PHARMACOLOGICAL STUDIES ON SUPERSENSITIZATION. IX. NON-SPECIFIC SUPERSENSITIVITY OF VAS DEFERENS OF GUINEA PIG INDUCED BY TRIPELENNAMINE, *N,N*-DIMETHYL-*N',N'*-DIBENZYLETHYLENEDIAMINE AND *N,N*-DIBENZYL-*N',N'*-DIMETHYL-1,2-PROPANEDIAMINE

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Effects of tripeleNNamine, *N,N*-dimethyl-*N',N'*-dibenzylethylenediamine (DBED) and *N,N*-dibenzyl-*N',N'*-dimethyl-1,2-propanediamine (DBPD) on the isotonic contractions of isolated vas deferens of guinea pig were examined. These ethylenediamines, except DBPD, induced slight but significant increase in sensitivity of vas deferens to K^+ . TripeleNNamine induced dose-dependent potentiation to epinephrine and norepinephrine, but tripeleNNamine-induced augmentation of acetylcholine-contractions was weak and dose-independent. DBED potentiated both catecholamines and acetylcholine in a dose-dependent way and in the same degree. The degree of DBPD-induced augmentation of epinephrine- and norepinephrine-induced contractions was dose-independent and much weaker than that of acetylcholine-contractions. TripeleNNamine and DBED did not affect the contractile response to tyramine, while DBPD potentiated the contractile response to tyramine. These results suggest the following possibilities: 1) tripeleNNamine affects both amine uptake mechanism and beyond receptor mechanism of contractile processes and induced supersensitivity of various stimulants. 2) DBED affects mainly beyond receptor mechanism of contractile processes and augments the contractile responses to various stimulants. 3) The mechanism of DBPD-induced supersensitivity remained obscure, but the inhibition of the metabolic degradation of stimulants was proposed as one of the possible mechanism of supersensitization.

Keywords—supersensitization; tripeleNNamine; ethylenediamines; epinephrine; norepinephrine; acetylcholine; potassium; tyramine; vas deferens of guinea pig

INTRODUCTION

Altura and Zwifach¹⁻⁴⁾ reported that anti-histamines elicited the contractile response of blood vessel and produced increase in sensitivity of small blood vessel of rat, rabbit and guinea pig to catecholamine. Johnson and Kahn⁵⁾ and Isaac and Goth^{6,7)} reported that tripeleNNamine, chlorpheniramine and diphenhydramine potentiated the pressor response to norepinephrine by blocking the uptake of norepinephrine into adrenergic nerve terminals.

In a previous report,⁸⁾ we examined 12 ethylenediamines and found that tripeleNNamine, *N,N*-dimethyl-*N',N'*-dibenzylethylenediamine (DBED) and *N,N*-dibenzyl-*N',N'*-dimethyl-

1,2-propanediamine (DBPD) were potent potentiators of the pressor response to epinephrine. Moreover, it was estimated that tripeleNNamine-induced augmentation of pressor response might involve different mechanism from the blockade of amine uptake and that DBED and DBPD did not inhibit the amine uptake mechanism. In the present study, the site and mode of potentiating action of tripeleNNamine, DBED and DBPD were investigated in isolated vas deferens of guinea pig.

MATERIALS AND METHODS

Male guinea pigs weighing between 280 and 380 g were killed by a blow on the head and vasa deferentia were isolated, dissected free from the

surrounding tissues and mounted in 10-ml organ baths containing aerated Tyrode solution at 31°C. Bath solution was prepared with demineralized and glass-distilled water. Tyrode solution included: NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.5, glucose 5.6 mM.

Preparations were allowed to equilibrate in Tyrode solution for 1 h before the application of drugs. The contractions were recorded isotonically with 8 times magnification and 0.6 g load. Acetylcholine, epinephrine, norepinephrine, tyramine and potassium were applied non-

cumulatively. Tripelennamine, *N,N*-dimethyl-*N',N'*-dibenzylethylenediamine (DBED), *N,N*-dibenzyl-*N',N'*-dimethyl-1,2-propanediamine (DBPD) and cocaine were applied 5 min before application of the stimulants. Cocaine was used as a reference standard of potentiators.

The drug used were: acetylcholine chloride (Daiichi Seiyaku Co.), epinephrine hydrochloride (Sankyo Co.), norepinephrine hydrochloride (Sankyo Co.), tyramine hydrochloride (Nakarai Chem., Ltd.), potassium chloride (Nakarai Chem., Ltd.), cocaine hydrochloride (Sankyo Co.) and tripelennamine (Ciba-Geigy). DBED (mp

TABLE I. *Effects of Ethylenediamines and Cocaine on Maximum Response and Sensitivity of Isolated Vas Deferens of Guinea Pig to Epinephrine and Norepinephrine*

Drug (M)	Increase in		Increase in	
	Maximum response ^{a)}	Sensitivity ^{b)}	Maximum response ^{a)}	Sensitivity ^{b)}
	Epinephrine		Norepinephrine	
Tripelennamine				
1.0 × 10 ⁻⁷	1.10(1.00–1.20) ^{c)}	1.84(1.56– 2.16)	0.99(0.93–1.05)	2.14(1.76– 2.59)
1.0 × 10 ⁻⁶	0.97(0.95–0.99)	3.03(2.16– 4.24)	0.95(0.90–1.00)	3.44(3.03– 4.12)
3.2 × 10 ⁻⁶			1.00(0.98–1.02)	4.20(3.00– 6.60)
1.0 × 10 ⁻⁵	0.95(0.89–1.01)	5.15(2.70– 9.82)	Inhibition	–
DBED ^{d)}				
3.2 × 10 ⁻⁷	1.07(1.02–1.12)	2.14(1.60– 2.68)		
1.0 × 10 ⁻⁶			1.05(0.92–1.18)	2.86(2.53– 3.23)
3.2 × 10 ⁻⁶	1.03(0.97–1.09)	4.75(3.10– 7.28)		
1.0 × 10 ⁻⁵			1.08(0.99–1.17)	5.26(4.32– 6.44)
DBPD ^{e)}				
1.0 × 10 ⁻⁷	1.04(0.97–1.12)	1.50(1.20– 1.87)	1.08(1.00–1.16)	1.46(1.12– 1.94)
1.0 × 10 ⁻⁶	1.04(0.98–1.19)	1.88(1.32– 2.60)	1.05(0.97–1.13)	1.33(1.01– 1.86)
1.0 × 10 ⁻⁵			0.99(0.92–1.06)	1.40(1.08– 1.83)
Cocaine				
1.0 × 10 ⁻⁷	1.00(0.95–1.05)	1.62(1.01– 1.85)	1.01(0.88–1.14)	2.01(1.72– 2.34)
1.0 × 10 ⁻⁶	0.98(0.88–1.08)	3.57(3.44– 3.71)	1.11(0.99–1.23)	4.16(3.41– 5.07)
3.2 × 10 ⁻⁶			0.98(0.95–1.01)	7.78(6.06–10.0)
1.0 × 10 ⁻⁵	1.10(1.00–1.20)	7.60(5.20–11.0)	1.05(0.95–1.15)	13.6 (11.2 –16.0)

a) Maximum response in the presence of ethylenediamine or cocaine / maximum response in the absence of ethylenediamine or cocaine.

b) Equipotent-dose ratio of the stimulant in the presence and absence of ethylenediamine or cocaine calculated by parallel-line assay.

c) Numbers in parentheses are confidence limits ($n = 8$, $p = 0.05$).

d) DBED: *N,N*-dimethyl-*N',N'*-dibenzylethylenediamine.

e) DBPD: *N,N*-dibenzyl-*N',N'*-dimethyl-1,2-propanediamine.

252–254°C,⁹⁾ found: 252–254°C) and DBPD (mp 190–190.5°C,¹⁰⁾ found: 190–190.5°C) were synthesized in our laboratory.

RESULTS

Effects of Dibenamine, Phentolamine and Atropine on the Contractile Response of Vas Deferens of Guinea Pig to Epinephrine, Norepinephrine, Acetylcholine and Potassium

The contractile responses to epinephrine and norepinephrine were decreased by 3.2×10^{-7} M

dibenamine and 1.0×10^{-9} M phentolamine, but were not affected by 1.0×10^{-9} M atropine. The contractile responses to acetylcholine were decreased by 1.0×10^{-9} M atropine, but were not affected by 3.2×10^{-7} M dibenamine and 1.0×10^{-9} M phentolamine. The contractile responses to K^+ were not affected by 3.2×10^{-7} M dibenamine, 1.0×10^{-9} M phentolamine and 1.0×10^{-9} M atropine.

Effects of Ethylenediamines and Cocaine on Epinephrine-, Norepinephrine-, Acetylcholine- and

TABLE II. *Effects of Ethylenediamines and Cocaine on Maximum Response and Sensitivity of Isolated Vas Deferens of Guinea Pig to Acetylcholine and Potassium*

Drug (M)	Increase in		Increase in	
	Maximum response ^{a)}	Sensitivity ^{b)}	Maximum response ^{a)}	Sensitivity ^{b)}
	Acetylcholine		Potassium	
Tripelennamine				
3.2 × 10 ⁻⁷	0.99(0.92–1.06) ^{c)}	1.14(0.80 – 1.59)	1.08(1.02–1.14)	1.55(1.51–1.59)
1.0 × 10 ⁻⁶	1.05(1.00–1.10)	1.51(1.45 – 1.56)		
3.2 × 10 ⁻⁶	1.03(0.93–1.13)	1.44(1.01 – 2.52)		
1.0 × 10 ⁻⁵	1.06(0.99–1.13)	1.66(1.03 – 3.28)		
DBED ^{d)}				
3.2 × 10 ⁻⁷	1.05(0.97–1.13)	2.20(1.77 – 2.73)	1.08(0.99–1.17)	1.37(1.36–1.38)
1.0 × 10 ⁻⁶	1.04(0.96–1.12)	2.93(2.34 – 3.68)		
3.2 × 10 ⁻⁶	1.03(0.98–1.08)	4.92(4.79 – 5.06)		
1.0 × 10 ⁻⁵	1.07(1.02–1.12)	6.32(4.40 – 8.88)		
DBPD ^{e)}				
3.2 × 10 ⁻⁷	1.00(0.98–1.02)	2.03(1.58 – 2.60)	1.02(0.98–1.02)	1.05(1.04–1.06)
1.0 × 10 ⁻⁶	1.05(1.01–1.08)	3.29(2.50 – 4.32)		
3.2 × 10 ⁻⁶	1.04(1.02–1.06)	3.63(2.15 – 6.12)		
1.0 × 10 ⁻⁵	1.00(0.98–1.01)	3.05(2.21 – 4.19)		
Cocaine ^{f)}				
3.2 × 10 ⁻⁷	1.03(0.86–1.20)	5.29(4.02 – 6.98)	1.07(1.04–1.09)	1.47(1.36–1.57)
1.0 × 10 ⁻⁶	0.98(0.88–1.08)	7.37(6.14 – 8.83)		
3.2 × 10 ⁻⁶	1.01(0.90–1.12)	13.8 (11.7 –16.2)		
1.0 × 10 ⁻⁵	1.05(0.91–1.23)	21.4 (17.6 –25.9)		

a) Maximum response in the presence of ethylenediamine or cocaine/ maximum response in the absence of ethylenediamine or cocaine.

b) Equipotent-dose ratio of the stimulant in the presence and absence of ethylenediamine or cocaine calculated by parallel-line assay.

c) Numbers in parentheses are confidence limits ($n = 8$, $p = 0.05$).

d) DBED: N,N-dimethyl-N',N'-dibenzylethylenediamine.

e) DBPD: N,N-dibenzyl-N',N'-dimethyl-1,2-propanediamine.

f) Cited from Araki *et al.*¹¹⁾

Potassium-contractions

Tripelennamine, DBED, DBPD and cocaine increased the sensitivity of vas deferens to epinephrine, norepinephrine and acetylcholine without affecting the maximum responses induced by these stimulants.

Tripelennamine induced dose-dependent potentiation to epinephrine and norepinephrine, but tripelennamine-induced augmentation of acetylcholine-contractions was weak and dose-independent. DBED potentiated both catecholamines and acetylcholine in a dose-dependent way and in the same degree. The degree of DBPD-induced augmentation of epinephrine- and norepinephrine-induced contractions was dose-independent and much weaker than that of acetylcholine-contractions. These ethylenediamines and cocaine also induced a slight but significant increase in sensitivity of vas deferens to K^+ (Table I, II).

Effects of Ethylenediamines and Cocaine on Tyramine-induced Contraction

Tyramine-induced contraction was attenuated or disappeared by injection of reserpine (a single subcutaneous injection of 5 mg/kg 24 h before experiment). Tripelennamine and DBED at the concentration of 3.2×10^{-6} M did not affect the contractile responses induced by 5.0×10^{-5} M tyramine, but DBPD and cocaine at the concentration of 3.2×10^{-6} M potentiated the contractile responses induced by 5.0×10^{-5} M tyramine to 228 ± 38 and $192 \pm 29\%$ of the control response, respectively.

DISCUSSION

Dibenamine and phentolamine at the concentration which inhibited epinephrine- and norepinephrine-contraction did not affect the contractile response to acetylcholine and K^+ , while atropine at the concentration which inhibited acetylcholine-contraction did not affect the contractile response to epinephrine, norepinephrine and K^+ . These results indicated that neither cholinergic-adrenergic nor adrenergic-cholinergic link was involved in the present experimental system.

Tripelennamine, DBED and DBPD increased the sensitivity of vas deferens to epinephrine, norepinephrine and acetylcholine without affecting maximum responses to these stimulants. Moreover, these ethylenediamines potentiated K^+ -contraction. This indicated that these ethylenediamines facilitated K^+ -induced depolarization and/or excitation-contraction coupling and suggested that these agents might nonspecifically increase the sensitivity of vas deferens to epinephrine, norepinephrine, acetylcholine and K^+ .

It was suggested that tripelennamine-induced potentiation of epinephrine and norepinephrine was caused by blockade of amine uptake mechanism.^{5-8,12} This mechanism of action might be probable, because the relative activities of tripelennamine to potentiate catecholamine-induced contractions were stronger than that to potentiate acetylcholine-contractions. Although tripelennamine did not inhibit the contractile response to tyramine, inhibition of amine uptake as a mechanism of tripelennamine-induced supersensitivity to catecholamine could not be excluded, because cocaine, a potent blocker of amine uptake mechanism and nonspecific potentiator, also augmented tyramine-induced contractions of vas deferens. Moreover, tripelennamine potentiated the contractile response to acetylcholine and K^+ . Thus, it was estimated that tripelennamine might affect both amine uptake me-

TABLE III. *The Slope of Dose-response Curve for Acetylcholine, Epinephrine, Norepinephrine and Potassium of Vas Deferens of Guinea Pig*

	Slope of dose-response curve
Acetylcholine	1.00 ± 0.14
Epinephrine	2.41 ± 0.28
Norepinephrine	3.81 ± 0.32
Potassium	6.07 ± 0.29

Slopes were estimated graphically and shown as the ratio to the slope of dose-response curve for acetylcholine (mean $\pm$ S.E., $n = 12$).

chanism and beyond receptor mechanism of contractile responses and induced supersensitivity to various stimulants.

DBED augmented K^+ -contractions and the dose-response relationship of DBED-induced potentiation to epinephrine, norepinephrine and acetylcholine was determined to be nearly equal. This suggested that site of action of DBED-induced supersensitivity of vas deferens was common pathway in the process of contraction induced by various stimulant.

The degree of DBPD-induced potentiation of the contractile responses to K^+ was very slight. This indicated that DBPD-induced supersensitivity was not caused by the effect of DBPD on depolarization of cell membrane or excitation-contraction coupling. DBPD potentiated acetylcholine- and tyramine-contractions stronger than that induced by other ethylenediamines. This suggested that DBPD possesses stronger activities on receptors or inactivation processes of these stimulants. Considering that DBPD induced relatively slight potentiation to epinephrine and norepinephrine than that to tyramine, DBPD-induced sensitization of adrenergic receptors might be improbable. Thus, as the mechanism of supersensitivity induced by DBPD, the inhibition of inactivation process of the stimulants was considered to be most probable. However, no experimental evidence which supported this speculation was obtained as yet.

Hudgins *et al.*¹³⁾ and Westfall¹⁴⁾ reported that for a heterogenous group of stimulants the magnitude of reserpine-induced supersensitivity (norepinephrine > histamine > K^+) was inversely correlated with the slope of the dose-response curve and speculated that reserpine changed preferentially the common pathway for these stimulants which mobilize bound calcium and gave a shallow dose-response curve. In the present study, the slope of dose-response curve was K^+ > norepinephrine > epinephrine > acetylcholine (Table III) and inversely correlated with the order of degree of cocaine- and DBED-induced potentiation to these stimulants. Since the mechanism of nonspecific supersensitization induced by

cocaine was estimated to be increase in Ca^{2+} -influx through superficial Ca^{2+} -binding sites,¹¹⁾ as proposed by Westfall in reserpine-induced supersensitization,¹⁴⁾ facilitation of the utilization of calcium in stimulant-induced contraction might be proposed as one of the possible mechanism of supersensitization induced by DBED.

In summary, following mechanisms were proposed that 1) tripeleannamine affects both amine uptake mechanism and beyond receptor mechanism of contractile processes and induces supersensitivity to various stimulants, 2) DBED may affect mainly beyond receptor mechanism of contractile processes and augments the contractile responses to various stimulants, and 3) DBPD-induced supersensitivity may be caused by the inhibition of the metabolic degradation of stimulants. Exact site and mode of action of these drug-induced supersensitivities are now under investigation.

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