Effect of evodiamine on catecholamine secretion from bovine adrenal medulla

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Abstract : The effect of evodiamine on catecholamine secretion from bovine adrenal medulla was investigated. Evodiamine, a bioactive component isolated from dry unripened fruit of *Evodia rutaecarpa* Bentham, was found to stimulate the secretion of catecholamine from perfused bovine adrenal medulla at a concentration of 10 μM and its effect persisted for at least 30 min. This stimulatory effect of evodiamine was abolished by omission of Ca^{2+} from the perfusion fluid. Evodiamine (0.1-10 μM) markedly enhanced the secretion of catecholamine from the adrenal medulla induced by acetylcholine (100 μM) or high K^+(56 mM). The secretion of catecholamine was promptly enhanced by acetylcholine or high K^+, but returned to the control level on treatment for 20 min. However, when evodiamine was added to the perfusion fluid after acetylcholine or high K^+ stimulation for 10 min, the secretion of catecholamine again increased greatly. These results indicate that evodiamine not only stimulated the secretion of catecholamine from bovine adrenal medulla but also reversed insensitivity of these cells to acetylcholine or high K^+ stimulation. J. Med. Invest. 44 : 79-82, 1997

Key Words : *evodiamine, catecholamine secretion, adrenal medulla*

INTRODUCTION

Evodiamine is a bioactive component isolated from dry unripened fruit of *Evodia rutaecarpa* Bentham(1). These fruit have long been used in Chinese medical practice for the treatments of headache, abdominal pain, dysentery, postpartum hemorrhage and amenorrhea(1). Pharmacological studies demonstrated that evodiamine has a positive inotropic action on isolated left atria of the guinea pig(1), an antianoxic action in anoxia induced by KCN in mice(2)and an effect of retaining the body temperature of rats treated with chlorpromazine(3).

Adrenal medulla contains neural crest-derived chromaffin cells. Therefore, the adrenal medulla is useful for studying the mechanism of stimulus-secretion coupling and is regarded as a model for catecholamine containing neurons. Physiological stimulations of the adrenal medulla such as acetylcholine cause an increase in the levels of intracellular free Ca^{2+}($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ leads to the stimulation of catecholamine secretion(4). However, the effects of evodiamine on the secretions of hormones and neurotransmitters have not yet been reported. In the present study, we examined the effect of evodiamine on catecholamine secretion from bovine adrenal medulla.

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MATERIALS AND METHODS

Fresh bovine adrenal glands were used throughout. The glands were perfused retrogradly(5)with a medium consisting of (in mM : NaCl 154, KCl 5.6, $CaCl_2$ 2.2, glucose 10 and Tris-HCl buffer 20 pH 7.4)saturated with 100% O_2 and maintained at 37℃. The glands were perfused at a rate of 4 ml/min for 40 min and then stimulated by changing to a medium containing high K^+(56 mM K^+ with equimolar reduction of Na^+), acetylcholine or evodiamine. Samples of 8 ml of perfusion fluid were collected at 2 min intervals. Catecholamine secretion was determined by HPLC with electrochemical detection (Yanaco model L-2000).

Evodiamine was obtained from Wako Pure Chemical Industries (Osaka, Japan). Other chemicals used were commercial products of reagent grade.

RESULTS

Table 1 shows the effect of evodiamine on secretion of catecholamine from perfused bovine adrenal medulla. Evodiamine at a concentration of 10 μM slightly, but significantly, stimulated the secretion of catecholamine from the adrenal medulla. This stimulatory effect of evodiamine was observed at concentrations of more than

1 μM (control, 24.5±2.8 μg/2 min ; 1 μM evodiamine, 27.6±2.9 μg/2 min, N=6) and continued for at least 30 min. To determine whether the stimulatory effect of evodiamine on catecholamine secretion depends on Ca^{2+} in the perfusion fluid, the adrenal medulla was perfused with Ca^{2+}-free fluid. As shown in Table 1, the slight effect of evodiamine on catecholamine secretion was not observed in Ca^{2+}-free fluid. Therefore, evodiamine may stimulate the secretion of catecholamine through increase in Ca^{2+} uptake into the cells of the adrenal medulla.

Catecholamine secretion from the adrenal medulla is known to be induced by stimulation of acetylcholine receptors or depolarization of the cell membrane by high K^+(6). As shown in Fig.1, the effect of evodiamine on acetylcholine-induced catecholamine secretion from perfused adrenal medulla was examined. Acetylcholine transiently increased the secretion of catecholamine from the adrenal medulla. This catecholamine secretion was potentiated by concentrations between 0.1 to 10 μM evodiamine. To exclude effects of evodiamine on acetylcholine receptors, we examined its effects on high K^+-induced catecholamine secretion from perfused adrenal medulla(Fig.2). High K^+ also transiently increased the secretion of catecholamine from the adrenal medulla. Evodiamine potentiated both the amount and the duration of catecholamine secretion induced by high K^+ stimulation.

There are reports that desensitization, in other words, insensitive to catecholamine secretion produced by frequent same stimulations, of stimulus secretion coupling in the bovine adrenal medulla is induced by physiological stimulations such as acetylcholine as well as high K^+(7-10). To determine whether evodiamine influences the process of desensitization of catecholamine secretion, we investigated the effects of evodiamine on catecholamine secretion after acetylcholine- or high K^+-induced desensitization (Fig.3 and 4). The secretion of catecholamine was promptly enhanced by acetylcholine or high K^+, but returned to the control level on treatment for 20 min. When evodiamine was added to the perfusion fluid after acetylcholine or high K^+ stimulation for 10 min, the secretion of catecholamine again increased greatly (Fig.3 and 4). Nifedipine, a typical voltage-dependent calcium channel blocker, did not change the effect of evodiamine on acetylcholine- or high K^+-stimulated catecholamine secretion in the same experimental protocol (Table 2). Moreover, 12-*O*-tetradecanoyl phorbol 13-acetate (TPA),

Table 1. Effect of evodiamine on catecholamine secretion from perfused bovine adrenal medulla

	Catecholamine Secretion (μg/2 min)	
	2.2 mM Ca^{2+}	0 mM Ca^{2+}
Control	24.5±2.8	24.3±2.2
Evodiamine(10 μM)		
(0-2 min)	32.3±3.5*	25.2±2.9
(8-10 min)	37.5±4.1*	25.1±2.6#
(28-30 min)	38.4±4.3*	24.8±2.7#

Adrenal medulla was stimulated by 10 μM evodiamine with or without Ca^{2+} in the perfusion fluid. Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.
*Significantly greater than control ($p<0.05$).
#Significantly less than with Ca^{2+} ($p<0.01$).

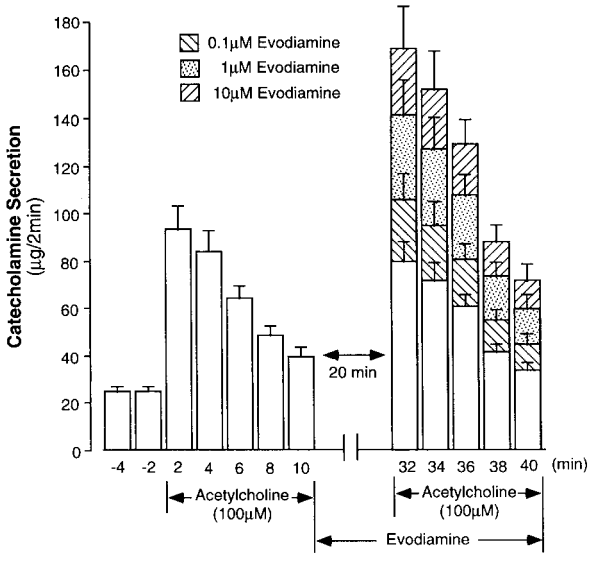

Fig.1. Effect of evodiamine on acetylcholine-induced catecholamine secretion from perfused bovine adrenal medulla.
Adrenal medulla was stimulated by 100 μM acetylcholine for 10 min without evodiamine, and then stimulated again by 100 μM acetylcholine with (hatched columns) or without (open columns) 0.1-10 μM evodiamine. Catecholamine secretion was significantly greater with 1 and 10 μM evodiamine than without evodiamine ($p<0.01$).
Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

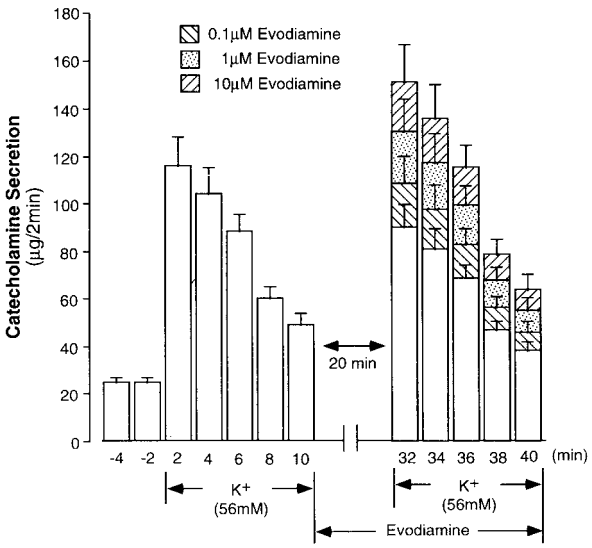

Fig. 2. Effect of evodiamine on high K^+-induced catecholamine secretion from perfused bovine adrenal medulla.
Adrenal medulla was stimulated by 56 mM K^+ for 10 min without evodiamine, and then stimulated again by 56 mM K^+ with (hatched columns) or without (open columns) 0.1-10 μM evodiamine. Catecholamine secretion was significantly greater with 1 and 10 μM evodiamine than without evodiamine ($p<0.01$).
Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

an activator of protein kinase C, did not mimic evodiamine which enhances the secretion of catecholamine from the bovine adrenal medulla, after stimulation by acetylcholine or high K^+ (Table 3). These results suggest that evodiamine may reverse the insensitivity of the bovine adrenal medulla to induction of catecholamine secretion that develops during acetylcholine or high K^+ stimulation.

DISCUSSION

Little is known about the effects of evodiamine or related compounds on stimulus-secretion coupling in several hormonal organs or nervous systems. In the present study, we demonstrated that evodiamine greatly increased acetylcholine- or high K^+-stimulated catecholamine secretion from the bovine adrenal medulla even after desensitization of the response (Fig. 3 and 4), although evodiamine alone had only a slight, but significant, effect on the spontaneous secretion (Table 1). Therefore, it is difficult to conclude that the effects of evodiamine and acetylcholine or high K^+ on catecholamine secretion were simply additive. In the experimental conditions for Fig.3 and 4, we found that the stimulatory effect of evodiamine on acetylcholine- or high K^+-induced catecholamine secretion was not affected by nifedipine, a typical voltage-dependent calcium channel blocker (Table 2). Therefore, the stimulatory effect of evodiamine on catecholamine secretion after acetylcholine or high K^+ stimulation does not seem to be regulated by activation of voltage-dependent calcium channels on the cell membrane.

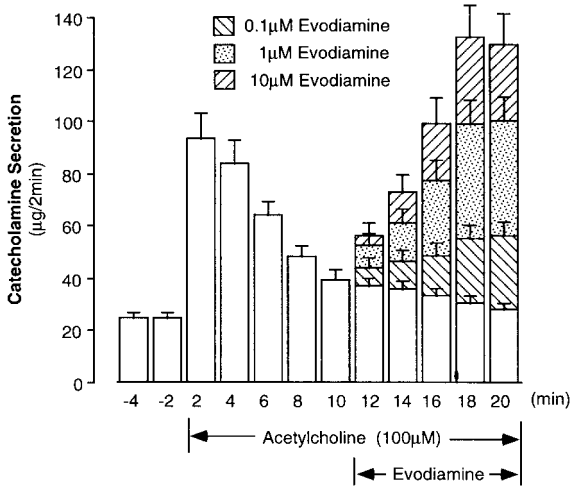

Fig. 3. Effect of evodiamine on catecholamine secretion after stimulation of acetylcholine from perfused bovine adrenal medulla.

Adrenal medulla was stimulated by 100 μM acetylcholine, and 1-10 μM evodiamine (hatched columns) was added after this stimulation for 10 min. Catecholamine secretion was significantly greater with 0.1, 1 and 10 μM evodiamine than without evodiamine ($p<0.01$).

Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

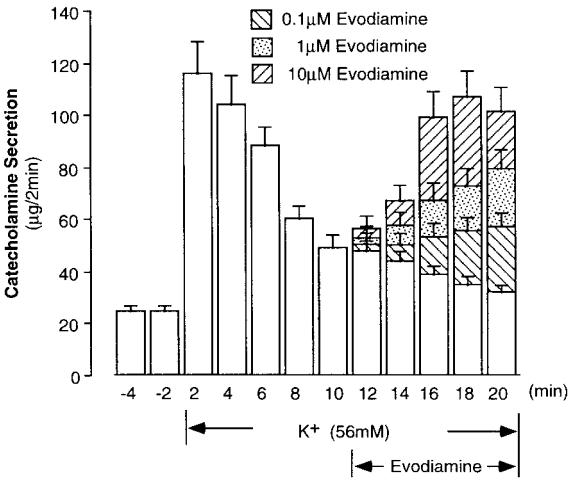

Fig. 4. Effect of evodiamine on catecholamine secretion after stimulation of high K^+ from perfused bovine adrenal medulla.

Adrenal medulla was stimulated by 56 mM K^+, and 1-10 μM evodiamine (hatched columns) was added after this stimulation for 10 min. Catecholamine secretion was significantly greater with 0.1, 1 and 10 μM evodiamine than without evodiamine ($p<0.01$).

Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

Table 2. Effects of nifedipine on evodiamine-induced catecholamine secretion treated with acetylcholine or high K^+ from perfused bovine adrenal medulla

	Catecholamine Secretion (18 to 20 min) (μg/2 min)	
Acetylcholine (100 μM) + Evodiamine (10 μM)	130±15	
Acetylcholine (100 μM) + Evodiamine (10 μM) + Nifedipine (100 μM)	125±13	NS
High K^+ (56 mM) + Evodiamine (10 μM)	100±13	
High K^+ (56 mM) + Evodiamine (10 μM) + Nifedipine (100 μM)	110±13	NS

Adrenal medulla was stimulated by 100 μM acetylcholine or 56 mM high K^+, and then 10 μM evodiamine and/or 100 μM nifedipine were added after this stimulation for 10 min. Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

Table 3. Effects of TPA on catecholamine secretion treated with acetylcholine or high K^+ from perfused bovine adrenal medulla

	Catecholamine Secretion (18 to 20 min) (μg/2 min)	
Acetylcholine (100 μM)	30± 4	
Acetylcholine (100 μM) + TPA (1 μM)	31± 5	NS
High K^+ (56 mM)	36± 4	
High K^+ (56 mM) + TPA (1 μM)	36± 5	NS

Adrenal medulla was stimulated by 100 μM acetylcholine or 56 mM high K^+, and then 1 μM 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) was added after this stimulation for 10 min. Catecholamine secretion was determined as described in Materials and Methods. Data are means±SE for 4-6 separate experiments.

Acetylcholine is known to stimulate nicotinic and muscarinic receptors in adrenal medullary cells (11). Activation of the nicotinic receptor induces Ca^{2+} influx through the cell membrane(11,12). However, activation of the muscarinic receptor stimulates the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol(13). Diacylglycerol is produced concurrently with IP_3 on breakdown of phosphatidyl inositol 4,5-bisphosphate (PIP_2) by phospholipase C and is thought to activate protein kinase C by increasing the affinity of the enzyme for calcium(14,15). Activation of protein kinase C using phorbol ester TPA increases secretion of catecholamine from leaking adrenal medullary cells(16). However, TPA did not mimic evodiamine which enhances the secretion of catecholamine from the bovine adrenal medulla, after stimulation by acetylcholine or high K^+ (Table 3). Therefore, the stimulatory effect of evodiamine on catecholamine secretion after acetylcholine or high K^+ stimulation may not be mediated by the activation of protein kinase C.

We are now studying the effects of evodiamine on the intracellular mechanisms of the catecholamine secretory response in bovine adrenal chromaffin cells in culture.

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