Synthesis and Some Pharmacological Properties of Oxytocin Analogues Having L-Thiazolidine-4-carboxylic Acid in Position 7[1]

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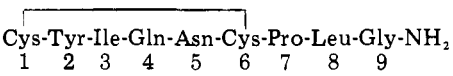
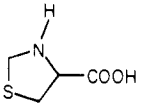
[7-(Thiazolidine-4-carboxylic acid)]oxytocin and [1-β-mercaptopropionic acid,7-(thiazolidine-4-carboxylic acid)]oxytocin have been synthesized by a solid-phase method. α-*N*-*tert*-Butoxycarbonyl- and *S*-ethylcarbamoyl-protecting groups were employed. The dipeptide Boc-Cys(Ec)-thiazolidine-4-carboxylic acid as well as individual residues was coupled to a H-Gly-dehydroalanine-resin with dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole. The appropriate protected polypeptide intermediates were cleaved from the resin by acidolysis, deprotected in NH_3, and oxidized to the cyclic disulfide analogues with ICH_2CH_2I. Purification was effected by partition chromatography and gel filtration. Relative to oxytocin and [1-β-mercaptopropionic acid]oxytocin, these analogues exhibit greatly enhanced oxytocic and avian vasodepressor potencies and unchanged rat pressor potencies.

Since the synthesis of oxytocin



by du Vigneaud et al.,[3] hundreds of analogues and homologues of this polypeptide hormone have been synthesized and tested for biological activity. However, the role of the proline residue in position 7 has not been extensively investigated. Formal substitution of D-proline,[4] glycine,[5-7] and hydroxyproline[8,9] for Pro in oxytocin has been reported to give compounds with substantial if somewhat lower oxytocic activity but little or no avian vasodepressor and rat pressor activities. As an extension of these studies, this paper reports the synthesis and some pharmacological properties of two oxytocin analogues resulting from the formal replacement of Pro with thiazolidine-4-carboxylic acid, which has the structure



Although thiazolidine-4-carboxylic acid (Thz) is readily available through the condensation of formaldehyde with cysteine,[10] Thz has been used in peptide synthesis as a Pro analogue in only a few instances. Polythiazolidine-4-carboxylic acid has been prepared[11] and shown to have a conformation like that of polyproline II, in which all the peptide bonds are trans. Thz has been employed as a Pro analogue in the synthesis of poly(alanylglycylthiazolidine-4-carboxylic acid) in a study of collagen models.[12] The formal replacement of the Pro residue at position 3 in bradykinin with Thz gave an analogue that possessed 26% of the rat blood pressure activity and 46% of the rat uterus activity of bradykinin itself.[13]

The incorporation of the Thz residue into a peptide requires special consideration because of the reactive nature of the thiazolidine ring. For example, the ring is opened on treatment with I_2, Hg^{2+}, aqueous alkali, and Na in liquid NH_3[14] but is stable to acid treatment. With these facts in mind, the *tert*-butoxycarbonyl (Boc) group was chosen for α-amino nitrogen protection as well as for Tyr side-chain protection, while the ethylcarbamoyl (Ec) group was chosen for S protection. The choice of the base-labile Ec protecting group precludes the use in the solid-phase technique of the usual chloromethylated polystyrene resin because of the lability of the Ec group[15] to the ammonolysis step generally used to cleave the peptide from the resin. A more desirable approach is one which allows for the cleavage of the C-terminal glycinamide peptide from a resin by acidolysis. Such an approach has been described by Gross et al.,[16] whereby a peptide amide results on cleavage of a peptide-dehydroalanine-resin at the dehydroalanine (Dha) residue, according to the generalized equation

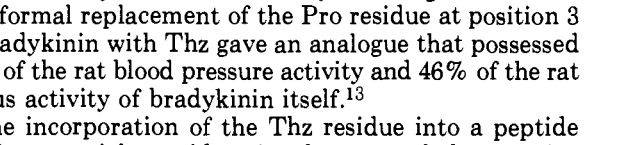


Table I.
Dicyclohexylcarbodiimide-1-Hydroxybenzotriazole Coupling Cycle

Step	Solvents or reagents	Repetitions	Time, min
1	CH_2Cl_2	3	2
2	TFA–anisole–CH_2Cl_2 (25:2:73)	1	2
3	TFA–anisole–CH_2Cl_2 (25:2:73)	1	30
4	CH_2Cl_2	4	2
5	*i*-Pr_2EtN–CH_2Cl_2 (7:93)	2	2
6	CH_2Cl_2	4	2
7[a]	Boc-AA and HBt in DMF–CH_2Cl_2 (1:1)	1	2
8[b]	DCC in DMF–CH_2Cl_2 (1:1)	1	240
9	CH_2Cl_2	3	2
10	EtOH	3	2
11[c]	Repeat steps 4–10		

[a] 2.5 equiv of Boc-AA and 3.75 equiv of HBt. [b] 2.5 equiv of DCC. This step is added to the reagents present from step 7. Only half of the normal solvent volume is used in steps 7 and 8, so that the total volume is the same as in all other steps. [c] 0.5 equiv of Boc-AA, 0.75 equiv of HBt, and 0.5 equiv of DCC and overnight coupling time. Abbreviations: DCC, dicyclohexylcarbodiimide; HBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; Boc, *tert*-butoxycarbonyl; AA, amino acid; and DMF, dimethylformamide.

The solid-phase synthesis of [7-(thiazolidine-4-carboxylic acid)]oxytocin ([Thz^7]oxytocin) and [1-β-mercaptopropionic acid,7-(thiazolidine-4-carboxylic acid)]oxytocin ([β-Mpa^1,Thz^7]oxytocin) was carried out on a manual device[17] starting from Boc-Gly-Dha-O-resin (1). The protected nonapeptide-resin Boc-Tyr(Boc)-Ile-Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (2), derived from 10 g of 1 (2.0 mmol of Gly), was synthesized (see Table I) by successive double couplings of each amino acid in reactions mediated by dicyclohexylcarbodiimide with 1-hydroxybenzotriazole.[18,19] The Thz residue was incorporated into the peptide-resin via the dipeptide Boc-Cys(Ec)-Thz-OH after preliminary experiments had shown that coupling of Boc-Cys(Ec)-OH to the N-terminal H-Thz-peptide-resin could not be carried to completion. The partially protected peptide-resins Boc-Cys(Ec)-Tyr-Ile-Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (3) and β-Mpa(Ec)-Tyr-Ile-

Table II. Active Ester Coupling Cycle

Step	Solvents or reagents	Repetitions	Time, min
1-6	As in Table I		
7	4.0 equiv of active ester in DMF-CH_2Cl_2 (1:1)	1	Overnight
8	1.0 equiv of *i*-Pr_2EtN	1	120
9	CH_2Cl_2	3	2
10	EtOH	3	2

Table III. Pharmacological Potencies[a] of [Thz^7] Analogues of Oxytocin

Compound	Oxytocic	AVD	Pressor
Oxytocin	546 ± 18[b]	507 ± 23[c]	3.1 ± 0.1[c]
[β-Mpa^1]-oxytocin	803 ± 36[d]	975 ± 24[d]	1.44 ± 0.06[d]
[Thz^7]oxytocin	1180 ± 47	720 ± 43	3.46 ± 0.05
[β-Mpa^1,Thz^7]-oxytocin	1538 ± 45	2039 ± 62	1.69 ± 0.08

[a] Expressed in units per milligram as mean potencies ± standard error. [b] W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *Endocrinology*, **72**, 279 (1963). [c] W. Y. Chan and V. du Vigneaud, *ibid.*, **71**, 977 (1962). [d] B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *J. Biol. Chem.*, 240, 4264 (1965).

Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (**4**) were prepared from the common intermediate **2** by the 2,4,5-trichlorophenyl (-$OPhCl_3$) active ester method (see Table II). A portion of **3** was treated with 1 N HCl in HOAc containing 1 equiv of H_2O[16] to effect hydrolysis of the Dha residue. The resulting nonapeptide amide was refluxed in NH_3 to remove Ec protection[15] and then oxidized to [Thz^7]oxytocin with ICH_2CH_2I.[20] [Thz^7]oxytocin was purified by partition chromatography[21] and gel filtration.[22] [β-Mpa^1,Thz^7]oxytocin was derived from **4** in a similar manner.[23]

The highly purified analogues were tested for oxytocic,[24] avian vasodepressor (AVD),[25] and rat pressor[26] activities against the U.S.P. posterior pituitary reference standard using the four-point assay design.[27] The results are shown in Table III. Surprisingly, [Thz^7]oxytocin has an oxytocic potency two times that of oxytocin and an AVD potency 1.4 times that of oxytocin. Similarly, [β-Mpa^1,Thz^7]-oxytocin possesses oxytocic and AVD potencies two times greater than those of its parent compound, [β-Mpa^1]-oxytocin. On the other hand, [Thz^7]oxytocin and [β-Mpa^1,Thz^7]oxytocin are observed to have rat pressor potencies that are relatively unchanged from those of the parent compounds oxytocin and [β-Mpa^1]oxytocin, respectively. It should be noted that [Thz^7]oxytocin and [Thr^4]oxytocin[28] are the only oxytocin analogues which have been found to possess enhanced potencies resulting from the replacement of a single amino acid with another amino acid.

Experimental Section

All melting points were determined in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC) of spots containing 10–40 μg of compound was performed on precoated glass plates of silica gel GF 254 (0.25 mm, E. Merck) in the following solvent systems: (A) $CHCl_3$–MeOH–HOAc, 9:1:1; (B) acetone–HOAc, 9:1; (C) BuOH–HOAc–H_2O, 4:1:1; (D) BuOH–pyridine–HOAc–H_2O, 15:10:3:6. Detection was made by uv, ninhydrin, and chlorination followed by KI–starch treatment. In all cases, unless otherwise noted, single symmetrical spots were observed for purified material. Amino acid analysis was performed on a Beckman Model 116 analyzer on a single column (0.9 × 58 cm) of AA-15 resin employing 0.066 M sodium citrate buffers at pH 3.28, 4.30, and 6.40 (Na^+ concentrations 0.2, 0.2, and 1.0 N, respectively) at 55°. At a flow rate of 68.8 ml/h with buffer changes at 85 and 140 min, thiazolidine-4-carboxylic acid emerges as a symmetrical peak with a retention time of 53 min and has an integration constant about 25% of that observed for leucine. A distinguishing feature of the peak is the ratio of the integration constant for the 570-nm line relative to that for the 440-nm line. For Thz this ratio is 1.6, while it is about 5.5 for Asp, Thr, and Ser. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Where analyses are indicated only by the symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

Boc-Gly-Dha-OH. Chloroacetyldehydroalanine[29] (5.77 g, 35.3 mmol) was dissolved in concentrated aqueous NH_3 (58 ml) and stirred overnight.[30] The filtered solution was concentrated to a low volume in vacuo, diluted with 1 N NaOH (40 ml) and H_2O (50 ml), and evaporated to dryness.

The residue, a mixture of glycyldehydroalanine and NaCl, was dissolved in H_2O (90 ml) containing Et_3N (12.4 ml, 88.6 mmol) and diluted with peroxide-free dioxane (45 ml). *tert*-Butyl azidoformate (7.36 ml, 53.0 mmol) was added and the mixture stirred vigorously for 3 days. The dioxane was removed in vacuo, and the filtered aqueous solution was adjusted to pH 2 by the addition of concentrated HCl. A precipitate formed which was collected after chilling in ice: 5.7 g. A second crop (0.4 g) was obtained on concentration of the mother liquor. Recrystallization of the combined materials from EtOH (150 ml)–H_2O (300 ml) gave 5.72 g (66%) of Boc-Gly-Dha-OH: mp 158° dec (lit.[16] mp 157–159°); TLC (A) 0.47.

Boc-Cys(Ec)-Thz-OH. Boc-Cys(Ec)-$OPhCl_3$[31] (471.8 mg, 1.0 mmol), thiazolidine-4-carboxylic acid[10] (199.8 mg, 1.5 mmol), and *i*-Pr_2EtN (172 μl, 1.0 mmol) were suspended in dimethylformamide (DMF, 3 ml) and stirred overnight. The suspension was evaporated to dryness in vacuo to leave a residue which was partitioned between Et_2O (25 ml) and 1 N HCl (5 ml). The Et_2O layer was washed with 1 N HCl (2 × 2 ml portions) and saturated NaCl (3 × 2 ml portions). Evaporation of the dried ($MgSO_4$) Et_2O solution in vacuo gave a syrup which was dissolved in Et_2O (10 ml) and diluted with hexane (50 ml). A precipitate formed which was collected and dried in vacuo: 298 mg (73%); TLC (B, 0.71) showed several minor impurities.

The solid was further purified through its dicyclohexylamine (DCHA) salt by dissolving 281 mg (0.69 mmol) in Et_2O (15 ml) and adding DCHA (135 μl, 0.69 mmol). A solid began to crystallize slowly and was collected after cooling in the freezer overnight, washed with Et_2O (15 ml), and dried: 346 mg (85%); mp 120–121°; $[\alpha]^{23}D$ −75.4° (*c* 1, 95% EtOH). Anal. ($C_{27}H_{48}N_4O_6S_2$) C, H, N, S.

Boc-Tyr(Boc)-Ile-Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (2). The solid-phase synthesis of the title protected nonapeptide resin was performed on a manual device.[17] The support used was a chloromethylated polystyrene resin (cross-linked with 2% divinylbenzene, Cl = 1.9 mmol/g) which had been treated with Boc-Gly-Dha-OH in DMF in the presence of Et_3N for 72 h. The resin contained 0.20 mmol of Boc-dipeptide/g, as determined by amino acid analysis of a small sample which had been hydrolyzed in propionic acid–concentrated HCl (1:1) at 130° for 2 h.[32] The peptide synthesis was run on a 2.0-mmol scale (10.0 g of resin). The cycles of deprotection, neutralization, and coupling were carried out for the introduction of each new residue in the peptide as described in Table I. Residues 6 and 7 were introduced as the dipeptide unit Boc-Cys(Ec)-Thz-OH in the same manner. All washes and reactions were carried out with 60-ml portions of solvent. Following the last coupling cycle, the resin was filtered and dried: 1.8 g increase (88% of theory).

Boc-Cys(Ec)-Tyr-Ile-Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (3). The cycle of deprotection, neutralization, and coupling was carried out for the introduction of Boc-Cys(Ec) via the -$OPhCl_3$ active ester in the nonapeptide resin **2** (6.0 g, assume 1.0 mmol) as described in Table II. All washes and reactions were carried out with 60-ml portions.

β-Mpa(Ec)-Tyr-Ile-Gln-Asn-Cys(Ec)-Thz-Leu-Gly-Dha-O-resin (4). In a similar fashion 4 was prepared from 6.0 g of **2** by coupling β-Mpa(Ec)-$OPhCl_3$[33] to the peptide resin.

[7-(Thiazolidine-4-carboxylic acid)]oxytocin. A partially protected nonapeptide amide was obtained from the decapeptide resin **3** (3.0 g, assume 0.5 mmol) by stirring the peptide resin with

1 N HCl in HOAc (30 ml) containing 0.5 mmol of H_2O at 50° for 30 min.[16] The solvent was filtered off and the resin was washed twice with HOAc (25-ml portions) at 50° for 5 min. Lyophilization of the combined filtrates gave 160 mg of powder. The air-dried resin was again treated as above to give 33 mg of powder. A third treatment gave 17 mg of powder. The Ec-protecting groups were removed by refluxing 121 mg (0.1 mmol) of the powder in NH_3 (150 ml, distilled from Na) for 4 h.[15] The solvent was evaporated under N_2 and the residue dissolved in 50% aqueous MeOH (200 ml, deaerated) to which ICH_2CH_2I[20] (30 mg, 0.11 mmol) dissolved in MeOH (5 ml) was added. The disappearance of the sulfhydryl groups was followed by the Ellman test.[34] After 5 min the reaction was judged complete. The solution was acidified with HOAc (6 ml) and concentrated to dryness by rotary evaporation. The residue was dissolved in 0.2 N HOAc (100 ml) and lyophilized. The lyophilizate was subjected to partition chromatography[21] on a 2.2 × 47 cm column of Sephadex G-25 (100–200 mesh) in the system BuOH–H_2O (1:1, the aqueous phase 1.5% pyridine and 3.5% HOAc). The product was eluted with upper phase and the eluate was monitored by the Folin–Lowry method.[35] The product emerged as a sharp peak at R_f 0.34, well resolved from an earlier peak. The fractions comprising the product peak were pooled, concentrated in vacuo, and lyophilized from 0.2 N HOAc (100 ml) to give 22 mg of powder. This material was dissolved in 2 ml of 0.2 N HOAc and further purified by gel filtration[22] on a 2.82 × 67.6 cm column of Sephadex G-25 (200–270 mesh) equilibrated with the same solvent. The peptide material was detected by reading the absorbency of the eluate at 280 nm and emerged as a single symmetrical peak at 84% of the column volume. The product was recovered by lyophilization: 18 mg; $[\alpha]^{23}D$ −26.1° (*c* 0.5, 1 N HOAc); TLC (C) 0.39, (D) 0.66, with trace impurities evident.

Amino acid analysis after hydrolysis in 6 N HCl[36] gave the following molar ratios: Asp, 0.99; Thz, 0.50; Glu, 0.98; Gly, 1.08; Cys, 1.77; Ile, 0.94; Leu, 1.01; Tyr, 0.32; NH_3, 2.99; as well as an undetermined amount of cysteine. The low Tyr ratio may have been caused by the condensation of Tyr with formaldehyde which results from partial hydrolytic cleavage[14] of Thz under the hydrolysis conditions. This problem was circumvented by hydrolysis in 3 N $HSCH_2CH_2SO_3H$,[37] after which ratios of 0.78, 1.00, and 0.95 were obtained for Thz, Glu, and Tyr, respectively. Performic acid oxidation by the method of Moore[38] converted Thz and Cys to cysteic acid. After 6 N HCl hydrolysis a cysteic acid to Glu ratio of 2.78:1.00 was obtained.

[1-β-Mercaptopropionic acid,7-(thiazolidine-4-carboxylic acid)]oxytocin. As in the preparation of [Thz^7]oxytocin, the partially protected peptide amide was cleaved from the peptide resin 4 (3.0 g, assume 0.5 mmol) to give 325 mg (56% of theory) of lyophilized powder which was further purified by precipitation from DMF (2 ml) with EtOAc (40 ml): 281 mg. Removal of the Ec-protecting groups from 115.4 mg (0.1 mmol) of this material with refluxing NH_3 followed by oxidative cyclization with ICH_2CH_2I gave crude [β-Mpa^1,Thz^7]oxytocin. The analogue was subjected to partition chromatography in the system BuOH–PhH–H_2O (1:1:2, the aqueous phase 1.5% pyridine and 3.5% HOAc). The product emerged as a sharp peak at R_f 0.35, well resolved from an earlier peak. The fractions comprising the product peak were pooled, concentrated in vacuo, and lyophilized from 0.2 N HOAc to give 30 mg of powder. This material was dissolved in 5 ml of 0.2 N HOAc and subjected to gel filtration in 0.2 N HOAc. The product eluted as a single symmetrical peak at 88.5% of the column volume and on lyophilization gave 29 mg of powder. Further purification of the material by partition chromatography in the system BuOH–PhH–HOAc–H_2O (1:2:2:2) gave a single symmetrical peak at R_f 0.17. The product was recovered from the peak tubes by concentration in vacuo and lyophilization from 0.2 N HOAc to give 23 mg of powder: $[\alpha]^{23}D$ −98.9° (*c* 0.5, 1 N HOAc); TLC (C) 0.52, (D) 0.68, with trace impurities evident.

Amino acid analysis after hydrolysis in 6 N HCl gave the following molar ratios: Asp, 1.09; Thz, 0.47; Glu, 1.02; Gly, 1.02; Cys, 0.61; β-Mpa Cys, 0.54; Ile, 0.97; Leu, 0.99; Tyr, 0.28; NH_3, 3.27; as well as an undetermined amount of cysteine. Following hydrolysis in 3 N $HSCH_2CH_2SO_3H$, ratios of 0.69:1.01:0.89 were obtained for Thz, Glu, and Tyr, respectively. Analysis following performic acid oxidation and hydrolysis in 6 N HCl gave a cysteic acid to Glu ratio of 1.94:1.03.

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References and Notes

(1) The symbols Thz, β-Mpa, and Dha are used to indicate the thiazolidine-4-carboxylic acid, β-mercaptopropionic acid, and dehydroalanine residues, respectively. All other symbols follow the recommendations (1971) of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). The optically active amino acids are of the L configuration unless otherwise noted.
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Synthesis and Antihypertensive Activity of Some Thienylethanolamines

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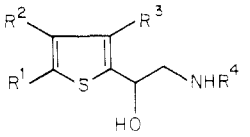
Synthesis of a series of thienylethanolamines having varying substituents on the thiophene ring and on the nitrogen atom is described using the general procedure reported earlier. In the determination of their pharmacological profile, some of the derivatives showed marked antihypertensive activity in the spontaneously hypertensive rat model. Tests are also reported which demonstrated that some of these derivatives antagonized α- and/or β-adrenoreceptor activities. The ability of this class of compounds to inhibit catecholamine-induced release of free fatty acids by adipose tissue was demonstrated. Structure–activity relationships in different tests were also determined.

The synthesis of analogues that mimic or block the effect of the adrenergic neurotransmitters represents a vast area of activity of medicinal chemists. We have reported recently[2a] the synthesis of some thiophene isosters of phenylethylamines. In this communication, we report the synthesis of some novel analogues of the same series and the biological profile of compounds of this class.

Chemistry. The thienylethanolamines of the type **1** were prepared as described[2a] starting with suitably substituted thiophenes according to the following scheme: thiophene → Friedel–Crafts type acylation → bromination of methyl ketone → reduction to alcohol → displacement of bromine by suitable amine. In our earlier work, because of the profound sympathomimetic properties of isoproterenol and sympatholytic properties of propranolol, both of which have the isopropylamine moiety as their partial structure, we chose to incorporate this amine in our analogues. In the present work, we have varied the amine moiety to explore the effect of this change on the pharmacological activity profile. The amines used for this purpose were either commercially available or synthesized by described procedures. Phenethylamine **2** was prepared by reducing the corresponding nitrile.[2b] More recently,[3] compounds having a 4-(*o*-tolyl)-1-piperazinyl group incorporated in aryloxyethylamines were reported to have vasodilator properties. Compounds **3** and **4** were therefore synthesized to investigate their cardiovascular effects.



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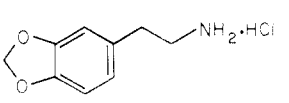

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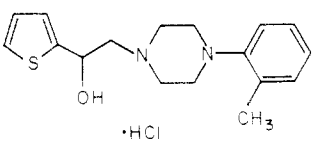

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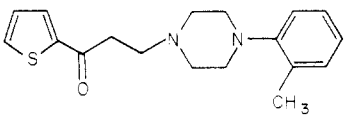

4

Pharmacology. Antihypertensive Activity. The blood pressure lowering property of a compound may be mediated in many different ways, e.g., (a) by direct actions on the central nervous system, via ganglionic blockade of impulses or by depletion of catecholamines from sympathetic postganglionic neurons; (b) by interfering with the adrenergic mechanisms by blocking α- or β-receptors; (c) by a direct effect on peripheral vascular smooth muscle;