PHARMACOLOGICAL STUDIES ON SUPERSENSITIZATION. VIII. DISSOCIATION OF INDUCTION OF SUPERSENSITIVITY FROM PRESYNAPTIC ACTION OF COCAINE ON ISOLATED VAS DEFERENS OF GUINEA PIG

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Effects of cocaine on the isotonic contractions of isolated vas deferens of intact and reserpinized guinea pigs to acetylcholine were examined. Both cocaine-induced increase in sensitivity to acetylcholine and cocaine-induced increase in maximum response of Ca^{2+} -contraction was attenuated remarkably by reserpine. However, cocaine-induced increases in acetylcholine- and K^{+} -contractions in Ca^{2+} -free Tyrode solution were not prevented by reserpine. Acetylcholine-contraction, but not K^{+} -contraction, in the preparation exposed for 5 min to Ca^{2+} -free Tyrode solution were inhibited by reserpine. Cocaine did not inhibit the cholinesterase activity of vas deferens of guinea pig. These results suggested that presynaptic mechanisms of cocaine-induced supersensitivity are excluded and that inhibitory effect of reserpine on cocaine-induced supersensitivity might be due to the inhibition of cocaine-induced increase in calcium-influx into smooth muscle cells of vas deferens.

Keywords — supersensitization; cocaine; reserpine; acetylcholine; potassium; calcium; cholinesterase; vas deferens of guinea pig

INTRODUCTION

In the previous papers,^{1,2)} the mechanism of cocaine-induced supersensitization of isolated vas deferens of guinea pig to acetylcholine was estimated to be the increase in transmembrane calcium influx from extracellular fluid and superficial calcium-binding site into smooth muscle cells. However, some possible mechanisms, such as inhibition of cholinesterase activity or reuptake of norepinephrine released by acetylcholine, remained to be examined. In the present study, these possibilities were investigated in isolated vasa deferentia of intact and reserpinized guinea pig.

MATERIALS AND METHODS

Experimental procedures were the same as described in the previous paper.¹⁾ Guinea pig was given a single subcutaneous injection of 5 mg/kg reserpine (Daiichi Seiyaku Co.) 24 h before experiment. The length of isolated vasa deferentia

of intact and reserpinized guinea pigs were made uniform as much as possible. Isolated vas deferens was suspended in aerated standard or modified Tyrode solution, kept at 31–32°C and isotonic contractions were recorded on a smoked drum. Acetylcholine-induced contractions in Ca^{2+} -free Tyrode solution were elicited every 5 min. Ca^{2+} -contractions were elicited in the preparations exposed to K^{+} -depolarizing Ca^{2+} -free Tyrode solution for 40 min.

Bath solution was prepared with demineralized and glass-distilled water. Standard Tyrode solution included: NaCl 136.9, KCl 2.7, CaCl_2 1.8, MgCl_2 1.1, NaHCO_3 11.9, NaH_2PO_4 0.5, glucose 5.6 mM. Ca^{2+} -free Tyrode solution was prepared by omitting CaCl_2 and K^{+} -depolarizing Ca^{2+} -free Tyrode solution was prepared by omitting CaCl_2 and substituting equimolar concentration of NaCl by KCl. Chelating agents were not used throughout the experiments.

Serum and homogenate of vas deferens of

guinea pig were used as the source of cholinesterase. Cholinesterase activity was determined by the method of Bullock.³⁻⁵⁾

Acetylcholine chloride (Daiichi Seiyaku Co.), CaCl_2 (Nakarai Chem., Ltd.), KCl (Nakarai Chem., Ltd.) and physostigmine salicylate (Merck) were dissolved in distilled water. Tyramine hydrochloride (Nakarai Chem., Ltd.) was dissolved in 0.1% NaHSO_3 . Cocaine hydrochloride (Sankyo Co.) was dissolved in distilled water and added into bath solution 5 min before application of the stimulants except Ca^{2+} . Ca^{2+} was applied 4 min after application of cocaine.

RESULTS

Effects of Reserpine on the Contractile Responses to Acetylcholine, Potassium and Tyramine in Stan-

dard Tyrode Solution

The concentration of acetylcholine and K^+ to induce maximum contraction (1.0×10^{-3} and 1.0×10^{-1} M, respectively), the absolute value of maximum response induced by acetylcholine and K^+ (Table I) and the dose-response curve for acetylcholine were not affected by reserpine. These results indicated that both reactivity and sensitivity of vas deferens of guinea pig to the stimulants was not affected by reserpine. The contractile response induced by 5.0×10^{-5} M tyramine was attenuated remarkably or disappeared by reserpine.

Effects of Cocaine on the Contractile Responses of Vas Deferens of Reserpinized Guinea Pig to Acetylcholine and Potassium in Standard Tyrode Solution

Cocaine-induced increase in sensitivity to

TABLE I. Contractile Response to 1.0×10^{-3} M Acetylcholine or 1.0×10^{-1} M Potassium Chloride of Vas Deferens in Intact or Reserpinized Guinea Pig in Standard Tyrode Solution

	Acetylcholine- contracture (mm)	Potassium- contracture (mm)
Intact	126.7 ± 1.7 ^{b)}	140.0 ± 3.0
Reserpinized ^{a)}	129.1 ± 2.1	140.4 ± 3.2

a) Given a single subcutaneous injection of 5 mg/kg 24 h before experiment.

b) Mean $\pm$ S.E. (n=72).

TABLE II. Effects of Cocaine on Maximum Response and Sensitivity of Isolated Vas Deferens of Intact and Reserpinized Guinea Pig to Acetylcholine in Standard Tyrode Solution

Cocaine (M)	Intact vas deferens ^{a)}		Reserpinized vas deferens ^{b)}	
	Increase in		Increase in	
	Maximum response ^{c)}	Sensitivity ^{d)}	Maximum response ^{c)}	Sensitivity ^{d)}
3.2×10^{-7}	1.03 (0.86–1.20) ^{e)}	5.29 (4.02–6.98)		
1.0×10^{-6}	0.98 (0.88–1.08)	7.37 (6.14–8.83)	1.05 (0.95–1.15)	1.40 (1.17–1.67)
3.2×10^{-6}	1.01 (0.90–1.12)	13.8 (11.7–16.2)	0.99 (0.94–1.04)	2.16 (1.60–2.92)
1.0×10^{-5}	1.05 (0.91–1.23)	21.4 (17.6–25.9)		

a) Cited from Araki et al.¹⁾

b) Given a single subcutaneous injection of 5 mg/kg 24 h before experiment.

c) Maximum response in the presence of cocaine/ maximum response in the absence of cocaine.

d) Equipotent-dose ratio of acetylcholine in the presence and absence of cocaine.

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

acetylcholine was attenuated by reserpine (Table II). Cocaine at the concentration of 3.2×10^{-6} M potentiated the contractile response to 3.0×10^{-2} M K^+ (1.82 ± 0.12 folds, $n=8$), and this increase in response to K^+ was attenuated by reserpine (1.08 ± 0.06 folds, $n=3$).

Effects of Cocaine on Calcium-induced Contraction of Depolarized Vas Deferens of Reserpinized Guinea Pig

The dose-response curve for Ca^{2+} was not affected by reserpine. Cocaine at the concentration of 1.0×10^{-5} M, but not at 3.2×10^{-6} M, increased the reactivity to Ca^{2+} in vas deferens of reserpin-

ized guinea pig in Ca^{2+} -free 60 mM- K^+ Tyrode solution, but the degree of cocaine-induced increase in reactivity to Ca^{2+} in vas deferens of reserpinized guinea pig was much smaller than those in intact vas deferens (Table III).

Effects of Cocaine on the Contractile Responses of Vas Deferens of Reserpinized Guinea Pig to Acetylcholine and Potassium in Calcium-free Tyrode Solution

Reserpine decreased contractile response to acetylcholine in the preparation exposed for 5 min to Ca^{2+} -free Tyrode solution to $39.1 \pm 2.6\%$ ($n=8$). Cocaine at the concentration of

TABLE III. *Effects of Cocaine on Maximum Response and Sensitivity of Isolated Vas Deferens of Intact and Reserpinized Guinea Pig to Calcium in 60 mM K^+ -depolarizing Ca^{2+} -free Tyrode Solution*

Cocaine (M)	Intact vas deferens ^{a)}		Reserpinized vas deferens ^{b)}	
	Increase in		Increase in	
	Maximum response ^{c)}	Sensitivity ^{d)}	Maximum response ^{c)}	Sensitivity ^{d)}
3.2×10^{-6}	1.66 (1.51–1.80) ^{e)}	1.67 (1.41–1.91)	1.06 (0.98–1.13)	1.00 (0.88–1.14)
1.0×10^{-5}	1.83 (1.71–1.95)	1.71 (1.52–1.95)	1.23 (1.13–1.34)	0.93 (0.81–1.07)

a) Cited from Araki et al.¹⁾

b) Given a single subcutaneous injection of 5 mg/kg reserpine 24 h before experiment.

c) Maximum response in the presence of cocaine/ maximum response in the absence of cocaine.

d) Equipotent-dose ratio of calcium in the presence and absence of cocaine.

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

TABLE IV. *Effects of Cocaine on the Contractile Response to 1.0×10^{-3} M Acetylcholine of Vas Deferens of Intact and Reserpinized Guinea Pig in Calcium-free Tyrode Solution*

	Response (%) ^{a)}	Half-time (min) ^{b)}
Intact		
Control	43.7 ± 6.3	5.18 ± 0.58
Cocaine 3.2×10^{-6} M	68.8 ± 5.6 ^{d)}	6.68 ± 1.27
Reserpinized ^{c)}		
Control	16.3 ± 3.5	3.30 ± 0.66
Cocaine 3.2×10^{-6} M	37.0 ± 7.4 ^{d)}	6.61 ± 1.83

a) Responses (mean $\pm$ S.E., $n=8$) were expressed as percentage of contractile response of preparation at 5 min after removal of Ca^{2+} from bath solution to the maximum contraction of the same preparation in standard Tyrode solution.

b) The half-time of decrease in contraction elicited every 5 min in Ca^{2+} -free Tyrode solution.

c) Given a single subcutaneous injection of 5 mg/kg reserpine 24 h before experiment.

d) Significantly different from control value ($p=0.05$).

TABLE V. *Effects of Cocaine on the Contractile Response to 1.0×10^{-1} M Potassium Chloride of Vas Deferens of Intact and Reserpinized Guinea Pig in Calcium-free Tyrode Solution*

	Response (%) ^{a)}	Half-time (min) ^{b)}
Intact		
Control	20.0 ± 5.2	1.92 ± 0.13
Cocaine 3.2×10^{-6} M	66.1 ± 3.3 ^{d)}	3.35 ± 0.30 ^{d)}
Reserpinized ^{c)}		
Control	19.1 ± 3.1	1.56 ± 0.06
Cocaine 3.2×10^{-6} M	42.8 ± 2.6 ^{d)}	2.24 ± 0.27

a) Responses (mean $\pm$ S.E., $n=8$) were expressed as percentage of contractile response of preparation at 5 min after removal of Ca^{2+} from bath solution to the maximum contraction of the same preparation in standard Tyrode solution.

b) The half-time of decrease in contraction elicited every 5 min in Ca^{2+} -free Tyrode solution.

c) Given a single subcutaneous injection of 5 mg/kg 24 h before experiment.

d) Significantly different from control value ($p=0.05$).

3.2×10^{-6} M potentiated the contractile responses induced by acetylcholine in vasa deferentia of intact and reserpinized guinea pigs, and the degree of the potentiation of acetylcholine-induced

contraction was not affected by reserpine (Table IV).

The contractile response to K^+ in the preparation exposed for 5 min to Ca^{2+} -free Tyrode solution was not affected by reserpine (1.22 ± 0.22 folds, $n=8$). Cocaine at the concentration of 3.2×10^{-6} M potentiated the contractile responses of vasa deferentia of intact and reserpinized guinea pig induced by K^+ , and the potentiation of K^+ -induced contraction was attenuated, but not abolished by reserpine (Table V).

The half-time of decrease in contractile response of vas deferens to acetylcholine and K^+ were not changed by reserpine and cocaine (Table IV, V).

Effects of Cocaine on the Contractile Response to Tyramine in Standard Tyrode Solution

The contraction of vas deferens of intact guinea pig elicited by tyramine was decreased by repeated application of tyramine at every 10 min. Cocaine at the concentration of 3.2×10^{-6} M potentiated the contractile response to 5.0×10^{-5} M tyramine and the degree of the potentiation of contractile response induced by 5.0×10^{-5} M tyramine was 1.92 (1.25–2.59) fold ($n=8$).

Effects of Cocaine and Physostigmine on Cholinesterase Activity of Serum and Vas Deferens of

FIG. 1. *Effects of Cocaine (●) and Physostigmine (○) on Cholinesterase Activities of Serum (—) and Homogenized Vas Deferens (---) of Guinea Pig*

Each value represents the mean $\pm$ S.E. ($n=8$ for serum; $n=4$ for vas deferens).

Guinea Pig

Both cocaine and physostigmine produced dose-dependent inhibition of cholinesterase activity of serum of guinea pig. Concentration of cocaine and physostigmine which produced 50 percent inhibition of cholinesterase activity of serum were $9.9 \pm 0.5 \times 10^{-4}$ M and $5.4 \pm 0.7 \times 10^{-7}$ M, respectively. Concentration of physostigmine which produced 50 percent inhibition of cholinesterase activity of vas deferens was $2.3 \pm 0.3 \times 10^{-8}$ M and lower than ED_{50} for the inhibition of cholinesterase of serum. The inhibitory activity of cocaine on cholinesterase of vas deferens was weaker than that on serum cholinesterase and ED_{50} of cocaine could not be determined (Fig. 1).

DISCUSSION

Cocaine was reported to have anticholinesterase activity,^{5,6)} and Westfall *et al.*⁷⁾ reported that the supersensitivity to acetylcholine in denervated vas deferens of guinea pig was due to the inhibition of cholinesterase. Moreover, Waterson *et al.*⁸⁾ reported that the inhibition of cholinesterase activity was produced by reserpine. Therefore, the observation that cocaine-induced increase in sensitivity was attenuated by reserpine suggested that cocaine-induced supersensitivity might be caused by the inhibitory activity of cocaine on cholinesterase. However, this mechanism of action may be improbable, since cocaine did not inhibit cholinesterase in vas deferens of guinea pig at the concentration which induced supersensitivity to acetylcholine.

It was reported that acetylcholine induced the antidromic firing of adrenergic nerve terminals^{9,10)} and released norepinephrine which elicited the contractile responses.¹¹⁻¹³⁾ These reports suggested the possibility that cocaine blocked the reuptake of norepinephrine released by acetylcholine from adrenergic nerve terminals and increased apparent sensitivity of isolated vas deferens to acetylcholine.

Vasa deferentia from reserpinized guinea pigs were considered to have depleted norepinephrine stores in adrenergic nerve terminals,¹⁴⁾

while Bell¹⁵⁾ reported that when neuronal norepinephrine stores in vas deferens of guinea pig had been depleted by reserpine, some norepinephrine was still available for release by postganglionic hypogastric nerve stimulation. Moreover, the pretreatment of reserpine was reported to induce supersensitivity to acetylcholine in rabbit aortae^{16,17)} and in nictitating membrane of cat.¹⁸⁾ However, in the present study, reserpinization of vas deferens did not change the sensitivity to acetylcholine, while the pretreatment of reserpine antagonized against the development of cocaine-induced supersensitivity to acetylcholine. If norepinephrine was released by acetylcholine from adrenergic nerve terminals, the dose-response curve for acetylcholine must be affected by reserpine. However, dose-response curve for acetylcholine was not affected by reserpine. These findings supported Ferry's observation¹³⁾ that norepinephrine was not released by acetylcholine from adrenergic nerve terminals in vas deferens of guinea pig. Thus the hypothesis that cocaine blocks the reuptake of norepinephrine released by acetylcholine and induces the supersensitization of vas deferens to acetylcholine must be excluded. This conclusion was supported by the experimental evidence that cocaine-induced supersensitivity to acetylcholine was inhibited by dibenamine at the lower concentration than those inhibiting acetylcholine-contraction of the same preparation.²⁰⁾ Moreover, the results that the tyramine-contraction attenuated or abolished by reserpine was increased by cocaine strengthen the hypothesis that cocaine acts postsynaptically and increases sensitivity to various stimulants.

In the present study, Ca^{2+} -contractions of partially depolarized vas deferens were increased by cocaine and this increase in Ca^{2+} -contraction was abolished by reserpine. However, cocaine-induced increases in acetylcholine- and K^{+} -contractions of vas deferens in Ca^{2+} -free Tyrode solution were not abolished by reserpine. These results suggested that both Ca^{2+} -influx and Ca^{2+} -release were increased by cocaine and the increase in Ca^{2+} -influx was selectively prevented

by reserpine.

Reserpine-induced inhibition of the contractile response to acetylcholine in preparation suspended in Ca^{2+} -free Tyrode solution might be due to the decrease in the intracellular calcium content as reported by Carrier *et al.*¹⁹⁾ and Carrier.²⁰⁾ However, K^{+} -contraction induced in Ca^{2+} -free Tyrode solution was not affected by reserpine. This indicated that K^{+} -contraction in Ca^{2+} -free Tyrode solution was mainly dependent upon superficially bound calcium and contribution of intracellular calcium to K^{+} -contraction might be negligible.

In contrast, dibenamine inhibited K^{+} -contraction, but not acetylcholine-contraction, induced in Ca^{2+} -free Tyrode solution.²⁾ This indicated that dibenamine did not affect the intracellular calcium-binding site and inhibited selectively Ca^{2+} -release or Ca^{2+} -influx from superficial Ca^{2+} -binding site. The experimental result that both reserpine and dibenamine antagonized against the development of cocaine-induced supersensitivity to acetylcholine and K^{+} in standard Tyrode solution, but not in Ca^{2+} -free Tyrode solution, strongly supported the hypothesis that cocaine-induced increase in Ca^{2+} -influx from extracellular fluid was selectively inhibited by reserpine and dibenamine. Moreover, these findings mentioned above indicated that reserpine-induced decrease in the intracellular calcium content did not involve in reserpine-induced inhibition of cocaine-induced increase in sensitivity to acetylcholine in standard Tyrode solution.

In conclusion, inhibitory effect of reserpine on cocaine-induced supersensitivity of isolated vas deferens to acetylcholine might be caused by the inhibition of increase in Ca^{2+} -influx and might not be due to the inhibition of cholinesterase activity or the blockade of reuptake of norepinephrine released by acetylcholine from adrenergic nerve terminals.

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