Receptor Binding Sites of Hypoglycemic Sulfonylureas and Related [(Acylamino)alkyl]benzoic Acids

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The blood glucose level lowering activity of [(acylamino)ethyl]benzoic acids, such as *p*-[2-(5-chloro-2-methoxybenzamido)ethyl]benzoic acid (HB699, **2**), is discussed in terms of binding at putative insulin-releasing receptor sites of pancreatic β cells. The hypoglycemic potencies found for synthetic analogues of **2** indicate that high hypoglycemic activity is only found when a carboxyl group or a group that is readily oxidized to carboxyl in vivo, such as methyl, is attached to the aromatic ring of the phenethyl group. It is proposed that this carboxyl group is able to bind at the same receptor site as the $SO_2NHCONH$ group of the sulfonylurea drugs, such as tolbutamide (**3**). The role of the benzamide group in **2** was attributed to protein binding.

Sulfonylurea derivatives are the most widely prescribed drugs for the treatment of the maturity onset form of diabetes mellitus. In consequence, information concerning their receptor binding and structure in relation to their ability to lower blood sugar levels is of considerable interest. It has been suggested[1] that the so called second-generation sulfonylurea drugs act at two distinct but related binding sites in pancreatic β cells. Gliburide (**1**), for example, is said to bind at site A (Figure 1) via the sulfonylurea moiety and at site B via the benzamide group. This view is strongly supported by structure–activity studies of first- [e.g., tolbutamide (**3**)] and second-generation sulfonylurea drugs, where the presence of both the benzamide CONH and the $SO_2NHCONH$ groups gives compounds with the greatest hypoglycemic potency. Recently, Rufer[2] has extended his hypothesis to include the related [(acylamino)ethyl]benzoic acids, such as **2** (HB 699), which show a hypoglycemic potency and profile[3] similar to that of the first-generation sulfonylurea drugs, such as tolbutamide (**3**). As part of this new hypothesis, compounds such as **2** were claimed[2] to lower blood sugar levels solely by occupation of site B. This conclusion assumes that the carboxyl group of **2** is not interacting with site A and that the benzamide group in **2** is involved in insulin release. We describe results that indicate that the [(acylamino)ethyl]benzoic acids may act at both sites A and B and suggest a role other than insulin release for the interaction of the benzamide group of **2** with site B.

Chemistry. The acid **2** was prepared from the known *p*-(2-aminoethyl)benzoic acid[4] and 5-chloro-2-methoxybenzoyl chloride.[5] This general method A was used in the preparation of **6**, **8–10** and **12–14** (Table I) by employing the appropriate amino compound. Reduction of **10** with stannous chloride gave **11**. The ketone **15** was prepared by Friedel–Crafts acylation of **8** with acetyl chloride.[5] Reduction of **15** with sodium borohydride, followed by dehydration and subsequent catalytic hydrogenation, afforded **16–18**. Alkylation[6] of **12** gave the phenoxyacetic acid **19**.

Biological Results and Discussion

The reduction of the blood sugar level of a rat by **2** following a glucose challenge was substantial (Table I). This hypoglycemic activity has been previously reported for **2** in rats and dogs, and there is evidence that it is based on an increase in circulating insulin levels.[3] The blood sugar lowering activity of the sulfonylurea drugs, such as **1** and **3** (Figure 1), in animals and man is also attributed mainly to insulin release.[7] Thus, **1–3** have similar actions with respect to glycemia, and their structures are now discussed in terms of ability to bind at the putative receptor sites A and B (Figure 1).

Comparison of the structure of gliburide (**1**) with **2** and the chiral derivative **4** with the chiral acid **5** shows that site B has precise structural and stereochemical requirements.[2] The position of the benzamidic NH group of **1**, **2**, **4**, and **5** is the same in all four molecules with respect to the two aromatic rings. In addition, if the aromatic ring of the benzoic acid moiety of **2** was replaced by three methylene groups as in **6**, activity was lost. Assuming that **2** and **6** both adopt strain-free conformations, this replacement will maintain closely the position of the COOH group of **6** relative to the benzamide portion of the molecule (Figure 1). Thus, this aromatic ring is an essential part of the hypoglycemic action of **2**, and **1**, **2**, **4**, and **5**, are very similar, apart from two drugs having a sulfonylurea moiety where the other two have a carboxyl group. The carboxyl group of **2** must lie exactly where the sulfonylurea group of **1** binds at the putative receptor site A. Examination of Dreiding stereomodels of **1** and **2** suggests that the acidic proton of the carboxyl group of **2** is 3.0 Å from the adjacent phenyl ring, and the acidic NH of the SO_2NHCO in **1**, similarly, lies 3.2 Å from its adjacent phenyl ring. We thus propose that the COOH group of **2** is an isostere of the SO_2NHCO group of **1**. The pK_a of **2** is 4.4 by potentiometric titration, which is within the known range of pK_a values found for the hypoglycemic sulfonylurea drugs,[8] e.g., 3.1–5.4. Thus, it appears likely that the [(acylamino)alkyl]benzoic acids, such as **2** and **5**, can bind at the putative receptor A, as well as at site B. If this is the case, removal of the carboxyl group or its replacement by other nonacidic groups should lead to a loss of hypoglycemic activity. Examination of the effect of compounds on rat blood sugar levels (Table I) shows that this was the case, as **8** was inactive. Similarly, when common aromatic substituents were used to replace the carboxyl group as in **9–12**, no significant hypoglycemic activity was observed. The methyl derivative **13**, however, was very hypoglycemic, in contast with the chloro compound **9**, which would have a similar log *P* value. This result was interpreted in terms of the known facile metabolism of aromatic methyl groups to carboxyl in vivo.

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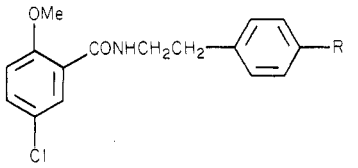
Table I. [(Acylamino)ethyl]benzenes



compd	R	method	mp, °C	recrystn solvent	mol formula	yield, %	% change in rat blood sugar after 1 g/kg of glucose[a]
2	CO_2H	A[b]	170-172[c]	EtOH	$C_{17}H_{16}ClNO_2$	37	−63
8	H	A	61-64[d]	Et_2O	$C_{16}H_{16}ClNO_2$	92	−7
9	Cl	A	106-107	toluene-petrol[e]	$C_{16}H_{15}Cl_2NO_2$	70	0
10	NO_2	A	157-159[f]	*i*-PrOH	$C_{16}H_{15}ClN_2O_4$	86	+2
11	NH_2	B[g]	100-101[h]	*i*-PrOH-petrol	$C_{16}H_{17}ClN_2O_2$	15	+7
12	OH	A	133-134[i]	toluene	$C_{16}H_{16}ClNO_3$	52	+2
13	Me	A	78-79[j]	petrol	$C_{17}H_{18}ClNO_2$	80	−55
14	CF_3	A	80-81	petrol	$C_{17}H_{15}ClF_3NO_2$	19	−3
15	COMe	C[k]	101-103	toluene	$C_{18}H_{18}ClNO_3$	38	−11
16	CH(OH)Me	B	97-99	toluene-petrol	$C_{18}H_{20}ClNO_3$	70	−11
17	$CH=CH_2$	B	72-73	petrol	$C_{18}H_{18}ClNO_2$	39	−6
18	Et	B	66-68[j]	petrol	$C_{18}H_{20}ClNO_2$	49	−4
19	OCH_2CO_2H	C	142-143	EtOH	$C_{18}H_{18}ClNO_5$	47	−9
6	*n*	A	117-118	*i*-PrOH	$C_{14}H_{18}ClNO_4$	55	+9
1 (gliburide)[o]					$C_{23}H_{28}N_3O_5S$		−44

[a] Reductions of blood sugar level in the range −1 to −15% are not significant. [b] Method A is general method A. [c] Reference 5: mp 170-172 °C. [d] Reference 13: mp 61-64 °C. [e] Petrol was petroleum ether, bp 60-80 °C. [f] Reference 14: mp 158-160 °C. [g] Method B indicates method is in Experimental Section. [h] Reference 14: no mp quoted. [i] Reference 6: mp 133-135 °C. [j] Reference 15: **13**, mp 77-78 °C; **18**, mp 66-68 °C. [k] Method C indicates preparation in cited reference. [l] Reference 5: mp 103-105 °C. [m] Reference 6: mp 142-143 °C. [n] Structure is in Figure 1. [o] Oral dose of 5 mg/kg.

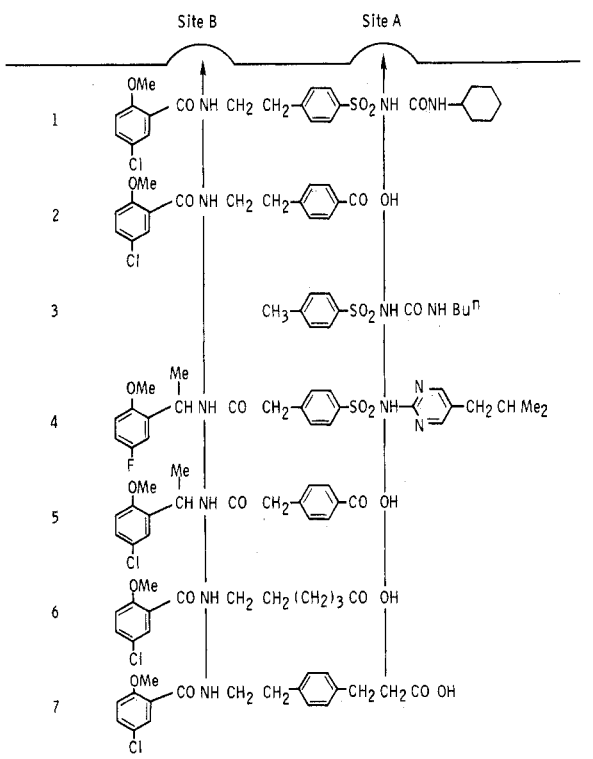


Figure 1. Relation of benzamide and acidic groups to putative receptor sites.

Tolbutamide (**3**), for example, is rapidly metabolized in vivo at the aromatic methyl group to give the carboxyl metabolite of tolbutamide.[9] A series of compounds was thus prepared, **14**–**18**, which either could not be metabolized to carboxyl or the conversion would be slow. None of these close analogues of the methyl compound **13** was active, suggesting that metabolic transformation of the methyl group was a prerequisite for hypoglycemic activity.

The potent hypoglycemic[3] acid **7** may also be metabolized in vivo to **2** by β-oxidation. In support of this view was the lack of hypoglycemic effect found for the phenoxyacetic acid derivative **19**, which cannot be β-oxidized.

Conclusions

The [(acylamino)alkyl]benzoic acids (Table I) only showed potent hypoglycemic activity when the aromatic ring of the phenethyl group was appropriately substituted. This optimum substituent was not determined by lipophilic character, steric size, or electronic effects on the aromatic ring but apparently needed to be an acidic group or a group that could be rapidly metabolized to carboxyl. Since the carboxyl group has similar acidity to the sulfonylurea moiety and could occupy the same position at a putative receptor, it is postulated that the carboxyl group interacts with the putative receptor site A to cause insulin release and, hence, a hypoglycemic response. The reported[10] potent plasma-binding properties of second-generation sulfonylureas, e.g., **4**, in contrast with tolbutamide, and the greater persistance[11] of bioelectrical effects of **1** over **3** on mouse pancreatic islet membrane suggest that the function of the benzamide CONH group in **2** involves an increase in protein binding. There is no clear evidence supporting a direct role in insulin release for the benzamide group. The recent hypothesis[12] that the insulin-releasing

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properties of the sulfonylureas drugs are related to an ability to assist the transport of calcium cations may suggest why an acidic group needs to be present in hypoglycemic [(acylamino)alkyl]benzoic acids.

Experimental Section

Melting points are uncorrected. IR and NMR were determined on Perkin-Elmer 157 and a Varian HA 100 spectrometer, respectively. Spectral data were consistent with the assigned structures and were supported by MS fragmentations from a Hitachi RMU-6E instrument. Where analyses are indicated only by symbols, elementary analyses are within ±0.4% of the theoretical values.

p-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzoic Acid (2) (General Method A). A solution of 5-chloro-2-methoxybenzoyl chloride (545 mg, 2.66 mmol) in acetone (1 mL) was added dropwise with stirring to a solution of *p*-(2-aminoethyl)benzoic acid (560 mg, 2.55 mmol) in 2 N NaOH (3.2 mL) and acetone (4.0 mL) maintained at 0–5 °C. The ice bath was removed, and the mixture was stirred for 2 h. The mixture was diluted with H_2O (40 mL) and acidified with 2 N HCl to pH 3. The solid product was collected and crystallized from EtOH to give **2** (330 mg, 37%).

5-Chloro-2-methoxy-N-[2-(p-aminophenyl)ethyl]benzamide (11). A solution of $SnCl_2 \cdot 2H_2O$ (7.0 g, 32.4 mmol) in EtOH (10 mL) was added to a stirred suspension of **10** (2.7 g, 8.0 mmol) in EtOH (8 mL) and 11 N HCl (8 mL), and stirring continued for 24 h. The mixture was diluted with H_2O (50 mL) and made alkaline with 30%, w/w, NaOH. The mixture was extracted with $CHCl_3$, and the $CHCl_3$ was shaken with an excess of 2 N HCl. The acid layer was made alkaline with 30%, w/w, NaOH and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$), and evaporated. Crystallization of the residue from *i*-PrOH–petroleum ether, bp 60–80 °C, gave **11** (355 mg, 14.5%).

5-Chloro-2-methoxy-N-[2-[p-(1-hydroxyethyl)phenyl]ethyl]benzamide (16). $NaBH_4$ (0.8 g, 21.6 mmol) was added in portions to a stirred suspension of **15**, (4.7 g, 14.2 mmol) in EtOH (100 mL). After 6 h, dilute HOAc was added, and the EtOH was evaporated. The residue was shaken with EtOAc and H_2O, and the EtOAc was dried ($MgSO_4$) before evaporation. The residue was crystallized from toluene–petroleum ether, bp 60–80 °C, to give **16** (3.3 g, 70%).

5-Chloro-2-methoxy-N-[2-(p-vinylphenyl)ethyl]benzamide (17). A solution of **16** (3.0 g, 9.0 mmol) and I_2 (100 mg, 0.39 mmol) in xylene (20 mL) was heated under reflux for 3 h. The cooled xylene was washed with $Na_2S_2O_3$ solution, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography on silica gel ($CHCl_3$), eluting with $CHCl_3$–EtOAc (40:1). Crystallization of the evaporated eluates from petroleum ether, bp 60–80 °C, gave **17** (1.1 g, 38.7%).

5-Chloro-2-methoxy-N-[2-(p-ethylphenyl)ethyl]benzamide (18). A solution of **17** (600 mg, 1.9 mmol) in EOH (20 mL) was hydrogenated at 1 atm over 5% Pd/C (100 mg) for 10 min. The catalyst was filtered and the EtOH was evaported. The residue was purified by preparative TLC on silica gel using 5% EtOAc in $CHCl_3$ as eluant. Evaporation of the eluates and crystallization of the residue from petroleum ether, bp 60–80 °C, gave **18** (300 mg, 49%).

Hypoglycemic Assay. Rats were fasted for 24 h. Compounds were ball milled in 0.5% Tween 80 overnight and dosed orally by gavage at 50 mg/kg to rats in groups of four. After 1 h, an oral glucose load (1 g/kg) was given by gavage. After an additional 1 h, blood samples were taken from the retro-orbital sinus under light ether anesthesia. Blood glucose levels were measured by a glucose oxidase method, and the effect of the compound on the ability of the rat to accommodate the glucose load was compared with control group rats given excipient. Compounds improving glucose tolerance by 15% or more were tested again in groups of six rats. The mean of the two results was taken, and if greater than 15%, the compound was considered active.

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Registry No. **2**, 54870-28-9; **6**, 87262-53-1; **8**, 33924-49-1; **9**, 87262-54-2; **10**, 25921-64-6; **11**, 63484-38-8; **12**, 41859-64-7; **13**, 64353-00-0; **14**, 87262-55-3; **15**, 60531-37-5; **16**, 64507-35-3; **17**, 87262-56-4; **18**, 64353-17-9; **19**, 41859-81-8; 5-chloro-2-methoxybenzoyl chloride, 29568-33-0; *p*-(2-aminoethyl)benzoic acid, 1199-69-5.

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Novel Tetracyclic Spiropiperidines. 4.[1] Synthesis and Pharmacological Evaluation of Spiro- and 6,7-Dihydrospiro[benzo[*b*]pyrrolo[3,2,1-*jk*][1,4]benzodiazepine-2(1*H*),4′-piperidine]s

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A previously described series of 1-arylspiro[indoline-3,4′-piperidine]s was reported by us to possess significant antidepressant properties. This biological activity was found to be at a maximum among those compounds bearing an ortho substituent (e.g., NH_2 as in **1**) in the pendant aryl ring. In order to explore further this "ortho effect", we synthesized cyclic analogues of type **3** and **4** in which the position of the *o*-NH_2-substituted aryl group is conformationally restricted and defined. When tested for antidepressant activity by tetrabenazine ptosis prevention in mice, it was found that restriction of rotation of the pendant *o*-aminophenyl group in these rigid analogues resulted in a loss of antidepressant properties. However, analgesic activity was retained and even improved by this molecular constraint.

We recently described the synthesis of a series of 1-arylspiro[indoline-3,4′-piperidine]s of formula I.[1] Some of the members of this class were shown to possess significant antidepressant activity, as measured by the in-

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(1) For paper 3 in this series, see Ong, H. H.; Profitt, J. A.; Fortunato, J.; Glamkowski, E. J.; Ellis, D. B.; Geyer III, H. M.; Wilker, J. F.; Burghard, H. *J. Med. Chem.* **1983**, *26*, 981.

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