Differential Effects of N-Terminal Modifications on the Biological Potencies of Growth Hormone Releasing Factor Analogues with Varying Chain Lengths

David H. Coy,* William A. Murphy, Valentine A. Lance, and Mark L. Heiman

Peptide Research Laboratories, Department of Medicine, Tulane University Medical Center, New Orleans, Louisiana 70112.
Received May 27, 1986

The excellent retention of biological potencies observed with human growth hormone releasing factor analogues with chains 29–44 amino acid residues long is suddenly lost when further amino acid residues are removed from the C-terminus. For instance, 1–27 and 1–24 exhibited little biological activity (<1%) in vivo and in vitro in the rat. Studies were made to determine whether this was due to conformational changes rather than simply the loss of amino acids needed for direct receptor interactions. These involved the introduction of mild conformational restraint in the N-terminal region by the introduction of D-amino acid residues previously shown to increase the potency of the 1–29 peptide. D-Ala in position 2, responsible for a 40- to 50-fold increase in activity in the 1–29 species, resulted in little increase in the potency of 1–27 or 1–24 sequences. However, N-terminal acetylation, responsible for a 12-fold increase in 1–29 in vivo potency, caused greater than 50-fold increase in 1–27 potency but had little effect on 1–24 potency. Likewise, D-Asn in position 8 was far more effective in increasing the potency of the 1–27 sequence compared to the 1–29 {[D-Asn^8]-GRF(1–29)NH$_2$, 220% vs. [D-Asn^8, Leu^{27}]-GRF(1–27)NH$_2$, 53%; in vivo}. This differential effect was even more clear in vitro. The highest in vivo potency in the 1–27 series was achieved with [D-Asp^3,D-Asn^8,Leu^{27}]-GRF(1–27)NH$_2$ (200%); however, this analogue was still far less potent than its 1–29 counterpart (3800%). None of the D-amino acid substitution strategies were effective in increasing 1–22 peptide potencies to detectable levels. The results indicate that the effect of N-terminal substitutions and resulting potencies of the GRFs is very much dependent on chain length, perhaps suggesting that C-terminal amino acids promote conformational effects at the N-terminus and/or vice versa.

Hypothalamic growth hormone releasing factors (GRF) are polypeptides ranging in size from 43 to 44 amino acid residues in the human,[1] pig,[2] cow,[3] sheep,[4] goat,[4] and rat[5]—species from which GRFs have thus far been sequenced (see Table I). It was soon determined, however, that elimination of amino acids from the C-terminus has little effect on biological activity as long as 29 or more residues remain.[6-8] Given its greater ease of synthesis, the 1–29 amide sequence was subjected to much structure–activity work whereupon it was determined that mild conformational restriction in the N-terminal decapeptide region results[7,9] in a number of analogues with much-enhanced potency. D-Amino acid substitutions in positions 1, 2, 3, 8, and 9 all gave increased GH releasing activity in the anesthetized rat[9] and also in a rat pituitary cell culture.[10] Further highly active analogues were obtained by combining several of these alterations within the same sequence.[9,11]

In this paper, we investigate the possibility that some of these N-terminal modifications could be used to boost the biological activity of sequences even shorter than 29 residues to useful levels. Thus, D-amino acid substitutions were made singularly and in combination in positions 2, 3, and 8 of the 1–27 and 1–22 sequences of human GRF and, to a lesser extent, in the 1–24 sequence. The effects of N-terminal acetylation, responsible for about a 12-fold increase in potency in the 1–29 peptide[9,10] on the biological activities of the shorter sequences, were also examined. A total of 16 new analogues are reported.

Table I. Amino Acid Sequence of A, hpGRF(1-44)NH$_2$;[1] B, Porcine GRF;[2] C, Bovine GRF;[3] D, Ovine GRF;[4] E, Caprine GRF;[4] and F, Rat GRF[5]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	Tyr	Ala	Asp	Ala	Ile	Phe	Thr	Asn	Ser	Tyr	Arg	Lys	Val	Leu	Gly
B															
C															
D													Ile		
E															
F	His											Arg	Ile		

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A	Gln	Leu	Ser	Ala	Arg	Lys	Leu	Leu	Gln	Asp	Ile	Met	Ser	Arg	Gln
B															
C													Asn		
D													Asn		
E													Asn		
F			Tyr						His	Glu			Asn		

	31	32	33	34	35	36	37	38	39	40	41	42	43	44
A	Gln	Gly	Glu	Ser	Asn	Gln	Glu	Arg	Gly	Ala	Arg	Ala	Arg	Leu-NH$_2$
B				Arg				Gln				Val		
C				Arg				Gln				Val		
D				Arg				Gln				Val		
E				Arg				Gln				Val		
F				Arg				Gln	Arg	Ser		Phe	Asn	OH

Experimental Section

Synthesis. Benzhydrylamine polystyrene resin cross-linked with 1% divinylbenzene was obtained from Bachem, Inc., Torrance, CA, and contained 0.5 mmol of free amino groups per gram of resin. All amino acids were coupled as their α-Boc derivatives, which were obtained from the same source. Reactive side chains were protected as follows: Ser and Thr, benzyl ether; Lys, 2-chlorobenzyloxycarbonyl; His and Arg, tosyl; aspartic and glutamic, benzyl ester; Tyr, 2-bromobenzyloxycarbonyl. The amino acids were coupled to the growing peptide chain in 3 M excess in the presence of diisopropylcarbodiimide with use of a Beckman 990B automatic synthesizer. Boc-Asn and Gln were coupled in the presence of equivalent amounts of 1-hydroxybenzotriazole. Boc protection was removed at each step by two treatments with 33% trifluoroacetic acid in methylene chloride for 1 and 25 min

(1) Guillemin, R.; Brazeau, P.; Bohlen, P.; Esch, F.; Ling, N.; Wehrenberg, W. B. *Science (Washington, D.C.)* **1982**, *218*, 585.
(2) Bohlen, P.; Esch, F.; Brazeau, P.; Ling, N.; Guillemin, R. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 726.
(3) Esch, F.; Bohlen, P.; Ling, N.; Brazeau, P.; Guillemin, R. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 772.
(4) Brazeau, P.; Bohlen, P.; Esch, F.; Ling, N.; Wehrenberg, W. B.; Guillemin, R. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 606.
(5) Spiess, J.; Rivier, J.; Vale, W. *Nature (London)* **1983**, *303*, 532.
(6) Rivier, J.; Spiess, J.; Thorner, M.; Vale, W. *Nature (London)* **1982**, *300*, 276.
(7) Lance, V. A.; Murphy, W. A.; Sueiras-Diaz, J.; Coy, D. H. *Biochem. Biophys. Res. Commun.* **1984**, *119*, 265.
(8) Ling, N.; Baird, A.; Wehrenberg, W. A.; Ueno, N.; Munegumi, T.; Brazeau, P. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 854.
(9) Coy, D. H.; Murphy, W. A.; Sueiras-Diaz, J.; Coy, E. J.; Lance, V. A. *J. Med. Chem.* **1985**, *28*, 181.
(10) Heiman, H. L.; Nekola, M. V.; Murphy, W. A.; Lance, V. A.; Coy, D. H. *Endocrinology (Baltimore)* **1985**, *116*, 410.
(11) Coy, D. H.; Murphy, W. A.; Lance, V. A.; Heiman, M. L. *Peptides, Suppl. 1* **1986**, *7*, 49.

0022-2623/87/1830-0219$01.50/0

Table II. Differential Effect of N-Terminal Modifications on Potency of Human GRF Analogues with Varying Chain Lengths in the Rat

no.	analogue	potencies,[d] % anesthetized rats (95% conf limits)	potencies,[d] % pituitary cells
	GRF(1–29)NH_2	100	100
1	Leu^{27}(1–29)NH_2	57 (40–80)	2
	D-Ala^2(1–29)NH_2	4454 (3099–6400)	5340[c]
2	*N*-Me-D-Ala^2(1–29)NH_2	1630 (1160–2280)	
	Ac-Tyr^1(1–29)NH_2	1200[a]	240[c]
	D-Asp^3(1–29)NH_2	670[a]	150[c]
	D-Asn^8(1–29)NH_2	220[a]	
	D-Asp^3,D-Asn^8,Nle^{27}(1–29)NH_2	3800[b]	
3	Leu^{27}-GRF(1–27)NH_2	<5	0.001
4	D-Ala^2,Leu^{27}(1–27)NH_2	7 (5–11)	0.0004
5	Ac-Tyr^1,Leu^{27}(1–27)NH_2	54 (39–75)	
6	D-Asn^8,Leu^{27}(1–27)NH_2	53 (37–76)	25
7	D-Asp^3,D-Asn^8,Leu^{27}(1–27)NH_2	199 (143–277)	83
	GRF(1–24)NH_2	<1[a]	0.008
8	D-Ala^2(1–24)NH_2	16 (12–23)	0.004
9	Ac-Tyr^1(1–24)NH_2	<5	
10	Ac-His^1(1–24)NH_2	<5	
11	Arg^{24}(1–24)NH_2	<1	
12	D-Ala^2,Arg^{24}(1–24)NH_2	20 (15–28)	
13	D-Asp^3,D-Asn^8(1–22)NH_2	<5	
14	D-Ala^2,D-Asn^8(1–22)NH_2	<5	
15	*N*-Me-D-Ala^2,D-Asn^8(1–22)-NH_2	<5	
16	D-Ala^2,D-Asp^3,D-Asn^8(1–22)-NH_2	<5	

[a] Taken from Lance et al. [b] Taken from Coy et al.[11] [c] Taken from Heiman et al.[10] [d] Relative to GRF(1–29)NH_2.

each. Couplings were monitored by the standard ninhydrin test[12] and repeated when necessary with use of preformed symmetrical anhydrides in dimethylformamide to which a catalytic amount of (dimethylamino)pyridine had been added.

The completed, protected peptide-resins (0.25 mmol) were treated with 20 mL of anhydrous hydrogen fluoride containing 10% anisole and 100 mg of dithiothreitol for 45 min at 0 °C. After removal of the hydrogen fluoride under a stream of nitrogen (ca. 15 min), the free peptide amides were precipitated with either ether or ethyl acetate, filtered, and extracted with 2 M AcOH. At this stage, an HPLC profile of the crude material was obtained as a reference for future purification steps.

The extract was applied to a column (2.5 × 100 cm) of fine Sephadex G-50 and eluted with 2 M AcOH. Fractions were collected and examined individually by TLC on silica gel plates developed in 1-butanol/pyridine/acetic acid/water (15:10:3:12). The spots were visualized by ninhydrin followed by Cl_2/starch reagent and fractions pooled accordingly. Material thus obtained was then purified to an estimated purity of >93% by preparative medium-pressure chromatography on a column (1.5 × 50 cm) of Vydac C-18 silica (15–20-μm particle size), which was eluted with a linear gradient (Eldex Chromatrol) of 10–35% acetonitrile/water (0.1% in TFA) at a flow rate of about 3 mL/min and pressure of 80 psi. Eluent was monitored at 280 nm, and fractions were examined by TLC and analytical HPLC under conditions described in the next section. Fractions were pooled for maximum purity rather than yield and lyophilized repeatedly from dilute AcOH as white, fluffy powders.

Purified materials were examined for homogeneity by analytical HPLC using a Rainin machine with Apple II+ controller, Gilson Model 231 autoinjector, and Hewlett-Packard integrator. The analysis was carried out on a column (0.24 × 25 cm) of Vydac C-18 silica (5-μm particle size). Solvent 1 consisted of 5% acetonitrile/0.1 M triethylammonium phosphate (pH 3.0), and solvent 2 was 80% acetonitrile/0.1 M triethylammonium phosphate. Peptide was eluted at a flow rate of 1.5 mL/min with use of a linear gradient of 20–45% of solvent 1 over 30 min. Peaks were detected at 215 nm with use of a Gilson Holochrome variable wavelength monitor. Retention times (minutes) and integrated purity estimates (percent) were as follows (for analogue number and structure, see Table II): **1**, 25.9, 100; **2**, 22.3, 96; **3**, 25.9, 100; **4**, 25.7, 97.9; **5**, 29.2, 96.7; **6**, 26.3, 96.5; **7**, 24.5, 91; **8**, 15.5, 100; **9** 20.2, 100; **10**, 15.9, 98.7; **11**, 13.8, 93.6; **12**, 14.8, 98.4; **13**, 11.9, 100; **14**, 12.0, 100; **15**, 13.7, 100; **16**, 12.9, 100.

Amino acid analyses of 6 M HCl hydrolysates (110 °C, 18 h) were carried out on a column-temperature-programmable LKB 4150 automatic analyzer equipped with a Shimadzu C-R3A computing integrator. Single column borate buffer methodology was employed, and amino acid ratios obtained were within 5% of expected values.

Biology. Peptides were tested in vivo as previously described[13] in male Charles River rats weighing 175–275 g, which were anesthetized with sodium pentobarbital (6 mg/100 g body weight). Animals (six to nine per group) were injected subcutaneously 30 min after the anesthetic with either saline, different doses of GRF(1–29)NH_2 (control), or two doses of an analogue in 0.5-mL

Table III. Effects of GRF Analogues on GH Release in the Nembutal-Anesthetized Rat

analogue[a]	dose, μg/100 g body weight	plasma GH,[b] ng/mL (mean ± SEM)
GRF(1–29)NH_2	10	1185 ± 74
	25	2569 ± 121
saline		495 ± 75
D-Ala^2-Grf(1–29)NH_2	0.2	1213 ± 202
	0.5	2182 ± 492
saline		425 ± 85
1	25	1410 ± 520
	62.5	1725 ± 322
saline		432 ± 61
2	0.4	761 ± 111
	1	1725 ± 322
saline		313 ± 56
3	40	741 ± 182
	100	507 ± 98
4	80	742 ± 198
	200	1501 ± 590
saline		439 ± 59
5	20	1267 ± 288
	50	2727 ± 564
6	20	1551 ± 362
	50	2368 ± 436
saline		384 ± 56
7	4	899 ± 239
	10	2174 ± 424
saline		333 ± 46
8	32	499 ± 47
	80	1370 ± 289
saline		303 ± 22
9	10	396 ± 50
	100	664 ± 137
10	10	358 ± 45
	100	719 ± 43
saline		350 ± 52
11	40	392 ± 65
	100	406 ± 62
12	50	1020 ± 485
	125	2792 ± 465
saline		332 ± 43
13	10	498 ± 94
	100	401 ± 99
14	10	417 ± 76
	100	305 ± 23
saline		369 ± 42
15	10	512 ± 99
	100	385 ± 62
16	10	314 ± 29
	100	340 ± 50

[a] See Table II for analogue structures. [b] Six to nine animals per group.

(12) Kaiser, E.; Colescott, R.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(13) Murphy, W. A.; Meyers, C. A.; Coy, D. H. *Endocrinology (Baltimore)* **1981**, *109*, 491.

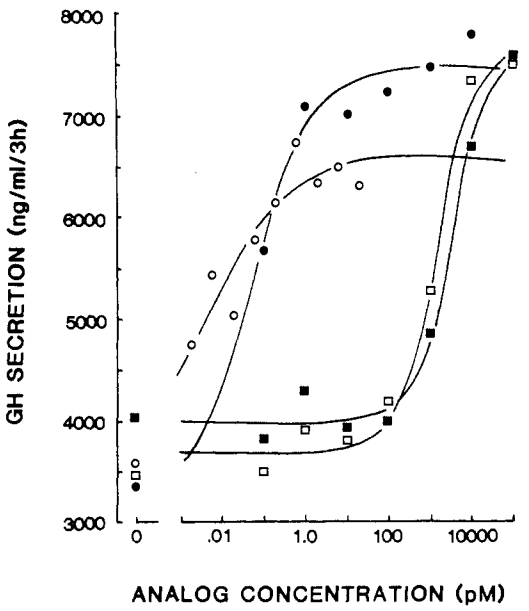


Figure 1. Effects of various concentrations of hGRF(1–29)NH_2 (○), [Leu^{27}]-GRF(1-29)NH_2 (●), [Leu^{27}]-GRF(1–27)NH_2 (□), and [D-Ala^2,Leu^{27}]-GRF(1–27)NH_2 (■) on GH release from primary cultured rat pituitary cells.

volumes. Fifteen minutes after injection of test substance, a blood sample was taken from the jugular vein, and plasma was analyzed for GH by radioimmunoassay using materials generously supplied by NIADDKD. Dose responses obtained are shown in Table III. Potencies and 95% confidence limits based on four-point assay design were calculated by the method of Pugsley[14] with use of an Applesoft BASIC computer program developed in this laboratory and are shown in Table II.

In vitro rat pituitary cell GH responses to the peptides were obtained by using the method of Heiman et al.[10] Multiple dose–response curves were analyzed simultaneously by the 4-parameter logistic equation of De Lean et al.[15] (the Applesoft program ALLFIT was modified and compiled in this laboratory to enhance operating speed). Typical dose–response curves for representative peptides with high and low GH releasing activities are shown in Figure 1. In vitro potency estimates are given in Table II.

Results and Discussion

The rapid decline in GH releasing activities when several amino acids are removed from the C-terminus of GRF(1–29)NH_2 has been noted before[8] and is particularly evident in our present study. For instance, [Leu^{27}]-GRF(1–27)NH_2 with just two amino acids deleted was more than 10 times less potent than [Leu^{27}]-GRF(1–29)NH_2 in vivo and about 100 times less potent in this in vitro assay system. No further activity was lost in going to the 1–24 sequences either in vivo or in vitro. The possibility that the C-terminal Arg residue was in some way necessary for high biological activity was checked by incorporating it at the C-terminus of the 1–24 sequence. No effect on biological activity was evident. The predominantly α-helical region of GRF as predicted by Chou–Fasman calculations[9,16] extends from position 14 through to the C-terminus. It is possible that it is the loss of some of this helical content that is not compatible with preserving the required conformation for receptor binding.

Replacement of Ala in position 2 of GRF(1–29)NH_2 by its D isomer results[7] in the most potent (5100%) analogue so far reported for the GRFs. This was resynthesized and retested for the present study whereupon a very similar potency of 4454% was obtained in the anesthetized rat (Tables II and III). In discussing the high potency of this analogue, it is important to realize that it was calculated relative to GRF(1–29)NH_2 rather than the longer 1–40 or 1–44 sequences, which are about twice as potent as the 1–29. The high activity of the D-Ala^2 analogues is not fully reflected in a recent human clinical study[17] where no more than a twofold increase in potency was observed. However, in the anesthetized pig[7] and conscious sheep and dairy cattle (D. Haines and R. DeLay, personal communication), about a 10-fold increase in GH releasing potency is evident. There is thus a considerable species difference in the potency of this analogue, which might be due to varying negative control of GH release exercised by endogenous somatostatin. The sodium pentobarbital anesthetized rat model is specifically designed to circumvent the somatostatin problem since this particular anesthetic eliminates secretion of somatostatin.[18] In our hands, it gives highly reproducible potency estimates and appears to be an excellent system for GRF structure–activity work. In general, the in vivo potencies match quite well with those obtained from primary cultures of rat pituitary cells (Table II) with the exception of several low-potency analogues that were far less active in vitro than in vivo. The reasons for this are hard to delineate but could be due to altered pharmacokinetics when very large doses of peptide are given subcutaneously.

The next most active reported peptide appears to be a [*N*-Me-D-Ala^2] analogue described[19] by Rivier et al., which we synthesized and tested in our in vivo system for comparison (Tables II and III). It had 1630% of the activity of GRF(1–29)NH_2 itself. Unlike its dramatic effects on 1–29 potency, D-Ala^2 in the 1–27 or 1–22 sequences displayed little effect on potencies either in vivo or in vitro. Likewise, *N*-Me-D-Ala^2 in the 1–22 sequence failed to increase potency. However, D-Ala^2 in the 1–24 peptide appeared to significantly increase biological activity from <1% to 16% in vivo.

Acetylation of the N-terminal Tyr α-amino group is another modification that has resulted in increased activity of the 1–29 chain length series.[9] Acetylation of [Leu^{27}]-GRF(1–27)NH_2 also resulted in more than a 10-fold increase in potency. Acetylation of either 1–24 or 1–22 sequences failed to affect observed activities.

Perhaps the most intriguing result was obtained with use of the D-Asn^8 modification, which in GRF(1–29)NH_2 results[9] in roughly a doubling of potency. In [Leu^{27}]-GRF(1–27)NH_2, however, at least a 10-fold increase in potency was produced. Therefore, in the 1–27 sequence, D-Asn^8 was far more effective than D-Ala in position 2. It is perhaps pertinent that the single β-turn predicted by Chou–Fasman calculations[9] occurs in residues 6–8. It may be that this folding is stabilized to some extent by interaction with the extreme C-terminal region of GRF(1–29). A lack of stabilization in GRF(1–27) might be compensated for by the inclusion of the D-amino acid in position 8, thus explaining some of the present structure–activity data. With the combination analogue, [D-Asp^3,D-Asn^8,Leu^{27}]-GRF(1–27)NH_2, potency was increased still further by a factor of 4 both in vivo and in vitro. None of the position

(14) Pugsley, L. I. *Endocrinology (Baltimore)* **1946**, *39*, 161.
(15) De Lean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
(16) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222.
(17) Barro, J. L.; Coy, D. H.; Millar, R. P. *Peptides* **1985**, *6*, 575.
(18) Chihara, K.; Arimura, A.; Schally, A. V. *Endocrinology (Baltimore)* **1979**, *104*, 1434.
(19) Rivier, J.; Pandol, S.; Perrin, M.; Seifert, H.; Galyean, R.; Yamamoto, G.; Rivier, C.; Vale, W. *Reg Peptides, Suppl. 3*, **1985**, S10.

8 analogues in the 1–22 series containing D-Asn alone or in combination with D-Asp[3], D-Ala[2], or *N*-Me-D-Ala[2] gave detectable levels of activity at the doses tested (Table III).

This is not the only report of differential effects of certain N-terminal modifications on the biological activity of GRF analogues with varying chain lengths. Baird et al.[20] found that whereas rat GRF(1–43) was 3.9 times more potent than human GRF(1–44)NH_2, rat GRF(1–27)NH_2 and (1–33)NH_2 were 6.15 and 9.72 times more potent than the human (1–27)NH_2 and (1–33)NH_2 sequences, respectively, using rat pituitary cells in culture. The major structural difference between the human and cat peptides is the presence of His rather than Tyr in position 1 of the rat hormone (see Table I). Thus, it appears that the biological effects of side-chain characteristics of amino acids in the N-terminal region of GRF are also dependent on amino acids present at the distantly removed C-terminus. This could indicate that both regions are involved with promoting the receptor binding conformation of GRF. Since very mild conformational restraint can enhance the activity of sequences shorter than 29 residues, it might be possible to eventually design far more rigid analogues of smaller chain lengths that retain useful levels of GH releasing activity.

(20) Baird, A.; Wehrenberg, W. B.; Ling, N. *Neurodendocrinology* **1986**, *42*, 273.

Acknowledgment. We thank Etchie Yauger, Lisa Mutty, and Vienna Mackey for their expert technical assistance. This research was supported by NIH Grant AM-30167.

Registry No. 1, 104874-07-9; 2, 104834-33-5; 3, 104874-08-0; 4, 104834-34-6; 5, 104848-48-8; 6, 104834-35-7; 7, 104834-43-7; 8, 104834-36-8; 9, 104834-37-9; 10, 104834-38-0; 11, 104848-62-6; 12, 104834-39-1; 13, 104834-40-4; 14, 104848-63-7; 15, 104834-41-5; 16, 104834-42-6; GRF(1–29)NH_2, 86168-78-7; D-Ala[2](1–29)NH_2, 89453-59-8; Ac-Tyr[1](1–29)NH_2, 89453-61-2; D-Asp[3](1–29)NH_2, 89453-60-1; D-Ash[8](1–29)NH_2, 94061-38-8; D-Asp[3],D-Asn[8],Nle[27]-(1–29)NH_2, 101366-32-9; GRF(1–24)NH_2, 95415-63-7; growth hormone, 9002-72-6.

3-[(2-Ethoxyphenoxy)methyl]piperidine Derivatives. Synthesis and Antidepressant Activity[1]

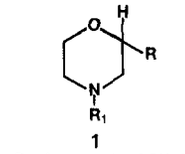
A. Balsamo,[†] I. Giorgi,[†] A. Lapucci,[†] A. Lucacchini,[‡] B. Macchia,*[†] F. Macchia,[§] C. Martini,[‡] and A. Rossi[∥]

Istituto di Chimica Farmaceutica, Istituto Policattedra di Discipline Biologiche, and Istituto di Chimica Organica, Facoltà di Farmacia, Università di Pisa, 56100 Pisa, Italy, and Ricerca Sviluppo Biologico Farmitalia-Carlo Erba, 20159 Milano, Italy.
Received April 7, 1986

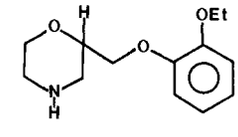
The 3-[(2-ethoxyphenoxy)methyl]piperidine derivatives **3–5** were synthesized and screened as potential antidepressant agents by the reserpine interaction test in mice and the evaluation of reuptake inhibition of biogenic amines in pig brain synaptosomal fractions. In addition, their anticonvulsant activity, tested by pentylenetetrazole antagonism, and approximate acute toxicity were evaluated. In vivo and in vitro tests showed that compounds **3** and **5** possess a biological activity comparable to that of the antidepressant drug viloxazine (**2**).

Recently, several antidepressants have been developed that are devoid of pharmacological and biochemical secondary effects typical of imipramine-like tricyclic antidepressants and of monoamine oxidase inhibitors.[1-8] Several of these new drugs reveal a common structural characteristic consisting of a morpholine ring to which an aromatic group is attached either directly or by an oxymethylene bridge, as indicated in 1.[2,4-7]

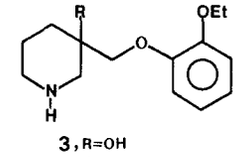
On the basis of this observation, investigations were designed to see whether the substitution of the morpholine ethereal oxygen of type 1 drugs with an alcoholic group could lead to new compounds in which the antidepressant activity is still present. We therefore synthesized 3-[(2-ethoxyphenoxy)methyl]-3-piperidinol (**3**), which exhibits in the 3-position the side chain of viloxazine (**2**),[2] one of the best known members of type 1 drugs. Subsequently, in order to evaluate the importance of the presence of the hydroxyl group in determining the activity of **3**, its 3-methoxy (**4**) and 3-desoxy (**5**) analogues were also prepared.



1
R = Ar, CH_2OAr, OCH_2Ar
R_1 = H, Alkyl group



2, Viloxazine



3, R=OH
4, R=OMe
5, R=H

Chemistry

The synthetic route to **3** and **4** is outlined in Scheme I. The treatment of 1-benzyl-3-piperidone (**6**)[9] with dimethyloxosulfonium methylide[10] afforded the epoxide **7**. Reaction of **7** with *o*-ethoxyphenol and NaOH yielded the 3-piperidinol **8**, which was catalytically hydrogenolyzed to

[†] Istituto di Chimica Farmaceutica.
[‡] Istituto Policattedra di Discipline Biologiche.
[§] Istituto di Chimica Organica.
[∥] Ricerca Sviluppo Biologico Farmitalia-Carlo Erba.

(1) A preliminary account of this work was presented at the 4th National Meeting of the Division of Medicinal Chemistry of the Italian Chemical Society, Palermo, Oct 1983, Abstract, p 87.
(2) Mallion, K. B.; Todd, A. H.; Turner, R. W.; Bainbridge, J. G.; Greenwod, D. T.; Madinaveitia, J.; Somerville, A. R.; Whittle, B. A. *Nature (London)* **1972**, *238*, 157.
(3) Christensen, J. A.; Squires, R. F. Ger. Offen. 2404113, 1974.
(4) Serradell, M. N.; Blancafort, P. *Drugs Future* **1978**, *3*, 667.
(5) Thomae, K. Ger. Offen. 2728898, 1977.
(6) Pifferi, G.; Nicola, M.; Gaviraghi, G.; Pinza, M.; Banfi, S. *Eur. J. Med. Chem.—Chim. Ther.* **1983**, *18*, 465.

0022-2623/87/1830-0222$01.50/0