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European Journal of Pharmacology 327 (1997) 135–141

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Apamin-sensitive SK_{Ca} channels modulate adrenal catecholamine release in anesthetized dogs

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Received 7 November 1996; revised 21 March 1997; accepted 25 March 1997

Abstract

We investigated the role of high conductance (BK_{Ca}) and small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels in adrenal catecholamine release in response to splanchnic nerve stimulation, acetylcholine, the nicotinic receptor stimulant 1,1-dimethyl-4-phenyl-piperazinium (DMPP), and muscarine in anesthetized dogs. The selective SK_{Ca} channel blocker apamin and the selective BK_{Ca} channel blocker charybdotoxin were infused into the adrenal gland through the phrenicoabdominal artery, and the cholinergic agonists were injected into the same artery. Splanchnic nerve stimulation (1, 2, 3 and 10 Hz), acetylcholine (0.75, 1.5 and 3 μg), DMPP (0.1, 0.2 and 0.4 μg) and muscarine (0.5, 1 and 2 μg) produced frequency- or dose-dependent increases in catecholamine output as measured in adrenal venous blood. Apamin infusion (1, 3 and 10 ng/min) enhanced the acetylcholine-, DMPP- and muscarine-induced increases in catecholamine output in a dose-dependent manner, but it did not affect the splanchnic nerve stimulation-induced catecholamine response. Charybdotoxin infusion (10, 30 and 100 ng/min) did not affect the increases in catecholamine output induced by the agonists and splanchnic nerve stimulation. Neither apamin nor charybdotoxin affected basal catecholamine output. These results suggest that apamin-sensitive SK_{Ca} channels located in adrenal medullary cells may play an inhibitory role in the regulation of adrenal catecholamine release mediated by extrasynaptic nicotinic and muscarinic receptors.

Keywords: Adrenal catecholamine; K^+ channel; Ca^{2+}-activated; SK_{Ca} channel; BK_{Ca} channel; Apamin; Charybdotoxin; Splanchnic nerve stimulation; Acetylcholine; DMPP (1,1-dimethyl-4-phenyl-piperazinium); Muscarine

1. Introduction

Acetylcholine released from splanchnic nerve terminal depolarizes the adrenal medulla chromaffin cell membrane through nicotinic receptors, and the subsequent opening of voltage-dependent Ca^{2+} channels results in an increase in Ca^{2+} influx (Cena et al., 1983; Corcoran and Kirshner, 1983). The elevation of intracellular Ca^{2+} triggers the exocytotic release of adrenal catecholamines (Garcia et al., 1984) and may simultaneously activate Ca^{2+}-activated K^+ channels (Marty, 1981), leading to hyperpolarization and inhibition of further influx of Ca^{2+}. Therefore, Ca^{2+}-activated K^+ channels, which act as a link between intracellular Ca^{2+} and the membrane potential, might control the release of adrenal catecholamines.

Apamin, a 18-amino-acid peptide isolated from the venom of the bee *Apis mellifera* (Habermann, 1972), is a selective and potent blocker of small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels in various cell types (Brown and Higashida, 1988; Lang and Ritchie, 1990), including bovine chromaffin cells (Artalejo et al., 1993). Blockade of SK_{Ca} channels by apamin is thought to inhibit the efflux of K^+ from the cell, and lead to changes in the membrane potential in a depolarizing direction. In fact, apamin has been shown to inhibit an afterhyperpolarization in rat sympathetic neurons (Kawai and Watanabe, 1986), bullfrog sympathetic ganglion cells (Pennefather et al., 1985) and rat adrenal chromaffin cells (Neely and Lingle, 1992). These studies provided evidence suggesting that SK_{Ca} channels play an important role in the hyperpolarizing phase of membrane potentials after depolarization. In bovine chromaffin cells, SK_{Ca} channels have been demonstrated and characterized (Artalejo et al., 1993). Furthermore, binding sites for apamin, distinct from high conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, have also been identified in bovine chromaffin cells (Lara et al., 1995). In

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PII S0014-2999(97)00089-7

perfused cat adrenal glands (Uceda et al., 1992, 1994), both catecholamine release and elevation of intracellular Ca^{2+} elicited by methacholine, which is pure muscarinic agonist, are markedly potentiated by apamin. On the other hand, BK_{Ca} channels have been reported to be present in bovine chromaffin cells (Marty, 1981). However, little is known about the contribution of BK_{Ca} channels to the regulation of adrenal catecholamine release (Montiel et al., 1995).

In the present study, we investigated the effects of apamin and charybdotoxin, a BK_{Ca} channel blocker, on the release of catecholamines evoked by splanchnic nerve stimulation, acetylcholine, 1,1-dimethyl-4-phenyl-piperazinium (DMPP) and muscarine in anesthetized dogs, in order to elucidate the functional role of SK_{Ca} and BK_{Ca} channels in the control of the release of adrenal catecholamines. Apamin, charybdotoxin and cholinergic agonists were administered intraarterially (i.a.) into the adrenal gland to eliminate their hemodynamic influences on adrenal catecholamine release.

2. Materials and methods

2.1. Animal preparation

Mongrel dogs of either sex, weighing 9–13 kg, were anesthetized with 30 mg/kg i.v. of sodium pentobarbital, and a constant level of anesthesia was then maintained by an i.v. infusion of sodium pentobarbital at a rate of 4–6 mg/kg per h with an infusion pump (201B, Atom, Tokyo, Japan). Artificial respiration was performed by means of a respiration pump (model 607, Harvard Apparatus, Millis, MA, USA), with room air being administered at 18 strokes/min (20 ml/kg tidal volume). The surgical procedure used in the present study was described previously (Kimura et al., 1988). The left adrenal gland was exposed by a retroperitoneal flank incision, and a polyethylene cannula was inserted into the left adrenolumbar vein for collection of the venous effluent blood from the adrenal gland. A thread was placed around the juncture of the adrenolumbar vein with the abdominal vena cava. Adrenal blood samples were obtained by pulling the thread, thus occluding the adrenolumbar vein and causing a retrograde flow of blood. The 1- or 2-ml blood samples were collected in chilled test tubes containing disodium EDTA. When not being sampled, adrenal venous blood was returned directly to the vena cava. Coagulation of blood was prevented by an initial i.v. injection of sodium heparin (500 U/kg) and hourly i.v. injections of 100 U/kg. Systemic blood pressure and heart rate were measured with a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) and a cardiotachometer (RT-5, Nihon Kohden), respectively, and recorded on a heat-writing oscillograph (RJG-4128, Nihon, Kohden).

2.2. Administration of drugs into the adrenal gland

The procedure for i.a. administration of drugs into the adrenal gland was reported previously (Kimura et al., 1992). The left phrenicoabdominal artery was dissected to expose its origin from the abdominal aorta. A needle connected to a Y-shaped polyethylene catheter was inserted into the phrenicoabdominal artery at its origin for i.a. infusion of 0.9% saline solution (as a vehicle), apamin and charybdotoxin. These drugs were infused into the adrenal gland by using an infusion pump (975E, Harvard Apparatus). Acetylcholine, DMPP and muscarine were injected for 3 s during saline, apamin and charybdotoxin infusion.

2.3. Splanchnic nerve stimulation

The left splanchnic nerves were dissected free from surrounding tissue and cut. A bipolar platinum electrode was placed in contact with the distal end of the splanchnic nerves. The splanchnic nerves were stimulated with rectangular pulses of 1 ms and 10 V (supramaximal voltage) delivered by an electronic stimulator (SEN-1101, Nihon Kohden) and an isolation unit (SS-101J, Nihon Kohden). In low-frequency stimulation experiments, stimuli were applied at 1 Hz for 2 min, subsequently 2 Hz for 2 min, and 3 Hz for 2 min during a 6-min stimulus period. In high-frequency stimulation experiments, stimuli were applied at 10 Hz for 20 s.

2.4. Experimental protocol

The dogs were divided into eight groups (groups 1–4 and groups 5–8 apamin and charybdotoxin experiments, respectively). In group 1 ($n = 12$), the effect of apamin on increases in catecholamine output induced by splanchnic nerve stimulation at low (1, 2 and 3 Hz) or high frequency (10 Hz) was examined. The low- ($n = 7$) and high- ($n = 5$) frequency stimulation experiments were carried out in separate animals. Splanchnic nerve stimulation was repeated 4 times at 30-min intervals. The first splanchnic nerve stimulation trial during saline infusion into the adrenal gland was regarded as a control. Apamin infusions (1, 3 and 10 ng/min) were started 5 min before the start of the second, third and fourth splanchnic nerve stimulation, respectively. In group 2 ($n = 7$), the effect of apamin on the acetylcholine-induced increase in catecholamine output was examined. A set of acetylcholine injections (0.75, 1.5 and 3 μg) into the adrenal gland was repeated 4 times at 40-min intervals. The interval between each dose of acetylcholine was 10 min. The first set of acetylcholine injections during the infusion of 0.9% saline solution was regarded as a control. Apamin infusion was started 5 min before the second, third and fourth set of acetylcholine injections, respectively. In groups 3 ($n = 7$) and 4 ($n = 8$),

the effects of apamin on increases in catecholamine output induced by DMPP (0.1, 0.2 and 0.4 μg) and muscarine (0.5, 1 and 2 μg) were examined, respectively, with the same protocol as used in group 2. The effects of charybdotoxin (10, 30 and 100 ng/min) on increases in catecholamine output induced by splanchnic nerve stimulation (group 5; $n = 6$), acetylcholine (group 6; $n = 9$), DMPP (group 7; $n = 6$) and muscarine (group 8; $n = 6$) were examined with the same protocol as used in the apamin experiments.

2.5. Blood sampling and determination of adrenal catecholamine output

In groups 1–8, adrenal venous blood was sampled before and during splanchnic nerve stimulation and injections of acetylcholine, DMPP and muscarine to determine basal catecholamine output and stimuli-induced increases in catecholamine output, respectively. Sampling during the basal state (during saline, apamin or charybdotoxin infusion) was performed 2 min before splanchnic nerve stimulation or sets of acetylcholine receptor agonist injections. The time required to collect 1 ml (during basal state or splanchnic nerve stimulation at 1–3 Hz) or 2 ml (during splanchnic nerve stimulation at 10 Hz or acetylcholine receptor agonist injections) of blood served to estimate adrenal venous flow rate.

Adrenal blood samples were centrifuged to obtain plasma samples. Catecholamines were extracted from plasma by the alumina adsorption method, and plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection (LC-304, Bioanalytical Systems, West Lafayette, IN, USA), as described previously (Kimura et al., 1988). Epinephrine and norepinephrine output (ng/min) was calculated by multiplying plasma catecholamine concentration (ng/ml) by adrenal plasma flow rate (ml/min), and the total output of epinephrine and norepinephrine was expressed as the catecholamine output. Adrenal plasma flow rate was calculated by multiplying adrenal venous blood flow by 1 − hematocrit. The basal catecholamine output was determined from samples collected before splanchnic nerve stimulation or injections of the cholinergic agonists. The splanchnic nerve stimulation-, acetylcholine-, DMPP- or muscarine-induced changes in catecholamine output were calculated by subtracting the basal catecholamine output from that obtained during the stimulus state.

2.6. Analysis of data

The results were expressed as means ± S.E.M. throughout the study. Analysis of variance was used for statistical analysis of multiple comparisons of data. P values smaller than 0.05 were considered to be statistically significant.

2.7. Drugs

The drugs used were apamin, charybdotoxin (Peptide Institute, Osaka, Japan), acetylcholine chloride (Daiichi Seiyaku, Tokyo, Japan), 1,1-dimethyl-4-phenylpiperazinium iodide (Aldrich, Milwaukee, WI, USA) and muscarine chloride (Sigma, St. Louis, MO, USA). All drugs were dissolved in 0.9% saline solution.

3. Results

3.1. Increases in catecholamine output in response to splanchnic nerve stimulation, acetylcholine, DMPP and muscarine

Splanchnic nerve stimulation at low (1, 2 and 3 Hz) and high frequency (10 Hz) or i.a. injections of acetylcholine (0.75, 1.5 and 3 μg), DMPP (0.1, 0.2 and 0.4 μg) and muscarine (0.5, 1 and 2 μg) into the adrenal gland pro-

Table 1
Effects of apamin infusion on adrenal plasma flow during the basal state and during splanchnic nerve stimulation (SNS) and injections of acetylcholine (ACh), 1,1-dimethyl-4-phenyl-piperazinium (DMPP) and muscarine (Mus)

Experiment	Adrenal plasma flow rate (ml/min)			
	Control	Apamin infusion (ng/min)		
		1	3	10
Group 1				
(1) Low frequency ($n = 7$)				
Basal state	2.6 ± 0.4	2.3 ± 0.3 [a]	2.2 ± 0.3 [a]	2.0 ± 0.3 [b]
SNS 1 Hz	2.1 ± 0.3	2.0 ± 0.3	1.9 ± 0.3 [b]	1.7 ± 0.3 [b]
SNS 2 Hz	2.2 ± 0.3	2.1 ± 0.4	2.0 ± 0.3 [a]	1.8 ± 0.3 [b]
SNS 3 Hz	2.5 ± 0.4	2.4 ± 0.4	2.1 ± 0.4 [b]	2.0 ± 0.3 [b]
(2) High frequency ($n = 5$)				
Basal state	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.2
SNS 10 Hz	1.7 ± 0.2 [c]	1.5 ± 0.2 [b]	1.4 ± 0.2 [b]	1.3 ± 0.1 [b]
Group 2 ($n = 7$)				
Basal state	2.0 ± 0.3	1.8 ± 0.2	1.8 ± 0.3	1.5 ± 0.3 [b]
ACh 0.75 μg	2.8 ± 0.3 [c]	2.6 ± 0.3	2.6 ± 0.4	2.4 ± 0.4 [b]
ACh 1.5 μg	2.9 ± 0.3 [c]	2.6 ± 0.3	2.7 ± 0.4	2.4 ± 0.4 [a]
ACh 3 μg	2.8 ± 0.4 [c]	2.7 ± 0.4	2.7 ± 0.4	2.6 ± 0.4
Group 3 ($n = 7$)				
Basal state	2.4 ± 0.4	2.3 ± 0.4	2.0 ± 0.3 [a]	1.9 ± 0.3 [b]
DMPP 0.1 μg	2.4 ± 0.4	2.3 ± 0.3	2.1 ± 0.3	1.8 ± 0.2 [b]
DMPP 0.2 μg	2.5 ± 0.4	2.3 ± 0.3	2.1 ± 0.3	1.8 ± 0.3 [a]
DMPP 0.4 μg	2.5 ± 0.4	2.3 ± 0.3	2.2 ± 0.3	2.0 ± 0.3 [a]
Group 4 ($n = 8$)				
Basal state	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	1.8 ± 0.2
Mus 0.5 μg	3.3 ± 0.3 [c]	3.3 ± 0.3	3.1 ± 0.3 [a]	3.0 ± 0.3 [b]
Mus 1 μg	3.5 ± 0.3 [c]	3.4 ± 0.3	3.2 ± 0.3 [b]	3.1 ± 0.3 [b]
Mus 2 μg	3.9 ± 0.4 [c]	3.7 ± 0.3	3.6 ± 0.3 [a]	3.3 ± 0.3 [b]

[a] $P < 0.05$, [b] $P < 0.01$ as compared with the corresponding control values. [c] $P < 0.01$ as compared with the values during the basal state under control conditions. Values represent means ± S.E.M.

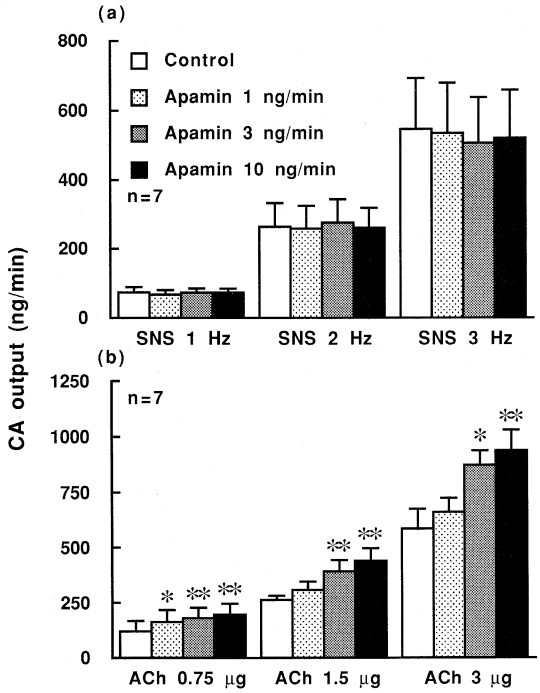

Fig. 1. Effects of apamin on catecholamine (CA) output from the adrenal gland in response to splanchnic nerve stimulation (SNS, a) and acetylcholine (ACh, b) injected into the phrenicoabdominal artery. Apamin was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. * $P < 0.05$; ** $P < 0.01$, compared with corresponding control values obtained before the apamin infusion.

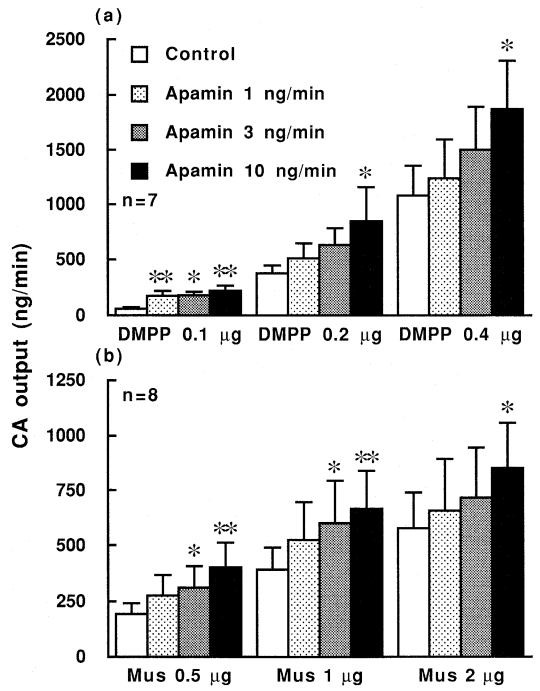

Fig. 2. Effects of apamin on catecholamine (CA) output from the adrenal gland in response to 1,1-dimethyl-4-phenyl-piperazinium (DMPP, a) and muscarine (Mus, b) injected into the phrenicoabdominal artery. Apamin was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. * $P < 0.05$; ** $P < 0.01$, compared with corresponding control values obtained before the apamin infusion.

duced frequency- or dose-dependent increases in adrenal venous plasma catecholamine concentration (data not shown). The 10 Hz splanchnic nerve stimulation-, acetylcholine- and muscarine-induced increases in catecholamine concentration were accompanied by increases in adrenal plasma flow rate (Table 1). Splanchnic nerve stimulation at 1, 2 and 3 Hz and DMPP had no effect on adrenal plasma flow rate. Catecholamine output, calculated from the catecholamine concentration and the adrenal plasma flow rate, was increased by splanchnic nerve stimulation, acetylcholine, DMPP and muscarine. The increases in catecholamine output induced by splanchnic nerve stimulation (3 and 10 Hz), acetylcholine (3 μg), DMPP (0.4 μg) and muscarine (2 μg) during the control stimulation periods were 505 ± 80 ($n = 13$), 1092 ± 229 ($n = 5$), 638 ± 109 ($n = 16$), 1187 ± 225 ($n = 13$) and 488 ± 95 ng/min ($n = 14$), respectively, in groups 1–8, in which basal catecholamine output during the resting state was 2.6 ± 0.7 ng/min ($n = 61$).

Splanchnic nerve stimulation produced small pressor and bradycardic responses. The increase in blood pressure produced by 10-Hz splanchnic nerve stimulation was 16 ± 3 mmHg ($n = 5$), and the decrease in heart rate was 12 ± 4 beats/min ($n = 5$). Both acetylcholine and muscarine decreased blood pressure slightly, but they did not modify

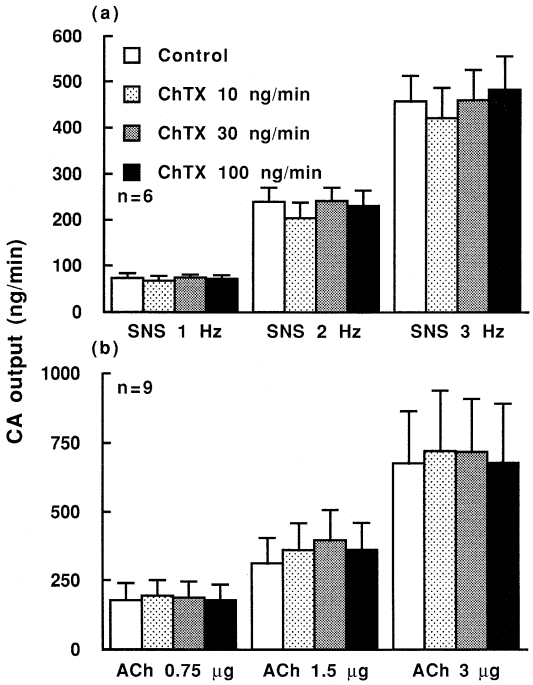

Fig. 3. Effects of charybdotoxin (ChTX) on catecholamine (CA) output from the adrenal gland in response to splanchnic nerve stimulation (SNS, a) and acetylcholine (ACh, b) injected into the phrenicoabdominal artery. ChTX was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. There were no significant differences ($P > 0.05$) in the splanchnic nerve stimulation- and acetylcholine-induced increases in catecholamine output before (control) and during the ChTX infusion.

heart rate. The decreases in blood pressure produced by 3-μg acetylcholine and 2-μg muscarine were 10 ± 2 (n = 16) and 25 ± 3 mmHg (n = 14), respectively. DMPP had no effect on blood pressure and heart rate.

3.2. Effects of apamin on the splanchnic nerve stimulation-, acetylcholine-, DMPP- and muscarine-induced increases in catecholamine output

Infusion of apamin (1, 3 and 10 ng/min) into the adrenal gland enhanced the acetylcholine-, DMPP- and muscarine-induced increases in catecholamine output in a dose-dependent manner (Figs. 1 and 2). The increases in catecholamine output induced by low-frequency splanchnic nerve stimulation at 1, 2 and 3 Hz were not affected even by the highest dose (10 ng/min) of apamin (Fig. 1a). The catecholamine response to high-frequency stimulation at 10 Hz was also not affected by apamin. The increases in catecholamine output induced by high-frequency stimulation before and during 1-, 3- and 10-ng/min apamin infusion were 1092 ± 229, 954 ± 182, 826 ± 144 and 905 ± 99 ng/min (n = 5), respectively.

Apamin did not affect basal catecholamine output. In groups 1–4 (n = 34), basal catecholamine output before and during 1-, 3- and 10-ng/min apamin infusion was 2.4 ± 0.7, 3.3 ± 1.4, 3.4 ± 1.7 and 3.0 ± 1.5 ng/min, respectively. Adrenal plasma flow rate was decreased by apamin (Table 1). Apamin did not affect blood pressure (mean pressure; 122 ± 3 mmHg) or heart rate (128 ± 6 beats/min) in groups 1–4 (n = 34).

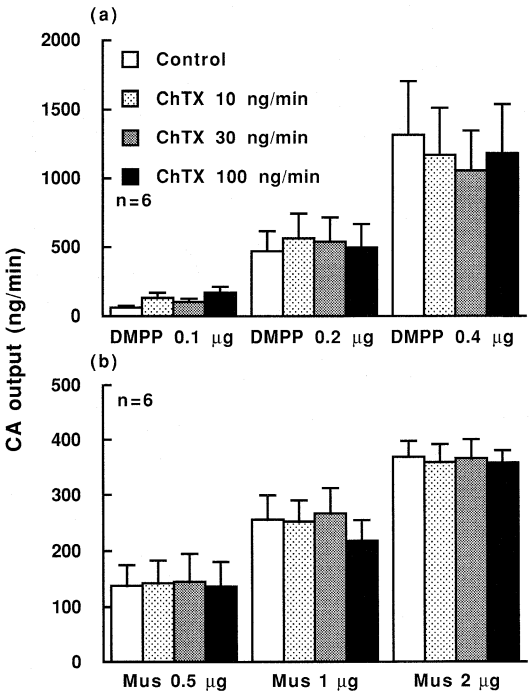

Fig. 4. Effects of charybdotoxin (ChTX) on catecholamine (CA) output from the adrenal gland in response to 1,1-dimethyl-4-phenyl-piperazinium (DMPP, a) and muscarine (Mus, b) injected into the phrenicoabdominal artery. ChTX was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. There were no significant differences ($P > 0.05$) in DMPP- and muscarine-induced increases in catecholamine output before (control) and during the ChTX infusion.

Table 2
Effects of charybdotoxin infusion on adrenal plasma flow during the basal state and during splanchnic nerve stimulation (SNS) and injections of acetylcholine (ACh), 1,1-dimethyl-4-phenyl-piperazinium (DMPP) and muscarine (Mus)

Experiment	Adrenal plasma flow rate (ml/min)			
	Control	Charybdotoxin infusion (ng/min)		
		10	30	100
Group 5 (n = 6)				
Basal state	2.3 ± 0.5	2.1 ± 0.4	2.1 ± 0.4	1.9 ± 0.4
SNS 1 Hz	2.1 ± 0.5	2.0 ± 0.5	1.9 ± 0.4	1.8 ± 0.4
SNS 2 Hz	2.3 ± 0.5	2.1 ± 0.5	2.2 ± 0.5	2.0 ± 0.5
SNS 3 Hz	2.4 ± 0.5	2.2 ± 0.5	2.3 ± 0.5	2.2 ± 0.4
Group 6 (n = 9)				
Basal state	2.0 ± 0.2	1.9 ± 0.2	1.8 ± 0.1 [b]	1.7 ± 0.1 [b]
ACh 0.75 μg	3.3 ± 0.3 [c]	3.3 ± 0.3	3.0 ± 0.2	3.0 ± 0.2 [b]
ACh 1.5 μg	3.5 ± 0.3 [c]	3.4 ± 0.3	3.3 ± 0.2	3.0 ± 0.2 [b]
ACh 3 μg	3.8 ± 0.3 [c]	3.6 ± 0.3	3.3 ± 0.3 [b]	3.2 ± 0.3 [b]
Group 7 (n = 6)				
Basal state	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2 [b]	1.1 ± 0.2 [b]
DMPP 0.1 μg	1.4 ± 0.2	1.4 ± 0.2	1.3 ± 0.2 [b]	1.2 ± 0.2 [b]
DMPP 0.2 μg	1.5 ± 0.2	1.4 ± 0.2	1.3 ± 0.2 [b]	1.2 ± 0.3 [b]
DMPP 0.4 μg	1.6 ± 0.3	1.5 ± 0.3	1.4 ± 0.2 [b]	1.2 ± 0.3 [b]
Group 8 (n = 6)				
Basal state	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1 [a]	1.6 ± 0.1 [b]
Mus 0.5 μg	3.2 ± 0.3 [c]	3.1 ± 0.3	3.0 ± 0.2 [a]	2.8 ± 0.2 [b]
Mus 1 μg	3.5 ± 0.3 [c]	3.3 ± 0.2 [a]	3.2 ± 0.2 [a]	2.9 ± 0.2 [b]
Mus 2 μg	3.8 ± 0.3 [c]	3.6 ± 0.3	3.4 ± 0.2 [a]	3.2 ± 0.3 [b]

[a] $P < 0.05$, [b] $P < 0.01$ as compared with the corresponding control values. [c] $P < 0.01$ as compared with the values during the basal state under control conditions. Values represent means ± S.E.M.

3.3. Effects of charybdotoxin

Infusion of charybdotoxin (10, 30 and 100 ng/min) into the adrenal gland did not affect the splanchnic nerve stimulation-, acetylcholine-, DMPP- and muscarine-induced increases in catecholamine output (Figs. 3 and 4).

Basal catecholamine output was not affected by charybdotoxin. In groups 5–8 (n = 27), basal catecholamine output before and during 10-, 30- and 100-ng/min charybdotoxin infusion was 2.6 ± 1.2, 2.3 ± 0.9, 2.0 ± 0.9 and 2.5 ± 1.4 ng/min, respectively. Adrenal plasma flow rate was decreased by charybdotoxin (Table 2). Charybdotoxin did not affect blood pressure (mean pressure; 121 ± 3 mmHg) or heart rate (125 ± 5 beats/min) in groups 5–8 (n = 27).

4. Discussion

Acetylcholine, DMPP and muscarine administered i.a. into the adrenal gland caused marked increases in cate-

cholamine output. The highest dose of acetylcholine (3 μg) or muscarine (2 μg) produced a fall in blood pressure. However, it is unlikely that baroreflex-mediated catecholamine release is involved in the catecholamine responses to both agonists, because the adrenal gland was decentralized by cutting the splanchnic nerves and because adrenal vein blood sampling was completed before the pressure change. The i.a. administration method made it possible to examine the direct action of apamin and charybdotoxin on adrenal catecholamine release in response to splanchnic nerve stimulation and cholinergic agonists under in vivo conditions.

Apamin infused into the adrenal gland enhanced the release of adrenal catecholamines in response to acetylcholine, DMPP and muscarine in a dose-dependent manner without affecting the basal catecholamine output. This indicates that apamin influences the release process induced by these stimuli, but that the toxin dose not stimulate the release process by itself. The enhancing effects of apamin on the DMPP- and muscarine-induced release of catecholamines indicate that apamin facilitates catecholamine release by affecting the process mediated by nicotinic and muscarinic receptors, respectively. Previously, we demonstrated, under the same experimental conditions as in this study, that acetylcholine stimulates the release of catecholamines by activating both nicotinic and muscarinic receptors (Kimura et al., 1992). On the basis of these findings, the enhancing effect of apamin on the acetylcholine-induced release of catecholamines is explained by its facilitatory actions on both nicotinic and muscarinic receptor-mediated pathways. These results suggest that SK_{Ca} channels play an inhibitory role in the adrenal catecholamine release mediated by both nicotinic and muscarinic receptors, as suggested in the perfused cat adrenal gland (Uceda et al., 1992, 1994) and in bovine adrenal chromaffin cells (Lara et al., 1995).

Nicotinic receptor activation promotes Na^+ and Ca^{2+} influx through receptor-linked ion channels, and the resulting depolarization produces Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Cena et al., 1983; Garcia et al., 1984). The elevation of intracellular Ca^{2+} triggers the release of catecholamines and simultaneously may activate SK_{Ca} channels. The activation of SK_{Ca} channels increases K^+ efflux, and the resulting hyperpolarization leads to inhibition of further Ca^{2+} influx. As a result, the release of catecholamines may be inhibited. Therefore, it is probable that apamin enhances the release of catecholamines mediated by nicotinic receptors by blocking the SK_{Ca} channel-mediated inhibition of Ca^{2+} influx. However, the elevation of intracellular Ca^{2+} mobilized from intracellular storage sites is thought to contribute to the muscarinic receptor-mediated release of adrenal catecholamines (Misbahuddin et al., 1985; Nakazato et al., 1988; O'Sullivan and Burgoyne, 1989). Furthermore, it has been shown that muscarinic receptor activation depolarizes the adrenal chromaffin cells of chickens (Knight and Baker, 1986), rats (Akaike et al., 1990) and guinea-pig (Inoue and Kuriyama, 1991). Therefore, the facilitatory effect of apamin on the muscarinic receptor-mediated release of catecholamines can be explained in the same manner as for the nicotinic receptor-mediated release.

Apamin did not affect the splanchnic nerve stimulation-induced increases in catecholamine output. Previously, we reported that splanchnic nerve stimulation-induced catecholamine release in the dog adrenal gland is mainly mediated by nicotinic receptors (Shimamura et al., 1991; Kimura et al., 1992). Therefore, the results of the present study indicate that SK_{Ca} channels have no role in nicotinic receptor-mediated catecholamine release in response to endogenous acetylcholine, and the different contribution of SK_{Ca} channels to catecholamine release elicited by endogenous and exogenous acetylcholine is surprising. This result is not consistent with the observation that in the perfused cat adrenal gland apamin enhances the transmural electrical stimulation-induced release of catecholamines without affecting the presynaptic release of acetylcholine (Montiel et al., 1995). These authors demonstrated that at lower frequencies (1 and 2 Hz) secretory responses were not significantly enhanced by apamin but at 5, 10 and 20 Hz secretion was significantly potentiated. In the present study, however, apamin did not affect catecholamine responses to low-frequency splanchnic nerve stimulation (1, 2 and 3 Hz) or to high-frequency stimulation (10 Hz). Thus, the contribution of SK_{Ca} channels to the release of catecholamines in response to sympathetic stimulation may differ from species to species.

The question arises as to why SK_{Ca} channels act on the nicotinic receptor-mediated release of catecholamines differently; an inhibition in the case of injections of acetylcholine and DMPP, and no effect in the case of splanchnic nerve stimulation. As a possible explanation for this, the different distribution of SK_{Ca} channels on the medullary cell membrane in synaptic and extrasynaptic regions can be considered. Endogenous acetylcholine released from the splanchnic nerves would predominantly activate nicotinic receptors located intrasynaptically. Exogenous acetylcholine and DMPP delivered through the arterial supply could diffuse into extrasynaptic regions and would predominantly activate nicotinic receptors located extrasynaptically. If SK_{Ca} channels are primarily concentrated in extrasynaptic regions but not in synaptic zones, they could affect the depolarization due to the activation of extrasynaptic nicotinic receptors but could not affect the depolarization due to the activation of intrasynaptic nicotinic receptors. However, the release of catecholamines under physiological conditions is caused by activation of the splanchnic nerves. Therefore, the physiological role of extrasynaptic SK_{Ca} channels remains to be resolved.

Charybdotoxin did not affect the increases in catecholamine output in response to splanchnic nerve stimulation, acetylcholine, DMPP and muscarine. Although the possibility remains that diffusion of charybdotoxin into

adrenal mudulla cells is restricted partially by its size (it is a large), these results are consistent with the observation that charybdotoxin has no effect on the transmural electrical stimulation-induced release of catecholamines in the perfused cat adrenal gland (Montiel et al., 1995). Thus, it is suggested that BK_{Ca} channels have no role in the release of catecholamines from the adrenal gland.

In conclusion, this study demonstrates that apamin facilitates adrenal catecholamine release in response to acetylcholine, DMPP and muscarine, but not to splanchnic nerve stimulation in the anesthetized dog. No effect of charybdotoxin on catecholamine secretion was observed. These results suggest that SK_{Ca} channels located in adrenal medulla cells may play an inhibitory role in the release of catecholamines mediated by extrasynaptic nicotinic and muscarinic receptors.

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