 \text{ pmol/min per mg of protein}$, but the apparent K_m was not significantly affected (Table 1), indicating that suramin was acting in a non-competitive manner to inhibit the hydrolysis, but not the binding, of GTP (Fig. 2). A further increase of suramin concentration to 100 $\mu\text{g/ml}$ further reduced the $V_{\max}$ to $11.2 \pm 1.3 \text{ pmol/min per mg of protein}$, with little effect on the estimated K_m for GTP ($0.34 \pm 0.08 \mu\text{M}$). Similar effects of suramin were noted on the basal GTPase activity of NG 108-15 cell membranes. With an IC_{50} of 30 $\mu\text{g/ml}$, suramin produced a reduction in $V_{\max}$, with no substantial effect on the apparent K_m for GTP (Table 1). As we have previously shown that two separate pertussis-toxin-sensitive G-proteins mediate the responses to opioid peptides and to foetal-calf serum in NG 108-15 cell membranes [23], we then decided to examine the effects of suramin on the receptor-stimulated GTPase activities associated with these two ligands. Stimulation of the high-affinity GTPase activity over the basal level was observed with the synthetic enkephalin DADLE (Fig. 3). The EC_{50} for this response was 21 nM, and the response was saturated at concentrations below 1 μM . However, in the presence of suramin at 100 $\mu\text{g/ml}$, no significant DADLE-stimulated high-affinity GTPase activity was observed at concentrations of the peptide up to 1 mM, a concentration approximately five orders of magnitude higher than the EC_{50} value noted in the absence of the inhibitor. To check that suramin was not acting as a potent antagonist at the δ -opioid-receptor-binding site, we performed binding studies with ^3H]DADLE on membranes of NG 108-15 cells in the presence and absence of suramin. The membranes contained some 250 fmol of δ -opiate receptors/mg of protein; however, no displacement of the opioid ligand was observed by concentrations of suramin up to 1 mg/ml.

In contrast with the opioid response, foetal-calf-serum-mediated stimulation of high-affinity GTPase activity over the basal levels in NG108-15 membranes was not decreased in the presence of suramin (Fig. 4). Two effects of suramin were, however, observed. Firstly,

Fig. 2. Suramin mediates non-competitive inhibition of basal GTPase activity of C6 BU1 membranes

Basal high-affinity GTPase activity in C6 BU1 membranes was measured as a function of GTP concentration in the absence (●) or presence of 30 μ g (○) or 100 μ g (▲) of suramin/ml. The data represent means $\pm$ s.d. for quadruplicate assays from a single experiment which was performed three times, with similar results in each case. The inset shows an Eadie-Hofstee transformation of the data.

the dose-response curve with foetal-calf serum was shifted to higher ligand concentrations and, secondly, a greater maximal response to the ligand was produced. Similar results with foetal-calf serum were noted in the C6 BU1 membranes (Fig. 5), where, in the presence of 30 μ g of suramin/ml, the estimated value of the EC_{50} for foetal-calf serum increased from $0.21 \pm 0.02\%$ to $1.50 \pm 0.21\%$ (mean $\pm$ s.d.) whereas the V_{max} values for receptor-stimulated high-affinity GTPase activity increased from 27.3 ± 0.9 to 39.5 ± 4.4 pmol/min per mg of protein (mean $\pm$ s.d.).

DISCUSSION

In the present study we demonstrate interactions of the anti-helminthic drug suramin with pertussis-toxin-sensitive G-proteins in both the neuroblastoma $\times$ glioma hybrid cell line NG 108-15 and the glioma cell line C6 BU1. NG108-15 cells express both δ -opioid receptors and poorly characterized growth-factor receptors which function by communicating with individual pertussis-toxin-sensitive G-proteins [23]. Whereas the C6 cell line

Fig. 3. Effect of suramin on opioid-peptide stimulation of basal GTPase activity in NG108-15 membranes

DADLE stimulation of basal high-affinity GTPase activity was assessed in membranes of NG108-15 cells in the absence (●) or presence (○) of 100 μ g of suramin/ml. The results, which represent means $\pm$ s.d. from a single experiment, were corrected for basal high-affinity GTPase activity and thus represent only the effect of the opioid peptide. In this experiment the basal GTPase activity was 18.3 ± 1.2 pmol/min per mg of protein in the absence of suramin and 4.8 ± 0.7 pmol/min per mg of protein in the presence of the inhibitor. This experiment is typical of four performed.

is one of the parental lines of the NG108-15 hybrid, it does not routinely express receptors for opioid peptides [24]. It does, however, respond to foetal-calf serum in a pertussis-toxin-sensitive manner [18]. Suramin equipotently inhibited the basal rate of hydrolysis of GTP by high-affinity GTPases in the membranes of both these cell lines (Fig. 1 and Table 1). In both cases this action was shown to occur via a non-competitive interaction. As suramin is able to interact in a non-selective manner with a wide range of proteins [15], then this observation would be of only very limited interest if we were unable to demonstrate a degree of selectivity of this drug in its effects on different receptor-G-protein interactions. This we have been able to achieve by comparing the ability of opioid peptides and foetal-calf serum to stimulate GTPase activity in NG 108-15 membranes. In this cell line these two ligands interact with separate pertussis-toxin-sensitive G-proteins which both have α -subunits of M_r near 40 kDa [23]. These conclusions were based on (1) the complete additivity of GTPase stimulations by

Fig. 4. Effect of suramin on the foetal-calf-serum-stimulated GTPase activity of NG 108-15 cell membranes

Foetal-calf-serum stimulation of basal high-affinity GTPase activity was measured in the absence (●) or presence (○) of suramin (30 µg/ml). Points represent means $\pm$ S.D. for quadruplicate samples. All results were corrected for basal high-affinity GTPase activity, which in this case was 21.7 ± 2.1 pmol/min per mg of protein in the absence, and 9.6 ± 1.2 pmol/min per mg of protein in the presence, of suramin. Three similar experiments were performed.

these ligands at maximally effective concentrations and (2) the ability of an anti-peptide antiserum which recognizes the C-terminal ten amino acids of G_i to prevent opioid-peptide-stimulated GTPase activity but not that stimulated by foetal-calf serum [23]. Accordingly, we have previously concluded that the opioid response is mediated via G_i and that of foetal-calf serum via an as-yet-uncharacterized pertussis-toxin-sensitive G-protein. The data presented here indicate (1) that receptor activation of G_i is completely prevented by suramin and (2) that receptor activation of the foetal-calf-serum-linked G-protein is not. These data provide further evidence in support of our contention that these responses are mediated via separate G-proteins and that the two pertussis-toxin-sensitive G-proteins have different and individual functions. Preliminary data suggest that high-affinity GTPase activity stimulated by both GABA and baclofen in rat brain cortex is also completely abolished by suramin (G. Milligan, unpublished work). As baclofen is a selective agonist at the GABA_B receptor, which is linked to inhibition of adenylate cyclase, then it may be that all productive G_i -receptor interactions will be abolished by suramin. Further studies are now required to test the validity of this hypothesis.

Fig. 5. Effect of suramin on foetal-calf-serum-stimulated GTPase activity in membranes of C6 BU1 cells

Foetal-calf-serum stimulation of high-affinity GTPase activity was assessed in membranes of C6 BU1 cells in the absence (●) or presence (○) of suramin (30 µg/ml). The results were corrected for basal GTPase activity, which was 18.4 ± 0.3 pmol/min per mg of protein in the absence of suramin and 6.2 ± 0.4 pmol/min per mg of protein in the presence of the inhibitor. As such, hydrolysis of GTP represents only that due to receptor stimulation. The inset shows an Eadie-Hofstee transformation of the data.

The foetal-calf-serum response was not blocked by suramin, but the dose-response curve was shifted to higher ligand concentrations. As suramin is frequently used as a competitive antagonist with a range of growth-factor receptors, then the observed results are not surprising. Indeed, increasing concentrations of suramin moved the dose-response curve with foetal-calf serum to progressively higher concentrations (results not shown). It is not clear why a greater maximal response to foetal-calf serum was obtained in the presence of suramin than in its absence (Figs. 4 and 5). It could be anticipated that a non-competitive interaction might produce inhibition, stimulation or no response. The mechanism(s) of this stimulation remain to be investigated. The effect of suramin on the opioid-peptide-stimulated GTPase activity could not be overcome by higher concentrations of the receptor ligand, and binding studies utilizing [3 H]DADLE indicated that suramin was not competing for the DADLE-binding site.

Although suramin possesses too many actions for it to be a very useful tool in studies of receptor-effector

coupling, it is enlightening that, despite the very large degree of homology between the α -subunits of different G-proteins, this drug can display a degree of discrimination between them. This raises the important possibility that drugs with a high degree of selectivity for different G-proteins and G-protein-receptor interactions could be designed.

This work was supported in part by a Medical Research Council project grant to G.M.

REFERENCES

1. Spiegel, A. M. (1987) *Mol. Cell. Endocrinol.* **4**, 1–16
2. Northup, J. K. (1985) *Mol. Aspects Cell Regul.* **49**, 91–116
3. Klee, W. A., Milligan, G., Simonds, W. F. & Tocque, B. (1985) *Mol. Aspects Cell Regul.* **4**, 117–129
4. Fung, B. K.-K. (1985) *Mol. Aspects Cell Regul.* **4**, 184–214
5. Hescheler, J., Rosenthal, W., Trautwein, W. & Schultz, G. (1987) *Nature (London)* **325**, 445–447
6. Gierschik, P., Milligan, G., Pines, M., Goldsmith, P., Codina, J., Klee, W. A. & Spiegel, A. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2258–2262
7. Masters, S. B., Stroud, R. M. & Bourne, H. R. (1986) *Protein Eng.* **1**, 47–54
8. Bourne, H. R., Masters, S. B. & Sullivan, K. A. (1987) *Biochem. Soc. Trans.* **15**, 35–38
9. Gill, D. M. & Meren, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3050–3054
10. Katada, T. & Ui, M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3129–3133
11. Milligan, G., Gierschik, P. & Spiegel, A. M. (1987) *Biochem. Soc. Trans.* **15**, 42–45
12. Milligan, G., Streaty, R. A., Gierschik, P., Spiegel, A. M. & Klee, W. A. (1987) *J. Biol. Chem.* **262**, 8626–8630
13. Milligan, G., Gierschik, P., Spiegel, A. M. & Klee, W. A. (1986) *FEBS Lett.* **195**, 225–230
14. Gawler, D., Milligan, G., Spiegel, A. M., Unson, C. G. & Houslay, M. D. (1987) *Nature (London)* **327**, 229–232
15. Bowman, W. C. & Rand, M. J. (1980) in *Textbook of Pharmacology* (Bowman, W. C. & Rand, M. J., eds.), 2nd edn., chapter 36, p. 36.18, Blackwell Scientific Publications, Oxford
16. Betsholtz, C., Johnson, A., Heldin, C.-H. & Westermark, B. (1986) *Proc. Natl. Acad. Sci. (U.S.A.)* **83**, 6440–6444
17. Williams, L. T., Tremble, P., Lavin, M. F. & Sunday, M. E. (1984) *J. Biol. Chem.* **259**, 5287–5294
18. Milligan, G. (1987) *Biochem. J.* **245**, 501–505
19. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560
20. Milligan, G. (1987) *Biochim. Biophys. Acta* **929**, 197–202
21. Sharma, S., Nirenberg, M. & Klee, W. A. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 590–594
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
23. McKenzie, F. R., Kelly, E. C. H., Unson, C. G., Spiegel, A. M. & Milligan, G. (1988) *Biochem. J.* **249**, 653–659
24. Koski, G. & Klee, W. A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4185–4189

Received 30 July 1987/15 October 1987; accepted 26 November 1987