5 mL of DMF were successively added triethylamine (1 mmol, 0.14 ml) and *N*-(*tert*-butyloxycarbonyl)-D-alanine *N*-hydroxysuccinimide ester (0.9 mmol, 257 mg). The reaction was allowed to proceed overnight at room temperature. The usual workup afforded the protected dipeptide ester as a clear oil, homogeneous by TLC (solvent A). This material was dissolved in 5 mL of 4 N HCl/dioxane. After 30 min, the solvent was evaporated and the residue triturated with 3 × 50 mL of ether, to yield the dipeptide methyl ester hydrochloride as a very hygroscopic white solid.

Physical data of these compounds are listed in Table I.

As an example, NMR data of **1** are as follows: NMR (CD_3OD) δ 4.00 (q, 1 H, C_taH D-Ala), 3.68 (s, 3 H, OCH_3), 1.52 (d, 3 H, CH_3 D-Ala), 1.50 (s, 6 H, CH_3 Aib).

General Method for the Synthesis of the Tripeptide Methyl Esters 10, 12, 13, 15, and 16. A solution of β-benzyl *N*-(benzyloxycarbonyl)-L-aspartate (1 mmol, 357 mg) in 10 mL of THF was cooled to −15 °C. *N*-Methylmorpholine (1 mmol, 0.101 mL) and isobutyl chloroformate (1 mmol, 0.130 mL) were successively added. The mixture was stirred for 5 min at −20 °C, and a solution of **2**, **4**, **6**, or **7** (1.1 mmol) and triethylamine (1.1 mol, 0.154 mL) in 10 mL of THF was added. The reaction was allowed to proceed for 1 h at 0 °C and 2 h at room temperature. The usual workup yielded the protected tripeptide ester as a clear oil, homogeneous by TLC (solvent A).

This material was dissolved in 20 mL or methanol and hydrogenated in the presence of palladium on charcoal 10% for 4 h at room temperature and under atmospheric pressure. The catalyst was then removed by filtration, the solvent evaporated under reduced pressure, and the residue dissolved in 2 mL of methanol. Upon addition of ether, the zwitterionic tripeptide ester precipitated. It was collected by filtration, washed with ether, and dried under vacuum over phosphorus pentoxide.

Physical data of compounds **10**, **12**, **13**, **15**, and **16** are listed in Table II.

General Method for the Synthesis of the Tripeptide Methyl Esters 9, 11, and 14. To a cold (0 °C) solution of **2**, **3**, or **6** (1 mmol) in 5 mL of DMF were successively added triethylamine (1 mmol, 0.14 mL) and *N*-(*tert*-butyloxycarbonyl)-β-*tert*-butyl-L-aspartic acid *N*-hydroxysuccinimide ester (0.9 mmol, 348 mg). The mixture was stirred for 1 h at 0 °C and 2 h at room temperature. The usual workup afforded the pure, homogeneous by TLC (solvent A) protected tripeptide ester as a clear oil.

This material was dissolved in 5 mL of trifluoroacetic acid. The reaction, monitored by TLC, was complete in 45 min. The trifluoroacetaic acid was removed under reduced pressure and the residue triturated with 3 × 20 mL of ether, to leave a white solid, which was dissolved in 20 mL of ethyl acetate. Upon addition of *N*,*N*-diisopropyl-*N*-ethylamine (1 mmol, 0.172 mL) the zwitterionic tripeptide ester precipitated. It was collected by filtration, washed with ethyl acetate (3 × 5 mL), and ether (3 × 10 mL), and dried under vacuum over phosphorus pentoxide.

Physical data of compounds **9**, **11**, and **14** are listed in Table II.

As an example, NMR data of **9** are as follows: NMR (CD_3OD) δ 4.33 (q, 1 H, C_α H D-Ala), 4.10 (m, 1 H, C_α H Asp), 3.65 (s, 3 H, OCH_3), 2.67 (m, 2 H, CH_2 Asp), 1.43 (s, 6 H, CH_3 Aib), 1.32 (d, 3 H, CH_3 D-Ala).

Acknowledgment. The authors gratefully acknowledge support of this research through a grant from the National Institutes of Dental Research (Grant DE 05476) and by a Grant-in-Aid by A. E. Staley Co. We also thank Dr. Piero-Andrea Temussi for his helpful discussions.

Registry No. 1, 97522-41-3; 2, 97522-42-4; 3, 97522-43-5; 4, 97522-44-6; 5, 97522-45-7; 6, 97522-46-8; 7, 97522-47-9; 8, 97522-48-0; 9, 97522-49-1; 10, 97522-50-4; 11, 97522-51-5; 12, 97522-52-6; 13, 97522-53-7; 14, 97522-54-8; 15, 97522-55-9; 16, 97522-56-0; H-Aib-OMe·HCl, 15028-41-8; H-DEG-OMe·HCl, 92398-54-4; H-AC_3C-OMe·HCl, 72784-42-0; H-AC_4C-OMe·HCl, 92398-47-5; H-AC_5C-OMe·HCl, 60421-23-0; H-AC_6C-OMe·HCl, 37993-32-1; H-AC_7C-OMe·HCl, 92398-50-0; H-AC_8C-OMe·HCl, 92398-52-2; Boc-D-Ala-OSu, 34404-33-6; Z-Asp-(OBzl)-OH, 3479-47-8; Boc-Asp(O-*t*-Bu)-OSu, 50715-50-9.

Synthesis of the C-Terminal Octapeptide of Pig Oxyntomodulin. Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala: A Potent Inhibitor of Pentagastrin-Induced Acid Secretion

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The synthesis of Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala representing the C-terminal octapeptide of oxyntomodulin isolated from pig intestine is described. Its structure was confirmed by its 360-MHz ^{1}H NMR spectra. The octapeptide was tested for its ability to inhibit pentagastrin-induced acid secretion, in the anaesthetized rat, in the conscious rat with chronic gastric fistula, and in the conscious cat with gastric chronic fistula. The octapeptide inhibits pentagastrin-induced acid secretion in all three models. Compared to oxyntomodulin, the parent hormone, the synthetic peptide was approximately 150 times less potent but has the same efficacy. Biological data are presented and discussed.

A porcine intestinal peptide that is able to display glucagon-like immunoreactivity was also shown to interact with the glucagon receptors and to activate the adenylate cyclase present in liver membranes.[1,2] On the basis of these criteria, Bataille et al.[3] isolated a peptide of 37 amino acid residues (G-37) representing the whole glucagon molecule (G-29) elongated at its C-terminal end by a basic octapeptide, Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala[4] (Figure 1). Biological activities of the G-37 were quite different from those of glucagon (G-29). The affinity of G-37 for antibodies directed against the C-terminal sequence of glucagon (such as K47 or 30K) was ≃1% that of glucagon. Indeed, the C-terminal extension is likely to hinder the

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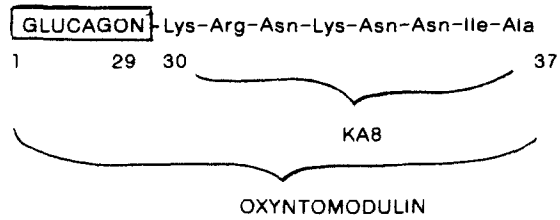

Figure 1. Amino acid sequences of pig oxyntomodulin and KA8.

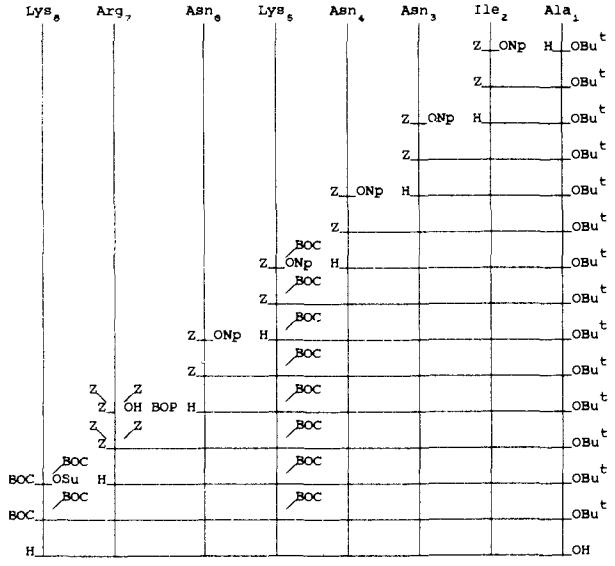

Figure 2. Synthesis of Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala.

interaction between the G-37 and the antibodies directed against the C-terminal portion of glucagon. The apparent potency of G-37 with the glucagon receptors present in rat liver membranes was ≃20% that of G-29. In stimulating hepatic adenylate cyclase, G-37 was ≃10% as potent as G-29, with the same efficacy.

In addition to its glucagon-like activities in liver, the main target tissue of G-37 is not the liver but the area that contains the oxyntic glands.[5] Owing to this specific action, the term "oxyntomodulin" has been proposed for the G-37.[5,6] Oxyntomodulin was shown to be ≃20 times more potent than glucagon in inhibiting pentagastrin-induced acid secretion, in rat in vivo.[7]

The basic octapeptide is thus most probably deeply involved in the interaction with the gastric receptor. These reasons prompted us to study its biological activities, if any, on pentagastrin-induced acid secretion.

Synthesis

The steps of the synthesis of KA8[16] are reported in Figure 2. The hydrochloride salt of the *tert*-butyl ester of L-alanine was allowed to react with Z-Ile-ONp[8] in the presence of 1-hydroxybenzotriazole as catalyst.[9] The chain was lengthened by stepwise acylations[10] with the active esters of the Z amino acids, up to the hexapeptide, with HOBt as catalyst. Partial deprotection of Z-Asn-Lys-(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu by hydrogenolysis in the presence of 1 equiv of HCl and coupling with $(Z\text{-Arg})_3$ with BOP reagent[11] yielded the heptapeptide Tri-Z-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu in good yield. After removal of the three benzyloxycarbonyl groups by hydrogenolysis, the resulting partially protected heptapeptide was allowed to react with Boc-Lys(Boc)-OSu[12] to produce the octapeptide Boc-Lys(Boc)-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu. This peptide was purified by chromatography on a column of silica gel with AcOEt/pyridine/AcOH/H_2O (80:20:6:10) as eluent. Complete deblocking of the pure peptide in TFA afforded Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala as a white powder. Its purity was ascertained by TLC (AcOEt/pyridine/AcOH/H_2O (20:20:5:15) and by HPLC (μ-Bondapak C_{18}; see Experimental Section). The structure of the peptide was confirmed by its 360-MHz ^1H NMR spectrum (Table I) and by amino acid analysis. Before biological tests, the peptide is dissolved in 0.5 N AcOH, filtered, and lyophilized.

Table I. ^1H NMR (360 MHz) Values of the Octapeptide Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala (Chemical Shifts, δ; Me_4Si as Reference)

AA residue	NH	CH_α	
Lys 8		3.826	$CH_{2\delta}$ 1.531
			$CH_{2\epsilon}$ 2.270
Arg 7	8.662 (d)	4.360	$CH_{2\beta}$ 1.710
			$CH_{2\gamma}$ 1.531
			$CH_{2\delta}$ 3.104
Asn 6	8.269 (d)	4.540	$CH_{2\beta}$ 2.574
			2.446
Lys 5	7.982 (d)	4.161	$CH_{2\delta}$ 1.531
			$CH_{2\epsilon}$ 2.270
Asn 4	8.154 (d)	4.540	$CH_{2\beta}$ 2.574
			2.446
Asn 3	8.076 (d)	4.540	$CH_{2\beta}$ 2.574
			2.446
Ile 2	7.510 (d)	4.161	CH_β 1.759
			$CH_{3\gamma}$ 0.838 (d)
			$CH_{2\gamma}$ 1.20
			$CH_{2\gamma'}$ 1.45
			$CH_{3\delta}$ 0.708 (t)
Ala 1	8.200 (d)	4.161	$CH_{3\beta}$ 1.265 (d)

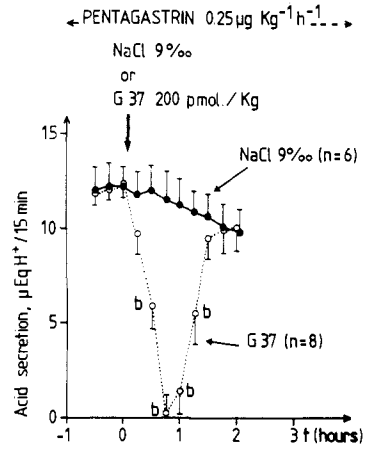

Figure 3. Antagonist activity of G-37 on pentagastrin-induced acid secretion in the anaesthetized rat: b, $p < 0.02$.

Biological Results and Discussion

The C-terminal octapeptide of oxyntomodulin was tested for its ability to inhibit pentagastrin-induced acid secretion, in vivo, in the anaesthetized rat according to the

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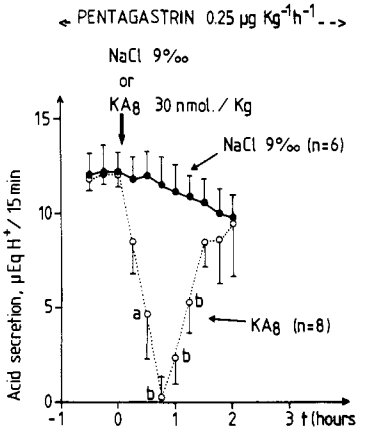

Figure 4. Antagonist activity of KA8 on pentagastrin-induced acid secretion in the anaesthetized rat: a, $p < 0.05$; b, $p < 0.02$.

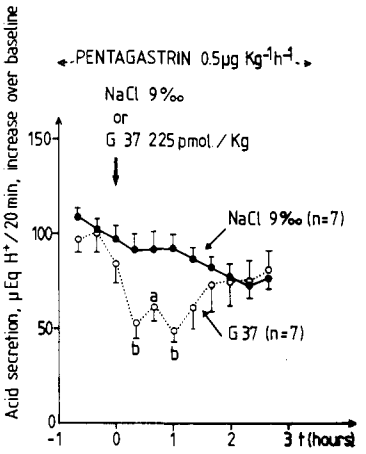

Figure 5. Antagonist activity of G-37 on pentagastrin-induced acid secretion in the rat with chronic gastric fistula: a, $p < 0.05$; b, $p < 0.02$.

method of Ghosh and Schild[13] modified by Lai[14] in conscious rat[7] and cat[15] with a chronic gastric fistula. In the anaesthetized rat, oxyntomodulin and its C-terminal octapeptide shared the ability to inhibit pentagastrin-induced acid secretion (Figures 3 and 4), the octapeptide being approximatively 150 times less potent but with the same efficacy. In fact, both peptides completely abolished the pentagastrin-induced acid secretion. In the conscious rat where the central nervous system is not depressed, oxyntomodulin and its C-terminal octapeptide inhibited pentagastrin-induced acid secretion with the same amplitude and kinetic. More than 50% of inhibition could not be reached, but the pentagastrin was perfused at a higher concentration (two times). The same difference in potency as that observed in anaesthetized rats was found between oxyntomodulin and its C-terminal octapeptide, in conscious rats with gastric chronic fistula (Figures 5 and 6). Duration of the inhibitory effect was somewhat longer. Preliminary experiments on the cat with a gastric fistula with the C-terminal octapeptide of oxyntomodulin showed also an inhibitory effect on pentagastrin-induced acid secretion (Figure 7). Three doses of the C-terminal octapeptide of oxyntomodulin were used (120, 90, and 60 nmol/kg). Again, on this model, we could not inhibit more than 50% of the pentagastrin stimulation with a perfusion

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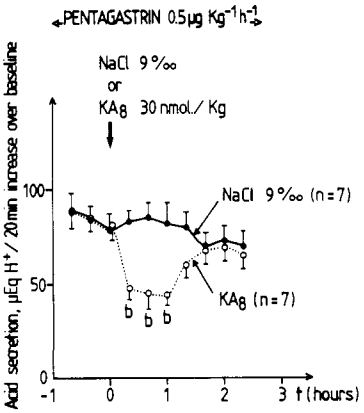

Figure 6. Antagonist activity of KA-8 on pentagastrin-induced acid secretion in the rat with chronic gastric fistula: b, $p < 0.02$.

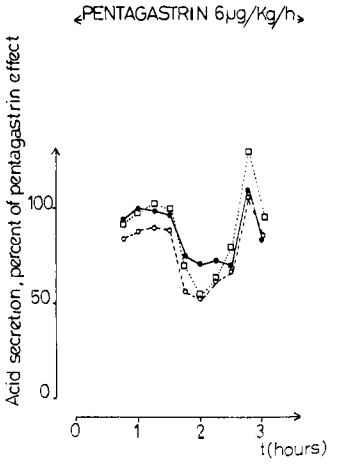

Figure 7. Antagonist activity of KA8 on pentagastrin-induced acid secretion, in the cat with gastric chronic fistula: □, 120 nmol/kg; ○, 90 nmol/kg; ●, 60 nmol/kg.

of 6 µg/kg per hour of the stimulant.

Conclusions

These preliminary results indicate that the C-terminal octapeptide of oxyntomodulin, Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala, KA-8,[16] is able to inhibit pentagastrin-induced acid secretion by itself. This peptide was found less potent than oxyntomodulin (≃150 times) but has the same efficacy and a quite similar dose–response curve. Further works on structure–activity relationships of this octapeptide are in progress in our laboratories. Particularly, the C-terminal hexapeptide of oxyntomodulin has been studied, and preliminary results showed that this peptide was also able to inhibit pentagastrin-induced acid secretion. These results will be reported separately.

Experimental Section

Pentagastrin-Induced Acid Secretion in the Anaesthetized Rat. Male Wistar rats weighing 300 ± 25 g were starved for 18 h before experiments but had free access to water. The rats were anaesthetized with urethane (0.6–0.7 mL of a 25% solution per 100 g) given by intramuscular injection.

A polyethylene catheter, introduced into the oesophagus and passed to the level of the cardia, was connected to a peristaltic pump (Desega) set to deliver a solution of 0.9% NaCl at a constant rate of 1.0 mL/min. The perfusate was collected through another catheter placed through the pylorus and secured with a ligature. Whenever necessary, the temperature of the rats was maintained

(16) KA8 represents the octapeptide starting by lysine (N-terminus amino acid, whose one letter code in K), ending by alanine (C-terminus amino acid whose one letter code is A).

at 34 °C with the aid of electric lamps.

The tests were begun after stabilization of the gastric perfusion, usually within 30–60 min after completion of the surgical preparation. The gastric secretion, diluted with the perfusate of 0.9% NaCl, was collected every 15 min, and the acidity was measured by titrating the entire sample with 0.01 N NaOH to the phenolphthalein end point. Pentagastrin (Peptavlon, ICI) was infused at a rate of 2.4 mL/h. The plateau stimulation was obtained 1.5 h after the beginning of infusion. KA-8 and oxyntomodulin were carefully administered as a 0.1-mL bolus, and the catheter was rinsed with 0.1 mL of saline. The injections, performed in random order, were separated by an interval of 2 h. Each experimental group consisted of five or six rats. Changes in gastric secretion were evaluated during the 30-min period following injection and expressed as percent of inhibition, the plateau value being taken as reference.

Pentagastrin-Induced Acid Secretion in the Rat with Chronic Gastric Fistula. Male Wistar rats weighing 300 ± 25 g on the day of operation were provided with a plastic cannula placed in the rumen as described by Brodie et al.[17] Secretory studies started 2 weeks or more after surgery. No animal was tested more than twice a week. After a 18-h fasting period with free access to water, the plug of the gastric cannula was removed and the stomach repeatedly washed with warm saline solution until clear. The rats were then placed in a Bollmann type cage for free drainage of the gastric juice. An intravenous perfusion was then placed in a tail vein, and saline (0.14 M NaCl solution) was continuously infused at a rate of 2 mL/h (Precidor, Switzerland) throughout the experiment. A resting period of 1 h or more was then allowed before starting the experiment. After collection of basal secretion during three consecutive 20-min periods, stimulant (pentagastrin, ICI) dissolved in saline solution was administered continuously through the perfusion system. Eighty minutes after the beginning of pentagastrin perfusion, compounds to be tested were bolus-injected iv. The volume of gastric juice collected in consecutive 20-min samples was measured to the nearest 0.01 mL and the acid concentration determined on aliquots of 0.01 mL by titration (NaOH, 0.01 N, phenolphthalein). All values include statistical comparison of means (±SEM) by the students t-test.

Pentagastrin-Induced Acid Secretion in the Cat with Chronic Gastric Fistula. Young cats (3–7 months) over 2-kg weight were cannulated according to Emas et al.[15] Secretories studies started 3 weeks or more after surgery. No animal was tested more than twice a week. Before any test, cats were kept from eating for 18 h with free access to water. Then, they were placed in a special type cage, leaving free access to their legs and to the gastric fistula. An intravenous perfusion was then placed in a vein of a posterior leg, and saline (0.9% NaCl solution) was continuously infused at a rate of 15 mL/h (Precidor, Switzerland) throughout the experiment. A 30-min resting period or more was then allowed before starting the experiment. After collection of the basal secretion during two consecutive 15 min, stimulant (Pentagastrin, ICI) dissolved in saline was administered continuously through the perfusion system. Ninty minutes after the beginning of pentagastrin perfusion, compounds to be tested were bolus injected (iv). The volume of gastric juice was collected in consecutive 15 min and the acid concentration determined by titration (NaOH, 0.01 N, phenolphthalein).

Experimental Procedures. Analytical Methods. Melting points were taken on a Buchi apparatus in open capillary tubes. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by le Service de Microanalyses de l'ENSCM (Montpellier, France). The amino acid composition of acid hydrolysates (HCl, 6 N, 12 h) was determined with a Beckman 120C (two hydrolyses, four separate analyses). Ascending TLC was performed on precoated plates of silica gel 60 F_{254} (Merck), using the following solvent systems (by volume): A, hexane/ethyl acetate (5:5); B, AcOEt/py/acetic acid/water (90:20:4:4); C, AcOEt/py/AcOH/H_2O (40:20:6:10); D, AcOEt/py/AcOH/H_2O (80:20:2:2); E, AcOEt/py/AcOH/H_2O (90:20:6:10); F, AcOEt/py/AcOH/H_2O (60:20:6:10); G, AcOEt/py/AcOH/H_2O (80:20:6:10). Peptide derivatives were located with charring reagent or ninhydrin. Column chromatographies were performed with silica gel 60, particle size 0.063–0.200 mm (Merck), or with neutral activated alumina (Prolabo). Analytical HPLC experiments were carried out with a Waters apparatus consisting of a 6000 A pump, a U6K injector, and a 441 detector. Samples were chromatographied on a 10-μm radial-pack C_{18} column using 0.1% trifluoroacetic acid as the mobile phase. ^1H NMR spectra (360 MHz) at 25 °C were recorded on a Bruker 360 spectrometer. Me_2SO for ^1H NMR was used as solvent, and samples were prepared as 4.9 μM solution. All L amino acids were from Bachem. All reagents and solvents were of analytical grade. BOP was recrystallized from acetone and ether. The following abbreviations were used: DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DIEA, *N,N*-diisopropylethylamine; BOP, benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate. Other abbreviations used were those recommended by the IUPAC-IUB Commission.[21]

Z-Ile-Ala-O-*t*-Bu (1). Z-Ile-ONp[8] (11.5 g, 30 mmol), HCl·Ala-O-*t*-Bu (5.81 g, 32 mmol), and HOBt (4.6 g, 30 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (11.2 mL, 65 mmol) was added. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated, and the residue was dissolved in ethyl acetate. The organic solution was washed twice with a $NaHCO_3$ saturated solution, once with water, citric acid (10%), and water again and dried over Na_2SO_4. After filtration and evaporation, the crude material was isolated from *p*-nitrophenol by filtration on a column of neutral alumina with dimethylformamide as eluent: yield 9.97 g (90%); mp ≃110 °C; $[\alpha]^{25}_D$ −16° (*c* 1, DMF); *Rf*(A) 0.83. Anal. ($C_{21}H_{32}N_2O_5$) C, H, N.

Z-Asn-Ile-Ala-O-*t*-Bu (2). Compound 1 (8.87 g, 22.6 mmol) was dissolved in ethanol and was hydrogenated for 3 h in the presence of HCl (1.5 equiv) and a 5% Pd/C catalyst. The catalyst was filtered on Celite and rinsed with ethanol. The filtrate was evaporated, and the residue was precipitated by addition of ether and washed twice with ether. HCl Ile-Ala-O-*t*-Bu (6.2 g, 21 mmol) was recovered, Z-Asn-ONp[18] (10.07 g, 26 mmol). HCl·Ile-Ala-O-*t*-Bu (6.8 g, 23.1 mmol), and HOBt (4 g, 26 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (8.95 mL, 52 mmol) was added. After stirring overnight at room temperature, the solvent was evaporated and the solid residue was washed twice with ethyl acetate, once with water, twice with a $NaHCO_3$-saturated solution, once with water, citric acid (10%), water, ethyl acetate, and ether: yield 9.7 g (85%); mp 222 °C dec; $[\alpha]^{25}_D$ −22° (*c* 1, DMF); *Rf*(B) = 0.8. Anal. ($C_{25}H_{38}N_4O_7$) C, H, N.

Z-Asn-Asn-Ile-Ala-O-*t*-Bu (3). Compound 2 (9.7 g, 19.15 mmol) was dissolved in a mixture of dimethylformamide, water, and ethanol and was hydrogenated for 12 h, under stirring, in the presence of HCl (1.5 equiv) and a 5% Pd/C catalyst. The catalyst was filtered on Celite and rinsed with water. The filtrate was evaporated and the residue precipitated by addition of ether and washed twice with ether. A 7.6-g (18.59 mmol) sample of HCl·Asn-Ile-Ala-O-*t*-Bu was recovered; *Rf*(C) 0.85. Z-Asn-ONp[18] (8.9 g, 23 mmol), HCl·Asn-Ile-Ala-O-*t*-Bu (7.5 g, 18.34 mmol), and HOBt (3.52 g, 23mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (7.9 mL, 46 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound 2): yield 9.4 g (82%); mp 245 °C dec; $[\alpha]^{25}_D$ −19° (*c* 1, DMF); R_f(D) 0.92. Anal. ($C_{29}H_{44}N_6O_9$) C, H, N.

Z-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (4). Compound 3 (9.4 g, 15.1 mmol) was dissolved in a mixture of dimethylformamide, H_2O, and EtOH and was hydrogenated for 12 h under stirring in the presence of acetic acid (10 mL) and a 5% Pd/C catalyst. The reaction mixture was treated according to the usual procedure (see hydrogenation of compound 2). An 8.45-g portion of CH_3COOH·Asn-Asn-Ile-Ala-O-*t*-Bu was recovered and purified by chromatography on a column of silica gel using the solvent system C as eluent; 3.52 g (6.44 mmol, 45%) of the pure peptide were recovered: R_f(E) 0.32. Z-Lys(Boc)-ONp[19] (4.37 g, 8 mmol),

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CH_3COOH·Asn-Asn-Ile-Ala-O-*t*-Bu (3.52 g, 6.44 mmol), and HOBt (1.23 g, 8 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (2.75 mL, 16 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**): yield 3.82 g (70%); mp 235 °C dec; $[\alpha]^{25}_D$ -26° (*c* 1, DMF); R_f(E) 0.14. Anal. ($C_{40}H_{64}N_8O_{12}$) C, H, N.

Z-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (5). Compound **4** (3.82 g, 4.5 mmol) was hydrogenated as previously described for compound **3**. A 3.21-g (4.14 mmol) portion of CH_3COOH·Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu was recovered; R_f(C) 0.42. Z-Asn-ONp[18] (2.2 g, 5.68 mmol), CH_3COOH·Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (3.21 g, 4.14 mmol), and HOBt (0.87 g, 5.68 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.42 g, 10 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**): yield 3.82 g (70%); mp 230 °C dec; $[\alpha]^{25}_D$ -27° (*c* 1, DMF); R_f(F) 0.73. Anal. ($C_{44}H_{70}N_{10}O_{14}$) C, H, N.

Z_3-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (6). Compound **5** (3 g, 3.12 mmol) was hydrogenated as previously described for compound **2**. A 2.5-g (2.89 mmol) portion of HCl·Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu was recovered; R_f(C) 0.32. Z_3-Arg-OH (0.75 g, 1.3 mmol),[20] HCl·Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (1 g, 1.16 mmol), and BOP (0.57 g, 1.3 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.42 mL, 2.47 mmol) was added. The reaction mixture was treated as already described (see compound **2**): yield 667 mg (40%); mp 225 °C dec; $[\alpha]^{25}_D$ -10° (*c* 1, DMF); R_f(G) 0.28. Anal. ($C_{66}H_{94}N_{14}O_{19}$) C, H, N.

Bis-Boc-Lys-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (7). Compound **6** (600 mg, 0.43 mmol) was hydrogenated as previously described for compound **3**. $(CH_3COOH)_3$·Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (356 mg, 0.31 mmol) was recovered; R_f(C) 0.20. Bis-Boc-Lys-OSu[12] (145 mg, 0.35 mmol) and $(CH_3COOH)_3$·Arg-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (346 mg, 0.3 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.06 mL, 0.35 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**) except that citric acid washings were deleted. Compound **7** was purified by chromatography on a silica gel column using G as the solvent system; yield 70 mg (12.5%) of pure compound. More fractions slightly impure were also recovered (280 mg, 50%): mp 220 °C dec; $[\alpha]^{25}_D$ -24° (*c* 1, DMF); R_f(G) 0.12.

TFA·Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala (8). Deprotection of compound **7** (50 mg, 0.036 mmol) in TFA was carried out during 3 h at room temperature, in an anhydrous medium. Then anhydrous ether (300 mL) was added, and compound **8** precipitated out. The residue was filtered and washed with ether: yield 33 mg (60%); R_f(D) 0.1; HPLC (C_{18} column, 0.1% TFA in a mixture of water/acetonitrile) showed single peak; amino acid anal. Asn 3.01, Ala 1.00, Ile 1.01, Lys 2.02, Arg 1.07. Anal. Calcd for $C_{39}H_{71}N_{15}O_{12}$·$4CF_3COOH$·$4H_2O$ (mol wt 1445): C, 39.22; H, 5.91. Found: C, 39.03; H, 5.74.

Registry No. **1**, 92779-28-7; **2**, 97391-52-1; **3**, 97391-53-2; **4**, 97391-54-3; **5**, 97391-55-4; **6**, 97391-56-5; **7**, 97403-51-5; **8**, 97391-57-6; H-Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala-Oh, 81117-26-2; Z-Ile-ONp, 2130-99-6; H-Ala-O-*t*-Bu·HCl, 13404-22-3; H-Ile-Ala-O-*t*-Bu·HCl, 97391-58-7; Z-Asn-ONp, 3256-57-3; H-Asn-Ile-Ala-O-*t*-Bu·Hchl, 97391-59-8; H-Asn-Asn-Ile-Ala-O-*t*-Bu·CH_3COOH, 97391-61-2; Z-Lys(Boc)-ONp, 2212-69-3; H-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·CH_3COOH, 97391-63-4; H-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·HCl, 97391-64-5; Z_3-Arg-OH, 14611-34-8; H-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·$(CH_3COOH)_3$, 97391-66-7; $(Boc\text{-}Lys\text{-}OSu)_2$, 30189-36-7; oxyntomodulin, 62340-29-8; pentagastrin, 5534-95-2.

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4-[2-(Di-*n*-propylamino)ethyl]-2(3*H*)-indolone: A Prejunctional Dopamine Receptor Agonist

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4-[2-(Di-*n*-propylamino)ethyl]-2(3*H*)-indolone (**1c**) (SK&F 101468) is a potent and selective prejunctional dopamine receptor agonist. It caused a dose-related inhibition of the constrictor response to electrical stimulation in the isolated perfused rabbit ear artery (EC_{50} = 100 nM), and this response was antagonized by (*S*)-sulpiride (K_B = 7 nM). Compound **1c** did not stimulate or block dopamine-sensitive adenylate cyclase and did not produce stimulation of the central nervous system in rats. It was prepared from (2-methyl-3-nitrophenyl)acetic acid in a multistep sequence based on the Reissert indole synthesis.

Goldberg and Kohli[1] have demonstrated that the peripheral dopamine receptors that modulate the release of norepinephrine from postganglionic sympathetic nerves are activated by a structurally diverse range of compounds. These include ergot and ergoline derivatives, 4-(aminoethyl)indoles, and apomorphine as well as phenethylamine, aminotetralin, and tetrahydro-3-benzazepine derivatives. Within these series, optimal prejunctional potency frequently occurs when the compound contains a tertiary amine and at least one *N*-*n*-propyl substituent. In contrast, only a few agonists are known that activate postjunctional dopamine receptors mediating relaxation of smooth muscle, and almost without exception these are catechol derivatives.

We recently described[2] two phenolic indolone derivatives, **1a** and **1b** that exhibited potent prejunctional dopamine agonist activity as determined in the isolated perfused rabbit ear artery (REA) assay.[3] Compound **1b** was unusually potent in this assay with an EC_{50} of 1.8 ± 0.3 nM (N = 10) compared to an EC_{50} of 110 ± 20 nM for

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0022-2623/85/1828-1533$01.50/0