Table V. Tools for the Investigation of the Role of Receptors for ATP or Adenosine in Physiological Functions

1.	Structure–activity correlations for responses to adenosine analogues: Profile with adenosine, 2-chloroadenosine, N^6-cyclohexyladenosine, L- and D-N^6-(phenylisopropyl)adenosine, N^6-benzyladenosine, N^6-phenyladenosine, adenosine 5′-cyclopropyl(ethyl)carboxamide, 2′,5′-dideoxyadenosine, 5′-(methylthio)adenosine, 8-bromoadenosine, inosine, adenine.
2.	Structure–activity correlations for alteration in physiological function or in blockade of adenosine responses by alkylxanthines: Profile with 8-phenyltheophylline, 1,3-dipropylxanthine, 3-isobutyl-1-methylxanthine, theophylline, caffeine, 1,7-dimethylxanthine, theobromine, isocaffeine.
3.	Structure-activity correlations for responses to ATP analogues: Profile with ATP, 2-Cl-ATP, 8-Br-ATP, 2′-(MeS)-ATP, ITP, GTP.
4.	Alteration in function by exogenous adenosine deaminase: Reduction in adenosine-mediated but not ATP-mediated response.
5.	Alteration in function or responses by inhibition of adenosine deaminase: Potentiation of effects of endogenous adenosine by 2′-deoxycoformycin or *erythro*-9-(2-hydroxy-3-nonyl)adenine.
6.	Alteration in function or responses by inhibitors of ecto-ATPases and nucleotidases: Blockade of conversion of ATP to adenosine with α,β-methyleneadenosine diphosphate.
7.	Alteration in function or responses by inhibitors of adenosine uptake: Potentiation of adenosine-mediated but not ATP-mediated responses by dipyridamole, dilazep, hexobendine, 6-(*p*-nitrobenzyl)thioguanosine, papaverine.
8.	Control of intracellular levels of adenosine due to action of *S*-adenosylhomocysteine: Reduction of intracellular adenosine levels with homocysteine.
9.	Characterization of membrane receptors with radioactive ligands. Comparison of structure–activity for binding of [^{3}H]adenosine analogues and [^{3}H]alkylxanthines with physiological or biochemical responses.

[a] See text for discussion.

tivity in different brain membranes showed no correlations. Thus, it appears likely that the specific binding of 2′,5′-dideoxy[^{3}H]adenosine is not to P sites but is instead to some theophylline-insensitive site to which adenosine also binds with high affinity. The significance of such sites is unknown. It was suggested that they might be intracellular membrane sites and might serve a role in "facilitated" uptake of adenosine into cells.

Future Strategies for Investigation of Adenosine Functions

It is apparent that 1980 represents a turning point in research on the physiological functions of adenosine. The advent of binding assays for adenosine receptors of the A_1 high-affinity class provides a new approach to the investigation of the nature, distribution, and control of such functional receptors. Further research on radioligands and binding assays for A_2-adenosine receptors, P sites, and ATP receptors must now assume a high priority. A wide range of research tools for the investigation of physiological functions for ATP and adenosine have been delineated and profitably used to advance our knowledge in this field. Some of these tools and approaches are listed in Table V. Undoubtedly, their diligent use will provide not only further definition of physiological functions controlled by the adenine nucleotides and nucleosides, but will reveal further classes of receptors and further complexities.

Communications to the Editor

Inhibitors of Gastric Acid Secretion: 3,4-Diamino-1,2,5-thiadiazole 1-Oxides and 1,1-Dioxides as Urea Equivalents in a Series of Histamine H_2-Receptor Antagonists

Sir:

The discovery of burimamide by Black and associates[1] provided the first example of a specific antagonist of the histamine H_2 receptor. This prototype, although of low intrinsic inhibitory activity, constituted a lead for the development of the more potent inhibitors metiamide[2] and cimetidine.[3] The clinical efficacy of the latter as a gastric antisecretory drug stimulated a search for agents with improved potency, longer duration of action, and a lower potential for side effects.

Recently, highly potent nonimidazole H_2 inhibitors, such as ranitidine[4] and tiotidine,[5] have been described. Structural comparison of these drugs reveals three fundamental units: a substituted heterocycle joined by a 2-thiabutyl connecting chain to an acyclic end group or "urea equivalent" such as cyanoguanidine or diaminonitroethene.

This report describes a new class of histamine H_2 receptor antagonists wherein 3,4-diamino-1,2,5-thiadiazole oxides function as the "urea equivalent". Representative examples (**1a–f**)[6] are presented to illustrate structure–activity relationships within the series and for comparison with reference drugs (Table I).

Chemistry. Carmack and co-workers[7] described the

(1) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *Nature (London)* **1972**, *236*, 385.
(2) Black, J. W.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R. *Nature (London)* **1974**, *248*, 65.
(3) Brimblecombe, R. W.; Duncan, W. A. M.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E. *J. Int. Med. Res.* **1975**, *3*, 86.
(4) Bradshaw, J.; Brittain, R. T.; Clitherow, J. W.; Daly, M. J.; Jack, D.; Price, B. J.; Stables, R. *Br. J. Pharmacol.* **1979**, *66*, 464P.
(5) Yellin, T. O.; Buck, S. H.; Gilman, D. J.; Jones, D. F.; Wardleworth, J. M. *Life Sci.* **1979**, *25*, 2001.
(6) An abstract describing **1d** (BL-6341, South African Patent 80/5250) was published during the preparation of this manuscript: Cavanagh, R. L.; Usakewicz, J. J.; Buyniski, J. P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 693.
(7) Wen, R. Y.; Komin, A. P.; Street, R. W.; Carmack, M. *J. Org. Chem.* **1975**, *40*, 2743.

0022-2623/82/1825-0207$01.25/0

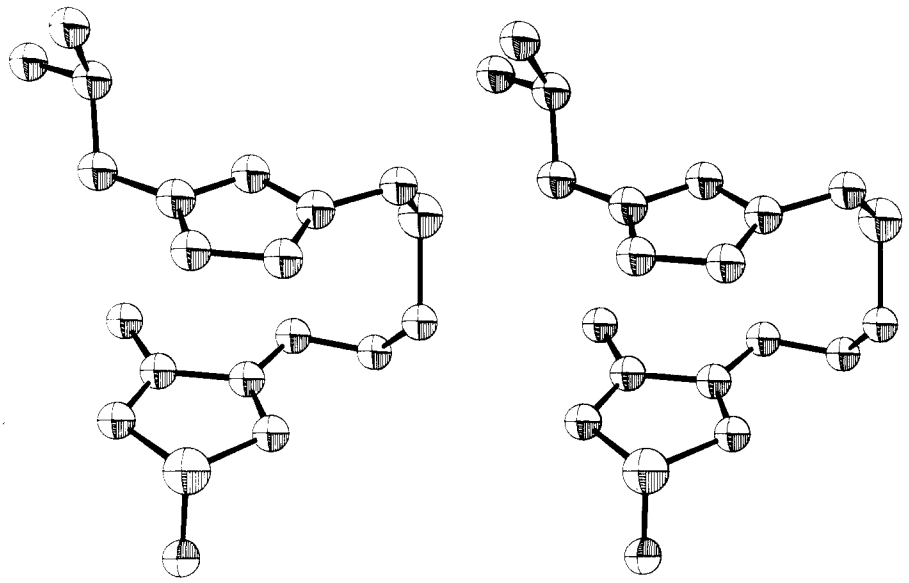
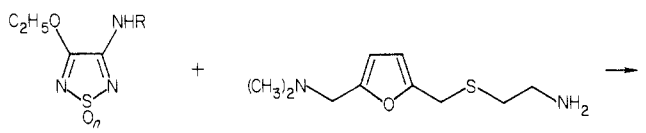
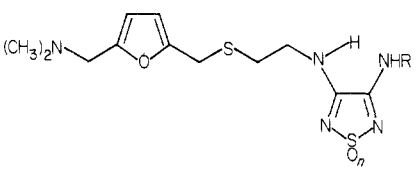


Figure 1. A computer-generated stereoscopic drawing of the X-ray determined crystal structure of **1b**. Hydrogen atoms are omitted for clarity.

Scheme I



2a, R = CH_3; n = 1
b, R = H; n = 1
c, R = H; n = 2



1a, R = CH_3; n = 1
b, R = H; n = 2
c, R = H; n = 2

synthesis of 3,4-diamino-1,2,5-thiadiazole 1,1-dioxides and their weakly acidic amide-like properties; more recently, the chemistry of the corresponding "sulfoxide analogues" or 1-oxides was developed by Weinstock and co-workers[8] in these laboratories. Compounds **1a–f** were synthesized by reacting the known amines R^1NH_2 (Table I) with the appropriate synthon **2** in a polar solvent such as acetonitrile (Scheme I). Analytically pure products **1a–f** crystallized from the reaction mixture in greater than 80% yield. These were characterized by standard methods. In particular, ^{13}C NMR (Me_2SO-d_6) chemical shifts for the thiadiazole ring C atoms at 157.4 (SO) or 155.9 ppm (SO_2) were characteristic for all compounds. Also, single-crystal X-ray determination of **1b**[9] unequivocally established its structure and revealed a high degree of intramolecular ordering into a "hairpin-shaped" conformation (Figure 1) similar to that found for cimetidine.[10]

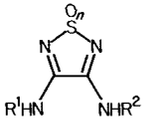
Since the weakly acidic property of the urea equivalent in the cyanoguanidine series was identified as a possible contributor to H_2-receptor affinity,[11] the acidities of the 3,4-diaminothiadiazole 1-oxide moiety of **1b** ($pK_A \simeq 11.0 \pm 0.2$)[12a] and the corresponding 1,1-dioxide moiety of **1c** ($pK_A \simeq 8.85$)[12b] were determined for comparison with *N,N'*-dimethyl-*N''*-cyanoguanidine ($pK_A \simeq 14$).[11] The greater acidity of the thiadiazole oxides may contribute to their enhanced receptor affinities (vide infra).

Results and Discussion

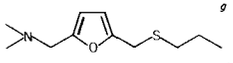
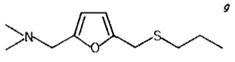
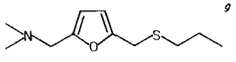
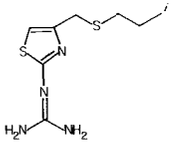
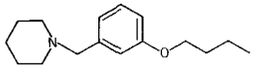
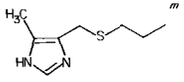
Thiadiazoles **1a–f** and reference drugs were evaluated in vitro for their ability to block the histamine-stimulated adenylate cyclase of guinea pig hippocampal homogenates[13] and the dimaprit-stimulated chronotropic response of guinea pig atrium strips[14] (Table I). For the former, Schild plots[15] of the data gave slopes within error of 1.0 for all compounds studied except ranitidine. However, in the guinea pig atrium model, Schild slopes significantly different from 1.0 were obtained for **1b,c,e**, and the maximum response to dimaprit was suppressed. In the adenylate cyclase preparation, the abscissa intercept (pA_2) and slope values given for cimetidine in Table I agree well with those reported by Green et al.[16a] and by Kanof and Greengard.[16b] The data in Table I show that the thiadiazoles are up to 2 orders of magnitude more potent than cimetidine.

(8) Karady, S.; Amato, J. S.; Dortmund, D.; Weinstock, L. M. *Heterocycles* **1981**, *16*, 1561.
(9) Determined on a Syntex $P2_1$ diffractometer with monochromatic Cu radiation (λ = 1.5418 Å). Crystals grown in 2-propanol had unit cell dimensions a = 10.514 (3) Å, b = 15.002 (6) Å, c = 10.251 (3) Å, β = 95.08 (2)°, and V = 1611 (1) Å^3 in the centric space group $P2_1/c$ (Z = 4). Observed reflections (1566, 71.2%) at the level $I \geq 3\sigma(I)$ were obtained and the data refined using anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors for hydrogen atoms with a residual index (R factor) of 4.4%.
(10) Hadicke, E.; Frickel, F.; Franke, A. *Chem. Ber.* **1978**, *111*, 3222.
(11) Ganellin, C. R. *J. Appl. Chem. Biotechnol.* **1978**, *28*, 183.
(12) (a) Estimated by nonlinear least-squares analysis of the change in UV absorbances (A) at 272 [conjugate acid (ca)] and 292 nm [conjugate base (cb)] in pH 9.95–12.2 buffers (λ isosbestic = 284 nm) using the relationship $A = A_{ca}10^{pK_A-pH} + A_{cb}/1 + 10^{pK_A-pH}$. (b) Determined by potentiometric titration in water (proton lost).
(13) Green, J. P.; Johnson, C. L.; Weinstein, H.; Maayani, S. *Proc. Natl. Acad. Sci. U.S.A* **1977**, *74*, 5697; Johnson, C. L.; Mizoguchi, H. *J. Pharmacol. Exp. Ther.* **1977**, *200*, 174. Salomon, Y. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 35–55.
(14) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; Durant, G. J. *Agents Actions* **1977**, *7*, 31.
(15) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
(16) (a) Green, J. P.; Johnson, C. L.; Weinstein, H. In "Histamine Receptors"; Yellen, T. O., Ed.; S. P. Medical and Scientific Books: New York, 1979; p 185. (b) Kanof, P. D.; Greengard, P. *J. Pharmacol. Exp. Ther.* **1979**, *209*, 87.

Table I. Chemical and Biological Data for Diamino-1,2,5-thiadiazole Oxides



1

compd	R¹	R²	n	mp,[d] °C	in vitro inhibn of H_2 receptor: adenylate cyclase[a] −log K_I[e]	adenylate cyclase[a] slope	guinea pig atrium[b] −log K_B[e] (95% CL)	guinea pig atrium[b] slope (95% CL)	inhibn of acid output in dogs[c] ED_{50}, mg/kg (95% CL)[f] iv	ED_{50}, mg/kg (95% CL)[f] po[r]	ratio po/iv
1a		CH_3	1	97–98.5			7.85 (7.71, 8.06)	1.03 (0.69, 1.36)	0.05 (0.02, 0.08)	0.30 (0.15, 0.58)	6.0
1b		H	1	145.5–146.5	8.2 ± 0.6	1.1 ± 0.2	8.25 (7.93, 9.31)	0.55[h] (0.23, 0.86)	0.007 (0.003, 0.015)	0.05 (0.02, 0.15)	7.1
1c		H	2	159–160	8.2 ± 0.4	1.3 ± 0.3	8.94 (8.38, 10.6)	0.53[h] (0.23, 0.83)	0.005 (0.003, 0.009)	0.09 (0.05, 0.13)	18.0
1d		H	1	186–188			8.17 (7.86, 8.92)	0.85[j] (0.47, 1.22)	0.004 (0.002, 0.008)	0.05 (0.02, 0.11)	12.5
1e		H	1	152.5–154.5	8.4 ± 0.9	1.4 ± 0.2	7.79[l]	1.73[s]	0.013 (0.009, 0.012)	0.32 (0.20, 0.50)	24.6
1f		H	1	191–193			5.98 (5.54, 7.62)	0.85[j] (0.34, 1.36)	>5.0[n]	>10.0[o]	
cimetidine[p]					6.3 ± 0.1	0.9 ± 0.2	6.31 (6.06, 6.78)	0.78[j] (0.50, 1.05)	1.84 (1.37, 2.46)	2.86 (2.43, 3.38)	1.5
ranitidine[q]					6.3 ± 0.1	1.6 ± 0.2	7.32 (7.08, 7.77)	0.76 (0.50, 1.03)	0.04 (0.02, 0.10)	0.42 (0.15, 1.14)	14.0

[a] Method of ref 13: data analyzed by Schild plot.[15] [b] Method of ref 14; data analyzed by Schild plot,[15] corrected for suppression of maximum. [c] See Method; acid output (mequiv) was calculated as the product of output (L) and acid concentration (mequiv/L). [d] Uncorrected. Microanalyses (C, H, N) were within 0.4% of theoretical values, and NMR and ^{13}C NMR were consistent with assigned structures. [e] Schild plot intercept with abscissa; this value is the pA_2 when the slope is not significantly different from unity. [f] Gastric secretion measured essentially by the method of ref 17. The ED_{50} and confidence limits were calculated by analysis of variance (D. J. Finney, "Statistical Method in Biological Assay," 2nd ed., Hafner, NY, 1964, Chapter 4) from four dose levels with 3–5 dogs per dose. [g] R^1NH_2; U.S. Patent 4 128 658, 1978. [h] Significantly different from unity. [i] R^1NH_2; U.S. Patent 4 165 378, 1979. [j] Not significantly different from unity. [k] R^1NH_2; Belgian Patent 875 846, 1979. [l] Confidence limits not established. [m] R^1NH_2; *Chem. Abstr.* 1972, *77*, 164 704*y*; *J. Med. Chem.* 1977, *20*, 901. [n] 8% ± 6 inhibition at 5 mg/kg; $N = 3$. [o] 30% ± 14 inhibition at 10 mg/kg; $N = 4$. [p] Synthesized in the Merck Sharp & Dohme Research Laboratories, Hoddesdon, Herts, England. [q] Sample kindly supplied by Glaxo, Ltd. [r] Intragastric administration via fistula. [s] Confidence limits could not be determined.

Specificity for the H_2 receptor was established through in vitro models. In the guinea pig atrium model, **1a–e** did not antagonize the chronotropic response to isoprenaline (β-adrenergic receptor) at concentrations up to 3×10^{-6} M. Likewise, the contractile responses to histamine (H_1 receptor) and methacholine (muscarinic receptor) in the guinea pig ileum were not blocked by **1a–e**.

Gastric antisecretory activity, after oral adminstration, was determined in dogs with a gastric fistula under histamine stimulation, as previously described.[17] For intravenous evaluation, the compounds were given immediately prior to histamine, and secretion was then measured as previously described.[17] ED_{50} values and confidence limits for the period of maximum effectiveness (0–30 min after histamine administration) are recorded in Table I. The test compounds were solubilized in aqueous vehicle with 1 equiv of HCl for both routes of administration.

The 1-oxide **1b** was similar in potency after intravenous administration to the corresponding 1,1-dioxide **1c**. Examples **1b,c** were some 300 times more potent iv and 30–50 times more potent po than cimetidine and about 8 times more potent than ranitidine both iv and po. The tiotidine analogue, **1d**, was approximately 450 times as potent as cimetidine. Surprisingly, the cimetidine analogue **1f** was significantly less potent than cimetidine, suggesting that H_2-receptor affinity depends on cooperative and dependent interactions of the three molecular substructures (vide supra) with the receptor. The approximately tenfold lower activity of the NCH_3 analogue **1a** compared with **1b** illustrates a trend of superior potency for unsubstituted analogues in this series.

Compounds **1a–e** were studied also for their ability to displace [^{3}H]-5α-dihydrotestosterone from rat prostate cytosol in vitro[18] and were found to have significantly less affinity than cimetidine[18,19] for these androgen receptors. In contrast to cimetidine,[20] these compounds did not inhibit mixed-function hepatic oxidases as assessed by potentiation of hexabarbital sleeping time in mice.[21] The compounds **1a–e** were negative in the Ames bacterial mutagenicity test using five *S. typhimurium* strains with and without metabolic activation by liver microsomal enzymes from rats pretreated with Aroclor 1254.[22]

Furthermore, since the most potent analogues in this series lack the *N*-methyl substituent in the urea equivalent group, they cannot give rise to a methylating species if nitrosated gastrointestinally. In addition, under WHO[23] and other nitrosating conditions, **1b** yields only the acid hydrolysis product, the corresponding 4-OH derivative.[24] In contrast, cimetidine in vitro yields an isolatable, mutagenic *N*-nitrosoguanidine derivative.[25]

In summary, members of the series of thiadiazole oxide histamine H_2 receptor antagonists show potential for clinical development as potent gastric antisecretory agents with a reduced likelihood of cimetidine-type side effects.[26]

Acknowledgment. The authors are indebted to Dr. B. E. Evans, Dr. G. S. Ponticello, Mr. K. E. Rittle, and Dr. S. H. B. Wright[27a] for synthetic chemical assistance, Mr. J. P. Moreau for chemical analyses, Dr. D. W. Cochran and Ms. J. S. Murphy for spectral data, Mr. Y. C. Lee for potentiometric studies, Ms. P. G. Cook, Ms. C. A. Hanson, Ms. C. A. Wesley, Ms. S. R. Weise, and Mr. J. M. Stavorski for expert technical assistance with in vitro and in vivo evaluations, Dr. N. R. Bohidar for statistical analyses, and Ms. T. H. Brunner for manuscript preparation.

(17) Bolhofer, W. A.; Hoffman, J. M.; Habecker, C. N.; Pietruszkiewicz, A. M.; Cragoe, E. J., Jr. Torchiana, M. L. *J. Med. Chem.* **1979**, *22*, 301.
(18) Liang, T., Merck Sharp & Dohme Research Laboratories, Rahway, NJ, private communication.
(19) Funder, J. W.; Mercer, J. E. *J. Clin. Endocrinol. Metab.* **1979**, *48*, 189.
(20) Patwardhan, R. V.; Yarborough, G.; Desmond, P. V.; Schenker, S.; Speeg, K. V., Jr.; *Gastroenterology* **1980**, *78*, 1316; Serlin, M. J.; Challiner, M.; Park, B. K.; Turcan, P. A.; Breckenridge, A. M. *Biochem. Pharmacol.* **1980**, *29*, 1971.
(21) MacDonald, J., Merck Sharp & Dohme Research Laboratories, West Point, PA., private communication.
(22) Bradley, M. O.; Cook, M. M., Merck Sharp & Dohme Research Laboratories, West Point, PA, private communication.
(23) Dunne, J. F., Ed. "The Potential Carcinogenicity of Nitrosatable Drugs," WHO Symposium, Geneva, June 1978; Coulston, F.; Ablex Publishing Corp.: Norwood, NJ 1980; Chapter 1, p 9.
(24) Characterized as the potassium salt monohydrate, $C_{12}H_{17}N_4O_3S_2K \cdot H_2O$ (C, H, N), mp 91–94 °C uncorrected.
(25) Foster, A. B.; Jarman, M.; Manson, D. *Cancer Lett. (Shannon, Irel.)* **1980**, *9*, 47; Jensen, D. E.; Magee, P. N. *Cancer Res.* **1981**, *41*, 230.
(26) For a discussion of adverse effects associated with cimetidine administration, see McGuigan, J. E. *Gastroenterology* **1981**, *80*, 181.
(27) (a) Merck Sharp & Dohme Research Laboratories, Hoddesdon, Herts, England. (b) Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

William C. Lumma, Jr.,* Paul S. Anderson
John J. Baldwin, William A. Bolhofer
Charles N. Habecker, Jordan M. Hirshfield[27b]
Adolph M. Pietruszkiewicz, William C. Randall
Mary Lou Torchiana, Susan F. Britcher
Bradley V. Clineschmidt, George H. Denny
Ralph Hirschmann, Jacob M. Hoffman
Brian T. Phillips, Kermit B. Streeter
Merck Sharp & Dohme Research Laboratories
West Point, Pennsylvania 19486
Received August 17, 1981

1,2,5-Thiadiazole 1-Oxide and 1,1-Dioxide Derivatives. A New Class of Potent Histamine H_2-Receptor Antagonists[1]

Sir:

The discovery of histamine H_2-receptor antagonists led to the development of cimetidine, which is widely used as an effective inhibitor of gastric acid secretion in the treatment of duodenal ulcers and related conditions.[2,3] More recently, the development of ranitidine,[4] tiotidine,[5] etintidine,[6] and oxmetidine[7] has demonstrated the po-

(1) Portions of this work have been presented: see "Abstracts of Papers", 181st National Meeting of the American Chemical Society, Atlanta, GA, Mar 29–Apr 3, 1981; American Chemical Society: Washington, DC, 1981; Abstr MEDI 42.
(2) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. *Nature (London)* **1972**, *236*, 385.
(3) Fordtran, J. S.; Grossman, M. I., Eds. *Gastroenterology* **1978**, *74*, 337–488.
(4) Bradshaw, J.; Brittain, J. W.; Clitherow, J. W.; Daly, M. J.; Jack, D.; Price, B. J.; Stables, R. *Br. J. Pharmacol.* **1979**, *66*, 464P.
(5) Yellin, T. O.; Buck, S. H.; Gilman, D. J.; Jones, D. F.; Wardleworth, J. M. *Life Sci.* **1979**, *25*, 2001.
(6) Cavanagh, R. L.; Usakewicz, J. J.; Buyniski, J. P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 768.
(7) Blakemore, R. C.; Brown, T. H.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E.; Rasmussen, A. C. *Br. J. Pharmacol.* **1980**, *70*, 105P.

0022-2623/82/1825-0210$01.25/0