응용약물학회지, The Journal of Applied Pharmacology, **10**, 142-151(2002)

Influence of Apamin on Catecholamine Secretion from the Rat Adrenal Medulla

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(Received September 3, 2002 ; accepted September 26, 2002)

Abstract–The present study was attempted to investigate the effect of apamin on catecholamine (CA) secretion evoked by ACh, high K^+, DMPP, McN-A-343, cyclopiazonic acid and Bay-K-8644 from the isolated perfused rat adrenal gland and to establish the mechanism of its action. The perfusion of apamin (1 nM) into an adrenal vein for 20 min produced greatly potentiation in CA secretion evoked by ACh (5.32×10^{-3} M), high K^+ (5.6×10^{-2} M), DMPP (10^{-4} M for 2 min), McN-A-343 (10^{-4} M for 2 min), cyclopiazonic acid (10^{-5} M for 4 min) and Bay-K-8644 (10^{-5} M for 4 min). However, apamin itself did fail to affect basal catecholamine output. Furthermore, in adrenal glands preloaded with apamin (1 nM) under the presence of glibenclamide (10^{-6} M), an antidiabetic sulfonylurea that has been shown to be a specific blocker of ATP-regulated potassium channels (for 20 min), CA secretion evoked by DMPP and McN-A-343 was not affected. However, the perfusion of high concentration of apamin (100 nM) into an adrenal vein for 20 min rather inhibited significantly CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, cyclopiazonic acid and Bay-K-8644. Taken together, these results suggest that the low concentration of apamin causes greatly the enhancement of CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors as well as by membrane depolarization.These findings suggests that apamin-sensitive SK (Ca^{2+}) channels located in rat adrenal medullary chromaffin cells may play an inhibitory role in the release of catecholamines mediated by stimulation of cholinergic nicotinic and muscarinic receptors as well as membrane depolarization. However, it is thought that high concentration of apamin cause the inhibitory responses in catecholamine secretion evoked by stimulation of cholinergic receptors as well as by membrane depolarization from the rat adrenal gland without relevance with the SK channel blockade.

Key words □ Apamin, adenal gland, catecholamine secretion, apamin-sensitive SK (Ca^{2+}) channels.

Apamin is known to be an octadecapeptide found in the venom of the honeybee (Habermann, 1992), which is a hydrophilic peptide and has a very high specific activity. It has been reported that apamin has the ability to selectively block the small conductance Ca^{2+}-activated K^+(SK) channels of rat sympathetic neurones (Kawai and Watanabe, 1986), cultured rat skeletal muscle fibers (Balatz and Magleby, 1986) and mammalian hepatocytes and red cells (Burguess *et al.*, 1981). It has been also found that membrane K^+ channels in various cells are responsible for controlling the membrane potential and excitability of cells (Petersen & Maruyama, 1984; Cook, 1988; Watson & Abbott 1991). The opening (activation) of these channels causeshyperpolarization, and conversely, their closing (inhibition) causes depolarization of the cell membrane. Electrophysiological studies using voltage- and patch-clamp technique have shown the presence of many different types of K^+ channels, which are sensitive to neurotransmitters, hormones, toxins and drugs (Cook, 1988; Watson & Abbott, 1991; Uceda *et al.*, 1992; Attwood *et al.*, 1991; Edwards & Weston, 1990).

Masuda and his coworkers (1994) have shown that in cultured bovine adrenal chromaffin cells, the K^+ channel openers, cromakalim and pinacidil, selectively inhibit CA secretory responses induced by moderate depolarization or by stimulation of nicotinic ACh receptors. Under physiological conditions, small-conductance, Ca^{2+}-activated K^+ channels are known to control some of the electrical activity of bovine chromaffin cells (Lara *et al.*, 1995). Ca^{2+}-dependent K^+ channels, probably of the small-conductance type have been reported to be involved in the modulation of muscarinic-evoked CA secretory responses in cat adrenal chromaffin cells (Uceda *et al.*, 1992). More recently, Lim and his colleagues

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(2000) have reported that pinacidil inhibits CA secretion greatly in the isolated perfused rat adrenal gland.

In the present study, it was attempted to investigatethe effect of apamin on the CA secretory responses evoked by stimulation of cholinergic receptors and membrane depolarization in the isolated perfused rat adrenal glands, and to clarify whether SK channels can regulate the secretory responses of CA.

MATERIALS AND METHODS

Experimental Procedure

Male Sprague-Dawley rats, weighing 180 to 250 grams, were anesthetized with thiopental sodium (40 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, andthe left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 ± 1°C.

Perfusion of Adrenal Gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co., U.S.A.) at a rate of 0.3 ml/min. Theperfusion was carried out withKrebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95 % O_2 + 5% CO_2 and the final pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of catecholamine.

Drug Administration

The perfusions of DMPP (100 M) and McN-A-343 (100 M) for 2 minutes and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock, and Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were also perfused for 4 min, respectively. In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, Bay-K-8644 and cyclopiazonic acid returned to pre-injection level in about 4 min, but the responses to DMPP in 8 min.

Collection of Perfusate

As a rule, prior to stimulation with various secretagogues, perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's was collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted from the stimulated sample to obtain thenet secretion value of CA, which is shown in all of the figures. To study the effects of apamin on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing apamin for 20 min.The perfusate was collected for a certain minute (background sample), and then the medium was changed to the one containing the stimulating agent and the perfusates were collected for the same period as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of Catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co., Italy). A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretogogues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in

terms of norepinephrine (base) equivalents.

Statistical Analysis

The statistical significance between groups was determined by utilizing the Student's *t*- and ANOVA-test. A P-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given inthe text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida and Murray (1987).

Drugs and Their Sources

The following drugs were used: glibenclamide, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K-8644) (Sigma Chemical Co., U.S.A.), cyclopiazonic acd, (3-(m-cholro-phenyl-carbamoyl-oxy)-2-butynyl trimethyl ammonium chloride [McN-A-343] (RBI, U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which were dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effect of 1 nM apamin on CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 22 ± 3 ng/2 min ($n = 8$). It has been shown that in anesthetized dogs, apamin infusion enhances CA output evoked by Ach, DMPP and muscarine in dose-dependent manner (Nagayama *et al.*, 1997). Therefore, it was decided initially to examine the effects of apamin on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion from perfused rat adrenal glands. Secretagogues were given at 20 min-intervals. Apamin was present for 15 min including stimulation with each secretagogue. In the present study, it was also-found that apamin itself did not produce any effect on basal CA output (data not shown), as shown in the previous report

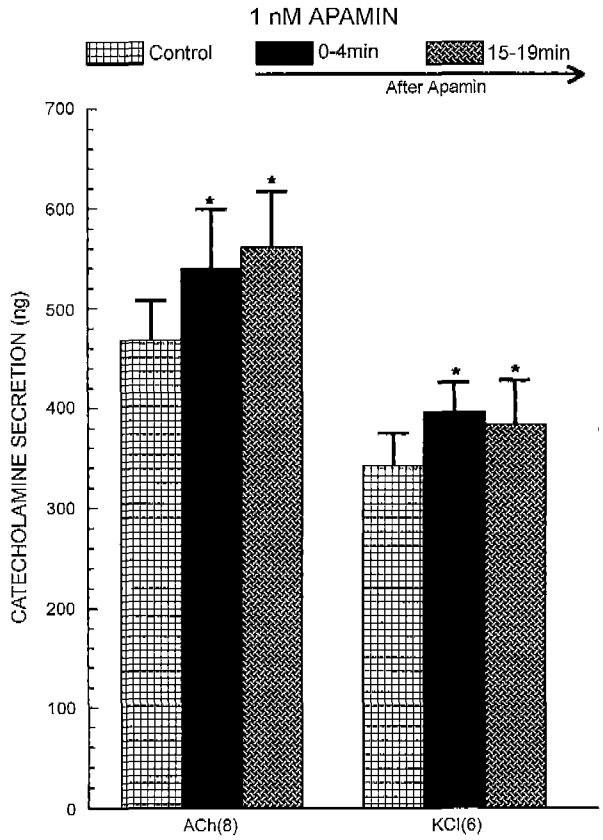

Fig. 1. Effects of 1 nM apamin on secretory responses of catecholamines (CA) evoked by ACh and high potassium from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) and excess K^+ (5.6×10^{-2} M) was induced before (Control) and after preloading with 10^{-9} M of apamin for 15 min, respectively. Numbers in the parenthesis indicate number of experimental rat adrenal glands. The columns and vertical bars represent the mean and the standard error of the mean, respectively. Ordinate: The amounts of CA secreted from the adrenal gland (ng). Abscissa: Secretagogues. The statistical difference was obtained by comparing the corresponding control with groups of 0-4 min and 15-19 min after pretreatment with apamin. Perfusates were collected for 4 minutes at 15 min interval, respectively. The numbers in the parentheses denote number of the rat adrenal glands. ACh: acetylcholine. *: $P < 0.05$.

(Nagayama *et al.*, 1997).

When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 468 ± 60 ng for 4 min. However, after the simultaneous perfusion with 1 nM apamin, ACh-stimulated CA secretion was significantly increased to 115% (0-4 min, $P < 0.05$) and 120% (15-19 min, $P < 0.05$) of the corresponding control (100%) from 8 adrenal glands as shown in Fig. 1. Also, it has earlier been found that a depolarizing agent such as KCl enhances sharply CA secretion. In the present work, high K^+