Compounds Inhibiting Insulin Degradation

Robert H. Williams, M.D., and Frances L. Martin, M.S.,† Seattle*

In diabetics there is insufficient endogenous insulin action, yet in these patients rapid destruction of insulin-I^{131} has been demonstrated.[1, 2] Provision of exogenous insulin is inferior to an adequate endogenous supply since it is not subject to the same homeostatic responses and since it commonly provokes increased binding of insulin by plasma protein and occasionally marked insulin resistance. Inhibition of the degradation of insulin with consequent increase in the effectiveness of the supply available is a justifiable major goal, particularly if it can be accomplished by the oral administration of a safe compound acting for a sufficient interval. Many compounds have been demonstrated to inhibit insulin degradation in vitro[3, 4, 5, 6, 7] but relatively little has been reported relative to their effect in vivo, except for carbutamide and tolbutamide. Although these compounds have been shown to inhibit insulin degradation under certain conditions, the importance of this action clinically, compared to other actions, has not been established.

In this paper are reported the investigations, in vitro, of the inhibitory effect of a variety of compounds, most of which were sulfonamides, disulfides, thiazoles, thioureas or indoles.

METHODS

The details of the methods employed have been published;[7] they have been modified since an earlier report by Tomizawa et al.[4] The main phase of the study consisted of incubating insulin-I^{131} with a liver enzyme system which rapidly degraded insulin, as noted by measuring the increase in the amount of radioactivity soluble in trichloroacetic acid, and of determination of the extent to which various compounds inhibited insulin degradation.

RESULTS

The major results are presented in the tables. It is observed in table 1 that 9 of the 10 sulfonamides, in the concentrations indicated, caused significant inhibition of insulin degradation. Most of the sulfonamides tested also had a thiazole radical. The greatest effect of the sulfonamides was shown by 6-ethoxy-2-benzothiazolesulfonamide. Butylaminobenzenesulfonylurea, butyltoluenesulfonylurea, isopropylaminobenzenesulfonamidothiadiazole and acetylaminothiadiazolesulfonamide (Diamox), all compounds that have been shown to have a hypoglycemic effect,[9, 10, 11, 12] showed a significant inhibitory effect. Another diuretic compound with a closely related structure (propionylaminothiadiazolesulfonamide) inhibited insulin degradation.

Of three nonsulfonamide thiazoles tested, aminomethoxyphenylthiadiazole was the most active (table 2). It is of note that the action of thiamine was comparable to that of the two sulfonylureas often used clinically for treating diabetics. Thiolthiazoline was inactive.

Seven of the eight disulfides tested were very active (table 3), the most active being biscarboxypropionylaminophenyldisulfide. Other very active disulfides were bisdimethylcarbamyldisulfide, bisaminophenyldisulfide, bisdimorpholinothiocarbamoyldisulfide, cystine, and lipoic acid. Among the four thioureas (table 4), dithiouracil and methoxybenzylthiosemicarbazide were definitely active. Other effective sulfur compounds were phthalazinedithiol sodium diethyldithiocarbamate, thiolhistidine, sodium heparinate and decamethylene-bisthiopseudourea (table 5).

The most active nonsulfur compound (table 6) was decamethylenebisguanidine (Synthalin A). This compound has been known for years to produce hypoglycemia.[13] The other more active nonsulfur compounds were: indolepropionic acid, methylindole, arbutin, indole, phenylbutyramide, phenylpropionic acid, and phenylacetic acid.

DISCUSSION

Previous reports have shown[3, 4, 5, 6, 7] that insulin degradation was inhibited by copper, zinc, p-chloromercuribenzoate, iodosobenzoate, iodoacetate, isopropylidene-2, 4-dithiohydantoin, various amino acids, auxins, peptides

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From the Department of Medicine, University of Washington School of Medicine, Seattle 5, Washington.

* Executive Officer and Professor of Medicine, Department of Medicine, University of Washington School of Medicine, Seattle.

† Senior Laboratory Technician.

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TABLE 1

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors
(see footnote on page 454)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Sulfonamides			
6-Ethoxy-2-benzothiazolesulfonamide (U)	1.0	89	0.5
	<0.4	44	
$C_2H_5OC_6H_3SC(SO_2NH_2)N$	<0.1	13	
2-Acetylamino-1,3,4-thiadiazole-5-sulfonamide	30.0	97	6
	12.0	79	
	1.4	0	
$H_2NSO_2CNNC(NH\,COCH_3)S$			
2-Ethyl-5-p-aminobenzenesulfonamido-1,3,4-thiadiazole (SK)	10.0	46	11
	5.0	24	
	2.0	9	
$H_2NC_6H_4SO_2NHCNNC(C_2H_5)S$			
2-Isopropyl-5-p-aminobenzenesulfonamido-1,3,4-thiadiazole (Sch)	9.4	42	11.5
	1.0	14	
$H_2NC_6H_4SO_2NHCNNC(C_3H_7)S$			
2-Propionylamino-1,3,4-thiadiazole-5-sulfonamide (L)	4.0	35	12
	1.0	23	
$H_2NSO_2CNNC(NHCOC_2H_5)S$			
2-n-Butyrylamino-1,3,4-thiadiazole-5-sulfonamide (SK)	<2.5	30	ca.12
$H_2NSO_2CNNC(NHCOC_3H_7)S$			
N′-(6-dimethoxymethyl-4-methyl-2-pyrimidyl) sulfanilamide (S)	10.0	32	ca.12
$(CH_3O)_2CHCCHC(CH_3)NC(NHSO_2C_6H_4NH_2)N$			
1-n-Butyl-3-p-aminobenzenesulfonylurea§ (L)	46.0	76	15
	11.0	44	
$H_2NC_6H_4SO_2NHCONHC_4H_9$	2.0	24	
1-n-Butyl-3-p-toluenesulfonylurea§ (U)	11.0	100	2.5
	6.6	93	
$CH_3C_6H_4SO_2NHCONHC_4H_9$	2.2	47	
	0.9	13	
	0.2	0	
N′-(2-Thiazolin-2-yl)sulfanilamide (S)	<1.6	3	
$S(CH_2)_2NCNHSO_2C_6H_4NH_2$			

TABLE 2

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors
(see footnote on page 454)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Thiazoles			
2-Amino-5-(p-methoxyphenyl)-1,3,4-thiadiazole (S)	1.0	58	0.8
$CH_3OC_6H_4CNNC(NH_2)S$	0.25	20	
Thiamine hydrochloride	30.0	60	15
$HClNH_2CNC(CH_3)NCHCCH_2N(Cl)CHSC(CH_2CH_2CH)CCH_3$	10.0	45	
2-Thiol-2-thiazoline (U)	2.5	5	
$(CH_2)_2NC(SH)S$	1.0	2	

TABLE 3

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors
(see footnote on page 454)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Disulfides			
Bis[2-(β-carboxypropionylamino)phenyl]disulfide (M)	0.01	100	0.002
	0.005	94	
	0.001	20	
$(HO_2C(CH_2)_2CONHC_6H_4S-)_2$	0.0001	17	
Bis(dimethylcarbamyl)disulfide (U)	0.025	100	0.011
	0.012	98	
$[(CH_3)_2NCOS-]_2$	<0.01	26	
Bis(2-aminophenyl)disulfide dihydrochloride (U)	<0.025	92	<0.015
	<0.01	30	
$(NH_2C_6H_4S-)_2 \cdot 2HCl$	<0.005	10	
	<0.002	4	
Bis(dimorpholinothiocarbamoyl)disulfide (U)	<0.2	100	<0.04
	<0.04	52	
$[O(CH_2CH_2)_2NNHCSS-]_2$			
Cystine	0.5	88	0.1
	0.13	54	
$(-SCH_2CHNH_2COOH)_2$			
DL-Lipoic acid	30.0	100	1.1
	10.0	95	
$CH_2CH_2CH_2(CH_2)_4COOH$	5.0	88	
S——S	2.5	73	
	0.5	33	
Bis(1-piperidinothiocarbamoyl)disulfide (U) $(C_5H_{10}NNHCSS-)_2$	<1.0	27	
Bis(p-nitrophenyl)disulfide (U) $(O_2NC_6H_4S-)_2$	<1.0	4	

TABLE 4

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors
(see footnote on page 454)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Thioureas			
2,4-Dithiouracil (S)	1.0	98	0.5
	0.5	57	
NC(SH)NC(SH)CHCH	0.1	0	
1-p-Methoxybenzyl-3-thiosemicarbazide (S) $CH_3OC_6H_4CH_2NHNHCSNH_2$	10.0	52	10
1,3-Bis(p-methoxyphenethyl)-2-thiourea (S) $(CH_3OC_6H_4(CH_2)_2NH)_2CS$	<1.0	11	
3,5-Diiodo-4-hydroxybenzaldehyde,3-thiosemicarbazone (S) $HOI_2C_6H_2CHNNHCSNH_2$	<1.6	13	

TABLE 5

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors (see footnote below)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Miscellaneous sulfur compounds			
1,4-Phthalazinedithiol (S)	0.5	100	0.04
	0.1	89	
$C_6H_4C(SH)NNCSH$	0.05	71	
	0.025	25	
Sodium diethyldithiocarbamate	10.0	100	<0.5
	2.0	91	
$(C_2H_5)_2NCS_2Na$			
L-2-Thiolhistidine	3.3	100	0.7
	0.45	32	
$NC(SH)NHCHCCH_2CH(NH_2)COOH$	0.1	25	
	0.06	0	
Sodium heparinate (U)	7.5	45	9
	2.5	23	
$C_{24}H_{33}N_2Na_7O_{35}S_5$	1.0	0	
2,2′-Decamethylenebis(2-thiopseudourea)dihydrobromide (S)	40.0	54	40
$HN{=}C(NH_2)S(CH_2)_{10}SC(NH_2){=}NH \cdot 2HBr$			
2-Thiol-4-imidazoline (U)	<1.0	11	
$HSCHN(CH_2)_2NH$			
1-p-Methoxyphenyl-1,2,3-propanetrithiol (SK)	<0.2	13	
$CH_3OC_6H_4CH(SH)CH(SH)CH_2SH$			

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†In many instances concentrations greater than those shown were tested but are not recorded either because lower concentrations caused maximal inhibition or because of very poor solubility in the media tested. Indeed, even a few of the compounds listed were not completely soluble at certain concentrations; the following sign < was used where a saturated solution of the inhibitor or a dilution prepared from the saturated solution was used. (In a similar study[7] with amino acids, 10^{-5} moles/ml. was erroneously labeled 10^{-2} moles/ml.)

‡The concentration of inhibitor necessary to give 50 per cent inhibition under the conditions of these experiments was obtained from a bi-logarithmic plot of per cent inhibition versus concentration of inhibitor. In most cases a linear relationship was observed and the 50 per cent value was read from the line. Where the data did not yield a straight line an approximation was made to obtain an estimate of the order of effectiveness of the various inhibitors. For the weakest inhibitors no such evaluation was attempted.

§Results of study of these two compounds have been reported previously.[8]

TABLE 6

Results of incubation of insulin-I^{131} plus insulin-degrading enzyme system with and without test inhibitors
(see footnote on page 454)

Inhibitor	mM/L.†	Average per cent inhibition	mM/L. causing 50 per cent inhibition‡
Nonsulfur compounds			
Decamethylene-bis(guanidine)	7.0	66	5
	2.8	30	
$NH_2CNHNH(CH_2)_{10}NHNHCNH_2$	1.0	28	
Indole-3-propionic acid	10.0	84	7
	6.0	39	
$C_6H_4C(CH_2CH_2COOH)CHNH$			
5-Methylindole	2.5	34	8
	0.8	24	
$CH_3C_6H_3CHCHN$	0.27	6	
Arbutin	10.0	53	9
	2.5	25	
$HOC_6H_4OCH(CHOH)_3CHCH_2OH$	1.0	20	
Indole	<30.0	70	11
	3.0	32	
$C_6H_4CHCHNH$			
2-Phenylbutyramide (U)	10.0	42	14
	5.0	28	
$C_2H_5CH(C_6H_5)CONH_2$	2·0	17	
3-Phenylpropionic acid (U)	30.0	60	22
	10.0	34	
$C_6H_5CH_2CH_2COOH$	2.5	10	
Phenylacetic acid	30.0	49	30
	10.0	22	
$C_6H_5CH_2COOH$			
α-(Dimethylaminomethyl)-1-ethyl-3-indolemethanol (U)	<1.0	15	
$C_6H_4CH[CH(OH)CH_2N(CH_3)_2]CH{-}NC_2H_5$			
3-(2-Aminoethyl)-5-indanol · creatine sulfate · hydrate (U)	6.2	14	
	1.2	0	
$HOC_6H_3C(CH_2CH_2NH_2)CHNH$			
Phenylalanine	23.0	23	
	7.8	13	
$C_6H_5CH_2CHNH_2COOH$			
3-Acetamido-5-methyltetronic acid (U)	10.0	18	
	5.0	6	
$H_3CCHC(OH)C(NHCOCH_3)COO$			
L-Leucine	25.0	2	
	5.0	0	
$(CH_3)_2CHCH_2CHNH_2COOH$			
5-Ethyldialuric acid (S)	10.0	0	
$C_2H_5C(OH)CONHCONHCO$			
L-Proline	30.0	0	
$NH(CH_2)_3CHCOOH$			

and proteins. The mechanism by which these compounds and the ones reported here inhibit the degradation of insulin is not known. Since reduction may play an important role in inactivation of the hormone it may be that some of the agents, particularly the disulfides, may spare insulin by acting as substrates for reducing agents. Another explanation may be that of de Barbieri and Grassi[14] who postulated that the insulin-degrading enzyme is a peptidase in which sulfhydryl groups play an essential part for activity and that when its sulfhydryl groups are oxidized by disulfides, the enzyme is inactivated. In case of compounds like phenylacetic acid it may be postulated that by combining with certain components of the insulin-degrading enzyme system degradation of the hormone is inhibited.

Some compounds may inhibit markedly the degradation of insulin in vitro yet exert no significant effect in vivo. Therefore in evaluating their possible usefulness in the treatment of diabetes it is important to determine their capacity to lower the blood sugar; such studies are in progress.

SUMMARY

Using an in vitro system consisting of a liver enzyme preparation which rapidly degrades insulin-I^{131}, it has been found that many different compounds inhibit the degradation of the hormone. Among the inhibitors are certain sulfonamides, disulfides, thiazoles and thioureas. Other types of sulfur compounds had a significant activity in the following order: phthalazinedithiol, sodium diethyldithiocarbamate, thiolhistidine, sodium heparinate, and decamethylenebisthiopseudourea. The most active nonsulfur compounds were, respectively: decamethylenebisguanidine, indolepropionic acid, methylindole, arbutin, indole, phenylbutyramide, phenylpropionic acid and phenylacetic acid.

The mechanisms by which the compounds inhibit degradation and their effectiveness in lowering blood sugar are being investigated.

SUMMARIO IN INTERLINGUA

Compositos Que Inhibi le Degradation de Insulina

Per medio de un systema in vitro consistente de un preparato de enzyma hepatic que produce un rapide degradation de insulina a I^{131}, il esseva constatate que multe differente compositos inhibi le degradation del hormon. Le inhibitores include certe sulfonamidos, bisulfidos, thiazoles, e thioureas. Altere compositos de sulfure possede significative grados de activitate in le sequente ordine: Phthalazinedithiol, diethyldithiocarbamato de natrium, thiolhistidina, heparinato de natrium, e decamethylenebisthiopseudourea. Le plus active compositos non-sulfuric esseva: Decamethylenebisguanidina, acido indolepropionic, methylindol, arbutina indol, phenylbutyramido, acido phenylpropionic, e acido phenylacetic.

Le mechanismo per que le compositos inhibi le degradation de insulina e lor efficacia in reducer le nivellos de sucro sanguinee es sub investigation.

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