Communications to the Editor

Cardioselective Anti-Ischemic ATP-Sensitive Potassium Channel Openers

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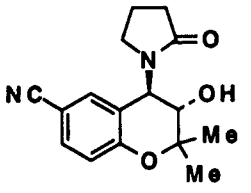
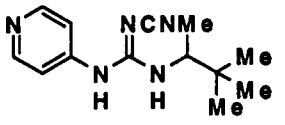
The ATP-sensitive potassium channel (K_{ATP}) openers cromakalim (1), pinacidil (2), aprikalim (3), and diazoxide (4) are potent antihypertensive agents acting via peripheral



Cromakalim (1)



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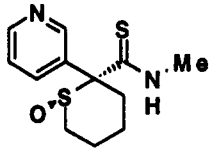
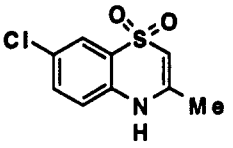


Aprikalim (3)



Diazoxide (4)

vasodilation.[1] The original excitement about the discovery of this class of agents is somewhat dampened as no clear advantages of K_{ATP} openers over the existing antihypertensive drugs have yet been demonstrated. This is in part due to the ability of these agents to open potassium channels in several tissue types.[2] Tissue-selective K_{ATP} openers are clearly required to explore the potential of these agents in disease states. We and others have shown that reference K_{ATP} openers have direct cardioprotective properties independent of their vasodilator effects.[3] However, the use of these potent vasodilators for the treatment of acute myocardial ischemia is somewhat limited due to the possibility of hemodynamic alterations with systemic administration. Therefore, cardioselective agents are required to exploit the potential of K_{ATP} openers for the treatment of myocardial ischemia. This is especially important in light of the hypothesis that K_{ATP} opening may be involved in the phenomenon of myocardial preconditioning, forming part of the heart's own response to minimize injury.[4] The objective of our studies was to find cardioselective anti-ischemic K_{ATP} openers with a lower level of vasodilator activity. In this communication, we wish to disclose such agents which, in spite of being equipotent to the reference agents (e.g., cromakalim) as anti-ischemic agents, are significantly less active as vasorelaxant agents.

For the discovery of selective anti-ischemic agents, we employed *in vitro* test systems to compare vasorelaxant and anti-ischemic potencies. The vasorelaxant potencies

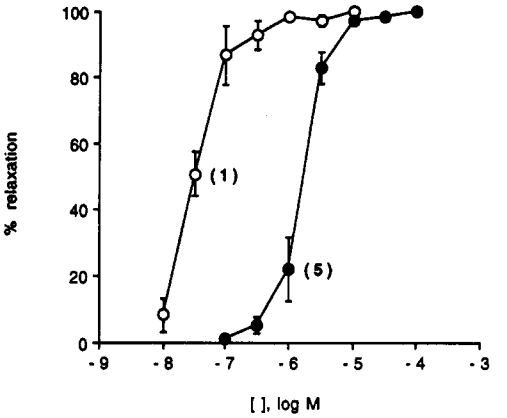


Figure 1. Concentration–response relationships of cromakalim (1) and phenylcyanoguanidine 5 for relaxation of the methoxamine-contracted rat aorta. Cromakalin (1) was 40-fold more potent than 5 as a vasorelaxant agent.

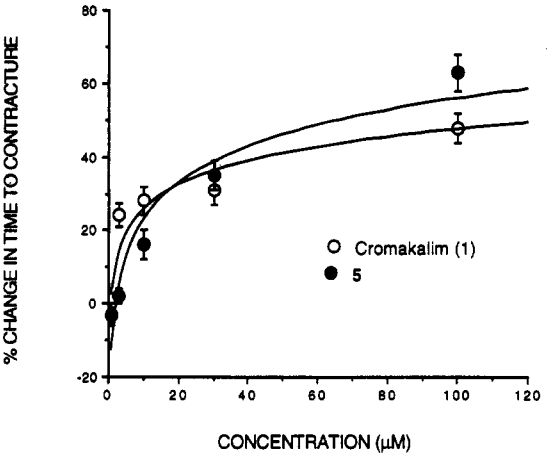


Figure 2. Concentration–response relationships for time to contracture in globally ischemic isolated perfused rat hearts for cromakalim (1) and phenylcyanoguanidine 5. Time to contracture is defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Cromakalim (1) and 5 were equipotent as anti-ischemic agents.

were determined by measurement of IC_{50} values for relaxation of the methoxamine-contracted rat aorta, as described previously.[5] The anti-ischemic potencies *in vitro* were determined by measurement of EC_{25} values for increase in time to contracture in globally ischemic isolated rat hearts.[6] Time to contracture is defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg.[6] As exemplified by the comparison between phenylcyanoguanidine analog 5 and cromakalim (1), we found no correlation between vasorelaxant and anti-ischemic potencies (Table I). The phenylcyanoguanidine analog 5 was equipotent to cromakalim as an anti-ischemic agent *in vitro*, despite being 40-fold less potent as a vasorelaxing agent. The concentration–response relationships comparing cromakalim (1) and 5 for vasorelaxant and anti-ischemic potencies are shown in Figures 1 and 2, respectively. To confirm its mechanism of action, we studied the effect of the K_{ATP} blocker glyburide on the anti-ischemic effects of 5.[7] While glyburide (0.1 μM) had no effect of its own, it completely

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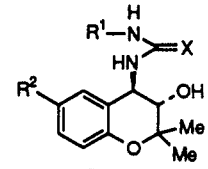
Table I. Vasorelaxant and Anti-Ischemic Potencies of Potassium Channel Openers



compd	R^1	R^2	X	mp, °C	time to contracture[a] EC_{25}, μM	vasorelaxant potencies[b] IC_{50}, μM (95% C. I.)	ratio EC_{25}/IC_{50}
5	Ph	CN	NCN	247–9	11.0	1.4 (0.98, 1.93)	7.9
6	Ph	C≡CH	NCN	220–2 (dec)	>30	5.4 (3.9, 7.5)	
7	Ph	H	NCN	220–1	*c*	72.7 (60.2, 87.9)	
8	Ph	NO_2	NCN	223–230	7.2	2.0 (1.3, 3.1)	3.6
9	$(CH_3CH_2)(CH_3)_2C$	CN	NCN	*d*	20.1	29.8 (23.2, 38.3)	0.7
10	Ph	CN	O	*e*	5.1	0.8 (0.51, 1.28)	6.4
11	Ph	CN	S	*e*	7.7	1.7 (0.97, 3.02)	4.5
12	Ph	CN	NCN	216–7	6.1	1.3 (0.95, 1.89)	4.7
13	Ph	CN	NCN	215–7	>30[f]	4.2 (2.94, 5.97)	
14	4-OHC_6H_4	CN	NCN	171–3	33.4	2.2 (1.65, 2.95)	15.2
15	4-ClC_6H_4	CN	NCN	170–2	2.5	1.8 (0.78, 4.25)	1.4
16	4-FC_6H_4	CN	NCN	158–160	3.5	1.4 (1.1, 1.83)	2.5
17	4-$CF_3C_6H_4$	CN	NCN	209–210	13.4	8.1 (5.28, 12.4)	1.7
1	(cromakalim)				8.9	0.032 (0.021, 0.049)	278.1

[a] Anti-ischemic potency was determined by measurement of EC_{25}, the concentration necessary for increase in time to contracture by 25%, in globally ischemic rat hearts. Time to contracture was defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Each value is an average of three determinations. [b] Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine-contracted rat aorta. IC_{50} is presented as a mean with 95% confidence intervals in parentheses, $n \geq 4$. [c] No significant activity at 10 μM. [d] See ref 10. [e] See ref 15. [f] EC_{25} could not be determined due to its proischemic effects at concentrations higher than 30 μM.

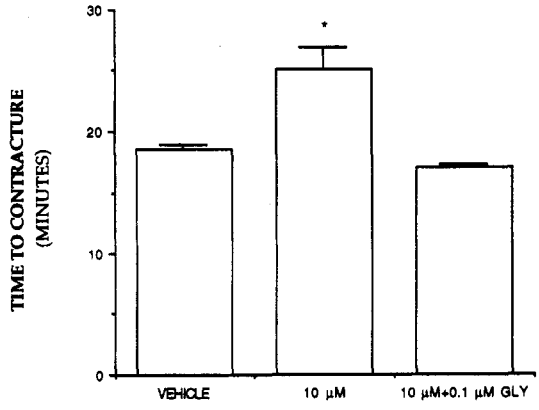


Figure 3. Reversal of antiischemic effects (time to contracture) of phenylcyanoguanidine **5** (10 μM) by the K_{ATP} blocker glyburide (GLY, 0.1 μM). Gluburide completely inhibited the cardioprotective effects of **5**.

abolished the cardioprotective effects of phenylcyanoguanidine **5** (Figure 3), indicating that K_{ATP} opening was involved in its mechanism of action. Glyburide was also able to cause a parallel shift (K_B = 0.11) in the concentration–response relationships for vasorelaxant activity of compound **5**. These results support the involvement of K_{ATP} opening in mediating the vasorelaxant effects of **5**.

We also explored preliminary structure–activity relationships for the anti-ischemic activity of **5** (Table I). The electron-withdrawing group at C6 of the benzopyran ring is required, as the isosteric acetylene **6** and the protio analog **7** showed diminished anti-ischemic potencies. This conclusion is also supported by the anti-ischemic potency of the nitro analog **8**. Replacement of the phenyl group with a bulky isoamyl group (**9**) was detrimental to both anti-ischemic and vasodilator potencies. Isosteric urea (**10**) and thiourea (**11**) replacements for cyanoguanidine were tolerated for anti-ischemic and vasodilator potencies. To investigate stereoselectivity for anti-ischemic activity, the single enantiomers **12** (3*S*,4*R*) and **13** (3*R*,4*S*) of the lead compound **5** were prepared and evaluated for biological activity. As shown in Table I, most of the anti-ischemic activity resides in the 3*S*,4*R*-enantiomer **12**. The similar vasorelaxant potencies of the two enantiomers **12** and **13** demonstrate there is no stereoselectivity for vasorelaxant activity. These data indicate that distinct structure–activity relationships exist for anti-ischemic and vasodilator activities.

Before investigating the potential of single enantiomer **12** (BMS-180447) for anti-ischemic efficacy *in vivo*, we studied its metabolism in rats.[8] While **12** had good oral bioavailability (70%), its plasma half-life was somewhat limited (1.7 h) due to rapid hydroxylation at the 4-position of the unsubstituted phenyl ring (**12** → **14**). While similar in vasorelaxant potency to **12**, the 4-hydroxyl metabolite **14** showed lower anti-ischemic potency *in vitro*. These results indicated that the generation of **14** *in vivo* could potentially compromise the anti-ischemic profile of **12**. Therefore, further efforts were devoted to preparing the substituted analogs **15**–**17** of **12** with the aim of suppressing its metabolism *via* 4-hydroxylation. While similar in vasorelaxant potencies, the 4-chloro (**15**) and 4-fluoro (**16**) analogs showed slightly improved anti-ischemic potencies over **12**. The 4-(trifluoromethyl) analog **17** was less active than **12** in both assays. On the basis of their anti-ischemic potencies, **15** and **16** were selected for metabolism studies in rats.[8] As shown in Table II the half-life of 4-chloro analog **15** was longer than those of the 4-fluoro (**16**) and the unsubstituted (**12**) analogs, although all compounds showed good oral bioavailabilities. On the basis of these pharmacokinetic studies, as well as its antiischemic potency and selectivity, the 4-chloro analog **15** (BMS-180448) was selected for further studies to determine its efficacy and selectivity *in vivo*.

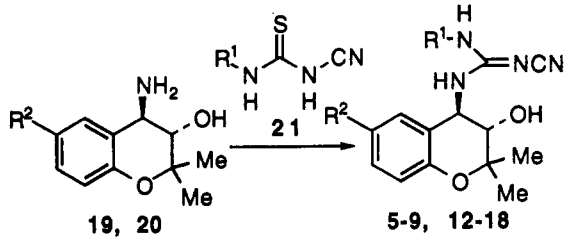
BMS-180448 was shown to be efficacious in several canine models of myocardial ischemia and reperfusion at a dose of 1.5–3.5 mg/kg (iv).[9] There was no effect on blood pressure or other hemodynamic variables at the anti-

Table II. Oral Bioavailabilities and Plasma Half-Lives of BMS-180448 and Its Analogs in Rats[a]

compound	% bioavailability[b]	elimination half-life, h
12 (BMS-180447)	70	1.8 ± 0.18
15 (BMS-180448)	61	7.0 ± 0.66
16	64	1.7 ± 0.07

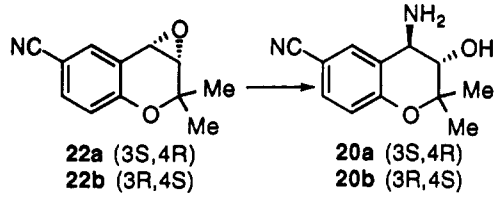
[a] For pharmacokinetic studies, compounds were administered as 22 mM solutions in 50% water/50% poly(ethylene glycol)-400. Rats were dosed either intravenously (n = 3) by injection into the jugular vein, or orally (n = 3) by gavage. Plasma concentrations were determined by reverse-phase HPLC.[8] [b] An average of three determinations.

Scheme I[a]



[a] Reagents: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, dimethylformamide, rt.

Scheme II[a]



[a] Reagents: ammonium hydroxide, ethanol, rt, 90%.

ischemic doses. In separate studies in an anesthetized canine model, it was shown that as much as 15–30 mg/kg (iv) of BMS-180448 is required to cause a reduction in blood pressure.[9] In the same model, cromakalim (1) was nearly 150-fold more potent than BMS-180448 in lowering blood pressure. Furthermore, we were unable to find a cardioprotective dose of cromakalim without affecting hemodynamic variables.[9] These studies validated our *in vitro* work aimed at finding cardioselective agents and provided BMS-180448 as a clinical candidate for the treatment of acute myocardial ischemia and related disorders.

We have demonstrated that distinct structure–activity relationships exist for vasorelaxant and anti-ischemic effects of K_{ATP} openers. Further, we have identified a selective anti-ischemic K_{ATP} opener in BMS-180448 with comparable or better antiischemic potency than the reference agents (e.g., cromakalim). BMS-180448 has 60-fold lower vasorelaxant potency than cromakalim, making it 200-fold more selective for the myocardium (Table I). With its high oral bioavailability and long plasma half-life, BMS-180448 offers the opportunity to explore the anti-ischemic potential of this class of compounds in humans without potential complications due to hemodynamic alterations with the currently available K_{ATP} openers. To our knowledge, BMS-180448 and its congeners are the first class of compounds to show a clear distinction between vasorelaxant and anti-ischemic effects of K_{ATP} openers. Following the initial disclosure of this class of agents in 1989 and their anti-ischemic actions without vasodilation,[10] compound 9 (BMS-189365), renamed U-89232, was reported to possess anti-ischemic effects without vasodilation.[11] However, no structure–activity studies leading to 9 were reported. Although slightly more cardioselective than BMS-180448, the lower anti-ischemic potency of 9 compared to BMS-180448 precluded its development as an anti-ischemic agent.

Chemistry. The cyanoguanidines **5–9** and **12–18** were prepared by treatment of the amino alcohols **19**[12] and **20** with thioureas **21**, themselves prepared from the corresponding isothiocyanates and sodium cyanamide (Scheme I). Details of this method are described.[13] The hydroxy analog **14** was obtained from the corresponding *O*-benzyl compound **18** by hydrogenolysis using 10% palladium on charcoal catalyst. The chiral amino alcohols **20a,b** used for the preparation of individual enantiomers **12** and **13** of **5**, were obtained by ammonolysis of the nonracemic epoxides **22**[14] (Scheme II). The urea (**10**) and thiourea (**11**) analogs were prepared according to published procedures.[15]

Supplementary Material Available: Experimental procedures and spectral data for **5–8**, **12–19** and procedures for the biological assays (8 pages). Ordering information is given on any current masthead page.

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(8) The test compounds were administered individually to male rats as 22 mM solutions in 50% water/50% poly(ethylene glycol)-400. Rats were dosed either intravenously (n = 3 for each compound), by injection into the jugular vein, or orally (n = 3 for each compound) by gavage at a dose of 45–50 μmol/kg body weight. Serial blood samples were obtained at 0, 5, 10, 20, and 40 min and at 1, 2, 4, 6, 8, 12, and 24 h after dosing. Plasma was prepared from each blood sample by centrifugation and analyzed by HPLC on a μBondapak reverse-phase C_{18} column (3.9 mm i.d. × 30 cm; Waters Chromatography Division, Millipore Corp., Milford, MA) using 0.02 M ammonium acetate, pH 5.1/acetonitrile (65:35 v/v) as the mobile phase, with detection by UV absorbance at 250 nm. Pharmacokinetic parameters were calculated by linear regression to determine the equation of the biexponential curve which best fit the data. The values for the plasma concentration *vs* time courses were extrapolated to infinity by use of the equation $AUC_{0\to\infty} = AUC_{0\to t} + (C_t/\beta)$, where $AUC_{0\to t}$ is the area under the plasma concentration *vs* time curve from time zero to time t, the last time point for which concentrations could be measured, C_t is the concentration of drug in plasma at time t, and β is the terminal elimination rate constant. The systemic oral bioavailability was estimated by dividing the mean $AUC_{0\to\infty}$ value for the oral doses by the mean $AUC_{0\to\infty}$ value for the intravenous doses.

(9) Part of this work was presented at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics in San Francisco, July 30-August 3, 1993. See: Grover, G. J.;

Sargent, C. S.; McCullough, J. R.; Atwal, K. S. Anti-ischemic Profile of Activity a Novel Cardioselective K_{ATP} Opener, BMS-180448. *Pharmacologist* **1993**, *35*, 178. Additional *in vivo* studies on BMS-180448 will be the subject of forthcoming publications.

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