British Journal of Pharmacology (1997) 120, 476–480 

# Pharmacological characterization of the sulphonylurea receptor in rat isolated aorta

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**1** The binding of the sulphonylurea [$^3$H]-glibenclamide, a blocker of adenosine 5′-triphosphate (ATP)-sensitive $K^+$ channels ($K_{ATP}$ channels), was studied in endothelium-denuded rings from rat aorta.

**2** [$^3$H]-glibenclamide labelled two classes of binding sites with $K_D$ values of $20 \pm 5$ nM and $32 \pm 1$ $\mu$M. The high affinity component, which comprised 17% of total binding at 1 nM [$^3$H]-glibenclamide, had an estimated binding capacity of 150 fmol mg$^{-1}$ wet weight.

**3** Other sulphonylureas such as glipizide and glibornuride and the sulphonylurea-related carboxylate, AZ-DF 265, inhibited high affinity [$^3$H]-glibenclamide binding with the potencies expected from their $K^+$ channel activity. At very high concentrations, AZ-DF 265 and glipizide started to interact also with the low affinity component of [$^3$H]-glibenclamide binding.

**4** Openers of the ATP-sensitive $K^+$ channel belonging to different structural groups inhibited only the high affinity [$^3$H]-glibenclamide binding; the potencies in this assay were similar to those obtained in functional (i.e. vasorelaxation) studies.

**5** High affinity [$^3$H]-glibenclamide binding was abolished by prolonged hypoxia combined with metabolic inhibition.

**6** The data indicate that the high affinity component of [$^3$H]-glibenclamide binding mediates the block of the $K_{ATP}$ channel by the sulphonylureas in rat aorta; hence, it represents the sulphonylurea receptor in this vessel. The pharmacological properties of this binding site resemble those of the binding site for the openers of the $K_{ATP}$ channel; present evidence suggests that these two classes of sites are negatively allosterically coupled.

**Keywords:** Glibenclamide binding; sulphonylurea receptor; $K_{ATP}$ channel; $K_{ATP}$ channel openers; [$^3$H]-P1075; metabolic dependence of binding; rat aorta

## Introduction

Adenosine 5′-triphosphate (ATP)-sensitive $K^+$ channels ($K_{ATP}$ channels) are a heterogeneous group of $K^+$ channels that couple membrane potential to the metabolic state of the cell as given by the intracellular concentrations of ATP and adenosine 5′-diphosphate (ADP) (Ashcroft & Ashcroft, 1990; Edwards & Weston, 1993). In addition to their regulation by nucleotides, these channels are blocked by sulphonylureas like glibenclamide and tolbutamide and activated by the $K_{ATP}$ channel openers, a structurally heterogeneous group of compounds (Ashcroft & Ashcroft, 1990; Quast, 1992; Edwards & Weston, 1993). Recent studies have shown that the $K_{ATP}$ channels in pancreatic B-cells (Inagaki *et al*., 1995; Sakura *et al*., 1995) in heart and skeletal muscle (Inagaki *et al*., 1996) and the principal cells of the renal cortical collecting duct (McNicholas *et al*., 1996) are heteromeric proteins composed of $\alpha$- and $\beta$-subunits. The structure of the $\alpha$-subunits is typical of inwardly rectifying $K^+$ channels; the $\beta$-subunits are members of the ATP-binding cassette protein superfamily and confer on the native channel the sensitivity to sulphonylureas and, perhaps with the exception of the kidney channel, also to nucleotides (Inagaki *et al*., 1995; 1996; Sakura *et al*., 1995; McNicholas *et al*., 1996). The molecular identity of the components making up the $K_{ATP}$ channel in (vascular) smooth muscle is presently unknown.

One way of characterizing the components of the $K_{ATP}$ channel is to use radiolabelled blockers, such as [$^3$H]-glibenclamide, or openers, such as [$^3$H]-P1075, a compound structurally related to pinacidil (Manley *et al*., 1993). In smooth muscle, only few such studies have been performed (Gopalakrishnan *et al*., 1991; Zini *et al*., 1991; Kovacs & Nelson, 1991; Bray & Quast, 1992; Quast *et al*., 1993; for review see Ashcroft & Ashcroft, 1992). In membrane fragments prepared from guinea-pig ileum (Gopalakrishnan *et al*., 1991; Zini *et al*., 1991) including the myenteric plexus (Zini *et al*., 1991), [$^3$H]-glibenclamide labelled two classes of binding sites with $K_D$ values of 0.06 nM (Gopalakrishnan *et al*., 1991) and 0.4 nM (Zini *et al*., 1991) for the high affinity sites, and 2 nM (Gopalakrishnan *et al*., 1991) and 83 nM (Zini *et al*., 1991) for the low affinity sites, respectively. The binding capacities of the two components ranged from 20 to 3000 fmol mg$^{-1}$ protein (Gopalakrishnan *et al*., 1991; Zini *et al*., 1991). The potencies of different sulphonylureas in inhibiting the low affinity component of [$^3$H]-glibenclamide binding showed some correlation with the potencies of the sulphonylureas in inhibiting opener-induced relaxation of guinea-pig small intestine; however, the openers did not inhibit [$^3$H]-glibenclamide binding (Zini *et al*., 1991). In membranes from canine aorta, a single class of [$^3$H]-glibenclamide binding sites was found with $K_D = 10$ nM. However, the pharmacological properties of this binding site were not characterized further (Kovacs & Nelson, 1991).

Binding studies with the opener [$^3$H]-P1075 in rings of rat aorta have shown the existence of a high affinity site (Bray & Quast, 1992; Quast *et al*., 1993) which was abolished by metabolic inhibition of the preparation (Quast *et al*., 1993). [$^3$H]-P1075 binding was inhibited by other openers with the affinity expected from functional (vasorelaxation) assays. Sulphonylureas also inhibited [$^3$H]-P1075 binding with the correct rank order of potencies. However, the affinities determined in the binding assay were 10–20 times lower than those calculated from the inhibition of $K_{ATP}$ channel opening (Quast *et al*., 1993); in these experiments, opener-induced $^{86}Rb^+$ efflux was used as a qualitative measure of channel opening (reviews Edwards & Weston, 1994; Quast, 1996). Kinetic experiments suggested a negative allosteric coupling between the sites for openers and sulphonylureas (Bray & Quast, 1992).

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In view of this coupling between the sites for openers and sulphonylureas found in the [$^3$H]-P1075 assay (Bray & Quast, 1992; Quast *et al*., 1993) we have now investigated the binding of [$^3$H]-glibenclamide in metabolically intact rings of rat aorta. It was hoped that under these conditions a functionally relevant binding site for the sulphonylureas in vascular smooth muscle could be detected.

## Methods

### *[$^3$H]-glibenclamide binding assay in rat aortic strips*

The [$^3$H]-glibenclamide binding experiments were performed essentially according to the protocol developed for [$^3$H]-P1075 binding (Bray & Quast, 1992; Quast *et al*., 1993). Briefly, the thoracic aorta was removed from male Sprague-Dawley rats (350–500 g) and immersed in warm HEPES-buffered PSS containing (in mM): NaCl 139, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11 and HEPES 5; the buffer was gassed with 95% $O_2$/5% $CO_2$ and titrated to pH 7.4 with NaOH at 37°C. Adherent fat and the adventitia were removed, the aorta was cut open, the endothelium removed by a moistened cotton bud and the organ was cut into strips of 1–2 mg wet weight.

The strips were incubated at 37°C for 90 min under light gassing with 95% $O_2$/5% $CO_2$ in PSS containing [$^3$H]-glibenclamide (1 nM) and the unlabelled test substance. They were then washed in ice-cold quench solution (154 mM NaCl, 50 mM Tris, pH 7.4) for 1 min, blotted, weighed and dissolved over 2 h in 0.3 ml of Soluene-350 at 37°C. The samples were supplemented with 50 $\mu$l 1 M HCl and 3 ml Ultima Gold and counted for $^3$H. Under these conditions, $B_{tot}$ was $42 \pm 2$ fmol mg$^{-1}$ wet weight and nonspecific binding, determined in the presence of unlabelled glibenclamide (300 $\mu$M), was $14 \pm 3\%$ of $B_{tot}$.

[$^3$H]-glibenclamide bound in the presence of I (unlabelled inhibitor) was fitted to a Hill equation taking into account two binding components:

$$B = B_{tot} - A_1 \, {}^*I^{n_{H1}}/(IC_{50,1}{}^{n_{H1}} + I^{n_{H1}}) - A_2 {}^*I^{n_H}/(IC_{50,2}{}^{n_{H2}} + I^{n_{H2}}) \quad (1)$$

Here B and $B_{tot}$ denote the binding of [$^3$H]-glibenclamide in the presence and absence of I; $A_1$ and $A_2$ are the extents (amplitudes) of the two components, $IC_{50.1}$ and $IC_{50.2}$ the concentrations of I producing half maximum inhibition and $n_{H1}$ and $n_{H2}$ the Hill coefficients of the respective components. In these experiments, the concentration of [$^3$H]-glibenclamide was much smaller than $K_{D,1}$ and $K_{D,2}$ of glibenclamide binding; hence, the $IC_{50}$ values can be equated with the respective inhibition constants, $K_i$ (Tallarida, 1995). The $K_{ATP}$ channel openers interacted only with the first component and $A_2$ was set to 0.

In the case of homologous displacement and a Hill coefficient of 1, the binding capacity, $B_{max}$ of a component may be estimated from the amplitude, A, by taking into account the degree of saturation, y:

$$B_{max} = A/y, \text{ with } y = L/(L + K_D) \quad (2)$$

where L denotes the label concentration (1 nM) and $K_D$ the equilibrium dissociation constant (approximated by the $K_i$ value of the component).

### *Drugs and solutions*

[$^3$H]-glibenclamide (specific activities 84 and 47 Ci mmol$^{-1}$) was purchased from Amersham Buchler (Braunschweig, Germany) and NEN (Bad Homburg, Germany), respectively; the unlabelled compound was from Sigma (Deisenhofen, Germany). The following drugs were kind gifts of the pharmaceutical companies given in parentheses: AZ-DF 265 (4-([N-(α-phenyl-2-piperidino-bentyl)carbamoyl]methyl)benzoic acid, Thomae, Biberach, Germany); glibornuride (Hoffmann-La Roche, Basel, Switzerland); glipizide (N-cyano-N′-(1,1-dimethylpropyl)-N″-3-pyridylguanidine, Pfizer, Sandwich, U.K.); aprikalim (Rhône-Poulenc Rorer, Paris, France); diazoxide (Essex Pharma, München, Germany); levcromakalim (SmithKline-Beecham, Harlow, U.K.); P1075 (Leo Pharmaceuticals, Ballerup, Denmark); pinacidil and minoxidil sulphate were synthesized by Dr W.P. Manley (Sandoz, Basel, Switzerland). Oligomycin and 2-deoxyglucose were from Fluka (Neu-Ulm, Germany). Drugs were dissolved in ethanol and dimethyl sulphoxide (1 : 1) and further diluted into the same solvent or into PSS; the final solvent concentration in the assays was always below 0.3%.

### *Calculations and statistics*

Results are expressed as mean ± s.e.mean. Individual inhibition curves were fitted to equation (1) according to the method of least squares by use of the Figure P programme (Biosoft, Cambridge, U.K.). Errors in the fitting parameters were estimated by assuming that the amplitudes were normally distributed and $IC_{50}$ values log-normally distributed. In calculations involving two mean values with standard errors, propagation in errors was taken into account according to Bevington (Bevington, 1969). Statistical significance of differences was assessed by ANOVA followed by the Dunnet's or the Newman-Keuls test as *post hoc* tests by use of the Instat programme (Graphpad Software, San Diego, U.S.A.).

## Results

### *Effect of glibenclamide on [$^3$H]-glibenclamide binding*

Figure 1 illustrates the inhibition of [$^3$H]-glibenclamide binding (1 nM) in rat aortic rings by unlabelled glibenclamide and other sulphonylureas. The inhibition curve obtained with glibenclamide was biphasic with a high affinity component comprising $17 \pm 1\%$ of $B_{tot}$, a $K_{i,1}$ value of $20 \pm 5$ nM and a Hill coefficient of $1.0 \pm 0.2$; the low affinity component accounted for $69 \pm 2\%$ of $B_{tot}$ with $K_{i,2} = 32 \pm 1$ $\mu$M and $n_{H,2} = 2.1 \pm 0.2$. Homologous displacement experiments were also performed at label concentrations varying from 0.3 nM to 5 nM (not shown). $B_{tot}$ increased linearly with the [$^3$H]-glibenclamide concentration but the relative amplitudes and $K_i$ values of the two components were not changed significantly. This would be expected if the binding parameters derived from the data in

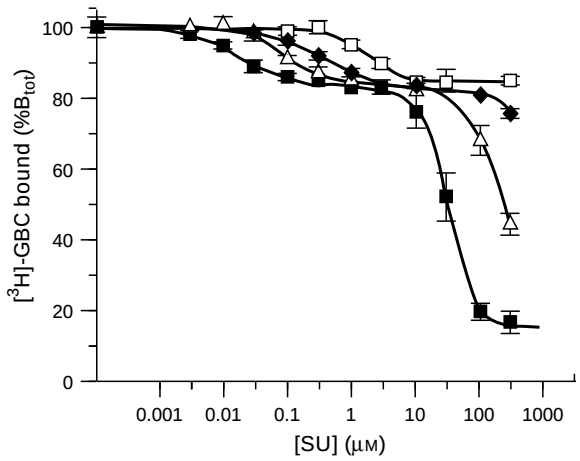


**Figure 1** Inhibition of [$^3$H]-glibenclamide (GBC) binding (1 nM) in rat aortic rings by the sulphonylureas (SU) glibenclamide (■), glipizide (◆), glibornuride (□) and the sulphonylurea-related carboxylate, AZ-DF 265 (△). Pooled data from 4–8 experiments for each compound are shown; individual inhibition curves were fitted to equation (1) yielding the fitting parameters given in the text and in Table 1. $B_{tot}$ was $42 \pm 2$ fmol mg$^{-1}$ wet weight.

Figure 1 were correct since even at 5 nM [$^3$H]-glibenclamide, the high affinity component would be saturated only to 20%.

### *Effect of other sulphonylureas on [$^3$H]-glibenclamide binding*

Other sulphonylureas such as glipizide (long chain sulphonylurea) and glibornuride (short sulphonylurea) as well as the sulphonylurea-related carboxylate AZ-DF 265 (Garrino & Henquin, 1988), inhibited high affinity [$^3$H]-glibenclamide binding; at very high concentrations, AZ-DF 265 and glipizide also started to interact with the low affinity component (Figure 1). Analysis of the inhibition curves shows that the amplitudes of the high affinity component for these compounds are not different from that obtained with glibenclamide (Table 1); the corresponding p$K_i$ ($=-\log K_i$) values and the Hill coefficients are listed in Table 1.

### *Openers interact with high affinity [$^3$H]-glibenclamide binding*

Representatives of the major structural families of the openers (Edwards & Weston, 1994) like P1075 and pinacidil (cyanoguanidines), levcromakalim (a benzopyran), aprikalim (a thioformamide), minoxidil sulphate (a pyrimidine) and diazoxide (a benzothiadiazine, chemically related to the thiazide diuretics) inhibited the high affinity component of glibenclamide binding completely but did not interfere with the low affinity component even at the highest concentrations used (Figure 2). Analysis of the curves yielded the p$K_i$ values compiled in Table 1; in general, Hill coefficients were not different from 1 with the exception of aprikalim and diazoxide.

### *Modulation of [$^3$H]-glibenclamide binding by metabolic inhibition*

We have shown previously that under hypoxia and metabolic poisoning, binding of the opener [$^3$H]-P1075 in rat aortic strips disappears concomitantly with the reduction in intracellular ATP levels (Quast *et al*., 1993). Severe metabolic blockade was produced by inhibition of glycolysis (substitution of glucose by deoxyglucose) combined with hypoxia (gassing with 95% $N_2$/5% $CO_2$) or with the inhibition of oxidative phosphorylation by oligomycin for 90 min. Figure 3 shows that under these conditions, total binding of [$^3$H]-glibenclamide was reduced by 15–16%, i.e. by exactly the amount of high affinity binding. Addition of glibenclamide (1 $\mu$M) or P1075 (100 $\mu$M), concentrations which saturate the high affinity component, did not decrease $B_{tot}$ further; in addition, non-specific binding was not affected. This indicated that severe metabolic inhibition abolished the high affinity component of [$^3$H]-glibenclamide binding but had no effect on low affinity binding.

## Discussion

### *Low affinity glibenclamide binding*

To our knowledge, this study presents the first investigation of [$^3$H]-glibenclamide binding in intact vascular smooth muscle. In homologous competition experiments two binding components were identified with $K_i$ values of 20 nM and 32 $\mu$M. The low affinity component was detectable only at glibenclamide concentrations $\geqslant 10$ $\mu$M; in this concentration range glibenclamide is known to bind to several proteins, be they membraneous or cytosolic (Panten *et al*., 1989; Ashcroft & Ashcroft, 1992; Sheppard & Welsh, 1992; Rabe *et al*., 1995). This heterogeneity of binding sites in the low affinity component is in obvious contradiction to the observed Hill coefficient

**Table 1** Inhibition of high affinity [$^3$H]-glibenclamide binding by $K_{ATP}$ channel modulators[1]

| *Substance* | $pK_i$ (−log M) | $A_1$[2] (% $B_{tot}$) | $n_{H,1}$ |
|---|---|---|---|
| Glibenclamide | 7.69±0.10 | 17±1 | 1.0±0.2 |
| AZ-DF 265 | 7.00±0.10 | 17±2 | 1.2±0.3 |
| Glipizide | 6.44±0.08 | 18±1 | 0.9±0.1 |
| Glibornuride | 5.75±0.05 | 15±1 | 1.6±0.2 |
| P1075 | 7.95±0.05 | 16±1 | 1.1±0.1 |
| Minoxidil sulphate | 7.27±0.05 | 17±1 | 1.2±0.1 |
| Pinacidil | 7.09±0.02 | 16±1 | 1.1±0.1 |
| Levcromakalim | 6.74±0.05 | 17±1 | 1.2±0.2 |
| Aprikalim | 6.74±0.02 | 15±1 | 1.4±0.1 |
| Diazoxide | 4.38±0.03 | 16±1 | 1.5±0.1 |

[1]Individual inhibition curves for the $K_{ATP}$ channel modulators were fitted to equation (1) yielding the p$K_i$ ($=-\log K_i$) values, amplitudes ($A_1$), and Hill coefficients ($n_{H,1}$) listed below. Pooled data are shown in Figures 1 and 2 ($n=4-8$). [2]$A_1$ values not different from that for glibenclamide (ANOVA followed by Dunnet's test).

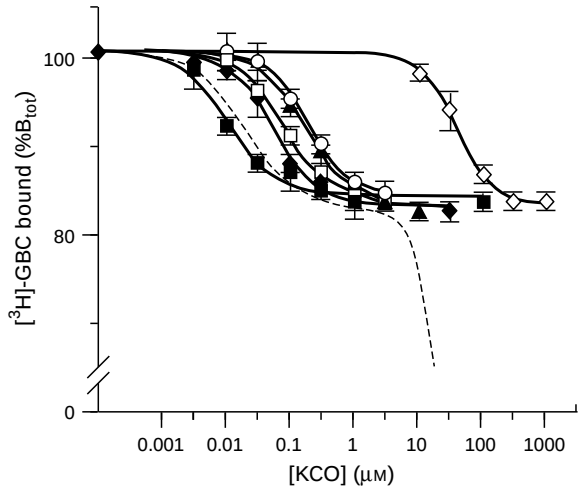


**Figure 2** Inhibition of [$^3$H]-glibenclamide (GBC) binding (1 nM) by $K_{ATP}$ channel openers: (■) P1075, (◆) minoxidil sulphate, (□) pinacidil, (▲) levcromakalim, (○) aprikalim and (◇) diazoxide. Data are means and vertical lines s.e.mean from 4–6 experiments; individual data were fitted to equation (1) with $A_2=0$; for fitting parameters see Table 1. The dashed curve represents glibenclamide.

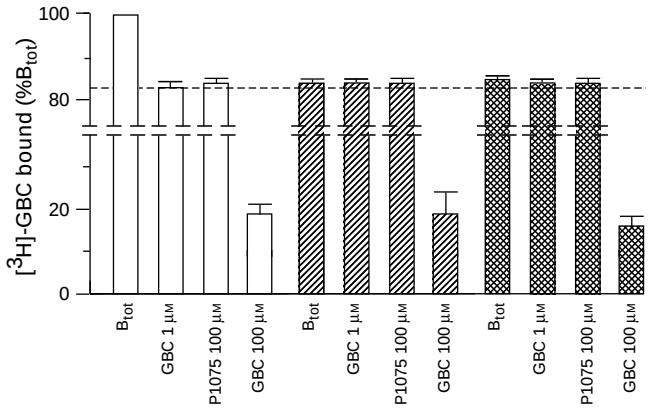


**Figure 3** Effects of metabolic inhibition on [$^3$H]-glibenclamide binding. Binding is given as % $B_{tot}$ under control conditions. Open columns: control; hatched columns: hypoxia combined with inhibition of glycolysis (i.e. bubbling with 95% $N_2$/5% $CO_2$ in the presence of 2-deoxyglucose (1 mM) and absence of glucose); cross-hatched columns: inhibition of oxidative phosphorylation (oligomycin (1 $\mu$M) in the presence of 2-deoxyglucose (1 mM)). The dashed line represents the contribution of the high affinity component to $B_{tot}$ under control conditions.

**Table 2** Correlation of the potencies of $K_{ATP}$ channel modulators in the [$^3$H]-glibenclamide binding assay with those obtained in functional and [$^3$H]-P1075 assays[1]

| | *x vs y* | *Correlation coefficient* | *Slope* | $d^2$ |
|---|---|---|---|---|
| Blockers: | $pA_2$ ($^{86}Rb^+$efflux)[3] vs $pK_i$ ([$^3$H]-GBC binding) | 0.992 | $1.1 \pm 0.1$ | $-0.14$ |
| | $pK_i$ ([$^3$H]-P1075 binding)[4] vs $pK_i$ ([$^3$H]-GBC binding) | 0.989 | $1.0 \pm 0.1$ | $-1.22$ |
| Openers: | $pD_2$ (vasorelaxation)[5] vs $pK_i$ ([$^3$H]-GBC binding) | 0.979 | $0.9 \pm 0.1$ | 0.43 |
| | $pK_i$ ([$^3$H]-P1075 binding)[4] vs $pK_i$ ([$^3$H]-GBC binding) | 0.993 | $1.0 \pm 0.1$ | 0.32 |

[1]$K_{ATP}$ channel blockers were glibenclamide (GBC), AZ-DF 265, glipizide and glibornuride; openers were P1075, aprikalim, diazoxide, levcromakalim, minoxidil sulphate and pinacidil. [2]d denotes the mean logarithmic distance of the correlation line from the line of identity in the region of interest; $d > 0$: shift upwards. [3]$pA_2$ values were obtained from inhibition of P1075 (60 nM)-induced $^{86}Rb^+$ efflux from rat aorta (Quast *et al*., 1993); this efflux is taken as a qualitative measure of $K_{ATP}$ channel activation (Edwards & Weston, 1994; Quast, 1996). $pK_i$ values were calculated from the $IC_{50}$ values in (Quast *et al*., 1993) after correction for the presence of the agonist, i.e. by dividing the $IC_{50}$ values by a factor of 4 (Quast, 1996); in view of the allosteric coupling between openers and sulphonylureas (Bray & Quast, 1992), the values calculated in this manner should be regarded as pseudo-$pA_2$ ('$pA_2$')-values. [4]Data from Bray & Quast (1992). [5]$pD_2$ values for opener-induced vasorelaxation were determined in endothelium-denuded rat aortic rings precontracted by noradrenaline (0.1 $\mu$M); data from Quast *et al*. (1993).

of 2.1. However, glibenclamide is known to partition into the membrane (Zünkler *et al*., 1989) and, at the high concentrations needed to saturate the low affinity component, non-specific effects of glibenclamide are likely to occur. At concentrations $\geqslant 100$ $\mu$M, glipizide and AZ-DF 265 also started to interact with these sites whereas the $K_{ATP}$ channel openers did not. The low affinity component was not investigated further.

### *High affinity glibenclamide binding: pharmacological characterization*

Although the high affinity component of glibenclamide binding was only 17% of total binding it was possible to characterize this component in detail. The Hill coefficient was close to unity ($n_{H,1} = 1.0 \pm 0.2$) and the $K_{i,1}$ value of glibenclamide was 20 nM. This $K_i$ value is in excellent agreement with the potency of glibenclamide for block of the vascular $K_{ATP}$ channel opened by guanosine 5′-diphosphate (GDP) (Beech *et al*., 1993) or by low concentrations of $K_{ATP}$ channel openers (Xu & Lee, 1994; Quast, 1996). It is also close to the inhibition constant of glibenclamide against the vasorelaxant effect of the openers calculated from the observed rightward shifts ($K_i = 10-30$ nM; Bray & Quast, 1991; Quast *et al*., 1993). Similarly, other sulphonylureas and the carboxylate derivative, AZ-DF 265, inhibited high affinity glibenclamide binding completely. The $K_{i,1}$ values determined from these curves agree within a factor of 2 with the respective inhibition constants determined previously against P1075-induced $^{86}Rb^+$ efflux (Table 2). These data strongly suggest that the high affinity component of [$^3$H]-glibenclamide binding mediates the block of the $K_{ATP}$ channel by the sulphonylureas.

Previous binding studies with [$^3$H]-glibenclamide in smooth muscle membranes have failed to show a meaningful inhibition by $K_{ATP}$ channel openers (Zini *et al*., 1991). However, in the intact aortic rings representatives of the major structural classes of $K_{ATP}$ channel openers inhibited high affinity glibenclamide binding completely. Table 2 shows that the midpoints of these inhibition curves agree within a factor of 3–4 with those of the vasorelaxation-concentration curves determined previously in rat aorta (Quast *et al*., 1993). Similarly, the potencies of the openers in the binding assay correlate very well with the potencies to induce $^{86}Rb^+$ efflux from rat aorta (data from Bray & Quast, 1992; Quast *et al*., 1993; correlation not shown). Hence, the high affinity sulphonylurea binding site described here also recognizes the $K_{ATP}$ channel openers in a pharmacologically relevant way.

Severe metabolic inhibition selectively and completely abolished the high affinity component of [$^3$H]-glibenclamide binding. The manoeuvres used here have previously been shown to reduce ATP levels in rat aortic rings by >90% (Quast *et al*., 1993); this could possibly result in inadequate phosphorylation of the drug receptor and/or in the disruption of the cytoskeleton (Hall & Haworth, 1996). Phosphorylation has indeed been shown to be necessary for functional binding of [$^3$H]-glibenclamide to membraneous or solubilized preparations from an pancreatic B-cell line (Niki & Ashcroft, 1991; Schwanstecher *et al*., 1992a) and from cerebral cortex (Schwanstecher *et al*., 1992b); an intact cytoskeleton has been shown to be necessary for proper functioning of the $K_{ATP}$ channel in the principal cells of the rat cortical collecting duct (Wang *et al*., 1994). It is well possible that both inadequate phosphorylation of the drug receptor and disassembly of the actin cytoskeleton prevent meaningful binding of $K_{ATP}$ channel modulators in membranes from smooth muscle (Gopalakrishnan *et al*., 1991; Zini *et al*., 1991; Quast *et al*., 1993).

### *Comparison of high affinity [$^3$H]-glibenclamide binding with [$^3$H]-P1075 binding*

In their ability to recognize both sulphonylureas and openers and in their sensitivity to metabolic blockade, the high affinity binding sites for [$^3$H]-glibenclamide strongly resemble the binding sites labelled by the opener, [$^3$H]-P1075, in rat aorta (Bray & Quast, 1992; Quast *et al*., 1993). When the results of the binding assays were compared, the openers were two times more potent in the [$^3$H]-P1075 assay whereas the sulphonylureas were 17 times more potent in the [$^3$H]-glibenclamide binding assay (Table 2). Collectively, the data of Table 2 show that the high affinity sulphonylurea sites recognize binding of the openers with almost the correct potency whereas the openers sense binding of the sulphonylureas in a more remote fashion. These shifts may reflect a negative allosteric coupling between the binding sites for openers and sulphonylureas. A negative allosterism between the sites for openers and blockers is supported by our earlier observation that glibenclamide, at high concentrations, enhanced the dissociation of the [$^3$H]-P1075-receptor complex (Bray & Quast, 1992).

A requirement for such an allosteric coupling is that the densities of binding sites for openers and sulphonylureas are of similar magnitude. The $B_{max}$ value for [$^3$H]-P1075 in rat aortic strips has been determined to be $21 \pm 3$ fmol mg$^{-1}$ wet weight (Bray & Quast, 1992). However, the lability of the binding, probably due to the strong dependence on cell metabolism, and more recent experiments suggest that this represents a lower limit and that the real value may be considerably higher. The $B_{max}$ value of the [$^3$H]-glibenclamide sites may be estimated from the amplitude of the high affinity component ($7.1 \pm 0.5$ fmol mg$^{-1}$ wet weight) by considering that at the label concentration used ([$^3$H]-glibenclamide = 1 nM) the high affinity sites are only saturated to 5%. By use of equation 2 in the Methods section and respecting propagation of errors, a $B_{max} = 150 \pm 38$ fmol mg$^{-1}$ wet weight was obtained. Considering the uncertainty in these numbers and taking into ac-

count that the stoichiometry between the two classes of sites is unknown, these estimates are compatible with the notion of an allosteric coupling between the sites for sulphonylureas and openers.

In conclusion, we have characterized a high affinity [$^3$H]-glibenclamide binding component in rat aortic rings which recognizes both sulphonylureas and $K_{ATP}$ channel openers with the potencies expected from functional assays; hence, these sites may represent the sulphonyl-urea receptor. The properties of this binding component, including its sensitivity to metabolic inhibition, resemble the properties of sites for the openers, indicating a strong coupling between the two classes of sites.

**Note added in proof**

While this paper was in press, cloning of a smooth muscle type sulphonylurea receptor was reported [Isomoto *et al*. (1996). *J. Biol. Chem*., **271,** 24321 – 24324].

We thank F. Metzger and Dr U. Ruß for helpful discussion. The study was supported by Deutsche Forschungsgemeinschaft, grants Qu 100/1-1 and Qu 100/2-1.

## References


ASHCROFT, S.J.H. & ASHCROFT, F.M. (1990). Properties and functions of ATP-sensitive $K^+$-channels. *Cell Signal*, **2,** 197 – 214.

ASHCROFT, S.J.H. & ASHCROFT, F.M. (1992). The sulfonylurea receptor. *Biochim. Biophys. Acta*, **1175,** 45 – 59.

BEECH, D.J., ZHANG, H., NAKAO, K. & BOLTON, T.B. (1993). $K^+$ channel activation by nucleoside diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. *Br. J. Pharmacol*., **110,** 573 – 582.

BEVINGTON, P.R. (1969). *Data Reduction and Error Analysis for the Physical Sciences*. pp. 55 – 65 and 92 – 118. New York: McGraw-Hill.

BRAY, K.M. & QUAST, U. (1991). Tedisamil (KC 8857) differentially inhibits the $^{86}Rb^+$ efflux-stimulating and vasorelaxant properties of cromakalim. *Eur. J. Pharmacol*., **200,** 163 – 165.

BRAY, K.M. & QUAST, U. (1992). A specific binding site for $K^+$ channel openers in rat aorta. *J. Biol. Chem*., **267,** 11689 – 11690.

EDWARDS, G. & WESTON, A.H. (1993). The pharmacology of ATP-sensitive $K^+$ channels. *Ann. Rev. Pharmacol. Toxicol*., **33,** 597 – 637.

EDWARDS, G. & WESTON, A.H. (1994). Effect of potassium channel modulating drugs on isolated smooth muscle. In *Handbook of Experimental Pharmacology*, Vol. 111, ed Szekeres, L.J. & Papp, G. pp. 469 – 531. Heidelberg: Springer.

GARRINO, M.G. & HENQUIN, J.C. (1988). Highly potent and stereo-selective effects of the benzoic acid derivative AZ-DF 265 on pancreatic $\beta$-cells. *Br. J. Pharmacol*., **93,** 61 – 68.

GOPALAKRISHNAN, M., JOHNSON, D.E., JANIS, R.A. & TRIGGLE, D.J. (1991). Characterization of binding of the ATP-sensitive potassium channel ligand, [$^3$H]glyburide, to neuronal and muscle preparations. *J. Pharmacol. Exp. Ther*., **257,** 1162 – 1171.

HALL, S.M. & HAWORTH, S.G. (1996). Effect of cold preservation on pulmonary arterial smooth muscle cells. *Am. J. Physiol*., **270,** L435 – L445.

INAGAKI, N., GONOI, T., CLEMENT IV, J.P., NAMBA, N., IAZAWA, J., GONZALES, G., AGUILAR-BRYAN, L., SEINO, S. & BRYAN, J. (1995). Reconstitution of $I_{KATP}$: An inward rectifier subunit plus the sulfonylurea receptor. *Science*, **270,** 1166 – 1170.

INAGAKI, N., GONOI, T., CLEMENT IV, J.P., WANG, C.-Z., AGUILAR-BRYAN, L., BRYAN, J. & SEINO, S. (1996). A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. *Neuron*, **16,** 1011 – 1017.

KOVACS, R.J. & NELSON, M.T. (1991). ATP-sensitive $K^+$ channels from aortic smooth muscle incorporated into planar lipid bilayers. *Am. J. Physiol*., **261,** H604 – H609.

MANLEY, P.W., QUAST, U., ANDRES, H. & BRAY, K. (1993). Synthesis of and radioligand binding studies with a tritiated pinacidil analogue: receptor interactions of structurally different classes of potassium channel openers and blockers. *J. Med. Chem*., **36,** 2004 – 2010.

MCNICHOLAS, C.M., GUGGINO, W.B., SCHWIEBERT, E.M., HEBERT, S.C., GIEBISCH, G. & EGAN, M.E. (1996). Sensitivity of a renal $K^+$ channel (ROMK2) to the inhibitory sulfonylurea compound, glibenclamide, is enhanced by co-expression with the ATP-binding cassette transporter CFTR. *Proc. Natl. Acad. Sci. U.S.A*., **93,** 8083 – 8088.

NIKI, I. & ASHCROFT, S.J.H. (1991). Possible involvement of protein phosphorylation in the regulation of the sulphonylurea receptor of a pancreatic $\beta$-cell line, HIT T15. *Biochim. Biophys. Acta*, **1133,** 95 – 101.

PANTEN, U., BURGFELD, J., GOERKE, F., RENNIKE, M., SCHWANSTECHER, M., WALLASCH, A., ZÜNKLER, B.J. & LENZEN, S. (1989). Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. *Biochem. Pharmacol*., **38,** 1217 – 1229.

QUAST, U. (1992). Potassium channel openers: pharmacological and clinical aspects. *Fundam. Clin. Pharmacol*., **6,** 279 – 293.

QUAST U. (1996). Effects of potassium channel activators in isolated blood vessels. In *Potassium Channels and their Modulators: From Synthesis to Clinical Experience*. ed. Evans, J.M., Hamilton, T.C., Longman, S.D. & Stemp, G. pp. 173 – 195. London: Taylor & Francis.

QUAST, U., BRAY, K.M., ANDRES, H., MANLEY, P.W., BAUMLIN, Y. & DOSOGNE, J. (1993). Binding of the $K^+$ channel opener [$^3$H]P1075 in rat isolated aorta: relationship to functional effects of openers and blockers. *Mol. Pharmacol*., **43,** 474 – 481.

RABE, E., DISSER, J. & FRÖMTER, E. (1995). $Cl^-$ channel inhibition by glibenclamide is not specific for the CFTR-type $Cl^-$ channel. *Pflügers Arch*., **429,** 659 – 662.

SAKURA, H., ÄMMÄLÄ, C., SMITH, P.A., GRIBBLE, F.M. & ASHCROFT, F.M. (1995). Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic $\beta$-cells, brain, heart and skeletal muscle. *FEBS Lett*., **377,** 338 – 344.

SCHWANSTECHER, M., BEHRENDS, S., BRANDT, C. & PANTEN, U. (1992a). The binding properties of the solubilized sulfonylurea receptor from a pancreatic B-cell line are modulated by the $Mg^{++}$-complex of ATP. *J. Pharmacol. Exp. Ther*., **262,** 495 – 502.

SCHWANSTECHER, M., SCHAUPP, U., LÖSER, S. & PANTEN, U. (1992b). The binding properties of the particulate and solubilized sulfonylurea receptor from cerebral cortex are modulated by the $Mg^{2+}$ complex of ATP. *J. Neurochem*., **59,** 1325 – 1335.

SHEPPARD, D.N. & WELSH, M.J. (1992). Effect of ATP-sensitive $K^+$ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. *J. Gen. Physiol*., **100,** 573 – 591.

TALLARIDA, R.J. (1995). Receptor discrimination and control of agonist-antagonist binding. *Am. J. Physiol*., **269,** E379 – E391.

WANG, W., CASSOLA, A. & GIEBISCH, G. (1994). Involvement of actin cytoskeleton in modulation of apical K channel activity in rat collecting duct. *Am. J. Physiol*., **267,** F592 – F598.

XU, X. & LEE, K.S. (1994). Characterization of the ATP-inhibited $K^+$ current in canine coronary smooth muscle cells. *Pflügers Arch*., **427,** 110 – 120.

ZINI, S., BEN-ARI, Y. & ASHFORD, M.L.J. (1991). Characterization of sulfonylurea receptors and the action of potassium channel openers on cholinergic neurotransmission in guinea pig isolated small intestine. *J. Pharmacol. Exp. Ther*., **259,** 566 – 573.

ZÜNKLER, B.J., TRUBE, G. & PANTEN, U. (1989). How do sulfonylureas approach their receptor in the B-cell plasma membrane? *Naunyn-Schmiedeberg's Arch. Pharmacol*., **340,** 328 – 332.


*(Received September 18, 1996*
*Accepted October 21, 1996)*